JOURNAL
OF THE
SCIENCE OF FOOD AND AGRICULTURE
(INCLUDING ABSTRACTS)

Published by the Society of Chemical Industry

Volume 21 | No. 9 | September, 1970

SOCIETY OF CHEMICAL INDUSTRY

Offices of the Society: 14 Belgrave Square, London, S.W.1
Telephone: 01-235 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*
£18 post free, single copies £1 17s. 6d. post free

Subscriptions (Non-members) to:
S.C.I. Publications, The Chemical Society Publications Sales Office,
Blackhorse Road, Letchworth, Hertfordshire
Telephone: 04626 5817

EFFECT OF pH, CONCENTRATION, AND TEMPERATURE ON THE STRENGTH OF CYTOPLASMIC PROTEIN FOAMS

By J. H. BUCKINGHAM

The strength of foams generated from solutions of red clover cytoplasmic protein is dependent on pH, protein concentration, and temperature. The variation of foam strength as a function of these three parameters has been studied and results indicate that the onset of foam stability occurs over very short concentration and temperature ranges.

A phase diagram for these foams is proposed which defines the differences between stable and unstable states in terms of protein concentration and temperature. The results show that above 40°C only unstable foams are generated, irrespective of protein concentration. Below 40°C, stable foams can be generated provided the protein concentration is greater than a certain critical concentration for the system. A possible implication in bloat studies is suggested.

Introduction

Foam is a phenomenon commonly found in nature, and has been well documented in the past. However, in spite of this, certain aspects of foam formation and stabilisation are not completely understood. Adamson[1] has stated that no very rigorous analysis of the inter-relation of factors determining film stability, and hence foam lifetimes, is possible. Consequently it is difficult to define precisely the differences between stable and unstable foams.

Adamson adds that qualitative experiments indicate that foam life-time depends on the drainage rate and this in turn depends on some combination of the fluid and film viscosities, and on the elasticity of the film which determines how extensively drainage must occur before rupture becomes possible. Burcik[2] considers that low equilibrium surface tension, a moderate rate of attainment of equilibrium surface tension, and high surface viscosity are the most important properties leading to high foam stability.

For protein foams, Cumper[3] has shown that stability and surface viscosity both pass through a maximum at the same pH for any given protein. This suggests that the mechanical properties of the surface films are of considerable importance in determining foam stability. It is significant that the optimum pH is often close to the iso-electric point of the protein when the repulsion is zero and the net cohesion greatest.

Mangan,[4,5] working on the problem of bloat in cattle, has undertaken foam strength measurements on cytoplasmic protein foams, foam strength being defined by him as the inverse of the rate of fall of a perforated weight through a column of foam. However, since no attempt was made to define foam strength in terms of the basic physical properties of the foam, such measurements are not absolute and merely give a qualitative indication as to whether one foam is mechanically weaker or stronger than another.

Mangan[5] was, however, able to demonstrate that the strengths of clover cytoplasmic protein foams defined in this manner exhibit a very marked pH dependence, with maximum foam strengths occurring at a pH of about 5·4. The falling weight technique does, therefore, possess considerable merit in that it conveniently indicates any sudden changes in the strength of a foaming system.

The present paper extends Mangan's work and considers the effects of protein concentration and temperature on the strength of foams generated from solutions of red clover cytoplasmic protein.

Experimental

Crude, unfractionated, clover protein was obtained by the method of Lyttleton.[6] Red clover (*Trifolium pratense* L.) was cut and immediately ground in an end-runner mill at 2°C with an equal mass of 0·2 M phosphate buffer, pH 7·5. Cell debris was removed by squeezing through muslin followed by centrifuging at 10,000 × *g* for 30 min. Anhydrous sodium sulphate (11% wt./vol.) was added at room temperature and the mixture was re-centrifuged at 10,000 × *g* to remove precipitated chloroplast fragments. The whole cytoplasmic protein was then precipitated by the addition of 25% wt./vol. sodium sulphate. This precipitate was spun off and dissolved in phosphate buffer, pH 7·5, dialysed against water at 2°C until free from salt, and freeze-dried. The material was then stored in a dessicator at −20°C until required for use. The nitrogen content of the material was approximately 15%, indicating fairly pure protein. The bulk of this cytoplasmic protein (~50%) is homogeneous protein with a molecular weight of 600,000 (fraction I). The remainder is heterogeneous protein with an average molecular weight of about 133,000 (fraction II).

The protein solutions to be foamed were prepared by dissolving carefully weighed quantities of the freeze-dried material in 0·1 M sodium acetate. The pH of each solution was then adjusted with acetic acid. Fresh protein solutions were made up for each foam experiment. It was decided not to use aliquots from a stock solution since protein denaturation (as a function of time) could affect the results. Each solution was aged at room temperature for 30 min prior to foaming to ensure a constant degree of bulk denaturation.

The foam strength apparatus enabled three solutions to be examined simultaneously. It was essentially that described by Mangan[4] and is derived from the apparatus of Clark[7] and Foulk & Miller.[8] It consisted of a temperature-controlled Perspex bath (Fig. 1(a)) in which were mounted three glass tubes 64 cm long × 3·5 cm. Temperature control of the liquid to be foamed was better than ± 0·01°C. Foam height was indicated by horizontal lines scored at 1 cm intervals into the front and back walls of the bath to avoid parallax errors. The bottom ends of the glass tubes were closed with rubber bungs, each carrying a glass sinter (porosity 2), together with an inlet tube for the solution to be foamed. The solutions were foamed by passing nitrogen gas through the sinters. The gas flow was monitored with a Platon Gapmeter Lab-kit and maintained constant at 50 ml/min.

The solution under examination was run in up to the 0-cm mark and was maintained at this level during the foaming process by additions from a reservoir. Foaming was begun 10 min after adding the solution. This delay allowed the temperature of the solution to come to that of the water bath. Using a gas flow rate of 50 ml/min, foam columns 45 cm in height were obtained from most solutions after foaming for approximately 10 min. This time increased as foam stability decreased (in cases where very unstable foams are generated the 45 cm foam height may never be attained). After 45 cm of foam had been generated, the gas supply was turned off and the foam regime was allowed to come to equilibrium for 1 min before the strength of the foam was measured.

Foam strength determinations were carried out by allowing a perforated brass weight to fall through the foam column. The weight (120 g) was in the form of a piston drilled with 35 2 mm diameter holes as shown in Fig. 1(b). There was a nominal 1 mm clearance between the weight and the wall of the tube, but stabilising pips and legs were added to the weight to prevent any tendency for it to rock (and hence stick) in the tube.

Experiments indicated that the strength of the foam column was not uniform throughout its length and tended to be less at the bottom than at the top. This is because the protein is extracted preferentially from solution during foaming with the net result that the concentration of protein in solution decreases with time. Because of this factor, foam strength measurements are always taken while the weight falls through the entire length of the column. This ensures uniformity in the experimental results.

Prior to any foam strength measurements, the weight was gently lowered on to the top of the foam column until bubbles appeared through the holes in the top surface of the weight. The clock was then started, and the weight released and allowed to fall freely through the foam. For the purpose of this experiment, foam strength is defined as the time in seconds taken for the weight to fall through the complete foam column divided by the distance fallen.

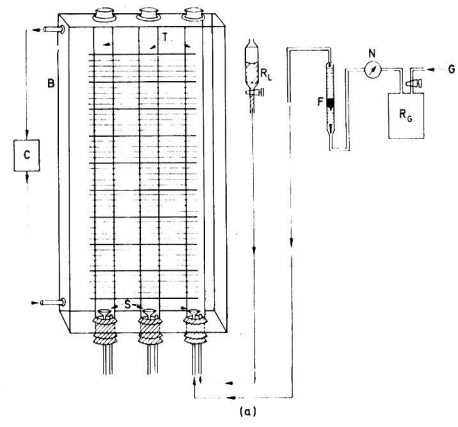

FIG. 1 (a) *Foam strength measuring apparatus* and (b) *brass weight*

B, Temperature-controlled bath; C, temperature controller (± 0·01°C); T, glass tubes; S, sinters; R_L, liquid reservoir; F, flowmeter; N, needle valve; R_G, gas reservoir (5 lb/in² (gauge)); G, gas supply (nitrogen)

Results

Effect of pH

All protein solutions in this investigation contained 60 mg of freeze-dried material/100 ml of solution (i.e., approximately 9 mg nitrogen/100 ml). The temperature of the water bath was maintained constant at 37°c and the pH of the solutions varied from 6·0 to 5·0.

The rigidity of the resulting foams exhibited a very marked pH dependence, with maximum foam strength occurring at a pH of about 5·5 (Fig. 2). This is in agreement with results obtained by Mangan,[5] although the present work suggested a much sharper maximum than hitherto realised. The turnover point has been omitted as the curve tends to become asymptotic on either side of the discontinuity. Below pH 5·5, the protein began to precipitate out of solution with the result that the concentration of protein remaining in solution was reduced. These results illustrate the importance of maintaining constant pH in any investigation of the effects of other parameters (such as protein concentration and temperature) on foam strength. In such experiments, therefore, the pH of the solutions has been maintained constant to better than ± 0·01 pH unit.

Effect of protein concentration

The concentration of protein in solution was varied from 30 mg (4·5 mg N) to 200 mg (30 mg N) of freeze-dried material/100 ml of solution (i.e. 0·03 – 0·2% wt./vol.). Two samples were taken at each concentration step and the pH of these was adjusted to 5·7 and 5·5. The temperature of the water bath was maintained constant at 37°c.

The variation of foam strength with concentration is shown in Fig. 3. It will be noticed that foams produced from solutions containing less than 0·05% wt./vol. freeze-dried material possess little or no strength. Above 0·05% protein, foam strength increases rapidly with increasing protein concentration and then levels off at 0·1% and above. The effect of pH seems merely to be a scaling one since the positions of the discontinuities in the curves are largely independent of pH. The overall increase in foam strength resulting from changing pH from 5·7 to 5·5 supports the statement of Cumper[3] that the cohesion of the molecules at the bubble surfaces is increased. This could indicate that the surface molecules in foams at pH 5·5 are more ordered than those in foams at pH 5·7.

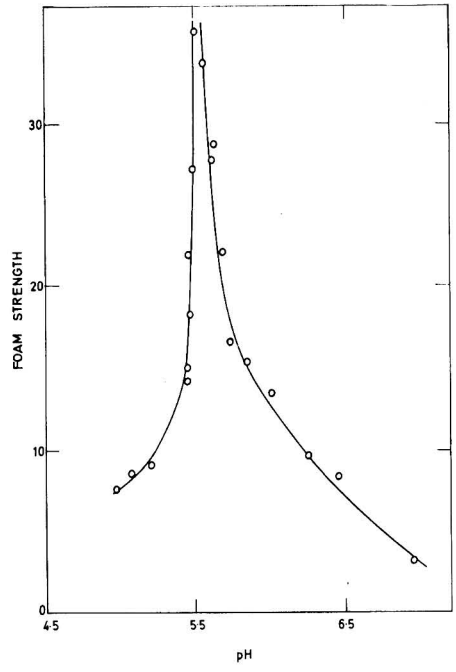

FIG. 2. *Foam strength as a function of solution pH*
Concentration: 0·06% wt./vol. freeze-dried material; temperature: 37°C

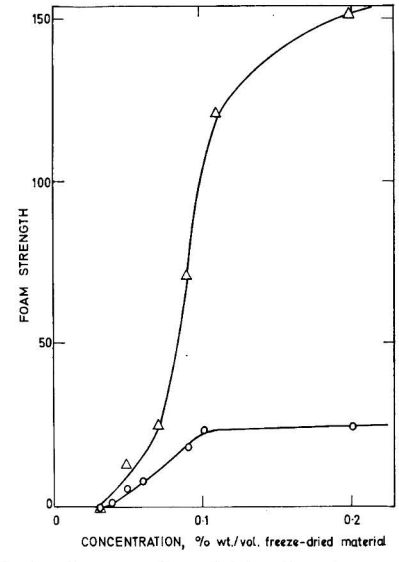

FIG. 3. *Foam strength as a function of protein concentration*
○ pH 5·7; △ pH 5·5
Temperature: 37°C

Fig. 3 would seem to indicate that the concentration of surfactant in solution has a very definite bearing on the stability of the foaming system. For this particular material at 37°C, the 0·05% concentration represents the point at which foam strength acquires sufficient magnitude to be measurable by the means used in this investigation. For present purposes, therefore, a stable foam is defined as one having measurable strength. The magnitude of the strength figure obtained is not necessarily related to foam lifetime, but clearly zero strength implies a relatively short life.

Although mechanical strength must give some indication as to the stability of the foaming system, it is clearly not the only feature of stable foams. This is evidenced by the scaling effect of changing pH. The protein concentration at which the system becomes stable appears independent of pH although stable foams generated at pH 5·5 are some five times as strong as those generated at pH 5·7. This indicates that foam strength and stability are not necessarily the same thing and possibly explains why two foams of apparent similar appearance and lifetime may exhibit very different mechanical strengths.

Effect of temperature

The effect of temperature variation on foam strength has been studied at various concentrations in the range 0·03–0·07% wt./vol. freeze-dried material. A series of solutions at each concentration was foamed over the temperature range 25 – 45°C. All these experiments were undertaken at a pH of 5·6. The 0·07% solutions were produced from a different batch of clover protein from that used for the 0·03 – 0·06% solutions.

The results of these runs are shown in Figs 4(a) and 4(b). The most significant aspect of these curves is that, at all concentrations studied, foam strength rises rapidly (and then levels off) as the temperature is reduced. Moreover, the very rapid onset of strength occurs over a narrow temperature range (37–40°C) in all concentrations greater than 0·04%. Above 40°C, all foams possess little or no strength and are very unstable, breaking up in a matter of minutes under no applied stress.

If the results contained in Fig. 4(b) are replotted on a linear scale (see Fig. 5) the discontinuities in the curve become more apparent. Between 40°C and 37°C, the strength increases rapidly and then levels off to form the 'knee' of the curve at about 35°C. The significance of this knee will be discussed later. Below 35°C, there is a further increase in strength down to temperatures around 25°C. Below 25°C there appears to be a levelling off in foam strength. In some solutions studied, an actual drop in foam strength was noticed at about this temperature.

Discussion

The dependence of foam strength on the pH of the protein solution suggests that, in the first instance, foam strength and consequently stability, depend on the relative magnitudes of the protein–protein and protein–water interactions. Clearly, if the protein is completely insoluble, it will not be available for foam formation. If, on the other hand, the cohesion between protein molecules is so weak that each molecule is effectively surrounded by a 'sea' of free water, there will be little tendency for film formation, and any foam which is generated will be short lived.

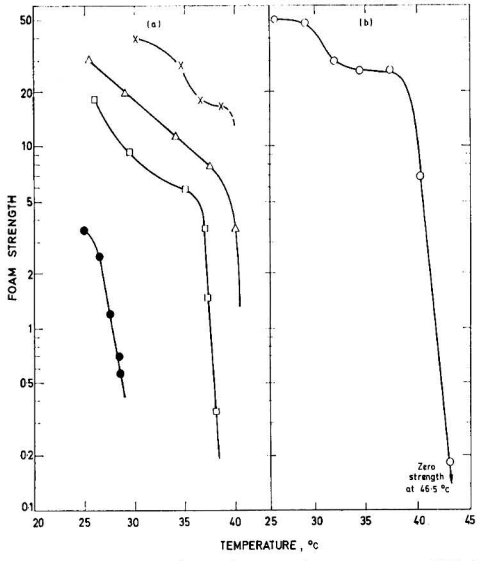

FIG. 4. *Foam strength as a function of temperature at pH 5·6*
(a) ●, 0·03%; □, 0·04%, △, 0·05%; ×, 0·06%, wt./vol. freeze-dried material; (b) ○, 0·07% wt./vol. freeze-dried material

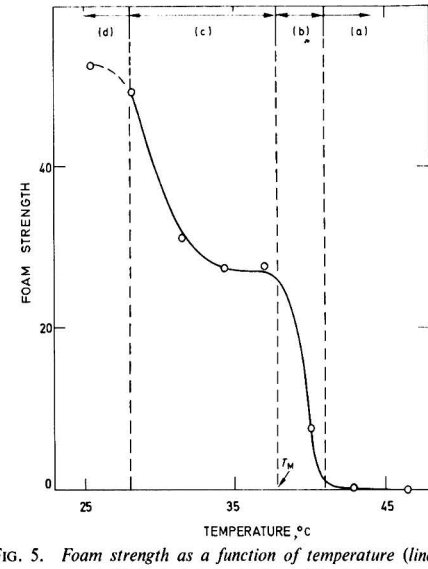

FIG. 5. *Foam strength as a function of temperature (linear plot)*
0·07% wt./vol. freeze-dried material, pH 5·6
See text for explanation of T_m and regions (a), (b), (c) and (d)

It is thought that the magnitude of the protein–protein interactions at pH 5·5 is somewhat less than that required for complete insolubility, such that each protein molecule is surrounded by a certain amount of bound water, but at the same time intimately associated with neighbouring protein molecules. The fall off in foam strength below pH 5·5 is undoubtedly due in part to protein precipitation and resulting reduction in solution concentration. However, the rate of protein precipitation is not sufficient to be the sole cause of this rapid reduction in foam strength.

Recent work on bovine serum albumin (Buckingham, in preparation) has shown that solutions of this normally extremely soluble protein produce weak, short persistence foams unless the solubility is deliberately reduced by irradiation or some chemical means. This reduction in solubility is accompanied first by an increase and then a decrease in foam strength as the precipitation point is approached.

As stated earlier, foams produced from solutions containing less than 0·05% protein possess little or no strength, irrespective of solution pH. Above 0·05%, foam strength rises rapidly and then levels off at protein concentrations of 0·1% and greater.

Examination of the solutions being foamed has shown that, for concentrations below 0·05%, there is a very rapid removal of protein from the bulk liquor, most of it being contained in the first 5 or 10 cm of foam. In addition, the solution carried up into the foam is trapped for only a short time before running back into bulk liquor. Because of this, very little solution needs to be added from the reservoir in order to maintain the level at the 0 cm mark. This results in a foam column in which only 5 or 10 cm of fine foam is 'supported' by a very weak open foam. In other words, the density of the foam column decreases very rapidly from top to bottom.

As the concentration of protein in solution is increased, the ratio of dense foam to open foam increases until, at concentrations of 0·1% and above, only dense foam is present and there is little variation in bubble size throughout the column. Much more liquid remains trapped in the foam, necessitating repeated additions of fresh solution from the reservoir and, consequently, the protein concentration in the solution remains fairly high throughout the foaming process.

The 0·1% concentration thus represents some saturation point for the system in that it probably provides for the maximum packing density of protein at the air/liquid interfaces themselves and in the adsorbed layer immediately beneath the bubble surfaces throughout the total length of the foam column.

The reduction in foam drainage rate is most likely due to increased bulk viscosity of the solution, which would explain the more gradual increase in foam strength at concentrations above 0·1% protein.

Adamson[9] states that, when compressed to the collapse point, protein films compact into bundles of insoluble fibres. It is likely that some similar process occurs during the present foam strength measurements. When the foam columns are broken down under pressure from the falling weight, insoluble material can be seen streaming back into the liquor at the bottom of the tube. This effect is particularly noticeable at concentrations of 0·1% and above, and probably explains why these foams cannot be refoamed successfully after having once been broken down.

The end result is similar to thermal denaturation of this material. Above 41–42°c, fibrous material was observed in the bulk solutions prior to any foaming or agitation of the liquid. One immediate effect of thermal denaturation therefore appears to be increased cohesion of the protein molecules and probably explains the very low foam strengths observed at these temperatures.

When the temperature is reduced below 41°c, foam strength rises rapidly (Fig. 5). Consideration of the pH effects makes it unlikely that this rise is due merely to an effective increase

in protein concentration, and it is more likely to be due to an increase in protein–water adhesion. Since thermal denaturation is highly time dependent, a small lowering in temperature probably results in a marked reduction in the rate of denaturation. It is also possible that, below 41°C, the denaturation process is different from that occurring above this temperature.

When the temperature is further lowered, the rate of increase in foam strength slows to form the 'knee' of the strength/temperature curve. For 0·07% protein solutions, this knee occurs at about 37°C. The more gradual increase in foam strength below this temperature is again probably due to an increase in the viscosity of the solution. Consideration of the slopes of the curve on either side of the knee indicates that, if the drop off in foam strength is a function of some denaturation process, the onset of that process occurs at this point. It is as if the foam suddenly melts at the temperature corresponding to the knee. Analysis of the foam strength/temperature curves indicates that this 'melting point' (T_m) tends to a constant for all concentrations above 0·05% protein.

The levelling off and, in some cases, reduction in foam strength at temperatures below about 25–28°C may be due to a reduction in the elasticity of the bubble membranes. Several workers[10–14] have shown that when single bubbles are introduced beneath fatty acid or protein monolayers at the air/liquid interface, bubble persistence increases with film compression provided the film remains elastic in nature. Once the condensed region of the pressure/area curve is reached, the film loses its elasticity and bubble persistence is reduced.

It is thus possible to produce a 'phase' diagram (Fig.6) from the experimental results shown in Figs 4(a) and 4(b), the differences between strong and weak foams now being solely dependent on protein concentration and temperature provided the pH conditions are satisfied. It can be seen from Fig. 6 that the transition from weak to strong (and hence from unstable to stable) foam requires that: the concentration of protein in solution is above some critical level, C_C. For clover cytoplasmic protein this would appear to be about 0·03% wt./vol. freeze-dried material; and the foam is at a temperature below the critical temperature T_C. It may also need to be above some lower temperature, T_T (~25°C) below which there is some indication of a reduction in foam strength. If either condition is not met, the foam will be unstable. The T_C line can therefore be considered as the variation in foam 'melting point' with protein concentration.

Bloat in cattle is thought to be caused by the presence of a stable foam in the rumen of the animal. Mangan[5] was able to show that the strength of foams produced from rumen liquor exhibits a pH dependence very similar to that of cytoplasmic protein foams, the maxima of both curves occurring at the same pH. It has been suggested, therefore, that cytoplasmic protein is the major surface agent responsible for the production of stable foams in the rumen.

If this is correct, then the present study suggests that bloat in cattle might be influenced by the concentration of cytoplasmic protein in rumen liquor and the temperature of the rumen contents. This in turn may be directly related to the state of the pasture and the prevailing climatic conditions. Since rumen temperatures are normally about 39°C, there would not need to be a great drop in temperature to stabilise any protein foam present and this may explain why there appears to be some evidence that animals can bloat following ingestion of cold water. The fact that there are at least three critical variables (pH, concentration and temperature) governing the strength of cytoplasmic protein foams may therefore have great significance in experimentation aimed at understanding the bloating process in animals.

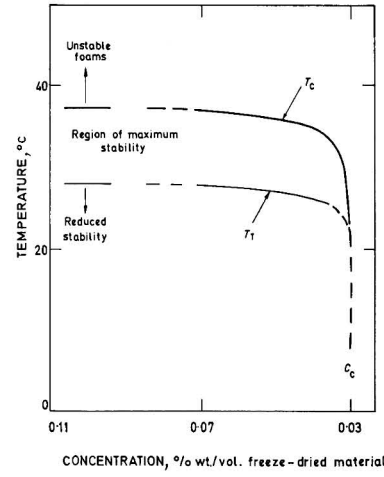

FIG. 6. *Phase diagram for red clover cytoplasmic protein foams*
C_C, critical concentration; T_C, critical temperature line

Acknowledgments

The author wishes to thank Dr. C. S. W. Reid for his continuous help and encouragement during this investigation. He also thanks Dr. J. W. Lyttleton for providing the clover cytoplasmic protein.

Department of Scientific and Industrial Research,
Physics and Engineering Laboratory,
Lower Hutt,
New Zealand

Received 2 March, 1970;
amended manuscript 4 May, 1970

References

1. Adamson, A. W., 'Physical Chemistry of Surfaces', 1960, p. 419 (New York: Interscience)
2. Burcik, E. J., *J. Colloid Sci.*, 1953, **8,** 520
3. Cumper, C. W. N., *Trans. Faraday Soc.*, 1953, **49,** 1360
4. Mangan, J. L., *N.Z. Jl agric. Res.*, 1958, **1,** 140
5. Mangan, J. L., *N.Z. Jl Agric. Res.*, 1959, **2,** 47
6. Lyttleton, J. W., *Biochem. J.*, 1956, **64,** 70
7. Clark, N. O., 'Mechanically Produced Foams for combating Petrol Fires', 1947 (London: H.M.S.O.)
8. Foulk, C. W., & Miller, J. N., *Ind. Engng Chem.*, 1931, **23,** 1283
9. Adamson, A. W., 'Physical Chemistry of Surfaces', 1960, p. 152 (New York: Interscience)
10. Hardy, W. B., *Proc. R. Soc.* (*A*), 1912, **86,** 610
11. Hardy, W. B., *J. chem. Soc.*, 1925, **127,** 1207
12. Talmud, D. L., & Sukhovolskaya, S. D., *Z. phys. Chem.*, 1931, **154,** 277
13. Trapeznikov, A. A., *Acta phys.-chim. URSS.* 1940, **13,** 265
14. Trapeznikov, A. A., & Rebinder, P. A., *Dokl. Akad. Nauk SSSR*, 1938, **18,** 427

STUDIES ON RUMEN METABOLISM

VI.*—*In vitro* hydrolysis of triglyceride and isolation of a lipolytic fraction

By D. G. CLARKE† and J. C. HAWKE

Hydrolysis of groundnut oil and synthetic glyceryl tri(oleate-1-^{14}C) by bovine rumen contents was demonstrated under *in vitro* conditions. Fractionation by differential centrifugation of total rumen contents obtained from a cow grazing on fresh pasture showed that the organisms responsible for hydrolysis of triglycerides were closely associated with the particulate material in the rumen and homogenisation released much of the lipolytic activity. Cell-free extracts of mixed rumen bacteria were prepared by subjecting mixed rumen bacteria to high frequency sonication or osmotic shock followed by isolation of the released lipolytic enzymes by high-speed centrifugation.

A method for assaying lipase activity is described which uses glyceryl tri(oleate-1-^{14}C) as substrate, and thin-layer chromatography to separate the free fatty acids, mono-, di- and tri-glycerides prior to radiochemical determination. The method was developed using pancreatic lipase and was applied to the isolation of lipases from rumen micro-organisms.

Introduction

Although early investigations on lipid metabolism in the rumen established the importance of biohydrogenation in changing the composition of the dietary lipids,[1] it has only recently been realised that no hydrogenation of unsaturated fatty acids takes place unless the constituent fatty acids of triglycerides are first released in free form by the activity of the lipolytic micro-organisms.[2] The relationship between the lipolysis and biohydrogenation of other dietary constituents has not been studied in such detail but from a comparison of the fatty acid composition of neutral and polar lipids and of free fatty acids in the rumen and in the diet it would seem that the inability to hydrogenate esterified fatty acids is a general phenomenon.[3] Since the first reports by Garton and co-workers on the lipolysis of linseed oil and other triglycerides,[4,5] it has been shown that rumen micro-organisms possess lipases which remove fatty acids from sterol and methyl esters,[6] galactosylglycerides,[6] lecithin and lysolecithin,[7] Tween 80,[8] and ethyl esters.[9]

Despite the importance of lipases in the sequence of reactions leading to the complete or partial hydrogenation of dietary lipids in the rumen, the microbial lipases in the rumen are poorly characterised. Hobson & Mann[10] isolated a pure bacterial culture, which could hydrolyse linseed oil, from sheep rumen contents on both a saliva-based and rumen-based medium. The bacteria appeared as Gram-negative rods which were strictly anaerobic and although morphologically similar to many types of rumen bacteria, they differed from all known species in their limited fermentation reactions. The authors pointed out that these bacteria were probably not the only ones in the rumen which hydrolyse glycerides, but because of the large numbers present (approximately 10^8/ml) they probably played a large part in this action. Garton *et al.*[5] were not successful in preparing a cell-free extract which possessed lipase activity although Dawson[7] prepared a soluble enzyme preparation possessing lysolecithinase activity. The present paper describes the preparation of a cell-free extract, from rumen contents, which was active in hydrolysing triglyceride.

The assay of lipase activity of rumen micro-organisms included the use of glyceryl tri(oleate-1-^{14}C) as substrate. This method was sufficiently sensitive to be used in following an enzyme purification and also enabled the formation of partial hydrolysis products to be followed. The assay was developed using pancreatic lipase so that comparisons with existing lipase assays could be made.

Experimental

Rumen sampling

Rumen samples were obtained from a lactating Jersey cow which had been fitted with a rumen fistula. The cow was a member of the milking herd, grazing on a pasture consisting essentially of ryegrass with some clover. Samples were taken from a central position in the rumen after the morning milking, so that the animal had not eaten for 3 h. In preliminary studies, rumen contents were strained immediately after sampling through two layers of cheese-cloth to remove coarser food particles. In later experiments, total rumen contents were subjected to homogenisation for 10 min before straining through cheese-cloth.

Preparation of fractions of rumen contents for lipase assay

During fractionation of rumen contents, each fraction was made up to the original volume with clarified rumen liquor (*CRL*) to enable valid comparisons of lipase activity to be made between fractions. The temperature was maintained at 39°c during the fractionation procedures and samples were held in an atmosphere of N_2.

Strained rumen contents were centrifuged at 500 × *g* for 10 min, in a Sorvall Model SS-3 centrifuge with a GSA rotor, to precipitate protozoa and finer food particles. Separation of the protozoa from the plant debris material was achieved by a sedimentation technique.[11] The supernatant from the 500 × *g* centrifugation was subjected to a further centrifugation at 14,000 × *g* for 30 min. The supernatant was re-centrifuged at 36,000 × *g* for 30 min in a Spinco Model L Ultracentrifuge to provide *CRL*. The sediment from the 14,000 × *g* centrifugation was suspended in *CRL*, centrifuged as before, and the sediment rewashed at least a further two times. The washed micro-organisms were suspended in the original volume of *CRL* for incubation.

* Part V: *J. Sci. Fd Agric.*, 1966, **17**, 241

† Present address: Biochemistry Department, Lincoln College, Canterbury, New Zealand

Incubation procedures and extraction of lipid

The incubation procedure varied according to the method used to assay the lipolytic activity. When using a colorimetric assay, 200 ml of the rumen fraction were incubated at 39°c under a stream of N_2 and with constant shaking. Groundnut oil (Kempthorne, Prosser and Co.) was added as a 50% emulsion with water and 1% Lissapol (Imperial Chemical Industries), to give a final concentration of 1·0% (by vol.). 5 ml samples were withdrawn from single incubation mixtures at 30 min intervals up to a total incubation time of 4 h. In the radiochemical method, radioactive glyceryl tri(oleate-1-^{14}C) (35·4 μCi/μM) (Radiochemical Centre, Amersham, England) was added in 5 μl hexane, with or without unlabelled carrier, to give a final count of 2 μCi/20 ml of incubation mixture. Incubation conditions were the same as those described above except that the incubation times were not extended beyond 1 h.

Anaerobic conditions were not maintained after cell-free extracts had been prepared and 5 ml aliquots of enzyme preparation were incubated with about 0·05 μCi glyceryl tri(oleate-1-^{14}C).

The assays were terminated by addition of an equal volume of ethanol and by boiling the mixture for 5 min. The lipid components were extracted by shaking with three 50-ml portions of chloroform and the combined extracts evaporated to dryness *in vacuo* in a rotary evaporator. The lipid extracts were dissolved in chloroform (colorimetric analysis) or hexane (radiochemical analysis).

Preparation of cell-free bacterial extracts

High-frequency sonication and osmotic shock were used to prepare cell-free extracts; these treatments were followed by centrifugation at 90,000 × *g* to give clear supernatants. Approximately 1 g of washed cells was used in each preparation.

Sonication

The bacterial cells were suspended in phosphate buffer (M/15, pH 7·0) to give a thick slurry. The slurry was cooled in an ice-bath before and during the 2-min sonication which was carried out at a frequency of 20 kc/sec (100 watt ultrasonic disintegrator, MSE Ltd., London).

Osmotic shock

The method of Nossal & Heppel[12] was used.

Analysis of hydrolysis products

Colorimetric determination of fatty acids

The method used was that of Duncombe.[13] Dilutions of the chloroform extracts were made so that 5 ml chloroform contained 0·05–0·5 μmole of free fatty acid.

Radiochemical method

The assay of lipases by estimation of the fatty acids released from triglyceride substrates by titration,[14] colorimetry[13] or manometry[15] is too insensitive for following enzyme purification. Chino & Gilbert[16] described a sensitive assay which uses ^{14}C-labelled triolein with added unlabelled carrier as substrate followed by separation of the hydrolysis products by column chromatography and measurement of radioactivity in the column effluent. Thin-layer chromatography of the reaction mixture used in the present study, followed either by a radiochromatogram scan or by removal of the appropriate areas of the chromatogram and measurement of radioactivity by liquid scintillation spectrometry, is more rapid than the column technique, and at the same time retains the advantages of following the formation of diglyceride and monoglyceride as well as of free fatty acids.

The method was tested with hog pancreatic lipase (Type II, Sigma Chemical Co., St. Louis, Mo., U.S.A.). The incubation procedure of Brockerhoff[17] was scaled down to use 0·02 mg pancreatic lipase and 0·05 μCi glyceryl tri(oleate-1-^{14}C).

The reaction was stopped by adding an equal volume of ethanol and the lipids were extracted as described above. The lipid was dissolved in a suitable volume of hexane and a small proportion removed for the determination of radioactivity in toluene by liquid scintillation spectrometry.

An aliquot of the hexane solution of lipids (containing about 3,000 dpm) was applied to a thin-layer of silica gel H (E. Merck, A.-G., Darmstadt, Germany) and chromatographed in hexane–diethyl ether–acetic acid (70:30:1 by vol.). The distribution of radioactivity between the lipid components was determined with a gas-flow chromatogram scanner (Packard Model 7200) followed by the planimetric determination of peak areas. The most suitable conditions for preparing the radiochromatogram scan were: voltage, 300 V; time constant, 30 sec; chart speed, 6 cm/h; gas flow (1·3% isobutane, 98·7% helium), 110 ml/min. Alternatively, the radioactivity of the lipid components was determined by removing the appropriate zones on the chromatogram and measuring the radioactivity of the silicic acid suspension in toluene containing 0·6% 2,5-diphenyloxazole and 0·05% *p*-bis 2′(5′-phenyloxazolyl)benzene, with a liquid scintillation spectrometer (Packard Model 3375). Standards were chromatographed on guide strips and sprayed with 0·2% 2′,7′-dichlorofluorescein in ethanol, in order to locate the position of the radioactive components. Both these methods of radioassay were used in the results cited below and always gave consistent values for percentage fatty acid released. For routine assays in enzyme purification the latter procedure was followed.

Fig. 1 shows the distribution of radioactivity between monoglyceride, diglyceride, free fatty acid (*FFA*) and triglyceride on a thin-layer chromatogram after incubation of ^{14}C-triolein with pancreatic lipase for 5 min. Under the conditions used about 45% of the fatty acids were released in 6 min, the release being linear over this period (Fig. 2). The accumulation of diolein, followed by a decrease in concentration with longer incubation times, was observed also by Chino & Gilbert[16] although in their experiments the dilution of radioactive triolein with unlabelled carrier would contribute to the slower release of labelled fatty acid (about 10% of the radioactivity in 5 min).

Evaluation of the radiochemical method for assaying lipolytic activity in rumen fluid.

The reproducibility of the procedure of scintillation spectrometry combined with thin-layer chromatography was tested on four aliquots of the same lipid extract prepared from an incubation mixture. The areas corresponding to the location of each component on a thin-layer chromatogram were transferred directly into counting vials and counted in scintillation fluid (Table I). The mean value for *FFA* is 52·03% with a standard error of ± 0·22. For routine assays in enzyme purification this method of radiochemical determination was used in preference to the scanning method. The latter method was very useful in preliminary assays during a fractionation.

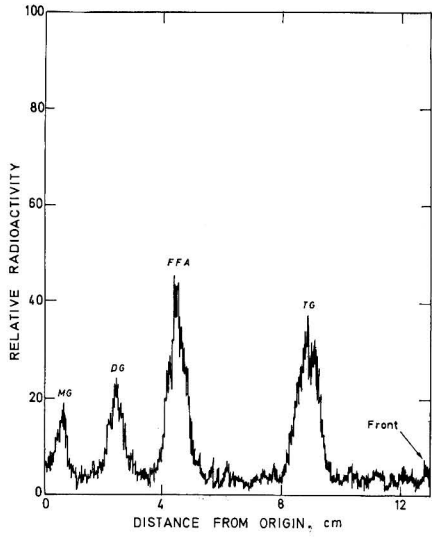

FIG. 1. *Radiochromatogram scan of t.l.c. separations on silica gel G in hexane–diethyl ether–acetic acid* (70:30:1 *by vol.) of the products of hydrolysis of glyceryl tri(oleate-1-*^{14}*C)*

Conditions of incubation: 5 μl substrate in hexane containing $1{\cdot}4 \times 10^{-3}$ μmole triolein ($1{\cdot}1 \times 10^6$ dpm), pancreatic lipase (0·02 mg) suspended in 1 ml 1 M Tris–HCl buffer (pH 7·5), 5 μl 4% $CaCl_2$, and incubated for 5 min at 39°C with constant shaking. *MG* = monoglyceride, *DG* = diglyceride, *FFA* = free fatty acid, *TG* = triglyceride

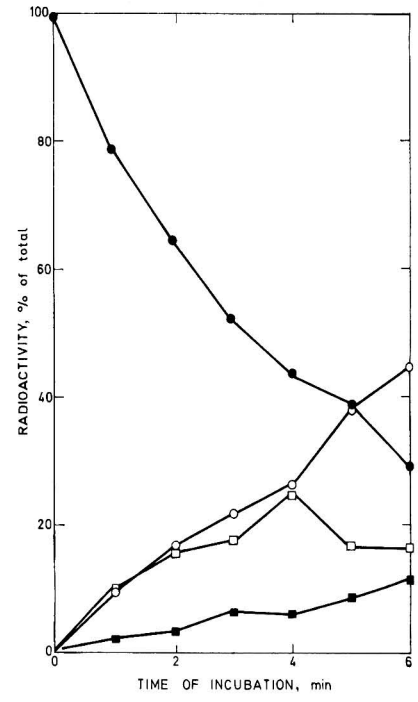

FIG. 2. *Changes in proportions of* ^{14}*C-components when glyceryl tri(oleate-1-*^{14}*C) was incubated with pancreatic lipase*

Conditions of incubation given in Fig. 1

■ Monoglyceride; □ diglyceride; ○ free fatty acid; ● triglyceride

TABLE I

Reproducibility of thin-layer radiochemical procedure for determining hydrolysis products in rumen fluid

Aliquot no.	Amount of radioactivity in each lipid compound, % of total radioactivity			
	Triglyceride	Fatty acid	Diglyceride	Monoglyceride
1	40·3	51·7	5·9	2·2
2	39·4	52·8	5·8	2·1
3	39·8	51·9	6·8	1·5
4	40·0	51·8	6·4	1·8

The release of ^{14}C-fatty acid was linear over a 4 h incubation period when 20 ml of strained rumen liquor were incubated with 10 μl glyceryl tri(oleate-1-^{14}C) in 0·4 ml of emulsified groundnut oil (oil–water–Lissapol, 50:50:1 by vol.) [Fig. 3(a)]. Furthermore, a linear relationship existed between the amount of enzyme extract and the release of *FFA* over a 4 h incubation period [Fig. 3(b)] which enables the method to be used to compare the lipolytic activity of different fractions.

Results

Lipolytic activity of fractions of strained rumen liquor

The formation of *FFA* from emulsified groundnut oil by fractions prepared from strained rumen fluid is summarised in Table II. The differences in the levels of *FFA* in each fraction at zero time indicate the way in which endogenous *FFA* is distributed in the contents of the rumen. The production of 2·26 μmole of *FFA* by the control after a 4 h incubation showed that some hydrolysis of endogenous lipid occurred in the absence of added lipid. However, when 4 ml of emulsified groundnut oil was added per 200 ml of strained rumen fluid, there was a net formation of 25·12 μmole of *FFA* after 4 h which represents the release of approximately half of the esterified fatty acids added. The rate of formation of *FFA* was linear in both the control and with added substrate over the 4 h incubation period. This rate of lipolysis was almost equalled by the fraction precipitated by the centrifugation of strained rumen liquor at 500 × *g* for 10 min. 20·04 μmole of *FFA* were released at a linear rate during a 4 h incubation following removal of protozoa from the 500 × *g* centrifugate by sedimentation compared with 22·24 μmole by the original 500 × *g* centrifugate. Furthermore, the protozoal fraction and the 14,000 × *g* supernatant did not exhibit any lipolytic activity. Microscopic examination of the incubation media after the 4 h incubation showed that the protozoa had remained viable in the 500 × *g* centrifugate but viability had declined to almost zero in the protozoal fraction itself. Similar trends to those given were observed in a number of experiments, although absolute values varied slightly with different rumen samples.

Appreciable variation did, however, occur in the formation of *FFA* by the 14,000 × *g* centrifugate prepared from the 500 × *g* supernatant. In the experiment cited above, *FFA* formation by this fraction increased at a linear rate from 4·96 μmole to 11·40 μmole. However, in several other experiments only about 2·00 μmole of *FFA* were produced.

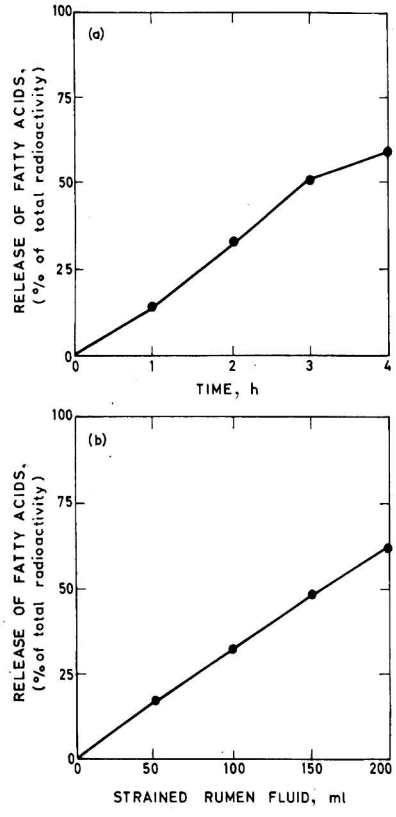

FIG 3. *Release of ^{14}C-labelled fatty acid from glyceryl tri(oleate-1-^{14}C) by rumen micro-organisms*

Fatty acid released with (a) time of incubation (b) amount of rumen fluid

Conditions of incubation given in text

Effect of substrate concentration on the amount of *FFA* produced and the nature of the hydrolytic products formed by strained rumen fluid

It is apparent from Fig. 4 that, at a substrate concentration of 200 mg triglyceride/100 ml strained rumen fluid, the hydrolytic capacity of the micro-organisms was almost saturated. Optimum rates of lipolysis occurred with substrate levels up to 100 mg triglyceride/100 ml rumen fluid and appropriate substrate levels for assaying lipolytic activity were in the range 50–100 mg triglyceride/100 ml strained rumen fluid.

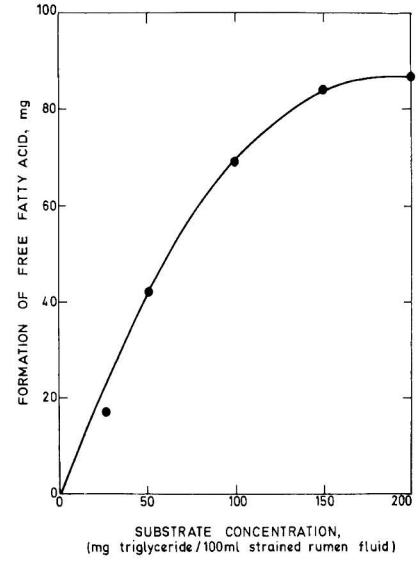

FIG. 4. *Effect of substrate concentration on the formation of free fatty acid by strained rumen fluid*

Conditions of incubation: glyceryl tri(oleate-1-^{14}C) (35·4 μCi/μM) was added to the unlabelled triglyceride to give 2 μM/20 ml of incubation mixture. Incubation was for 4 h at 39°C under N_2 with constant shaking

TABLE II

Formation of free fatty acids from emulsified groundnut oil by strained rumen fluid and from fractions prepared from strained rumen fluid

Time of incubation, h	Free fatty acids, μmole						
	Strained rumen fluid		500 × *g* centrifugate			500 × *g* supernatant	
	No substrate added	Substrate added	Total fraction	Total fraction less protozoa	Protozoa	14,000 × *g* centrifugate	14,000 × *g* supernatant
0·0	11·34	11·28	11·52	8·60	1·13	4·96	0·15
0·5	11·48	15·20	14·28	11·24	1·13	5·84	0·15
1·0	11·56	17·04	17·12	14·00	1·12	7·12	0·15
1·5	12·08	20·40	20·00	14·42	1·13	7·04	0·15
2·0	12·20	24·24	22·40	19·20	1·13	8·32	0·15
2·5	12·20	26·96	24·96	21·92	1·12	9·04	0·15
3·0	12·60	29·92	28·56	24·32	1·13	9·20	0·15
3·5	13·44	33·84	30·96	26·88	1·13	10·88	0·15
4·0	13·60	36·40	33·76	29·44	1·13	11·40	0·15

Conditions of incubation: 200 ml rumen fluid or fraction; 4 ml emulsified groundnut oil; N_2 atmosphere; constant shaking at 39°C

Under saturating conditions of substrate, achieved by increasing the substrate concentration as outlined in Table III, diglycerides accumulated up to about 25% of the *FFA* level (Table III). A similar accumulation of diglycerides resulted when the concentration of the rumen fluid was decreased and the substrate levels held constant or when shorter times of incubation were used. For example with 200 mg triglyceride/100 ml rumen fluid, the *FFA* and diglycerides contained 14·4% and 3·3%, respectively, of the total radioactivity after 1 h, whereas the same components comprised 59·1% and 1·1%, respectively, after 4 h, the remainder in each case being in triglyceride.

Effect of homogenisation of total rumen contents on lipolytic activity

Since it was apparent that most of the lipolytic activity in strained rumen fluid was associated with the particulate material, i.e. the fraction sedimented at 500 × *g* and to a lesser extent with the fraction sedimented at 14,000 × *g* after removal of the 500 × *g* precipitate, it seemed likely that many lipolytic organisms were adhering to the solid digesta in the rumen. Consequently, in a further experiment a sample of total rumen contents was homogenised in a Waring Blendor for approximately 10 min and the lipolytic activities of the fractions prepared by the above procedures were compared with those obtained from strained rumen liquor.

TABLE III

Effect of triglyceride concentration on the composition of the hydrolysis products after incubation with strained rumen fluid for 4 h at 39°C

Triglyceride level,* mg/100 ml rumen fluid	Amount of each lipid component, % of total radioactivity			
	Triglyceride	Fatty acid	Diglyceride	Monoglyceride
25	32·7	61·4	3·3	2·4
50	9·5	85·4	2·2	3·0
100	25·8	68·6	3·7	2·0
150	38·5	55·2	4·4	2·0
200	49·3	43·3	6·1	1·3

* Groundnut oil + 1·4 × 10^{-3} μmole glyceryl tri(oleate-1-^{14}C) (1·1 × 10^5 dpm)

Although there was about twice the amount of endogenous fatty acid present in the strained rumen contents used in these experiments compared with those presented in Table II, the *FFA* formation over 4 h was of the same order both in the presence (32·80 and 25·12 μmole) and in the absence (2·72 and 2·26 μmole) of added substrate (Table IV). 27·92 μmole of *FFA* (about 50% hydrolysis) were formed after 4 h when the fraction sedimented at 500 × *g* was incubated with groundnut oil whereas the corresponding supernatant only formed 2·30 μmole of *FFA*. This latter fraction is equivalent to the fraction sedimented at 14,000 × *g* in Table II.

On the other hand, the 500 × *g* supernatant, prepared from homogenised rumen contents, formed 12·32 μmole of *FFA* and a corresponding decrease was observed in the formation of *FFA* by the 500 × *g* centrifugate. It appeared that homogenisation had released lipolytic micro-organisms from the particulate material, so that fewer were sedimented at 500 × *g*. Further centrifugation of this fraction at 14,000 × *g* for 30 min resulted in the lipolytic activity being confined to the sedimented fraction. This distribution of lipolytic activity was confirmed by the formation of radioactive *FFA* and diglycerides when these rumen fractions were incubated for 60 min with emulsified groundnut oil (200 mg/20 ml rumen sample) to which ^{14}C-triolein had been added. The fraction sedimented at 14,000 × *g* transferred 10·2% of the ^{14}C from the triglycerides to the free fatty acids when prepared from rumen contents which had been homogenised but no activity was detected in the corresponding fraction prepared from the unhomogenised strained rumen fluid.

Lipolytic activity of cell-free extracts of rumen bacteria

The fraction from homogenised rumen contents sedimented at 14,000 × *g* was considered to be the most suitable starting material for the preparation of cell-free bacterial extracts because it was relatively uncontaminated with plant debris. The bacterial pellet was washed several times with M/15 phosphate buffer (pH 7·0) without a resulting loss of lipolytic activity. Following a 1 h incubation with ^{14}C-triolein as a suspension in M/15 phosphate buffer at pH 7·0, 47·5% and 3·0% of the total radioactivity was in the *FFA* and diglyceride components, respectively, compared with 44·3% and 7·8% before washing (see Table V for activities of subsequent fractions).

TABLE IV

Effect of homogenisation of total rumen contents on the lipolytic activity of fractions prepared by differential centrifugation

Time of incubation, h	Free fatty acids, μmole					
	Strained rumen liquor				Homogenised rumen contents	
	Total fraction		500 × *g* centrifugate	500 × *g* supernatant	500 × *g* centrifugate	500 × *g* supernatant
	No substrate added	Substrate added				
0·0	24·00	24·00	22·96	2·50	17·84	6·40
0·5	24·24	28·16	26·40	2·54	19·68	7·72
1·0	24·56	31·44	29·92	2·60	22·00	9·60
1·5	24·96	34·64	31·20	3·10	23·84	10·96
2·0	25·36	39·04	36·56	2·94	25·60	12·40
2·5	25·60	42·24	40·08	3·50	27·52	13·92
3·0	25·92	45·92	43·68	3·50	29·28	15·12
3·5	26·16	49·12	47·52	4·34	31·36	17·36
4·0	26·72	52·80	50·88	4·80	33·36	18·72

See Table II for conditions of incubation

TABLE V

Formation of ^{14}C-labelled hydrolysis products from ^{14}C-triolein by cell-free extracts of rumen bacteria

% radioactivity in each lipid component

Lipid component	Control		Sonicated bacterial suspension		Osmotic shock treatment	
	90,000 × *g* centrifugate	90,000 × *g* supernatant	90,000 × *g* centrifugate	90,000 × *g* supernatant	90,000 × *g* centrifugate	90,000 × *g* supernatant
Triglyceride	29·3	100·0	66·0	60·7	23·9	47·4
Fatty acid	65·0	—	21·1	22·6	23·2	33·8
Diglyceride	0·8	—	8·7	14·3	52·9*	13·8*
Monoglyceride	4·9	—	4·3	2·4	—	—

Conditions of incubation: enzyme in 5 ml M/15 phosphate buffer at pH 7·0, 5 ml glyceryl tri(oleate-1-^{14}C) in hexane, 1 h at 39°C with shaking

* Includes an unidentified component which ran between diglycerides and free fatty acids on a thin-layer chromatogram

High frequency sonication of a thick suspension of the bacteria in buffer, or osmotic shock treatment, resulted in the transfer of appreciable lipolytic activity to the 90,000 × *g* supernatant (Table V). In both treatments this lipolytic activity was retained following passage of the supernatants through a Zeitz filter. An unidentified radioactive component, which was a contaminant of the diglyceride fraction, was formed when ^{14}C-triolein was incubated with the cell-free fraction prepared by osmotic shock of the suspended bacteria, and contributed substantially to the proportion of ^{14}C occurring in the diglyceride fraction.

Discussion

It has been established by numerous workers,[18–21] that cellulolytic micro-organisms in the rumen are associated with the particulate material and, from the above studies on the distribution of lipolytic activity in rumen contents, it is likely that lipolytic bacteria have, in part at least, a similar association. The components of the rumen contents not sedimented at 500 × *g* were, at best, weakly lipolytic and in many fractionations they did not possess a detectable lipolytic activity. When the total rumen ingesta were homogenised some of this activity was transferred to the 500 × *g* supernatant and centrifugation of this fraction at 14,000 × *g* precipitated almost all of the lipolytic activity. While a relationship between cellulolytic and lipolytic activity has yet to be established it is of interest to note that the cellulolytic bacterium *Butyrivibrio fibrisolvens* is the only rumen micro-organism on which detailed studies of fatty acid hydrogenases have been made.[22]

The lipolytic activity of strained rumen contents obtained at standardised sampling times from a fistulated cow maintained on a controlled dietary regime was remarkably constant despite the variable levels of endogenous *FFA* found in different samples. Apparently the nature and the level of micro-organisms in strained rumen contents were reasonably reproducible and, as a consequence, constant amounts of added triglycerides were hydrolysed in a predictable manner. In contrast, earlier workers[5,8] experienced extreme variability in the rates and extent of hydrolysis of triglyceride by rumen fluid.

If the amounts of triglycerides added were sufficient to saturate the capacity of the micro-organisms to hydrolyse the ester groups, di- and mono-glycerides were consistently found among the hydrolysis products. However, if the substrate/micro-organism ratio was decreased, either by altering the amount of substrate or micro-organisms, little or no partial hydrolysis products were isolated from the reaction media.

Unlike pancreatic,[23] milk lipases[24] and many other bacterial lipases,[25] the lipases of rumen micro-organisms do not appear to exhibit any specificity with respect to the hydrolysis of glycerol esters. However, there is a possibility that individual species of micro-organisms have lipases of different specificity which exhibit a symbiotic relationship similar to that found in carbohydrate degradation in the rumen.

The lipolytic activity of the 90,000 × *g* supernatant following exposure of rumen bacteria to sonic oscillation or osmotic shock is evidence for the solubilisation of lipases. However, these experiments must be regarded as only partly successful because of the small proportion of the original activity which was transferred to the soluble fraction. The detection of lipase activity was made using much more sensitive assay techniques than earlier workers who reported unsuccessful attempts to prepare cell-free extracts.[4] Since both osmotic shock and sonication solubilised the enzymes to a similar extent no information has been obtained about the possible cellular location of the lipases. However, from the absence of active lipases in clarified rumen liquor it appears that these enzymes are cell-bound and not released into the surrounding medium.

Because biohydrogenation of unsaturated fatty acids only takes place if fatty acids are in a free state, a slow rate of release of free fatty acids into the rumen from dietary lipids should lead to a lower level of hydrogenation in the rumen. However, from the rates of lipolysis found in the present experiments lipolysis would not appear to be the rate limiting step in the metabolic sequence leading to biohydrogenation of dietary lipids.

Acknowledgment

This work was supported by a grant from the Nuffield New Zealand Research Fund.

Department of Chemistry and Biochemistry
Massey University,
Palmerston North,
New Zealand

Received 23 February, 1970

References

1. Willey, N. B., Riggs, J. K., Colby, R. W., Butler, O. D., & Reiser, R., *J. Anim. Sci.*, 1952, **11,** 705
2. Hawke, J. C., & Silcock, W. R., *Biochem. J.*, 1969, **112,** 131
3. Patton, S., & Kesler, E. M., *Science, N.Y.*, 1967, **156,** 1365
4. Garton, G. A., Hobson, P. N., & Lough, A. K., *Nature, Lond.*, 1958, **182,** 1511

5. Garton, G. A., Lough, A. K., & Vioque, E., *J. gen. Microbiol.*, 1961, **25,** 215
6. Garton, G. A., 'Metabolism and Physiological Significance of Lipids', (Eds, Dawson, R. M. C., & Rhodes, D. N.), 1964, p. 335 (London: John Wiley & Sons (Inc.) Ltd.)
7. Dawson, R. M. C., *Nature, Lond.*, 1959, **183,** 1822
8. Wright, D. E., *N.Z. Jl agric. Res.*, 1961, **4,** 216
9. Hill, F. D., Saylor, J. H., Allen, R. S., & Jacobson, N. L., *J. Anim. Sci.*, 1960, **19,** 1266
10. Hobson, P. N., & Mann, S. O., *J. gen. Microbiol.*, 1961, **25,** 227
11. Heald, P. J., Oxford, A. E., & Sugden, B., *Nature, Lond.*, 1952, **169,** 1055
12. Nossal, N. G., & Heppel, L. A., *J. biol. Chem.*, 1966, **241,** 3055
13. Duncombe, W. G., *Biochem. J.*, 1963, **88,** 7
14. Dole, V. P., & Meinertz, H., *J. biol. Chem.*, 1960, **235,** 2595
15. Wills, E. D., *Biochem. J.*, 1954, **57,** 109
16. Chino, H., & Gilbert, L. I., *Analyt. Biochem.*, 1965, **10,** 395
17. Brockerhoff, H., *Archs Biochem. Biophys.*, 1965, **110,** 586
18. King, K. W., *Tech. Bull. Va agric. Exp. Stn*, 1956, **127,** 3
19. King, K. W., *J. Dairy Sci.*, 1959, **42,** 1848
20. Gouws, L., & Kistner, A., *J. agric. Sci., Camb.*, 1965, **64,** 51
21. Hungate, R. E., 'The Rumen and its Microbes', 1966, p. 384 (New York: Academic Press)
22. Kepler, C. R., & Tove, S. B., *J. biol. Chem.*, 1967, **242,** 5686
23. Savary, P., & Desnuelle, P., *Biochim. biophys. Acta.*, 1956, **21,** 349
24. Jensen, R. G., Duthie, A. H., Gander, G. W., & Morgan, M. E., *J. Dairy Sci.*, 1960, **43,** 96
25. Lawrence, R. C., *Dairy Sci. Abstr.*, 1967, **29,** 59

CONSERVATION OF WILTED HERBAGE ENSILED IN SEALED PLASTIC CONTAINERS, WITH AND WITHOUT THE ADDITION OF UREA, AND ITS INTAKE BY SHEEP

By N. JACKSON and B. K. ANDERSON

Timothy–meadow fescue grass was wilted to 27·7–33·9% dry matter content and two silos were filled with the wilted material alone and two with the wilted herbage to which urea, at a level of 1·38% of the dry matter ensiled, was added. The mean dry matter loss from the control silages was 16·7% and from the urea-treated silages 11·4%. No urea could be detected in the silage made from the urea-treated herbage. There was no evidence for any major differences in the fermentation pathways between the control and urea-treated silage. Voluntary intake of control and urea-treated silage by sheep was similar (1·7 times the maintenance metabolisable energy requirement) so that nitrogen intake was higher when the urea-silage was fed. No toxic effects of the treated silage were observed when assessed by blood urea and ammonia levels.

Introduction

Fingerling *et al.*[1] and others have shown that simple nitrogenous compounds can be converted to protein by micro-organisms in the rumen. Harris & Mitchell[2] have also shown that the digestibility of a low protein ration can be improved by supplementing it with urea. The use of urea in ruminant diets is at present receiving more attention because urea can now be produced at a price which is favourable in the light of current animal production costs. The value of urea as a nitrogen supplement in ruminant diets has recently been reviewed[3] and the use of urea alone or with limestone in silage fed to beef cattle has also been reviewed by Essig.[4] Urea is converted by the urease activity of the rumen micro-organisms to ammonia which can then be converted to microbial protein, this latter being subsequently digested and the products absorbed. If the formation of ammonia occurs too rapidly the efficiency of urea as a source of nitrogen decreases, since the ammonia is absorbed into the blood stream and transported to the liver where it is converted to urea, most of which is subsequently eliminated via the kidney. In addition, if high levels of urea are consumed, acute toxicity may result due to the presence of excess levels of ammonia or ammonium carbamate in the peripheral blood. Attempts have been made to produce feeds in which the urea is not too rapidly converted to ammonia.[5]

The use of urea in silage is not a recent development. In 1928 Brigl & Windheuser[6] added 0·5–2% urea to ensiled maize. The resulting silage was consumed by dairy cattle and appeared to be satisfactorily utilised. Davis *et al.*[7] added urea to ensiled sorghum and analysis indicated that comparatively little of the urea was broken down during the ensilage period. The silage was readily eaten by cattle at the 0·5% inclusion of urea, but at 1·5% urea addition intake was depressed and at 2·5% of added urea the silage was refused.

Several workers[8–12] have investigated the effect of adding urea to ensiled maize both with regard to fermentation changes and to silage intake by cattle. Cullison[13] added urea at a level of 0·5% to silage made from sweet sorghum. Cows fed this silage lost weight.

Polan *et al.*[14] investigated the effect of the dry matter content of ensiled maize with added urea and found a direct relationship between the dry matter content of the silages and the ratio of ammonia to urea in the silage.

Owen *et al.*[15] reported that there was no detectable urea in laboratory silos when urea or limestone and urea were added at the time of ensiling, and that the addition of urea resulted in a large increase in ammoniacal nitrogen.

Most of the experiments cited above have referred to urea added to maize at the ensiling stage. In view of the lack of information on the fate of urea added to grass it was decided to investigate this, and to study the feeding value of the resulting silage for sheep. The study was designed to integrate with the long-term conservation programme of the Department in which chemical changes in herbage conserved in the wilted form in sealed containers are being investigated. Wilting of herbage for ensiling in farm-scale partial gas-tight towers is much more satisfactory under conditions where the herbage is more mature and can be more easily wilted in the field. There is also the possibility that the nutritive value of lower protein herbage of this type may be improved by the addition of non-protein nitrogen in the form of urea and that under self-feeding or automatic-feeding systems a more uniform intake of urea may be attained through its incorporation in a bulky feed such as silage.

Experimental

The herbage was the second cut of a timothy–meadow fescue sward and was cut on 31 July, 1969. A flail mower was used for cutting, and, after wilting for 24–36 h, the herbage was collected with a forage harvester and filled into four trench silos, each fitted with a rectangular container (35 × 2·5 × 3 m) constructed of 500-gauge polyethylene film. During the cutting, wilting and ensiling period, the weather was satisfactory, being dry with 9·8 h of sunshine. The maximum air temperature was 20°c and the minimum 10°c. On filling, no addition was made to the grass in silos 1 and 3, while urea was evenly distributed throughout the grass ensiled in silos 2 and 4 at a level of approximately 1·38% of the dry matter ensiled. The silo contents were consolidated by trampling and the polyethylene was sealed using a PVC waterproof adhesive tape.

The silos were opened after 3 months and the silage was taken out and weighed. Preservation appeared to be highly satisfactory, although some waste was observed at the front and top of the mass. Fungi present in the waste were examined.

Grass and silage dry matters were determined by drying in a forced-air oven at 85°c for 18 h and bulked samples of the dry matter were kept for chemical analyses. Samples of the silage were taken for the determination of volatile constituents and for lactic acid determination. The volatile acids and amino acids were determined by the Woodman[16] method and individual volatile fatty acids were determined by gas–liquid chromatography. The acids in the steam distillate obtained by Woodman's[16] method were neutralised, the solution was concentrated by evaporation, and then acidified. The sample was then injected directly onto the column. The columns were packed with 15% Tween 80 (polyoxyethylene sorbitan mono-oleate) coated on 70/80 mesh 'Embacal' and used in conjunction with a Pye Series 104 Dual Flame Ionization Chromatograph. Nitrogen was used as the carrier gas and the operating temperature was 105°c. Lactic acid was determined by a modification of the microdiffusion method as described by Conway.[17] The soluble carbohydrate content of the grass and silages was determined by the method of McDonald & Henderson.[18]

Volatile ammonia was determined by the Conway[17] microdiffusion method. Urea was also determined by the microdiffusion method by first converting it to ammonia with the enzyme urease. In computing the losses, the volatile acids lost on drying were added to the N-free extractives fraction, and the ammonia lost was added to the total dry matter. Digestibilities were carried out on the grass, grass with added urea and the silages, using two wethers fed at the maintenance level for each determination. The grass was preserved in deep freeze prior to the digestibility determinations. The metabolisable energies (*ME*) of the ensiled material and the silages were also determined. The procedure used was that described by Anderson & Jackson.[19] Allowance was made for the energy contribution from the volatile acids.

In addition to the digestibility determinations, six sheep were maintained on each of the two types of silage for a period of 2 weeks and then voluntary feed intake was assessed over the next 10 days. The level of food offered was adjusted so that the refusal amounted to approximately 15%. The food was offered in 2 equal feeds at 9.30 a.m. and 5.00 p.m. The possible effect of the urea inclusion in the ensiled material on the blood urea and ammonia was investigated in sheep over a period of 5 days. Six sheep were starved overnight and the blood urea and ammonia were determined in blood samples taken from the jugular vein prior to feeding at 9.30 a.m. Blood urea was determined by the method of Wheatley[20] and ammonia by the method of McCullough.[21] Then three sheep were fed the control silage and three the urea silage *ad libitum*. Urea and ammonia levels were then determined on blood samples taken at 11.30 a.m. and 1.30 p.m.

Results

The chemical composition of the herbages and of the silages, including the soluble carbohydrates, is shown in Table I. The nitrogen fractions have been tabulated at the bottom of the Table since the urea nitrogen cannot validly be expressed as crude protein. Wilting raised the dry matter content of the grass, which ranged from 27·7 to 33·9% at the time of ensiling. The fibre and nitrogen contents are consistent with the stage of growth of the grass at the time of cutting. The mean crude fibre increases of 3·3% in the control silage and 2·5% for the urea silage compared with the grass ensiled indicate a loss of other constituents, although this loss cannot have been large. The soluble carbohydrates data show considerable losses as a result of ensiling. On average, the loss is lower from the urea silage than from the control silage.

The level of ammonia in the control silage is of the order expected if the observation by Jackson[22], that approximately 97% of the volatile base nitrogen in silage is present as ammonia, is accepted. In the urea silage, the results show that none of the added urea nitrogen could be detected as urea after the material had been ensiled for 3 months. The data suggest that a large proportion, if not all of the urea nitrogen has been converted to ammonia. There was a large increase (9-fold) in the content of amino acids with ensiling and this was not affected by the addition of urea at the time of ensiling; nor did the presence of urea or its decomposition products affect the level of other forms of non-protein nitrogen present in the silage.

The volatile organic acid and lactic acid contents of the silages are given in Table II, together with the pH values.

TABLE I

Composition and gross energy content of grass and edible silage

	Control				Urea-treated			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Dry matter, %	27·69	25·38	33·90	28·86	31·82	28·24	33·21	30·40
Ether extract, %	2·21	3·79	2·23	3·79	2·25	3·51	2·17	3·86
Crude fibre, %	23·21	26·53	22·63	25·84	22·37	25·06	22·93	25·21
Ash, %	9·27	10·68	8·91	10·02	9·12	10·15	9·40	10·03
N-free extractives, %	51·19	43·82	52·80	46·33	51·21	45·71	50·31	44·75
Organic matter, %	90·73	89·32	91·09	89·98	90·88	89·85	90·60	89·97
Gross energy, kcal/g	4·36	4·60	4·36	4·60	4·35	4·59	4·35	4·65
Soluble carbohydrates as glucose, %	11·91	2·76	14·65	3·61	13·37	2·47	12·58	2·56
Total N	2·27	2·61	2·15	2·44	2·83	2·98	2·85	3·43
Urea N	—	—	—	—	0·65	—	0·64	—
Ammonia N	—	0·22	—	0·25	—	0·61	—	1·05
True protein N	2·01	1·17	1·86	1·24	1·84	1·25	1·94	1·32
Amino acid N	0·06	0·52	0·06	0·45	0·06	0·49	0·06	0·50
Non-protein N*	0·21	0·92	0·23	0·75	0·94	1·25	0·86	1·60
Non-protein N* excluding urea and ammonia	0·21	0·70	0·23	0·51	0·29	0·64	0·21	0·55

* Non-protein nitrogen = total nitrogen—(true nitrogen N + amino acid N)

The pH values reflect the low organic acid production normally associated with wilted silage from which air is efficiently excluded, and the effect of the urea addition has been to raise the pH value, presumably as a result of ammonia production. The average total volatile acids level in the urea silage is 11% greater than in the control silages, while the within treatment values for lactic acid are such that no between treatments effect could be detected. There are no differences in the proportions of the individual volatile acids which can definitely be attributed to urea addition. The levels of butyric acid in both types of silage are fairly high, and are higher in the urea-treated silage than in the control.

TABLE II

pH values, percentage volatile acids and lactic acid in the silage dry matter

	Control		Urea-treated	
	Silo 1	Silo 3	Silo 2	Silo 4
pH	4·33	4·57	4·67	4·97
Acetic acid	1·990	1·445	1·477	1·592
Propanoic acid	0·075	0·087	0·110	0·118
Butanoic acid	1·379	2·093	2·440	2·342
Pentanoic acid	0·024	0·045	0·028	0·016
Hexanoic acid	0·004	0·042	0·018	0·072
Others (as iso-pentanoic)	0·024	0·021	0·032	0·043
Total volatile acids	3·49	3·74	4·10	4·18
Lactic acid	6·16	4·93	6·14	5·32

TABLE III

Weights of dry matter filled into and removed from silos

	Control		Urea-treated	
	Silo 1	Silo 3	Silo 2	Silo 4
Dry matter put in as grass, kg	763·4	832·3	708·7	717·1
Dry matter put in as urea, kg	0·0	0·0	10·0	10·0
Total dry matter ensiled, kg	763·4	832·3	718·7	727·1
Wt. of edible silage taken out (corrected for volatiles), kg	485·99	578·22	536·15	509·62
Wt. of inedible silage taken out (corrected for volatiles), kg	159·91	103·23	108·11	127·15
Total weight of dry matter taken out, kg	645·90	681·45	644·26	636·77
Loss of edible dry matter, %	36·34	30·53	25·40	29·91
Total dry matter loss, %	15·39	18·12	10·36	12·42

The dry matter losses from all four silos (Table III) are relatively low, although they are not as low as the losses found for larger quantities of material ensiled under similar conditions.[23,24]

The presence of urea had a marked effect on dry matter losses, the mean loss from the control silage being 16·7% and that from the urea silages being 11·4%. The presence of urea did not appear to result in any difference in the percentage of the ensiled material which was classified as being inedible, although this was relatively high, partly because of the strictness of the classification of material as inedible.

The digestibility data were subjected to analysis of variance. The only significant difference ($P < 0·05$) found was between the nitrogen digestibility of the control silage (mean N digestibility coefficient = 71·2) and the urea silage (mean N digestibility coefficient = 79·0).

The contents of digestible nutrients for the grass and silages are presented in Table IV, together with the metabolisable energy values determined at the maintenance level of feeding.

The percentage losses of individual total and digestible constituents are presented in Table V. The major loss of the total constituents was from the N-free extractives fraction, the mean value being 28·1% from the control silage and 21·8% from the urea silages. The loss of true protein from the total constituents was greater from the control silage than from the urea-silage. The total nitrogen loss from both types of silage was low, the mean loss being 5% from the control silages and 0·2% from the urea-treated silages. The gross energy loss from the urea silage was less than half of that from the control silage.

A white fungus present in the waste of all silos was identified as a species of *Paecilomyces*, and a red fungus found in the waste of the control silos was *Malbranchea pulchella* var. *sulfurea*.

The mean daily feed intakes/kg of metabolic bodyweight (i.e. $kg^{0·73}$) expressed as dry matter, digestible dry matter, digestible organic matter, digestible nitrogen and metabolisable energy are presented in Table VI, together with the level of voluntary feed intake expressed as a multiple of the maintenance *ME* requirement which was calculated using the A.R.C.[25] standards.

The voluntary intake of the silages was quite high, being 1·7 times the maintenance *ME* requirement for both types of silage. The only significant difference in the intake data

TABLE IV

Digestible nutrients and metabolisable energy values of grass and silage

	Control				Urea-treated			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Dry matter, %	75·02	74·61	75·02	73·86	74·55	74·46	74·55	76·51
Ether extract, %	0·63	2·29	0·64	2·44	0·90	2·05	0·86	2·13
Crude fibre, %	18·62	22·13	18·23	21·03	17·96	20·93	18·41	21·45
Ash, %	5·55	5·91	5·33	5·78	5·44	5·62	5·60	5·93
N-free extractives, %	40·15	33·36	41·30	35·25	39·92	34·17	39·22	34·48
Organic matter, %	69·47	68·70	69·69	68·08	69·11	68·84	68·94	70·58
Total nitrogen, %	1·612	1·903	1·524	1·694	2·084	2·284	2·096	2·795
True protein, %	8·66	3·04	8·02	3·32	7·65	3·41	8·07	4·14
ME, kcal/g	2·52	2·70	2·52	2·69	2·52	2·65	2·52	2·77

TABLE V

Percentage losses of total and digestible constituents from silos

	Control				Urea-treated			
	Silo 1		Silo 3		Silo 2		Silo 4	
	Total	Digestible	Total	Digestible	Total	Digestible	Total	Digestible
Dry matter	15·4	36·7	18·1	31·6	10·4	25·5	12·4	28·1
Ether extract	+45·0	+131·4	+39·4	+167·0	+40·1	+70·9	+56·2	+73·0
Crude fibre	3·0	24·4	6·4	19·9	+0·6	13·1	3·4	18·4
Ash	2·6	32·2	7·8	24·7	0·1	22·9	6·3	25·9
N-free extractives	27·8	47·1	28·4	40·7	20·5	36·1	23·1	38·8
Organic matter	16·7	37·1	19·1	32·1	11·4	25·7	13·1	28·3
Total nitrogen	3·0	24·9	7·0	22·8	5·7	18·2	+5·3	6·5
True protein	50·8	77·7	45·2	71·3	39·2	66·8	40·1	64·1
Gross energy	10·8	—	13·8	—	5·5	—	6·4	—
ME	—	31·9	—	25·9	—	21·4	—	22·8

TABLE VI

Metabolic bodyweight, daily voluntary intakes of dry matter, digestible dry matter, digestible organic matter, digestible nitrogen and metabolisable energy intakes and the voluntary feed intake level of sheep (mean ± S.E.)

	Control silages	Urea-treated silages	
Bodyweight, $kg^{0.73}$	15·72 ± 0·50	15·17 ± 0·38	
Dry matter intake, $g/kg^{0.73}$	60·9 ± 3·50	58·5 ± 4·01	n.s.
Digestible dry matter intake, $g/kg^{0.73}$	45·2 ± 2·53	44·1 ± 2·94	n.s.
Digestible organic matter intake, $g/kg^{0.73}$	41·7 ± 2·34	40·7 ± 2·73	n.s.
Digestible nitrogen intake, $g/kg^{0.73}$	1·09 ± 0·047	1·48 ± 0·102	P <0·01
ME intake, $kcal/kg^{0.73}$	162 ± 8·95	156 ± 9·98	n.s.
Feed intake level*	1·7	1·7	

$$\text{* Feed intake level} = \frac{ME \text{ intake}}{\text{maintenance } ME \text{ requirement}}$$

presented in Table VI is in the digestible nitrogen intake, this being some 38% higher for the urea-treated than for the control silage. This increased nitrogen intake is a function both of the higher nitrogen content and of the higher digestibility of nitrogen in the urea-treated silage.

Although there was in general an increase in the blood urea level with feeding, this increase was of the same order for sheep fed both the control and urea silages. The mean blood urea figures for the three control sheep over a five-day period were 34, 36 and 43 mg%, respectively, for the pre-feeding, the 2 h and 4 h post-feeding samples. For the sheep fed the urea silage the corresponding values were 34, 37 and 44 mg%. The corresponding mean blood ammonia figures were 149, 190 and 154 μg% for the control sheep, and 154, 169 and 147 μg% for the sheep fed the urea-treated silages.

Discussion

The results of this experiment again confirm that fairly low losses of dry matter can be attained when heavily wilted herbage is conserved in a completely sealed plastic container. In comparing the results of the present experiment with other experiments in which herbage was ensiled with urea, regard must be paid to the fact that in none of the experiments reported was a sealing method used. The losses in the present experiment are, however, much lower than those normally encountered in lined trench silos.

Although the loss as inedible material reported in Table III is high, this figure should be interpreted carefully, since when partitioning this material into edible and inedible portions, the extremely high quality of the majority of the silage mass caused any material, however slightly contaminated with mould, to be classified as inedible, even though the material was frequently of a much higher quality than that fed and found to be palatable in farm practice. This also causes the figure for percentage loss of digestible constituents (Table V) to be exaggerated. The loss of material as waste is also attributable to the relatively small bulk of the material ensiled, causing the surface area to volume ratio to be large. In addition, the consolidation was poor since because of the small bulk it was not possible to consolidate using a tractor.

The fact that treatment with urea reduced dry matter losses is at variance with Huber *et al.*[10] who found the opposite effect, and Woodward & Shepherd[8] who found no effect of urea on dry matter losses.

The mean apparent digestibility of the nitrogen in the grass was 70·9 and of the control silage was 71·2 and the figures for the grass plus urea and the corresponding silage were 73·5 and 79·0, respectively. Assuming the A.R.C.[25] value of 5 mg N/kg of dry matter intake for the metabolic faecal nitrogen of ruminants, calculation of true digestibility for the silage suggests that the effect of urea addition on nitrogen digestibility is due not only to the higher nitrogen intake, but also to the high digestibility of nitrogen from the urea source and possibly also to an effect of urea addition on the digestibility of other forms of nitrogen. This effect is not present in the case of urea addition to the grass.

The true protein figures show the considerable breakdown of protein normally found in silage. This breakdown was less when urea was added, the mean losses from this fraction being 48% for the control silos and 40% for the urea-treated silos.

The apparent complete conversion of the added urea to ammonia is in agreement with Bentley *et al.*[12] and Owen *et al.*[15] but is contrary to the observations of Davis *et al.*,[7] Polan *et al.*[14] and Huber *et al.*[10] Presumably, much of the ammonia nitrogen was present as the ammonium salts of the volatile acids, lactic acid and other organic acids, otherwise losses would have been greater, since volatile nitrogen would have escaped on opening the silos.

The volatile organic acid data do not indicate any major difference in the fermentation pathways although a quantitative difference is indicated. The level of butyric acid was high in both types of silage, especially in the urea-treated silage. This high level of butyric acid in good quality high dry matter silage has been observed when the material was ensiled under conditions of complete air-tightness.[26]

The voluntary feed intakes on both types of silage were similar and hence the sheep eating urea silage had a higher nitrogen intake than those on the control silage. The experiment would have been more satisfactorily carried out using grass of lower nitrogen content if it had been available and it cannot be assumed that the fate of urea or the other nitrogen fractions will be the same when urea is added to low protein silage as when it is added to high protein silage.

One of the drawbacks to the utilisation of urea in ruminant diets is its rapid hydrolysis, resulting in excess ammonia which may then be followed by a deficiency of ammonia production. Feed efficiency and urea utilisation have been reported to increase with an increase in the frequency of feeding. Raleigh & Wallace[27] found that sheep fed once a day excreted more nitrogen than those fed three times a day. It should not be difficult in feeding urea-treated silage to ruminants to devise a husbandry system which permits a controlled pattern of silage intake.

The concentration of ammonia in the blood up to 4 h after giving free access to the silages indicated that the level of urea used on the silage was too low to have any effect on the peripheral blood concentration and certainly there was no danger of toxicity, since it has been shown that toxic symptoms do not appear in sheep until the blood ammonia nitrogen rises above 1 mg%.[28–30] The blood urea figures were unaffected by treatment. Lewis[30] is of the opinion that fluctuations in blood urea are the result of variations in the quantity of ammonia produced in the rumen and that such variations affect the blood urea after a latent period ranging from 4 to 8 h. In the light of this fact it would have been useful to continue observations on blood urea at intervals for a further 4 or more hours to see if the blood urea value had attained a maximum. When blood urea exceeds 50 mg% there is a considerable loss of nitrogen which may reach 15–30%.[31]

In the present experiment the level of urea added is below that in the other experiments cited, and it is possible that in those experiments, in which urea addition amounted to perhaps 8% of the dry matter, the presence of the high level urea could have affected the ability of the bacteria present on the ensiled material to hydrolyse urea. Also, in the present experiment, sealing the ensiled material in plastic created anaerobic conditions in which anaerobes and facultative anaerobes were capable of flourishing, and these may have had a greater urease activity than bacteria present under more aerobic conditions.

Acknowledgments

The authors wish to thank Professor W. O. Brown for useful discussion during the course of this work. They also thank the Directors and Trustees of the Agricultural Research Institute, Hillsborough, for providing facilities for the work, Mr. R. Thompson of the Veterinary Research Division who provided the blood urea data, and C. Black and W. Crawford for technical assistance.

Department of Agricultural Chemistry,
The Queen's University of Belfast,
Belfast, BT9 6BB
and
Ministry of Agriculture,
Northern Ireland

Received 13 January, 1970;
amended manuscript 26 May, 1970

References

1. Fingerling, G., Hientzsch, B., Kunze, H., & Heifgerst, K., *Landwn VersStnen*, 1937, **128,** 221
2. Harris, L. E., & Mitchell, H. H., *J. Nutr.*, 1941, **22,** 167
3. Briggs, M. H., 'Urea as a protein supplement', 1957 (London & New York: Pergamon Press Ltd.)
4. Essig, H. W., *J. Anim. Sci.*, 1968, **27,** 730
5. Bartley, E. E., Doyce, C. W., & Pfost, H. B., *Feedstuffs*, 1968, **40,** (17), 9
6. Brigl, P., & Windheuser, C., *Tierernährung*, 1931, **3,** 220
7. Davis, G. K., Becker, R. B., Dix Arnold, P. T., Comar, C. L., & Marshall, S. P., *J. Dairy Sci.*, 1944, **27,** 649
8. Woodward, T. E., & Shepherd, J. B., *J. Dairy Sci.*, 1944, **27,** 648
9. Wise, G. H., Mitchell, J. H., LaMaster, J. P., & Roderick, D. B., *J. Dairy Sci.*, 1944, **27,** 649
10. Huber, J. T., Thomas, J. W., & Emery, R. S., *J. Dairy Sci.*, 1968, **51,** 1806
11. Harris, R. R., Anthony, B., & Brown, V. L., in 'Highlights of Agricultural Research', Vol. 14, No. 2, (Auburn University); *Feedstuffs*, 1967, (June 3), p. 41
12. Bentley, O. G., Klosterman, E. W., & Engle, P., *Res. Bull. Ohio agric. Exp. Stn*, 1955, No. 766
13. Cullison, A. E., *J. Anim. Sci.*, 1944, **3,** 59
14. Polan, C. E., Huber, J. T., & Miller, C. N., *J. Dairy Sci.*, 1967, **50,** 616
15. Owen, F. N., Meiske, J. C., & Goodrich, R. D., *Minn. Beef Cattle Feeders Day Res. Rep. B*-74, 1966
16. Woodman, H. E., *J. agric. Sci., Camb.*, 1925, **15,** 343
17. Conway, E. J., 'Microdiffusion analysis and volumetric error', 1957 (London: Crosby & Lockwood)
18. McDonald, P., & Henderson, A. R., *J. Sci. Fd Agric.*, 1964, **15,** 395
19. Anderson, B. K., & Jackson, N., *J. Sci. Fd Agric.*, 1970, **21,** 228
20. Wheatley, V. R., *Biochem. J.*, 1948, **43,** 420
21. McCullough, H., *Clinica Chim. Acta*, 1967, **17,** 297
22. Jackson, R. B., *J. Sci. Fd Agric.*, 1964, **15,** 308
23. Brown, W. O., & Kerr, J. A. M., *J. Br. Grassld Soc.*, 1965, **20,** 227
24. Jackson, N., *J. Br. Grassld Soc.*, 1969, **24,** 17
25. Agricultural Research Council. 'The nutrient requirements of farm livestock No. 2. Ruminants'. 1965. Technical Reviews and Summaries (London: Agricultural Research Council)
26. Anderson, B. K., & Jackson, N., *J. Sci. Fd Agric.*, 1970, **21,** 235
27. Raleigh, R. J., & Wallace, J. D., *J. Anim. Sci.*, 1965, **24,** 595
28. Repp, W. W., Hale, W. H., Cheng, E. W., & Burroughs, W., *J. Anim. Sci.*, 1955, **14,** 118
29. Lewis, D., Hill, K. J., & Annison, E. F., *Biochem. J.*, 1957, **66,** 587
30. Coombe, J. B., & Tribe, D. E., *Nature, Lond.*, 1958, **182,** 116
31. Lewis, D., *J. agric. Sci., Camb.*, 1957, **48,** 438

SURVEY OF SOME FARM STORES OF VENTILATED GRAIN

By N. J. BURRELL and S. J. HAVERS

A short survey was carried out at 10 farms on low-rate ventilation systems in 15 grain bins and floor stores, on two on-floor driers and on two drying bins. The population density and the frequency of the mite genera present were recorded. Heavy infestations were mainly limited to the upper surface or to layers of damper grain in the bulks. The density of infestation in grain dried slowly in bins or on the floor was sometimes as heavy as in damp grain cooled by low-rate ventilation. Air distribution measurements showed that improvements to existing stores of ventilated grain could be obtained by repositioning the ducts, by using peak-loading instead of level-loading, by extending the length of ducting used, and in some instances by radically altering the duct systems. Further improvements could be made using automatic methods of controlling the cooling fans. The terms 'path ratio', 'drying ratio', 'velocity ratio' and 'cooling ratio' are defined and discussed to provide the basis for future improvements to the planning and efficiency of duct systems.

Introduction

Aeration at low rates of airflow (3–10 ft^3 air/min/ton of grain) is used in the U.K. to cool bulks of dry grain and so to prevent insect infestations arising.[1] This method, and systems using refrigeration,[2,3] have recently been used for damp grain storage in order to reduce drying costs, to increase the tonnage of saleable grain and to remove the bottleneck often created during a damp harvest by limited drying capacity. Frequent ventilation removes the heat generated by the grain and so prevents 'hot spots' arising. It does, however, permit infestation by mites which may be accompanied by musty odours and sometimes by mould growth.[4]

The results of ventilation on farms were likely to vary widely according to the moisture content of the grain and its temperature. The temperature depends upon the method used to select suitable ventilation periods, the rate of airflow and duration of ventilation periods, the geometry and size of the grain bulk and the duct spacing chosen. A short survey was, therefore, carried out in November and December, 1968 on the condition of grain held mainly at high moisture contents by low-rate ventilation in 15 bins and floor stores. The results were compared with those from two on-floor dryers and two drying bins. The study covered moisture content, temperature, mite infestation and air distribution through the grain; the variations of these conditions were measured in order to forecast the degree of success of the various systems and to provide a basis for recommending improvements to existing and future ventilation systems in grain stores.

Survey

Questionnaire

Ten farmers completed a questionnaire giving details of their grain stores and ventilation systems. Descriptions of how each operated and what results were expected from the systems were included.

Stores

Thirteen floor stores ranging from 70 to 315 tons in capacity and six bins of 30–45 tons were investigated. Not all stores on each farm were studied; where several identical bins or stores were in use, samples were taken from those reported to contain the dampest grain. The ventilation methods varied; 15 out of 19 stores were cooled by low-volume systems with low-powered fans and widely spaced ducting (Tables I and III), and of these all but two were aerated by drawing the air downwards to the duct. On farm IX the grain was dried before being loaded into the ventilated bins. Grain in two of the three stores on farm X was floor-dried using a high-powered fan and closely spaced ducting; on farm VII the 8 bins of grain were dried individually, or in batches, by a high-powered fan.

Sampling

Grain samples were normally taken at the centre of each floor store or bin, from the surface downwards, at 1 ft intervals. Where no power supply was available, duplicate samples were taken with a gravity sampler holding approximately 170 g of grain; a suction sampler[5] was used wherever possible. An initial sample was taken and discarded to clear the sampling tube of residual grain and mites. A 400 g sample was then taken, shaken 30 times in a sieve (30 mesh per in, 12 per cm), and the dust and mites were transferred to a polyethylene tube. The mites were later counted under a binocular microscope, and the genera were identified. Where mite numbers were extremely heavy, a counting disc, similar to that described by Solomon,[6] was used to estimate the numbers present. Sub-samples of the grain were retained for moisture determination, the grain being milled and then dried in a ventilated oven at 113°C for 4 h.

Airflow and air distribution

The mean rates of airflow (Table I) were based on measurements, where possible, and sometimes on fan characteristics. Air distribution and flow were assessed using a simple, calibrated, soap-film tube[7] constructed to measure velocities of 0·5–30·0 ft/min (0·2–9·0 m/min). Air passing up or down through the grain surface was led through a glass tube containing a detergent film. The time taken for the film to travel 1 ft (0·3 m) was measured by a stop watch. Readings were taken at intervals of 1–3 ft in rows selected as being likely to demonstrate the greatest variations in air velocities through the surface. Readings were not taken in all stores as in some the fans were not available for aeration, and unloading had started in others.

TABLE I

Details of farm stores investigated during survey

Farm stores investigated	Floor store (F) or bin (B)	Wheat (W) or barley (B)	Capacity, tons	Up (U) or down (D) ventilation	Fan (b.h.p.) per ton of grain stored*	Approx. rate of airflow (ft^3min/ft^3 grain)	Initial moisture content, %	Fan hours run, h	Moisture content at time of survey, %			Mites/kg			Mite surface density (% of total population)
									Max.	Min.	Mean	Max.	Min.	Mean	
I A	F	B	120	D	0·003	0·14		750	17·0	16·4	16·7	1030	260	700	11
B	F	B	150	D	0·003	0·14	14–22	750	16·7	15·8	16·3	4630	1580	3040	17
C	F	W	190	D	0·002	0·14		900	17·7	16·2	16·9	1090	120	550	50
D	F	B	170	D	0·002	0·14		900	19·5	17·4	18·6	5890	800	2370	62
III	F	B	250	D	0·0018	0·15	15–17	350	21·1	14·0	16·1	1128	0	330	30
IV A	B	W	30	D	0·03	0·35		370	17·7	17·3	17·4	12500	25	2600	97
B	B	B	30	D	0·03	0·35	Mean 17	370	17·9	15·2	15·8	962	35	212	68
V	F	B	224	D	0·001	0·12	13·7–18	260	19·1	15·6	17·1	2362	12	420	66
VI	F	B	140	D	0·07	0·25	14–25	404	22·5	15·6	19·3	5275	1287	2235	58
VII A	B	W	32	U	0·11	0·15	17–21	200	18·0	14·5	15·3	6000	5	330	80
B	B	B	32	U	0·11	0·2		200	15·2	13·1	13·9	4750	0	220	95
VIII A	B	B	45	D	0·004	0·09	15·5–24	720	22·7	18·7	20·7	6350	375	2180	73
B	B	W	45	D	0·004	0·09		720	20·7	19·5	20·0	31250	18750	27000	40
IX A	F	W	250	U	0·001	0·04	Mean 14	280	16·7	12·6	13·8	413	0	121	87
B	F	B	250	U	0·001	0·04		280	16·6	13·8	14·7	15	0	2	66
X A	F	W	70	U	0·21	1·0	16–22	404	17·4	14·1	15·0	562	8	200	95
B	F	B	260	U	0·21	1·1		404	18·9	14·0	15·9	62000	8	12500	95
C	F	B	300	D	0·0025	0·2	16–28	2400	21·5	17·7	18·6	111000	802	7670	77
XI	F	W	315	D	0·002	0·12	16·5–22·7	723	21·7	17·4	18·5	4750	37	970	88

* On some farms the fans were shared between up to 8 ducts

Temperatures

Temperature records were obtained from two farmers but the majority of temperatures, given later, were based on measurements made in a 70-ton farm floor store and in a nest of 6 bins, each of 20 tons, ventilated under various schedules at the laboratory (Figs 1 and 2). The method chosen by each farmer to govern fan operation was then used as a basis for estimating probable temperatures within those farm bins for which temperature records were not available.

Methods of operating fans and the results on grain temperature

Initially, at farms I, V, VI, VII, VIII, X and XI, the freshly harvested grain was aerated continuously for 2–10 days. The grain at farm III was cooled at night only (avoiding foggy periods), that at farm IV was cooled automatically when the air temperature fell below the temperature measured by a sensor near the centres of the bins; at farm IX, aeration was mainly during the day and during periods either of low relative humidity or of low temperature. Eight of the ten farmers expected some drying as well as cooling. Some used experience to decide when the weather was suitable for drying and, at farms V and VI, fans were operated when the R.H. fell below 70 and 84% respectively. At farms III and IV no attempt was made to select air of low R.H. and temperature only was considered. At farms V, VIII and X, selection was by relative humidity only, slow drying being the main object, but incidental cooling was considered to be useful.

The variations in the method of selecting suitable periods of weather for aeration lead to temperature differences in different stores (Figs 1 and 2) and within individual stores. During continuous ventilation, warm layers of grain were introduced during the day and were followed by cooler bands at night. Following the 1968 harvest initial grain temperatures were assessed at 18–21°c. In stores where the farmer considered cooling to be most important, these temperatures would be expected to fall to 10–13°c during September and to remain near this level until the end of October when the temperatures would fall to 2–10°c at the time of sampling in November and December. On the farms attempting drying only, the mean temperatures were about 3° higher except in those stores where grain heating occurred.

Results

Moisture contents

Differences between the moisture contents reported by the farmers on loading the bins and those found by the authors on sampling (Table I) were due to various factors. These include moisture exchange between the grain and the ventilating air, and the differences in methods of sampling and moisture determination used by the farmers and by the authors. It was not possible, therefore, to determine definitely whether the moisture content of the grain had changed during storage.

Mites

The density of the mite population in relation to the moisture content of the grain is shown in the scatter diagram (Fig. 3). There is a positive correlation coefficient of 0·4. The surface layers, usually a little damper than the rest of the grain bulk, supported the heaviest infestation, sometimes as much as 97% of the total mite population (Table I).

Mite density varied from a few *Glycyphagus* spp. found in barley (previously dried to 14·5% moisture content) to up to 111,000 mites/kg on grain at 21·5% moisture content. Of the eight genera identified, *Glycyphagus* spp. and *Acarus* spp. were dominant, occurring in 90% and 60% of samples, respectively (Table II). Barley and wheat were equally infested by these genera.

The predatory mite, *Cheyletus* spp. occurred only in the 14–17% moisture content range. It was present in such small numbers as to have little effect on the acarid population. More frequent predators were the gamasid mites, which were found over a wider range of moisture contents (14–22%). This genus was more common on barley than wheat.

Tarsonemus spp., present in the moisture content range of 15–19%, were found in 10% of the samples. This genus occurred in 19% of samples of Japanese grain[8] and in 9% of samples of Canadian grain.[9]

Tydeus spp. occurred in 7% of the samples, and were found in the moisture content range of 16–20%. Sinha[8,9] found *Tydeus* spp. occurring in 6% and 22%, respectively, of samples of Japanese and Canadian grain. He found, however, that this genus preferred wheat to barley whereas in this

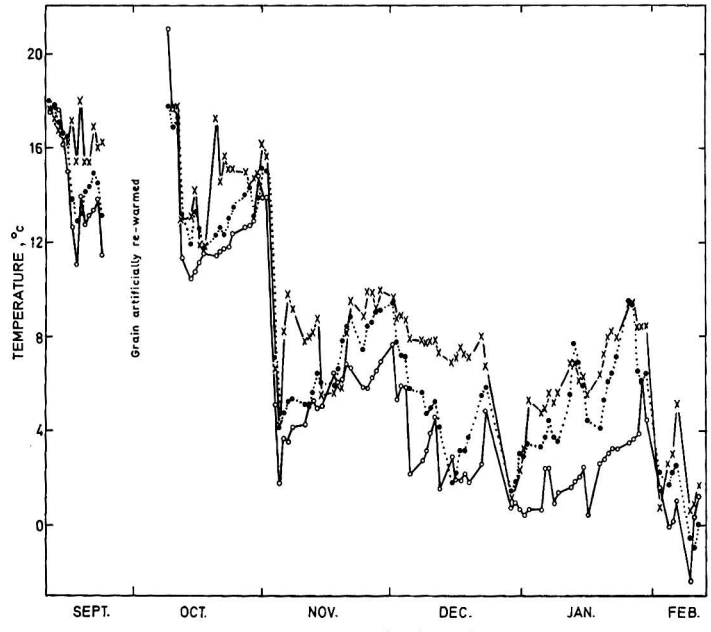

FIG. 1. *Temperatures in ventilated grain bins*, 1968–69

Bin II fan operated by thermostat; bin III fan operated by time proportioning controller; bin V fan operated by humidistat
○ Bin II (mean temp. 5·4°C); ●, bin III (mean temp. 7·0°C.) × bin V (mean temp. 8·5°C)

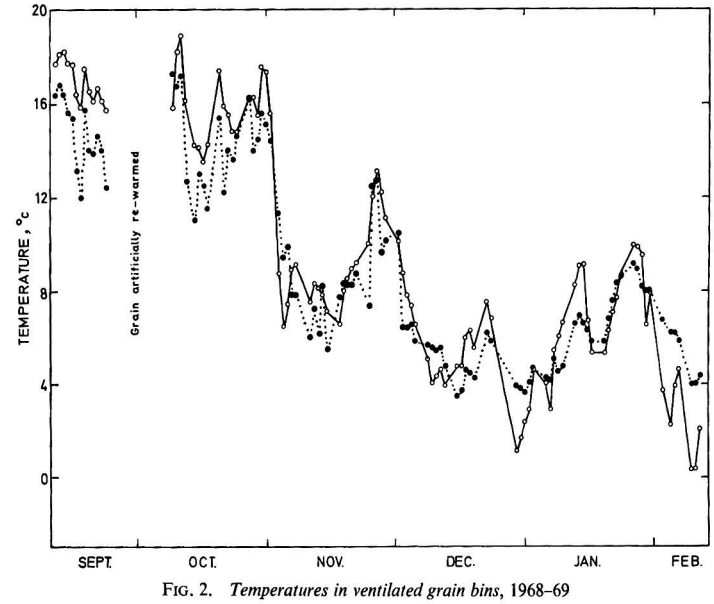

FIG. 2. *Temperatures in ventilated grain bins*, 1968–69

Bin I fan operated by time switch (8 a.m.–12 noon; 4 p.m.–7 p.m.); bin VI fan operated by time switch (12 midnight to 7 a.m.)
○ Bin I (mean temp. 8·6°C); ● bin VI (mean temp. 8·3°C)

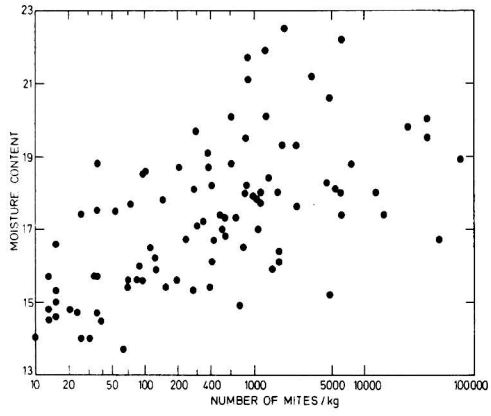

FIG. 3. *Scatter diagram showing relationship between moisture content and number of mites in 92 samples from 19 grain stores*
There is a positive correlation with a coefficient of 0·4

survey, *Tydeus* spp. and *Tarsonemus* spp. were present only in barley samples.

Chortoglyphus spp., infesting grain mainly at 17–22% moisture content, were more frequent on wheat. The remaining genus, *Acaropsis*, was only found in one surface sample of barley.

Airflow measurements and air distribution

The measurements (Table III) showed air channelling in the 2 bins with perforated floors (farm VII) and in the bins with single ducts at the base (farms IV and VIII). The ratios of fastest to slowest air velocities (the velocity ratio) out of the top surface of the two bins at farm VII were 1·7 and 1·9:1. The velocity ratios in the shallow floor stores varied from 1·5:1 to 2·4:1 with the exception of farm I where a greater variation was found. The importance of such variations in bins has been noted elsewhere;[2] the greater the velocity ratio the greater is the variation in time taken to cool different parts of a bulk of grain.

There was a large difference between the airflows through apparently identical bins of wheat and barley on farm VII; this difference may have resulted from several factors

TABLE II

Percentage distribution of mites in relation to moisture content, frequency of appearance in the samples, and apparent preference for wheat or barley

Genus	Percentage distribution in relation to moisture content, %											Frequency (%) in in total no. of samples	Preference (if any) to wheat or barley
	12	13	14	15	16	17	18	19	20	21	22		
Glycyphagus	100	43	47	100	100	100	84	58	100	100	59	90	—
Acarus	—	29	53	47	75	56	68	43	100	100	100	60	—
Chortoglyphus	—	—	5	—	—	18	47	29	75	20	100	20	W
Gamasids	—	—	5	—	—	6	26	43	50	40	100	14·5	B
Tarsonemus	—	—	—	18	25	6	10	29	—	—	—	10	B
Tydeus	—	—	—	—	8	6	—	43	25	—	—	7	B
Cheyletus	—	—	10	12	—	18	—	—	—	—	—	7	—
Acaropsis	—	—	—	—	8	—	—	—	—	—	—	1	B
No. of samples	1	7	19	17	12	18	19	7	4	5	2		

TABLE III

Results of duct spacing, grain depth and ratios of longest to shortest air paths on air distribution and rate of cooling

Farm	Store	Airpath		Path ratio (a/b):1	Air velocities through surface, ft/min		Velocity ratio (c/d):1	Cooling* and drying ratio $\left(\frac{a}{b} \times \frac{c}{d}\right)$:1
		Longest (a), ft	Shortest (b), ft		Max. (c)	Min. (d)		
I	C	23	7	3·3:1	1·6	0·38	5:1	16:1
III		16	9	1·7:1	2·1	1·3	1·6:1	2·7:1
IV	A	18	13	1·4:1	3·5	2·2	1·6:1	2·2:1
	B	18	13	1·4:1	2·6	2·1	1·2:1	1·7:1
V		16	17	2·3:1	1·2	0·8	1·5:1	3·5:1
VI		19	7	2·7:1	1·6	0·8	2·0:1	5·4:1
VII	A	16	16	1:1	3·1	1·6	1·9:1	1·9:1
	B	16	16	1:1	6·0	3·5	1·7:1	1·7:1
VIII		22	17	1·3:1	2·0	0·8	2·5:1	3·3:1
IX		10	6	1·7:1	1·8	1·2	1·5:1	2·6:1
X	A	6	4	1·5:1	8·5	3·5	2·4:1	3·6:1
	B	8	6	1·3:1	12·0	6·3	1·9:1	2·5:1
XI		17	7	2·4:1	2·1	1·0	2·1:1	5·0:1

* The theoretical conception of a cooling ratio can only be considered relevant to grain bulks of infinite size. In small bulks the time taken to cool all the grain in a store will be reduced by natural cooling, particularly at the surfaces

including partial blockage of the perforated tiles or excessive dust in the bin of wheat.

Where ducts were blanked-off for the first few feet from the wall, in level-loaded stores (e.g., farms I and XI), reduced rates of airflow were found towards the corners but rates were adequate elsewhere in the bins. On farm I, for example, the air movement became immeasurably low several feet from the corner.

Where mounds of grain protruded only 1–2 feet above the levelled surface in a bin, lower air velocities were found through the mound than occurred around it. At farm VII a mound 1 ft high in a bin resulted in a 30% drop in airflow, but where mounds were present directly above the ducts in level-loaded floor stores, the air distribution was improved as the mounds reduced excessive passage of air through the grain immediately above the ducts, causing greater lateral air movement.

Mustiness and mould growth

Musty odours have been reported to be noticeable after about 6 months on cooled grain stored at over 18% moisture content, and at 20–21% moisture there was often visible mould growth in a similar period.[4] These moisture levels were exceeded on some farms but at the time of sampling (2–4 months after harvest) little mustiness or mould was apparent. Mustiness was, however, noticeable in one bin of wheat at up to 21·7% moisture and in barley at 20·1–22·7% moisture content. Limited mould growth was seen in samples taken from a store of floor-dried wheat and in damp ventilated wheat at 21·5% moisture content.

Discussion and Conclusions

The main aims of this short survey were to find whether low-rate ventilation, as carried out on farms, was satisfactory for the safe storage of damp grain and to find methods of improving ventilation systems. The majority of grain stored at above 16% moisture content, by low-rate ventilation, is likely to be used for stockfeed, since at higher moisture contents other changes, e.g., loss of viability,[3,4] are probable after 6–8 months storage.

Mite infestation

The ventilation of damp grain may be expected to lead to the increasing occurrence of mite infestations but the results showed that slow-drying in bins could result in similar levels of infestation. Only where grain was dried to below 14% moisture content were insignificant numbers of mites found. Whether it is safe for humans and animals to consume mite-infested grain can only be decided by future medical and veterinary evidence, since although published information suggests that mites themselves may not be a great danger to animal health,[10–13] contradictory reports are common.

Physical damage produced by mites on whole grain has been measured by Solomon[14] who found a relationship between the product (no. of mites/100 ml [63–69 g grain]) × (time in weeks) and the degree of damage to grain. Solomon[14] calculated a mean density × time value corresponding to 1% loss, as being 526,000/100 ml of grain at 25°c and 1,765,000 at 5–9°c. He also found a consumption of up to 3% by wt. of the grain before the infestation died out, the damage being confined almost entirely to the germ. Based on these figures the densities of the mite populations encountered in the survey were unlikely to have caused serious physical damage to the grain. Freeman & Turtle[15] found, however, that 7500 mites/kg of wheat produce a taint which may persist throughout the milling processes. On this basis, 7500 mites/kg could be regarded as a heavy infestation and the surface of four of the nineteen bins and stores examined may be said to be heavily infested; however, only one of these stores (farm VIII) was similarly infested from top to bottom.

Path ratio, velocity ratio, cooling ratio and drying ratio

In an ideal bin system, with a perforated floor, air might be expected to pass evenly through the grain. In practice, dust deposits and differences in packing prevent this[2] and measurements at farm VII (Table III) showed air-channelling to occur in bins with perforated floors. Although the ratio between the longest and shortest airpaths i.e., the 'path ratio,' cannot be improved by changes in the duct system in such bins, since the ratio is already 1:1, nevertheless the variation between fastest and slowest air velocities i.e., the 'velocity ratio', out of the top surface of the two bins was 1·7 and 1·9:1, respectively. For the purpose of this discussion, therefore, a velocity ratio of up to 1·9:1 is described as 'good' since it is unlikely to be improved apart by changes in the cleaning and loading methods employed.

Following a similar argument, the path ratio of 1·3:1 found in a bin on farm VIII provided a velocity ratio of 2·5:1. It is suggested, therefore, that in floor stores a path ratio producing a velocity ratio of up to 2·5:1 is 'adequate' for grain cooling purposes.

The importance of the velocity ratio was stressed by Burrell & Laundon[2] whose measurements showed that a velocity ratio of 1·7:1 in a bin of grain 33 ft deep was associated with a cooling time of 34 h in the most rapid cooling area and 60 h in the slowest, i.e., a cooling ratio of 1·8:1.

Holman[16] suggested that for ventilated stores, the longest airpath through the grain should not exceed the shortest by a ratio of more than 1·5:1 (Fig. 4(a)). This guide gives entirely satisfactory results but others,[1] although agreeing in principle with the lower ratio, found satisfactory cooling in peak-loaded stores with path ratios of 1·7 or 1·8:1. In order to achieve a path ratio of 1·5:1 in level-loaded stores, the spacing between ducts would equal the grain depth and the distance from duct to wall would be half the grain depth. The path ratios in the six bins covered by the survey, were below 1·5:1 but in all the floor stores this ratio was exceeded. The air distribution in floor stores with path ratios of from 1·7:1 to 2·7:1 (Fig. 4(d)) seemed adequate when based on the evenness of airflow out of the surface. It should be remembered, however, that the slowest air velocity is usually found at the end of the longest airpath. In farm VI, for example, the ratio of longest and shortest airpath was 2·7:1 and the air velocity above the duct was twice that near the wall. Assuming no natural cooling to occur, the time necessary to cool the grain near the longest airpath was, therefore, likely to be 2·0 × 2·7, i.e., 5·4 times longer than the column of grain above the duct, i.e., along the shortest airpath. In practice, natural cooling intervenes to reduce the temperature of the slowest cooling area but if the grain is damp and requires to be dried there may be a 'drying ratio' of up to 5·4:1 between the slowest and the most rapid drying grain in the bulk.

Scale effects of grain ventilation

In addition to the geometric ratios of duct spacings in a grain bulk, the size of the bulk affects the rate of natural cooling particularly at the centre of the heap; the smaller the bulk the faster it cools. Ducts placed in small bulks of grain

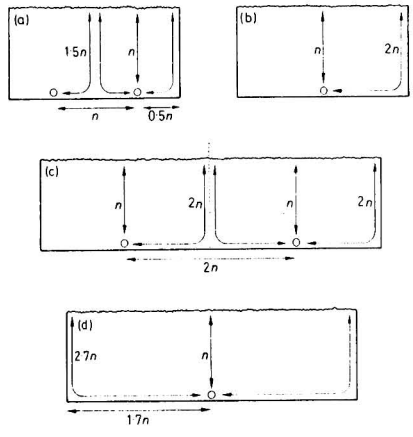

FIG. 4. *Effect of the distance ratio on air distribution in a level-loaded store*

(a) Good air distribution under all conditions of ventilated grain storage at a ratio of 1·5 *n*:*n* (where *n* = grain depth above the duct, where the space between duct centres is equal to the grain depth and where the distance from duct to wall = 0·5 *n*). Suitable for small bulks of grain over 18% moisture content; (b), (c) a distance ratio of 2*n*:*n* gives adequate air distribution in small bulks of grain at up to 18% moisture content; (d) a distance ratio of up to 2·7*n*:*n* gives adequate air distribution is small bulks of dry grain

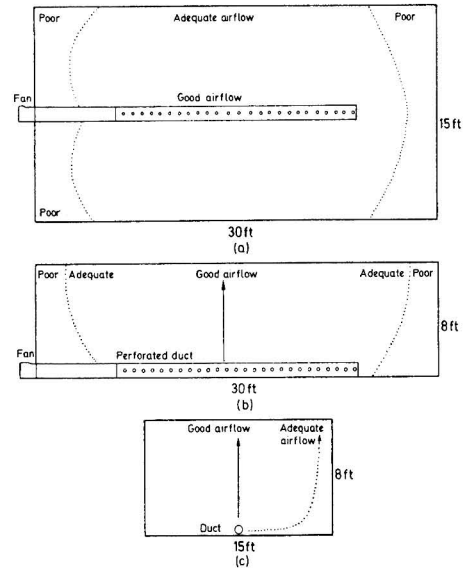

FIG. 5. *Air distribution from a single perforated duct in a level-loaded, ventilated bin of grain*

(a) Plan of 75 ton bin; (b) longitudinal section; (c) median section
Note the poor air flow near the corners due to the inadequate length of perforated duct which should extend from one end of the bin to the other in a level-loaded store.

may, therefore, be spread relatively further apart (Fig. 4) than in large bulks since, in the latter, natural cooling is mainly peripheral.[1] With grain above 16% moisture, however, even the periphery of the bulk must be adequately ventilated since the heat produced by respiration may exceed the heat losses by natural cooling.[1] For grain above 18% moisture content it is also necessary to provide an even air distribution of sufficient magnitude to produce a drying effect if the damp grain is to remain satisfactory for stockfeed.

Biological factors of the ventilation of damp grain

The survey showed that the choice of duct spacing, the rate, and the periods of ventilation in the grain stores should not be determined only by physical factors. The effect of temperature and moisture content on the biology of stored grain is well-established;[1] for example, samples from the farm stores showed that mites increased rapidly with increasing moisture contents above 13%. In addition, at the temperatures achieved in ventilated grain stores, Burrell *et al.*[4] found that in grain stored at over 16% moisture content, the percentage germination was likely to be reduced owing to death of the grain, to maintenance of dormancy, or to the imposition of a secondary dormancy. Musty odours often occurred in cool grain above 18% moisture content, after about 6 months, and visible mould colonies appeared on grain above 20% moisture content. The duct spacing and rate of ventilation should, therefore, be capable of conditioning the grain to an extent which would vary according to the intended use of the grain and to its moisture content.

Selection of duct position

As described earlier, the simplest method of determining duct position in all stores is to arrange that the longest airpath from the duct to the grain surface does not exceed the shortest airpath by much more than a ratio of 1·5:1. This ratio was, however, often exceeded in small bulks of grain where natural cooling was substantial. In addition the biological factors which depend on the moisture content and future use of the grain should also be taken into account. It is suggested that the following ratios may serve as a useful guide for future installations.

For damp grain likely to exceed 18% moisture, the path ratio should be between 1·5 and 1·7:1; for grain at 15–18% moisture content the ratio may be as high as 2:1; and for bulks of dry grain up to 200 tons, path ratios of up to 2·7:1 seem acceptable. For very large bulks of grain, however, e.g., over 600 tons, the path ratio should be kept to 1·5 or 1·7:1.

Improvements to existing ventilation systems

The air distribution showed that large improvements to many existing stores of ventilated grain loaded to an even depth could be obtained by repositioning the ducts (Fig. 4). Further improvements could be achieved by extending the perforated ducting completely across the store instead of following the usual practice of using a length of unperforated ducting under the grain near the fan and ending the ducting several feet from the wall away from the fan (Fig. 5). Airflow measurements also confirmed previous unpublished work showing more even airflow in those stores where the grain is peak-loaded above the ducting than in level-loaded stores.[4] One disadvantage of peak-loading over level-loading lies in the increased grain pressures that are exerted against the walls of a peak-loaded store. However, if the capacity of each store was maintained at the original tonnage by increasing the depth at the centre and by reducing the depth against the walls, the wall pressures could be reduced by 'peak-loading'.

The practice of leaving mounds of grain in a haphazard fashion over the surface of a level-loaded grain bulk produced greater variation in air flow, but where the heaps were above the duct more even air distribution resulted. An increase in grain depth over the duct would be expected to increase resistance to airflow but the graphs given by Holman[16] showed that the increase in static pressure provided by 3–4 ft more grain would be insignificant in bins ventilated at a low rate of airflow.

The uneven cooling that occurred in many stores was corrected by long periods of aeration (Table I). On at least 3 of the 10 farms some grain heating or mould growth was experienced. This happened on farm I which had the poorest air distribution, on farm V where distribution was good but where, as a result of aeration during fine weather only, the hours of ventilation were lower and temperatures were higher, and on farm XI where the path ratio was 2·4:1 and where the moisture content remained high.

Improvements in methods of controlling fan operation were possible in most instances. Even on the only farm where thermostatic control was used, low grain temperatures were not easily obtainable as the thermostat could not be set to operate at a sufficiently low temperature. A thermostat calibrated to 27°F (−3°C) or below is necessary to achieve sub-zero temperatures. Fan operation at low relative humidities, e.g., using a humidistat, is unlikely to provide sufficient drying to prevent increase in mite population, because of the high prevailing relative humidities during November to March. The achievement of low temperatures for mite control during the coldest months, however, seems practicable.

Acknowledgments

The authors would like to thank all the farmers for their co-operation in the survey, and Mr. J. K. Grundey of the National Agricultural Advisory Service, Norwich for his kindness in supplying their names. The authors also thank Mr. S. Gordon for his help with the experimental work.

Agricultural Research Council,
Pest Infestation Laboratory,
London Road,
Slough,
Bucks.

Received 6 February, 1970

References

1. Burges, H. D., & Burrell, N. J., *J. Sci. Fd Agric.*, 1964, **15,** 32
2. Burrell, N. J., & Laundon, H. J., *J. stored Prod. Res.*, 1967, **3,** 125
3. Burrell, N. J., *Ceres*, 1969, No. 5, p. 15
4. Burrell, N. J., *et al.*, *Pest Inf. Res. Rep.*, 1965, 1966, 1967, 1968 (London: H.M.S.O.)
5. Burges, H. D., *Bull. ent. Res.*, 1960, **51,** 1
6. Solomon, M. E., *Ann. appl. Biol.*, 1945, **32,** 71
7. Burrell, N. J., Dacke, C. G., & Laundon, J. H. J., *Res. Develop. Ind.*, 1963, **27,** 32
8. Sinha, R. N., *Ann. ent. Soc. Am.*, 1968a, **61,** 938
9. Sinha, R. N., & Wallace, H. A. H., *Ann. ent. Soc. Am.*, 1966, **59,** 1170
10. Szwabowicz, A., & Miedzobrodzki, K., *Medycyna wet.*, 1957, **13,** 475
11. Szwabowicz, A., Miedzobrodzki, K., & Donigiewicz, K., *Med. vet.*, 1957, **13,** 724
12. Szwabowicz, A., Miedzobrodzki, K., & Schmid, W., *Med. vet.*, 1958, **14,** 344
13. Szwabowicz, A., Miedzobradzki, K., Pankowa, J., & Holnicka, B., *Med. vet.*, 1958, **14,** 556
14. Solomon, M. E., *Ann. appl. Biol.*, 1946a, **33,** 280
15. Freeman, J. A., & Turtle, E. E., 'Insect pests of food: the control of insects in flour mills,' 1947 (London: H.M.S.O.)
16. Holman, L. E., *U.S. Dept. Agric. Marketing Res. Rep.*, 1960, No. 178

A POSSIBLE RAPID METHOD OF DETERMINING THE MOISTURE CONTENT OF HIGH-MOISTURE GRAIN

By S. W. PIXTON

A linear relationship was shown to exist between the moisture content of barley and of tick beans and the temperature rise obtained when a quantity of concentrated sulphuric acid was added to the grain. This relationship can be used to determine the moisture content of grain, particularly at the higher moisture levels which are often outside the upper limit of many existing rapid methods of moisture measurement.

The 'acid' method is reasonably rapid, taking about 15 minutes, and gives a reproducibility of approximately 1·0% moisture content. If the initial temperature of the grain differs widely from the calibration temperature, a temperature correction must be applied.

Introduction

Rapid determination of the moisture content of high-moisture grain with reasonable precision is becoming increasingly necessary. Storage of 'wet' grain under airtight conditions is a well established farming practice, and, at the recommended upper moisture limit, about 24% for this form of storage, the present rapid methods of measuring moisture are adequate. However, more recently, treatment of wet grain with propionic acid, at a dosage rate related to the moisture content, has enabled much wetter grain to be stored in air without mould growth and subsequent heating. For use on grain so treated, a rapid method for measuring moisture content of up to, say 40%, reproducible to about ±1% is required, as the rapid methods generally used have an upper limit of measurement of only 24–28%.

Of the various rapid methods that were practicable at high moisture contents an exothermic method investigated by Visyagin & Lisina[1] seemed to be the most promising. They found that by treating whole grain of up to 18% moisture content with concentrated sulphuric acid the temperature rise obtained in 5 minutes was linearly related to the moisture content.

These results seemed sufficiently encouraging to warrant further study at higher moisture contents than those used by Visyagin & Lisina.[1] The present paper describes an investigation of the addition of concentrated sulphuric acid (sp.gr. 1·84) to barley and to tick beans and measuring the rise in temperature obtained at a range of moisture content from 14 to 35%.

Experimental

Barley

Six samples of barley were prepared to a range of moisture content from 14 to 35%. After an equilibration period of 14 days at 5°C, the moisture contents were determined by oven-drying ground samples in a mechanically ventilated oven for 4 h at 113°C,[2,3] using a two-stage procedure if the moisture content exceeded 20%.

Determinations using concentrated sulphuric acid were made on the prepared samples of barley in triplicate. Three grammes of barley were put into a Pyrex test-tube of i.d. 5/8 in (1·6 cm) and approximately 6 in (15·2 cm) long. A thermometer graduated in intervals of 0·1°c was inserted into the barley in the tube and the initial temperature recorded. Three ml of concentrated acid were added, this quantity being just sufficient to cover the grain in the test-tubes, and the rise in temperature was recorded at intervals of 1 min up to a total time of 15 min. In order to increase the temperature rise obtained, which would be an advantage, the test was repeated with the test-tubes insulated by being placed in holes in a 2 in thick block of polystyrene. The ambient temperature in this experiment was 15°c. Each set of determinations was repeated and the mean temperature rise of six observations at each moisture content after 5, 10 and 15 min in insulated tubes, and after 5 min in non-insulated tubes, was related to the moisture content of the grain as determined by the oven method.

Beans

A similar investigation was carried out on tick beans (*Vicia faba*). The ambient temperature for this series was 20°c. Determinations were first made using the whole bean, and later, in an attempt to obtain a more rapid temperature rise, on the samples of beans split in half to increase the surface area exposed to the acid.

Results and Discussion

Fig. 1 shows the relationship between the moisture content of barley and the rise in temperature obtained when the grain was immersed in the acid for 5, 10 and 15 min in insulated tubes, and for 5 min in non-insulated tubes. The relationship between moisture content and temperature rise for tick beans, whole and split, is shown in Fig. 2. The calculated structural relationship of the different lines, together with the standard error of the observations, is given in Table I. The structural relationships were examined by the method of Lefkovitch & Pixton[4] and it was found that there was no reason to doubt a linear relationship between moisture content and the rise in temperature obtained in each set of results, i.e., the estimated constant in each equation satisfactorily calibrated that particular set of conditions.

Insulation of the test-tubes is desirable. There appears to be no advantage in extending the exposure time beyond 10 min for either barley or beans. In barley, the temperature rise of 1·2°c for each 1% increase in moisture is twice that of beans, both in insulated and non-insulated tubes. The lines for whole and split beans (Fig. 2) run almost parallel to each other, a greater temperature rise being obtained when the beans are split. The temperature rise obtained is insufficient to justify the labour of splitting the beans, as splitting does not increase the rise in temperature for each 1% moisture. The standard error of the method for both barley and beans, whether in insulated tubes or not, and for times of 5, 10 and 15 min, is about the same (Table I).

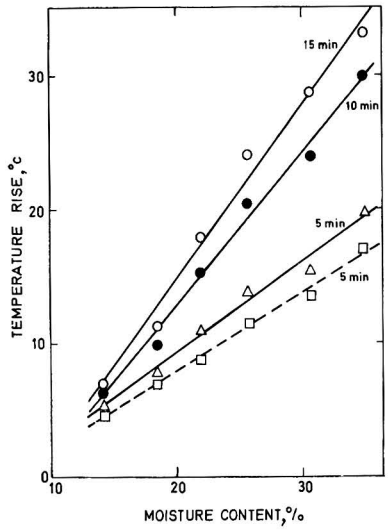

FIG. 1. *Relationship between the moisture content of barley and the temperature rise obtained when 3 g of barley are immersed in concentrated sulphuric acid for 5, 10 and 15 min in insulated tubes (———), and for 5 min in non-insulated tubes (- - - - -)*

Initial temperature of barley approximately 15°C

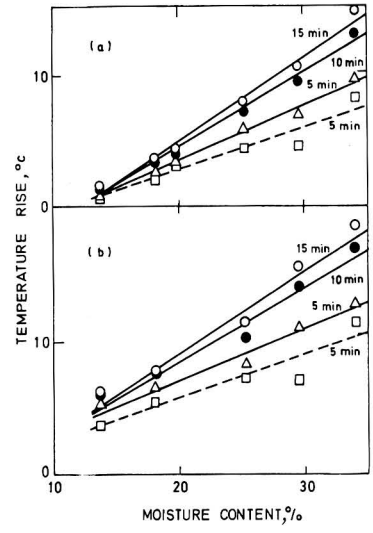

FIG. 2. *Relationship between the moisture content of whole beans* (a) *and split beans* (b) *and the temperature rise obtained when 3 g of the beans are immersed in concentrated sulphuric acid for 5, 10 and 15 min in insulated tubes (———), and for 5 min in non-insulated tubes (- - - - -)*

Initial temperature of beans approximately 20°C

TABLE I

Summary of relationship between temperature rise, moisture content and time of exposure to concentrated sulphuric acid, for barley, whole and split beans

	Product	Ambient temperature (approx), °C	Time, min	Relationship of temperature rise (y) °C to moisture content (x) % wet weight	S.E. of y	S.E. of x
Insulated tubes:			5	$y = 0{\cdot}6716x - 4{\cdot}2439$	0·83	1·24
	Barley	15	10	$y = 1{\cdot}1295x - 9{\cdot}9262$	1·21	1·22
			15	$y = 1{\cdot}2902x - 11{\cdot}1595$	1·62	1·26
			5	$y = 0{\cdot}4112x - 4{\cdot}6734$	0·49	1·22
	Whole beans	20	10	$y = 0{\cdot}5626x - 6{\cdot}7585$	0·75	1·22
			15	$y = 0{\cdot}6321x - 7{\cdot}7088$	0·76	1·19
			5	$y = 0{\cdot}3880x - 0{\cdot}8086$	0·49	1·26
	Split beans	20	10	$y = 0{\cdot}5621x - 2{\cdot}9218$	0·70	1·25
			15	$y = 0{\cdot}6155x - 3{\cdot}3547$	0·81	1·31
Non-insulated tubes:	Barley	15	5	$y = 0{\cdot}5764x - 3{\cdot}7555$	0·72	1·25
	Whole beans	20	5	$y = 0{\cdot}3102x - 3{\cdot}3679$	0·40	1·28
	Split beans	20	5	$y = 0{\cdot}3227x - 0{\cdot}8596$	0·42	1·31

The heat change accompanying any process, physical or chemical, generally varies with temperature. Results of a test, with barley, at initial temperatures of 10°, 20° and 30°C, at one moisture content (18%), in both insulated and non-insulated tubes, are shown in Fig. 3. It will be seen that the temperature rise obtained in a given time increases with increasing initial grain temperature, particularly in the insulated tubes. In the non-insulated tubes there was an apparent increase in the temperature rise obtained as the initial temperature of the grain was increased; the greatest increase was at 30°C, the highest initial temperature that was used. If the effect of time is considered at a given initial grain temperature it will be seen that the rate of evolution of heat increases with increased initial temperature of the grain, particularly in the insulated tubes.

Conclusions

There is a satisfactory linear relationship between the moisture content of barley, and that of tick beans, over the range 14 to 35%, and the temperature rise obtained when the grain is immersed in concentrated sulphuric acid. The standard error of approximately $\pm 1{\cdot}0\%$ is considered acceptable in this moisture range. Insulation of the test containers protects them from rapid changes in the ambient

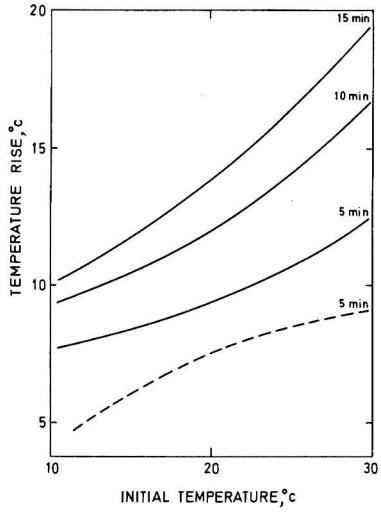

FIG. 3. *Effect of varying the initial temperature of barley, a approximately* 18% *moisture content, on the temperature rise obtained after* 5, 10 *and* 15 *min in insulated tubes* (———), *and after* 5 *min in non-insulated tubes* (- - - - -)

conditions and also enables a greater rise in temperature to be obtained.

The temperature rise obtained at a given moisture content is influenced by the initial temperature of the grain. If determinations are to be made at temperatures which differ widely from the calibration temperatures, the results obtained require correction.

If the conditions of the test procedure are carefully standardised it is possible to obtain a reasonably rapid estimate of the moisture content of wet whole grains.

Acknowledgments

The author would like to thank Miss S. Warburton for her assistance throughout.

Ministry of Agriculture, Fisheries and Food,
Pest Infestation Control Laboratory,
London Road,
Slough,
Bucks.

Received 13 February, 1970;
amended manuscript 20 April, 1970

References

1. Visyagin, N. I., & Lisina, A. I., *Selekts. Semenov.*, 1950, **17**, 53; *Chem. Abstr.*, 1951, **48**, 8438
2. Oxley, T. A., Pixton, S. W., & Howe, R. W., *J. Sci. Fd Agric.*, 1960, **11**, 315
3. Pixton, S. W., *J. stored Prod. Res.*, 1967, **3**, 35
4. Lefkovitch, L. P., & Pixton, S. W., *J. stored Prod. Res.*, 1967, **3**, 81

DETERMINATION OF DIMETHYL SULPHIDE IN BEER AND LAGER

By A. SINCLAIR, R. D. HALL, D. THORBURN BURNS* and W. P. HAYES*

A gas chromatographic method is described for the determination of dimethyl sulphide in beer. The peak height of the dimethyl sulphide is compared with that of an n-butanol standard and the concentration calculated using their relative response factors.

When authentic dimethyl sulphide was added to beer good recoveries (99% average) were obtained. Determination of the reproducibility from duplicate analyses of beer samples gave an average coefficient of variation of 7·7% for the concentration range of 0–35 μg/l.

Introduction

Whereas the presence of dimethyl sulphide in beer has been reported by Ahrenst-Larsen & Hansen[1] and Kepner *et al.*,[2] its quantitative determination has received scant attention. A recent paper by Drews *et al.*[3] describes a method dependent upon expelling the dimethyl sulphide from the sample by a current of nitrogen. The dimethyl sulphide is oxidised to dimethyl sulphone which is assayed by gas chromatography. Since sample preparation by this method is time consuming, a procedure using direct injection of the beer vapour is now described in which the dimethyl sulphide is determined using n-butanol as internal standard. When this method was used to analyse a variety of beers the reproducibility of the results had a coefficient of variation of 7·7% obtained by duplicate analyses of seventeen samples.

The identity of the dimethyl sulphide peak has been confirmed by comparison of both the retention data on two different stationary phases and the reaction of the dimethyl sulphide in the sample and a similar sample containing added dimethyl sulphide, with a variety of reagents.

Experimental

Reagents

All reagents were of Analar quality unless otherwise specified.

Aqueous ethanol: absolute ethanol (3·5 ml) diluted to 100 ml with de-ionised water.

n-Butanol standard: n-butanol (200 mg) diluted to one litre with aqueous ethanol.

Dimethyl sulphide standards in aqueous ethanol solution: dimethyl sulphide (100 mg) was diluted to one litre and an aliquot (10 ml) of this solution was further diluted to 100 ml. Subsequent dilutions of this latter solution gave the necessary working standards. These should be freshly prepared each day.

Sodium chloride.

Apparatus

F & M Model 810 research chromatograph with dual flame ionisation detectors. The operating conditions were as follows:

Columns	:	10 ft × ¼in coiled stainless steel (18 : 8 : 1)
Solid support	:	Embacel 60–80 mesh (May and Baker Ltd.)
Stationary phase	:	20% Polyethylene glycol 1500 (Shell Chemicals Ltd.)
Column temperature	:	Isothermal (50°c) for seven minutes then programmed at 60°/min to 130°c
Carrier gas	:	Argon at 100 ml min.
Detector temperature	:	210°c
Injection temperature	:	110°c
Attenuation	:	1 × 4 changing to 1 × 16 after 7 min.

Method

Sodium chloride (8 g) was placed in a clean, dry conical flask (100 ml, B24 Q & Q) which was sealed immediately with a Quickfit & Quartz screwcap adaptor and silicone rubber septum. To the flask was added: beer (20 ml at 0°c) by a hypodermic syringe; n-butanol standard solution (0·500 ml) by a micrometer burette. The flask was left in a water bath (30° ± 1°c) for 90 min. A sample (10 ml) of the equilibrated vapour was withdrawn with a gas syringe and injected on to the gas chromatograph.

The concentration of dimethyl sulphide in the sample was calculated from the heights of the dimethyl sulphide and n-butanol peaks (Fig. 1) and the relative response factor for n-butanol and dimethyl sulphide.

Results and Discussion

According to recommended gas chromatographic practice[4] the measurement of peak area provides the best relationship with concentration. In this instance it was found that measurement of peak height afforded both the necessary accuracy and reproducibility. For quantitative analysis the relationship between dimethyl sulphide and n-butanol was expressed by the relative response factor, F:

$$F = \frac{Cs}{Hs} \cdot \frac{Hx}{Cx}$$

where Cs, Cx are the concentrations in μg/l of the standard and unknown and Hs and Hx are the peak heights. The average value for F obtained for the range of concentration 10–100 μg dimethyl sulphide, was found to be 11·50 (Table I) and the linearity of detector response is illustrated in Fig. 2.

The identity of the dimethyl sulphide found in beer (Fig. 1) was confirmed by comparing:

1. The retention volumes on two different stationary phases (Table II)—the effect of the adjacent acetaldehyde peak was measured by chromatographing model systems of aqueous ethanol (3% wt./vol.) containing acetaldehyde (20–30 μg/l) and dimethyl sulphide (10–20 μg/l);

* Present address: Loughborough University of Technology, Leics.

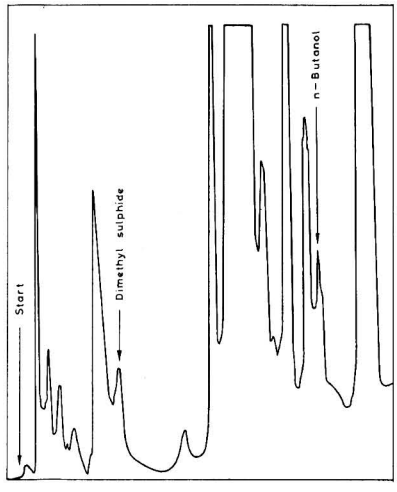

FIG. 1. *Typical gas chromatogram of beer vapour*

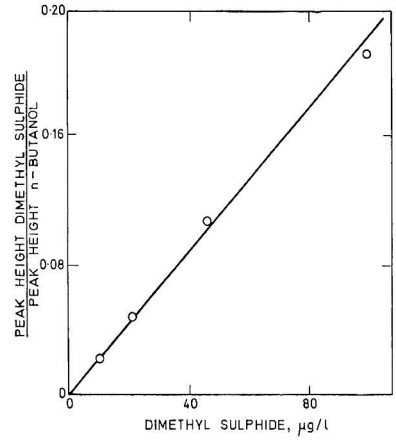

FIG. 2. *Calibration curve showing linearity of detector response*

TABLE I

Determination of the response factor of dimethyl sulphide and n-butanol

	Dimethyl sulphide concentration, µg/l			
	10	20	46	100
Response factor	10·72	13·43	10·82	11·82
	13·19	11·26	10·97	10·56
	13·19	11·57	10·49	9·57
	13·21	13·16	12·49	9·87
	11·08	13·22	11·66	11·19
	12·68	10·87	11·19	10·78
	—	—	—	10·43
	—	—	—	10·69
	—	—	—	10·34

Average response factor, $F = 11{\cdot}50$
Standard deviation = 1·16

TABLE II

Retention volume* for dimethyl sulphide

Sample	Stationary phase	
	PEG 1500	Tris CNEP†
Control beer	268	303
Dimethyl sulphide + acetaldehyde in 3% by vol. ethanol	277	306
Control beer + added dimethyl sulphide	268	300

* Retention volume = carrier gas flow (ml/min) × retention time (min)
† 1,2,3-Tris-(2-cyanoethoxy) propane

TABLE III

Dimethyl sulphide (DMS) content of beer

Beer quality	Original gravity, °Sacch	DMS, µg/l
Lager (British 6 samples)	44–74	18–27
Average DMS for bottom fermentation = 24 µg/l		
Lager (Continental, 11 samples)	31–67	44–114
Average DMS for Continental lager = 74 µg/l		
Light ale (3 samples)	32	4–13
Pale ale (5 samples)	48	0–10
Average DMS for top fermentation = 11 µg/l		

2. The reactions with silver nitrate, hydrogen peroxide and mercuric chloride of both the sample and authentic dimethyl sulphide.

The standard deviation (S) of the replication error obtained from duplicate analyses of bottled beer (Table III) was calculated from:

$$S = \left[\frac{\Sigma d^2}{2n}\right]^{\frac{1}{2}}$$

where each d is the difference for a pair of duplicate results and n is the number of samples. This provides an average value for the standard deviation (2·12) and coefficient of variation (7·7%) which is applicable to the method used under routine conditions.

The detection limit of the method (i.e. twice the average baseline noise) was found to be approximately 3 µg/l under normal working conditions.

Interference from acetaldehyde which was experienced in some samples of beer was easily removed by adding a pre-column of FFAP (12 in × ¼ in, 20% FFAP on Chromosorb W 80–100 mesh). Alternatively, the addition of a small quantity of hydroxylamine hydrochloride (0·05–0·1 g) to the sample prior to equilibration was found to be satisfactory.

The average recovery of dimethyl sulphide added to beer was found to be 99% over a concentration range from 10–90 µg/l (Table IV). The upper limit of 90 µg was taken as three times the organoleptic threshold of 30 µg/l for dimethyl sulphide reported by Harrison.[5]

TABLE IV

Recovery of dimethyl sulphide (DMS) added to beer

Sample	Added DMS, μg/l	Total DMS found, μg/l			Average DMS recovered, μg/l	Recovery, %
	0		28			
1	9	38	36	36	8·7	97
	0		30			
2	20	48	50	48	18·7	94
	0		33			
3	59	94	94	92	60·3	102
	0		32			
4	89	125	124	125	92	104

TABLE V

Effect of temperature on assay of dimethyl sulphide

Sample	Dimethyl sulphide, μg/l	
Control (lager beer, OG = 1044)	21	22
Control after 60 min at 20°c	22	22
Control after 60 min at 60°c	23	21
Control after 60 min at 80°c	18	19

The levels of dimethyl sulphide found in the various beers analysed (Table III) demonstrate two noteworthy features. First, all the results obtained for British lagers (0–35 μg/l) were considerably lower than the values obtained by Drews *et al.*[3] for Pilsner beers (120–140 μg/l). Since in the technique described by Drews *et al.*[3] the beer sample is heated briefly at 65°c, it is possible that the differences in these analytical results may be due not only to the type of beer and brewing process but also the the effect of temperature on the sample when it is assayed by the method of Drews *et al.*[3]

Formation of dimethyl sulphide, presumably by thermal decomposition of sulphur compounds, was demonstrated by Drews *et al.* to occur on distillation of the sample and significantly lower, but constant, results were obtained for distillation temperatures of 75°, 65° and 55°c; however, no attempt was made to obtain results at temperatures lower than 55°c. The possibility of formation of dimethyl sulphide with increasing temperature within the range 20–80°c was tested by analysing bottled lager beers (OG 1044) previously incubated at 20, 60 and 80°c for one hour and cooled to room temperature (20°c). Comparison of results for the heated beer vs. its control (Table V) showed clearly that increased temperature had no effect on the assay, thus confirming the findings of Drews *et al.*[3] for samples not subjected to distillation.

There is a significant difference in concentration of dimethyl sulphide in ales (average 11 μg/l) and lagers (average 24 μg/l). The differences in the dimethyl sulphide content of beers analysed by the present method and those quoted by Drews *et al.*[3] may therefore reflect the differences in British and Continental brewing practice. Nevertheless, since a figure of 30 μg/l has been reported to be the flavour threshold for dimethyl sulphide and since high concentrations of dimethyl sulphide impart an undesirable flavour it is, in the authors' opinion, difficult to completely reconcile the results obtained by Drews *et al.*[3] with normal production beer. A series of Continental lagers were examined (Table III) and the results obtained, although invariably higher than for the British brewed product, were nevertheless lower than the values quoted by Drews *et al.*[3]

This difference between ales and lagers might be attributed to a number of factors, e.g. the different kilning procedures used in the production of lager and ale malt, the metabolic differences between *Saccharomyces cerevisiae* and *S. carlsbergensis*, and thirdly the purely physical effect due to the influence of carbon dioxide washout of volatiles during fermentation. This effect would be more efficient at the higher temperatures used in top fermentations (~ 20°c) than at the lower temperature (~ 7°c) used in bottom fermentations combined with the prolonged lagering period at 0°c. An investigation of these suggested mechanisms is currently being undertaken.

Conclusions

A gas chromatographic method is described for the quantitative determination of dimethyl sulphide in beer. Analytical precision illustrated by replicate results for a variety of beers over a wide concentration range (0 to 90 μg/l) has shown that the derived relative response factor is valid for this range of concentration.

The concentration of dimethyl sulphide found in normal production British ales and lagers has been found to be less than 30 μg/l compared with the range of 120–140 μg/l found by Drews *et al.*[3] for Pilsner type beers.

Acknowledgments

The authors express their thanks to the Directors of Allied Breweries Limited for permission to publish this paper and to Mr. A. C. Smith for technical assistance.

Allied Breweries Ltd.
Research Department,
Burton-upon-Trent

Received 2 February, 1970;
amended manuscript 28 April, 1970

References

1. Ahrenst-Larsen, B., & Hansen, H. L., *Brauwissenschaft*, 1963, **16,** 393
2. Kepner, R. E., Strating, J., & Weurman, C., *J. Inst. Brew.*, 1963, p. 399
3. Drews, B., Specht, H., & Kühl, E. D., *Mschr. Brau.*, 1966, **19,** 239
4. Primavesi, G. R., McTaggart, N. G., Scott, G. C., Snelson, F., & Wirth, M. M., *J. Inst. Petrol.*, 1967, **53,** 367
5. Harrison, T., *Brewer's Dig.*, 1967, **42, 6,** 74
6. Toon, T., Barrett, R., & Claydon, T., *J. Dairy Sci.*, 1965, **48,** 1174

MALO-LACTIC FERMENTATION IN AUSTRALIAN DRY RED WINES

By B. C. RANKINE, J. C. M. FORNACHON,* D. ANNETTE BRIDSON and K. M. CELLIER†

Malo-lactic fermentation occurred in 62% of 466 Australian dry red wines of 1968 vintage at 6 months of age. In a further 9% of wines the fermentation was still in progress. Wines which had undergone the fermentation were less acid than those which had not. The difference was greater in wines made from Cabernet Sauvignon than from Shiraz or Grenache grapes.

The winery in which the wine was made strongly influenced the occurrence of malo-lactic fermentation in a particular wine-growing area, and was the most important factor involved.

Incidence of malo-lactic fermentation was closely correlated with the presence of *Leuconostoc* and *Coccus* in the wine, but less so with *Lactobacillus*. *Leuconostoc*, *Lactobacillus* and sometimes *Coccus* occurred together, but no wines contained both *Lactobacillus* and *Coccus*.

Diacetyl content of the wines ranged from 0 to 7·5 ppm. In wines which had undergone malo-lactic fermentation the mean diacetyl content was 2·8 ppm, compared with 1·3 ppm for wines which had not undergone this. Diacetyl content of wines made from Grenache was not significantly different, with respect to the occurrence or absence of malo-lactic fermentation.

The statistical significances of the various inter-relationships between malo-lactic fermentation, type of bacteria present and certain wine constituents are assessed.

Estimates of variance components of 11 constituents of Australian dry red wines are given, and these may be used in designing future sampling surveys.

Introduction

Malo-lactic fermentation of wine is a bacterial decarboxylation of L-malic acid to lactic acid with liberation of carbon dioxide. It was first recognised as such by Müller-Thurgau in 1891, and is widespread in the various wine-producing areas of the world. The fermentation has been recently reviewed.[1–4] Although much attention has been paid to the bacteria involved, metabolic pathways, energetics and control, more information is needed on the extent of the fermentation in particular wine-growing areas and the effect of wine-making procedures.

Malo-lactic fermentation is frequently observed in young Australian dry red wines, and in some areas is regarded as very important in influencing wine quality. Since malo-lactic fermentation results in reduced acidity, its occurrence may be either desirable or undesirable, depending on the original acidity of the wine. In the warmer wine-growing areas of Australia, the acidity of table wines tends to be low, and any further reduction, such as that produced by malo-lactic fermentation, may be undesirable. On the other hand, in cooler areas where the grapes ripen more slowly and the natural acidity of the wine is higher, a reduction in acidity is usually desirable. Since the malo-lactic fermentation occurs naturally and is sporadic in incidence, the Australian Wine Research Institute decided to carry out an investigation of its frequency in Australian dry red wines. As well as providing basic information on the incidence of the fermentation, it was intended that the results could be used to decide whether to proceed with artificial induction of the fermentation by bacterial inoculation, a process which is in an advanced stage of development by the Institute and which has shown considerable promise.

This paper reports the results of the investigation and an assessment of the significance of grape varieties, viticultural areas, wineries, bacteria and certain other factors on the incidence of the fermentation and composition of the wines.

Experimental

A total of 466 dry red wines of the 1968 vintage were obtained on request from as many wineries as possible (48) in the principal viticultural areas of South Australia, New South Wales and Victoria. The areas sampled represented over 95% of the total wine-growing area in Australia.

The wines were obtained between 3 and 5 months after making, and were stored at 15°C and examined as soon as possible. The following measurements were carried out: incidence of malo-lactic fermentation by paper chromatography;[5] diacetyl content by head-space gas chromatography;[6] pH by glass electrode, titratable acidity by electrometric titration to pH 8·4; presence of *Leuconostoc*, *Lactobacillus* and *Coccus* by microscopic examination, with an assessment of the relative numbers of each class of micro-organism; colour measurements of optical density by recording spectrophotometer at wavelengths of maximum and minimum absorption over the range 400–700 nm. Colour density (O.D. $\lambda_{min.}$ + O.D. $\lambda_{max.}$ for 1 cm cell) and tint $\left(\frac{\text{O.D. } \lambda_{min.}}{\text{O.D. } \lambda_{max.}}\right)$ were calculated; potassium and sodium were determined by flame photometry.

A total of nearly 5000 measurements and observations were made, and the quantitative results were statistically analysed by analysis of variance. The tests for association between malo-lactic fermentation and the type of bacteria present and among the three types of bacteria were made by calculating χ^2 in contingency tables.

Since the main interest in this work was to determine the effect of malo-lactic fermentation (m.l.f.) on the several constituents of dry red wines, an analysis of variance using the following model was carried out on each constituent:

$$Y_{ijkl} = u + \alpha_i + \beta_{ij} + \gamma_{ijk} + S_{ijkl}$$

where Y_{ijkl} = the amount of constituent obtained from the i^{th} bottle in the k^{th} winery from the j^{th} area on the l^{th} m.l.f. class (i.e. m.l.f.$^+$ or m.l.f.$^-$).

The effects β_{ij}, γ_{ijk} and S_{ijkl} are assumed to be normally and independently distributed with mean = 0 and variances = σ_b^2, σ_c^2 and σ_d^2 where σ_b^2 = variance between areas, σ_c^2 = variance between wineries within areas and σ_d^2 = variance between samples within wineries within areas. This model

* Now deceased

† Present address: Division of Mathematical Statistics, C.S.I.R.O., Adelaide, South Australia

is commonly referred to as 'nested sampling' and allows the testing of the significance of areas and wineries in the total variation and the estimation of the corresponding variance components. Since the numbers of areas, wineries and samples were different in the m.l.f. classes, the standard errors were calculated by methods proposed by Ganguli.[7] Sufficient wines were obtained from the grape varieties *Vitis vinifera* cv. Shiraz (the Syrah of the Rhône Valley and the Petite Sirah of California), Grenache and Cabernet Sauvignon to enable these to be considered separately for statistical analysis.

Results

Influence of acidity on wines

A total of 289 wines out of 466 (62%) had undergone malo-lactic fermentation and the fermentation was still in progress in a further 43 wines (9%). The overall relationship between occurrence of the fermentation and pH is shown in Fig. 1, and for wines made from the three main grape varieties used, the mean pH values and titratable acidity are shown in Table I. Malo-lactic fermentation increased the pH and decreased the titratable acidity of wines made from all three varieties, but the difference was considerably greater in wines made from Cabernet Sauvignon than in those made from the other two varieties. This indicates that Cabernet Sauvignon grapes contain more malic acid than Shiraz and Grenache grapes, but there are no data on the composition of the musts before fermentation.

Bacteria present in the wines

The distribution of bacteria is shown in Table II. It can be seen that only 16 of the wines (3%) were free from bacteria, whilst 178 (38%) contained only *Leuconostoc*, having the general microscopic appearance described by Fornachon.[8]

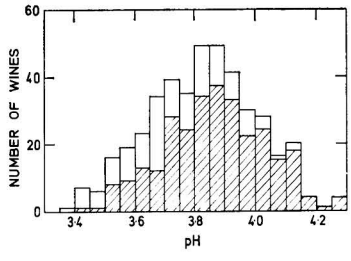

FIG. 1. *Relationship between malo-lactic fermentation and pH for different numbers of wine*
▨ Positive; □ Negative

The numbers of wines containing only *Lactobacillus* (19) and only *Coccus* (2) were much less than those containing only *Leuconostoc*, but 202 of the wines (43%) contained a mixture of *Leuconostoc* and *Lactobacillus*.

A total of 429 (92%) contained *Leuconostoc*, either pure or with other bacteria. Similarly, 258 wines (55%) contained *Lactobacillus* and 51 wines (11%) contained *Coccus*. It is of interest that no wines were encountered which contained both *Lactobacillus* and *Coccus*.

The numbers of bacteria were counted and, for *Leuconostoc* and *Lactobacillus*, classified into four levels, viz., very few (up to 5 million cells/ml), few (5–10 million cells/ml), plentiful (10–100 million cells/ml) and very plentiful (above 100 million cells/ml). For the purpose of simplifying the presentation of data in Table III, the classifications were limited to none, few (incorporating very few and few) and many (incorporating plentiful and very plentiful). For *Coccus*, three levels of occurrence were recorded: none, few and many, but these are listed only as absence and presence in the Table, because insufficient wines of the three population levels were available to justify further separation. Table III is divided into two parts to show the relationship between malo-lactic fermentation and the type and number of bacteria present, and interrelationship between different bacteria.

In wines made from the three grape varieties, there is a clear relationship between the occurrence of malo-lactic fermentation and the presence of *Leuconostoc*. Between 80 and 95% of wines with large numbers of *Leuconostoc* present had undergone m.l.f. compared with 10–50% of the wines in which they were absent. Similarly, a higher proportion

TABLE II

Distribution of different types of bacteria in 466 Australian dry red wines (1968 vintage) at 6 months of age

Bacteria	Number of wines
Nil	16
Leuconostoc	178
Lactobacillus	19
Coccus	2
Leuconostoc and *Lactobacillus*	202
Leuconostoc and *Coccus*	12
Lactobacillus and *Coccus*	0
Leuconostoc and *Lactobacillus* and *Coccus*	37
Total	466

TABLE I

Relationship between malo-lactic fermentation (m.l.f.), pH and titratable acidity in wines made from three grape varieties
Titratable acid as g/l tartaric acid

Grape variety	pH		Sig. diff.	Titratable acidity		Sig. diff.
	m.l.f.⁻	m.l.f.⁺		m.l.f.⁻	m.l.f.⁺	
Shiraz	3·78 ± 0·033 (64)†	3·88 ± 0·027 (109)	*	5·66 ± 0·17 (64)	4·91 ± 0·14 (109)	**
Grenache	3·60 ± 0·089 (19)	3·78 ± 0·086 (53)	n.s.	5·51 ± 0·16 (19)	4·75 ± 0·13 (53)	**
Cabernet Sauvignon	3·64 ± 0·075 (14)	3·94 ± 0·052 (30)	**	6·46 ± 0·37 (14)	4·70 ± 0·28 (30)	**

† Figures in brackets refer to the number of wines in each group

TABLE III

Relationship between types and relative numbers of bacteria present in the wines, and the incidence of malo-lactic fermentation (m.l.f.)
Figures in the Table refer to the number of wines; arrows indicate pooling of adjacent classes in order to keep sufficient numbers in each cell of contingency table

A. Malo-lactic fermentation

	Shiraz				Grenache				Cabernet Sauvignon			
	Nil	Few	Many	Total	Nil	Few	Many	Total	Nil	Few	Many	Total
						Leuconostoc						
M.l.f.+	2	37	68	107	3	18	32	53	1 →	10	19	30
M.l.f.−	18	31	14	63	6	12	1	19	1 →	10	2	13
Total	20	68	82	170	9	30	33	72	2	20	21	43
	$\chi^2_2 = 40{\cdot}19$ (P < 0·001)				$\chi^2_2 = 19{\cdot}65$ (P < 0·001)				$\chi^2_1 = 8{\cdot}35$ (P < 0·01)			
						Lactobacillus						
M.l.f.+	48	34	15	97	13	15	17	45	15	7 ←	2	24
M.l.f.−	35	22	2	59	15	4	0	19	13	1 ←	0	14
Total	83	56	17	156	28	19	17	64	28	8	2	38
	$\chi^2_2 = 5{\cdot}63$ (n.s.)				$\chi^2_2 = 15{\cdot}51$ (P < 0·001)				$\chi^2_1 = 4{\cdot}20$ (P < 0·05)			

	Shiraz			Grenache			Cabernet Sauvignon		
	Absence	Presence	Total	Absence	Presence	Total	Absence	Presence	Total
					Coccus				
M.l.f.+	90	19	109	53	0	53	28	2	30
M.l.f.−	62	2	64	19	0	19	14	0	14
Total	152	21	173	72	0	72	42	2	44
	$\chi^2_1 = 7{\cdot}74$ (P < 0·01)			not significant			not significant		

B. Interrelationships between bacteria

		Shiraz				Grenache				Cabernet Sauvignon			
		Nil	Few	Many	Total	Nil	Few	Many	Total	Nil	Few	Many	Total
							Leuconostoc						
Lactobacillus:	nil	7	37	43	87	6	16	7	29	2 →	13	14	29
	few	11	25	25	61	3	7	10	20	0 →	4	6	10
											↑	↑	
	many	2	6	8	16	1	7	10	18	0	2	0	2
	total	20	68	76	164	10	30	27	67	2	19	20	41
		$\chi^2_4 = 3{\cdot}62$ (n.s.)				$\chi^2_4 = 6{\cdot}36$ (n.s.)				$\chi^2_1 = 0{\cdot}06$ (n.s.)			
							Leuconostoc						
Coccus:	absence	19	66	76	161	9	30	33	72	2	18	25	45
	presence	1	10	12	23	1	2	0	3	0	3	0	3
	total	20	76	88	184	10	32	33	75	2	21	25	48
		$\chi^2_2 = 1{\cdot}16$ (n.s.)				not significant				not significant			
							Lactobacillus						
Coccus:	absence	77	55	13	145	28	20	17	65	29	9	2	40
	presence	10	8	5	23	1	1	1	3	1	1	0	2
	total	87	63	18	168	29	21	18	68	30	10	2	42
		$\chi^2_2 = 3{\cdot}43$ (n.s.)				not significant				not significant			

of the wines with large numbers of *Lactobacillus* present had undergone m.l.f. than when they were absent. There were too few wines containing *Coccus* for a statistical analysis to be carried out for the varieties Grenache and Cabernet Sauvignon. For the Shiraz variety there was a significant positive association between m.l.f. and the presence of *Coccus*.

There was no association between any pair of the three types of bacteria, i.e., the occurrence of any one type of bacteria was independent of the other though the majority of wines had more than one type of bacteria present.

Diacetyl formation

Diacetyl is an important product of malo-lactic fermentation. It can be produced by certain bacteria and yeasts. The mean diacetyl content of wines which had not undergone m.l.f. was 1·3 ppm, whilst the mean level in the fermented wines was 2·8 ppm. The range of values was from 0 to 7·2 ppm.

The diacetyl content is shown in Table IV for wines made from the three most important grape varieties. It can be seen that the increase in diacetyl content after malo-lactic

TABLE IV

Relationship between malo-lactic fermentation (m.l.f.) and mean diacetyl content (ppm) in 289 wines made from three grape varieties

Grape variety	Diacetyl content		S.D.
	M.l.f.$^-$	M.l.f.$^+$	
Shiraz	$1{\cdot}5 \pm 0{\cdot}34$ (64)†	$3{\cdot}1 \pm 0{\cdot}30$ (109)	**
Grenache	$0{\cdot}9 \pm 0{\cdot}58$ (19)	$1{\cdot}3 \pm 0{\cdot}54$ (53)	n.s.
Cabernet Sauvignon	$1{\cdot}2 \pm 0{\cdot}75$ (14)	$3{\cdot}6 \pm 0{\cdot}50$ (30)	*

† Figures in brackets refer to the number of wines in each group

fermentation is considerably less in wines made from Grenache than in those made from the other two grape varieties.

Influence of winery practice

The incidence of malo-lactic fermentation was examined in relation to the winery in which the wine was made, as well as the grape variety and viticultural area. Winery practice clearly had a profound influence on the incidence of the fermentation. This is shown in Table V, from which it can be seen that wines made in different wineries in the same viticultural area differed widely with respect to m.l.f.

Composition of wines examined

Considerable data, obtained during the investigation, on certain aspects of the composition of the wines are summarised in Table VI, which presents the mean, minimum and maximum amounts of the constituents in wines made from the three main grape varieties; the composition of the wines obviously varied over a very wide range.

Besides information directly related to malo-lactic fermentation the investigation has revealed interesting differences in the composition of wines made from the different grape varieties, and this is shown in Table VII of the Appendix. With only minor exceptions the pattern of variation in wines made from Grenache differs from that of wines made from Shiraz and Cabernet Sauvignon. The wines made from Grenache from different areas varied more in pH, potassium, sodium and $\lambda_{min.}$ and less in diacetyl content, O.D.$_{min.}$, O.D.$_{max.}$ and colour density than did wines made from Shiraz and Cabernet Sauvignon. The viticultural area in which the wines were produced did not influence titratable acid and $\lambda_{max.}$ for wines made from Grenache and Cabernet Sauvignon. Considering the variation between wineries within a viticultural area, wines made from Grenache showed less variation in potassium, sodium and O.D.$_{max.}$ than wines from the other two varieties.

Discussion

The investigation is of particular interest in relation to a previous study of malo-lactic fermentation in Australian wines[9] in which 64% of dry red wines (total 112) and 30% of dry white wines (total 179) had undergone m.l.f. before they were eight months old. The most important factors influencing the incidence of the fermentation (apart from ethanol content) were (*a*) pH value (greater incidence at higher levels) and (*b*) length of time the wines were in contact with their yeast deposit (higher incidence with longer contact time). The principal organisms responsible were heterofermentative lactobacilli, but Fornachon[8] subsequently identified a *Leuconostoc* spp. which he also found was widely responsible for the fermentation.

TABLE V

Relationship between viticultural area, winery and incidence of malo-lactic fermentation (m.l.f.)

Area	Winery	No. of wines	M.l.f.$^-$	M.l.f.$^+$
Barossa (S.A.)	A	13	10	3
,, ,,	B	16	4	12
,, ,,	C	14	7	7
Southern Vales (S.A.)	D	23	2	21
,, ,, ,,	E	11	10	1
,, ,, ,,	F	19	0	19
M.I.A.,* (N.S.W.)	G	8	8	0
,, ,,	H	8	1	7

* Murrumbidgee Irrigation Area

The present investigation involved a much greater number of dry red wines than did the previous work, but the overall results obtained were very similar. Apart from the extent of the occurrence of malo-lactic fermentation, a very important practical finding in the present study has been the dominant influence of the individual winery. The vinification practices of the individual wineries largely dictated the occurrence of m.l.f., and certain wineries appeared to possess somewhat distinct microflora. Also, in 4 out of 20 wines from one winery, tartaric acid was found to be absent. These wines were all above pH 4 and contained large numbers of lactobacilli, and this was the first occasion in which evidence of bacterial destruction of tartrate has been detected in Australia.

It appears that the most important single factor influencing malo-lactic fermentation is the amount of sulphur dioxide in the wine, and this is clearly the main reason why m.l.f. occurs to a much greater extent in dry red than in dry white wines. In Australian wineries it is now the usual practice to withhold addition of sulphur dioxide to dry red wines, if the winemaker desires m.l.f. to occur. Many winemakers check their wines routinely for m.l.f. by paper chromatography, and use the results to decide on subsequent treatment of the wine and time of bottling. It is generally undesirable for the malo-lactic fermentation to occur in bottled wine, because the wine evolves carbon dioxide and the bacteria present produce a slight haze. In some cases such wines are temporarily unpalatable, owing to secondary products of m.l.f. formed by some bacteria, but they usually recover after the fermentation is completed.

Although the malo-lactic fermentation was more prevalent in wines of high pH values (Fig. 1), 23 out of 144 wines of pH 3·9 and above had not undergone it. Whilst this may have been fortuitous in some cases, in others it represented deliberate policy by the winemaker to retain the natural malic acid by addition of sulphur dioxide to inhibit bacterial growth. In extreme cases, the m.l.f. of wines of low acidity constitutes spoilage, as in the case of bacterial decomposition of tartrate, and it is sound winemaking practice to prevent malo-lactic fermentation of low acid wines.

Wines which had undergone malo-lactic fermentation tended to be slightly lighter in colour, but not significantly so, than wines which had not undergone it. The effect was far less than that found by Vetsch & Lüthi,[10] who found reduction in colour of up to 38% in Swiss red wines. They attributed this to bacterial degradation of citric acid, but citric acid was not detected in any of the Australian wines examined.

TABLE VI

Summary of all data measured in wines made from varieties Shiraz, Grenache and Cabernet Sauvignon

Variate	Variety	Number of samples	Mean	Minimum value	Maximum value
pH	Grenache	76	3·75	3·40	4·28
	Shiraz	188	3·84	3·50	4·26
	Cab. Sauvignon	49	3·86	3·37	4·22
Titratable acid, g/l tartaric acid	Grenache	75	4·90	3·60	6·80
	Shiraz	185	5·18	3·70	8·20
	Cab. Sauvignon	49	5·19	2·50	7·70
Diacetyl content, ppm	Grenache	71	1·40	0·10	5·60
	Shiraz	188	2·52	0·40	7·20
	Cab. Sauvignon	49	2·86	0·60	6·30
Potassium, g/l	Grenache	76	1·19	0·80	1·90
	Shiraz	188	1·49	0·80	2·50
	Cab. Sauvignon	49	1·60	1·10	2·15
Sodium, ppm	Grenache	76	113·4	10·00	335·0
	Shiraz	188	126·0	10·00	971·0
	Cab. Sauvignon	49	85·7	13·00	228·0
$\lambda_{min.}$	Grenache	76	447·6	425·0	485·0
	Shiraz	188	437·7	415·0	480·0
	Cab. Sauvignon	49	443·7	422·0	480·0
$O.D._{min.}$	Grenache	76	0·30	0·12	0·61
	Shiraz	188	0·57	0·11	1·24
	Cab. Sauvignon	49	0·62	0·31	1·22
$\lambda_{max.}$	Grenache	76	527·7	515·0	535·0
	Shiraz	188	530·5	515·0	540·0
	Cab. Sauvignon	49	529·4	522·0	538·0
$O.D._{max.}$	Grenache	76	0·39	0·11	0·87
	Shiraz	188	0·85	0·14	2·16
	Cab. Sauvignon	49	0·90	0·34	2·16
Colour tint	Grenache	76	0·82	0·57	1·43
	Shiraz	188	0·70	0·51	0·97
	Cab. Sauvignon	49	0·73	0·47	0·95
Density	Grenache	76	3·47	1·20	7·20
	Shiraz	188	7·08	1·30	16·80
	Cab. Sauvignon	49	7·60	3·20	16·90

The occurrrence of malo-lactic fermentation in Australian table wines appears to be rather similar in pattern to that found in California. Ingraham & Cooke[11] found that 75% of dry red bottled wines (total 80) and 32% of dry white wines (total 56) had undergone the fermentation, and a higher proportion of dry red wines from the Napa Valley underwent the fermentation than elsewhere. Radler[3] reported extensive data on 1217 Germany quality table wines, in which 77% of dry red wines (total 145), 21% of rosé wines (total 38) and 11% of white wines (total 1034) underwent complete m.l.f. The fermentation is common in red wines from Bordeaux[1] and it occurs, suprisingly, in colder areas such as Switzerland,[12] the Niagara peninsula in Ontario, Canada[13] and New York State[14] where the natural acidity of the wines is high. This high acidity is sometimes a barrier to growth of the bacteria, and may result in the absence of m.l.f. in wines which would benefit greatly by such biological deacidification. There are numerous reports of malo-lactic fermentation occurring in wines from other wine-growing areas, which have been reviewed by Kunkee.[4]

Acknowledgments

Grateful acknowledgment is made to the many Australian wine-makers who kindly provided the wines which made this investigation possible. Mr. T. C. Somers and Mrs. P. M. Seal kindly carried out the colour measurements on the wines.

The Australian Wine and Research Institute,
Adelaide,
South Australia

Received 17 March, 1970

References

1. Ribéreau-Gayon, J., & Peynaud, E., 'Traite d'Oenologie', 1960–61 (Paris and Liege: Beranger)
2. Radler, F., *Vitis*, 1962, **3**, 144; 1963, **3**, 207; 1963, **4**, 62
3. Radler, F., *Z. Lebensmittelunters. u.-Forsch.*, 1968, **138**, 35
4. Kunkee, R. E., *Adv. appl. Microbiol.*, 1967, **9**, 235

5. Rankine, B. C., *Aust. Wine Brew. Spirit Rev.*, 1969, **88**, 46
6. Rankine, B. C., *et al Vitis*, 1969, **8**, 129
7. Ganguli, M., *Sankhya*, 1941, **5**, 449
8. Fornachon, J. C. M., *Am. J. Enol. Vitic.*, 1964, **15**, 184
9. Fornachon, J. C. M., *Aust. J. appl. Sci.*, 1957, **8**, 120
10. Vetsch, U., & Lüthi, H., *Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1964, **55**, 93
11. Ingraham, J. L., & Cooke, G. M., *Am. J. Enol. Vitic.*, 1960 **11**, 160
12. Lüthi, H., *Revue Ferment. Ind. aliment.*, 1957, **12**, 15
13. Adams, A. M., *Rep. Hort. Res. Inst., Ontario*, 1967, p. 92
14. Rice, A. C., *Am. J. Enol. Vitic.*, 1965, **16**, 62

APPENDIX

Application to future surveys

Although differences between the presence and absence of malo-lactic fermentation were not significant for several constituents of the wine and the mean values were not presented, the results of the present study can be of use in planning future surveys of wines from different viticultural areas in Australia, since estimates of the variance components for areas, wineries within areas and samples within wineries within areas have been obtained on an Australia-wide basis.

The model used for this study is slightly different from that used in the first part of this paper since, in general, interest will be in sampling areas, and wineries, irrespective of malo-lactic fermentation. Analyses of variance on each constituent were carried out using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \gamma_{ijk}$$

where Y_{ijk} = the amount of constituent obtained from the k^{th} sample in the j^{th} winery from the i^{th} area. The effects α_i, β_{ij}, γ_{ijk} are assumed to be normally and independently distributed with mean = 0 and variances σ_b^2, σ_c^2, and σ_d^2 where σ_b^2 = variance between areas, σ_c^2 = variance between wineries within areas, σ_d^2 = variance between samples within wineries within areas. Estimates of the variance components and mean values are given for the three main varieties in Table VII. The results in Table VII can be used to determine the number of areas, wineries and samples needed to obtain any desired precision. In the general case when data are obtained from n_3 samples from each of n_2 wineries in n_1 areas then the variance of the mean is:

$$V(\bar{y}) = \frac{\sigma_b^2}{n_1} + \frac{\sigma_c^2}{n_1 n_2} + \frac{\sigma_d^2}{n_1 n_2 n_3}$$

If costs for travelling, sampling, etc., are known, then the number of wineries per area and samples per winery to be sampled could be optimised in the usual way (Yates, F., 'Sampling methods for censuses and surveys', 1960 (3rd edition) (London: Griffin)). Where unequal numbers of wineries in each area and samples from each winery are taken, the variance of the mean value for each constituent can be obtained by methods described by Ganguli.[7]

TABLE VII

Estimates of variance components* and mean values of 10 constituents of dry red wines made from three grape varieties in Australia

Constituent	Estimate of component variance	Variety		
		Shiraz ($n=184$)	Grenache ($n=76$)	Cabernet Sauvignon ($n=49$)
pH	σ_b^2	0·0000	0·0254	0·0000
	σ_c^2	0·0107	0·0109	0·0296
	σ_d^2	0·0141	0·0152	0·0056
	Mean	3·842	3·750	3·859
Titratable acid	σ_b^2	0·0798	0·0000	0·0000
	σ_c^2	0·1180	0·1733	0·8332
	σ_d^2	0·3990	0·2632	0·2369
	Mean	5·18	4·90	5·19
Diacetyl	σ_b^2	0·7665	0·0000	0·7472
	σ_c^2	0·8197	1·8820	1·8183
	σ_d^2	0·6650	0·3228	0·6024
	Mean	2·52	1·308	2·86
K	σ_b^2	0·0158	0·0337	0·0241
	σ_c^2	0·0358	0·0000	0·0460
	σ_d^2	0·0337	0·0155	0·0134
	Mean	1·488	1·188	1·596
Na	σ_b^2	0·00	2309·38	0·00
	σ_c^2	7587·07	551·12	2403·42
	σ_d^2	1492·24	896·54	194·30
	Mean	126·0	113·4	85·7
λ_{min}	σ_b^2	9·3525	62·1605	0·0000
	σ_c^2	54·8102	29·0246	116·7932
	σ_d^2	114·2601	205·2941	142·7833
	Mean	437·7	447·6	443·7
λ_{max}	σ_b^2	3·1583	0·0000	0·0000
	σ_c^2	4·8404	3·4251	16·9952
	σ_d^2	6·7462	15·5333	6·9653
	Mean	530·5	527·7	529·4
O.D.$_{min}$	σ_b^2	0·0213	0·0030	0·0251
	σ_c^2	0·0185	0·0044	0·0000
	σ_d^2	0·0170	0·0075	0·0170
	Mean	0·572	0·305	0·620
O.D.$_{max}$	σ_b^2	0·0650	0·0093	0·0563
	σ_c^2	0·0483	0·0064	0·0577
	σ_d^2	0·0629	0·0194	0·0506
	Mean	0·848	0·392	0·905
Colour tint	σ_b^2	0·0017	0·0101	0·0000
	σ_c^2	0·0023	0·0055	0·0104
	σ_d^2	0·0044	0·0090	0·0040
	Mean	0·705	0·816	0·726
Colour density	σ_b^2	4·0282	0·5747	4·1208
	σ_c^2	3·0626	0·5027	1·9703
	σ_d^2	3·5957	1·2462	3·0293
	Mean	7·08	3·47	7·60

* σ_b^2 = variance between areas
σ_c^2 = variance between wineries within areas
σ_d^2 = variance between samples within wineries, within areas

EFFECT OF PASTEURISATION ON THE CHEMICAL COMPOSITION OF LIQUID WHOLE EGG

IV.*—Further resolution of certain fractions of the soluble proteins

By T. L. PARKINSON

Amino acid analyses confirmed previous electrophoretic evidence that two particular chromatographic fractions from the soluble proteins of liquid whole egg were mixtures of proteins. Further resolution of these fractions has been effected by ion-exchange chromatography on carboxymethyl cellulose, using sodium acetate buffer solutions containing increasing concentrations of sodium chloride followed by certain alkaline solutions. Some distinctions between sub-fractions obtained from raw and pasteurised egg have been noted.

Introduction

In a previous paper in this series[1] it was stated that the only consistent quantitative change in soluble proteins produced by pasteurisation was a reduction in those fractions eluted from diethylaminoethyl cellulose by 0·05 M and 0·08 M salt buffer. Examination by starch-gel electrophoresis had shown that these fractions each contained several proteins and it was considered that useful information would be obtained if they were resolved still further, with the ultimate object of ascertaining which of these proteins were most effected by pasteurisation. Exploratory experiments indicated that re-fractionation on carboxymethylcellulose from an acidic buffer solution offered a promising means of achieving this further resolution. Such a system has been successfully applied to egg white by Rhodes *et al.*[2] and although it gave very poor results with whole egg in these laboratories it would be expected to prove more successful with the two fractions under consideration.

Experimental and Results

The methods of examination have mostly been described in previous papers in this series.[3,4] Amino acid analyses were obtained with a Technicon amino acid analyser; details of the method have been given by Ewart.[5]

Egg was pasteurised on the laboratory scale by the method described in Part II.[4]

Amino acid composition of fractions

In a previous paper[3] it was indicated that conalbumin and ovalbumin were probably the major components in the fractions of soluble proteins eluted by 0·05 M salt buffer and 0·08 M salt buffer, respectively. It was considered that amino acid analyses of these mixtures might provide further evidence of their composition. Fractions derived from raw shell egg were dialysed against distilled water and were then freeze-dried in an Edwards centrifugal freeze dryer Model 30 P2/686. Nitrogen contents of the freeze-dried materials were: 0·05 M salt buffer fraction, 13·1%; 0·08 M salt buffer fraction, 13·3%. Amino acid analyses of the two fractions are given in Table I, together with corresponding values, calculated from figures given in the literature, for vitellenin[6,7], α- and β-vitellin,[6] phosvitin,[8] lysozyme,[9] conalbumin,[10] ovalbumin,[11] ovumucoid,[12] ovomucin (Robinson, D. S., personal communication) and ovomacroglobulin.[13] In order that the results could be compared, they were all calculated in terms of moles of anhydro amino acid per 1000 mole residues.

Comparison of the 0·08 M salt buffer fraction with ovalbumin reveals that of the seventeen amino acids given in the Table, eight are within two moles of each other, a further six differ by 4–6 moles and only three differ by more than 6 moles per 1000, suggesting that ovalbumin is the predominant protein in this fraction as previously indicated by zone electrophoresis. Zone electrophoresis of these 0·08 M fractions has also revealed bands in the ovomucoid position, and the presence of this protein would explain the discrepancies in cysteine and methionine, and to some extent that in isoleucine as well. The discrepancies between the 0·05 M fraction and conalbumin are much greater, being 8 or more moles for ten out of the seventeen amino acids and it can be assumed that this protein is much less predominant in the 0·05 M fraction than is ovalbumin in the 0·08 M fraction.

Sub-fractionation on carboxymethylcellulose

Exploratory tests showed that these two fractions could be further resolved by dialysing against acetate buffer, placing the equilibrated dialysate on a column of carboxymethylcellulose (Whatman CM 52) and eluting with a series of acetate buffers containing increasing concentrations of sodium chloride, followed by a series of more alkaline solutions.

One of the early experiments merits a more detailed description. It arose from observations that dialysis of the 0·05 M fraction from raw shell egg against 0·02 M acetate buffer, pH 5·2, caused a flocculent precipitate to form, whereas this did not occur with the corresponding fraction from pasteurised egg. Some shell egg was mixed and pasteurised for seven minutes at 150°F, which is rather longer than the average time in commercial practice, and 40-g portions of the raw and pasteurised egg were dialysed, centrifuged and fractionated on DE 52, giving patterns similar to those obtained on previous occasions,[4,1] with the quantitative reduction in the 0·05 M and 0·08 M salt buffer fractions being well marked. These fractions were separately dialysed against the acetate buffer for several days, in a refrigerator. The 0·05 M fraction from raw egg, after dialysis, contained a fair amount of flocculent precipitate whereas only a trace formed in the corresponding fraction from the pasteurised egg.

* Part III: *J. Sci. Fd Agric.*, 1968, **19**, 590

TABLE I

Amino acid composition of soluble protein fractions from raw shell egg
moles/1000 mole residues

	0·05 M	0·08 M	Vitellenin		α-Vitellin	β-Vitellin	Phos-vitin	Lyso-zyme	Con-albumin	Ov-albumin	Ovo-mucoid	Ovo-mucin	Ovo-macro-globulin
			Ref. 6	Ref. 7									
Asp	98	83	110	109	81	87	63	171	116	84	159	90	96
Thr	54	47	55	64	47	47	23	57	54	42	75	101	67
Ser	88	95	70	70	73	68	555	81	67	95	60	105	75
Glu	120	134	110	109	110	112	60	41	104	138	79	103	115
Pro	41	43	36	33	58	57	15	16	38	37	33	70	54
Gly	62	56	50	52	51	49	24	98	73	50	82	61	52
Ala	85	95	74	80	85	79	36	98	89	93	60	55	58
Val	75	72	67	69	75	79	15	49	73	74	84	62	80
½ Cys	25	15	6	8	16	17	0	65	33	5	97	29	17
Met	20	23	21	10	26	27	3	16	16	43	12	19	21
Ile	47	54	65	65	61	64	8	49	33	67	18	43	65
Leu	82	85	109	109	94	95	11	65	67	84	62	75	92
Tyr	26	26	36	39	31	33	3	24	29	24	32	30	40
Phe	43	50	44	40	36	33	8	24	35	56	25	38	51
Lys	73	58	83	76	67	68	76	49	107	52	69	60	61
His	19	19	15	13	26	20	47	8	18	19	27	22	18
Arg	42	41	50	54	63	68	52	90	50	39	32	33	38

TABLE II

Amino acid composition of precipitates from dialysis of soluble protein fractions
moles/1000 mole residues

	0·05 M salt fraction		0·08 M salt fraction	
	Raw	Pasteurised	Raw	Pasteurised
Asp	105	102	98	105
Thr	74	64	72	69
Ser	97	86	97	98
Glu	123	136	120	108
Pro	47	48	49	33
Gly	62	64	53	65
Ala	76	82	78	91
Val	64	67	64	67
½ Cys	18	15	19	14
Met	12	11	16	7
Ile	47	51	47	41
Leu	95	98	99	98
Tyr	27	16	32	30
Phe	39	38	37	39
Lys	54	58	56	58
His	10	14	13	14
Arg	51	51	50	63

The 0·08 M fractions from both raw and pasteurised egg contained fair amounts of precipitates after dialysis. All four dialysates were centrifuged at 21,000 rev/min and the insoluble matter from each was separated and dried in a desiccator. The amino acid compositions of these precipitates are given in Table II, which reveals a marked similarity among the four substances. Comparison of these figures with those in Table I shows that these precipitates cannot be exactly identified with any of the individual egg proteins for which the amino acid composition is known, but they are closest in composition to vitellenin, which is the protein moiety of the low density lipoprotein of egg yolk. These and similar precipitates from other experiments, after drying, were bulked together for a determination of total fat (by acid hydrolysis), which yielded a figure of 57% by wt. Their composition will be discussed further in Part V.[14]

The clear supernatant solutions of soluble proteins obtained after the precipitates had been centrifuged off were then separately adsorbed on columns of CM 52, equilibrated with acetate buffer, and the columns were eluted with solutions of 0·02 M sodium acetate, pH 5·2, containing successively 0·08, 0·10, 0·11, 0·12, 0·15, 0·18, 0·20 and 1·0 M sodium chloride followed by 0·001 M and 0·02 M disodium hydrogen phosphate, 0·02 M sodium carbonate and 0·5 N sodium hydroxide. The patterns obtained are shown in Fig. 1 (the middle portions being omitted, as no fractions were eluted in that range). It will be noted, particularly in the case of 0·08 M fractions, that there is considerable overlapping of the peaks eluted by acetate buffer containing 0·10 M and 0·11 M sodium chloride. Samples taken from these peaks were examined by starch-gel electrophoresis and gave the patterns shown in Fig. 2. It is relevant to note that the fastest-running band present in each of the peaks derived from the 0·08 M salt buffer fraction of raw egg was absent in the corresponding peaks derived from the pasteurised sample. From its position on the gel it is possible that this may be the ovalbumin, designated A1, containing two phosphorus atoms per molecule.

Clearly, the greatest need at this stage was to find some way of resolving the overlapping peaks eluted by acetate buffer containing 0·10–0·11 M sodium chloride, and attention was given to the effect of reducing the concentration of the acetate buffer. A similar experiment to that described above was carried out using 0·001 M acetate buffer in place of 0·02 M (for convenience, thawed frozen egg was used in this instance). This had the effect of resolving the peaks concerned and also of delaying their elution until the 0·18, 0·20 and 1·0 M salt buffers were passed through the column. There was a disadvantage in this change, however, as the 0·18 M salt buffer did not remove its material as a sharp peak but as a flat plateau spread over a comparatively large number of tubes. This led to a further series of experiments in which the concentration of the acetate buffer was gradually increased. As an example, use of 0·004 M acetate buffer gave sharper peaks, of which the first two were eluted by 0·15 M and 0·18 M salt buffer, respectively. When examined by starch-gel electrophoresis, the 0·15 M salt buffer fraction derived from raw egg showed two clearly separated bands in the ovalbumin region whereas that from the pasteurised egg showed only one. Both 0·18 M salt buffer fractions showed only one band in this region.

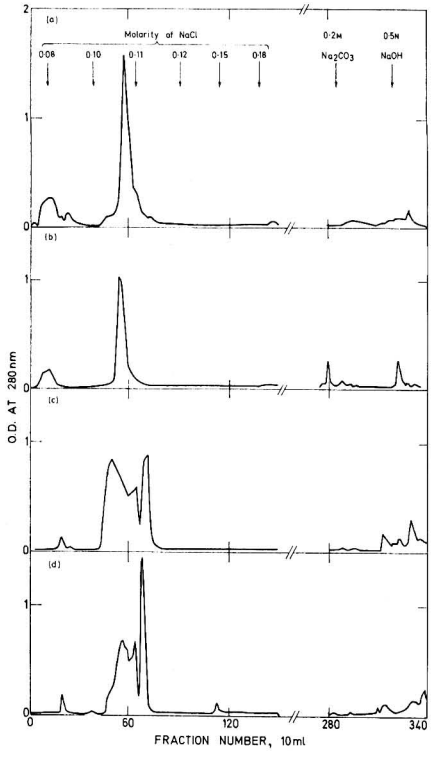

FIG. 1. *Sub-fractionation on carboxymethyl cellulose of 0·05 M and 0·08 M salt buffer fractions from diethylaminoethyl cellulose chromatography of soluble proteins from raw egg and egg pasteurised for 7 min at 150°F*

(a) 0·05 M salt buffer fraction from raw egg
(b) 0·05 M salt buffer fraction from pasteurised egg
(c) 0·08 M salt buffer fraction from raw egg
(d) 0·08 M salt buffer fraction from pasteurised egg

When the concentration of acetate buffer was increased to 0·008 M, overlapping of peaks occurred again in the 0·11 M salt buffer fraction, in a similar manner to that shown in Fig. 1.

The next experiment tested the use of 0·005 M sodium acetate buffer. A batch of new-laid eggs was homogenised and five 10 ml portions were pasteurised on the laboratory scale for 8 min at 150°F, and bulked and dialysed against 0·02 M glycine solution for four days. 50 ml of the unpasteurised egg were similarly dialysed, both samples were centrifuged and the supernatant liquids were fractionated on DE 52 columns, again giving results similar to those obtained previously, although the quantitative differences between the raw and pasteurised samples were less than those in the previous experiment, in spite of the heating time being a little longer. The 0·05 M and 0·08 M salt buffer fractions were each bulked and dialysed against 0·02 M sodium acetate buffer, pH 5·2, and on this occasion only small amounts of precipitates formed during the dialysis. The dialysed samples were re-fractionated on columns of CM 52, using 0·005 M sodium acetate (pH 5·2) buffer solutions containing increasing concentrations of sodium chloride, followed by the more alkaline solutions used previously, and the results are shown in Fig. 3.

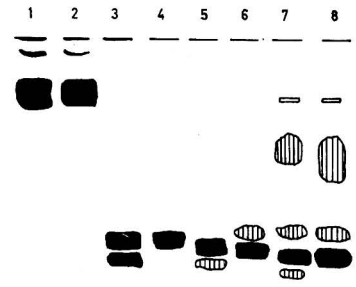

FIG. 2. *Starch gel electrophoretogram (0·024 M barbiturate buffer*[15] *for 20 h at 100 V) of sub-fractions shown in Fig. 1*

Slot 1, sub-fraction eluted by 0·10 M salt buffer from the 0·05 M fraction from raw egg; 2, corresponding sub-fraction from pasteurised egg; 3 and 5, sub-fractions (from successive peaks) eluted by 0·10 M salt buffer from the 0·08 M fraction from raw egg; 4 and 5, corresponding sub-fractions from pasteurised egg; 7, sub-fraction eluted by 0·11 M salt buffer from the 0·08 M fraction from raw egg; 8, corresponding sub-fraction from pasteurised egg.

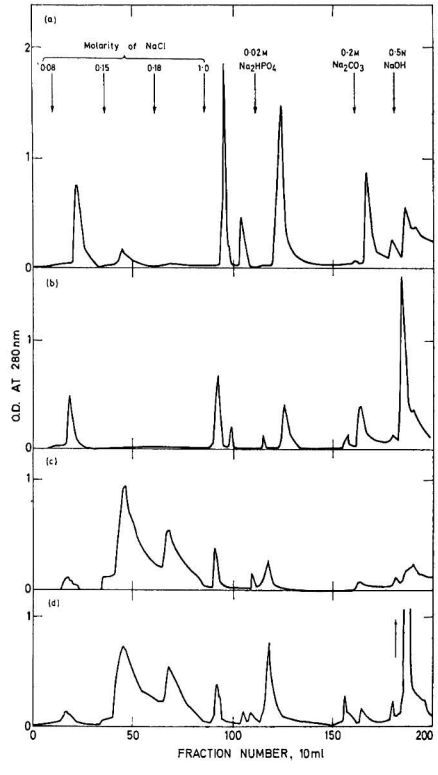

FIG. 3. *Sub-fractionation on carboxymethyl cellulose of 0·05 M and 0·08 M salt buffer fractions from diethylaminoethyl cellulose chromatography of soluble proteins from raw egg and egg pasteurised for 8 min at 150°F*

(a) 0·05 M salt buffer fraction from raw egg
(b) 0·05 M salt buffer fraction from pasteurised egg
(c) 0·08 M salt buffer fraction from raw egg
(d) 0·08 M salt buffer fraction from pasteurised egg

It will be noted that on this occasion re-fractionation on CM52 of the 0·05 M and 0·08 M salt buffer fractions showed some marked differences. In particular, the 0·05 M fraction from pasteurised egg generally gave smaller peaks than the corresponding raw egg fraction, with the exception of that eluted by sodium hydroxide; also, the sodium hydroxide eluant brought out a very large peak from the 0·08 M fraction from pasteurised egg in comparison with only a small peak from the corresponding raw egg fraction. This sub-fraction from pasteurised egg was dialysed against distilled water and freeze-dried, and its amino acid composition was determined. This is given in Table III and comparison with the figures given in Table I show that it cannot be identified with any of the individual proteins given in that Table. One possibility is that it is a complex protein that formed as a result of association when the egg was pasteurised.

TABLE III

Amino acid composition of sub-fraction eluted by sodium hydroxide from 0·08 M salt buffer fraction of pasteurised egg

moles/1000 mole residues

Asp	89
Thr	51
Ser	74
Glu	142
Pro	83
Gly	79
Ala	81
Val	60
½ Cys	32
Met	20
Ile	38
Leu	72
Tyr	31
Phe	33
Lys	42
His	21
Arg	52

Discussion

Amino acid analyses of 0·05 M and 0·08 M salt buffer fractions from the soluble proteins of whole egg confirmed the electrophoretic evidence that these fractions are mixtures of proteins.

The scheme developed for further resolution of these two fractions appears to be adequate for demonstrating differences between raw and pasteurised egg and should also prove useful for studying the effects of other treatments, such as staling and freezing and thawing.

A significant finding was the occasional presence in these fractions, especially those derived from raw egg, of small amounts of a substance that precipitated out when the fractions were dialysed against sodium acetate buffer of pH 5·2. This material had a high fat content and its amino acid composition was similar to that of vitellenin, indicating that it was probably derived from a yolk lipoprotein and not from any of the major proteins of egg white. Its relationship to a lipoprotein isolated from the insoluble protein fraction of whole egg will be discussed in Part V.[14]

Acknowledgments

This work was done under the sponsorship of the British Egg Marketing Board, to whom thanks are due. The author also thanks Dr. G. A. H. Elton and colleagues for helpful discussion, Mrs. P. J. Walker for skilled experimental assistance, Dr. D. G. Redman for amino acid analyses and Dr. D. S. Robinson for disclosing his results on the amino acid composition of ovomucin in advance of publication.

Flour Milling & Baking Research Association,
Chorleywood,
Rickmansworth,
Herts.

Received 19 February, 1970;
amended manuscript 8 May, 1970

References

1. Parkinson, T. L., *J. Sci. Fd Agric.*, 1968, **19,** 590
2. Rhodes, M. B., Azari, P. R., & Feeney, R. E., *J. biol. Chem.*, 1958, **230,** 399
3. Parkinson, T. L., *J. Sci. Fd Agric.*, 1967, **18,** 208
4. Parkinson, T. L., *J. Sci. Fd Agric.*, 1968, **19,** 582
5. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1967, **18,** 111
6. Cook, W. H., Burley, R. W., Martin, W. G., & Hopkins, J. W., *Biochim. biophys. Acta*, 1962, **60,** 98
7. Steer, D. C., Martin, W. G., & Cook, W. H., *Biochemistry, N.Y.*, 1968, **7,** 3309
8. Allerton, S. E., & Perlman, G. E., *J. biol. Chem.*, 1965, **240,** 3892
9. Canfield, R. E., *J. biol. Chem.*, 1963, **238,** 2691
10. Azari, P. R., & Baugh, R. F., *Archs Biochim. Biophys.*, 1967, **118,** 138
11. Neuberger, A., & Marshall, R. D., 'Glycoproteins' (Ed. Gottschalk, A.), 1966, p. 299 (Amsterdam: Elsevier)
12. Melamed, M. D., 'Glycoproteins' (Ed. Gottschalk, A.), 1966, p. 317 (Amsterdam: Elsevier)
13. Miller, H. T., & Feeney, R. E., *Biochemistry, N.Y.*, 1966, **5,** 952
14. Parkinson, T. L., *J. Sci. Fd Agric.*, 1970, **21,** 481
15. Baker, C. M. A., *Comp. Biochem. Physiol.*, 1964, **12,** 389

EFFECT OF PASTEURISATION ON THE CHEMICAL COMPOSITION OF LIQUID WHOLE EGG

V*.—Isolation and examination of a complex lipoprotein from pasteurised liquid egg

By T. L. PARKINSON

A high molecular weight substance isolated from the insoluble proteins of pasteurised liquid whole egg was found to be a complex lipoprotein containing 70% lipid and 30% protein. The lipid portion was a mixture of several different groups and the apoprotein was an extremely insoluble material with an amino acid composition that did not exactly match that of any egg protein reported in the literature but closely resembled that of vitellenin. It is postulated that this lipoprotein was derived from the low density fraction of egg yolk. A substance of lower molecular weight that was also separated from the inert fraction of insoluble proteins by gel filtration was tentatively identified as one of the lipovitellins.

Introduction

In a previous paper in this series[1] it was stated that the insoluble protein fraction of pasteurised liquid whole egg contained a much higher proportion of high molecular weight material than did that from raw egg. As the formation of this material is one of the few changes resulting from pasteurisation it was decided to isolate this high molecular compound and investigate its structure.

Experimental and Results

The methods used have mostly been described in previous reports.[2-4] Lipid fractionation followed the method described by Evans *et al.*[5] The starting material was obtained by gel filtration of the inert fractions of insoluble proteins obtained from several fractionations of pasteurised liquid whole egg that had not been frozen at any stage.

Isolation of the high molecular weight fraction

Those fractions eluted from the Sepharose column immediately after emergence of the bed volume and giving high spectrophotometer readings at 254 nm or 280 nm were bulked and dialysed against distilled water in a refrigerator for several days. The resulting precipitate was centrifuged for three hours at 21,000 rev/min and 0–3°C, the supernatant liquid was poured off and the insoluble residue was then dried over silica gel in a desiccator for several days.

The material from four experiments, in which a total of 320 g of pasteurised egg was used, was combined to give a total weight of 1·25 g, i.e. approximately 0·4% of the original liquid egg.

Examination of the high molecular weight compound

Analysis gave the following results: fat (acid hydrolysis), 68·3% by wt.; nitrogen, 4·5% by wt.; protein (N × 6·68), 30·1% by wt.

The remaining material (1·1277 g) was extracted, by stirring at room temperature, with a chloroform–methanol mixture (2:1 by vol.) and centrifuging at 2000 rev/min. The solvent mixture was decanted off and the extraction repeated twice. The combined extracts were evaporated to small bulk and dried over silica gel, yielding 0·7885 g (70%) of lipid. The unextracted residue was dried over silica gel, yielding 0·3211 g (28·5%) of apoprotein.

Examination of lipid moiety

The most detailed fractionation procedure for egg lipids to be found in the literature is that of Evans *et al.*,[5] in which the lipids are separated into seven groups by column chromatography on silicic acid. The lipid extract was dissolved in hexane and adsorbed on a narrow column of Mallinckrodt silicic acid (10 g). Elution was carried out successively with 200-ml portions of the following solvents: *A*, hexane; *B*, hexane containing 15% benzene; *C*, hexane containing 5% diethyl ether; *D*, hexane containing 20% diethyl ether; *E*, diethyl ether; *F*, chloroform containing 30% methanol; *G*, chloroform–methanol–water (15:4:1 by vol.).

In each case the entire eluate was collected, evaporated to small bulk, dried over silica gel in a desiccator and weighed. The weights obtained, and their percentages of the total, are recorded in Table I.

Evans *et al.*[5] claim that the groups eluted by the solvent mixtures given are: *A*, hydrocarbons; *B*, sterol esters; *C*, triglycerides; *D*, cholesterol; *E*, mono- and di-glycerides; *F*, cephalin and *G*, lecithin. However, examination of the fractions by thin-layer chromatography showed the situation to be considerably more complex in this instance, as exemplified in Fig. 1. Indeed, a short survey of published work in which lipid mixtures have been separated on silicic acid[6-8] indicated that free fatty acids would be eluted with mixture *C* as well as triglycerides and that diglycerides would be eluted with mixture *D* as well as cholesterol.

TABLE I

Fractionation of lipid moiety of high molecular weight fraction

Eluant*	Wt., g	% of total
A	0·0044	0·6
B	0·0677	9·2
C	0·2137	29·0
D	0·1308	17·7
E	0·1645	22·3
F	0·0835	11·3
G	0·0738	10·0

* See text for details

* Part IV: Preceding paper

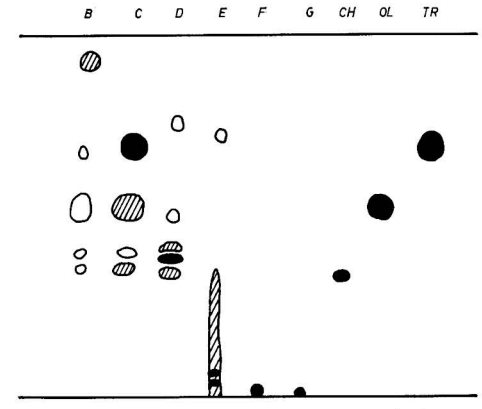

FIG. 1. *Thin-layer chromatography of fractions of lipid portion of high molecular weight lipoprotein from pasteurised egg, following separation on silicic acid*

For details of fractions *B* to *G*, see text
Markers denoted as follows: *CH*, cholesterol; *OL*, oleic acid; *TR*, tristearin. Adsorbent: Kieselgel G. Solvent; petroleum ether (b.p. 60–80°C)–diethyl ether–glacial acetic acid (70:30:3 by vol.). Spots made visible by charring with sulphuric acid. Strong spots are denoted by solid areas, medium spots by shaded areas and weak spots by open areas

Nevertheless, the results obtained do show that the lipid moiety of the lipoprotein is a very complex mixture in which a number of lipid groups are represented, including cholesterol and its esters, free fatty acids and mono- and diglycerides as well as the expected triglycerides and phospholipids. It is possible that some of the weaker spots in Fig. 1 represent degradation products formed during the removal of solvent by evaporation.

Examination of the apoprotein

The amino acid compositions of the apoprotein and the original lipoprotein from the first of the four experiments are recorded in Table II and compared with the composition of vitellenin, as calculated from published data.[4] Reference to the figures for other egg proteins, calculated from published data and tabulated in Part IV of this series,[4] showed that the apoprotein was nearer in composition to vitellenin than to any other protein, and this is well illustrated in Fig. 2. For ten out of the seventeen amino acids the difference between the apoprotein and vitellenin is not greater than five moles, but for six amino acids the difference is fifteen or more. Except for lysine, the difference between the lipoprotein and the apoprotein are small and, as the sample of lipoprotein examined was derived from one experiment and so represented only a portion of the material from which the apoprotein was extracted, these differences are probably insignificant. There was another difference between these two substances, the recorder trace showing a large peak in the glucosamine position for the lipoprotein hydrolysate and only a small one for the apoprotein hydrolysate.

Fig. 3 reveals that there is a very close correspondence in the amino acid compositions of the apoprotein and of the material that was obtained as a precipitate when the 0·05 M and 0·08 M salt buffer eluants from the fractionation of whole-egg soluble proteins on diethylaminoethyl cellulose were dialysed against sodium acetate buffer pH 5·2, as described in Part IV of this series.[4]

TABLE II

Amino acid content (moles/1000 mole residues) of the lipoprotein and its apoprotein,* and of vitellenin

	Lipoprotein	Apoprotein	Vitellenin[4]	
Asp	100	103	110	109
Thr	63	59	55	64
Ser	83	90	70	70
Glu	112	109	110	109
Pro	47	41	36	33
Gly	66	68	50	52
Ala	80	79	74	80
Val	62	66	67	69
½ Cys	24	26	6	8
Met	19	22	21	10
Ile	45	50	65	65
Leu	85	90	109	109
Tyr	31	32	36	39
Phe	41	40	44	40
Lys	75	57	83	76
His	17	15	15	13
Arg	51	53	50	54

* See text for details

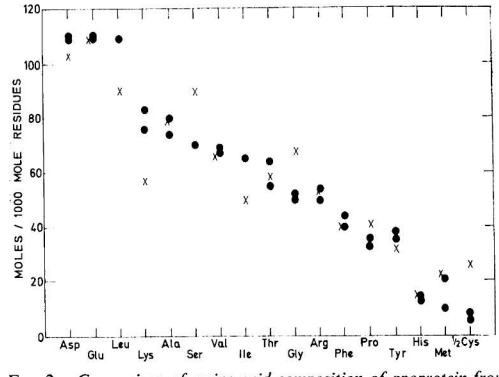

FIG. 2. *Comparison of amino acid composition of apoprotein from high molecular weight lipoprotein* (×) *with literature values*[9,10] *for composition of vitellenin* (●)

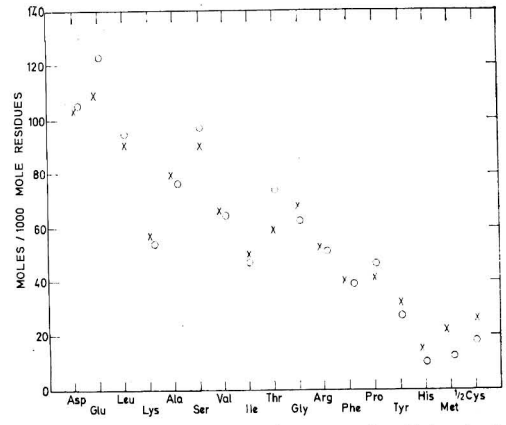

FIG. 3. *Amino acid compositions of apoprotein from high molecular weight lipoprotein* (×) *and precipitate from dialysis of 0·05 M salt buffer eluate of soluble proteins* (○)

It was considered desirable to ascertain whether the apoprotein was homogeneous and to obtain some idea of its molecular size. However, all attempts to get it into solution failed (even with anhydrous formic acid) and it was found impossible to solubilise any of it by reduction with mercaptoethanol in guanidine hydrochloride solution, no peaks being observed when this mixture was examined in a Beckman Model E analytical ultracentrifuge.

A Sepharose eluant containing the high molecular weight lipoprotein, isolated from another batch of pasteurised egg, was examined directly in a Beckman Model E analytical ultracentrifuge at 24,000 rev/min. A negative peak was observed to move rapidly through the cell in the first four minutes, but the initial turbidity and rapid clearing of the solution made it difficult to get satisfactory photographs. When the solution had cleared, no further peaks were observed, even when the centrifuge speed was increased to 56,000 rev/min, and at the end of the run the lipoprotein was seen to form a narrow band at the top of the cell.

Examination of a lower molecular weight fraction from insoluble proteins of pasteurised egg

During the isolation of high molecular weight material from one batch of pasteurised egg, a later peak was also observed when the inert fraction was passed through the Sepharose column. The appropriate eluate was bulked and dialysed against distilled water and the resulting insoluble material was separated by centrifuging at 21,000 rev/min and then dried in a desiccator at room temperature.

Analysis of the dried material gave the following results: fat (acid hydrolysis), 15·2% by wt.; nitrogen, 12·2% by wt.; protein (N × 6·68), 81·5% by wt.

The amino acid composition is recorded in Fig. 4, together with those of α-vitellin and β-vitellin, the latter being calculated from values given by Cook *et al.*[9] The most recent values for lipid contents of α- and β-lipovitellin recorded in the literature[11,12] are 14·6% and 17·0% for α-lipovitellin and 16·5% and 18·5% for β-lipovitellin, and the figure of 15·2% found for this preparation is sufficiently close to suggest that the material isolated is predominantly a lipovitellin, especially when the close agreement of amino acid composition is considered. Analogy with the findings of Radomski & Cook[11] and Wallace[12] suggest it is more likely to be β-lipovitellin than α-lipovitellin because it was not adsorbed on the diethylaminoethyl cellulose column.

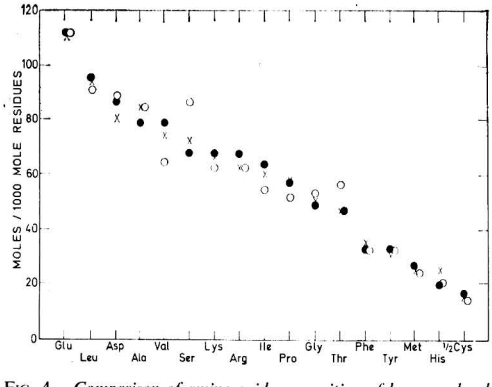

FIG. 4. *Comparison of amino acid composition of lower molecular weight fraction from separation of insoluble proteins on Sepharose* (○) *with literature values*[9] *for compositions of α-vitellin* (×) *and β-vitellin* (●)

Discussion and Conclusions

Examination of the high molecular weight protein fraction isolated from pasteurised liquid whole egg has shown it to be a lipoprotein, containing approximately 70% lipid and 30% protein, and appearing to be a single compound. The apoprotein is an extremely insoluble material with an amino acid content that suggests a fairly close relationship to vitellenin. It is known that vitellenin is also an insoluble material, although less so than the apoprotein isolated in the present work, and that the 'low density fraction' (*LDF*) of egg yolk, which contains nearly 90% of lipid, becomes less soluble as its lipid content is progressively reduced. It is therefore considered possible that the lipoprotein fraction isolated in this work could have arisen from association of *LDF* with other (probably lipid-free) proteins, from a partial removal of lipid from *LDF*, or from a combination of both mechanisms in which removal of lipid was followed by association. The lipid moiety of the isolated lipoprotein is a complex mixture of different compounds and contains sterols, sterol esters, mono- and di-glycerides as well as the expected triglycerides and phospholipids. Taking total neutral lipids to be the sum of all fractions eluted by the different hexane, benzene and ether mixtures used, the values found for neutral lipids, lecithin and cephalin are compared in Table III with values calculated from figures given in the literature[13,14] for samples of *LDF* from egg yolk. These suggest that if lipid removal occurred during pasteurisation it affected the lecithin fraction more than the other lipids.

It is relevant to note that the amino acid composition of this lipoprotein, isolated from the insoluble proteins, was so close to that of a small amount of lipoprotein that was precipitated by dialysis of certain fractions of the soluble proteins against acetate buffer at pH 5·2, and it is reasonable to assume that both were derived from the same source, namely the *LDF* of egg yolk.

A fraction that was partly retained on the Sepharose column, and consequently had a molecular weight well below the exclusion limit, had a lipid content and amino acid composition similar to those of the lipovitellins and was tentatively identified as one of these, probably β-lipovitellin. These compounds have molecular weights (in their dimer forms) of 400,000[15] and are components of the yolk granules and high density fraction, so their presence among the insoluble proteins would be expected.

TABLE III

Comparison of composition of lipid moiety of lipoprotein from pasteurised egg with published values for lipids of low density fraction (*LDF*) from egg yolk

	Lipid from pasteurised egg lipoprotein, %	Lipids from egg yolk *LDF*, %	
		Martin *et al.*[13]	Saari *et al.*[14]
Neutral lipids*	79	74	72 ; 68
Lecithin	10	19	24 ; 27
Cephalin	11	5	4 ; 5

* See text

Acknowledgments

This work was done under the sponsorship of the British Egg Marketing Board, to whom thanks are due. The author also thanks Dr. G. A. H. Elton and colleagues for helpful discussion, Mrs. P. J. Walker for skilled experimental assistance, Dr. D. G. Redman for amino acid analyses and the Analytical Section for fat and nitrogen determinations. He is especially grateful to Prof. S. D. Elsden for permission to use the analytical ultracentrifuge at the Food Research Institute, Norwich, and to Dr. D. S. Robinson for obtaining and discussing results given by this apparatus.

Flour Milling & Baking Research Association,
Chorleywood,
Rickmansworth,
Herts.

Received 19 February, 1970;
amended manuscript 10 April, 1970

References

1. Parkinson, T. L., *J. Sci. Fd Agric.*, 1968, **19,** 590
2. Parkinson, T. L., *J. Sci. Fd Agric.*, 1967, **18,** 208
3. Parkinson, T. L., *J. Sci. Fd Agric.*, 1968, **19,** 582
4. Parkinson, T. L., *J. Sci. Fd Agric.*, 1970, **21,** 477
5. Evans, R. J., Bandemer, S. L., & Davidson, J. A., *Poult. Sci.*, 1967, **46,** 151
6. Barron, E. J., & Hanahan, D. J., *J. biol. Chem.*, 1958, **231,** 493
7. Hirsch, J., & Ahrens, E. H., *J. biol. Chem.*, 1958, **233,** 311
8. Worth, H. G. J., & MacLeod, M., *J. Chromat.*, 1969, **40,** 31
9. Cook, W. H., Burley, R. W., Martin, W. G., & Hopkins, J. W., *Biochim. biophys. Acta.*, 1962, **60,** 98
10. Steer, D. C., Martin, W. G., & Cook, W. H., *Biochemistry, N.Y.*, 1968, **7,** 3309
11. Radomski, M. W., & Cook, W. H., *Can. J. Biochem.*, 1964, **42,** 395
12. Wallace, R. A., *Analyt. Biochem.*, 1965, **11,** 297
13. Martin, W. G., Tattrie, N. H., & Cook, W. H., *Can. J. Biochem. Physiol.*, 1963, **41,** 657
14. Saari, A., Powrie, W. D., & Fennema, O., *J. Fd Sci.*, 1964, **29,** 307
15. Cook, W. H., 'Egg Quality' (Ed. Carter, T. C.), 1968, p. 109 (Edinburgh: Oliver & Boyd)

REDUCTION OF 3,4-BENZOPYRENE CONTENT IN CURING SMOKE BY SCRUBBING

By I. M. MOODIE

A procedure has been developed for the detection and estimation of 3,4-benzopyrene in smoke and smoked fish. Results indicate that the use of a smoke scrubber in the industrial smoking process is instrumental in removing at least 70% of this carcinogenic material from curing smoke. Results from a complementary study of samples of smoked fish confirm this finding.

Introduction

In recent years, increasing attention has been focused upon the carcinogens or carcinogenic agents (cancer-inducing compounds). A widespread awareness of their presence both naturally and artificially in the human environment has led to much work on the assessment of levels and toxicology of these materials with a view ultimately to defining acceptable tolerance levels. Of particular importance has been the analysis of both smoked food products and curing smoke for the polycyclic aromatic hydrocarbons (PAH) and their derivatives of which more than 200 have been found to be carcinogenic. Research in this field was initiated following observations of a relatively high incidence of carcinoma of the alimentary tract in those populations where smoked foods are consumed in large quantities; for example, Bailey & Dungal[1] have isolated 3,4-benzopyrene from fish and mutton smoked in Iceland.

However, the significance of the amount of carcinogenic PAH in smoke for actual carcinogenesis appears at present to be unknown.[2] Boutwell *et al.*[3] and Roe *et al.*[4] independently have also drawn attention to a significant factor, particularly in the case of wood smoke, namely that the phenolic fraction of smoke condensate has a synergistic effect upon carcinogenesis, even though the phenolic fraction alone does not itself produce tumours.

Following its discovery as the first carcinogenic component of coal tar, 3,4-benzopyrene has received much attention and has come to be considered as a useful general indicator of carcinogenicity where PAH are concerned. Its potency as a carcinogen and its outstanding spectrophotometric properties have also contributed to its widespread acceptance as an indexing compound.

The level of PAH in smoke is believed[2,5-7] to be dependent upon three principal factors: burning zone temperature; quantity and size of solid particles in the smoke; and lignin content of wood used in smoke production.

The thermal conditions under which the pyrolysis of wood takes place will determine the quantity of carcinogenic PAH in the curing smoke generated. Conflicting reports[8-10] have appeared concerning the threshold furnace temperature below which the carcinogenic content may be reduced but it seems likely that a burning zone temperature of 450°C or lower leads to such a reduction.

Solid particular material in smoke acts as a transporting agent for PAH,[2,7] by surface adsorption, between furnace and food product. Electrostatic precipitation of these solids from whole smoke has been shown to reduce the level of 3,4-benzopyrene in the resultant vapour phase by some 66%.[2]

Pyrolysis of lignin at elevated temperatures gives rise to PAH and therefore those woods exhibiting the lowest lignin content should be selected for smoke generation.[7] Hardwoods (e.g. oak) contain approximately 24%, whilst soft woods (e.g. pine) may contain as much as 35% lignin. Tilgner,[5] however, is of the opinion that all organic substances are potentially capable of forming carcinogenic products on thermal decomposition at high temperatures in the presence of air.

These observations, together with the advantages already demonstrated (removal of undesirable particular material leading to a clean smoke chamber interior and absence of product discoloration) of a smoke scrubber designed and developed in these laboratories[11] motivated the present investigation into the effectiveness of scrubbing as a means of removing PAH from smoke.

Of the carcinogenic PAH, those most often reported to be present in smoke are 3,4-benzopyrene and 1,2,5,6-dibenzanthracene, although an equally potent possible compound is 9,10-dimethyl-1,2-benzanthracene.

All are neutral compounds and should be readily isolated from condensates by extraction following initial treatment with base to remove acidic components. The neutral fraction prepared in this fashion was shown by thin-layer chromatography to be made up of at least eleven components.

Howard *et al.*[12] have recently developed a method for the isolation and determination of PAH in smoked foods. The procedure consists of extraction and saponification, followed by an involved and lengthy clean-up process; estimation is effected using ultra-violet spectroscopy to a reported accuracy of the order of 1 in 10^9.

In the present study, preliminary results from clean-up and estimation procedures based on those of Howard *et al.* were not encouraging; the regions in which absorption due to 3,4-benzopyrene was expected were obliterated with that of other strongly absorbing components, rendering measurement of small absorption maxima impossible.

Gas chromatography was used in view of the encouraging results reported by Wilmshurst[13] on the analysis of PAH mixtures. Using similar conditions to those described, the system was calibrated and a suitable programme developed so that extracts from a chromatographic clean-up process could be evaluated for 3,4-benzopyrene content on a semi-quantitative basis. Under the same conditions, chromatograms of 9,10-dimethyl-1,2-benzanthracene and 1,2,5,6-dibenzanthracene showed peaks with associated retention times which did not correspond to any of those from the sample under examination.

As an extension to this investigation an examination of smoked hake was undertaken to ascertain the 3,4-benzo-

pyrene levels in fish smoked with scrubbed and unscrubbed smoke. Results were expected to reflect a similar reduction to that already observed in the study of smoke.

Samples were drawn from three batches of hake smoked in these laboratories under different conditions and from two batches of commercially smoked hake.

Experimental

Analysis of smoke

Smoke production

Smoke condensates were prepared on a laboratory pilot-scale apparatus[11] designed and manufactured at this Institute and shown diagrammatically in Fig. 1.

Smoke produced in the generator by slow smouldering was diverted, by appropriate valve adjustments, alternately through the scrubber (scrubbed smoke) or the by-pass (unscrubbed smoke) and thence to the condensers cooled by circulation of ice-chilled water, so as to condense tars, high molecular weight and non-volatile products. Other more volatile materials were removed to the atmosphere via the extraction unit. Scrubbing was effected by passage through a spray chamber incorporating a continuous mechanically circulated stream of reservoir water which had previously been saturated with smoke and filtered to remove sediment containing PAH.

Liquid smoke condensates which collected at the condenser outlets, consisted principally of water together with a relatively high proportion of phenolics, responsible for the flavour properties in smoked foods, and the high molecular weight wood pyrolysis products including PAH and polymeric materials (e.g. tars).

Using a mixture of oak chips and sawdust (45 kg), scrubbed and unscrubbed smoke condensates were collected alternately at approximately equal rates (455 ml/h) over a total period of 33h, giving 7·5 l of each condensate.

Extraction and clean-up procedure

The crude condensates were treated with sufficient (approximately 1500 ml) 10% sodium carbonate followed by warming to ensure complete reaction of acidic components and resulting in ~pH 9. On cooling, the mixture was filtered to remove solid material which might have precipitated, both filtrate and solid (if any) then being exhaustively extracted with 2,2,4-trimethylpentane (iso-octane).

The combined extracts were then concentrated to approximately 10 ml and chromatographed on grade II–III alumina (80×18 mm column), eluting initially with iso-octane. The column was then eluted with benzene (250 ml), thereby dissolving small traces of material rendered insoluble by the initial concentration step and removing the 3,4-benzopyrene from the column. (This elution procedure was developed in a control experiment in which 3,4-benzopyrene was first applied to the column in iso-octane. A band of this material was observed, under ultra-violet light, to be stationary 35 mm from the starting point and it resisted further elution with iso-octane. Subsequent elution with benzene gave an eluate from which the 3,4-benzopyrene was recovered quantitatively.) The total eluate was concentrated, further chromatographed on a Florisil column (80×18 mm) and eluted with benzene (250 ml). The solvent in this final eluate was removed and replaced with iso-octane, and the final solution was made up to 10 ml.

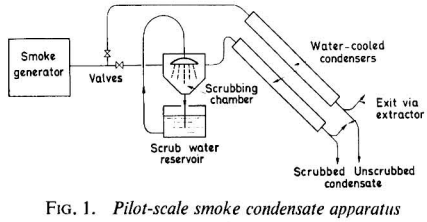

FIG. 1. *Pilot-scale smoke condensate apparatus*

Extraction of material in condensers

Following the run, each condenser was disconnected from the circuit, refilled with methanol (~ 6 l) and warm water was circulated through the jacket to facilitate dissolution of condensed material. After approximately 16 h, the methanol solution was drained, the condenser was washed with methanol and the combined extracts were filtered. The filtrate was concentrated to 500 ml, treated with excess 10% sodium carbonate solution (to pH 9) and exhaustively extracted with iso-octane. The filtered solid was also extracted with the same solvent and the total combined extract was subjected to the chromatographic clean-up procedure outlined above.

Extraction of sediment from wash water reservoir

Sediment, obtained by filtration, was treated with warm 10% sodium carbonate solution and the resulting suspension centrifuged. The precipitated solid was repeatedly washed with water, dried *in vacuo* over phosphorus pentoxide and finally exhaustively extracted with iso-octane. This solution was subjected to the chromatographic clean-up procedure outlined above.

Estimation by gas chromatography

Analyses were carried out on a Hewlett Packard Research Gas Chromatograph 5750, using 2 mm i.d. stainless-steel columns 6 ft long, packed with stationary phase W98 (10% by wt.) on Diatoport (80–100 mesh). The carrier gas was nitrogen at an assay/reference flow ratio of 4:1. Heating was programmed between 180° and 300°c at various rates (specified below) after 1 min initial delay. Sensitivity range was set at 10^{-10}, a full-scale deflection. Detection was by flame ionisation.

Calibration of system

Three iso-octane solutions, each containing different proportions of anthracene and 3,4-benzopyrene, were chromatographed (heating rate 15°/min). Peak height ratios were compared with corresponding weight ratios in each mixture. The results are shown in Table I. These indicated that there was a close correlation between the component weight ratio and peak height ratio and enabled the 3,4-

TABLE I

Comparison of component weight and peak height ratios in standard solutions

	Solution A	Solution B	Solution C
Weight ratio of anthracene: 3,4-benzopyrene	1:1	5:1	1:3
Peak height ratio of anthracene: 3,4-benzopyrene	152:143	100:20	55:163

benzopyrene content of a more complex mixture to be determined. Thus, having applied a sample (a) of smoke condensate extract to the column under the same or closely similar conditions and compared the chromatogram obtained with that from a similar sized sample (b) to which had been added a known quantity of 3,4-benzopyrene, the peak, if any, due to 3,4-benzopyrene was identified and, by simple ratio of peak heights, the original level of 3,4-benzopyrene was calculated. Small deviations in sample size at injection were nullified by direct comparison of convenient peaks arising from a further component in the sample, having adjusted the value for 3,4-benzopyrene in (b), if necessary, so that the further component peak heights were equal.

Analysis of smoked fish

PAH are deposited on the surface of the fish during smoking, no diffusion deep into the flesh taking place. Samples for extraction were obtained, therefore, by carefully removing the surface flesh to a depth of approximately 5 mm, dehydrating and milling the solids. The extraction procedure differs somewhat from that employed for smoke and is essentially that described by Genest & Smith[14] in their method for detection of 3,4-benzopyrene in smoked food products. Pre-analysis clean-up was by column chromatography as for smoke extracts, the final solutions being suitable for analysis by gas chromatography.

Smoking conditions

Three batches of hake fillets were smoked in a pilot-scale smoker[11] under the following conditions: (i) washed smoke in a chamber temperature of 39°C; (ii) unwashed smoke in a chamber temperature of 53°C, smouldering being controlled by initial and intermittent addition of water to the sawdust (burning zone ~450°C); (iii) unwashed smoke in a chamber temperature of 73°C, with uncontrolled burning at ~625°C. In each case the smoking period was 3½ h. The total fillet surface of each batch was removed and extracted as follows.

Extraction and clean-up procedure

A layer (~5 mm) from the fillet surface was removed by careful dissection, minced and dried *in vacuo* over phosphorus pentoxide at 55–60°C for 24 h. After milling to pass a 32-mesh sieve, the dried material was exhaustively extracted (Soxhlet) with iso-octane; the extract was reduced to 100–150 ml and was shaken separately with four 50 ml aliquots of dimethyl sulphoxide (DMSO), any emulsion being broken by centrifugation. Combined DMSO extracts were then treated with an equal volume of water and, on cooling, thoroughly extracted with benzene, emulsions again being broken with centrifugation. Combined benzene extracts were concentrated to 20 ml and dried over anhydrous sodium sulphate; the solvent was replaced with iso-octane. This solution (~10 ml) was chromatographed on a Florisil column (150 × 20 mm), eluting initially with iso-octane (100 ml) and then with benzene (250 ml). The latter eluate was concentrated and adjusted to a total volume of 5 ml, samples of which were examined by gas chromatography.

Estimation by gas chromatography

The conditions used in determining levels of 3,4-benzopyrene in the various samples of smoked fish were identical to those described previously with the exception of the programme heating rates shown in Table III. Included in this study were samples of fish smoked commercially with scrubbed and unscrubbed smoke.

Results

Smoke analysis

Analysis of the condensate samples were performed using the pre-selected heating rates indicated along with the results in Table II.

Smoked fish analysis

Results of the analysis of fish samples are shown in Table III together with burning zone temperature and smoking chamber temperature where available.

TABLE II

Levels of 3,4-benzopyrene detected in smoke condensate and other samples

Source	Level
'Scrubbed' smoke condensate	0·32 mg/7·5 l*
'Unscrubbed' smoke condensate	1·1 mg/7·5 l*
Condenser† in 'scrubbed' smoke line	0·89 mg**
Condenser† in 'unscrubbed' smoke line	4·20 mg**
Total solid residue in scrub water reservoir	3·34 mg/5·5 g**

* Programme heating rate 15°c/min
** Programme heating rate 6°c/min
† Internal surface area 5315 cm²

TABLE III

Levels of 3,4-benzopyrene detected in samples of smoked hake

Source	Smoking conditions	Burning zone temperature, °C	Smoking chamber temperature, °C	Level, mg/kg
F.I.R.I.†	Scrubbed smoke	—	39	0·09*
	Unscrubbed smoke	450	53	0·25**
	Unscrubbed smoke	625	73	0·36**
Industry	Scrubbed smoke	—	—	0·05***
	Unscrubbed smoke	—	—	0·63***

† Fishing Industry Research Institute
* Programmed heating rate 15°c/min
** Programmed heating rate 8°c/min
*** Programmed heating rate 6°c/min

Discussion

The levels of 3,4-benzopyrene detected in smoke condensate indicate that there has been a 71% reduction of this material in the scrubbed smoke. This is further corroborated by the observation that the scrubber was shown to contain 3·34 mg of 3,4-benzopyrene, representing some 73% of the total detected carcinogen in that section of the apparatus. Thus it appears that a further attribute of the scrubber, in addition to those already mentioned, is its ability to reduce very significantly the carcinogenic content of smoke, comparing in this respect very favourably with reported performances of the electrostatic precipitator. Whilst the results indicate the effectiveness of the small laboratory scrubber (a spray chamber approximately $10{\cdot}2 \times 10^3$ cm^3 coping with smoke generated from 45 kg of wood) used in these experiments, enhanced removal of 3,4-benzopyrene might be expected by the use of a larger scrubber, relative to the volume of smoke, as would be used industrially. This would lead to less contamination not only of the product but also of smoke emerging from the smoke chamber into the atmosphere. These conclusions are supported by results from a complementary study on the smoked product.

Fish smoked in the laboratory apparatus with scrubbed smoke (39°c) contained 0·09 mg 3,4-benzopyrene/kg of smoked product, representing a 64% reduction on the amount contained in fish exposed to unscrubbed smoke (53°c). This result is in broad agreement with that derived from measurements on the efficiency of the scrubber. Uncontrolled burning at temperatures > 625°c in the smoke generator led, as expected, to considerably increased production of 3,4-benzopyrene.

A similar but more marked reduction was observed in two commercially smoked samples, probably attributable to use of a relatively larger scrubber per unit volume of smoke generated.

Attention must be drawn, however, to the extreme care which should be exercised before attempting further interpretations of these results, full cognisance being given to observations from tests on animals in connexion with threshold doses of carcinogenic PAH required for tumour induction and the relevance of such observations to practical circumstances. In this light it is perhaps interesting to recall an earlier work by Sulman & Sulman[15] who reported the conflicting finding that whilst a carcinogenic response is observed following parenteral treatment there is a noticeable absence of carcinogenesis after oral administration.

Acknowledgments

The author wishes to thank the Director of the South African Fishing Industry Research Institute for permission to publish this work.

Fishing Industry Research Institute,
University of Cape Town,
Rondebosch,
Cape Province,
South Africa

Received 16 February, 1970;
amended manuscript 23 April, 1970

References

1. Bailey, E. J., & Dungal, N., *Br. J. Cancer*, 1958, **12,** 348
2. Rhee, K. S., & Bratzler, L. J., *J. Fd Sci.*, 1968, **33,** 626
3. Boutwell, R. K., *et al.*, *Cancer Res.*, 1959, **19,** 413
4. Roe, F. J. C., *et al.*, *Br. J. Cancer*, 1959, **13,** 623
5. Tilgner, D. J., *Fd Mf. J.*, 1968, **43,** 37
6. Tilgner, D. J., & Kazimirz, M., *Przem. spozyw.*, 1963, **17,** 17
7. Dobes, M., *et al.*, *Čslká. Onkol.*, 1954, **1,** 254
8. Tilgner, D. J., *Fleischwirtschaft*, 1958, **10,** 649
9. Dikun, P. P., *et al.*, *Chem. Abstr.*, 1966, **65,** 12406b
10. Wynder, E. L., *et al.*, *Cancer*, 1958, **11,** 1140
11. Dreosti, G. M., & Pienaar, A. G., *A. Rep. S. Afr. Fishg Ind. Res. Inst.*, 1959, **13,** 15
12. Howard, J. W., *et al.*, *J. Ass. off. analyt. Chem.*, 1966, **49,** 545
13. Wilmshurst, J. R., *J. Chromat.*, 1965, **17,** 50
14. Genest, C., & Smith, D. M., *J. Ass. off. analyt. Chem.*, 1964, **47,** 894
15. Sulman, E., & Sulman, F., *Cancer Res.*, 1946, **6,** 366

FOOD AND AGRICULTURE ABSTRACTS

SEPTEMBER, 1970

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

SEPTEMBER, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Genetic and effective classification of soils. E. SCHLICTING (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 220–231. Ger., 15 ref.).—A review, discussing the basis of classification and its interpretation. M. Long.

General soil map of the United States. J. F. DOUGLAS, M. E. AUSTIN and G. D. SMITH (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 746–749. 15 ref.).—A table presents the 1938 classification as revised to 1959 together with the equivalents in the present system (7th approximation). A. H. Cornfield.

The vertical soil zonality of the Bohemian massive. J. PELÍŠEK (*Z. PflErnähr. Bodenk.*, 1969, **124** (2), 148–156. 4 ref.).—Soil types for each elevation range are given from 115 m to 1600 m. Soil characteristics and properties, and vegetation for each soil zone are described. M. Long.

Weathering of montmorillonite during formation of a solodic soil. II. Nature of the mixed layer products. M. G. KLAGES (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 543–546. 20 ref.).—The most important effect of weathering on clay mineralogy was a change from relatively pure mineral crystals to particles with several 2 : 1 layer lattice minerals in a highly random arrangement of various degrees of interlayer expansion. A. H. Cornfield.

Chemical and biological weathering in vermiculite from Transvaal. B. L. SAWHNEY and G. K. VOIGT (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (4), 625–629. 16 ref.).—Changes in micromorphology, crystal structure, and chem. compn. of vermiculite flakes resulting from salt, acid and biological weathering processes are reported. A. H. Cornfield.

Chelating ability of fumarprotocetraric acid and *Parmelia conspersa*. J. K. SYERS (*Pl. Soil*, 1969, **31** (1), 205–208. 8 ref.).—Fumarprotocetraric acid, a lichen depsidone, and ground *Parmelia conspersa*, a saxicolous lichen, reacted with ground granite in pH 7·4 buffer to produce sol. coloured complexes, indicating that chem. weathering of the rock had taken place. Lichens may be significant as pedogenic agents, particularly in the early stages of soil formation. A. H. Cornfield.

Radiocarbon chronology of the Florida Everglades peat. L. L. MCDOWELL, J. C. STEPHENS and E. H. STEWART (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 743–745. 14 ref.).—The rate of formation of peat, which started about 4400 yr ago, was slow for the first 500–1000 yr, more rapid (7·3 cm per century) up to 1200 yr ago, and slower thereafter. By 1914 the peat had developed to a depth of 3·65 m (8·4 cm per century). Since drainage 50 yr ago, about 1·8 m of the profile has disappeared. A. H. Cornfield.

Physical Properties of Soils

Profile modification of a slowly permeable soil. H. V. ECK and H. M. TAYLOR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 779–783. 9 ref.).—Profile modification by mixing the permeable and impermeable layers of a silty clay loam to depths of 90 and 150 cm, increased grain sorghum yields by 66–80% over 3 yr, when irrigation water was applied only before planting. Under adequate irrigation, profile modification had little effect on yields. Efficiency of water use was increased under limited and adequate water supply. A. H. Cornfield.

Effects of petroleum mulch on soil water content and soil temperature. A. KOWSAR, L. BOERSEMA and G. D. JARMAN (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 783–786. 14 ref.).—Petroleum mulch added to a silty clay loam resulted in higher soil temp. (up to 5°) compared with unmulched soil. Bare soil lost water from the upper 4 cm; mulched soil lost water from the upper 1 cm, but gained water below this zone. Beneficial effects of mulch on germination and seedling growth can be attributed not only to higher soil temp., but also to improved soil moisture status. A. H. Cornfield.

Prediction of evaporation, drainage, and soil water storage for a bare soil. T. A. BLACK, W. R. GARDNER and G. W. THURTELL (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 655–660. 18 ref.).—On a bare sand, cumulative evaporation at any stage was $\propto \sqrt{\text{time}}$ following each heavy rainfall. The drainage rate was an exponential function of water storage. Both relations can be predicted from flow theory with knowledge of soil capillary conductivity, diffusivity and moisture retention characteristics. A. H. Cornfield.

Theoretical consideration of the calcium sulphate–bicarbonate–carbonate interrelation in soil solution. F. S. NAKAYAMA (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 668–672. 13 ref.).—The probable distributions of the ion-pairs $CaSO_4^\circ$ and $CaCO_3^\circ$, and complex $CaHCO_3^+$ in soln. are discussed thermodynamically and graphically in terms of SO_4^{2-}, CO_3^{2-}, HCO_3^-, and H^+ activities. A. H. Cornfield.

Evaluating the use of Na^+, Ca^{2+}, and divalent cation electrodes in some soil extracting solutions. S. A. EL-SWAIFY and M. N. GAZDAR (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 665–667. 17 ref.).—The performance of a Na electrode was more satisfactory than that of a Ca or 'divalent' cation (Ca + Mg) electrode in buffered or unbuffered soln. Observed e.m.f. responses of the latter electrodes to changes in ion activities in buffered systems were generally less than predicted theoretically. A. H. Cornfield.

Chemical potentials in the transport of soil solutions through the solum. B. ULRICH (*Z. PflErnähr. Bodenk.*, 1969, **123** (3), 181–186. Ger., 3 ref.).—Potentials were calc. on the basis of Schofield potentials. Positive values indicated a tendency towards an excess of the ion concerned in the pool of exchangeable ions, whilst negative values were connected with an excess of the exchangeable form in the soil soln. M. Long

Relationships among adsorbed phosphate, silica, and hydroxyl during drying and re-wetting of kaolinite suspension. B. BAR-YOSEF, U. KAFKAKI and N. LAHAV (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 672–677. 14 ref.).—Concn. of P, Si and OH^- in the soln. of a kaolinite suspension, drying out isothermally, and a comparison of adsorption characteristics of re-wetted clay suspension, after oven- and air-drying, were studied. Si played an important part in the mechanism of phosphate adsorption by kaolinite. Drying induced an increase in the retention power of phosphate on the surface of kaolinite. A. H. Cornfield.

Conductivity instrumentation for *in situ* measurement of soil salinity. C. G. ENFIELD and D. D. EVANS (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 787–789. 5 ref.).—A transducer constructed of porous glass and Pt electrodes is described. The method is accurate to within 10% over the conductivity range 1–20 mmho per cm^3 and temp. range of 5–45°C. A. H. Cornfield.

Biological Aspects, Available Nutrients, Analysis

Major oxygen-containing functional groups present in humic and fulvic acid fractions from contrasting marine environments. M. A. RASHID and L. H. KING (*Geochim. cosmochim. Acta*, 1970, **34** (2), 193–201. 28 ref.).—These acids, extracted from different clays, contained carboxylic (*C*), phenolic (*P*), carbonyl -CO and alc. hydroxyl O groups. The total acidity arises mainly from the *C* grouping rather than from *P*. The -CO content appears to be high in relation to other groups, and also high as compared to the -CO content in soil org. matter. It is suggested that the environment is conducive to the accumulation and preservation of -CO. The O-content of overlying water does not appear to influence the findings as similar results were found in org. matter from reducing and non-reducing environments. C. V.

Decomposition of plant litter in Montane and alpine soils on Mt Kosciusko, Australia. T. G. WOOD (*Nature, Lond.*, 1970, **226** (5245), 561–562. 10 ref.).—These acid, cold and wet alpine-humus soils have a high content of org. matter, 20–35% of clay, and a C/N ratio of 12–17. Rates of decomp. of *Eucalyptus pauciflora* and *E. delegatensis* (leaves) and of *Poa caespitosa* (leaves and roots) during 1 yr were generally much greater in these soils than in podsolic and transitional alpine-humus soils. This trend is opposite to the usual decrease in rate of decomp. and increased thickness of the A_0 layer with altitude. Biol. factors responsible for the rapid breakdown of litter are probably earthworms, Enchytraeidae and micro-organisms, although a low-temp. tolerant fauna and flora is not excluded. W. J. Baker.

Production by species of *Allium* of alkyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum*, Berk. J. R. COLEY-SMITH and J. E. KING (*Ann. appl. Biol.*, 1969, **64** (2), 289–301. 23 ref.).—A no. of sulphides which stimulated germination of sclerotia of *S. cepivorum* in soil were evolved by chopped garlic cloves and onion bulbs, and by their extracts and distillates and condensates prepared from extracts. Sulphides produced by garlic were Me and allyl compd., whilst those of onion were Pr^n and Me deriv. A. H. Cornfield.

Production of volatile alkyl sulphides by microbial degradation of synthetic alliin and alliin-like compounds, in relation to germination of sclerotia of *Sclerotium cepivorum* Berk. J. E. KING and J. R. COLEY-SMITH (*Ann. appl. Biol.*, 1969, **64** (2), 303–314. 20 ref.).—Distillands of onion bulb juice were more stimulatory than distillates towards germination of sclerotia of *Sclerotium cepivorum*. Synthetic allyl- and Pr^n-cysteine and their sulphoxides stimulated germination of sclerotia when added to soil. Soln. of these compd., in presence of unsterile soil, produced gaseous alkyl mercaptans, sulphides and disulphides which stimulated sclerotial germination when drawn through soil containing sclerotia. The atm. over Pr^n- and allyl-cysteines was also slightly stimulatory. Six common soil bacteria were able to degrade the synthetic precursors, causing the evolution of appropriate volatile alkyl sulphides. A. H. Cornfield.

Biological nitrogen fixation. J. R. POSTGATE (*Nature, Lond.*, 1970, **226** (5240), 25–27. 69 ref.).—Reviews the many proposed systems and pathways involved in the anaerobic reduction of N to NH_3 in soils, i.e., enzymatic, physiol., microbial-ecological, control processes and chem. aspects. There is no direct biochem. evidence that a metal complex is formed during fixation, but some hypotheses are based on dinitrogen complexes or heavy-metal nitrides and others on electron transport in anaerobes. W. J. Baker.

Uptake of initially available soil potassium by ryegrass [on Rothamsted and Woburn soils]. T. M. ADDISCOTT (*J. agric. Sci., Camb.*, 1970, **74** (1), 123–129. 15 ref.). M. Long.

Use of quantity/potential relationship to provide a scale of the ability of extractants to remove soil potassium. T. M. ADDISCOTT (*J. agric. Sci., Camb.*, 1970, **74** (1), 119–121. 13 ref.).—Quantity/potential relationships between gain and loss of K by the soil, K potential and K extracted by neutral NH_4OAc (**I**), H-resin and 0·5M-$NaHCO_3$ were detd. on 27 soils. The ability of an extractant to remove soil K was related to a K potential derived from the quantity/potential curve. The extracting ability of **I** and $NaHCO_3$ soln. was found to be less in rich than in poor soils. The extracting ability of **I** was not affected by variations in NH_4^+ concn. between 0·1 and 1N. M. Long.

Potassium-supplying power of some soils of Ghana cropped to cacao. YAW AHENKORAH (*Soil Sci.*, 1970, **109** (2), 127–135. 19 ref.).—To clarify a report of K-deficiency in Ghana soils cropped to early and high-yielding varieties of cacao, the K-supplying power of the soils was examined. In pot tests, the soils, after cropping with cacao for 10 years, were cropped continuously with perennial ryegrass to determine the release of (previously) non-exchangeable K. Such releases varied from < 5 to > 1200 kg of K/ha. Addn. of P increased the K uptake of ryegrass in some soils but caused K fixation in others. In some cases 'fixed' K served as an additional source of soil K. Most of the soils appeared to be well buffered against K-depletion. A. G. Pollard.

Sporulation of *Bacillus subtilis* as a possible measure of available manganese in calcareous soils. H. ROSENBURG, J. NAVROT and Y. HENIS (*Pl. Soil*, 1969, **31** (2), 337–344. 19 ref.).—Sporulation (measured by staining and microscopic counts) of *Bacillus subtilis* ATCC 9799 was correlated with Mn^{2+} concn. over the range 10^{-7} to 10^{-6} M in nutrient broth. Available Mn, determined by % sporulation in culture soln. treated with sterilised soil, was well correlated with response in growth of oats to applied Mn in 5 of 6 soils containing 8–58% $CaCO_3$. A. H. Cornfield.

Actions of various organic materials on the solubilisation of boron in calcareous soil. P. CORNILLON (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1970, **56** (2), 134–141. Fr., 4 ref.).—The org. materials added were pure powdered cellulose, cereal straw (37% cellulose) and dry clover (21% cellulose), and the soil was an alluvial one from the lower Durance valley. During a period of 4 yr, straw had no apparent effect, whilst clover caused a slight increase; after 10 days, the pure cellulose, which contained no B, caused an increase in B concn., 0·60 ppm being reached after 21 days. This level remained more or less const. for 3 yr after which it began to fall again. M. T. Rawnsley.

Evaluation of indices of available soil nitrogen. R. R. STORRIER, A. T. HANLY, and H. I. NICOL (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 89–94. 25 ref.).—Perennial ryegrass was grown under lab. conditions in 12 different soils. These were then analysed for: total N (micro-Kjeldahl), org. C, NH_3-N (Waring and Bremner), total mineral-, incubation-, water-sol.- and 'glucose'-N. It is concluded that chem. tests are preferable to biol tests. Soil N tests are not satisfactory. M. T. Rawnsley.

Fertilisers

Sulphur transformation in a saline-sodic soil of the Lajas Valley. R. P. ESCOLAR and M. A. LUGO LOPEZ (*J. Agric. Univ. P. Rico*, 1969, **53** (2), 118–123. 3 ref.).—The extent of transformation of elementary S (applied to a saline sodic clay, pH 8·0) to SO_4^{2-} over 6 months, ranged from 40% of that applied at 4 tons to 29% of that applied at 12 tons per acre. Soil pH decreased with rise in level of applied S, with a max. decrease of 0·55 units 3 months after application. A. H. Cornfield.

Effect of sulphur in fertilisers, rainwater, and soils on crop nutrition. P. A. GALLAGHER (*Scient. Proc. R. Dubl. Soc. B*, 1969, **2** (20), 191–204. 41 ref.).—Levels of S, C, org. matter and N in soils are tabulated. The survey indicates that S deficiency could become a problem in certain areas (in Ireland), if non-S fertilisers continue to be used, and if crops demanding high S nutrition (e.g., onions), are grown. More work is needed on leaching, absorption of SO_2 from the air etc. M. T. Rawnsley.

Bench-scale studies of sulphate recycle nitric phosphate process. G. M. BLOUIN, O. W. LIVINGSTON and J. G. GETSINGER (*J. agric. Fd Chem.*, 1970, **18** (2), 313–318. 12 ref.).—Studies were carried out to determine the effects of operating conditions on various steps of a fertiliser production process, using the acidulation of phosphate rock with HNO_3. The sulphate recycle phosphate process was reported as a method for Ca removal. $(NH_4)_2SO_4$ soln. was added to the acid–rock acidulate and the resulting $CaSO_4$ ppt. was filtered off and washed with $(NH_4)_2SO_4$ which was regenerated by treating the $CaSO_4$ with NH_3 and CO_2 and filtering off the resultant $CaCO_3$. M. J. Rawlins.

Efficacy of sources of phosphorus in relation to phosphorus availability in three coffee soils. A. A. RAMAMURTHY and T. R. SUBRAMANIAN (*Indian Coff.*, 1970, **34** (2), 41–43, Engl., 8 ref.).—Three incubation studies were carried out with soils of pH 6·2, 5·2 and 5·6. Nitrophosphate was superior to other sources of P in maintaining availability of P in all three soils. At soil pH > 5·6, there was very little release of P from rock phosphate. W. J. G.

Fertiliser and soil phosphorus uptake by maize, as affected by soil phosphorus level, granule size, and solubility of phosphate sources. G. L. TERMAN and S. E. ALLEN (*J. agric. Sci., Camb.*, 1969, **73** (3), 417–424. 15 ref.).—Pot trials were carried out with labelled dicalcium phosphate and labelled conc. superphosphate, applied to soils low, high and very high in available phosphate. On the low-P soil, forage yield, total P uptake and fertiliser P uptake increased linearly with applied P up to 80 ppm. All three factors were found suitable for evaluating the efficacy of P fertilisers. On the other soils, only uptake of fertiliser P was suitable. As soil P increased, total fertiliser P uptake decreased, although relative differences between P sources remained similar. Calc. values of limiting yields or uptakes proved to be of less value for evaluation of P sources than actual uptakes of fertiliser P or % recoveries of labelled P with soils high in P. Labelling, essential with soils of high P status, was of little advantage with soils of low P status. M. Long.

Influence of phosphate manuring and phosphate condition of the soil on under-water weight of industrial potatoes. H. J. PRUMMEL

(*Landbouwvoorlichting*, 1969, **26** (12), 418–421. Dut., 2 ref.).—Results show that both phosphate manuring and condition of the soil increase the under-water weight (detd. by the starch %); a large phosphate supply is important for starch forming.
J. C. T. Nieuwenhuis.

Neutralising acidity under Bermudagrass sod. F. Adams and R. W. Pearson (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 737–742. 6 ref.).—Surface application of dolomitic limestone to Bermudagrass sod, which had been treated for 2–3 yr with acid-forming N fertilisers, was more effective in increasing subsoil pH in a loamy sand than in a clay loam. $NaNO_3$ and $Ca(NO_3)_2$ were effective in increasing subsoil pH, and there was no accumulation of Na due to $NaNO_3$. Ca gluconate was also effective but caused an undesirable fungal bloom. A. H. Cornfield.

Residual effects of liming volcanic ash soils in the humid tropics. B. C. Mahilum, R. L. Fox and J. A. Silva (*Soil Sci.*, 1970, **109** (2), 102–109. 29 ref.).—Effects of liming these highly weathered soils are examined experimentally, over a 7-yr period, using surface and sub-surface samples to depths of 4 ft. Following addn. of moderate or heavy dressings of coral lime (2–17 tons/acre), recovery of Ca from a 4 ft profile 5 yr later was ~30%. Leaching of Ca was rapid and application of $CaCO_3$ or $CaSiO_3$ lowered the exchangeable Al content of the soils drastically, Ca replacing Al in approx. equiv. proportions. Al thus displaced did not readily re-occupy exchange positions even when Ca disappeared from the soil. An Akaka soil having a permanent charge equiv. to 4 mequiv./100 g, and normally being ~12% Ca-saturated, supported near-normal crops of sugar-cane. A. G. Pollard.

The potassium Q/I relationships of soils given different K manuring. T. M. Addiscott (*J. agric. Sci., Camb.*, 1970, **74** (11), 131–137. 11 ref.).—The K quantity/intensity (Q/I) relationships, relating changes in exchangeable K content (Q) to changes in activity ratio ($I = a_K/\sqrt{a_{Ca+Mg}}$) were measured in soils from manuring trials. The Q/I curves within a trial were superimposable on each other and on the exchangeable K/I_0 curves, where K/I_0 is the activity ratio at which the soils neither gain nor lose K. The buffer capacity dQ/dI was related to the K satn. of the cation exchange capacity for the soils investigated. M. Long.

[Effects of] forms of K fertilisers and soil moisture content on potassium status of a Trinidad soil. N. Ahmad and C. E. Davis (*Soil Sci.*, 1970, **109** (2), 121–126. 13 ref.).—The K status of a Trinidad loam soil treated with K as Cl^-, NO_3^-, PO_4^{3-} or SO_4^{2-} in concn. 0–1000 ppm was examined. Fixation of K occurred when K saturation was > 3% of the *CEC* and was not affected by soil moisture contents between 0·5 and 1·6 × field capacity. Fixation was influenced appreciably by associated Cl^- or PO_4^{3-}; SO_4^{2-} and NO_3^- had less effect. Repeated wetting and drying of the soil did not increase fixation. Increased amt. of K added to the soil increased the proportion of water-sol. extractable K which, for a given rate of application, was inversely related to soil moisture content during equilibration. A. G. Pollard.

Selenium concentrations from several sources applied to a low-selenium alkaline soil. D. L. Carter, M. J. Brown and C. W. Robbins (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 715–718. 17 ref.).—Application of $CuSeO_3$ or two 'ferric hydroxy bi-selenites' to an alkaline silt loam provided slowly available Se to lucerne, resulting in Se concn. adequate but not toxic for livestock. Se from $BaSeO_4$, $BaSeO_4$–$BaSO_4$ mixture, or $CuSeO_4$ was absorbed by lucerne in concn. toxic to livestock. Elemental Se provided adequate Se only during the early stages of growth. A. H. Cornfield.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Effects of nitrogen, phosphorus and potassium fertilisers on assimilation capacity of *Beta vulgaris* chloroplasts. L. Tombesi, M. T. Cale and B. Tiborne (*Pl. Soil*, 1969, **31** (1), 65–76. 17 ref.).—The following aspects of non-cyclic photophosphorylation and NADP photoreduction were studied in *B. vulgaris* and chloroplasts: effects of varying levels of N, P and K on these factors and on catalase activity before and after exposure to light; correlations between photosynthetic activity in leaf tissue discs and the ability of chloroplast suspensions to synthesise ATP and reduce NADP.
A. H. Cornfield.

Relationship between the effects of water deficiency, saline or cold rooting media and the proline, pipecholic acid and total amino acid contents of plants. G. Pálfi and Julia Juhász (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 36–42. Ger., 11 ref.).—Proline content in leaves of plants grown with insufficient water was several times higher than in normal plants. The effect could be simulated by increasing the salt content of the growing medium. At temp. of the rooting medium much lower than air temp, the root system was unable to compensate for transpiration losses and the proline content of the leaves increased. Disturbance of the protein synthesis of the plant was also characterised by the appearance of pipecholic acid amongst the amino acids. M. Long.

Determination of need for potato irrigation using refractometric index of sap from frozen leaves. E. E. Ewing and L. Farkas (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 163–167. 19 ref.).—A technique for detecting moisture stress in potato leaves by refractometric measurement of sap expressed from frozen tissue is described. Irrigation based on a 'deficiency index' (difference in readings between unwatered and watered plants) increased potato yields in 2 of 3 yr. A. H. Cornfield.

Survival of plants under prolonged flooded conditions. P. T. Yu, L. H. Stolzy and J. Letey (*Agron. J.*, 1969, **61** (6), 844–847. 16 ref.).—Young plants of those species and varieties (maize, sunflower and Pato wheat) which were able to withstand severe damage due to flooding for 7–31 days showed greater increases in root porosity due to flooding than did those (barley, tomato and Inia wheat) which were severely damaged. Tolerance to excess moisture may be related to increased internal supply of O_2 resulting from increased root porosity. A. H. Cornfield.

Polar and lateral transport of water in an apple tree. D. W. West, W. K. Thompson and J. D. F. Black (*Aust. J. biol. Sci.*, 1970, **23** (1), 231–234. 3 ref.).—Root systems and lower 20 cm of the trunks of 2-yr old apple trees, were divided into 4 by 2 saw cuts normal to each other. Each part of the root system was established in a separate container, and the water level in each was allowed to fall. It was then increased to field capacity with tap water in zones 1–3 and tritium-enriched tap water in zone 4. Whilst the soil in 1–3 zones was allowed to dry out, that in zone 4 was replenished. Trees were weighed at 1 or 2 day intervals. Tritium activity was plotted. Polar transport of water was shown to occur and be maintained against a soil water potential gradient on different parts of the root system of the order of 2 atm. M. J. Rawlins.

Plant Nutrition and Metabolism

Uptake and translocation of iron by bean plants as influenced by transpiration and metabolic activity. R. Schulz and H. Marschner (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 1–12. Ger., 28 ref.).—Uptake and translocation of Fe by bean plants from $FeCl_3$ (**I**), FeEDTA (**II**) and FeEDDHA (**III**) in nutrient soln. at pH 5·0 was investigated. The three sources of Fe were labelled with ^{59}Fe. Transpiration was controlled by varying r.h. from 40–95%. The order of uptake was **II** < **III** < **I**. Increasing soln. pH to 7·0 almost stopped uptake from **I** and **III** with little effect on **II**. Fe taken up from **III** was largely translocated to the shoots compared with that from **I** and **II**. There was no direct relationship between translocation and transpiration. Reduction of metabolic activity by N_2 or 2,4-DNP greatly reduced translocation of Fe to the shoots. M. Long.

Intake and export of nutrients in cacao leaves. M. Wessel (*Trop. Agric. Trin.*, 1970, **47** (2), 167–170. Engl., 3 ref.).—Amelonado seedlings were studied in a glasshouse expt. Leaves of previous flushes contributed most to the development of new leaves and were the main reservoirs of mobile leaf P and K.
E. G. Brickell.

Effects of silica and nitrogen supply on some leaf characteristics of the rice plant. S. Yoshida, S. A. Navasero and E. A. Ramirez (*Pl. Soil*, 1969, **31** (1), 48–56. 14 ref.).—Increasing Na_2SiO_3 supply (40–200 ppm SiO_2) in the nutrient resulted in increased erectness and thickness of rice leaves, whilst increasing NH_4NO_3 (5–200 ppm N) had the reverse effect. Increasing N markedly increased leaf length and width, whilst increasing SiO_2 had no effect on these characteristics. A. H. Cornfield.

Rate and efficiency of nutrient absorption by tomato seedlings. G. M. Ward (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 128–130. 8 ref.).—Application of KNO_3 to pre-starved tomato seedlings increased uptake of K and N by 20–90% within 6 h and up to 400% within 7 days. Plants raised in a relatively rich nutritional environment were more efficient in absorbing the applied nutrients than were those raised at a lower nutritional level. Vigorous plants did not absorb more than a certain amount of KNO_3 applied at high levels, indicating a control mechanism in the plant.
A. H. Cornfield.

Molybdenum deficiency symptoms and their development. W. BUSSLER (*Z. PflErnähr. Bodenk.*, 1970, **125** (1), 50–64. Ger., 25 ref.).—Various species were grown in nutrient solutions freed of Mo, using peat, sand or soil as substrates. In all the species investigated, similar tissue changes were induced by Mo deficiency, development of the deficiency being dependent on the age of the plant part. Early damage in the embryonic region leads to the development of whiptail leaves and leaf development is modified by irregular vein growth. Later necrosis appears but with the older leaf does not affect its development. With the growing leaf, necrosis leads to distortion. On incompletely developed leaves the necrosis appears as 'yellow spot' and is known as such. Microscopic examination shows that both yellow spot and whiptail are similar. The reduction of laminae, known as whiptail in Mo-deficient cauliflower, is a typical Mo deficiency symptom and applies to all species. M. Long.

Development of molybdenum deficiency symptoms in cauliflower. W. BUSSLER (*Z. PflErnähr. Bodenk.*, 1970, **125** (1), 36–50. Ger., 16 ref.).—Cauliflower were grown in sand culture with Mo-free nutrient soln. in order to determine the order of appearance of Mo deficiency symptoms. M. Long.

Effect of excess boron supply on germination and seedling growth of groundnut. N. H. GOPAL and I. M. RAO (*Pl. Soil*, 1969, **31** (1), 188–192. 14 ref.).—Treatment of groundnut seed with 25 ppm B (H_3BO_3) hastened germination and growth up to 12 days even though slight chlorotic (toxic) symptoms occurred. Growth, compared with the control, then declined and very severe chlorotic symptoms occurred by 21 days of age. A. H. Cornfield.

Influence of variable manganese and silicon on nutrition, sugar production, and enzyme activity of immature sugar-cane. G. SAMUELS and A. G. ALEXANDER (*J. Agric. Univ. P. Rico*, 1969, **53** (1), 14–27. 32 ref.).—Uptake of Mn by sugar-cane in sand cultures decreased whilst that of Si increased with increasing Si (Na_2SiO_3) in the culture. Increasing nutrient Mn ($Na_2MnEDTA$) resulted in increased uptake of Si. High Si + Mn supply decreased growth, but increased tissue sugar % and suppressed starch phosphorylase and phosphatases. Of the enzymes assayed, polyphenol oxidase showed the greatest sensitivity to variable Mn and Si supply. Rôles of the two elements in the mechanisms of auxin and protein synthesis are discussed. A. H. Cornfield.

Suspected copper deficiency in Radiata pine. J. H. RUITER (*Pl. Soil*, 1969, **31** (1), 197–200. 9 ref.).—Acute deformity in the terminal and uppermost lateral shoots followed by yellowing, bronzing and necrosis of the needles of Radiata pine in the 3rd yr of growth were associated with very low (< 1 ppm) Cu in the foliage. The symptoms were most severe where N + P was applied, but were absent where Cu + other trace elements were applied. A. H. Cornfield.

Effects of copper on composition and anatomy of tobacco. B. E. STRUCKMEYER, L. A. PETERSON and F. HSI-MEI TAI (*Agron. J.*, 1969, **61** (6), 932–936. 9 ref.).—When the Cu content of a nutrient soln. for tobacco seedlings was increased above 0·16 ppm at two weeks of age, growth was decreased and chlorotic symptoms appeared within 10 days. Cu% in tops increased slightly and in roots considerably with increasing nutrient Cu concn. Toxic Cu concn. were associated with abnormal cellular growth and development throughout the plant. A. H. Cornfield.

Effect of manganese on tobacco leaf quality and on the inorganic cation levels of tobacco leaves. A. D. JOHNSON and R. W. KNOWLTON (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 118–123. 19 ref.).—Sand-culture, pot and field expt. were carried out and leaf areas, leaf thickness, Mn, Mg, K, Na and Ca were detd. Results show that const. K nutrient supply and increased Mn caused increased K uptake in leaves, but decreased Ca intake. This occurred in both substrate and foliar application. Mg uptake was not affected, but the grey appearance of leaves is probably related to the K:Ca:Mg ratio. Caution is needed in using Mn-based sprays. M. T. Rawnsley.

Detection of molybdenum in a drop of nutrient solution by the deficiency symptoms shown by *Aspergillus niger*. W. BUSSLER (*Z. PflErnähr. Bodenk.*, 1970, **125** (1), 16–23. Ger., 9 ref.).—The nutrient solution was freed of Mo by co-pptn. with added Cd or Cu. Fresh spores of Mo-deficient *Aspergillus niger* culture were added to purified nutrient soln. and one drop of the suspension placed on a microscope slide. After incubation for 3 days at 30°, mycelium development could be seen under a microscope. The presence or otherwise of Mo could be detected by the spore formation and by the amt. of mycelium. In deficient cultures, hyphae cells suffered marked malformation, the cells remaining short and showing irregular thickening. M. Long.

Growth of higher plants in a mineral nutrient solution determined as optimal for *Aspergillus niger*. M. FOROUGHI and W. BUSSLER (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 19–22. Ger., 9 ref.).—Behaviour of oats, tomato and sunflower was studied in nutrient soln. detd. as optimal for *Aspergillus niger* at 4 concn. ranging from 2 to 5 mg equiv./40 ml. At the highest concn. the plants died due to salt damage, whilst at the lower ones, except for oats, the plants suffered from B deficiency. Fe and Mn concn. were too low, and Cu and Zn concn. too high for successful growth. M. Long.

Investigations on the use of the method of systematic variations for determining nutrient requirements of maize (*Zea mays*). L. A. WATSON and C. C. WEIR (*Trop. Agric. Trin.*, 1970, **47** (2), 93–101, Engl., 8 ref.).—From sand culture expt., approx. optimum ratios of 3:1 for N:P and 1:1 for K:Mg were found to be compatible with highest dry wt. yield. Generally, tissue contents were found to reflect nutrient treatments. E. G. Brickell.

Germination, Growth Regulation, Senescence

Purine metabolism in germinating wheat embryos. C. E. PRICE and A. W. MURRAY (*Biochem. J.*, 1969, **115** (2), 129–133. 30 ref.).—The acid-sol. fraction and the nucleic acid fraction of the wheat embryo are extensively labelled after incubation for 6 h with [8-^{14}C] adenine. Subsequently, in absence of labelled adenine, there is a continuous increase in ^{14}C in the acid insol. fraction of root and leaf tissue relative to that in the coleorhiza and coleoptile. During incubation there is 26-fold increase in the 3′-nucleotidase activity between 4 and 24 h. Thus the purines released during the degradation of RNA by this enzyme in the coleorhiza and coleoptile regions of the wheat embryo during the first 2 days of germination are utilised by the embryo, probably by the neighbouring root and leaf tissue. J. N. Ashley.

Effects of kinetin, salt concentration, and temperature on germination and early seedling growth of *Lactuca sativa* (lettuce). O. A. ODEGBARO and O. E. SMITH (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 167–170. 24 ref.).—Increasing salinity (0–0·12 M-NaCl) and temp. (20–30°C) decreased germination and early seedling growth of lettuce. The deleterious effects of increasing salinity were least at the lowest temp. Soaking seed in 10 ppm kinetin for 3 min largely overcame the effects of high salinity and temp. in decreasing germination. Kinetin treatment increased seedling growth only at high temp. and salinity. A. H. Cornfield.

Hormonal regulation of cereal grain weight. G. MICHAEL, P. ALLINGER and E. WILBERG (*Z. PflErnähr. Bodenk.*, 1970, **125** (1), 24–35. Ger., 33 ref.).—It suggested that cytokinins play an active part in the ripening of grains by intensifying the accumulation processes in growing kernels, prolonging the filling period and increasing grain wt. Ears of pot grown barley were sprayed with kinetin soln. contg. 20 mg kinetin/l. Awns were removed from others. Spraying with kinetin increased grain wt. slightly. The removal of awns had a slight adverse effect except at high r.h. M. Long.

Effects of nonionic surfactants on monocotyledons. R. M. ENDO, J. LETEY, N. N. VALORAS and J. F. OSBORN (*Agron. J.*, 1969, **61** (6), 850–854. 13 ref.).—Two nonionic surfactants (polyoxyethylene ester-ether and polyoxyethylene ethanol) applied in soln. at 330–4000 ppm usually reduced germination and shoot and root growth of barley and grasses, particularly at the higher rates of application. Toxicity was higher in soln. culture than in solid media. A. H. Cornfield.

Plant growth-regulating substances. XXX. The plant growth-regulating activity of substituted phenols. D. B. HARPER and R. L. WAIN (*Ann. appl. Biol.*, 1969, **64** (3), 395–407. 41 ref.).—High auxin activity in 58 substituted monophenols and related compd. occurred where electron-attracting substituents having certain steric properties were substituted in the 2- and 6-positions. It was also necessary for at least one of the *o*-substituents to be able to bond intramol. with the H of the phenolic OH group. Activity was reduced by substitution in the *m*- and completely lost by substitution in the *p*-position. A. H. Cornfield.

Use of centrifugation to obtain auxin extracts from cuttings treated with terminal applications of indoleacetic acid (IAA). J. J. MCGUIRE, L. S. ALBERT and V. G. SHUTAK (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 41–43. 5 ref.).—A refrigerated centrifuge procedure is described for extracting sap from segments of *Ilex crenata* cuttings which had been treated terminally or basally with 2-^{14}C-labelled

IAA. Movement and changes in levels of IAA and its metabolised forms could be studied. At least 35% of the label was in the form of IAA 48 h after treatment. A. H. Cornfield.

Uptake of indoleacetic acid by cuttings of *Ilex crenata*. J. J. McGuire, L. S. Albert and V. G. Shutak (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 44–45. 3 ref.).—Tests with 2-^{14}C-labelled IAA showed that it was absorbed through intact tissue, leaf scars, wounds or cut apical or basal ends of cuttings. A. H. Cornfield.

Effect of Cycocel and abscisic acid on bud growth of Redblush grapefruit. R. Young and W. C. Cooper (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 8–11. 14 ref.).—Bud growth of normal and defoliated grapefruit seedlings was delayed by spraying with 100–1000 ppm abscisic acid or 500–3000 ppm Cycocel. The effect increased with concn. and no. of applications and was greater at low than at high temp. Abscisic acid was the more toxic, and both compd. were more toxic to defoliated than to normal plants. A. H. Cornfield.

Abscisic acid, a component of the beta-inhibitor complex in the *Prunus* endocarp. K. Ryugo (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 5–8. 7 ref.).—Aq. and ethanolic extracts of the *Prunus* endocarp delayed germination and subsequent growth of peach, almond, cucumber and plantago embryos. Abscisic acid was tentatively identified in the inhibitor complex and most of the inhibitors were fixed on anion exchange resin. The extractives markedly decreased O_2 uptake by germinating pea seedlings and inhibited growth of cucumbers, the latter effect being reversed by gibberellic acid. A. H. Cornfield.

Determination of the growth regulators CCC (chlorocholine chloride) and CMH (*N*-dimethyl-*N*-(β-chloroethyl)-hydrazonium chloride) in biological materials. J. Jung and G. Henjes (*Z. PflErnähr. Bodenk.*, 1969, **124** (2), 97–107. Ger., 2 ref.).—The method depends on the extraction of CCC and CMH with MeOH. The compd. are then isolated by chromatog. on an Al_2O_3 column, followed by passage through a cation exchange column (Dowex WX8, 200–400 mesh). The two compd. are then detd. photometrically as their dipicrylamine complexes. The lower level of detection for CCC is 0·1–0·3 and for CMH is 0·2–0·5 ppm. Added recovery of the two compd. in a wide range of biol. materials was found to be between 90–100%. M. Long.

Fruit and vegetative responses of the highbush blueberry to gibberellic acid under greenhouse conditions. C. M. Mainland and P. Eck (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 19–20. 9 ref.).—Application of 5–500 ppm gibberellic acid (GA) to flowers and foliage or foliage only over 3 yr, increased % fruit set only where 50–500 ppm was applied, but decreased the no. of flower buds formed for the next crop. The treatments had no effect on shoot no. or length or on stem dia. (Cf. *Idem., ibid.*, 1969, **94** (1), 21). A. H. Cornfield.

Comparative study of the ability of methionine or linolenic acid to act as precursors of ethylene in plant tissues. L. W. Mapson, J. F. March, M. J. C. Rhodes and L. S. C. Wooltorton (*Biochem. J.*, 1970, **117** (3), 473–479. 14 ref.).—Discs of apple peel and of tissue from tomato walls, and cauliflower florets rapidly incorporate ^{14}C from L-[1,2,3,4-^{14}C] methionine and from the corresponding oxo-acid, 4-methylmercapto-2-oxobutyric acid into C_2H_4. There is no incorporation of ^{14}C from uniformly labelled linolenate. J. N. Ashley.

Other Aspects

Comparison of sampling methods for determination of seasonal changes in the nutrient content of apple leaves. D. C. Zeiger and T. R. Konsler (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 73–77. 14 ref.).—Four methods of taking leaf samples in different sequence from the same shoot for analysis were compared with a standard method. A. H. Cornfield.

Effects of root sectioning and chemicals on sweetpotato (*Ipomoea batatas*) plant production. B. T. Whatley (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 179–180. 8 ref.).—Treatment of sectioned roots of sweetpotato with mixtures of dimethylsulphoxide, captan, and Semesan Bel resulted in the production of more plants than did the use of any chemical alone. Whole roots produced more plants with higher av. wt. than did individual root sections. A. H. Cornfield.

Electrophoretic separation of proteins from roots and root exudates. P.-S. Juo and G. Stotzky (*Can. J. Bot.*, 1970, **48** (4), 713–718. 12 ref.).—Exudates from intact roots show positive reaction with the Folin phenol reagent. These were subjected to acrylamide gel electrophoresis and changes during growth were followed. Proteins were present, but the concn. fluctuated at different growth stages; the type of protein varied according to species. Protein exudation did not occur by injury to roots. M. T. Rawnsley.

Effect of berseem (*Trifolium alexandrinum*) on the growth of maize crop on artificially compacted soil. R. P. R. Sharma and A. Singh (*Pl. Soil*, 1969, **31** (1), 11–21. 7 ref.).—In pot expt., compaction of a sandy loam to a bulk *d* of 1·6–1·8 g per cm^3 decreased growth of maize tops and roots, but growth of a previous crop of berseem largely eliminated the effects of compaction. A. H. Cornfield.

Seasonal fluctuations of amino acids, organic acids, and simple sugars in 'Elberta' peach and 'Chinese' apricot flower buds during and after test. H. I. El-Mansy and D. R. Walker (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 184–192. 46 ref.).—Changes in the content of 15 amino acids, 5 org. acids and 3 sugars over the period Sept.–Mar. are reported. A. H. Cornfield.

Survey of maize strains for lysine content. A. V. Paez, J. P. Ussary, J. L. Helm and M. S. Zuber (*Agron. J.*, 1969, **61** (6), 886–889. 12 ref.).—The lysine %, on whole kernel basis, of 429 inbred lines ranged from 0·16 to 0·45 and of 169 plant introduction strains from 0·22–0·41. For a selected group of high-lysine lines there was a significant correlation between lysine % and protein %. Lysine % in 6 inbred lines varied little due to location and year growth. A. H. Cornfield.

Pharmacological studies on varieties of tobacco: *Nicotiana sylvestris, N. tabacum* × *N. sylvestris* F_1, *N. tabacum* × *N. sylvestris* amphidiploid and tobacco from cigarettes. T. Shipochliev and A. Manolov (*Qualitas Pl. Mater. veg.*, 1969, **18** (4), 360–366. Engl., 3 ref.).—It was found that crossing of *N. tabacum* with *N. sylvestris* resulted in nornicotine tobaccos with less expressed hypertensive effects and lower toxicity. The cross *N. tabacum* × *N. sylvestris* was less toxic than the *N. tabacum* × *N. sylvestris* amphidiploid hybrid. W. J. G.

***N*-substituted succinamides.** Uniroyal Inc. (Br. Pat. 1,187,533, 13.5.68. U.S., 25.5.67).—The compd. are substituted on one N by NR^1R^2 and on the other by R^3 and R^4. R^1 and R^2 are alkyl of 1–12 C or NR^1R^2 is, e.g., pyrrolidyl, piperidyl or morpholin-4-yl and R^3 and R^4 are as R^1 and R^2 or one of them is H and the other alkyl, hydroxyalkyl, amino, aryl, etc., of up to 12 C. They are prep. by reacting 1-NR^1R^2-succinimide with an amine or hydrazine R^3R^4NH. An example is *N*-dimethylamino-*N'*-(2-chlorophenyl)-succinamide. The compd. are plant growth regulators. For example, 1 gal/tree of a spray contg. 2000 ppm of the compd. when used on apple trees at full bloom time produced larger apples. Vegetative growth is retarded. R. J. M.

Crops and Cropping

Field Crops

Effect of nitrogen, phosphorus, and potassium on per cent and yield of oil in maize. L. F. Welch (*Agron. J.*, 1969, **61** (6), 890–891. 6 ref.).—Maize grain oil % was increased 8, 3 and 2% by application of N, P and K resp. Oil yield per acre was increased 43, 54 and 11% resp.; this was due more to increased grain yield than to increased oil % in the grain. A. H. Cornfield.

Yield of maize on Frederick silt loam as affected by slope and fertility treatments. J. A. Lutz, jun., G. W. Hawkins, P. H. Hoepner, D. C. Martens and H. C. Porter (*Agron. J.*, 1969, **61** (6), 945–947. 4 ref.).—Higher rates of application of both N and K were required for max. grain and forage yields of maize on a silt loam with slope of 7·5–15% than with slope of 2·5–7·5%. Grain and forage yields on both slopes were similar the first year, but were higher on the 2·5–7·5% slope in the next 2 yr. A. H. Cornfield.

Dry-matter accumulation in maize plants: comparisons among single-cross hybrids. J. J. Hanway and W. A. Russell (*Agron. J.*, 1969, **61** (6), 947–951. 10 ref.).—Developmental characteristics, dry matter yields and seed characteristics of 11 maize hybrids grown in different years and at different plant populations are reported. A. H. Cornfield.

Response of maize to Zn-EDTA and zinc sulphate in field investigations. M. G. Schnappinger, jun., D. C. Martens and G. W. Hawkins (*Agron. J.*, 1969, **61** (6), 834–836. 25 ref.).—Maize yields on a silt loam (pH 7·2) were increased to a greater extent by

$ZnSO_4$ (14 kg Zn per ha) than by Zn-EDTA (4·48 kg Zn per ha). On a loamy fine sand (pH 6·3) maize yields were increased slightly by $ZnSO_4$ (14 kg Zn per ha). Highest grain yield in both soils occurred with 14 kg $ZnSO_4$ per ha, broadcast.
A. H. Cornfield.

Combined analysis of yield data from fertiliser experiments. R. J. LAIRD and F. B. CADY (*Agron. J.*, 1969, **61** (6), 829–834. 8 ref.).—Maize yield data in relation to level of applied N at 76 locations were combined to give a general equation as a function of other production variables. Particular attention is paid to error terms employed in testing the significance of regression coeff. and procedures for obtaining prediction equations. A. H. Cornfield.

Effect of urea, treated with nitrification inhibitors, on nitrogen uptake and yield of rice in relation to placement and timing of the application. B. A. LAKHDIVE and RAJENDRA PRASAD (*Z. PflErnähr. Bodenk.*, 1969, **124** (1), 23–29. Ger., 15 ref.).—2-Chloro-6-(trichloromethyl)pyridine- or 2-amino-4-chloro-6-methylpyrimidine (**I**)-treated urea increased N uptake and grain and straw yields regardless of method of application. Single applications of urea treated with **I** led to higher uptake of N than did split applications of untreated urea. Placement of urea and pelleted urea led to increased rice yield but split applications had no effect, With 3 split applications, differences in placement and form of urea disappeared. M. Long.

Experiments with CCC (2-chloroethyltrimethylammonium chloride) on wheat: effects of spacing, nitrogen and irrigation. E. C. HUMPHRIES and W. BOND (*Ann. appl. Biol.*, 1969, **64** (3), 375–384. 10 ref.).—Application of CCC (2·8 kg per ha) to reduce lodging increased yields of spring and winter wheat at 2 locations over 4 yr with close spacing (10 cm) more than with normal spacing (20 cm). Yields were not increased with higher than usual rates of N when CCC was applied. Irrigation increased yields to a greater extent than did CCC, but the combined treatments were little more effective than irrigation alone. Data on yield components and leaf area are also presented. A. H. Cornfield.

Spikelet number, its control and relation to yield per ear in wheat. H. M. RAWSON (*Aust. J. biol. Sci.*, 1970, **23** (1), 1–15. 16 ref.).—12 wheat varieties, ranging from early to late maturing, were grown under a 21° day/16°-night or 15°/10° regime. Three plants of each wheat were harvested every few days and particular attention was paid to the development of the stem apex. The effect of day length on spikelet no. (*S*) and the response to several weeks of vernalisation were studied. For each of the 12 varieties, *S* was related to grain no., but individual grain wt. differed between varieties. When *S* was varied by day length or vernalisation treatment, grain yield per ear was dependent on *S* with each variety. M. J. Rawlins.

Effect of ammonium sulphate, treated with a nitrification inhibitor, and calcium nitrate, on growth and N-uptake of spring wheat, ryegrass and kale. E. D. SPRATT and J. K. R. GASSER (*J. agric. Sci., Camb.*, 1970, **74** (1), 111–117. 14 ref.).—Wheat (*W*), ryegrass (*R*) and kale (*K*) were given $(NH_4)_2SO_4$ [treated with nitrification inhibitor, 2-chloro-6-(trichloromethyl)-pyridine] or $Ca(NO_3)_2$ each, supplying 50 and 100 lb of N/acre. The plants were sampled at various stages and dry wt., %N and N uptake were detd. Initially, *W* and *R* grew better and took up more N with $(NH_4)_2SO_4$ than with $Ca(NO_3)_2$ but final yields were similar. *K* grew better with $Ca(NO_3)_2$. Fertilised crops contained max. N 109 days after sowing. 50 lb of N/acre led to the highest *W* grain/lb N. *W* and *R* grew fastest and took up most N from $(NH_4)_2SO_4$ during the fastest growing period, whilst *K* fared best on $Ca(NO_3)_2$.
M. Long.

Undersowing wheat with annual legumes: effects on wheat yields and legume seed yields in the south-eastern wheat belt of Western Australia. M. L. POOLE and J. W. GARTRELL (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 84–88. 4 ref.).—Varieties of *Trifolium* and *Medicago* were undersown with wheat at 1–6 or 3–18 lb/acre. In all cases, yields of both wheat and legume seed were reduced, sometimes severely. It is suggested that if undersowing is necessary, higher rates of legume sowing would be better.
M. T. Rawnsley.

Autumn-saved Coastal Bermudagrass, *Cynodon dactylon*: effects of age and fertilisation on quality. R. H. HART, W. G. MONSON and R. S. LOWREY (*Agron. J.*, 1969, **61** (6), 940–941. 8 ref.).—Dry matter digestibility (*DMD*) and protein % were highest in the November cut, but decreased with delay in cutting to February. Rate of N fertilisation after the last summer cut did not affect *DMD* or protein % in later cuts. A. H. Cornfield.

Effect of nitrogen, phosphorus, and potassium on yield and chemical composition of bluejoint (*Calamagrostis canadensis*) forage. W. M. LAUGHLIN (*Agron. J.*, 1969, **61** (6), 961–964. 18 ref.).—Native bluejoint grass responded well to application of N and K only when adequate P was applied to a silt loam (pH 4·2–4·9). The effect of varying rates of application of N, P and K in various combination on tissue contents of N, P, K, Ca, Mg, Al, Ba, Sr, Cu and Zn are reported. A. H. Cornfield.

Seasonal tiller populations of grass and grass/clover swards with and without irrigation. E. A. GARWOOD (*J. Br. Grassld Soc.*, 1969, **24** (4), 333–344. 19 ref.).—The no. of tillers per unit area of pure swards of S23 and S24 perennial ryegrass, S37 cocksfoot, S48 timothy and an S23 perennial ryegrass/white clover sward in relation to season over 2 yr and effects of irrigation were studied. The relationship between seasonal fluctuations in tiller no. and root growth of swards is discussed. A. H. Cornfield.

Comparisons of liquid and solid fertilisers and anhydrous ammonia for sugar-beet. A. P. DRAYCOTT and R. HOLLIDAY (*J. agric. Sci., Camb.*, 1970, **74** (1), 139–145. 2 ref.).—Solid (*SF*) and liquid fertilisers (*LF*) were compared in a set of field trials and the interaction between depth of placement and soil moisture was examined in glasshouse trials. Sugar yields were the same for both *SF* and *LF*, although N uptake by the crop was less from *SF*. Placement at 6 in gave higher yields than at 2 in with the *LF* but these were not significantly higher than those with broadcast *SF*. Pot trials showed that deep placement led to higher *DM* yields when the surface soil was watered infrequently. Injection of anhydr. NH_3 in the seed bed gave the same sugar yield and N uptake as did *SF* and was superior to injection during early spring. M. Long.

Fertiliser requirements of sugar-beet on fen peat soils. P. B. TINKER (*J. agric. Sci., Camb.*, 1970, **74** (1), 73–77. 11 ref.).—Factorial fertiliser trials were carried out with beet on deep peat soils with various combinations of N, P, K and Na. Responses were small and rarely economic except towards Na. N led to increased yields on some sites but depressed sugar %. Previous heavy P and K dressings are thought to account for the lack of response to nutrients. M. Long.

Horticultural Crops

Chemical thinning of apple trees using concentrated sprays. B. L. ROGERS and A. H. THOMPSON (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 23–25. 9 ref.).—Conc. sprays (3–33 lb per 100 gal) of Sevin (= carbaryl) were usually as effective as normal spray for thinning apples, but sprays of naphth-1-yl acetic acid at 3–6 times their normal concn. were not as effective as were normal sprays.
A. H. Cornfield.

Effect of cytokinins and gibberellins on shape of Delicious apple fruits. M. W. WILLIAMS and E. A. STAHLY (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 17–19. 13 ref.).—Application of cytokinins and gibberellins, alone or in combination, to apples just after full bloom increased the length/dia. ratio of the fruit. Cytokinins were more effective. The appearance of the fruit due to treatments was similar to that of untreated trees grown with cool early-season temp.
A. H. Cornfield.

Effect of cultivation and nitrogen levels on storage quality, yield and colour grade of 'Starking Red Delicious' apple grown under grass sod. J. L. MASON (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 78–80. 4 ref.).—The effects over 6 yr of 0·165 to 3·0 lb N (NH_4NO_3) per tree annually, and cultivation (shallow rototilling) once or twice per year, compared with no cultivation, on yields, skin and flesh colour, firmness, sol. solids, titratable acidity, leaf N%, trunk growth and incidence of storage rot and scald were studied. The grass sod, with or without moderate cultivation, largely overcame the effects of the wide range of N levels applied. Yields and quality were lower only with the very low N levels without cultivation.
A. H. Cornfield.

Comparison of maturation and composition of Valencia oranges in some major subtropical zones of the United States. W. REUTHER, G. K. RASMUSSEN, R. H. HILGEMAN *et al.* (*J. Am. Soc. hort. Sci.*, 1969, **94** (2), 144–157. 40 ref.).—Maturation indices and phys. and chem. characteristics of Valencia oranges in 6 climatic zones of the subtropical region of the U.S. are reported over 2 seasons.
A. H. Cornfield.

Influence of pre-harvest applications of malathion and indoleactic acid (IAA) on anthocyanin development in *Vaccinium macrocarpon* var. 'Early Black' (cranberry). R. M. DEVLIN, B. M. ZUCKERMAN and I. E. DEMORANVILLE (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 52–55. 19 ref.).—Application of malathion (100 gal per acre) to cranberry bushes 2 weeks before harvest increased anthocyanin % in the fruit

at harvest. 30–50 ppm IAA either had no effect on or slightly decreased anthocyanin %. Neither treatment affected colour development during storage. A. H. Cornfield.

Vegetative and reproductive responses of highbush blueberry to succinic acid mono (2,2-dimethyl hydrazide) (Alar). J. C. HAPITAN, JUN., V. G. SHUTAK and J. T. KITCHIN (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 26–28. 7 ref.).—Spray application of 5000 ppm Alar on July 22 and/or August 22 to highbush blueberry retarded new shoot elongation but increased the no. of flower buds per unit length of new growth. The treatments delayed blossom opening and berry ripening, but did not affect fruit size. July and July + August treatments increased the no. of berries per 5 in of new shoot growth. A. H. Cornfield.

Fruiting response of the highbush blueberry to gibberellic acid under field conditions. C. M. MAINLAND and P. ECK (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 21–23. 7 ref.).—The % fruit set of highbush blueberry was increased by 50–500 ppm gibberellic acid (GA), applied at bloom, only in the first of 2 yr. Berry yields were increased by 100 and 500 ppm GA only in the second year and highest yields were obtained with 500 ppm GA treatment of caged plants (to exclude pollinating insects). The treatments produced smaller berries that required a longer maturation period. The smallest and latest maturing fruit was seedless and was produced by caged plants receiving GA. GA treatment had no effect on flower bud formation in either year. (Cf. *Idem., ibid.*, 1969, **94** (1), 19). A. H. Cornfield.

Limestone gravel as a growth medium in hydroponics. M. SCHWARZ and Y. VAADIA (*Pl. Soil*, 1969, **31** (1), 122–128. 7 ref.).—Growth of vegetable crops on a limestone gravel medium was satisfactory providing extra PO_4^{3-} (0·1 mM) and Fe^{2+} (1 ppm) were supplied in the standard nutrient soln. Yields were comparable with those obtained using a basalt gravel medium. A. H. Cornfield.

A comparison of application methods and stages of plant development for applying Duraset on the tomato. A. BINCHY and J. V. MORGAN (*Scient. Proc. R. Dubl. Soc. B*, 1970, **2** (21), 205–215. 9 ref.).—Single-truss tomato plants, sown in March or June, were used; Duraset was applied either at cotyledon expansion, or at ½ in length of 1st, 2nd, 3rd, 4th, or 5th true leaves. Spray and tip application methods were used. Results confirm that the plant development stage is crucial, and the variety of plant must be known. Other previous recommendations for Duraset application are contradicted. Duraset does not seem to be suitable for general use to increase yield. M. T. Rawnsley.

Peat–sand substrates for plants grown in containers. I. Effect of base fertilisers. A. C. BUNT (*Pl. Soil*, 1969, **31** (1), 97–110. 13 ref.).—The performance of tomato and antirrhinum in peat–sand and loam–based composts was studied in relation to levels of applied N (hoof and horn grist + NH_4NO_3), superphosphate, K_2SO_4 and $CaCO_3$. A. H. Cornfield.

Influence of growth retardants on growth, nutrient content, and yield of tomato plants grown at various fertility levels. D. E. KNAVEL (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 32–35. 12 ref.).—Treatment of tomato plants at the 3-leaf stage with Cycocel or Alar reduced subsequent growth, Cycocel having the greater effect. Both treatments increased drought resistance, depth of green colour and stem and leaf N and P levels. Alar increased early fruit yields in one of three tests but decreased fruit size. Neither treatment affected total yields. A. H. Cornfield.

Production of seed beans for export. K. B. HANSSEN (*Rhodesia agric. J.*, 1970, **67** (2), 45–50. 13 ref.).—Preparative conditions, such as suitable areas for production, the varieties of bean most suited to the project, land prepn., irrigation and fertilisation were studied. Time of planting was considered in conjunction with plant population and sowing rates. Effects of pest and disease control on yield were considered together with the economics of production. M. J. Rawlins.

Fertiliser nitrogen and growth regulators for field beans (*Vicia faba* L.). I. Effects of seed bed applications of large dressings of fertiliser nitrogen and the residual effects on following winter wheat. II. The effects of large dressings of fertiliser nitrogen, single and split applications, and growth regulators. J. MCEWEN (*J. agric. Sci., Camb.*, 1970, **74** (1), 61–66. 3 ref.; 67–72. 4 ref.).—I. N was applied at 0, 1, 2 and 3 cwt N/acre as NH_4NO_3, $NaNO_3$ or $(NH_4)_2SO_4$. Basal P_2O_5 and K were applied as a compd. fertiliser (0:14:28) at 360 lb/acre. In a second trial 'Nitrochalk' was applied either broadcast or as compd. fertiliser (25:10:10) applied sideband with extra Nitrochalk to give 2 and 3 cwt/acre. Large applications of N lessened nodulation by as much as 50%. Highest yield increases were 1·9 cwt grain/acre in the first trial and 3·1 cwt grain/acre in the second.

II. Split plot applications increased grain yields from 0·2 to 4 cwt/acre. 1·5 cwt/acre applied in May increased grain yield by 5·5 cwt/acre. CCC at 5 lb/acre had no visible effect on plant growth and decreased yield by 1·9 cwt/acre. *N*-dimethylamino-succinic acid (B9) greatly affected growth and had inconsistent effects on yields; stems and pods/acre were increased in no. whilst 1000 grain-wt. decreased. Seasonal effects were large and greatly exceeded treatment effects. M. Long.

Plantation Crops

Effect of trace elements and magnesium upon growth, development, and yields of plantain (*Musa paradisiaca*). E. H. MEDINA and M. A. LUGO LOPEZ (*J. Agric. Univ. P. Rico*, 1969, **53** (1), 33–40. 10 ref.).—Treatment of a clay soil (pH 6) with Mg + Fe + Mn + B + Zn + Cu + Mo increased plantain growth and fruit no. and yield. When foliar sprays were applied, only Fe and Zn were effective in increasing fruit no. and yield. A. H. Cornfield.

Effect of fruit thinning on size, quality and ripening of 'Sakkoti' dates grown at Asswan. FATHI HUSSEIN (*Trop. Agric. Trin.*, 1970, **47** (2), 163–166. Engl., 11 ref.).—Moderate thinning (by removal of 30% of the strands from the centre, and cutting back the tips of the remainder to remove 30% of their fruits, making a total reduction of about 51%) is recommended for this variety. E. G. Brickell.

Fruit growth and composition of two dry date cultivars grown at Asswan. FATHI HUSSEIN (*Trop. Agric. Trin.*, 1970, **47** (2), 157–162. Engl., 10 ref.).—'Sakkoti' and 'Bartamuda' were studied. Changes in size and fresh wt. of fruit followed an S-shaped growth curve. The fruit reached its final (*Tamar*) stage of development in the second half of Sept. (28 weeks after pollination) when the moisture, size and wt. of fruit decreased and the % total sol. solids increased to a max. Total sugars increased as the fruit ripened. Sucrose was dominant at the *Tamar* stage and increased as the fruit matured. E. G. Brickell.

Leaf rust and leaf fall in Arabica coffee. C. C. CHINNAPPA and M. S. SREENIVAN (*Indian Coff.*, 1969, **33** (12), 374–379. Engl., 3 ref.).—Rust incidence and defoliation was recorded every 15 days for ~ 1 yr starting from May 1965 when there was practically no rust incidence in the field. The varieties used, Bourbon and Kents, were also treated regularly with Bordeaux spray. In spite of the spray treatment, defoliation and leaf rust still occurred. W. J. G.

Forest Crops

Leaf analysis of *Pinus silvestris* affected by lime induced chlorosis. W. ZECH (*Z. PflErnähr. Bodenk.*, 1970, **125** (1), 1–16. Ger., 12 ref.).—N, P, K, Ca, Mg, Mn, Fe, Cu, B and active Fe were detd. in pine needles. Chlorotic needles contained more N, P, K and Mn than green needles. At early stages of growth, chlorotic needles contained < 20 ppm of Fe, but as the season progressed the needles turned green and total Fe rose to 50 ppm. Chlorotic needles contained approx. half as much active Fe as did the green needles. The P/total Fe ratio in chlorotic needles was much higher than in green needles. M. Long.

Foliar and soil nutrients in relation to sugar maple (*Acer saccharum*) decline. D. L. MADER and B. W. THOMPSON (*Proc. Soil Sci. Soc. Am.*, 1969, **33** (5), 794–800. 19 ref.).—Trees exhibiting crown decline and poor growth had low foliar N levels and low or high levels of total soil N. Foliar Ca and Mg were closely correlated with soil levels of these elements. Application of N (224 kg per ha) increased foliar N and improved leaf appearance in the season following application. A. H. Cornfield.

Animal Husbandry

Feedstuffs

Influence of cutting frequency on the yield, composition and persistence of irrigated lucerne. P. JUDD and J. C. RADCLIFFE (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 48–52. 17 ref.).—Lucerne (*Medicago sativa*) was harvested during 3 summers at 3-, 4-, 5- and 6-week frequencies. In the 2nd summer, analyses for crude protein, digestibility and crude fibre were made. Results suggest that, when lucerne is intended for protein supplement, 5-week cutting is best, but for direct feeding, the 6-week cutting

gives higher yields. Standards of digestibility etc., if enforced, could affect this. The 3-week cutting frequency resulted in low density of plants, reduced protein, and weed invasion.
M. T. Rawnsley.

The cellulose–lignin complex in forages and its relationship to forage nutritive value. D. W. ALLINSON and D. F. OSBOURN (*J. agric. Sci., Camb.*, 1970, **74** (1), 23–36. 31 ref.).—Relationships between the cellulose–lignin complex, voluntary consumption and *DM* digestibility were examined using two Italian ryegrass varieties, lucerne and sainfoin. The cellulose, acid detergent lignin and acid detergent fibre contents and their resp. digestibilities were detd; lignins were extracted from the fibre fractions and the u.v. difference spectra detd. M. Long.

Digestibility and productivity of selected herbage varieties. R. D. HARKESS and R. H. ALEXANDER (*J. Br. Grassld Soc.*, 1969, **24** (4), 282–289. 30 ref.).—Primary growth *in vitro* digestibility and the effects of various cutting managements on digestibility and productivity were measured for S22 and Tetila Italian ryegrass, S37 cocksfoot, S170 tall fescue, S215 meadow fescue, Scots timothy, English broad red clover and S100 white clover grown in the west of Scotland. A. H. Cornfield.

Evaluation of dewatering and wilting as moisture reduction methods for hay-crop silage. J. C. DERBYSHIRE, C. H. GORDON, R. D. HOLDREN and J. R. MENEAR (*Agron. J.*, 1969, **61** (6), 928–931. 18 ref.).—Total dry matter losses after ensiling were greater for windrow-wilted lucerne than for direct-cut or dewatered forage. Silage temp. was similar for the 3 harvesting systems, but silage dry matter bulk *d* was greatest for the wilted method. Milking cow performance was similar on the three silages, in spite of small differences in energy consumption. A. H. Cornfield.

General pattern of silage fermentation in two subtropical grasses. V. R. CATCHPOOLE and W. T. WILLIAMS (*J. Br. Grassld Soc.*, 1969, **24** (4), 317–324. 12 ref.).—Results for previous expt. on the nature of the fermentation in *Setaria sphacelata* and *Chloris gayana* were re-analysed by numerical methods intended for the elucidation of pattern of sequential data. Suggestions for future experimental work, based on the pattern of the data obtained, are put forward.
A. H. Cornfield.

Sodium chloride in cattle nutrition. J. M. VAN LEEUWEN (*Versl. landbouwk. Onderz. Ned.*, 1970, (737), 218 pp. Dut., 246 ref.).—Physiol. aspects of the supplementation with NaCl, of rations with low and normal NaCl contents, are studied extensively.
J. C. T. Nieuwenhuis.

Feed intake by grazing ruminants. II. Grazing experiments with dairy cattle in which different exogeneous and endogeneous markers were compared. N. D. DIJKSTRA and A. KEMMINK (*Versl. landbouwk. Onderz. Ned.*, 1970, (738), 23 pp. 4 ref.).—The best results were obtained by use of polyethylene as the added exogeneous faecal marker and the digestible crude-protein content of the grass as the endogeneous marker. J. C. T. Nieuwenhuis.

Use of chromic oxide in the measurement of individual feed intake in cattle fed on silage and barley. J. M. WILKINSON and J. H. D. PRESCOTT (*Anim. Prod.*, 1970, **12** (1), 71–80. 22 ref.).—Cr_2O_3, as a component of shredded paper, was fed twice daily to Friesian steers on a silage/barley diet. Absolute recovery of Cr_2O_3 was 85–91%, whilst relative recovery was 97% in grab samples. The lowest concn. of Cr_2O_3 in the faeces during a 24 h period was found during the night. Day-to-day variation in faeces-Cr_2O_3 concn. was small and random. M. Long.

Comparison of pasture conserved as hay or as silage for feeding sheep. A. H. BISHOP and T. D. KENTISH (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 13–18. 14 ref.).—Over 3 yr of feeding fodder from equal areas of grass to adult sheep, the amt. of nutrients recovered, the length of time the crops lasted, and the wastage occurring, showed that hay is the better feed. M. T. Rawnsley.

Use of sheep fitted with oesophageal fistulae in grazing studies. J. HODGSON (*J. Br. Grassld Soc.*, 1969, **24** (4), 325–332. 21 ref.).—Studies are reported on the effects of diurnal variation, fasting before sampling and acclimatisation to a sward on the chem. compn. and *in vitro* org. matter digestibility of samples of extrusa collected from sheep fitted with oesophageal fistulae.
A. H. Cornfield.

Digestibility and nutritive value of byproducts from wheat flour production. N. D. DIJKSTRA (*Versl. landbouwk. Onderz. Ned.*, 1970, (730), 14 pp. Dut.).—Digestibility and nutritive value of some wheat flour byproducts with 1·34 to 13·45% crude fibre were estimated in trials with wethers and pigs. A sliding scale based on crude fibre content is proposed. J. C. T. Nieuwenhuis.

Protein requirement for maintenance of adult rams. MANOHAR SINGH and V. MAHADEVAN (*Anim. Prod.*, 1970, **12** (1), 185–189. 11 ref.).—Adult rams were fed diets contg. 4, 8 or 16% crude protein (*CP*), and which were nearly isocaloric. Daily digestible *CP* requirements for maintenance ($0{\cdot}875 \pm 0{\cdot}06$ g/kg $W^{0{\cdot}734}$ by the factorial method, and $0{\cdot}893 \pm 0{\cdot}03$ g/kg $W^{0{\cdot}734}$ from N balance and net utilisation of protein were similar but slightly higher than those estimated from N balance alone. All values are lower than conventional recommendations. M. Long.

Growth-promoting properties of crude soyabean phospholipids. P. VOHRA and J. R. HEIL (*Poult. Sci.*, 1969, **48** (5), 1661–1667. 8 ref.).—These phospholipids were growth-promoting for turkey poults when fed in a casein–gelatine diet but not when fed in a soyabean protein diet. Studies with and without addn. of EDTA to the diet showed that growth promotion was not due to any chelating properties of the material. A. H. Cornfield.

Effect of hydration, gelatinisation and ball milling of starch on growth and energy utilisation by the chick. E. C. NABER and S. P. TOUCHBURN (*Poult. Sci.*, 1969, **48** (5), 1583–1589. 7 ref.).—Water treatment of potato starch at 58°c significantly improved chick performance and starch utilisation; treatment at 68–72°, to induce complete gelatinisation, was even more effective. Performance on maize starch was improved only by water treatment at 78°. Ball milling of potato starch increased starch utilisation, but chick growth was poor due to low feed consumption caused by hydration of starch in mouth and beak. A. H. Cornfield.

Value of opaque-2 corn proteins for chicks. R. JARQUÍN, C. ALBERTAZZI and R. BRESSANI (*J. agric. Fd Chem.*, 1970, **18** (2), 268–272. 16 ref.).—Opaque-2 corn was compared with common corn for nutritional value of the protein. The corn was tested with other protein sources or as the sole source of dietary protein. Owing to high lysine content, opaque-2 corn was superior in protein quality only when fed as the sole source of protein, or when tested with lysine deficient protein sources. M. J. Rawlins.

Evaluation of distillers dried grains with solubles [*DDGS*] in diets of laying hens. R. H. HARMS, R. S. MORENO and B. L. DAMRON (*Poult. Sci.*, 1969, **48** (5), 1652–1655. 7 ref.).—*DDGS* contained 0·4% available methionine, 0·7% available total *S*-amino acids, and >2640 kcal metabolisable energy per kg. Providing the diet was formulated to allow for the amino acid content of *DDGS*, the addn. of 10% *DDGS* resulted in as satisfactory egg production and wt., and feed efficiency as did a maize–soyabean meal diet.
A. H. Cornfield.

Effects of Diet and Environment on Livestock

Nutrition of ruminants. IV. Use of ground straw of different particle sizes for cattle from twelve weeks of age. D. W. PICKARD, H. SWAN and G. E. LAMMING (*Anim. Prod.*, 1969, **11** (4), 543–550. 7 ref.).—Friesian calves were fed, from 12 weeks of age, 6 diets contg. 15 or 30% of barley straw, ground to pass a screen of 1/16, 3/16 or 5/16 in. There were no significant differences in performance (live wt. gain and slaughter data) between the 6 treatments. Differences in feed intake and in efficiency of feed conversion between initial, middle and final stages of fattening were highly significant for all treatments. M. Long.

Effect of wilting berseem and lucerne herbage on voluntary dry-matter intake by buffalo heifers. K. YOELAO, M. G. JACKSON and ISWHAR SARAN (*J. agric. Sci., Camb.*, 1970, **74** (1), 47–51. 19 ref.).—The *DM* content of lucerne was increased in two trials from 27 to 50 and from 26 to 44% by wilting lucerne (*L*) and from 21 to 31% for berseem (*B*). Intake of *L* was increased from 2·3 to 2·8 and from 2·1 to 3·5 kg/100 kg body-wt. by wilting. There was an increase in digestibility coeff. of org. matter, total carbohydrate and crude protein ranging from 6–20% over unwilted herbage. Nutritive values for *L* and *B* increased by 160 and 60% resp. Av. loss of *DM* due to wilting was 3·8%. Retention time was unaffected by wilting. M. Long.

Comparative studies of perennial ryegrass, timothy and meadow fescue. I. Herbage consumption and performance of calves fed on cut grass. F. E. ALDER (*J. Br. Grassld Soc.*, 1969, **24** (4), 308–316. 12 ref.).—Voluntary intake of cut forage of perennial ryegrass, timothy and meadow fescue by calves and live-wt. gains on the 3 species were similar in tests over 2 yr. Digestibility of all species and calf live-wt. gains and intake were higher with short (15–25 days) than with long (35–55 days) rest periods between cuts.
A. H. Cornfield.

Intake and live weight gain of beef cattle fed on maize silage and dried pelleted whole-crop beans (*Vicia faba* L.). C. R. LONSDALE

and J. C. TAYLOR (*J. Br. Grassld Soc.*, 1969, **24** (4), 299–301. 12 ref.).—Nine-month-old steers were fed *ad lib.* for 90 days on maize silage alone or with 29% or 57% of the total dry matter (*DM*) intake as dried and pelleted whole-crop beans. Total *DM* intakes were 3·7, 5·0 and 5·8 kg per head per day, resp.; daily live-wt. gains were 0·44, 0·74 and 0·97 kg per head and feed efficiency values were 12, 15 and 17 kg live wt. gain per 100 kg *DM* intake, resp. A. H. Cornfield.

Performance of sheep and cattle grazed separately and together. D. HAMILTON and J. G. BATH (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 19–26. 7 ref.).—Groups of 2 young steers and 8 autumn lambing ewes with one lamb each, were grazed together and separately in N.E. Victoria. Results show that, esp. in winter, combined grazing gives increased final wt. of lambs and cleaner ewe wool. Steers were not particularly affected; stocking rate was not significant. M. T. Rawnsley.

A urea feeding experiment with sheep in Iran. M. SARIF-SARBAN and K. H. MENKE (*Anim. Prod.*, 1970, **12** (1), 177–180. 9 ref.).—Four groups of sheep were given rations contg. barley, dried beet-pulp, wet beet-pulp silage, lucerne hay and straw with (*a*) no protein supplement, (*b*) 1·6% urea; (*c*) 21% Rumevite, supplying 1·3% urea and (*d*) 15% cottonseed cake. Average daily wt. gains were 143, 187, 190 and 189 g, resp. M. Long.

Influence of nutritional level in early pregnancy of the ewe. I. E. COOP and V. R. CLARK (*J. agric. Sci., Camb.*, 1969, **73** (3), 387–394. 14 ref.).—One group of ewes was fed at maintenance and another at half this level. Most ewes were either 2–3 weeks pregnant at the time of restriction or else 5–7 weeks pregnant. Restriction was maintained for 5 to 8 weeks. No significant effect on the reproductive performance of the ewes was found. M. Long.

Influence of sulphur-containing amino acids on the biosynthesis of high-sulphur wool proteins. A. BROAD, J. M. GILLESPIE and P. J. REIS (*Aust. J. biol. Sci.*, 1970, **23** (1), 149–164. 19 ref.).—Two sheep received semipurified, S-deficient diet of alkali extracted straw. One sheep consumed 500 g and the other 300 g/day for 18 weeks. During the last 6 weeks, one received 5 g/day L-cystine and the other, an equiv. amt. of elemental S. The extracted straw supplied the sheep with 80 and 50 mg of S/day, resp. S content of wool samples was detd. by an O_2 flask combustion technique and high-S proteins were extracted by alk. reduction. There was a linear relationship between S content of wool and its content of high-S proteins, but there was no evidence that wool of increased S content was more desirable. M. J. Rawlins.

Influence of dietary protein and methionine on the sulphur content and growth rate of wool in milk-fed lambs. P. J. REIS (*Aust. J. biol. Sci.*, 1970, **23** (1), 193–200. 15 ref.).—Three-day-old lambs were fed a range of diets: milk; low protein milk; high protein milk; methionine milk; and high protein methionine milk. All except the low protein diet supported satisfactory rates of body wt. gain. The S content of wool grown by lambs receiving 3 diets of protein content between 11 and 39% of dry matter, was lower than the wool of lambs fed a methionine supplemented diet. Wool growth rate was influenced by the level of protein diet. M. J. Rawlins.

Effects of experimentally produced local subdermal temperature changes on skin temperature and wool growth in the sheep. A. G. LYNE, M. JOLLY and D. E. HOLLIS (*J. agric. Sci., Camb.*, 1970, **74** (1), 83–90.).—A Perspex heat exchanger, 40 mm in dia. and 6 mm thick, was inserted beneath the skin of a black Merino sheep. The subdermal temp. was raised by ~4° for 4 days by passing warm water through it and lowered by ~5° for 4 days. The temp.-regulating mechanism of the animal was able to maintain a fairly const. subdermal temp. during the heating period but was less successful during the cooling. During cooling there was a 12% decrease in length growth rate of wool over the exchanger, with no change in dia. Heating had little effect, apart from causing a slight decrease in wool dia.; heating caused a slight reduction in pigmentation. There was no effect on crimping. M. Long.

Cold exposure of Southdown and Welsh Mountain sheep. IV. Changes in concentrations of free fatty acids, glucose, acetone, protein-bound iodine, protein and antibody in the blood. R. HALLIDAY, A. R. SYKES, J. SLEE *et al.* (*Anim. Prod.*, 1969, **11** (4), 479–491. 47 ref.).—Both breeds of female sheep were maintained on either a high- or low-plane diet and were subjected to temp. down to −20°. Before cold exposure the sheep were kept either at 8° or 30°. Effects on *FFA*, glucose etc. in the blood are described. M. Long.

Effect of supplementing a low-protein hay on the cellulolytic bacteria in the rumen of sheep and on the digestibility of cellulose and hemicellulose. N. O. VAN GYLSWYK (*J. agric. Sci., Camb.*, 1970, **74** (1), 169–180. 24 ref.).—The effects of supplementing *Eragrostis tef* hay with (*a*) egg albumin, (*b*) urea and (*c*) urea and C_4 and C_5 branched-chain volatile fatty acids, on the cellulolytic flora in the rumen of sheep and on the digestibility of cellulose and hemicellulose were investigated. Treatment (*b*) increased voluntary hay intake and %-digestibility of cellulose and hemicellulose without greatly affecting cellulolytic bacteria counts. With treatment (*c*), the numbers of cellulolytic bacteria and the proportion of ruminococci were greater than with (*b*). Voluntary hay intake was further increased with digestibilities similar to those with (*b*). Lower digestibilities and also lower % of total culturable counts were found for (*a*) compound with (*b*). M. Long.

Effect of cobalt supplementation, as an oral drench or pasture treatment, on growth of lambs. J. R. GRIFFITHS, R. J. BENNETT and R. M. R. BUSH (*Anim. Prod.*, 1970, **12** (1), 89–94. 13 ref.).—One group of twin lambs was reared on pasture sprayed with 2 lb $CoSO_4$/acre and another on untreated pasture. One partner of each twin set was drenched with $CoSO_4$ (100 mg in 25 ml of water) every two weeks. Up to weaning there were no significant live-wt. gain differences nor thereafter were the twins on the sprayed pasture found to be different. The drenched lambs on the unsprayed pasture gained an av. 9 lb more than their partners over 15 weeks, although they were still 7 lb lighter than the lambs on the sprayed pasture. Blood vitamin B_{12} concn. was not well correlated with the treatments and all values lay above critical levels. M. Long.

Protein, lysine and feed intake level effects on pig growth. II. Effects on carcass composition and quality. R. BLAIR, J. B. DENT, P. R. ENGLISH and J. R. RAEBURN (*J. agric. Sci., Camb.*, 1969, **73** (3), 395–415. 34 ref.).—Pigs were fed a combination of 16 diets contg. varying amt. of protein and lysine at different levels of intake. Carcass quality was affected by treatment and sex, increasing protein leading to increased leanness. Lysine content had little effect on carcass compn.; increasing feed intake produced fatter carcasses. Male carcasses were fatter and had a lower proportion of ham than did female carcasses. Chem. compn. of the *longissimus dorsi* was not affected by diet or sex but dry matter and intramuscular fat content rose with increasing wt. M. Long.

Influence of dietary protein intake during lactation on reproductive performance of sows. R. M. MACPHERSON, F. W. H. ELSLEY and R. I. SMART (*Anim. Prod.*, 1969, **11** (4), 443–451. 7 ref.).—Large White gilts were given a diet contg. 14% crude protein (*CP*) during 3 successive pregnancies. During a 6-week lactation one of 3 diets was given, contg. 19, 16·5 or 14% *CP*. With decreasing *CP* the av. 42-day gain in litter wt. from birth increased significantly in the 1st lactation, continuing into the 2nd lactation. Milk yield and compn. were unaffected by changes in dietary *CP*. In the 1st lactation there was greater loss in sow live-wt. with the lower levels of protein. M. Long.

Effect of dietary copper on the structure and physical properties of adipose tissue triglycerides in pigs. W. W. CHRISTIE and J. H. MOORE (*Lipids*, 1969, **4** (5), 345–349. 16 ref.).—The inner back fat (*BF*) of control pigs had a higher m.p. and stearic : oleic acid ratio than the outer *BF* of control animals or the inner or outer *BF* of pigs given a Cu supplemented diet (250 ppm). C. V.

Animal endogenous triglycerides: I. Swine adipose tissue. II. Rat and chicken. III. Swine, rat and chicken liver: comparison with adipose tissue. R. E. ANDERSON, N. R. BOTTINO and R. REISER (*Lipids*, 1970, **5** (2), 161–164. 18 ref; 165–170. 14 ref; 171–179. 14 ref.).—A study to determine the influences of diet and other environmental factors. C. V.

Protein requirements of layers per day and phase feeding. S. C. NIVAS and M. L. SUNDE (*Poult. Sci.*, 1969, **48** (5), 1672–1678. 18 ref.).—Egg production was higher when 18–20 g, than when 14–16 g protein, was supplied per hen per day. Birds given a const. 16·8% protein diet and those phase fed (16·8% protein to 40 weeks, 15% protein from 40 to 60 weeks, and 16·8% protein from 60 to 68 weeks of age) produced as well as did those receiving 18–20 g protein per hen per day. A. H. Cornfield.

Comparison of phosphorus assay techniques with chicks. VI. Development of a calcium standard curve for Curaçao Island phosphate. B. L. DAMRON and R. H. HARMS (*Poult. Sci.*, 1969, **48** (5), 1618–1621. 6 ref.).—Based on tests with broilers to 21 days of age, involving measurement of wt. gains and tibia ash, a curve is

presented relating the optimum total Ca levels in the feed for varying levels of P supplied by Curaçao Island phosphate. A. H. Cornfield.

Effect of a high level of lithium carbonate on egg shell formation of laying hens. N. MILLS, JUN., N. V. HELBACKA and R. D. CREEK (*Poult. Sci.*, 1969, **48** (5), 1766–1767. 7 ref.).—Addn. of Li_2CO_3 (326 ppm Li) to the diet of laying hens caused severe diarrhoea, excess salivation and regurgitation, and production of shell-less eggs. A. H. Cornfield.

Dietary interactions between zinc, manganese and copper for turkey poults. P. VOHRA and J. R. HEIL (*Poult. Sci.*, 1969, **48** (5), 1686–1691. 9 ref.).—Growth of turkey poults on a Zn- and Mn-deficient diet was improved by addn. of Zn, but not of Mn. The treatments did not affect bone ash or the Cu or Fe contents of the tibia, but increased tibia Zn and Mn. Liver Mn and Cu, but not Zn, were increased by the addn. of the resp. elements to the diet. There was an antagonistic effect of Zn on storage of Cu, and of Zn and Cu on storage of Mn in the liver. A. H. Cornfield.

Length of test for sulphur amino acid studies with laying hens. F. G. MARTIN, B. L. DAMRON and R. H. HARMS (*Poult. Sci.*, 1969, **48** (4), 1167–1168. 2 ref.).—In tests for determining the methionine requirement of laying hens there was no advantage in continuing the expt. after 6 months, since the coeff. of variation increased rapidly without any increased response to methionine level. A. H. Cornfield.

Nature and deposition of the carotenoids from lucerne and maize gluten meal in chicken skin. A. L. LIVINGSTON, D. D. KUZMICKY, R. E. KNOWLES and G. O. KOHLER (*Poult. Sci.*, 1969, **48** (5), 1678–1683. 11 ref.).—Lutein, zeaxanthin, cryptoxanthin, and zeinoxanthin were found in the shanks of broilers fed lucerne or maize gluten meal. The proportions of xanthophylls (*X*) found in the shanks were similar to those added in the diets. *X* present in org. solvent extracts of lucerne and maize gluten meals were more available to the birds than were those in the original meals. A. H. Cornfield.

Heat and moisture production of broilers. II. Winter conditions. J. W. DEATON, F. N. REECE and C. W. BOUCHILLON (*Poult. Sci.*, 1969, **48** (5), 1579–1582. 6 ref.).—Heat and moisture production and associated performance of broiler chickens on litter under winter conditions typical of the major broiler-producing area of the south-east U.S. are reported. A. H. Cornfield.

Analysis and Other Aspects

Urinary phosphate excretion in the dairy cow. R. MANSTON and M. J. VAGG (*J. agric. Sci., Camb.*, 1970, **74** (1), 161–167. 15 ref.).—About 10% of permanently housed cows excreted excessive amt. of phosphate in their urine; this effect was not observed in grazing cows except when these were temporarily housed. Urinary compn. was otherwise unaffected. The cause of this phosphaturia does not appear to be acidosis. M. Long.

Steroids in bovine muscle and adipose tissue. C. TU, W. D. DOWRIE and O. FENNEMA (*Lipids*, 1969, **4** (5), 369–379. 41 ref.).— C. V.

Relative precision of the tritiated water and slaughter techniques for estimating energy retention in grazing sheep. T. F. REARDON (*Anim. Prod.*, 1969, **11** (4), 453–460. 7 ref.).—A group of grazing sheep was deprived of feed and water for 48 h and used to compare errors of the two techniques. The mean energy retention estimated by the slaughter technique would have had a standard error of 3·64 Mcal compared with 1·56 Mcal using the tritiated water technique. The relationship between live-wt. and energy content is probably the most important factor determining the relative precision of the two methods. M. Long.

Estimation of plasma and red cell volumes in pigs. D. M. ANDERSON, I. MCDONALD and F. W. H. ELSLEY (*J. agric. Sci., Camb.*, 1969, **73** (3), 501–505. 15 ref.).— M. Long.

Effect of oestradiol-17β-monopalmitate (EMP) and surgical caponisation on production efficiencies, yields and organic characteristics of chicken broilers. L. R. YORK and J. D. MITCHELL (*Poult. Sci.*, 1969, **48** (5), 1532–1536. 3 ref.).—Birds treated with *EMP* (0·01 g subcutaneously) at 5 weeks of age gained more than controls to 11 weeks of age. Birds caponised at 4 weeks of age gained the least. Both treatments decreased feed efficiency and increased the fat content of light and dark meat and liver. Neither treatment affected thawing loss, cooking loss and cooking time. Although treated birds received slightly higher scores for juiciness, tenderness and flavour, the differences were not significant. A. H. Cornfield.

Effect of varying levels of ethoxyquin and vitamin E on reproduction in White Leghorn males fed diets high in linoleic acid. R. V. KUHNS and G. H. ARSCOTT (*Poult. Sci.*, 1969, **48** (5), 1646–1651. 11 ref.).—Sterility of males on a diet containing 7·3% linoleic acid (as safflower oil), without vitamin E addn., increased to a high level over a 38-week period. Sterility was reversed within 1–2 weeks of addn. of 0·075% ethoxyquin or vitamin E (0·0324 g per kg of feed) to the diet. A. H. Cornfield.

Influence of oat fractions on diethylstilboestrol-induced aortic ruptures of turkeys. C. F. SIMPSON and R. H. HARMS (*Poult. Sci.*, 1969, **48** (5), 1757–1761. 8 ref.).—Inclusion of whole ground oats, dehulled oats, and, in particular, oat hulls in the diet decreased mortality rate from aortic ruptures induced by diethylstilboestrol injections as compared with birds on an oat-free diet containing animal fat. The treatments also decreased atherosclerosis. A. H. Cornfield.

Fertility of chickens fed thiouracil prior to maturity. H. L. MARKS (*Poult. Sci.*, 1969, **48** (5), 1612–1618. 17 ref.).—Addn. of 0·1% thiouracil to the diet of chicken from 0–6 and again from 10–14 weeks of age in 3 trials increased fertility by 4·4 to 11%, but had no effect on hatchability of fertile eggs. A. H. Cornfield.

Collection and measurement of ^{14}carbon-labelled carbon dioxide from the fowl. L. B. COLVIN, L. J. FRAHM and J. L. MORRISON (*Poult. Sci.*, 1969, **48** (5), 1743–1747. 5 ref.).—A metabolism chamber (with feed and water containers and facilities for collection of excreta) for collection and measurement of expired labelled CO_2 is described. A. H. Cornfield.

Brood measurement as a valid index to the value of honey bees as pollinators. F. E. TODD and C. B. REED (*J. econ. Ent.*, 1970, **63** (1), 148–149. 2 ref.).—Pollen traps were operated on 170 *Apis mellifera* colonies for 20 days. The yield was related to the amount of brood up to 800 in^2. Above this level, no extra pollen was gathered. Pollen collection was a good measure of all field activity. Queenless colonies were the least valuable. C. M. Hardwick.

Food products. PILLSBURY CO., Assee of F. E. HALLECK (Br. Pat. 1,187,614, 7.4.67. U.S., 8.4.66).—The (animal) food products, useful in reducing the normal plasma cholesterol level, contain < 0·05 by wt. of a polysaccharide (mol. wt. > 1000) consisting of a polymeric chain of β-1,3-linked D-glucopyranose units having appendant β-1,6-linked D-glucopyranose units. S. S. Chissick.

2.—FOODS AND CROP CONVERSION

Cereals, Flours, Starches, Baking

Comparative studies on glutenins from different classes of wheat F. R. HUEBNER (*J. agric. Fd Chem.*, 1970, **18** (2), 256–259. 22 ref.).—Glutens from 2 g each of 11 wheat varieties were extracted in 0·05 N-HOAc. Electrophoretic patterns of reduced and alkylated glutenins showed significant variations among varieties of the same class, but the greatest differences were among different classes. Response of glutenins to pptn. with salt were studied. Varieties recognised for higher quality in bread and pastry making yielded steeper pptn. curves than poorer quality wheats. M. J. Rawlins.

Vital wheat gluten. H. E. HALE and W. A. CARLSON (*Baker's Dig.*, 1969, **43** (6), 52–56).—Vital wheat gluten, which is a tan-coloured, dry powder consisting essentially of wheat proteins, is manufactured by a continuous supply of fresh water on a slack dough; the wet gluten is dried under controlled conditions to preserve the vital nature of the gluten. In the bakery it reduces the no. of flour types required and increases production flexibility; it improves dough tolerance to processing and improves the quality of the finished product. I. Dickinson.

Lipid–protein interaction. J. G. FULLINGTON (*Baker's Dig.*, 1969, **43** (6), 34–38, 61. 35 ref.).—There are hydrophobic and ionic interactions, H-bonding, and mixed chelate formation. All lipid classes are capable of hydrophobic bonding to protein; other protein–lipid interactions involve more polar lipids such as glycolipids, or ionic lipids such as sphingolipids or phospholipids, which may be molecular, micellar, or lamellar. I. Dickinson.

Chemical bonds in dough. H. P. WEHRLI and Y. POMERANZ (*Baker's Dig.*, 1969, **43** (6), 22–26. 53 ref.).—Chem. bonds in wheat protein, and the involvement of other components are

discussed. Practically all types of bonds contribute to the structure of dough. While covalent and ionic bonds primarily increase cohesiveness of doughs, dipole-, H- and hydrophobic-bonds contribute to elasticity and plasticity. Van der Waals interactions are apparently of limited significance. I. Dickinson.

Relation of starch damage to the baking performance of flour. K. H. TIPPLES (*Baker's Dig.*, 1969, **43** (6), 28–32, 44. 11 ref.).—Increase in damaged starch results in an increase in flour water-absorption. The extent to which baking absorption increases depends on the baking method used and the level of α-amylase. Deterioration of bread quality occurs when flour damaged starch is increased above a certain max. value. The higher the protein content, the higher the damaged starch may be raised without bread quality deterioration. Starch damage cannot be increased indefinitely, because as the water-starch mass is increased, the air-dough interface becomes unstable during the oven stage thus resulting in loss of vol. and coarse texture; there will also be insufficient gluten to cover the surface area of the starch, which results in a loss of gas retention capacity, reduction in loaf vol. and a breakdown of cell structure. I. Dickinson.

Instantised flour. Physical properties. B. S. MILLER, H. B. TRIMBO and R. I. DERBY (*Baker's Dig.*, 1969, **43** (6), 49–51, 66. 14 ref.).—Typical data on the phys. properties are presented. These include definition of terms, particle size distribution, structure of parent and instantised flour, uniform cup wt., effect of instantising and particle size on the oil-binding capacity of flour, free-flow characteristics, dispersion of flour in water, disintegration of instantised flour in water and the oil-binding capacity. I. Dickinson.

Studies in composite flours. I. Use of sweet potato flour in bread and pastry making. G. M. SAMMY (*Trop. Agric., Trin.*, 1970, **47** (2), 115–125. Engl., 18 ref.).—Sweet potato flour from the cultivar '049' may be used without any difficulty as a substitute for wheat flour at a rate of up to 15% in bread making and at 20–30% in pastries; flour from cultivar 'C9' was of poorer quality. Na metasulphite treatment improved colour but a SO_2 content > 100 ppm affected baking properties. E. G. Brickell.

Factors affecting the grain and texture of white bread. P. W. KAMMAN (*Baker's Dig.*, 1970, **44** (2), 34–38).—The terms 'grain' and 'texture' are defined. The effect is studied of (*a*) different fermentation times on loaf characteristics, underfermented (2·5 h), normal (4·5 h) and overfermented (6 h), (*b*) mixing time on loaf characteristics, undermixed (3·5 min), normal (8·5 min) and overmixed (14 min), (*c*) variation in absorption on loaf characteristics, too low (56%) normal (63%) and too high (70%), (*d*) moulder head roll setting, loose, normal, too tight, (*e*) final proof time, short (48 min) normal (63 min) and excessive (72 min). Photographs of the cut loaves are presented. I. Dickinson.

Functions of dairy ingredients in baking. M. H. MERTENS (*Baker's Dig.*, 1969, **43** (6), 57–60).—Dairy products are used for their improving effects on flavour, nutrition, moisture absorption and retention, crumb and crust colour, and buffer condition. Since the functional characteristics of these products are detd. by their protein and lactose content, the relative levels of these constituents influence the effects the individual dairy products produce. I. Dickinson.

New developments in multifunctional dough conditioners. F. BARRETT (*Baker's Dig.*, 1970, **44** (1), 66–68. 10 ref.).—The formulation consists of distilled monoglyceride of glyceryl monostearate, ethoxylated mono- and diglycerides, hydroxylated lecithin and starch. It serves the dual role of dough strengthener and shelf life extender, giving the necessary strength to the dough to overcome variations in ingredients and to withstand the equipment abuse thus enabling const. production of uniform, high quality goods. I. Dickinson.

Soya protein products in commercial cake formulations. E. J. TURRO and E. SIPOS (*Baker's Dig.*, 1970, **44** (1), 58–64).—A processed soya protein, in the form of a cream-coloured free-flowing powder, is an effective additive for commercial cakes when used as a complete or partial replacement for non-fat dry milk. At a 50% replacement level, no formula changes are necessary; at 75% level, dextrose must be included in the total sugar content, and the leavening must be increased to obtain the desired vol. The added cost is more than offset by the increased yield of batter. I. Dickinson.

Biscuit-making techniques in the United Kingdom. P. WADE (*Chemy Ind.*, 1970, (20), 639–643).—Prepn. and measurement of ingredients, mixing, dough formation, baking, cooling, etc. are outlined, together with possible improvements of various unit processes. W. J. Baker.

Factors in the spread of coconut cookies during baking. M. E. RUEHRMUND (*Baker's Dig.*, 1969, **43** (6), 46–48).—A slow-acting Na acid pyrophosphate is a better agent for reaction with baking soda than tartaric acid, as it reduces cookie spread variation to a min. Rehydration of the coconut before incorporation into a cookie formula helps to achieve more uniform cookies in the final bake-out. Cookies are more tender if the coconut is premoistened. I. Dickinson.

Using sulphosuccinates and sulphosuccinamates in baking. AMERICAN CYANAMID CO. (Inventor: K. WHELAN) (Br. Pat. 1,187,876, 24.7.68. U.S., 7.8.67).—Monoglyceride softening agents can be replaced by dialkyl sulphosuccinate or carboxyethyl sulphosuccinamate wetting agents, giving increased through-put, decreased critical dependence on mixing speed/time and improved storage properties. Suitable compd. are, e.g., Na di(ethylhexyl)sulphosuccinate and Na_4 *N*-(1,2-dicarboxyethyl)-*N*-octadecylsulphosuccinamate. S. S. Chissick.

Sugars, Syrups, Confectionery

Dry honey and dry molasses. E. GLABE, P. W. ANDERSON, P. F. GOLDMAN and S. LAFTSIDIS (*Baker's Dig.*, 1970, **44** (1), 70–72, 81).—Dehydrated honey and molasses have product functions superior to those of their liquid counterparts, as a result of the unique sugar–starch relationship brought about by the dehydrating step. As well as wider latitude in use levels, it provides closer control on product uniformity and easier measurement and handling operation. I. Dickinson.

Manufacture of dessert praline with pectin. R. LOHMANN (*Fette Seifen AnstrMittel*, 1970, **72** (4), 315–317. Ger.).—Gel-forming properties of pectin soln. and dried pectin with sugar and other additives is discussed, together with the colloidal aspects of the reaction on heating. It is considered that 50% esterified mixtures of sugar/pectin are required for prepn. of dessert praline products. Detailed procedures are given for the prepn. of such gels, including the effect of time, temp. and mode of addn. of the ingredients. G. R. Whalley.

Malting, Brewing and Alcoholic Beverages

Histamine content of some Italian wines. G. VITALI (*Industrie aliment., Pinerolo*, 1970, **9** (5), 79–80. It., 4 ref.).— P. P. R.

Whisky production. L. VANOSSI (*Industrie aliment., Pinerolo*, 1970, **9** (5), 81–85. It.).— P. P. R.

Free-flowing hop acid complex. MILLER BREWING CO. (Br. Pat. 1,188,484, 29.1.69. U.S., 13.3.68).—The humulones and isohumulones (but not lupulones) are pptd. by treatment of a hop acid soln. (solvent has dielectric const. ⩾ 2·1, e.g., hexane) with anhyd. NH_3. The complex is separated and added to wort for beer production. S. S. Chissick.

Beer. SOCIETE EUROPEENNE DE BRASSERIE (Br. Pat. 1,189,075, 11.11.68. Fr., 28.11.67).—A high density beer is prep. by adding sugar to a mashed unhopped wort (*d* = 20–25° Balling) to give a *d* of 30–40°, followed by impregnation with yeast and fermentation at 25–30°C. S. S. Chissick.

Fruits, Vegetables and Their Products

Column chromatographic isolation of the anthocyanin-3,5-diglucosides from grapes. GEZA HRAZDINA (*J. agric. Fd Chem.*, 1970, **18** (2), 243–245. 29 ref.).—Ripe grapes of variety Seibel-9549 were processed immediately after harvesting. The juice was prep. by hot pressing and filtering, and was held frozen until used. Delphinidin, petunidin, malvidin, cyanidin and peonidin-3,5-diglucosides were isolated, by column chromatog. using Polyclar AT. M. J. Rawlins.

Volatile components of smooth Cayenne pineapple. R. A. FLATH and R. R. FORREY (*J. agric. Fd Chem.*, 1970, **18** (2), 306–309. 19 ref.).—Pineapple essence was satd. with NaCl, extracted with isopentane in a glass–Teflon liquid–liquid extractor for 24 h. Examina-

tion of the extracts was carried out by combination g.c.–mass spectrometry, using large bore, open columns and a membrane type interface. The 45 identified compd. are tabulated.
M. J. Rawlins.

Changes in amino acid composition during ripening and storing of tomatoes. E. I. Petropavlovsky and Z. A. Troyan (*Pishch. Tekhnol.*, 1970, [1 (74)], 21. Russ.).— C. V.

Lipoxygenase activity of various types of pea. J. Rigaud, P. Dupuy and R. Cousin (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1970, **56** (2), 142–148. Fr., 5 ref.).—Lipoxygenase (lipoxydase) catalyses the formation of conjugated double bonds from polyunsatd. fatty acids in which the double bonds are in the *cis*-position, separated by a CH_2 group. Linoleic acid in peas is very susceptible. The enzyme was extracted from peas quick-frozen to −20°c; three varieties of pea were tested for activity. Results show that if samples are taken at the same tenderometer index, wrinkled peas have a higher activity than smooth peas. The effects of this on the quick-freezing of peas, and the bad smell which sometimes occurs, are discussed. The time between shelling and blanching seems to be critical, as does the harvesting time. M. T. Rawnsley.

Non-alcoholic Beverages

Treatment of cocoa after fermentation: drying and storage trials in Uganda. F. Couprie (*Café-Cacao-Thé*, 1970, **14** (1), 39–46. 5 ref.).—Four treatments were compared, involving drying in the sun, direct drying in Samoa and soaking prior to drying. Results showed that in Uganda washing is necessary; an excellent cocoa is obtained after six months of stocking, the aroma developing in the first three months and the acidity being reduced in the last three.
W. J. G.

Treatment of roasted coffee products. Coffee Instants Inc. (Inventors: I. M. Reich and A. S. Cascione) (Br. Pat. 1,186,305, 20.8.68).—The flavour and aroma of the product is preserved by exposing the coffee to anhyd. SO_2 and NH_3 vapours during grinding. S. S. Chissick.

Milk, Butter, Other Dairy Products, Eggs

Modern dairy science. E. L. Crossley (*Jl. R. Soc. Arts*, 1970, **118** (5167), 402–413).—Discusses, mainly from the biol. and technological aspects, breeding, feeding and management of dairy herds, control of udder disease, methods of liquid milk processing (pasteurisation, sterilisation, ultra-heat treatment, packaging), and manufacture and nutritional value of conc. and dried milks. Probable trends in the form and packaging of domestic milk supplies are indicated, and the problem of packaging of all forms of milk is examined. Cheesemaking is briefly reviewed.
W. J. Baker.

Developments in sterilisation of milk and milk products. A. Balducci (*Fette Seifen AnstrMittel*, 1970, **72** (4), 308–309. Ger.).—A brief description of modern Italian equipment for the continuous and batch sterilisation of all types of milk and milk products. G. R. Whalley.

Effect of anionic ion exchange resin treatment of milk for removal of radio-iodine on its thiamine content. J. Barth, J. K. Avants, B. H. Bruckner and L. F. Edmondson (*J. agric. Fd Chem.*, 1970, **18** (2), 324–325. 6 ref.).—Raw whole milk (of normal pH), stored for 24 h, was passed through a column of 20–50 mesh anion exchange resin, previously charged with a salt soln. contg. NaCl, NaH_2PO_4 and Na citrate. Thiamine was detd. in the resulting fractions; there were no observed changes in the free, bound or total thiamine content of milk treated for ^{131}I removal by anion exchange resin. M. J. Rawlins.

Factors in milk affecting growth of yoghurt bacteria. H. K. Frank (*Milchwissenschaft*, 1969, **24** (5), 269–277. Ger., 20 ref.).—For practical milk processing purposes, variations in coagulation times may be reduced by long, high temp. heating of yoghurt milk.
P. P. R.

Preparation of water-insoluble derivatives of rennin and chymotrypsin and their use in the hydrolysis of casein and the clotting of milk. M. L. Green and G. Crutchfield (*Biochem. J.*, 1969, **115** (2), 183–190. 24 ref.).—Enzymically active insol. deriv. of chymotrypsin and rennin are prep. by coupling each enzyme to agarose, and also rennin to aminoethylcellulose. Agarose–chymotrypsin is stable from pH 2–9, but agarose–rennin progressively releases enzyme into soln. at pH > 2, and aminoethylcellulose–rennin is similarly unstable at pH > 4·1. Each deriv. catalyses clotting of milk at 30°; this is probably entirely due to release of sol. or covalently bound enzyme. The sp. activities of the bound enzymes are never > 20% of those of the free enzymes in soln.
J. N. Ashley.

Melting-salt action of Na trimetaphosphate and Na tetrametaphosphate. K. H. Ney and O. P. Garg (*Fette Seifen AnstrMittel*, 1970, **72** (4), 279–285. Ger., 25 ref.).—When 100 g of finely chopped cheese is placed on a water bath with 3 g of Na tetrametaphosphate (**I**) and stirred with 64 ml of water, a homogenous melt is obtained; with Na trimetaphosphate (**II**) a uniform melt is not obtained. The difference in behaviour is assumed to be due to the different Ca-binding behaviour, which is low in **II**, unlike **I**. Hydrolysis of both **I** and **II** under melting process conditions cannot be excluded however. G. R. Whalley.

Edible Oils and Fats

Survey on Greek olive oil. XII. Production 1967–1968. P. Katsoulis (*Greek Minist. Commerce gen. tech. Dir. 4th Div. chem. technol. Res.*, 1969, (63), 18 pp. Engl., 15 ref.).—Extensive tabular data are presented. Small kernel olives, in general, yield oils of low I-value and refractive index whereas medium kernel olives show higher values; those of the varieties Asprolia and Mavrolia, grown in the island Leucas, are intermediate. The ratio of satd. to linoleic acid was high (1·84–4·16) for low kernel olives, low (1·02–1·91) for medium ones and intermediate for the varieties Asprolia and Mavrolia. E. G. Brickell.

Odorous components of olive oil. E. Fedeli and G. Jacini (*Chimica Ind., Milano*, 1970, **52** (2), 161–164. 4 ref.).—The odorous constituents of a typical virgin olive oil were isolated by distillation with ethanediol, under mild conditions, followed by extraction with pentane. The conc. extract was examined by g.l.c., column chromatog. and combined g.c.–mass spectrometry and ~ 50 components were characterised. A series of satd. (C_7–C_{12}) and unsatd (C_{11}–C_{13}) aldehydes, together with acetals of ethanediol, methyl palmitate, oleate and linoleate and ethyl palmitate were identified in the extract. A group of substances thought to be of aromatic structure is also present. J. N. Brazier.

Sterol changes during the industrial processing of fats and oils. I. H. P. Kaufmann, E. Vennekel and Y. Hamza (*Fette Seifen AnstrMittel*, 1970, **72** (4), 242–246. Ger., 26 ref.).—When soyabean oil contg. 3·3% of β-sitosterol (**I**) is heated in the presence of O_2 for 3 h at 200°c, it is shown that 7-hydroxysterols (**II**) can be isolated from the unsaponified matter by t.l.c. Hydrocarbons and disterol ethers (**III**) are also formed by bleaching earth treatment of cholesterol, **I**, stigmasterol and brassicasterol; a similar treatment of **II** leads to the formation of hydrocarbons and keto-steroids. Sterols are partially oxidised during deodorisation under inadequate vac. conditions. Fresh rapeseed oil does not contain any **III**, although these substances are detected in the commercial products. Dicholesteryl ether does not have any carcinogenic properties in the rat. G. R. Whalley.

Modern technological developments in the industrial production of edible fats. II. J. Baltes (*Fette Seifen AnstrMittel*, 1970, **72** (3), 166–180. Ger., 3 ref.).—A review of modern industrial manufacturing processes for the continuous and discontinuous production of edible oils and fats, including de-acidification, deodorisation, earth bleaching, hardening and the use of antioxidants and plasticisers. Systems of batch weighing and blending are also considered.
G. R. Whalley.

Margarine for baking. J. B. Carden and D. F. Meisner (*Baker's Dig.*, 1970, **44** (2), 58–60).—Margarines vary in consistency from fluid, pumpable oils to firm, waxy, puff pastry type shortenings. Solid Fat Index values and crystal structure may be controlled so that margarines may have very narrow or very broad plastic ranges. They are suitable for a wide variety of bakery foods in a broad range of processing conditions. I. Dickinson.

Occurrence of ketones in fats which resemble butter. A. Huyghebaert, H. Hendrickx, N. Schamp and L. De Buyck (*Fette Seifen AnstrMittel*, 1970, **72** (4), 289–293. Fr., 20 ref.).—Examination of the unsaponified fraction of butter-like fats or butter substitutes indicates the presence of ketones (not Me ketones which are caused by oxidn.), which are separated by t.l.c. It is shown by g.l.c. and i.r.

analysis that the compd. consist of two homologous series of ketones of the type, $C_nH_{2n+1}CO \cdot C_nH_{2n+1}$ and $C_nH_{2n+1}CO \cdot C_nH_{2n-1}$, where n = 11 to 17. The relationship between fatty acid and ketone compn. indicates that ketones are formed during manufacture of the fats. G. R. Whalley.

Determination of trace sulphur in fat by combustion and reduction of sulphate to hydrogen sulphide. E. L. PIPPEN and E. P. MECCHI (*J. agric. Fd Chem.*, 1970, **18** (2), 301–305. 19 ref.).—Poultry fat samples (5–15 g) were burnt in 1 h at 1000°C in a vertical quartz tube contg. V_2O_5. Combustion products were passed through peroxide to convert SO_2 to SO_4. SO_2 was reduced to H_2S with HI and H_3PO_3 in HOAc. Distinction between samples which differed by only 0·5 μg of S per g of fat was readily achieved. M. J. Rawlins.

Meat, Poultry and Fish

Lighting conditions for evaluation of beef marbling and colour. K. E. HOKE and C. E. DAVIS (*Fd Technol., Champaign*, 1970, **24** (3), 283–285. 9 ref.).—Meat samples from beef ribs were studied under a range of incandescent and fluorescent lights. Colour scores for beef were affected by the type and intensity of light. It was not conclusively discovered that lighting conditions affected graders' scores for marbling. Further investigations were designed to establish requirements for type and intensity of light for accurate judgments of meat qualities. M. J. Rawlins.

Studies on textural stability of irradiated Bombay duck. M. S. GORE and U. S. KUMTA (*Fd Technol., Champaign*, 1970, **24** (3), 286–289. 19 ref.).—Freshly caught 'duck' was washed, eviscerated, cleaned, cut and stored frozen until use. Dip treatments to study optimum concn. of salt soln. and dip time in terms of drip loss caused by irradiation were carried out using 10% soln. of $Na_4P_2O_7$, $Na_3P_3O_{10}$ and NaCl. Pre-irradiation treatment conferred textural stability and increased storage life of duck, otherwise affected by textural damage and bacterial spoilage. M. J. Rawlins.

Analysis of fish cakes. G. H. O. BURGESS, T. MCLACHLAN, I. N. TATTERSON and M. L. WINDSOR (*Analyst, Lond.*, 1970, **95** (1130), 471–475. 2 ref.).—The fish content is detd. as follows: a sample is washed with Et_2O, mixed with trichloroacetic acid, sieved and washed. The residue plus water is heated to 85°C, cooled to 60°C and NaOAc added to give pH 4·2–4·6. Diastase soln. is added and the mixture heated (73–74°C, 30 min) this process being repeated until the I-test for starch is negative. The residue is washed, dried to const. wt. at 100°C and the N-content (C) detd. Protein content = 6·25C. S. S. Chissick.

Determination of magnesium, manganese, iron, copper and zinc in marine shrimp. G. A. KNAUER (*Analyst, Lond.*, 1970, **95** (1130), 476–480. 15 ref.).—The powdered desiccated whole-shrimp samples are charred (200°C), combusted (550°C, 4–16 h), and then dissolved in 1:1 HNO_3. The soln. are diluted ($\Rightarrow \times 10^3$) with water and introduced into an atomic-absorption spectrometer using a laminar flow burner and air/C_2H_2 fuel. The lamps and standards are chosen according to the element under investigation. S. S. Chissick.

Isopropyl alcohol extraction of oil and lipids in the production of fish protein concentrate from herring. B. DROZDOWSKI (*Stud. Fish Res. Bd Can.* [1969], 1970, (2), *J. Am. Oil Chem.* Soc., **46**, 269–274. 30 ref.).—Using the Halifax process, the largest portion of lipid is found in the first extract of isopropyl alcohol (IPA); high quality triglyceride oil is readily recovered by cooling this extract. Phospholipids are also extracted without degradation and, together with free fatty acids, are found mostly in the IPA-rich phase from the first extraction. E. G. Brickell.

Delicatessen fish products. R. MCLAY (*Torry advis. Note*, 1970, (43), 10 pp.).—Prepn. of sausages, crisps, chips, savoury fingers, pies, and pâte from fish, particularly from the less popular species, is described. E. G. Brickell.

Phosphate complexes of soluble fish proteins. Their formation and possible uses. J. SPINELLI and B. KOURY (*J. agric. Fd Chem.*, 1970, **18** (2), 284–288. 15 ref.).—Sarcoplasmic proteins were obtained by mincing rockfish flesh; soln. were prep. adjusting the protein concn. to 1% with distilled H_2O. Sufficient phosphate (*P*) was added to yield specific molar concn. of the *P*. Condensed *P* complexes were pptd. from the soln. The P:N ratio in the complexes depended on the concn. of condensed *P* in the soln., the type of *P* and the pH of the soln. The reaction between condensed *P* and sol. protein could be used to recover waste proteins from industrial effluents. M. J. Rawlins.

Food Additives

Preservatives, Colouring Matter

Spectrophotometric determination of diethyl pyrocarbonate with 4-amino-antipyrine. E. MONCELSI (*Chimica Ind., Milano*, 1970, **52** (4), 367–369. It., 10 ref.).—The method is based on the reaction of diethyl pyrocarbonate (**I**) with the primary amino group of 4-amino-antipyrine (**II**) giving 1-phenyl-2,3-dimethyl-4-urethan-5-pyrazolone (**III**). Reaction is complete in 5 min; excess of **II** is detd. by reaction with phenol, 6N-NH_3 and $K_3Fe(CN)_6$ to give a red colour which is measured spectrophotometrically at 460 nm. The amt. of **I** is then calc. The method is applicable to ether extracts of foods and beverages and is sensitive to 20 μg of **I** in the ether soln. with an error of 3%. J. I. M. Jones.

Composition of an ether-soluble fraction of a liquid smoke solution. W. FIDDLER, R. C. DOERR and A. E. WASSERMAN (*J. agric. Fd Chem.*, 1970, **18** (2), 310–312. 27 ref.).—An ether-sol. fraction was separated from a commercial liquid smoke soln. by g.l.c. The 34 components were identified by comparison of g.l.c. retention times and i.r. spectra, with those of known samples. 14 components were tentatively identified. Some of the compd. were also found in food flavours and their possible formation was discussed. M. J. Rawlins.

Food additives as insecticides. D. L. MILNE (*Jl S. Afr. chem. Inst.*, 1969, **22** (special issue), S28–34. Engl., 9 ref.).—Preliminary expt. suggest that the commercial food preservatives, sorbic and benzoic acids, will protect tobacco against *Lasioderma serricorne* and milled grain against *Tribolium castaneum* and *T. confusum*. E. G. Brickell.

Spices, Flavours, Other Additives

Colorimetric method of determination of pungent constituents of pepper. M. L. SHANKARANARAYANA, S. NAGALAKSHMI and C. P. NATARAJAN (*Flavour Ind.*, 1970, **1** (3), 173–175. Engl., 12 ref.).—Those compd. which are responsible for the pungency of pepper and oleoresin pepper, are hydrolysed (by boiling with 2N-KOH in aq. propylene glycol) to piperidine (**I**), which is then reacted with CS_2 to form piperidinium pentamethylene dithiocarbamate. This is then detd. spectrophotometrically at 435 nm as the yellow cupric deriv. Beer's Law is obeyed in the range 1–14 μg/ml of **I**. G. R. Whalley.

Synthesis of some 2-methoxy-3-alkylpyrazines with strong bell pepper-like odours. R. M. SEIFERT, R. G. BUTTERY, D. G. GUADAGNI, *et al.* (*J. agric. Fd Chem.*, 1970, **18** (2), 246–249. 12 ref.).—The synthesis, mass and i.r. spectra for 4 new 2-methoxy-3-alkylpyrazines with alkyl groups hexyl, Pr, Pr^i, and Et, are described. Their odour characteristics were compared with the known bell pepper component 2-methoxy-3-isobutylpyrazine. The hexyl and Pr were very similar, the Pr^i moderately similar, and the Et deriv. showed no similarity. M. J. Rawlins.

Process for artificially imparting a smoked colour to foodstuffs. NEDERLANDS ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TEN BEHOEVE VAN DE VOEDING (Inventor: P. J. GROENEN) (Br. Pat. 1,186,303, 2.4.68. Neth., 3.4.67).—The foodstuff (protein-rich) is coated with a γ-carbonyl compd. by immersion in an aq. soln. and then heating at $\leq$ 70°C. E.g., unsmoked Frankfurter sausages are immersed in a dil. soln. prep. by heating diethoxytetrahydrofuran and laevulinic aldehyde diethylacetal with H_3PO_4 at 80°C for 1 h, and then heated at 70°C for, e.g., 30 min. S. S. Chissick.

Elimination of nitrites in spinach. PRODUITS FINDUS S.A. (Inventors: S. I. W. BOSUND and B. L. BENGTSSON) (Br. Pat. 1,186,482, 27.8.68. Switz., 23.11.67).—At least one non-toxic acid (citric) or an ascorbate is added (0·5–2 g/kg) to (partially) cooked spinach to reduce the risk of nitrite poisoning. S. S. Chissick.

Food Processing, Refrigeration, Packaging and Storage

Energy requirements and heat transfer in scraped surface heat exchangers. O. MÖLLER and A. M. TROMMELEN (*Fette Seifen AnstrMittel*, 1970, **72** (4), 235–242. Ger., 15 ref.).—The use of scraped film heat exchangers (*SFHE*) in the foodstuffs industry is considered, together with a description of the theoretical principles involved in their design, considering esp. heat transfer and

overall energy requirements. Application of *SFHE* to margarine manufacture is briefly considered. G. R. Whalley.

Comparison of microwave with steam or water blanching of corn-on-the-cob. I. Characteristics of equipment and heat penetration. C. C. Huxsoll, W. C. Dietrich and A. I. Morgan, jun. **II. Peroxidase inactivation and flavour retention.** W. C. Dietrich, C. C. Huxsoll, J. R. Wagner and D. G. Gvadagni (*Fd Technol., Champaign*, 1970, **24** (3), 290–292. 13 ref.; 293–296. 15 ref.).—I. The microwave system was a Cryodry Model, continuous processing tunnel. Water blanching was by immersion in 40 gal of water in a steam jacketed kettle with automatic temp. control and stirrer. Steam blanching (210–212°F) was done in a pilot plant conveyorised blancher. A stainless steel thermocouple was inserted into the corn ear to measure heat penetration. There was inactivation of the peroxidase enzyme by 6 min in microwave, 20 min in steam or H_2O and by 4 min water-blanching followed by 2 min microwave blanching.

II. After blanching, various parts of the ear, top and base of kernels, ends and middle-cross section were qual. examined. Peroxidase tests showed a variation in activity from end to middle of the ear for underblanched samples. Sensory-panel results on samples stored at −20°, 0° and 20°F indicated better flavour retention in the 4 min microwave and 12 min steam-blanched samples. M. J. Rawlins.

Retention of organic volatiles in freeze-dried solutions of carbohydrates. J. Flink and M. Karel (*J. agric. Fd Chem.*, 1970, **18** (2), 295–297. 16 ref.).—5 ml aliquots of soln. prep. from carbohydrate, org. volatiles and H_2O were frozen by immersion in liquid N_2 and freeze-dried in flasks for 48 h. Volatile and water analyses were made by g.c. A proposed mechanism by which org. volatiles were retained by sol. carbohydrates after freeze-drying from soln. suggested that the dry material was organised on a microlevel and the microstructure retained the volatile. Microstructure development depended upon many processing parameters. M. J. Rawlins.

Physiological parameter as means for operational control of application of orange skin-coating in packing plants. S. Ben-Yehoshua, M. J. Garber and C. K. Huszar (*Trop. Agric. Trin.*, 1970, **47** (2), 151–155. Engl., 8 ref.).—Both rate of wt. loss and internal O_2 were correlated with storage life and amt. of coating material left on oranges dipped in Tag coating. Rate of wt. loss might serve as an indicator for the quality of the waxing operation in a packing plant. E. G. Brickell.

Drawn aluminium cans—a new type of container. M. Fischer (*Fette Seifen AnstrMittel*, 1970, **74** (4), 293–295. Ger.).—The use of light Al-coated cans is recommended for jams, soup concentrates, honey etc. and Al band, lined with polypropylene foil, for all products which must be sterilised. The materials are deep-drawn without folds by a suitable tool, into light cans of 20 to 600 cc capacity. They are odourless, tasteless, with easy opening, and have excellent sales-promoting appearance. G. R. Whalley.

Packaging treatments for the storage and export of Australian grapes. B. B. Beattie and N. L. Outhred (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 124–128. 7 ref.).—Varieties Gordo Blanco, Purple Cornichon, and Waltham Cross were subjected to one or more of the following: granulated cork packing contg. 12 or 8 g of $Na_2S_2O_5$ (**II**); tissue and wood wool impregnated with Na *o*-phenylphenate (**I**) (10 mg/tissue); tissue and wood-wool impregnated with 5 ml **II**/100 g; tissue and brown paper pads impregnated with 3 g **II**; pads with 3 g **II** and 0·2 g **I**; unwrapped, sprayed with thiabendazole; tablets or sachets of **II** instead of impregnation; and polythene film with sachets of **II**. All varieties can be stored for more than 3–5 weeks. The best pack is shown to be tissue, 100–150 g wood-wool and 20 tablets, each 0·19 g **II**. The cost is ~ 40% less than present export costs. M. T. Rawnsley.

Nutrition, Proteins, Amino Acids, Vitamins

Nutritional values of different milk-fat fractions. V. Antila and M. Antila (*Fette Seifen AnstrMittel*, 1970, **72** (4), 285–289. Ger., 7 ref.).—Milk fat was fractionated into four fractions, distinguished by their m.p. range. Liquid fractions contained mainly short-chain satd. and unsatd. fatty acids (*FA*); solid fractions contained chiefly palmitic and stearic acids. Vitamins were mainly conc. in the liquid fractions. Dietary expt. with albino rats, fed with milk-free diets, showed that the I-values and *FA* compn. of depot fats of the animals were independent of the feed, and that feeding caused no histological changes in the tissues of the heart, kidney and liver. G. R. Whalley.

Preparation of a colourless sunflower protein isolate. S. Gheyasuddin, C. M. Cater and K. F. Mattil (*Fd Technol., Champaign*, 1970, **24** (3), 242–243. 9 ref.).—Sunflower seed meal was extracted with 0·25% aq. Na_2SO_3 at pH 10·5, at room temp. for 1 h. This was followed by isoelectric pptn. and extraction of the ppt. with 50% aq. Pr^iOH. The nearly white isolate had an amino acid compn. with decreased cysteic acid value and increased tyrosine, phenylalanine and leucine. Other amino acids were unaffected. M. J. Rawlins.

Plant-leaf protein as source of food protein. J. E. Kinsella (*Chemy Ind.*, 1970, (17), 550–554. 41 ref.).—A review with emphasis on necessary requirements (compn., nutritional value, storage quality, flavour) and on recent research into production and costs. Although leaves are a potential source of future human food protein, international scientific and technical coordination is necessary to develop suitable formulations and processing methods and also to study the productivity and protein value of new species of leaves. W. J. Baker.

Availability of amino acids. Fructose-glycine as a source of non-specific nitrogen for rats. S. N. Hagan, M. J. Horn, S. H. Lipton and M. Womack (*J. agric. Fd Chem.*, 1970, **18** (2), 273–275. 15 ref.).—Fructose–glycine (*FG*) was prep. by the Heyns and Paulsen method. *FG* was microbiol. assayed for glycine using *Leuconostoc mesenteroides* P-60 and yielded 68% of the growth-stimulating ability of glycine. Growth was as good as when equivalent amt. of glycine, were fed to N deprived rats. M. J. Rawlins.

Unclassified, Tobacco

Studies on dehulling and screw pressing of soyabean to obtain optimally processed soyaflour. M. C. Shamanthaka, K. S. Srinivasan and R. Rajagopalan (*J. Fd Sci. Technol.*, 1969, **6** (3), 189–191. 13 ref.).—2, 4, 5, and 10% H_2O/seed wt. were sprayed on the seeds which were then dried after 15 min. 2% was observed to be the optimum. Drying and dehulling were achieved by the use of an electrically heated coffee roaster, and a plate type flour mill with an attached air blower. Screw press of 2 tons capacity/24 h was used. Edible quality of the soyaflour was good. M. J. Rawlins.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Man-made plant diseases. C. E. Yarwood (*Science, N.Y.*, 1970, **168** (3928), 218–220. 28 ref.).—In many cases the gain in crop production due to 'interference' is greater than the loss due to disease and therefore the control of these man-favoured plant diseases will be difficult. C. V.

Effect of mineral nutrition of plants on parasitic disease and pest attack. A. Krauss (*Z. PflErnähr. Bodenk.*, 1969, **124** (2), 129–147. Ger., 122 ref.).—A review. M. Long.

Chemical treatments for control of post-harvest diseases. J. W. Eckert, (1969, **8** (3), 116–135. 74 ref.).—A comprehensive review covering fumigation techniques, chem. treatments applied to fruit in water or wax formulations, e.g., Na *o*-phenylphenate, and fungicides such as dicloran, 2-aminobutane, 2-substituted benzimidazoles, etc. W. J. G.

Chemical control of root diseases of Douglas fir seedlings in relation to fungus and nematode populations. W. J. Bloomberg and W. R. Orchard (*Ann. appl. Biol.*, 1969, **64** (2), 239–244. 12 ref.).—Seed treatment with thiram reduced post-emergence damping-off, whilst fumigating forest nursery soils with MeBr or DD improved Douglas fir seedling emergence and shoot and root growth and decreased the incidence of root disease. These benefits were associated with decreased no. of pathogenic fungi and nematodes in the seedlings and soil. A. H. Cornfield.

Herbicides

Effect of ratio of soil to water on adsorption of linuron and atrazine. R. Grover and R. J. Hance (*Soil Sci.*, 1970, **109** (2), 136–138).—Expt. with soil slurries may not afford a true picture of the adsorptive capacity of a soil in natural field conditions, e.g., with limited water content. Freundlich adsorption isotherms were detd. for

the two herbicides, used in varying concn. Results are discussed with a view to clarifying the previously presumed anomalies.
A. G. Pollard.

Adsorption of *s*-triazines by soil organic matter. J. B. Weber, S. B. Weed and T. M. Ward (*Weed Sci.*, 1969, **17** (4), 417–421. 45 ref.).—Adsorption of *s*-triazines, including prometryne, prometon, propazine, by org. soil colloids from aq. soln. was studied. About half the prometon adsorbed by org. matter was extractable with 0·1 N-NaCl. Adsorption of the *s*-triazines was due to complexing by functional groups on the org. colloids and/or exchange adsorption of *s*-triazine cations. A. H. Cornfield.

Exchange adsorption of 3-amino-1,2,4-triazole by montmorillonite. D. C. Nearpass (*Soil Sci.*, 1970, **109** (2), 77–84. 6 ref.).—Adsorption of amitrole (**I**) by montmorillonite (*M*) is probably due to proton association and cation exchange; it followed the Langmuir isotherm and reached equilibrium quickly. The process was completely reversible and no mol. absorption occurred. Adsorption diminished with time due to conversion of H-clay into Al-clay. Adsorption of **I** on clays is dependent on pH and is not strong in pH ranges comparable with those in soil. A. G. Pollard.

Degradation of phenoxyalkanoic acid herbicides by soil micro-organisms. M. A. Loos (*Jl S. Afr. chem. Inst.*, 1969, **22** (special issue), S71–78. Engl., 29 ref.).—A review. E. G. Brickell.

Herbicide concentrations in live oak (*Quercus virginiana*) treated with mixtures of picloram and 2,4,5-T. J. R. Baur, R. W. Bovey and J. D. Smith (*Weed Sci.*, 1969, **17** (4), 567–570. 13 ref.).—Recovery of 2,4,5-T was greater in live oak tissue treated with mixtures of 2,4,5-T ester and picloram than in tissue treated with 2,4,5-T alone. Most of the herbicide recoverable 1 month after treatment was gone 6 months after treatment. Brush control 2 years after treatment showed that mixtures of picloram (K salt) and 2,4,5-T were better than mixtures of picloram (iso-octyl ester) and 2,4,5-T or 2,4,5-T alone. A. H. Cornfield.

Mode of action of foliage-applied translocated herbicides with particular reference to the phenoxy-acid compounds. II. Mechanism and factors influencing translocation, metabolism and biochemical inhibition. M. M. Robertson and R. C. Kirkwood (*Weed Res.*, 1970, **10** (2), 94–120. over 200 ref.).—A review.
W. J. G.

Biochemical effects [on leaf protein and total RNA] in apple leaf tissue associated with the use of simazine and amitrole. M. Solecka, H. Profic and D. F. Millikan (*J. Am. Soc. hort. Sci.*, 1969, **94** (1), 55–57. 9 ref.).—Although there was no enhancement of effects when both materials were applied together, there were indications that the depressing effects on leaf protein and RNA of amitrole were moderated when used with simazine.
A. H. Cornfield.

Annual bluegrass (*Poa annua*) control in overseeded Bermudagrass putting green turf. S. W. Bingham, R. E. Schmidt and C. K. Curry (*Agron. J.*, 1969, **61** (6), 908–911. 9 ref.).—Overseeding Bermudagrass golf greens with cool season grasses, particularly annual ryegrass, reduced annual bluegrass cover and gave a good surface. Effects of DCPA and Bensulide [*N*-(2-di-isopropyldithiophosphoryl)ethylbenzenesulphonamide] are also discussed.
A. H. Cornfield.

Selective control of prickly pear (*Opuntia polyacanthia*) in rangeland (U.S.A.) with herbicides. G. A. Wicks, C. R. Fenster and O. C. Burnside (*Weed Sci.*, 1969, **17** (4), 408–411. 12 ref.).—Silvex, picloram, dicamba, and 2,4,5-T were the most effective (of 13 herbicides tested) 1–2 yr after application. Rotary hoeing prickly pear cladodes just before spraying improved control.
A. H. Cornfield.

Effects of four herbicieds on pasture yield and composition. A. A. McGowan (*Aust. J. exp. Agric. Anim. Husb.*, 1970, **10** (42), 42–47. 12 ref.).—Herbicides 2,4-D, 2,2-DPA, paraquat and diquat were sprayed at recommended rates in April, May or July on a pasture in N.E. Victoria. The pasture was *Phalaris tuberosa*, *Lolium rigidum* (Wimmera ryegrass), *Hordeum leporinum* (barley grass), *Vulpia bromoides* (silver grass), subterranean clover, and *Arctotheca calendula* (capeweed). Clover was temporarily affected by 2,2-DPA, while the amt. of capeweed was much increased. May applications of 2,2-DPA and paraquat reduced the amt. of barley and silver grass, and increased the ryegrass. M. T. Rawnsley.

Fungicides

Fungicidal activity and chemical constitution. XVI. The use of partition data for an analysis of the activities of alkyldinitrophenol fungicides against apple mildew. D. R. Clifford, A. C. Deacon and M. E. Holgate (*Ann. appl. Biol.*, 1969, **64** (1), 131–137. 13 ref.).—Structure/activity relationships for members of 5 homologous 4-(1-substituted alkyl)-2,6-dinitrophenols and apple mildew, *Podosphaera leucotricha*, are discussed, with special reference to their hydrophobic bonding constants π; π values may be of use in predicting potential anti-mildew fungicides with modes of action similar to those of the compd. studied. A. H. Cornfield.

Effect of mulches on southern blight (*Sclerotium rolfsii*) in dwarf bean (*Phaseolus vulgaris*). S. G. Reynolds (*Trop. Agric. Trin.*, 1970, **47** (2), 137–144. Engl., 23 ref.).—Mulching delayed the onset of southern blight so that three good harvests were obtained and there was a very close relationship between bean yield and the incidence of wilt. A mulch of coconut fronds produced a 396% increase in yield over the control. E. G. Brickell.

Control of fungal seed-borne disease by means of a thiram seed soak. R. B. Maude, A. S. Vizor and C. G. Shuring (*Ann. appl. Biol.*, 1969, **64** (2), 245–257. 16 ref.).—Eleven of 13 seed-borne pathogens in 11 species of seed were completely controlled by soaking seed in 0·2 aq. suspension of thiram for 24 h at 30°. This treatment was much more effective than dusting with fungicides and generally more effective and less damaging than hot-water treatment. A. H. Cornfield.

Rate of fungicidal action. N. J. Turner (*Contr. Boyce Thompson Inst. Pl. Res.*, 1970, **24** (10), 227–229. Engl., 1 ref.).—Rate of action of all fungicides tested, although varying at 20°, increased with temp. though with less evidence in the very fast acting compd. such as thiram. E. G. Brickell.

Relationship between chemical reactivity, oil : water partitioning, and temperature on the rate of fungicidal action of tetrachloroisophthalonitrile and some of its analogues. N. J. Turner and R. Battershell (*Contr. Boyce Thompson Inst. Pl. Res.*, 1970, **24** (10), 203–212. Engl., 18 ref.).—5-Chloro-2,4,6-trifluoroisophthalonitrile (the most active member) required < 10 sec to cause a 50% inhibition in germination of *Monilinia fructicola* spores; the least active required > 5 h. In general, activity increased with temp. but varied with compd. Relative activity of tetrachloroisophthalonitrile and its compd. may depend on differences in ability to overcome the detoxifying effects of fungus sulphydryl compd.
E. G. Brickell.

Efficacy *in vitro*, of new fungicides against *Fusicoccum amygdali* del., cause of peach tree canker. I. Palazon (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1970, **56** (2), 122–128. Fr., 7 ref.).—With ziram as control, thiabendazole, benomyl, carboxine, and 1,2-bis(3-methoxycarbonyl-2-thioureidobenzene) (**I**), were tested on mycelia (*M*) and pycniospores (*P*) of *F. amygdali* in lab. conditions. Benomyl gave good control on both *M* and *P*, as did thiabendazole. Carboxine was inferior to ziram; **I**, while effective against mycelia, had no effect on conidia. The mechanism of the fungicidal action is discussed, esp. with regard to protein synthesis. Caution must be used in interpreting these results for field use.
M. T. Rawnsley.

Seed treatments with Vitavax for control of loose smut of wheat and barley. R. B. Maude and C. G. Shuring (*Ann. appl. Biol.*, 1969, **64** (2), 269–253. 5 ref.).—Seed treatment of spring barley seed by dusts or soaks with Vitavax (2,3-dihydro-carboxanilido-6-methyl-1,4-oxathiin) eliminated *Ustilago nuda*, whilst treatment of winter wheat seed greatly reduced infection due to *U. tritici*.
A. H. Cornfield.

Insecticides

Effect of rice ear-bugs on grain yields. G. H. L. Rothschild (*Trop. Agric. Trin.*, 1970, **47** (2), 145–149. Engl., 6 ref.).—An av. of four fully milk-ripe grains were probed by an adult of *Leptocorisa oratorius* per day, increasing to nearly eight per day when the ears were at the early post-flowering stage. It is suggested that a 25% yield loss could result from the feeding activities of 100,000 adults per acre. E. G. Brickell.

Infestation of sour cherries by the apple maggot: confirmation of a previously uncertain host. L. J. Shervis, G. M. Bousch and C. F. Koval (*J. econ. Ent.*, 1970, **63** (1), 294–295. 11 ref.).—Pupae were obtained from sour cherries picked from an abandoned orchard. Of the 22 flies that emerged, 18 were *Rhagoletis pomonella* and 4 were *R. fausta*. C. M. Hardwick.

***Orthosia hibisci* a new pest of azaleas, rhododendrons and roses.** F. F. Smith and T. L. Bissell (*J. econ. Ent.*, 1970, **63** (1), 335–336. 3 ref.).—*O. hibisci* larvae have been found damaging rosebuds.

This species was also probably responsible for azalea and rhododendron damage. It may have originated in nearby deciduous woodland. C. M. Hardwick.

Production of concentrated pyrethrum extract: effect of concentration of pyrethrum oleoresin and water. S. PRASAD and RAJKUMARI JAMWAL (*Chemy Ind.*, 1970, (20), 650–651. 2 ref.).—When the extract is prep. by the extraction-freeze method described previously (*Idem, ibid.*, 1969, **23**, 756), the max. concn. of pyrethrins (46–48%) is obtained only if the ethylene glycol monomethyl ether extractant contains 5% (w/w)H_2O and if the oleoresin content of the soln. is 18–23% w/w (4–5%, w/w, of pyrethrins). At this concn. of oleoresin, separation of gums and resins is max.
W. J. Baker.

Persistence, degradation and bioactivity of phorate and its oxidative analogues in soil. I. W. GETZIN and J. H. SHANKS, JUN. (*J. econ. Ent.*, 1970, **63** (1), 52–58. 17 ref.).—The use of g.l.c., t.l.c. and assay with *Acheta pennsylvanicus* is described with reference to the detn. of phorate and its 5 oxidative analogues in silt loam.
C. M. Hardwick.

Influence of the protozoan *Mattesia grandis* McLaughlin on the toxicity to the boll weevil of four insecticides. M. R. BELL and R. E. MCLAUGHLIN (*J. econ. Ent.*, 1970, **63** (1), 266–269. 7 ref.).—*Anthonomus grandis* infected with *M. grandis* were susceptible to lower doses of malathion, azinophos-methyl, DDT or carbaryl. Susceptibility increased with the extent of the disease until it was 5 to 7-fold by the 12th day. Fat content also decreased.
C. M. Hardwick.

Latent and differential toxicity of insecticides to larvae and adults of six fly species. J. R. YATES and M. SHERMAN (*J. econ. Ent.*, 1970, **63** (1), 18–23. 12 ref.).—Of the 5 insecticides tested, dieldrin was the only one to exhibit latent toxicity against 3rd instar larvae and 3–4 day old females. C. M. Hardwick.

Review of pesticides. II. Biological methods of pest control. L. CHALMERS (*Mfg Chem. Aerosol News*, 1970, **41** (4), 23–29. 41 ref.).—A review of the recent methods of pest control includes the use of sterilisation by chemicals and irradiation, insect sex attractants and baited lures, microbial and viral control of insects, biol. control by predators, parasites and nematodes, hormone metabolism interferents, molluscides and general repellents.
G. R. Whalley.

Methyl cyclohexanepropionate and related chemicals as attractants for the Japanese beetle. T. P. MCGOVERN, M. BEROZA, P. H. SCHWARTZ *et al.* (*J. econ. Ent.*, 1970, **63** (1), 276–280. 4 ref.).—Of 88 chemicals tested alone or with eugenol, 5 were more attractive than the standard phenethyl butyrate + eugenol (9:1). Their relative attractiveness was 1·1–3·1 times increased. 4 of the 5 were cyclohexane-substituted esters. The best was methyl cyclohexanepropionate. C. M. Hardwick.

Response of female Mediterranean fruit flies to male lures in the relative absence of males. S. NAKAGAWA, G. J. FARIAS and L. F. STEINER (*J. econ. Ent.*, 1970, **63** (1), 227–229. 6 ref.).—Sexually mature female *Ceratitis capitata* responded only to trimedlure, medlure and angelica seed oil when males were absent. This was a useful indication of infestation levels. C. M. Hardwick.

Propyl 1,4-benzodioxan-2-carboxylate, a new attractant for the European chafer. T. P. MCGOVERN, B. FIORI, M. BEROZA and J. C. INGANGI (*J. econ. Ent.*, 1970, **63** (1), 168–171. 6 ref.).—Of 340 lures tested, the most effective were esters of 1,4-benzodioxan-2-carboxylate. Their synthesis is described. Relative attractiveness was based on numbers of chafers captured for 9 days. All were at least 1·5 times as effective as butyl sorbate. The most attractive compd. had limited volatility and so was more persistent.
C. M. Hardwick.

Response of some predators and parasites of *Ips confusus* (Lec.) (Coleoptera; Scolytidae) to olfactory attractants. R. E. RICE (*Contr. Boyce Thompson Inst. Pl. Res.*, 1969, **24** (9), 189–194. 20 ref.).—Olfactory response by *Enoclerus lecontei* and *Temnochila virescens chlorodia* to monoterpenes serves as a distinct mechanism of host finding by these predators; also the aggregating pheromones of *Ips* spp. play a role in host finding by the predators and particularly for the parasite *Tomicobia tibialis*. A tentative order of relative attractiveness was α-pinene = β pinene > △-3-carene = myrcene > limonene. E. G. Brickell.

Insect hormones and insect pheromones as possible agents for insect control. P. KARLSON (*Jl S. Afr. Chem. Inst.*, 1969, **22** (special issue), S41–55. Engl., 46 ref.).—A critical review.
E. G. Brickell.

Eradication of oriental fruit fly from the Mariana islands by the methods of male annihilation and sterile insect release. L. F. STEINER, W. G. HART, E. J. HARRIS *et al.* (*J. econ. Ent.*, 1970, **63** (1), 131–135. 5 ref.).—The release of 16 million irradiated pupae on Guam, following a hurricane which destroyed host plants, eradicated the population of *Dacus dorsalis*. Five subsequent occurrences of *D. dorsalis* in traps, were controlled by the release of 20 million more sterile flies. Similar releases on Saipon and Tinian were not successful and reasons are suggested. The distribution of fibreboard squares impregnated with methyl eugenol and nailed every 2 weeks for 4½ months was successful in eradication. No flies have been found for 4 yr. C. M. Hardwick.

Sterile male technique for insect-population control. L. A. BUSCARLET (*Rapp. CEA*, 1970, No. 3980, 29 pp. Fr., 42 ref.).—The review emphasises radiation effects on insect sterilisation and the depressive effect of a sterile population on a fertile population under both lab. and field conditions. Applications to the control of screw-worm, fruit fly, olive fly and apple codling-moth are summarised. 58 insect species used in international control programmes are tabulated together with data on crops or animals attacked, optimum irradiation dosage, and relative effectiveness of treatment. Economic aspects are considered. W. J. Baker.

Normal development of *Anophes flavipes* in cereal leaf beetle eggs killed with *X*-radiation, and potential field use. L. C. BARTON and F. W. STEHR (*J. econ. Ent.*, 1970, **63** (1), 128–130. 12 ref.).—Irradiation with 1000–5000 R gave complete mortality of *Oulema melanopus* but viability of the parasite *Anaphes flavipes* was not affected. The release of a sufficient quantity of parasites to give beetle control is impracticable but the addition of sterile eggs to increase the numbers of *A. flavipes* might be useful.
C. M. Hardwick.

1-Carboxybenzimidazole derivatives. FISONS PEST CONTROL LTD. (Inventors: D. HUGHES and D. W. POUND) (Br. Pat. 1,185,159, 14.10.66).—Pesticidal 1-carboxybenzimidazoles of Br. Pat. 1,104,936, are prep. in improved yield in aq. medium. Eg., $ClCO_2Ph$ is quickly stirred at 23° into a mixture of 5,6-dichloro-2-trifluoromethylbenzimidazole, NaOH, and aq. Pr^iOH while keeping at pH 9·5–9·8 with N-NaOH. Ppt. is collected after 13 min and comprises pure Ph 5,6-dichloro-2-(trifluoromethyl)-benzimidazole-1-carboxylate, m.p. 101–104° F. R. Basford.

Polychloroketoalkenoic acids. AIR PRODUCTS AND CHEMICALS INC. (Br. Pat. 1,185,200, 7.4.67. U.S., 8.4.66).—The acids (5–10 C) and their inner anhydrides contg. C:C and ≮ 4 Cl or Br are prep. by reacting a satd. monocarboxylic ketoalkanoic acid or ester, amide, etc., with Cl_2 and/or Br_2 at room temp. and then heating to 170–260°. E.g., 2,3,4,5,5,5-hexachloro-2-pentenoyl-4-lactone, useful as a herbicide or insecticide, is obtained by reacting Et levulinate with Cl_2. S. S. Chissick.

Heterocyclic compounds. SHELL INTERNATIONALE RES. MIJ., N.V. (Br. Pat. 1,186,880, 7.12.67. U.S., 9.12.66).—Compd. with herbicidal or defoliant activity, esp. useful in rice paddies, comprise 2,1,3-benzo(thia- or selena-)diazoles substituted by CN or $CSNH_2$ and optionally 1–3 Cl, Br, NO_2, alkyl, NH_2 etc. An example is 4,7-dicyano-2,1,3-benzothiadiazole, m.p. 187–189° (COMeEt), prep. by heating a mixture of 4,7-dibromo-2,1,3-benzothiadiazole $Cu_2(CN)_2$ and $HCONMe_2$ at 150–155° for 19 h.
F. R. Basford.

Substituted heterocyclic compounds. FISONS PEST CONTROL LTD. (Inventors: L. T. ALLAN, G. T. NEWBOLD, and A. PERCIVAL) (B.P. 1,186,504, 15.10.66).—Compd. with biocidal activity, esp. as herbicides, are 2-R-1-R^I-4-R^{II}-5-R^{II}-imidazoles and quaternised deriv. thereof, wherein R is alkyl, alkenyl, aryl, etc., opt. subst. by alkyl, halogen, or NO_2; R^I is H, e.g., alkyl, aryl, $CY^IY^{II}R^{III}$ or COR^{III}(Y^I, Y^{II} are O or S; R^{III} is opt. subst. alkyl, or aryl), SR^{IV}(R^{IV} is polyhalogenoalkyl); the $2R^{II}$ together represent a Z^I:Z^{II}·Z^{III}:Z^{IV} chain; one of Z^I–Z^{IV} is N and the remainder are CR^V wherein R^V is, e.g., H, alkyl, OH, alkoxy, NO_2, halogen, CO_2H, SH or heterocyclyl. An example is 6-chloro-2-isopropyl-imidazo[4,5-*b*]pyridine, m.p. 190–192°C, prep. in 70% yield by boiling a soln. of 5-chloro-2,3-diaminopyridine in isobutyric acid for 24 h. F. R. Basford.

2-Benzimidazolecarbamic acid esters, and related compounds. E. I. DU PONT DE NEMOURS & CO. (Br. Pat. 1,185,237, 14.11.67. U.S., 15.11.66).—A cheaper, less hazardous process for the prepn. of fungicidal 2-NH·CO_2R-benzimidazoles (and halogeno, nitro and alkyl deriv.) comprises reacting NH_2·CN (or a salt) with an alkyl chloroformate on carbonate in neutral to basic aq. medium at

0–130°C, then treating the intermediate alkyl cyanocarbamate salt (I) with o-$(NH_2)_2C_6H_4$ (II) in acidic aq. medium at 40–130°. E.g., $ClCO_2Me$ is added to aq. Ca cyanamide at 40–50°, then, after 1 h, solid is filtered off and washed with water. The filtrate is treated with II, conc. HCl is added to pH 3·5, then the mixture is quickly heated to 90°. After 30 min. at 90–98°/pH 2·5–3·1, pptd. Me benzimidazol-2-ylcarbamate is recovered in 83·3% yield. The compd. and their I–II salt intermediates are active against phytotoxic fungi. F. R. Basford.

Fluorinated benzothiophen derivatives. IMPERIAL SMELTING CORP. (N.S.C.) LTD. (Inventors: G. M. BROOKE, W. K. R. MUSGRAVE and M. A. QUASEM) (Br. Pat. 1,187,671, 26.1.67).—The compd., with insecticidal and fungicidal activity, are 2,3-dicarboxy-4,5,6,7-tetrafluorobenzo(*b*)thiophen and its esters and are made by reacting Li pentafluorothiophenate with a diester of acetylene dicarboxylic acid (I) at < 0°. E.g., pentafluorothiophenol (prepn. described) in tetrahydrofuran is treated with BuLi in hexane followed by I (Et_2 ester) at < −55°. After 4 h the mixture is refluxed. The product is 4,5,6,7-tetrafluoro-2,3-diethoxycarbonylbenzo(*b*)thiophen, m.p. 37–38°C. A. Gordon.

Phosphorus-containing 2-imino-1,3-dithioles. AMERICAN CYANAMID CO. (Inventor: R. W. ADDOR) (Br. Pat. 1,186,911, 6.4.67. U.S., 21.4.66).—Compd. with pesticidal properties (e.g., against *Prodenia eridania*, *Aphis fabae*, *Tribolium confusum*) have the formula $RR^IPX{\cdot}N{:}C{\cdot}S{\cdot}CR^{III}{:}CR^{II}$ wherein X is O or S; R-R^I are alkyl, alkylthio, alkoxy, aryl or $NR^{IV}R^V$; R^{IV}-R^V are H or alkyl; and R^{II}-R^{III} are H, alkyl or Ph. In an example, a mixture of 2-imino-1,3-dithiole hydrochloride, 2:1-benzene–water, $KHCO_3$ and $(OEt)_2POCl$ is stirred at room temp. overnight, then worked up to give a 78% yield of 2-diethoxyphosphinylimino-1,3-dithiole, m.p. 46·6–48° (Et_2O). F. R. Basford.

Substituted vinyl esters of acids of phosphorus. SHELL INTERNATIONALE RESEARCH MIJ N.V. (Inventors: D. E. POEL, D. MEDEMA and R. VAN HELDEN) (Br. Pat. 1,185,112, 25.1.68).—The compd., which have insecticidal, acaricidal and nematocidal properties, have formula $R^1(O)_mR^2(O)_nP(X){\cdot}OC(R^3){:}CR^4R^5$, where X = O or S; R^1, R^2 are (halogenated) hydrocarbon groups or together form an aliphatic group; R^3 is aryl; R^4, R^5 are H, Cl, Br, alkyl; m and n are each 0–1. They are prep. from the compd. $R^1(O)_mR^2(O)_nP(X){\cdot}H$, and an α-haloketone $R^3C(O)C(Y)R^4R^5$ (where Y = Cl or Br) by reacting in presence of a base. E.g., diethyl 2-chloro-1-(2,4-dichlorophenyl)vinyl phosphate is obtained by reacting Et_2 phosphite with 2,2,2′,4′-tetrachloroacetophenone in presence of NH_3. S. S. Chissick.

Diseases and Pests in Livestock; Veterinary Treatments

Control of Exogenous Pests

Vanadium compounds as inhibitors of reproduction of the screw worm fly. M. M. CRYSTAL (*J. econ. Ent.*, 1970, **63** (1), 321–323. 7 ref.).—Of 14 inorg. and org. compd. administered orally to flies for 5 days, 7 were completely effective chemosterilants, 5 had some antifertile effect and 2 had no effect. In 1 day oral or topical tests, no compd. was more than moderately effective. C. M. Hardwick.

Dichlorvos-impregnated resin strands for control of chicken lice on laying hens. S. E. KUNTZ and B. F. HOGAN (*J. econ. Ent.*, 1970, **63** (1), 263–266. 3 ref.).—Hens were banded with resin bands impregnated with dichlorvos. One band was nearly as effective as two, and 3% dichlorvos gave lice-free hens at 3 days as did 5% and 10%. Bands fastened to the cages reduced infestations slowly. C. M. Hardwick.

Other Treatments

Effect of lincomycin and spectinomycin water medication on chickens experimentally infected with *Mycoplasma gallisepticum* and *Escherichia coli*. A. H. HAMDY and C. J. BLANCHARD (*Poult. Sci.*, 1969, **48** (5), 1703–1708. 10 ref.).—Lincomycin (1 g) + spectinomycin (2 g per gal of drinking water for 7 days) increased the survival rate of chickens infected with *M. gallisepticum* from 40 to 100%, of birds with *E. coli* infection from 33 to 80%, and of birds with combined infection from 27 to 80%. A. H. Cornfield.

Antibacterial activity of sulphadimethoxine potentiated mixture (Ro 5–0013) in chickens. M. MITROVIC, G. FUSIEK and E. G. SCHILDKNECHT (*Poult. Sci.*, 1969, **48** (4), 1151–1155. 2 ref.).—The activity of Ro 5–0013 [sulphadimethoxine + 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl)pyrimidine] against fowl cholera, colibacillus, infectious coryza, and salmonellosis in chickens was studied. A. H. Cornfield.

Ethopabate tolerance in growing chickens. J. KOBOW, M. AKKILIČ and H. LÜDERS (*Poult. Sci.*, 1969, **48** (3), 1019–1021).—When supplied in a ration containing 100 ppm amprolium, chickens tolerated 80–100 ppm ethopabate (4-acetamido-2-ethoxyl-benzoic acid methyl ester) (I) to 8 weeks of age without significant effects. 200–400 ppm I markedly depressed growth. A. H. Cornfield.

1,3-Bis(*p*-chlorobenzylideneamino)guanidine hydrochloride (Robenzidene): a new poultry anticoccidial agent. K. A. HOSSMANN and K. SATO (*Science, N.Y.*, 1970, **168** (3929), 373–376).—In a concn. of 66 ppm in the feed, this compd. was highly effective in the prevention of chicken coccidiosis caused by any one of eight Eimeria spp. Three times this dose is safe for broiler chickens. In dogs and rats toxicity was low over a 90-day period. In lab. trials oocyst production by 7 species was completely prevented. In *E. maxima* it was greatly reduced. C. V.

Thiol in liver and kidneys of chickens: effect of injection with three mercury compounds or starvation. V. L. MILLER, G. E. BEARSE, T. S. RUSSELL and E. CSONKA (*Poult. Sci.*, 1969, **48** (5), 1736–1743. 22 ref.).—Effects of injecting $HgCl_2$, Ph-Hg acetate, and Me-Hg-Cl, and of starvation and protein depletion on liver and kidney thiol levels in leucosis-resistant and -susceptible chicks were studied. A. H. Cornfield.

Toxic effects of different mercury compounds on young White Leghorn cocks. A. SWENSSON and U. ULFVARSON (*Poult. Sci.*, 1969, **48** (5), 1567–1574. 31 ref.).—The least dose of Hg, as a single i.v. injection, causing toxicity was much lower for $Hg(NO_3)_2$ than for Ph-Hg-OH, methoxy-Et-Hg-OH or Me-Hg-OH. When the compd. were added to wheat to supply 80 ppm Hg in the feed, only Me-Hg-OH was toxic, while at 16 ppm Hg, none of the compd. caused ill effects. A. H. Cornfield.

Sanitation, Hygiene and Safety

General Sanitation, Pollution

Enhancement by glycerol of phototropic growth of marine planktonic algae and its significance to the ecology of glycerol pollution. J. Y. CHENG and N. J. ANTIA (*J. Fish. Res. Bd Can.*, 1970, **27** (2), 335–346. 16 ref.).—Apart from a chrysomonad (*Prymnesium parvum*) and a cryptomonad (*Chroomonas salina*), none of 18 species of marine phytoplankters belonging to 5 algal classes showed any significant growth on glycerol in the absence of light. However, in the presence of light, glycerol enhanced the growth of 16 species, in particular members of the Chrysophyceae and Cryptophyceae, one diatom (*Phaeodactylum tricornutum*), one rhodophyte (*Porphyridium cruentum*), and one chlorophyte (*Nannochloris oculata*). Some species showed obvious cytological and metabolic changes. E. G. Brickell.

Control of schistosomiasis. S. OLDHAM (*Mfg Chem. Aerosol News*, 1970, **41** (4), 31–32. 9 ref.).—Recent progress in the control of schistosomiasis is reported, and includes the early larval stages of the miracidia and its ability to locate the host aquatic snail, migration of the sporocysts, chem. and biol. methods of snail control and the use of new drugs for mass chemotherapy. G. R. Whalley.

***Salmonella* spp. and serotypes of *Escherichia coli* isolated from the lesser mealworm collected in poultry brooder houses.** P. K. HAREIN, E. DE LAS CASAS, B. S. POMEROY and M. D. YORK (*J. econ. Ent.*, 1970, **63** (1), 80–82. 20 ref.).—1000 *Alphitobius diaperinus* were collected, surface disinfected, macerated and cultured. From 2·2% of the sample, pathogenic *Salmonella* spp. were isolated. 26 of the *E. coli* serotypes isolated were pathogenic to man or animals. These bacteria represent a potential infection source. C. M. Hardwick.

Control of houseflies in swine-finishing units by improved methods of waste disposal. R. C. DOBSON and F. W. KUTZ (*J. econ. Ent.*, 1970, **63** (1), 171–174. 3 ref.).—Three special and one standard system for disposal of pig waste are described. Each unit was screened and no insecticides were used. Greatly reduced fly populations resulted. C. M. Hardwick.

Laboratory evaluation of insecticides as contact sprays against adult houseflies. D. L. BAILEY, G. C. LABRECQUE and T. L.

WHITFIELD (*J. econ. Ent.*, 1970, **63** (1), 275–276. 3 ref.).—Results are given for 22 of 81 compd. tested against a susceptible and a resistant strain of flies. The degree of resistance was ascertained by the ratio of LC_{50} values for the resistant colony to that of the susceptible one. Four compd. were more effective than ronnel against both strains, and 16 others were more effective against the resistant one only. C. M. Hardwick.

Circadian rhythm in susceptibility of houseflies and Madeira cockroaches to pyrethrum. W. N. SULLIVAN, B. CAWLEY, D. K. HAYES *et al.* (*J. econ. Ent.*, 1970, **63** (1), 159–163. 17 ref.).—Groups of newly emerged houseflies and *Leucophaea maderae*, mostly large nymphs, were treated each hour with pyrethrum aerosol and then kept under const. conditions. Both species showed circadian rhythm in their susceptibility. Both were most susceptible during the last quarter of the light span–about mid-afternoon. C. M. Hardwick.

Mothproofing investigations with imidazole. R. E. BRY and L. L. MCDONALD (*J. econ. Ent.*, 1970, **63** (1), 71–74. 10 ref.).—Boric acid was more effective in protecting wool cloth from attack by *Attagenus megatoma* than was 2% imidazole alone or with boric acid. Deposits were not resistant to washing but were fairly resistant to dry cleaning. An increase in pick up of 50% was obtained when immersion time was increased from 5 min to 16 h. Both levels were greater than the calc. deposit. C. M. Hardwick.

Pesticides. CIBA LTD. (Br. Pat. 1,184,950, 27.7.67. Switz., 28.7.66).—Pests may be combatted in open water/muddy, moist soil, by applying a compd. of formula $R_3SnX^1{\cdot}C(:X^2)NR^1{}_2$, where R is opt. substd. phenyl, X^1, X^2 are O or S and R^1 is as R or opt. substd. aliphatic group, or one R^1 is H, or $NR^1{}_2$ is a heterocyclic ring. E.g., a soln. of $Ph_3SnSC(S)NMe_2$ in $COMe_2$–DMF is active against *Australorbis glabratus* (100% mortality) at a dilution of 0·1 ppm. The compd. are also active against mosquito larvae. S. S. Chissick.

Control of mosquito larvae and pupae. REGENTS OF THE UNIVERSITY OF CALIFORNIA (Inventor: M. S. MULLA) (Br. Pat. 1,185,989, 1.5.67. U.S., 22.8 and 13.10.66).—The control of mosquitoes in egg, larval and pupal form is effected by applying (e.g., to the water) 0·2–2 lb/acre of surface of a long chain mono- or di-amine (salt) such as 2-aminononane, 4-aminopentadecane oleate or *N*-dodecane-4-(1,4-butylene diamine), dissolved in a water-immiscible solvent (aromatic petroleum distillate, b.p. 420–630°F). The compd. cause morphological defects in adults emerging from such pupae as are not killed outright. S. S. Chissick.

Food Hygiene and Safety

Carcinogens in smoked meat products. L. TÓTH (*Fleischwirtschaft*, 1969, **49** (12), 1611–1614. Ger., 23 ref.).—The amt. of 3,4-benzpyrene and other polycyclic compd. found in meat and other foods by a no. of workers are listed. The use of smoke soln. and smoke concentrates is briefly discussed and it is concluded that these are not necessarily safer than smoke itself. P. P. R.

Bacterial counting in dried milk [by three methods]. B. VON BOCKELMANN (*Milchwissenschaft*, 1969, **24** (8), 468–472. Ger., 11 ref.).— P. P. R.

Treatment of large grain stores in Kenya with dichlorvos slow release strips for control of *Cadra cautella*. J. A. MCFARLANE (*J. econ. Ent.*, 1970, **63** (1), 288–292. 4 ref.).—Dichlorvos slow release strips at 1 strip to 25 m^3 free space, gave satisfactory control of *C. cautella* for 18–20 weeks. The grain had previously been fumigated with MeBr and the warehouse had little ventilation. The strips were less effective in a more ventilated warehouse. C. M. Hardwick.

Contamination by Pesticides, Pesticide Toxicity

Parathion degradation in lake sediments. D. A. GRAETZ, G. CHESTERS, T. C. DANIEL *et al.* (*J. Wat. Pollut. Control Fed.*, 1970, **42** (2), Pt. 2, R76–R94. 18 ref.).—Mechanisms and rates of degradation of parathion (**I**) were detd. in a sterile sediment, in aerobic and anaerobic peptone soln., in a peptone-enriched sediment system and in intact sediments from two Wisconsin lakes. **I** is highly resistant to chem. degradation, yielding diethylphosphoric acid and *p*-nitrophenol on hydrolysis, but is readily susceptible to microbial degradation in aerobic and anaerobic environments, the major pathway being reduction of the $-NO_2$ group to $-NH_2$ with formation of aminoparathion. Without microbial activity, **I** remains in the environment for several months, but in biol. active environments it is degraded in a matter of weeks. J. M. Jacobs.

Organochlorine insecticide residues in soils and soil invertebrates from agricultural lands. C. D. GISH (*Pestic. Monitoring J.*, 1970, **3** (4), 241–252. 30 ref.).—Tabular data for the states of Alabama, Arkansas, Illinois, Kansas, Louisiana, Maryland, Mississippi and Missouri show that organochlorine insecticides in soils averaged 1·5 ppm dry wt. and in earthworms 13·8 ppm. Residues in earthworms averaged nine times that of soils. Coeff. of correlation were significant for DDE, DDD, and DDT regardless of crop or soil type. E. G. Brickell.

Dimethoate residues in winter spinach. R. E. MENZER and E. D. THOMAS (*J. econ. Ent.*, 1970, **63** (1), 311–312. 10 ref.).—Residues from winter application of dimethoate declined to 2 ppm in 3 days. The disappearance rate was not related to temp. C. M. Hardwick.

Herbicide residues in tobacco leaves and their transfer into the smoke. R. CORBAZ, A. ARTHO, P. CESCHINI *et al.* (*Beitr. Tabakforsch.*, 1969, **5** (2), 80–91. Engl., 27 ref.).—Residues of Patoran (*P*) (= metobromuron) and Molipan (*M*) (= monolinuron + linuron) in air-cured tobacco, after field application at 4 and 2·5 kg/ha, resp., were ~ 1–2%; *P* levels remained unchanged after fermentation but *M* residues were reduced by ~ 40%. When the tobacco was smoked as cigarettes, ~ 4% of the *P* residue was found in the mainstream smoke; another 10% was recovered as 4-chloroaniline from the smoke condensate. The corresponding rates for *M* were 2·5% plus 10% (as 4-chloroaniline) from monolinuron and 6% plus 20% (as 3,4-dichloroaniline) for linuron. P. P. R.

Distribution of residues from atrazine, ametryne, and pentachlorophenol [PCP] in sugar-cane. H. W. HILTON, Q. H. YUEN and N. S. NOMURA (*J. agric. Fd Chem.*, 1970, **18** (2), 217–220. 2 ref.).—Atrazine, ametryne and PCP, all labelled with ^{14}C were applied in nutrient soln. to sugar-cane roots. Atrazine-^{14}C and PCP-^{14}C were also applied to leaves. Samples of treated and untreated leaves, dry leaf trash, stalk, roots and suckers were taken at intervals from 2 weeks after treatment. By 13 weeks, 60% atrazine-^{14}C and 16% PCP-^{14}C were lost from the foliar treated plants. PCP-^{14}C applied to roots was not translocated but atrazine-^{14}C and ametryne-^{14}C were translocated readily from roots to green leaves. All radioactivity was lost with natural abscission. M. J. Rawlins.

Residues in apples subsequent to ground sprays of Endrin. F. HORSFALL, JUN., R. E. WEBB, N. O. PRICE and R. W. YOUNG (*J. agric. Fd Chem.*, 1970, **18** (2), 221–223. 10 ref.).—Red Delicious and Golden Delicious apple trees were used. Four different treatments of endrin as a ground spray were applied at two different seasons. Both picked and dropped fruit were analysed for endrin. Treatment in November produced 0–0·005 ppm of endrin in the harvested apple compared with 0·005–0·028 ppm when spray was applied after petal fall as leaves and fruits were forming. M. J. Rawlins.

Effects of commercial processing on residues of aldrin and dieldrin in tomatoes and residues in subsequent crops grown on the treated plots. A. J. B. POWELL, T. STEVENS and K. A. MCCULLY (*J. agric. Fd Chem.*, 1970, **18** (2), 224–227. 15 ref.).—Tomatoes were treated with 3 aldrin levels, harvested and commercially processed within 30 h of last spray application. Commercial canning and juicing removed ~ 80% of aldrin and dieldrin residues. Residues in the soil declined rapidly; after overwintering, 10% of former levels remained. Subsequent crops did not accumulate appreciable residues from the soil. M. J. Rawlins.

Chemical and biological evaluation of the release of aldicarb from granular formulations. R. A. STOKES, J. R. COPPEDGE and R. L. RIDGWAY (*J. agric. Fd Chem.*, 1970, **18** (2), 195–198. 24 ref.).—Granular formulations of 10% aldicarb included granules of charcoal, corn cobs, compacted flour, $CaSO_4$ and cellulose acetate. Relative rates of aldicarb release into H_2O and soil were measured. The effective use of binders was studied. Bioassay of new growth of treated cotton plants with boll weevils, showed prolonged uptake of toxicant in the plant, with formulations which had slow rates of release in lab. H_2O immersion tests. Mixtures of fast and slow rate formulations gave a greater initial uptake which was more prolonged. M. J. Rawlins.

Colorimetric method for the microdetermination of 2-phenylphenol. A. RAJZMAN (*Analyst, Lond.*, 1970, **95** (1130), 490–497. 12 ref.).—The fungicide 2-phenylphenol (**I**) is extracted from the surface of fruit with $CHCl_3$ and the soln. mixed with excess glacial

AcOH contg. HCHO (1–2 moles HCHO/2 moles **I**). A soln. of Fe^{3+} (> ~150 μg/ml) in conc. H_2SO_4 is added and the intensity of the colour produced measured at 520 nm and referred to that of known standards. The method is specific and useful at the 1 μg **I** level. S. S. Chissick.

Biological effects and persistence of Dursban in freshwater ponds. S. H. HURLBERT, M. S. MULLA, J. O. KEITH, W. E. WESTLAKE and M. E. DÜSCH (*J. econ. Ent.*, 1970, **63** (1), 43–52. 28 ref.).—Dursban (*O,O*-Et_2O-(3,5,6-Cl_3-2-pyridyl)phosphorothioate) was applied 4 times at 2-week intervals to small ponds at dosages of 0·01, 0·5, 0·10 and 1·0 lb/acre. There was considerable mortality amongst mallard ducks but only slight mortality of caged mosquito fish. Extensive investigation of 4 zooplankton species was carried out and residues found in the mud, vegetation and animals are described. C. M. Hardwick.

Investigation of the toxicity of insecticides to birds' eggs using the egg-injection technique. J. F. DUNACHIE and W. W. FLETCHER (*Ann. appl. Biol.*, 1969, **64** (3), 409–423. 17 ref.).—Injection of 25 insecticides into the hen egg yolk showed that organo-P compd. were much more toxic to hatching than were organo-Cl compd. The insecticides were generally more toxic when dissolved in maize oil than in acetone. The results indicate that most insecticides are harmless to eggs either because they are not toxic in the concn. so far found in U.K. or because they are unlikely to pass through the mother bird to the egg. A. H. Cornfield.

Possible selective mechanisms in the development of insecticide-resistant fish. M. T. FINLEY, D. E. FERGUSON and J. L. LUDKE (*Pestic. Monitoring J.*, 1970, **3** (4), 212–218. 14 ref.).—Data from bioassays and g.c. analyses suggest that insecticide contamination resulting from runoff is a major selective factor in the development of resistant fish populations. DDT and toxaphene were the major agents found. E. G. Brickell.

Influence of body weight on chronic oral DDT toxicity in coho salmon. D. R. BUHLER and W. E. SHANKS (*J. Fish. Res. Bd Can.*, 1970, **27** (2), 347–358. 22 ref.).—Median survival time was directly proportional to body wt. in young coho salmon of the same age that were fed a diet contg. technical DDT. E. G. Brickell.

4.—MISCELLANEOUS

Radiochemical determination of caesium-137, strontium-89, 90 and barium-140 in milk and bone ash. M. SENEGAČMK, Š. PALJK and J. KRISTAN (*Z. analyt. Chem.*, 1970, **249** (1), 39–41. Engl., 6 ref.).—After alk. fusion of the ash, ^{137}Cs is separated by leaching with H_2O. The residue, dissolved in HCl, is passed through Dowex 50W, X-8. Radio-Y, and rare earths fission products, Mg and Ca are eluted with 1M-NH_4 lactate at pH 7·5; ^{90}Sr, ^{140}Ba and ^{224}Ra are separated by elution with 0·15M-NH_4 citrate at pH 7·5. Radio-Cs is isolated as Cs-dipicrylaminate, $^{89}Sr/^{90}Sr$ as $SrCO_3$, ^{140}Ba as $BaCO_3$. The elements are then counted on a low-background β-counter. With 80% av. chem. yield for all detd. radionuclides, the limits of detection are ~0·02 pCi/g for milk ash (10 g) and ~0·04 pCi/g for bone ash (4 g). P. J. Herbert.

Automated method for determination of lactic acid. J. D. CAMERON and B. J. FRANCIS (*Analyst, Lond.*, 1970, **95** (1130), 481–484. 3 ref.).—A method is described for detn. of L (+) lactic acid in protein-free extracts at concn. > 540 μg/ml at a rate of 20 samples/h. Biol. material is homogenised with $HClO_4$, centrifuged, and the supernatant liquid neutralised with aq. K_2CO_3. The soln. is mixed with buffered lactic dehydrogenase soln., nicotinamide adenine dinucleotide and air in a Technicon AutoAnalyzer and, after 12 min, passed through a spectrophotometer flow cell and the absorbance measured at 340 nm. A special washing device is described to prevent carry-over between successive samples. S. S. Chissick.

5.—RECENT BOOKS AND JOURNALS

Chemical and botanical guide to lichen products. C. C. CULBERSON. 1969, 628 pp. (Chapel Hill, U.S.A.: University of North Carolina Press). S. C. H.

Nutritional disorders in cucumbers and gherkins under glass. J. P. N. L. ROORDA VAN EYSINGA and K. W. SMILDE. 1969, 46 pp. (Wageningen: Centre for Agricultural Publishing & Documentation). S. C. H.

Since Silent Spring. F. GRAHAM, JUN. 1970, 297 pp., 40/–. (London: Hamish Hamilton).—This book (22 chapters) describes the genesis of Rachel Carson's book 'Silent Spring' and the progress of the controversy aroused by it. Alternatives to conventional pesticides (insect and plant hormones, sex attractants, breeding of resistant plants, etc.) are briefly discussed. References are given for each chapter and six of the chapters are largely based on the papers and correspondence of R. Carson. P. P. R.

Sorghum (Tropical Agric. Series). H. DOGGETT. 1970, xvi + 403 pp., 52 illustr., 14 colour plates., 130/–. (London: Longmans Group Ltd.).—After a historical introduction, the morphology, reproduction, cytology and genetics of sorghum are discussed in detail. The next two chapters are concerned with breeding. Uses of grain sorghum as food, in brewing, as stockfeed for cattle, pigs and poultry, as a source of starch and of a natural red dye are given, also the production of syrup and sugar from sorgo and the use of Sudangrass as forage. Prepn. of, e.g., warehouse or barn brooms from broomcorn (broom-millet) is described. The succeeding chapters deal with plant, bird and insect pests of sorghum and fungal diseases (several colour plates). There are many references, and a good index. P. P. R.

Phenolic substances in grapes and wine and their significance. V. L. SINGLETON and P. ESAU. 1969, 282 pp. (New York & London: Academic Press).—Advances in Food Research, Supplement 1. S. C. H.

The cheese handbook. B. H. AXLER. 1970, 110 pp., 30/–. (London: Cassell & Co. Ltd.). S. C. H.

Refrigeration, air-conditioning and cold storage: principles and applications. R. C. GUNTHER. 1969, 2nd rev. and enlarged edn, 1398 pp. (Philadelphia, etc.: Chilton Book Co.). S. C. H.

Aflatoxin: scientific background, control and implications. Ed. L. A. GOLDBLATT. 1969, 472 pp. (New York & London: Academic Press).—**Introduction.** L. A. GOLDBLATT. (24 ref.); **Aflatoxin formation by *Aspergillus flavus*.** V. L. DIENER and N. D. DAVIS. (176 ref.).; **Structure and chemistry.** G. BUCHI and I. D. RAE. (29 ref.).; **Physicochemical assay.** W. A. PONS, JUN. and L. A. GOLDBLATT. (75 ref.).; **Biological assay.** M. S. LEGATOR, (68 ref.); **Metabolism and biochemical effects.** G. N. WOGAN. (64 ref.); **Types of mycotoxins in foods and feeds.** A. J. FENELL. (131 ref.); **Aflatoxicosis in laboratory animals.** W. H. BUTLER. (50 ref.); **Aflatoxicosis in farm animals.** R. ALLCROFT. (59 ref.); **Aflatoxicosis and trout hepatoma.** J. E. HALVER. (50 ref.); **Fungal spoilage in stored crops and its control.** C. GOLUMBIC and M. M. KULIK. (115 ref.); **Processing to ensure wholseome products.** C. J. KENSLER and D. J. NATOLI. (18 ref.); **Detoxification of aflatoxins in foods and feeds.** F. G. DOLLEAR. (80 ref.); **Regulatory aspects of control of mycotoxins in foods and feeds.** B. L. OSER. (6 ref.); **Implications of fungal toxicity to human health.** H. F. KRAYBILL and R. E. SHAPIRO. (194 ref.). S. C. Haworth.

Pathogenic anaerobic bacteria. L. D. SMITH and L. V. HOLDERMANN. 1968, 423 pp. (Springfield, Ill.: Charles C. Thomas Publisher). S. C. H.

Pictorial keys: arthropods, reptiles, birds and mammals of public health significance. 1969, 192 pp., $2.25. (Atlanta, Ga.: U.S. Dept. of Health Education & Welfare).—Public health service publication no. 1955. S. C. H.

Microbial contamination control facilities. Ed. R. S. RUNKLE and G. B. PHILLIPS. 1969, 198 pp. (New York, etc.: Van Nostrand Reinhold Co.).—Van Nostrand Reinhold Environmental Engineering Series, sponsored by Biological Contamination Control Committee, American Association for Contamination Control. S. C. H.

Recommended procedures for measuring the productivity of plankton standing stock and related oceanic properties. BIOLOGICAL METHODS PANEL, COMMITTEE ON OCEANOGRAPHY. 1969, 59 pp. (Washington, D.C.: National Academy of Sciences, Division of Earth Sciences, National Research Council). S. C. H.

Marine biology: an introduction to its problems and results. H. FRIEDRICH; transl. G. VEVERS. 1969, 474 pp., 70/–. (London: Sidgwick & Jackson). S. C. H.

Eutrophication: causes, consequences, correctives. 1969, 661 pp. (Washington, D.C.: National Academy of Sciences).—Proceedings of Symposium held at Madison, Wisc., June 1967. S. C. H.

The environmental revolution: a guide for the new masters of the earth. M. NICHOLSON. 1970, 366 pp., 84/–. (London: Hodder & Stoughton). S. C. H.

AUTHOR INDEX

J. Sci. Fd Agric., 1970, Vol. 21, September

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

Printed in England by Staples Printers Limited at their Kettering, Northants, establishment