A TECHNIQUE FOR THE APPROXIMATE QUANTITATIVE PREDICTION OF FLAT SOURING IN CANNED PEAS

By G. G. KNOCK

A technique for the survey of pea-canning lines is described. It is shown that the percentage of canned peas that will sour on incubation at 131° F (55·0° C) may be predicted approximately by enumerating the flat-sour spores in unprocessed cans, expressing the cannery process in terms of decimal reduction times for flat-sour spores, calculating the number of surviving spores per can and from this the percentage spoilage. Graphs have been drawn to simplify the expression of processing times as decimal reduction times, and for calculating the percentage spoilage from the number of surviving spores per can.

In the Union of South Africa, the Regulations of the Department of Agriculture provide for the incubation at 131° F (55·0° C) of samples from each day's production of canned vegetables intended for export; if souring (or gas production) is found in more than $2\frac{1}{2}\%$ of the samples incubated at this temperature, the export of the product is prohibited. In S. Africa flat-sour spores of high average heat-resistance are found in large numbers on vined peas, and in-plant multiplication is seldom the primary cause of flat-sour spoilage. The existence of this Regulation therefore imposes a difficult task on canners, and has created a need for a technique that will not only detect the presence of 'flat-sour' organisms in the canning plant, but give an indication of the final percentage spoilage that would result from a given level of contamination. Incubation of processed cans for 5–10 days is too slow, since, by the time the presence of flat-sour organisms is detected in this way, and the results of the appropriate remedial measures are known, the production from at least two full working weeks will already have been packed; moreover, if the initial attempts to reduce contamination are found to have been unsuccessful, nearly half the pea-canning season may be over before the results of any further changes are known.

Few detailed descriptions of techniques for the bacteriological survey of vegetable-canning plants appear to have been published, but, during a visit to Washington in 1947, Dr. Yesair, of the National Canners' Association, kindly supplied the author with details of the method used there; these methods formed the starting point for the work described in this paper and they provide a valuable guide to the general bacteriological condition of the canning plant. However, they are not designed to provide data from which the percentage of souring could be predicted in processed cans.

Johnson *et al.*[1] drew attention to the fact that the number of flat-sour spores needed to sour a can of peas was very small, and that it was quite possible that the plating of a few millilitres of the washings from the peas in an unprocessed-line can would fail to detect the presence of small numbers of spores. The authors employed a flask test in which 100 g. of peas covered with brine was processed for 35 minutes at 240° F (115·5° C), incubated at 131° F (55·0° C) and tested for acid production daily for 5 days. By this method smaller numbers of flat-sour spores could be detected, but here again the results could not be interpreted quantitatively.

Recently, a method has been described by Murdock & Folinazzo[2] in which line samples are processed in small (202 × 202) cans, to which a nutrient medium is added where necessary, and 'spore-tested' by processing to an F_0 value of 1 or 2 minutes. The cans are then incubated for 48 hours at 131° F (55·0° C) and tested for souring. The authors, who compared the results obtained by their own technique with a plating method described by Wilson & Tanner[3] and a modification of Johnson's flask test, concluded that a combination of the 'plate' and 'can' methods showed the most promise, since the plate method indicated the numbers of spores present and the can method detected the presence of small numbers of spores. None of the counts, or positive and negative results, obtained by these methods are related to the processing conditions or to actual spoilage in the processed cans.

It is claimed for the method described in this paper that small numbers of spores can be detected, and that the spore counts obtained on the unprocessed-line cans may be used to estimate the percentage of cans souring on incubation. The work involved is not too much for a cannery control-laboratory to undertake, and has been carried out in several canneries in S. Africa for the past two years by qualified chemists without specialized bacteriological training.

Media and methods

The only culture-medium required is dextrose-tryptone agar[4] made in single and double strength.

The standard formula is: Bacto-tryptone (10 g.), agar (20 g.), dextrose (5 g.), bromocresol purple (0·04 g.) and water (1000 ml.). The ingredients are steamed until dissolved and adjusted to pH 6·8–7·0 after which the medium is filtered if necessary, filled into screw-cap bottles in 100-ml. amounts, and sterilized at 15 lb. pressure for 20 minutes. It is advisable to test for sterility by incubating the bottles at 131° F (55·0° C) for 48 hours, as some agar powders have been found to contain large numbers of flat-sour spores.

The double-strength medium is made by using half the quantity of water. 50 ml. is filled into screw-cap bottles graduated at the 100-ml. mark, sterilized at 15 lb. pressure for 25 minutes, and tested for sterility.

In the cannery surveys for flat-sour organisms made by this Laboratory, samples of blancher water, washed blanched peas, brine and unprocessed-line can are collected in clean cans at the beginning and end of one or more shifts; they are examined by the following method.

Blancher water.—Blancher water (50 ml.) is poured into a six-ounce screw-cap bottle and autoclaved for 10 minutes at 240° F (115·5° C). A volume (3 ml.) of the sample is pipetted into each of two Petri dishes and poured with dextrose–tryptone agar.

Washed blanched peas.—Peas (75 g.) are weighed into a clean washed can or screw-cap jar and an equal weight of water is added. The container is closed and shaken vigorously for exactly 1 minute; 50 ml. of washings is then poured off into a six-ounce screw-cap bottle graduated at the 100-ml. mark and containing 50 ml. of molten double-strength dextrose–tryptone agar. While still hot the mixture is autoclaved for 10 minutes at 240° F (115·5° C), after which four plates are poured. If it is necessary to examine samples of peas from other points in the line they are treated in the same way.

Brine.—The brine is poured into a six-ounce screw-cap bottle graduated at the 100-ml. mark and containing 50 ml. of molten double-strength dextrose–tryptone agar. While still hot, the mixture is autoclaved for 10 minutes at 240° F (115·5° C); four plates are poured.

Unprocessed-line can.—The closed, unprocessed-line can is shaken vigorously for 1 minute, after which it is opened and the liquid poured off; this liquid is treated in the same way as the washings from the blanched peas.

The small domestic-type pressure-cooker is convenient for autoclaving the samples but the pressure gauge must be checked for accuracy, and it is advisable to fit a thermometer. All cultures are examined after incubation at 131° F (55·0° C) for 48 hours, when all the acid-producing colonies are counted.

Incubation tests.—At least 12 processed cans are collected to correspond with each sampling time; these are incubated at 131° F (55·0° C) for 7 days and checked by pH measurements, for evidence of souring, supplemented if necessary by microscopical and cultural tests.

Interpretation of results

Blancher-water flat-sour counts are graded by the National Canners' Association as follows: 0–15 colonies per 6 ml.—low contamination; 15–30 colonies per 6 ml.—moderate contamination; more than 30 colonies per 6 ml.—heavy contamination.

This grading of blancher-water contamination is useful. A 'low' blancher-water count never causes serious contamination of the washed blanched peas unless the blancher-exit sprays are obviously inadequate in volume, distribution or pressure, but when the blancher-water count rises to 'moderate', a significant number of flat-sour organisms may pass forward on the peas to the cans.

Moderate to heavy blancher-water contamination is often caused by accumulation of spores carried there on the peas; this indicates that the washing is inefficient or insufficient in relation to the spore-load carried by the peas. The condition is aggravated if the overflow from the blancher is small. Actual multiplication of flat-sour bacteria in the blancher during canning operations practically never occurs, because the temperature is above the thermophilic growth range, but multiplication is extremely common in short shut-down periods, during which the spore-count may be doubled, even if the blancher is drained and refilled before sampling. Multiplication has also been noted during periods of non-operation if the steam-inlet valve is faulty or not tightly closed.

The flat-sour counts found in the brine and washed blanched peas are considered in relation to the count of the unprocessed-line can. If the count of the unprocessed-line can is high, then one of the other two counts usually indicates the source of the spores, but if the count in the unprocessed-line cans exceeds either of the other counts, then the filler bowl and

valves require investigation, in spite of the theoretical relationship between the counts in the unprocessed-line can, brine and washed blanched peas (see below).

With a good sample of canner's grade of sugar the brine count is usually 0 per 50 ml., and under satisfactory operating conditions the washed blanched-pea count does not exceed 1 or 2 spores per 50 ml. of washings.

Prediction of the percentage of infected cans after processing

The prediction of the probable percentage of infected cans in the processed pack is based on three considerations: (1) the number of flat-sour spores per unprocessed-line can; (2) the decimal reduction time of flat-sour organisms; and (3) the lethal effect of the processing time expressed in terms of decimal reduction times. In addition, it is assumed that one surviving spore is sufficient to sour a can.

Calculation of flat-sour spores per can

Taking the A1 tall (301 × 411) can as an example, the minimum drained weight of peas is, in South Africa, 10·4 oz., or approximately 300 g., and the volume of brine is about 150 ml. The 50-ml. sample from the unprocessed-line can therefore represents one-third of the brine in the can, so that the count must be multiplied by three. (In other can-sizes it is of course necessary to multiply by the denominator of whatever fraction of the brine content the 50-ml. sample represents.) The figure thus obtained is then multiplied by 5/2 to allow for the spores still adhering to the peas.[5] Again, the number of spores surviving 10 minutes at 240° F (115·5° C) is approximately one-tenth of the numbers originally present, since, with the coming-up and coming-down times in a small retort, the heating is equivalent to 1 decimal reduction time (see below). If, therefore, one spore is found in 50 ml. of the brine in an A1 tall can, the number of spores per can is 75 (3 × 5/2 × 10).

Theoretically an error occurs if the brine count is high; it should be possible to correct this by subtracting the brine count from the count of the unprocessed-line can, multiplying the remainder by 5/2, adding the brine count and then multiplying by three. In practice, however, the brine count is (if it contains any spores at all) usually higher, volume for volume, than the count of the unprocessed-line can; the reason for this is not clear but is presumably connected with the adhering of spores to the peas. Again, in theory, the total number of spores in an unprocessed A1 tall can amounts to three times the brine count per 50 ml. plus six times the washed blanched-pea count, since this is made on 50 g. of peas, but in practice the numbers found in the cans are always fewer, indicating that, in passage from the blancher to the filler, spores are lost or germinate sufficiently to become heat-labile.

Calculation of decimal reduction time

Numerous strains of flat-sour organisms present in canned peas that had soured on incubation at 131° F (55·0° C) were identified by the methods described by Smith, Gordon & Clark;[6] all were found to conform to the description of *Bacillus stearothermophilus*.

The death rate of the spores of one such strain that had caused extensive spoilage in canned peas (No. 014) was determined in canned-pea brine (pH 5·90) at temperatures of 250° F (121·1° C), 245° F (118·3° C), 240° F (115·5° C), 230° F (110·0° C) and 220° F (104·4° C) (see Fig. 1) and expressed in terms of the decimal reduction time (D.R.T.). This term was introduced by Katzin *et al.*,[7] who defined it as the time required at a given temperature to reduce the population to 10% of the original number; such a conception of the death rate has the great advantage of being directly applicable to processing problems.

From Fig. 1 it will be seen that the D.R.T. is 4 minutes at 250° F, 12 minutes at 240° F, 36 minutes at 230° F, and 124 minutes at 220° F. This equals the D.R.T. reported by Gillespy[8] for thermophilic anaerobes in pea brine.

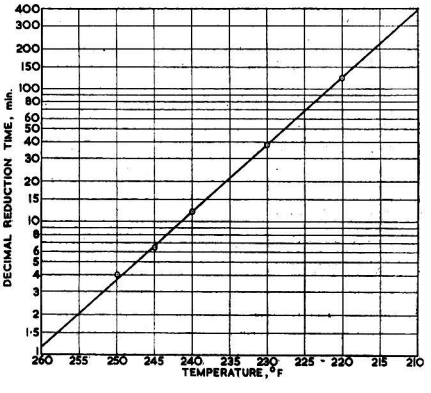

FIG. 1.—*Death rate of spores of* Bacillus stearothermophilus *(strain* 014)

As a check on this laboratory determination of the decimal reduction time, an estimate of the D.R.T. under practical canning conditions was obtained by counting the number of spores in unprocessed-line cans and observing the percentage of cans that soured on incubation after a given process.

A calculation based on an actual example may be taken as an illustration. In a batch of peas processed at 240° F for a time integrating to 33 minutes at 240° F there were 104 spores per unprocessed-line can, and 10 out of 60 of the processed cans soured on incubation.

Let N = the total number of samples examined (60), M = the number of defective samples (10), A = the mean initial number of spores per sample (104), and S = the mean number of surviving spores per sample. The most probable mean number of surviving spores per sample is given by the natural logarithm of $N/(N - M)$, that is $S = \ln [60/(60 - 10)] = \ln 1{\cdot}2 = 0{\cdot}18$. The D.R.T. may be estimated from the equation $\log S = \log A - t \log K$, where $1/K$ is the fraction that survives unit time. For the derivation of this equation see Gillespy.[9]

Since the decimal reduction time is required, we have $K = 10$ and t = the number of D.R.T. values attained by the process; therefore $\log S = \log A - t \log 10$ or $\log 0{\cdot}18 = \log 104 - t$, from which $t = 2{\cdot}0170 - \bar{1}{\cdot}2553 = 2{\cdot}7617$.

The process is therefore equivalent to $2{\cdot}7617 \times$ D.R.T. and D.R.T. $= 33/2{\cdot}7617 = 11{\cdot}9$. Similar calculations on other data have given values ranging from 11 to 13¼ minutes.

Lethal effect of the processing time expressed in terms of D.R.T.

Fig. 2 shows the relationship between decimal reduction times and actual processing at 260° F (126·6° C), 250° F (121·1° C), 245° F (118·3° C) and 240° F (115·5° C) in a still retort; an allowance of 42% was made for the 'come-up' time.[10] The D.R.T. values were calculated in a manner analogous to the method of Taggart & Farrow,[11] which is a simplification of Bigelow's[12] 'area under the curve' method. A special graph paper was prepared on which the reciprocals of the decimal reduction times determined at various temperatures under laboratory conditions were marked off on the vertical scale; the temperature lines are drawn at distances proportional to the decimal reduction times at the corresponding temperatures. The heat-penetration data were then plotted on this paper, and the decimal reduction time values determined by measuring the area under the curve. The rate of heat transfer in peas packed in A1 (211 × 400), A1T (301 × 411), A2 (307 × 408) and A2½ (401 × 411) cans is very nearly the same, so that Fig. 2 may be used for any of these can-sizes.

Prediction of percentage souring from the unprocessed-line-can count

The method by which the percentage of cans souring may be predicted is as follows:

Step 1. Find r, the number of spores recovered from 50 ml. of unprocessed-line can, by the method described, i.e. after heating for one D.R.T.

Step 2. From Fig. 2 find t, the D.R.T. value of the cannery process. The cannery process should be timed to include 42% of the retort 'come-up' time.

Step 3. Calculate $10^{-t}Cr$, where Cr is the estimated number of spores per unprocessed-line can. The values of C are: for A1 (211 × 400) cans, 45; A1T (301 × 411) cans, 75; A2 (307 × 408) cans, 90; and A2½ (401 × 411) cans, 135; provided that the ratio of peas to brine is about 2 : 1.

Step 4. From Fig. 3 find the estimate of the most probable percentage of infected cans.

As an example, assume that in an A1T can $r = 2$ and that the cannery process is 40 minutes at 240° F (115·5° C). From Fig. 2 it will be seen that this process is equivalent to 2·6 D.R.T. values. The value of $10^{-t}Cr$ is, therefore, $75 \times 2 \times 10^{-2{\cdot}6} = 0{\cdot}375$, which, from Fig. 3, is equivalent to 32% of infected cans.

Explanatory note

The calculation of the percentage of infected cans from the number of spores detected in the unprocessed-line can is based on the following considerations:

If N is the total number of cans, and x is the mean number of surviving spores per can, then the most probable number of cans souring is given by $N\{1 - [(N - 1)/N]^{Nx}\}$.

When N is large, a close approximation is given by $N(1 - e^{-x})$. If r spores are found in 50 ml. of the unprocessed can by the standard method, there are Cr spores in the whole can; C varies, as already stated, with the size of can and the ratio of pea to liquor.

If t is the D.R.T. equivalent of the cannery process, then the mean number of surviving spores per can is given by $x = 10^{-t}Cr$. Thus the estimate of percentage spoilage is given by

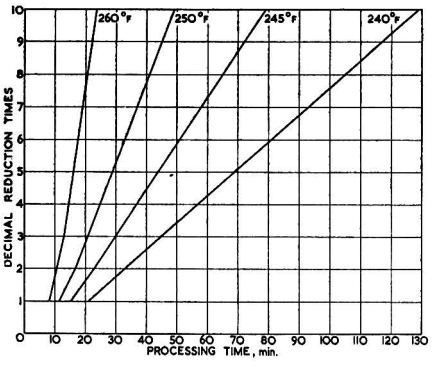

FIG. 2.—*Relationship between decimal reduction times and processing times at various temperatures*

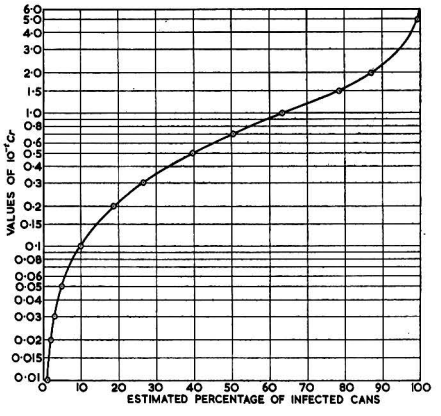

FIG. 3.—*Plot of* $10^{-t}Cr$ *against percentage spoilage*

$100[1 - e^{-10^{-t}Cr}]$. The calculation is facilitated by the use of Fig. 3, in which the values of $10^{-t}Cr$ are plotted against percentage spoilage.

Some practical results

It may be of interest to give the results of some cannery surveys which illustrate the method; all the figures given refer to actual counts on A1T cans examined by the technique described in this paper.

1. Low contamination

	Process: 40 minutes at 240° F Flat-sour counts			
	a.m. Start-up	a.m. Shut-down	p.m. Start-up	p.m. Shut-down
Blancher water, 6 ml.	3	12	8	15
Washed blanched peas	0	2	0	0
Brine, 50 ml.	0	0	0	0
Unprocessed-line can	0	0	0	0
Proportion of incubated cans showing souring	0/12	0/12	0/12	0/12
Predicted souring, %	0	0	0	0

2. Blancher-water contamination: over-night build-up

		Process: 50 minutes at 240° F		
Blancher water, 6 ml.	over 300	29	7	21
Washed blanched peas	79	11	2	3
Brine, 50 ml.	0	0	0	0
Unprocessed-line can	16	0	0	0
Proportion of incubated cans showing souring	8/12	0/12	0/12	0/12
Predicted souring, %	38	0	0	0

3. Blancher-water contamination: persistent

		Process: 50 minutes at 240° F		
Blancher water, 6 ml.	over 300	94	247	over 300
Washed blanched peas	51	12	16	25
Brine, 50 ml.	0	0	0	0
Unprocessed-line can	17	2	7	13
Proportion of incubated samples showing souring	3/12	2/12	3/12	3/12
Predicted souring, %	40	6	19	32

4. Contaminated brine and blancher water

		Process: 45 minutes at 240° F		
Blancher water, 6 ml.	73	26	35	31
Washed blanched peas	31	26	17	19
Brine, 50 ml.	29	5	16	11
Unprocessed-line can	16	2	9	3
Proportion of incubated cans showing souring	6/12	3/12	5/12	3/12
Predicted souring, %	87	15	49	37

5. Heavy infection of vined peas and blancher

	Process: 40 minutes at 240° F Flat-sour counts
Peas as received at cannery	673
Peas after first reel-washer	474
Peas after size-grader (sprays fitted)	266
Blancher water, 6 ml.	720
Blanched peas	535
Washed blanched peas	235
Brine, 50 ml.	0
Unprocessed-line can	549
Proportion of incubated cans showing souring	12/12
Predicted souring, %	99

Effect of can size on processing requirements of convection-heating packs

Since the rate of heat penetration in convection-heating packs such as peas is nearly the same in, for example, 10-oz. and 30-oz. cans, it is usually assumed that the same processing time will suffice for both. This assumption is logical only when the process for each can-size is based on the 'absolute' destruction time of a particular organism, for in this instance the degree of initial infection is then not considered. However, since the death-rate is in fact logarithmic, it is clear that after any given process the percentage sterility attained will be less in the larger cans because the initial infection is proportional to their capacity; in fact, the number of surviving spores is directly proportional to the volume of the can. The difference is small when no organisms are present that have a D.R.T. exceeding a few minutes, but when thermophilic organisms are involved it may be considerable. For example, if peas carrying the same load of spores are filled into A1 and A2½ cans and processed for 45 minutes at 240° F (equivalent to 3 D.R.T. values), and $r = 2$, then in A1 cans $C = 45$ and $10^{-t}Cr = 45 \times 2 \times 0{\cdot}001 = 0{\cdot}09$, and the percentage spoilage is therefore 8·5%; in A2½ cans, $C = 135$ and $10^{-t}Cr = 135 \times 2 \times 0{\cdot}001 = 27$, and the percentage spoilage = 24%.

The extra processing required to reduce 27 surviving spores per 100 cans to 9 spores per 100 cans may be calculated from the equation $\log S = \log A - t$. If $A = 27$ and $S = 9$, then $\log 9 = \log 27 - t$, that is $0{\cdot}9542 = 1{\cdot}4314 - t$, therefore $t = 0{\cdot}4772$; but D.R.T. at 240° F is 12 minutes, the extra time is therefore $12 \times 0{\cdot}4772 = 6$ minutes (approx.).

Discussion

Prediction of the percentage of potential flat-sour cans in a pack by counting the spores in unprocessed-line cans is unlikely to be very exact, but neither is an estimate based on incubating only 12 cans.

Fiducial limits for an observation of M soured cans out of 12 (and out of 24) incubated cans are given in Table I. They were calculated by means of Table VIII, Fisher & Yates.[13] A practical interpretation of these limits is that the true percentages lie within the ranges tabulated with a probability of 0·95. In only one of the 16 examples above does the prediction lie outside the range estimated by incubating 12 cans.

Table I

Fiducial limits of defective cans, %, when M cans are observed to be defective in a sample of N cans

	Fiducial limits ($p = 0{\cdot}025$)	
M	$N = 12$	$N = 24$
0	(0)–26	(0)–14
1	0·21–38	0·11–21
2	2·1–48	1·0–27
3	5·5–57	2·7–32
4	9·9–65	4·8–37
5	15–72	7·1–42
6	21–79	9·8–47
7	28–85	13–51
8	35–90	16–55
9	43–94	19–59
10	52–98	22–63
11	62–99·8	26–67
12	74–(100)	29–71

For $N = 24$ and $M > 12$, enter the Table at $N - M$ and subtract the tabulated limits from 100

Technically, the counting of flat-sour spores on samples of peas depends upon careful adherence to a standardized technique, especially the shaking of the samples in order to free the spores from the peas, and the autoclaving before culturing. Again, spores may deviate in heat resistance from the values on which the percentage survivals are calculated, and any serious maldistribution of spores in the cans would greatly affect the accuracy of the method.

There are, no doubt, less-heat-resistant strains of flat-sour organisms than that on which the calculations in this paper have been based, but since the purpose of cannery surveys is to give an early indication that souring is likely to exceed a small percentage, an over-optimistic forecast of the position would only be made if the flat-sour strains present had a D.R.T. greater than that which has

been taken as the standard. Such a strain has not so far been reported in South Africa, although an examination of the data given by Williams, Merrill & Cameron[14] for the flat-sour organism known as ' No. 1518 ' suggests that more-resistant strains might be encountered. The presence of such an exceptionally heat-resistant strain would soon be made apparent if the methods described in this paper were in use, in which event a rough estimate of its D.R.T. could be made by making use of the equation $\log S = \log A - t \log K$, as already described.

The number of spores on canned peas may rise and fall during the course of a shift as the level of contamination changes, but it is the author's experience that at any given time the distribution of spores varies little from can to can ; it is probable that this is a result of the bulk handling of the peas, which distributes the infection evenly both at the vining station and in the canning line.

However, in spite of the several possible sources of error, experience over the last two years has shown that the prediction of the percentage souring from the count of the unprocessed-line can is sufficiently accurate to be of considerable assistance in the control of flat-sour spoilage. It would be reasonable to expect some difficulty when the processing time is near the minimum normally used commercially—say 35 minutes at 240° F, for at this level of processing the detection of only one spore by the standard method predicts that 41% of processed A1T cans will be infected, but it is found in practice that souring rarely occurs in incubated samples when no spores are recovered from the unprocessed cans. The accuracy of the method may be increased by culturing more than one unprocessed-line can ; thus, if one spore were found in 10 cans by the standard method the number of infected cans would not exceed 6%. If this multiplication of the samples examined imposes too great a strain on the available laboratory facilities, the absence of flat-sour spores on the washed blanched peas, in the brine and in the unprocessed-line can, provides strong evidence that contamination is at a low level, especially when these counts are found to be low over a period of several days.

It is suggested that the procedure described in this paper may have more general application, since, by estimating the initial infection with the more-heat-resistant organisms in any particular pack and determining their decimal reduction times, it may be possible to apply a process that will give an acceptable predetermined percentage of sterile cans.

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VOLATILE FATTY ACIDS IN LABORATORY AND FIELD SILAGE

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The results of work conducted on the formation of volatile fatty acids in laboratory slurries of minced crop and water mixtures is presented. The absence of formic acid in such slurries has been noted. The occurrence of acids higher than C_6, under varying conditions of temperature, aeration or anaerobiosis, has not been demonstrated. Straight-chain volatile acids from C_1 to C_8 have been shown to occur in field silage but only occasionally has the presence of branched-chain acids been noted. The principal volatile acid present is acetic acid.

Introduction

In a classical work, Russell[1] steam-distilled 10 kg. of silage and collected 10 l. of distillate from which he isolated various lower fatty acids as their silver salts. There has been a tendency since then to consider that the most significant fatty acid present in silage is butyric acid and to believe that this acid occurs to a large extent only in material of high pH; a high total concentration of volatile fatty acids (V.F.A.) has also been looked upon as characteristic of high-pH silage. If the rigorous definition of silage as a material formed under precise conditions of fermentation resulting in the establishment of a pH of 3·8–4·2 throughout the mass is excluded, two questions arise. First, is butyric acid characteristic only of bad silage? Secondly, does butyric acid, if present, merely indicate poor-quality material or is it in some way toxic to the animal? The second question may be answered immediately. Butyric acid is a normal product of cellulose fermentation in the rumen[2–4] and the acid is probably a precursor of glycogen.[5] Again, Zelter[6] has shown that the feeding of calcium butyrate, or silage rich in the acid itself, results in an increase of the butter-fat content of the milk produced. There is no question of butyric acid itself producing a taint in milk or cheese, but silage containing much butyric acid will also contain a large number of the *Clostridium* spp. that produce it and contamination of milk may result.[7, 8] Thus, butyric acid is not harmful in silage, but large amounts of it may be characteristic of material not of the highest technical quality.

The older techniques for estimating volatile fatty acids in silage are due to Dyer,[9] Foreman,[10, 11] Wiegner[12] and Lepper.[13] Foreman's method gives the total volatile acids, but the others involve the use of formulae, based on the distillation constants of acetic and butyric acids, to arrive at estimates of these constituents. No account is taken of propionic acid, which is present in silage, and leads to a confusion in calculation by these methods. The work of Axellson[14] suggests that it is possible to calculate the butyric acid content of silage from its pH.

Paper-chromatographic methods include those of Hiscox & Berridge,[15] used by Virtanen & Miettinen,[16] and a modification of the Reid & Lederer[17] technique as evolved by Duncan & Porteous.[18] We have used Duncan & Porteous' method, as well as the column methods of Moyle, Baldwin & Scarisbrick[19] and of James & Martin.[20] The last is undoubtedly the best method for research work. Finally, the counter current-distribution method of Sato, Barry & Craig[21] may be mentioned; this has not been used in the present work.

Experimental

No attempt will be made here to describe in detail the use of the James & Martin[20] gas-phase chromatographic column or the Duncan & Porteous[18] paper-chromatographic method.

Materials

Slurries.—These have been prepared by means of the apparatus devised by Barnett[22] and incorporating minced grass or kale (1400 g. of fresh crop with 2 l. of water). The effects of various conditions of aeration, anaerobiosis and temperature have been studied by Barnett & Duncan.[23–26]

Field-silage samples.—These were collected in the field by cutting into a block from the silage mass. On arrival at the laboratory the material was well mixed and the pH determined on a portion (100 g.) in the usual way with a glass electrode and a Cambridge pH-meter.

Chromatography

Preparation of sample.—Slurry and field effluents may be used without further treatment in 5-μl. quantities when paper chromatograms are prepared by the Duncan & Porteous[18] technique. The results thus obtained are quantitative for C_3–C_6 acids but not for acetic acid, owing to interference by lactic acid.

In order to obtain full quantitative results, steam distillation of the effluents is performed in the following way. Of the effluent, 0·5–10 ml. is distilled from a buffer solution [1 ml. of 5% (w/v) oxalic acid + 1 ml. of 10% (w/v) potassium oxalate per g. or ml. of material used]. A few drops of a solution of 1% octyl alcohol in liquid paraffin (B.P.) are added before distillation. For the distillation, if a volume less than 10 ml. is used, a Markham[27] micro-Kjeldahl apparatus is suitable and about 80 ml. of distillate should be collected.

For field silage, 10 g. of minced material, from at least 200 g. of a minced preparation, is placed in a steam-distillation apparatus with 10 ml. of the oxalate buffer and a few drops of the octyl alcohol/paraffin solution. Steam distillation is conducted until no more volatile acid comes over as determined by titration with 0·1N-sodium hydroxide. It is found that about 300 ml. of distillate is sufficient to include all the V.F.A.

Paper method.—An aliquot of the neutralized distillate is evaporated to dryness; the residue is dissolved in 0·05 ml. of 60% (w/v) phosphoric acid and transferred to a column (20 mm. × 4 mm.) packed with AnalaR anhydrous sodium sulphate (which has been predried at 350° and cooled in a desiccator) and having at the top a 4-mm. layer of Celite 545 kieselguhr. The column is eluted with 0·6 ml. of anhydrous ether. To the ethereal solution of the free V.F.A. is added 0·1 ml. of ammonium hydroxide, and the ether is allowed to evaporate. The solution of ammonium salts is used in 5-μl. amounts for spotting on paper.

Column method.—The ethereal solution, prepared as described above, may be loaded directly on to the gas-phase chromatographic column and the run conducted according to the description of James & Martin.[20]

Results

In Table I are set forth the nature, initial composition and experimental conditions used in eleven of the slurries examined by us and the results obtained for the contents of individual V.F.A. present are shown in Table II.

Table I

Initial composition and experimental conditions of laboratory silage slurries

No.	Initial composition	Temp. of fermentation, ° c	Period of expt.	Conditions
1	1400 g. grass + 2 l. water	17–20	11 days	Anaerobic, continuous passage of nitrogen
2	,,	17–20	13 weeks	Anaerobic, nitrogen passed for 2 h. at start
3	,,	37	,, ,,	,,
4	,,	62	,, ,,	,,
5	,,	17–20	,, ,,	Aerobic, continuously aerated
6	,,	17–20	20 ,,	Anaerobic, nitrogen passed for 2 h. at start
7	,,	37	,, ,,	,,
8	,,	17–20	,, ,,	Aerobic, aerated for 2 h. at start
9	1400 g. kale + 2 l. water	17–20	,, ,,	Aerobic, continuously aerated
10	,,	17–20	,, ,,	Anaerobic, continuous passage of nitrogen
11	,,	17–20	,, ,,	6 g. SO_2 added

A study of these Tables indicates that the formation of higher acids is generally less at a lower than at a higher temperature and that exclusion of air is also involved in the inhibition of the formation of gross amounts of V.F.A. The effect of aeration is particularly notable in a kale slurry (No. 9), where a large production of fatty acids, accompanied by a complete loss of amino-acids,[28] is evident. In the kale slurry containing sulphur dioxide (No. 11) the preservative nature of this additive was evident, not only in V.F.A. formation, but also because amino-acid decomposition did not occur (Robertson & Barnett[28]). *n*-Caproic acid appeared in slurries Nos. 7 and 10 and appeared to be formed under conditions of aeration or high temperature (37°). Table III shows the average distribution of all the V.F.A. and their total amount in all the anaerobic and aerated grass slurries examined. High V.F.A. production occurs with rising pH and also with disappearance of lactic acid. In extreme cases the production of V.F.A. may be so great as to bring about a lowering of the pH, which is normally considered to indicate the degree of lactic acid content.

An examination of Table IV, in which are presented the results for the V.F.A. composition of a number of samples of field silage, shows that (*a*) acetic, propionic and butyric acids

Table II

The pH changes in the slurries mentioned in Table I and the different amounts of the various V.F.A. present at these different pH readings

Silage No.	Time of sampling	pH	Wt., mg./100 g. (dry-matter basis)					Total V.F.A. as ml. of N-NaOH
			Acetic acid	Propionic acid	*n*-Butyric acid	*n*-Valeric acid	*n*-Caproic acid	
1	Start	4·46	102					1·72
	1 day	4·88	258					4·30
	11 days	3·48	2424					40·50
2	Start	5·37	102					1·72
	1 day	4·74	258					4·30
	1 week	3·74	1140					19·04
	4 weeks	3·72	1738					28·90
	12 ,,	3·89	2510	578	3530			89·60
3	Start	5·37	102					1·72
	1 day	4·22	892		724			24·00
	1 week	3·59	1788		1570			47·60
	4 weeks	3·65	3160		2114			59·40
	12 ,,	3·95	4916	392	3074			121·50
4	Start	5·37	102					1·72
	1 day	4·50	102					1·72
	1 week	4·42	248					4·12
	4 weeks	4·44	413					6·87
	12 ,,	4·26	1304					22·00
5	Start	5·35	102					1·72
	1 day	5·26	146					2·72
	1 week	3·92	1570					31·50
	4 weeks	4·36	3180	482	804			82·70
	12 ,,	4·72	4570	1158	3580			160·00
6	Start	5·59	27·6					0·46
	1 day	5·46	528					8·80
	1 week	3·75	2590					43·10
	4 weeks	4·02	3430					57·00
	12 ,,	4·80	4100	589	233			79·40
	20 ,,	4·38	6150	1770	312			118·00
7	Start	5·59	27·6					0·46
	1 day	4·38	1414					23·40
	1 week	3·82	2690					44·70
	4 weeks	5·06	3850	1320	3560			121·60
	12 ,,	4·92	5300	1635	4470	543	310	170·00
	20 ,,	4·87	8480	1310	4200	723	616	220·00
8	Start	5·59	27·6					0·46
	1 day	5·59	467					7·94
	1 week	3·82	2220					36·80
	4 weeks	4·44	3990					74·00
	12 ,,	4·86	6410	1260	3030	361		162·00
	20 ,,	4·94	8520	1575	2180	633		195·00
9	Start	5·84	74	8				1·32
	1 day	5·60	58	16				1·14
	1 week	3·86	1150					19·10
	4 weeks	5·43	3550	650	3020	390		105·00
	12 ,,	6·48	4260	2156	252			106·00
	20 ,,	7·74	736					12·30
10	Start	5·84	74	8				1·32
	1 day	5·46	74	16				1·41
	1 week	3·94	1375					22·90
	4 weeks	3·90	2498	68	155			43·90
	12 ,,	5·20	5030	640	2760	662	313	115·00
	20 ,,	4·94	5710	961	3520	884	503	159·00
11	Start	3·70	74	8				1·32
	1 day	3·70	42					0·70
	1 week	3·84	53					0·88
	4 weeks	3·64	71					1·19
	12 ,,	3·60	84					1·41
	20 ,,	3·62	105					1·75

are present in all samples examined, whatever the pH range is. (*b*) Butyric acid is never present to the same extent as acetic acid. (*c*) Formic acid is present in many cases. (*d*) Of acids higher than butyric, caproic acid is of most frequent occurrence. (*e*) The only branched-chain acid observed is *iso*valeric acid. (*f*) Although in general, the higher the pH, the higher the V.F.A. content, this does not hold above about pH 5·5. It seems that material of a pH higher than that, whatever it may be, is not silage. (*g*) A study of the Tables, particularly

Table III

Average individual V.F.A. contents (mg.-% of D.M.) of the aerobic and anaerobic grass slurries mentioned in Table I

At the end of	1 day	7 days	28 days	84 days	140 days
Average pH	5·05	3·75	4·32	4·64	4·73
Average C_2 acids	512	2106	3238	4580	7717
,, C_3 ,,	0	0	360	1044	1552
,, C_4 ,,	0	0	873	2970	2230
,, C_5 ,,	0	0	0	181	452
,, C_6 ,,	0	0	0	62	205
Total V.F.A.	512	2106	4471	8837	12156

Table IV

The average V.F.A. contents of 42 field-silage (grass/clover) samples. Acid contents are expressed in mg.-% of D.M. except in the last column

pH range	C_1	C_2	C_3	n-C_4	n-C_5	*iso*-C_5	C_6	C_7	C_8	Total V.F.A. as % of D.M.
3·61–3·80	30	2073	314	247	9	0	0	0	0	2·67
3·81–4·00	49	2502	292	381	8	0	0	0	0	3·23
4·01–4·20	0	1330	304	376	0	0	0	0	0	2·01
4·21–4·40	16	1835	285	303	141	0	77	0	0	2·66
4·41–4·60	41	4058	663	1309	232	0	595	0	0	6·90
4·61–4·80	97	3850	645	2012	218	0	578	0	0	7·40
4·81–5·00	0	2030	224	535	84	0	770	108	358	4·11
5·01–5·20	59	5290	853	2084	464	0	563	0	0	9·31
5·41–5·60	234	5201	730	1485	716	490	1439	0	0	10·29
6·21–6·40	0	330	107	314	0	114	35	0	0	0·90
6·41–6·60	89	1610	346	377	99	0	0	0	0	2·52
6·81–7·00	0	266	14	16	0	0	0	0	0	0·30
7·01–7·20	0	333	20	28	0	0	0	0	0	0·38

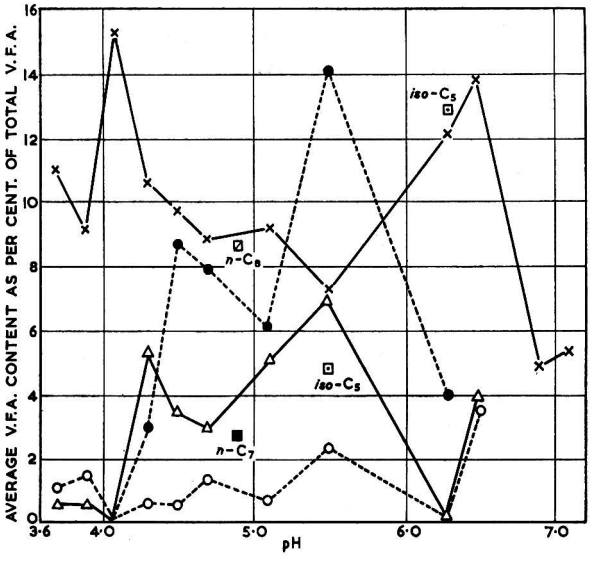
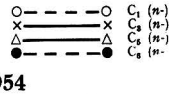


FIG. 1.—*Average amounts of the different volatile fatty acids, other than acetic and butyric acids, present in field silage, expressed as percentages of the total V.F.A. concentration and plotted against the corresponding pH*



when they are considered graphically (Fig. 1), reveals that there are generally two peaks of occurrence of these acids. It may be concluded that the peak at lower pH indicates carbohydrate fermentation and that a higher pH is related to deamination processes occurring in the mass.

The relative amount of the V.F.A. produced is of particular interest when compared with the results obtained in different fields where fatty acids have been formed and determined.

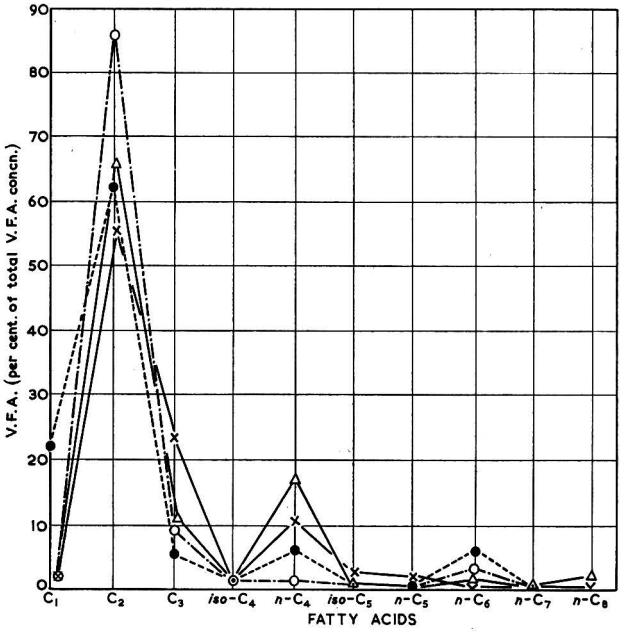


FIG. 2.—*The similarity between the concentrations of the different V.F.A. present in four systems where these concentrations have been studied*

×——× Per cent. average concentration of V.F.A. in rumen of sheep on various diets[4]
●– – –● Per cent. concentration of combined V.F.A. in 'oil' fraction of kale[23]
○—·—·—○ Per cent. concentration (approx.) of V.F.A. from lactic acid fused with KOH[29]
△——△ Per cent. concentration of V.F.A. in silage (see text)

Thus Fig. 2 shows the results obtained by different workers for the V.F.A. in rumen contents[4] combined in the 'oil' fraction of kale,[26] and obtained by heating lactic acid with potassium hydroxide,[29] compared with those noted in the present work. The explanation of the similarity between these findings is probably governed by the free energies of formation of the different acids.

Discussion

The presence of fatty acids in silage may be looked upon as the result of three main processes: (1) the breakdown of carbohydrate at moderately low pH, (2) the breakdown of lactic acid with rising pH, and (3) the deamination of amino-acids at high pH.

This classification is probably oversimplified but it will serve as a working basis for the following reasoning.

(1) Acetic acid is produced from carbohydrate by many organisms including *Escherichia coli* and is indeed present in fresh grass which has undergone no fermentation.[30] Propionic acid is produced by the action of the Lactobacteriaceae on pentosans and on malic and lactic acids.[31] It has been suggested by Van Beynum[32] that propionic acid is peculiarly characteristic of low-pH silage. The acid has been shown to be present in fresh kale, together with acetic acid.[26]

(2) The breakdown of lactic acid is usually associated with the actions of *Clostridium* spp.,

of which *Cl. sporogenes*, *Cl. butyricum* and even *Cl. welchii* occur in grass. Lactic acid is probably converted into pyruvic acid and thence into butyric and acetic acids.[33]

(3) Valeric acid might arise from a breakdown of arginine, which is present in rye-grass,[34] to ornithine, which is deaminated to δ-aminovaleric acid. This acid might further be deaminated to valeric acid. Again, *Streptococcus lactis* has been shown to be capable of breaking down arginine to ornithine.[35] Lysine, which is also present in rye-grass, may also be a starting point for the formation of valeric acid.

It is necessary to remember that certain micro-organisms are capable of synthesizing higher fatty acids from lower members of the series. Thus *Cl. kluyveri* has been shown by Bornstein & Barker[36] to be capable of synthesizing *n*-butyrate, *n*-pentanoate or *n*-hexanoate from ethanol in the presence of acetate, propionate or butyrate respectively. It is not suggested that *Cl. kluyveri* is present in grass but other micro-organisms, e.g. that referred to as LC, a Gram-negative coccus isolated from the rumen, by Elsden & Lewis,[37] also possess this type of biosynthetic capacity. The finding of Elsden & Lewis is of particular significance when it is remembered that the microfloral population of the rumen is partially derived from and sustained by that obtained from the crops eaten by the animal and used for making silage.

Conclusions

(1) The main conclusions reached in the present study seem to be in accord with the results obtained in previous work done in this Laboratory on the V.F.A. and lactic acid contents of silage.

(2) In general, the V.F.A. contents increase with rising pH and with the disappearance of lactic acid; however, acetic, propionic and butyric acids are present in all field-silage samples examined, acetic acid being the predominant acid in all. Other straight-chain acids formed vary from C_1 to C_8, but, of the branched-chain acids the presence only of *iso*valeric acid has been noted.

(3) A consistency has been observed between the relative contents of fatty acids formed in different systems, and it is suggested that this is based upon the free energies of formation of these individual acids.

(4) From this and preceding work it is clear that if a high total V.F.A. content indicates poor-quality silage, the best method of preventing the formation of these high acid contents is by compressing the mass to exclude air; this effect is readily obtainable by chopping the crop, or by sterilizing it with sulphur dioxide.

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PLANT PROTEINS. I.—Extraction of Hay Proteins and Nitrogen Distribution

By J. KOLOUŠEK and C. B. COULSON*

The distribution of the nitrogen that takes place after the isolation of protein from grass and legume hays is given. The samples were prepared by slow air-drying. The amount of protein nitrogen isolated is found to be related to the ' true protein ' (copper-precipitable protein) nitrogen and not to the sum of protein nitrogen and alkali-insoluble nitrogen. The amount of alkali-insoluble nitrogen is found to be of the same order as that of the nitrogen not solubilized by pepsin.

Introduction

Recently, extensive work has been carried out on the extraction of proteins from fresh grass, together with those from processed grass[1] and fresh grass alone[2] and the amino-acids have been estimated with the aid of paper chromatography. Other work has been published in which amino-acid estimation has been carried out directly on the hydrolysates of the dried material,[3, 4] despite the admitted shortcomings of this procedure as a method of protein analysis, though recent modifications may improve the method.[4a]

The importance of hay as an animal feeding-stuff is well recognized, but little work has been carried out to correlate the older methods of analysis used in routine operations of nitrogen estimation[5] with protein extraction and amino-acid analysis. Preliminary work on the amino-acids present in a number of hays[6] and on the routine chemical analyses of these hays[7] has been carried out.

The aim of the present paper is to extend the scope of the work already carried out by the authors as a preliminary to a quantitative amino-acid analysis. The results of classical nitrogen analyses[7] are compared with those from actual protein extraction. In the last few years some attention has been paid to the nature of the nitrogen remaining after extraction or removal of the protein fractions from plant materials.[17, 18] Bondi & Meyer[17] claim, from their experiments on lignin extraction, that the residual nitrogen is not protein. However, the similarity of the tyrosine and tryptophan content of the residual nitrogen fraction to that of the isolated protein has led de Man & de Heus[18] to deny this claim. The present account gives further information and discusses the problem.

Experimental

Material

The mature grasses were: red clover (*Trifolium pratense* L.), alfalfa (lucerne) (*Medicago sativa* L.), orchard grass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.). They were obtained from the State Agricultural Research Station, Měšic, near Tábor (450 m. above sea-level), which is in a potato–wheat area. The lucerne and clover were sown in 1949 with barley, the orchard grass and timothy with clover, so as to obtain samples from more natural conditions. The samples were obtained from the first cutting (23 June, 1950). The group

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of experimental plots were level, except for a slight S.S.W. slope, and were protected on two sides by woodland. The soil was shallow sandy-loam (degraded brown forest soil) with a significant amount of flinty pebbles [Depth of furrow: 20 cm.; subsoil: clay; fertilizers: 40% sulphate of potash (80 kg. of K_2O/hectare), basic slag (36 kg. of P_2O_5/hectare)]. Table I gives the climatic conditions that prevailed during growth.

Table I

Temperature and rainfall during growth

Month	Temp., °c	Rainfall, mm.	Month	Temp., °c	Rainfall, mm.
November	3·4	49·8	April	7·6	40·4
December	1·6	37·8	May	14·5	54·3
January	− 3·6	31·7	June	18·0	20·0
February	0·6	26·5	July	19·0	95·1
March	4·6	8·0	August	18·0	63·6

The different lucerne growth stages were obtained from the State Agricultural Research Station, Liběchov, near Mělník (200 m. above sea-level), which is in a beet–rye area [Soil: Deep Central European brown forest soil to chernozem (unmanured before use)] (see Table II). Climatological conditions prevailing were similar to those of Tábor. A certain amount of weed was mixed with the crop. The average samples were prepared by mixing samples collected from various parts of the plot, milling and mixing the powdered hay thoroughly.

The plants were botanically separated after cutting. The hay was prepared by allowing the freshly cut samples to dry in a well-ventilated and shady place. The resulting samples retained much of their original green colour.

Table II

Data for the growth stages of lucerne

No.	Growth stage	Height, cm.	Date, 1950
1	Very young	12	4 May
2	Young	14	11 ,,
3	Beginning of bloom	46	1 June
4	Full bloom	55	8 ,,
5	Beginning of seed formation	70	15 ,,

Extraction of protein

The extraction of the protein was carried out with weak sodium hydroxide solution (0·2%). This method offered the possibility of a high extraction in the cold through the disruption of the cell wall and ease of protein solution.[8] Although the method of alkali extraction had previously been used[9] the method utilized was essentially a modification of later work on seed proteins.[8] Higher alkali concentrations might lead to protein change[10] and xylan extraction.[11]

The finely ground sample (10 g.) was placed in a 500-ml. Stohmann flask (long-necked bottle used in soil extraction), together with 0·2% sodium hydroxide solution (200 ml.), shaken and left to stand at room temperature. The operation lasted about two hours. This was repeated five times (duration 15 minutes); each time the residue was filtered off by means of a Jena sintered-glass filter (porosity 2 or 3). After the fifth extraction, the extract gave no precipitate with trichloroacetic acid. The residue remaining after protein extraction was then dried at 100° and stored for nitrogen determination. The filters were cleaned with aqua regia and washed with alkali, then with water. The alkali extracts were then combined and the solution was adjusted to pH 4·5–5·1 with glacial acetic acid. The flocculated precipitate was allowed to settle overnight and the supernatant liquid was decanted, by means of a vacuum-assisted siphon, into a 2-litre bottle. The small amount of floating insoluble matter was filtered off with the aid of a sintered-glass filter, dissolved in a small quantity of 0·2% sodium hydroxide solution, reprecipitated and added to the rest of the precipitate, which was then centrifuged (3500 r.p.m., 30 min.). The supernatant liquid gave no precipitate with trichloroacetic acid. The extract was then evaporated to dryness on a water bath and the residue taken for nitrogen estimation.

The precipitate was repeatedly dissolved in 0·2% sodium hydroxide solution and the solution reprecipitated with glacial acetic acid (five times). The final precipitate was washed with 80% ethanol several times, and partly dried, followed by a Soxhlet extraction with ether for 24 hours, in order to remove chlorophyll and lipids. The protein was redissolved in 0·2% sodium hydroxide solution and filtered to removed paper particles. After addition of glacial

acetic acid, the precipitate was mixed repeatedly with distilled water slightly acidified with glacial acetic acid, followed by distilled water. Drying in a desiccator at room temperature was found to be more efficient than drying at 80°, as the latter produced horny material, whereas that dried in the desiccator could be powdered (Yield: 200–800 mg.).

A more convenient method is to wash with 96% ethanol and then ether and allow to dry in air; a fine powder is then obtained.

A suitable alternative filter was found in filtration through a Whatman Soxhlet cartridge standing on a perforated Buchner funnel (vacuum applied) of similar diameter. During the extraction, it was noticed that only the lucerne extracts tended to produce a strong foam. The weak alkali solutions of lucerne protein were light-brown in colour, in contrast with the darker brown of the others.

Estimation of moisture, ash and nitrogen

The determination of the moisture and ash content of the protein was that used by Chibnall & co-workers.[12] Similarly, the estimation of nitrogen was essentially the same. Although we employed the long digestion period, the results obtained by the Analytical Department of the Institute of Organic Chemistry (Prague), with normal short-period micro-Kjeldahl digestion times, were for several of the protein samples in close agreement with our own. A modified Parnas–Wagner–Pregl apparatus, with silver delivery-tube and valve in the steam generator to prevent 'sucking-back', was employed for distillation. The addition of equal quantities of the methyl-red indicator to the acid and alkali used in the titration (before the standardization) enabled a clear end-point to be obtained.

Routine methods

The routine methods[5] used were:

Total organic nitrogen (*Kjeldahl*).—The material (1–2 g.) was digested with a selenium catalyst. The normal distillation methods were used.

Undigested nitrogen (*Sjollema & Wedermeyer*).—The sample (2 g.) and pepsin (1 g.) were placed in a beaker (800 ml.). Distilled water (480 ml.) at 40° and 25% hydrochloric acid (10 ml.) were added. After mixing, the beaker was placed in a thermostat at 39° and stirred from time to time. After 24 hours a further quantity of 25% hydrochloric acid (10 ml.) was added. After 48 hours the residue was filtered off through a medium filter and washed with distilled water until the washings gave no chloride reaction. The nitrogen content of the residue was determined by the same method as for total nitrogen.

True protein-nitrogen (*Barnstein*).[5]—The sample (2 g.) was placed in a beaker (400 ml.) and boiled with distilled water (100 ml.) for 1–2 min. Copper sulphate solution (25 ml.: 25 g./l., w/v) was added, then sodium hydroxide (25 ml.: 12·5 g./l., w/v) with constant stirring. After the precipitate had settled, the supernatant liquid was decanted and the precipitate filtered off. The precipitate was washed with distilled water until the washings gave no reaction for copper and the precipitate was dried; the nitrogen content was determined as before.

The method of estimating the nitrogen content of the residues was essentially that previously employed.[5]

Results

The results of the protein analyses are given in Tables III and IV together with those of casein (as prepared by Hammarsten and marketed by the Merck Co.) for comparison. The analysis of the casein was carried out by the same methods used for the other proteins.

The three nitrogen fractions analysed after protein extraction were protein nitrogen, alkali-insoluble nitrogen and alkali-soluble nitrogen.

Protein nitrogen (*PN*) is the nitrogen of the isolated protein expressed as a percentage of the original hay sample (dry basis) (Figs. 1 and 2*a*). It is also expressed as a percentage (i) of total nitrogen (Figs. 2*b* and 6); (ii) of the isolated protein (dry basis) (Tables III and IV; Figs. 4*b* and 5); (iii) of true protein nitrogen (Fig. 3, graph No. 1, and Fig. 4*a*) and (iv) of protein nitrogen plus alkali-insoluble nitrogen (Figs. 3 and 4*a*).

Alkali-insoluble nitrogen (*AIN*) is the nitrogen of the residue remaining after protein extraction with dilute alkali expressed as a percentage of the original hay sample (dry basis) (Figs. 1 and 2*a*). It is also expressed as a percentage of total nitrogen (Figs. 2*b* and 6).

Alkali-soluble nitrogen (*ASN*) is the nitrogen remaining in the extract after precipitation of the protein by acidification of the dilute sodium hydroxide solution extract with glacial

Table III

Moisture, ash and nitrogen contents of the isolated proteins of mature grasses, with figures for casein for comparison

	Red clover	Lucerne	Orchard grass	Timothy	Casein
Water, %	6·93	8·23	4·41	5·68	8·37
Ash (dry basis), %	1·43	2·72	1·75	1·86	1·80
Nitrogen (dry basis), %	8·68	7·73	9·71	9·62	16·09

Table IV

Moisture, ash and nitrogen contents of the isolated proteins of lucerne at various growth stages

Growth stage	1 (12 cm.)	2 (14 cm.)	3 (46 cm.)	4 (55 cm.)	5 (70 cm.)
Water, %	5·21	5·38	5·86	4·99	1·72
Ash (dry basis), %	3·76	2·67	1·20	3·75	0·24
Nitrogen (dry basis), %	13·20	16·46	13·89	12·04	13·03

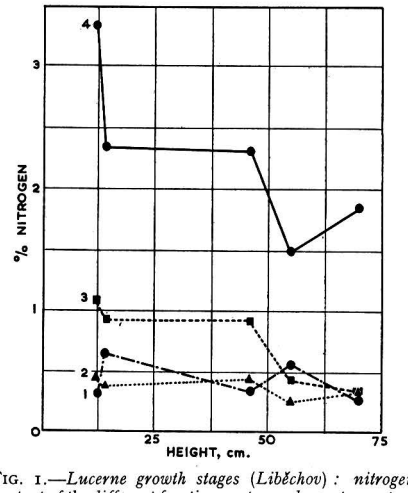

FIG. 1.—*Lucerne growth stages (Liběchov): nitrogen content of the different fractions expressed as a percentage of the hay (dry basis)*

1. Alkali-insoluble nitrogen (AIN)
2. Pepsin-undigested nitrogen
3. Protein nitrogen (PN)
4. Alkali-soluble nitrogen (ASN)

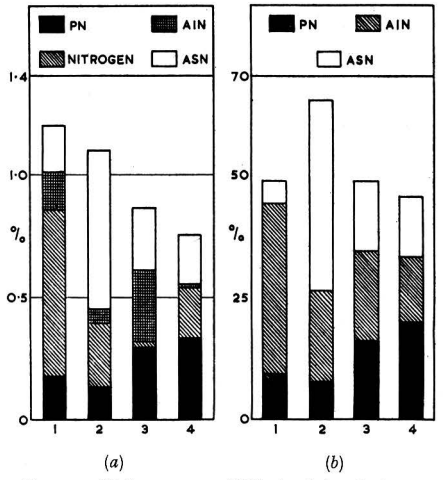

FIG. 2.—*Mature grasses (Tábor): (1) red clover, (2) lucerne, (3) orchard grass, (4) timothy*

(a) Nitrogen content of the different fractions expressed as a percentage of the hay (dry basis)

(b) Nitrogen content of the different fractions expressed as a percentage of total nitrogen

(Amount is indicated by height above base line)

acetic acid. It was found that evaporation of the extract to dryness on a water bath resulted in varying nitrogen losses. The alkali-soluble nitrogen was found by difference between total nitrogen and the sum of protein nitrogen and alkali-insoluble nitrogen. It is expressed as a percentage of the original hay sample (dry basis) (Figs. 1 and 2*a*) and as a percentage of the total nitrogen (Figs. 2*b* and 6).

Total nitrogen (Kjeldahl), copper-precipitable-protein nitrogen and 'indigestible protein' (Sjollema & Wedermeyer) were determined by routine methods,[5] and the results previously reported[7] are given in Table V.

Discussion

In Fig. 1, AIN (No. 1) is found to be of the same order as nitrogen not solubilized in the pepsin digestion (No. 2). A somewhat similar picture is presented by the mature grasses (cf. Fig. 2*a*). Again, Fig. 1 shows that the course of the fall in ASN through the different

Table V

Results of routine analyses for the mature grasses (Tábor) and lucerne growth stages (Liběchov)

Hay	Total nitrogen (Kjeldahl), %	True protein-nitrogen (Barnstein), %	Undigested nitrogen (i.e. pepsin-insoluble), %
Red clover	2·45	2·04	0·86
Lucerne	1·68	1·18	0·39
Orchard grass	1·77	1·07	0·32
Timothy	1·64	1·32	0·54
Lucerne growth stages:			
1 (12 cm.)	4·73	2·97	0·44
2 (14 cm.)	3·89	2·40	0·38
3 (46 cm.)	3·57	2·92	0·43
4 (55 cm.)	2·52	1·70	0·25
5 (70 cm.)	2·47	1·69	0·34

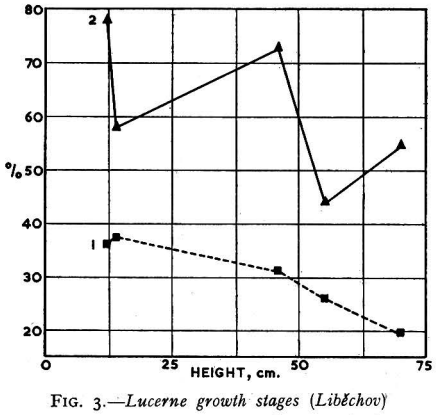

FIG. 3.—*Lucerne growth stages (Liběchov)*

1. Protein nitrogen as a percentage of true protein nitrogen
2. Protein nitrogen as a percentage of PN plus AIN

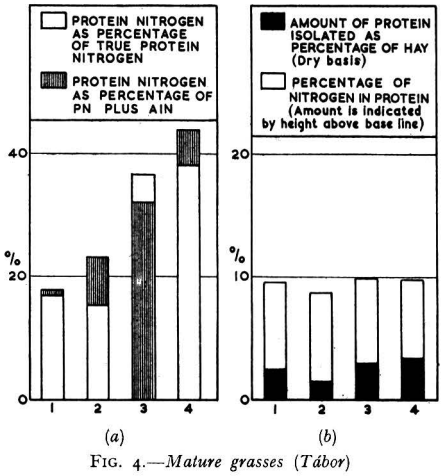

FIG. 4.—*Mature grasses (Tábor)*

stages of growth is similar to that of the fall in PN. The ASN (Fig. 1, No. 4) corresponds roughly to the total nitrogen (Table V). The PN (Fig. 1, No. 3) is only a fraction of the 'true protein' nitrogen (cf. Fig. 3, No. 1).

There is similarity between the change of PN and the change in copper-precipitable-protein nitrogen resulting in what is virtually a straight line (Fig. 3, No. 1), but the change in PN does not seem to be related to the sum of PN and AIN (Fig. 3, No. 2) which has been suggested as a more accurate value for PN.[13] A similar result has been obtained for mature grasses (Fig. 4*a*). A difference is seen between Leguminosae (1 and 2) and Graminae (3 and 4) of Fig. 4*a*.

The course of the variation of the amount of protein isolated from the lucerne growth stages (Fig. 5, No. 1) is similar to that of 'true protein' and 'digestible protein' nitrogen,[7] but the amount of protein isolated from grasses (Fig. 4*b*) does not seem to vary in the same way. Although there seems to be some variation in the PN content with the lucerne growth stages (Fig. 5, No. 2), there seems to be little variation between the different mature grasses (Fig. 4*b*).

Figs. 2*b* and 6 show how the total nitrogen of the plant materials is distributed between the three fractions ASN, AIN and PN.

From Fig. 6 it can be seen that although the PN component changes little until after the beginning of flowering, during early growth, AIN increases temporarily at the expense of the ASN component. After the onset of flowering, the AIN component again increases, this

time at the expense of both the ASN and PN. Seed formation leads to reversal of this: a fall in the amount of the AIN component and an increase in the ASN component.

Fig. 2*b* shows that the two grasses (orchard grass and timothy) resemble one another closely in their nitrogen components, but a distinct difference is to be seen between the two legumes. Whereas the red clover has roughly equivalent amounts of the ASN and AIN components, the lucerne shows a predominance of the ASN component over that of the AIN. This proportion of components corresponds to a growth stage later than full bloom (Fig. 6) and thus there is some indication that the lucerne is maturing earlier than the other plants under the same conditions.[14] It is also to be noted from Fig. 2*b* that the PN component that can be isolated does not necessarily increase with increasing nitrogen content.

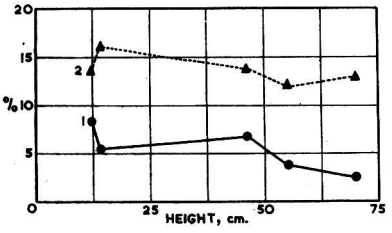

FIG. 5.—*Lucerne growth stages (Libĕchov)*

1. Amount of protein isolated expressed as a percentage of the hay (dry basis)
2. Percentage of nitrogen in the isolated protein (dry basis)

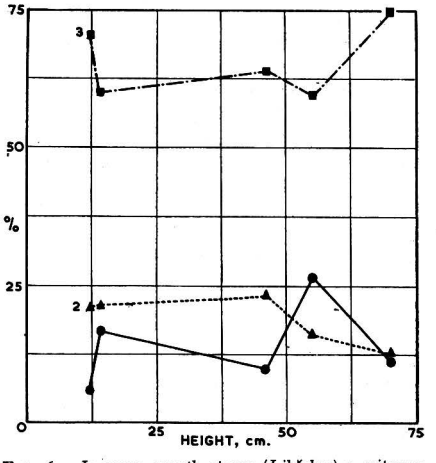

FIG. 6.—*Lucerne growth stages (Libĕchov): nitrogen content of the different fractions expressed as a percentage of the fractions*

1. AIN 2. PN 3. ASN

Conclusions

It is quite clear from the outset that dried plant material will contain protein so denatured by the drying process that it is difficult to dissolve even in such a solvent as weak caustic soda solution, as well as proteins affected by enzymic action.[6] It has been suggested that the intermixing in the fresh material of the slightly acid cell-sap and protoplasm during expression of juice leads to a partial denaturing and hence insolubilization of the proteins of the protoplasm.[15]

It has been suggested also that some of the insoluble high-molecular-weight non-protein nitrogen is linked with lignin[16, 17] (cf. Pirie *et al.*[10]); this has been strongly challenged,[18] because the tyrosine and tryptophan values of this nitrogenous material are very close to those for the isolated proteins.[18]

Both the ratio of protein nitrogen to copper-precipitable-protein nitrogen (Figs. 3 and 4*a*) and the actual protein isolated from the different lucerne growth stages (Fig. 5) indicate a definite relation between the protein isolated and the copper-precipitable protein, but no relation is indicated between PN and PN plus AIN (Figs. 3 and 4*a*). Thus there seems to be no basis for assuming that the insoluble nitrogen, together with the protein nitrogen, gives copper-precipitable-protein nitrogen. The claim that insoluble nitrogen represents only insolubilized protein has as little basis as that 'crude protein' (total nitrogen × 6·25) represents actual protein. It is possible that the insoluble residue is part protein, part non-protein high-molecular-weight nitrogenous material. It would seem that the insoluble nitrogen corresponds to the nitrogen that pepsin fails to solubilize (cf. Fig. 1, Nos. 1 and 2; Fig. 2*a*).

The general changes that take place in the lucerne plant throughout the various stages of growth (based on the analysis of the dried material) indicated by the nitrogen fractions follow a similar course to that indicated by the previous routine analyses.[7] The relative contribution of insoluble nitrogen to the total in these materials varies inversely with the relative contribution of the soluble nitrogen, since that of the protein nitrogen is, in the main, fairly constant (Fig. 6).

There is also a noticeable difference between the protein-nitrogen components of the grasses on the one hand and the legumes (for the mature plants) on the other (Fig. 2*b*).

These conclusions can be summarized as follows:

1. Nitrogen distribution, after protein extraction with weak alkali, between protein, soluble and insoluble fractions, supports, in general, the conclusions drawn from routine analyses.

2. Legumes and grasses show certain family nitrogen composition differences.

3. The alkali-insoluble nitrogen is found to be of the same order as the nitrogen not solubilized by pepsin digestion.

4. The change in protein-nitrogen content of the sample seems related to the change in copper-precipitable protein but not to the sum of protein nitrogen and alkali-insoluble nitrogen.

Acknowledgments

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THE COMPOSITION OF *AGROSTIS SETACEA*, THE BRISTLE-LEAVED OR HEATH BENT GRASS

By B. M. DOUGALL

A description of the bristle-leaved or heath bent grass is given and figures showing variations in its chemical composition over the growing season are presented. The probable value of this species as a food for hill livestock is discussed.

Agrostis setacea Curt. is a perennial, with densely tufted leaves that are mostly radical and usually of a pale-glaucous-green colour. The stems are erect, 12 to 24 inches high, with compound panicled inflorescence, usually upright and compact until in flower when it spreads only slightly. The flowers are pale purple; glumes, which are usually downy, are unequal and narrow and rather more taper-pointed than in other species of *Agrostis*. The plant flowers in July or August and its seed ripens at the beginning of September.

The bristle-leaved or heath bent grass, according to Bentham & Hooker,[1] is distributed over western Europe from Spain and Portugal to Germany and Holland. In the United Kingdom it is confined to the dry heaths and downs of the southern and south-western counties. Lowe[2] states that it is most abundant in Hampshire, Devon and Cornwall, in which counties it flourishes and provides food for sheep.

As information on the chemical composition of this species appeared to be non-existent it was considered desirable to ascertain the changes in composition that occur during the season.

For this purpose samples were obtained from open moorland near Teignhead, Devon, at an elevation of 1400 feet above ordnance datum on a podzolic mineral soil of granitic origin. The ground vegetation was dominantly *A. setacea* with smaller amounts of *Triodia*, *Carex*, *Calluna* and *Erica*.

Experimental

Sampling was carried out during 1950 at two-monthly intervals, except in May, June and July, when the interval was reduced to one month. Samples were obtained from a restricted area by cutting close to the ground with heavy scissors. On two occasions separate hand-plucked samples were collected for cobalt estimation.

The samples were conveyed in sealed containers to the laboratory where, after the determination of moisture, they were milled and subjected to the conventional feeding-stuffs analysis. True protein was determined by Stutzer's method and the *in vitro* digestibility coefficients for the crude protein by that due to Wedemeyer. Lime and phosphoric acid were estimated by the usual methods, potash and soda by Piper's modifications of the methods of Krugel & Retter[3] and Kahane[4] respectively, and cobalt by the procedure of Kidson & Askew.[5] The results are given in Table I.

Table I

Percentage composition of dry matter

Sampling date, 1950	1 Mar.	23 May	20 June	20 July	11 Sept.	2 Nov.
Crude protein (including true protein)	5·42	8·13	10·53	11·38	9·41	10·19
Ether extract	2·12	2·07	2·97	3·02	3·05	2·30
Nitrogen-free extractives	50·58	52·45	50·18	50·75	47·86	48·48
Crude fibre	35·29	31·49	29·34	27·40	34·79	32·78
Ash (including CaO, P_2O_5, K_2O, Na_2O and SiO_2)	6·59	5·86	6·98	7·45	4·89	6·25
True protein	4·87	6·98	8·21	9·10	7·96	8·95
True protein : crude protein	0·90	0·86	0·78	0·80	0·85	0·88
Percentage digestibility of crude protein (Wedemeyer)	41·13	59·66	69·80	64·12	62·00	57·54
Lime (CaO)	0·091	0·129	0·154	0·175	0·154	0·107
Phosphoric acid (P_2O_5)	0·145	0·180	0·206	0·291	0·232	0·277
Silica (SiO_2)	4·582	3·204	3·527	2·292	2·361	4·174
Silica-free ash	2·008	2·656	3·453	5·158	2·529	2·076
K_2O	0·597	0·939	1·474	1·719	1·546	1·194
Na_2O	0·093	0·109	0·110	0·166	0·146	0·153

Discussion

The results obtained indicate that *A. setacea* is a late-maturing species, which reaches its maximum crude-protein content in July. The percentage of crude protein decreases in August and September as seeding takes place; a slight increase, coincident with the growth of the aftermath, follows in early autumn. This slight increase is, however, of doubtful significance. Over the winter period there is a marked decline in the percentage of crude protein, which is at a minimum between January and March.

Throughout the growing season the digestibility of the crude protein is comparatively low; this may be partially explained by the rather narrow ratio of true : crude protein. The maximum coefficient of digestibility for the crude protein is obtained on the June herbage.

The crude-fibre content, although high, is not sufficiently so to entirely account for the somewhat low digestibility. There is a close inverse relationship between crude fibre and crude protein contents.

The ash of *A. setacea* is poor in lime and phosphoric acid and must at all times be considered inadequate as a source of these constituents. The lime and phosphoric acid contents increase as the season advances until, in July, they are almost 100% above the amounts present

in the March sample. Throughout late summer and autumn the proportion of lime present markedly declines and is at a minimum during the period November to March. In late summer there is a sharp decrease in the proportion of phosphoric acid present, followed by a notable increase in November, so that, over the whole season, this constituent follows the same trend as the crude protein.

The amount of silica-free ash increases to a maximum in July, at which time *A. setacea* is of maximum feeding value. Afterwards it decreases as the plant approaches maturity. Conversely the silica content increases as the plant matures.

The ratio of potash to soda in spring is approximately 6·4 : 1 ; this increases to about 13·4 : 1 in June and decreases again with the advancing age of the material.

The hand-plucked samples that were obtained on May 23 and July 20 were oven-dried but not, subsequently, milled. They contained respectively 0·06 and 0·09 p.p.m. of cobalt as Co. Examination of the soil disclosed a satisfactory cobalt level of 0·40 p.p.m.

Conclusion

The results presented in Table I do not suggest that *A. setacea* is of high feeding value. Indeed it must be regarded as a poor source of digestible protein and of the mineral nutrients.

This plant is an important component of the available herbage on certain areas of Dartmoor, but its value as a food for hill livestock is restricted to a comparatively short period, after which the foliage becomes brown and hard as the season advances. Its usefulness as a grazing plant is further minimized by reason of the fact that it develops late in the season. Although no yield data are available it is probable that, under moorland conditions, its productivity may be low.

Cattle, in general, find the bristle-leaved bent grass unpalatable, and it must be regarded, essentially, as food for sheep. Nevertheless, unless grazing is begun early the grass tends to become tough and fibrous, and may even be rejected by sheep.

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THE COMPOSITION AND PROBABLE FEEDING VALUE OF *TRIODIA DECUMBENS*

By B. M. DOUGALL

A description of *Triodia decumbens* is given and figures showing variations in the chemical composition of this species over the growing season are presented. Its probable value as a food for hill sheep is discussed.

Triodia decumbens Beauv. has been stated by Lowe[1] to be of little agricultural interest. Nevertheless it is selectively grazed by sheep, is widely distributed over Europe and Western Asia and extends from Spain and Italy into Scandinavia. According to Armstrong,[2] it is rather common on heaths and barren land in the British Isles, but its distribution is by no means confined to such habitats. Although it may be found on dry heaths and hilly pastures it is also frequently present on soils of imperfect drainage at lower elevations.

T. decumbens is a semi-prostrate tufted perennial, 6–12 inches high, with stringy roots. The leaves, which are narrow and rather long, bear soft hairs on their sheaths and edges. On the upper surface of the leaf is a distinctly defined groove and ridge on either side of the

slight mid-rib. The inflorescence is simple-panicled, the panicle consisting of 5 or 6 rather large erect spikelets containing 3 or 4 flowers. The plant flowers in July and the seed ripens in early August.

Experimental

In order to obtain information on the composition and probable feeding value of *Triodia* throughout the growing season samples were obtained from Teignhead, Devon, by courtesy of the late Sir Ralegh Philpotts, at approximately monthly intervals during the summer and at two-monthly intervals in the autumn. At all times they were taken by cutting close to the ground with heavy scissors (contaminated material being rejected) and conveyed immediately to the laboratory in tins. After the determination of moisture on a representative sample the remainder was oven-dried at 100° for 24 hours and ground in a Christy–Norris power mill over a $\frac{1}{32}$-inch screen.

Analytical methods subsequently employed were those prescribed by the Fertiliser and Feeding Stuffs Regulations 1932, the true protein being determined by Stutzer's procedure and the digestibility of the crude protein by the Wedemeyer method.

Table I

Percentage composition of dry matter

Sampling date, 1950	1 Mar.	23 May	20 June	20 July	11 Sept.	18 Nov.
Crude protein (including true protein)	7·07	10·88	10·30	9·09	8·06	8·33
Ether extract	2·43	2·36	2·59	2·92	2·58	3·20
Nitrogen-free extractives	52·64	46·16	47·69	50·59	51·81	52·10
Crude fibre	32·52	22·15	22·66	32·69	33·33	31·44
Ash (including CaO, P_2O_5 and SiO_2)	5·34	5·85	5·30	4·71	4·22	4·93
True protein	6·41	9·75	9·14	8·10	6·65	7·84
True protein : crude protein	0·90	0·90	0·89	0·89	0·83	0·94
Percentage digestibility of crude protein (Wedemeyer)	46·41	72·51	71·08	67·64	66·83	47·39
Lime (CaO)	0·139	0·164	0·137	0·122	0·104	0·161
Phosphoric acid (P_2O_5)	0·214	0·339	0·332	0·315	0·198	0·243
Silica (SiO_2)	3·784	2·598	2·136	1·731	1·658	2·270

Discussion

From figures in Table I it is seen that, in March, *Triodia* is of low feeding value and the date of sampling coincides approximately with the beginning of spring growth. The crude-protein content is low and of low digestibility and the silica content is rather high.

During spring there is a substantial increase in the percentage of crude protein found but the amount of this constituent declines rapidly in late summer; it is followed by a slight rise in November owing to the inclusion of some aftermath in this sample. Throughout spring and summer the ratio of true to crude protein remains fairly constant, but in September it widens sharply, and in November the amount of non-protein nitrogen is least.

The crude-fibre content, except in May and June, is relatively high, and, throughout the season bears a close inverse relationship to that of the crude protein.

From March to September the percentage digestibility of the crude protein, as determined by Wedemeyer's (pepsin/hydrochloric acid) procedure, and the percentage of crude fibre are inversely related. In late autumn, with a certain amount of aftermath present in the sample, the fall in the amount of non-protein nitrogen is probably responsible for the depression of the digestibility coefficients; the percentage of crude fibre also decreases at this time.

Over the whole season the phosphoric acid content of *Triodia* follows the same trend as the crude protein, and is at all times inadequate. Although there is an appreciable increase in the lime content of the late summer and autumn samples the amount of this constituent never reaches a satisfactory level. The silica content is quite high but diminishes steadily as the season advances; in November there is a slight increase in the amount present. As this species approaches maturity there is a decline in the percentage of silica-free ash.

Conclusion

From the results presented, *T. decumbens* does not contain unusual amounts of any single constituent. In composition it appears inferior to a number of other hill-species, e.g. *Molinia coerulea*, the flying bent,[3] and *Festuca capillata*, the fine-leaved sheeps fescue,[4] but is of approximately similar feeding value to *Eriophorum vaginatum*, the draw-moss.[5]

As a constituent of upland grazings dominated by *Agrostis* spp., *T. decumbens* is of undoubted value as a food for sheep, if only because it is available at a time of general scarcity.

Acknowledgment

The writer wishes to express his indebtedness to his colleague Mr. J. G. Jenkinson, M.Sc., for his help in the collection of samples.

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THE 1 : 1′-DIANTHRIMIDE METHOD FOR DETERMINATION OF BORON IN SOILS: FURTHER OBSERVATIONS

By E. GORFINKIEL and A. G. POLLARD

The 1 : 1′-dianthrimide method previously examined is further modified to increase its sensitivity for determining small amounts of boron in soils. By the use of a 20-g. sample of soil, quantities of water-soluble boron of the order of 0·3 p.p.m. (dry soil) can be determined. In this range the coefficient of variability of results is 5·8% and the standard deviation is 0·178. The sensitivity of the modified 1 : 1′-dianthrimide method is greater than that found for the Waxoline-purple method.

Work on the 1 : 1′-dianthrimide method, described in an earlier paper,[1] has been continued, and certain improvements in the procedure for the determination of water-soluble boron in soils have been made. The Waxoline-purple method[2] has also been examined for this purpose, and a comparison of the two methods seemed desirable.

Method

The following modifications of the procedure described earlier were finally adopted:

(*a*) The concentration of the stock 1 : 1′-dianthrimide solution was increased from 400 to 600 mg. in 100 c.c. of 98% sulphuric acid; as before, the working solution (freshly prepared for every batch of determinations) is made by diluting 5 c.c. of the stock solution to 100 c.c. with 98% sulphuric acid.

(*b*) A saturated solution of sodium chloride is added to the suspension of soil in water to facilitate clarification.

(*c*) The use of an orange filter in place of the original green (maximum absorption at 620 mμ) increased the sensitivity of the method.

The modified technique for the preparation of the water extract of soil is as follows: A 20-g. sample of soil (air-dried and passing a 20-mesh sieve) is extracted with 40 c.c. of water in a large tube made of boron-free glass, which is connected to a reflux condenser having an inner tube made of silica, and boiled for 5 minutes in a glycerin bath. To the cooled extract 5 c.c. of a saturated solution of sodium chloride is added and the mixture is centrifuged for about 15 minutes at 5500 r.p.m. Part of the supernatant liquid (20 c.c.) is poured into a silica dish, saturated calcium hydroxide solution (10 c.c.) is added, and the solution is evaporated to dryness. The procedure is then the same as described before, except that 2 c.c. of the final test-solution instead of 1 c.c. is used in the actual determination.

Results and discussion

The accuracy of the method is shown by results from six different batches of determinations made with a Wisley soil. The results are given in Table I.

Table I

Boron, p.p.m. of dry soil		
0·32	0·30	Mean = 0·31
0·33	0·28	Standard deviation = 0·178
0·31	0·30	Coefficient of variability = 5·8%

Comparison of the 1 : 1′*-dianthrimide and the Waxoline-purple reagents*

The Waxoline-purple method described by Higgons[2] was used, except that the same concentrations of sulphuric acid for dissolving the ignited samples were employed in both methods. Also the ratio of the reagent (either Waxoline purple or 1 : 1′-dianthrimide) to the test solution was made the same.

The absorption curves for each reagent alone and with different amounts of boron were measured with a Hilger spectrophotometer, and are shown in Figs. 1 and 2.

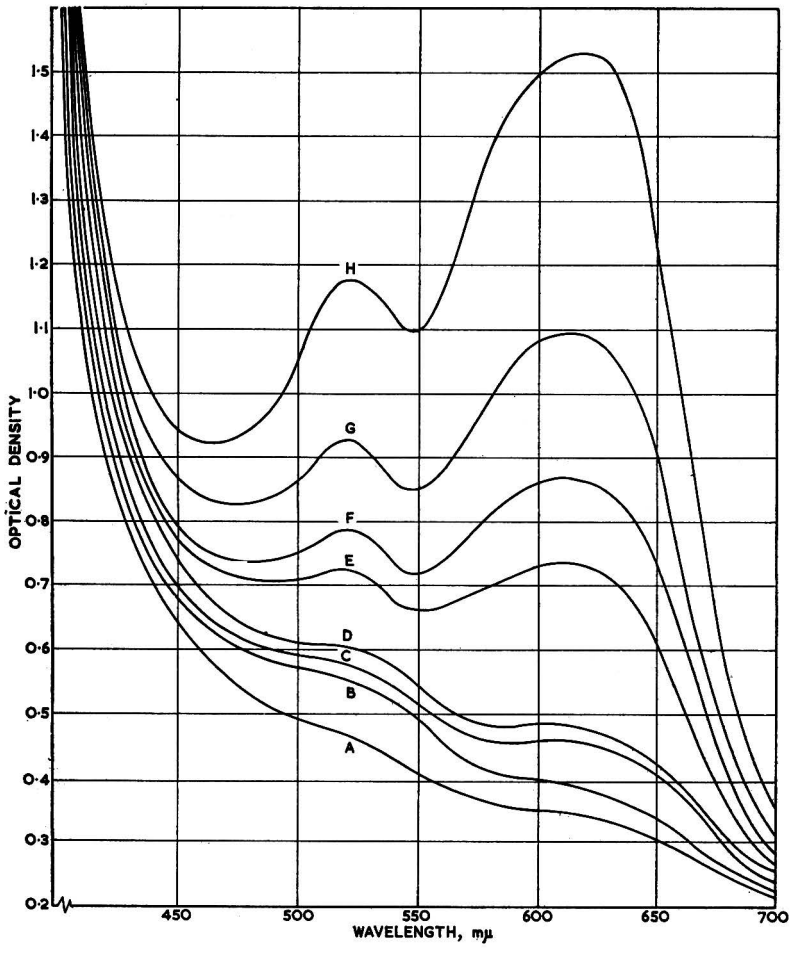

FIG. 1.—*Absorption spectra of* 1 : 1′*-dianthrimide with and without added boron*

Curves obtained with Hilger spectrophotometer and a 1-cm. cell; concentration of the reagent, 30 mg./100 c.c. of concentrated sulphuric acid

A No added boron
B 0·4 μg. of boron/10 c.c. of reagent
C 0·8 μg. „ „ „ „ „
D 1·6 μg. „ „ „ „ „
E 3·2 μg. of boron/10 c.c. of reagent
F 4·8 μg. „ „ „ „ „
G 6·4 μg. „ „ „ „ „
H 9·6 μg. „ „ „ „ „

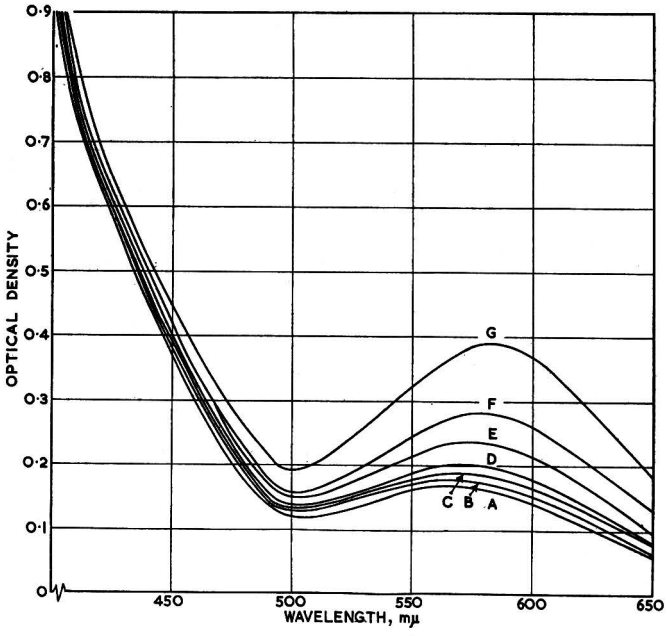

FIG. 2.—*Absorption spectra of Waxoline purple with and without added boron*

Curves obtained with Hilger spectrophotometer and a 1-cm. cell; concentration of the reagent, 7·5 mg./100 c.c. of 84% sulphuric acid

A No added boron
B 0·4 μg. of boron/10 c.c. of reagent
C 0·8 μg. „ „ „ „ „
D 1·6 μg. „ „ „ „ „
E 2·8 μg. of boron/10 c.c. of reagent
F 4·8 μg. „ „ „ „ „
G 9·6 μg. „ „ „ „ „

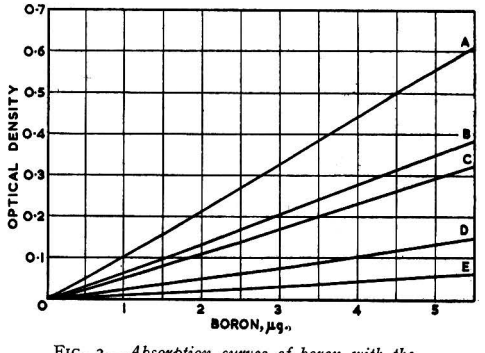

FIG. 3.—*Absorption curves of boron with the 1 : 1′-dianthrimide and the Waxoline-purple reagents*

A 1 : 1′-Dianthrimide with Spekker absorptiometer, 607 orange filter
B „ „ „ Biochem absorptiometer, OY2 filter
C „ „ „ „ „ OR2 filter
D Waxoline purple with Spekker absorptiometer, 606 yellow filter
E „ „ „ Biochem absorptiometer, OY2 filter

As can be seen from these Figures, the absorption curves for the Waxoline-purple reagent and the reagent plus boron overlap when small amounts of boron (0·4 μg., 0·8 μg.) are used, and are very near to each other with larger amounts of boron. Thus, the sensitivity of the Waxoline-purple method is much less than that of the 1 : 1′-dianthrimide method. The latter

reagent does not absorb very much light at the wavelength (600 mμ) at which a broad absorption is shown by the reagent plus boron. The measurement of the absorption curves permitted a choice of filters for use with the Spekker and the Biochem absorptiometers. Fig. 3 presents results obtained with solutions of different concentrations of boron, with each reagent and different filters, when measurements were made by means of the Spekker and by the Biochem absorptiometers.

The best results were obtained with 1 : 1'-dianthrimide when the Spekker absorptiometer was used with the orange 607 filter. Otherwise the results from Slough soils, treated in different ways, were in good agreement; the values obtained with the Waxoline-purple reagent were generally somewhat lower than those obtained when the 1 : 1'-dianthrimide was used (Table II).

Table II*

Slough soil treatment	Water-soluble boron, p.p.m.	
	Waxoline purple	1 : 1'-Dianthrimide
NPK + dung	1·00	1·16
Nil	0·40	0·45
Green manure	0·27	0·32
Dung	0·47	0·51
Lime	0·38	0·43

* All the values are averages of two determinations

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PHYSICOCHEMICAL STUDIES ON THE APPLICATION OF INSECTICIDES TO SHEEP FLEECE.

IV.*—The Influence of Physical Properties of Fleece on the Inactivation of Cationic Wetting Agents

By C. C. ADDISON and C. G. L. FURMIDGE

A survey has been made of the physical properties of Scotch Blackface fleeces from sheep of ages ranging from one to five years. These properties include grease and suint contents, pH, surface area and inactivation of cationic wetting agents. Surface area and grease and suint contents are found to be important properties determining the extent of inactivation, and by a combination of these the inactivation isotherms can be closely correlated.

It has been shown[1] in Parts I–III of this series that the surface activity of solutions of cetylpyridinium chloride is often diminished by the immersion of fleece samples, and this is regarded as an important factor in the stability of insecticidal emulsions and suspensions. This inactivation of wetting agent (expressed here in terms of the weight of wetting agent apparently removed from solution) was shown to result largely from reaction with the grease and suint deposits on the wool. All the inactivation measurements recorded in earlier papers were carried out on wool samples from a three-year-old ewe. This paper describes the manner in which fleece properties vary with the age of the sheep, and the extent to which the conclusions reached in the preceding papers may be applied to wool samples taken from any position on the body.

* Part III: *J. Sci. Fd Agric.*, 1952, **3**, 330

A number of samples were taken from various parts of each of the fleeces of Scotch Blackface ewes born in 1950, 1949, 1948 and 1946, and sheared in 1951. The grease and suint contents were measured as described in Part II, and the pH of a solution of given concentration of surface-active agent, after the immersion of a 1-g. sample of wool, was determined with a Marconi pH-meter and glass electrode assembly, as described in Part III.

It should be emphasized that the major aim of this work was to relate physical properties with wetting-agent inactivation, rather than to attempt a statistical survey of physical properties by the study of a large number of fleeces. Altogether four separate series of fleeces, each covering the age-range mentioned above, were examined, and each series showed similar variations with age. The properties given below apply to the series on which inactivation measurements were made, but they may be regarded as typical of all such fleeces. The measurements on 1948 fleeces were in close agreement with those already reported in Parts II and III; therefore they are not included in this paper, but are taken into account when correlating the results.

Grease and suint variations

Diagram I shows the general distribution of grease and suint content over the hogget (born 1950) fleece, and Diagrams II and III show the results for the ewes born in 1949 and 1946 respectively. For each sample, the upper figure gives the grease content, and the figure in parentheses the suint content; all results are percentages of the dry-fleece weight.

The grease and suint contents vary in a somewhat random manner over all the fleeces examined, but there are certain broad generalizations: (*a*) The grease contents of the back samples were always rather less than those of flank and breast samples. (*b*) The suint contents fluctuate more than the grease contents, though there is a tendency for the suint contents to

Diagram I

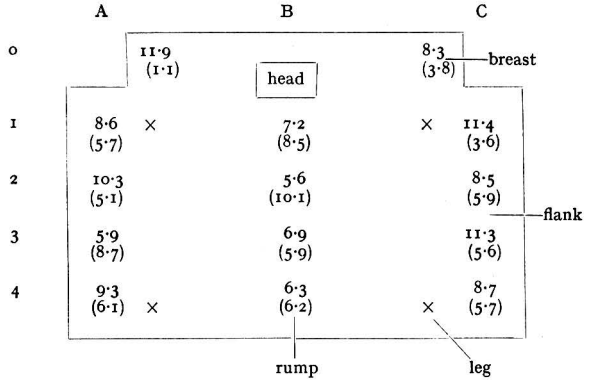


Diagram II

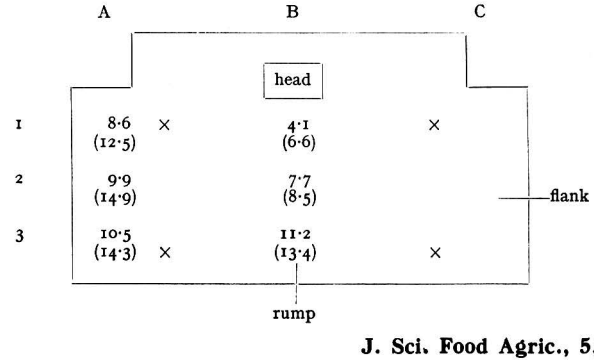
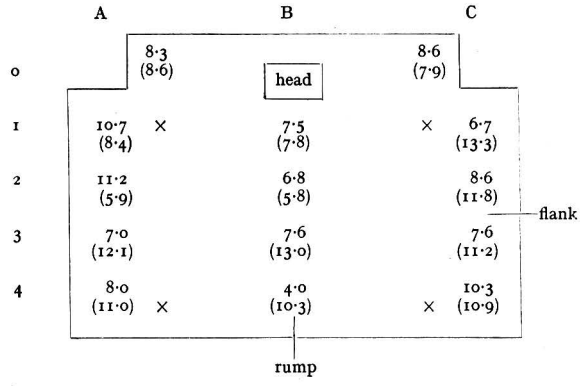


Diagram III



increase on moving from the back round the body. (*c*) The total grease and suint contents (employing whole staples) varies very little over the whole hogget fleece, the total lying between 12·5 and 15·7% for 12 samples. The variations become more pronounced with increasing age of ewe.

Variation of pH

The pH of an 0·002M-solution of cetylpyridinium chloride was measured before and after immersion of a 1-g. sample of fleece in 50 ml. of solution. This concentration lies in the α range (see Part III) where reactions between wool and wetting agent influence pH the least.

All pH changes were small; the original solutions had pH values between 6·1 and 6·4, and the change after immersion of the wool was seldom more than ± 1·0. Most of the back samples showed an acid reaction, which changed to an alkaline reaction on moving from the back round the body. Hobson[2] observed that the variation in pH of the fleece of Welsh ewes followed broadly the variation in suint content. The same broad correlation was to be observed in the present results, but in view of the narrow pH ranges involved it was not profitable to attempt close correlation.

Variation of inactivation

The inactivation of cetylpyridinium chloride solutions produced by the wool samples listed in Diagrams I, II and III has been measured as described in Parts II and III. In these papers it was shown that the inactivation/concentration curve could be divided into three distinct sections (designated α, β and γ) and that different mechanisms of reaction take place in each range of concentration.

α-Range inactivation.—This range, in which inactivation is produced by interaction of the long-chain cations with suint anions, coupled with the adsorption by the grease layer of both this cation–anion complex and the long-chain cations themselves, has been shown with the 1948 ewe samples (Part II) to be largely independent of grease and suint contents. Results of fleece samples from sheep of other ages, with a 0·002M-solution, are shown in Table I.

These values show very little fluctuation, and are largely independent of both grease and suint contents. The inactivation values obtained at this concentration with samples from the 1948 ewe were very similar to the values of the 1946 ewe given above. The age of the ewe has therefore only a small effect on inactivation in the α concentration-range, although the values do decrease somewhat with increasing age.

β-Range inactivation.—In this range of concentration, the α-range processes occur first, after which grease is removed from the fibre surface and the inactivation is reduced, in some instances to zero. This is due both to the interaction of some of the grease constituents with the long-chain ions, and to the partial liberation of surface-active material absorbed during the α-range processes. The results in Table I show inactivation values obtained with a 0·003M-solution.

The values for the fleece of the 1946 ewe fluctuate widely. In this range the inactivation isotherm is falling rapidly, and, in contrast to the α range, the processes involved are sensitive

Table I

Wool sample	Inactivation (mg./g. of dry wool): Hogget (1950)			1949 ewe			1946 ewe		
	α	β	γ	α	β	γ	α	β	γ
Ao (breast)	26·8	18·7	0·0	—	—	—	24·0	25·6	16·1
Co ,,	27·4	17·6	3·4	—	—	—	24·6	24·0	19·6
A1 (flank)	27·4	27·2	15·4	26·7	23·8	15·3	24·1	20·3	40·9
A2 ,,	26·5	27·3	13·7	28·2	28·6	18·7	24·6	14·6	17·5
A3 ,,	28·1	21·7	6·8	27·8	27·2	10·2	22·5	9·6	34·8
A4 ,,	27·4	19·5	−5·0	—	—	—	21·7	14·2	42·3
B1 (back)	27·7	25·3	3·4	25·7	17·0	6·8	25·6	0·0	−4·9
B2 ,,	24·7	16·6	4·1	29·4	30·2	23·8	23·0	2·0	8·4
B3 ,,	28·0	27·9	5·1	27·2	28·9	54·3	26·1	15·5	17·5
B4 ,,	27·2	21·2	7·6	—	—	—	23·4	18·6	23·4
C1 (flank)	27·7	18·4	0·0	—	—	—	24·2	21·6	41·1
C2 ,,	25·9	15·0	0·0	—	—	—	24·0	15·9	25·1
C3 ,,	27·9	22·0	3·5	—	—	—	24·6	21·6	25·5
C4 ,,	27·4	23·1	6·6	—	—	—	23·7	10·2	36·8

to grease and suint. However, the results for hogget and 1949 ewe are very little lower than those obtained in the α range. This suggests that the α range extends to higher concentrations in these instances, so that 0·003M is at the start of the β range.

γ-Range inactivation.—Two opposing mechanisms operate in this range. First, the flocculating effect of cationic wetting agents causes high inactivation by re-depositing suspended grease, dirt and insoluble cation–anion complex on the fibres. Secondly, interaction between long-chain cations and the exposed protein chains of the fibres produces activation of the solution. Figures for the inactivation produced with a 0·005M-solution of cetylpyridinium chloride are shown in Table I.

The inactivation produced by the wool samples from the 1949 and 1946 ewes show typical wide variation with grease and suint contents, although the amount of inactivation produced does not always follow closely the grease and suint contents of the samples. The inactivation values produced by the hogget-fleece samples vary within a much narrower range, and their small magnitude suggests that the β range here extends to higher concentrations than for the older ewes.

A comparison of all inactivation values given in this paper with the corresponding grease and suint contents shows that, although this content is an important factor, it is not the only major factor determining the magnitude of inactivation. It is shown later that the surface area of fleece is also of importance.

Inactivation isotherms

Full inactivation isotherms for immersion periods of 30 minutes have been determined on selected fleece-samples from the hogget, and from the 1949 and 1946 ewes (Figs. 1, 2 and 3 respectively).

The three isotherms for the hogget fleece (Fig. 1) are similar in general shape despite differences in grease : suint ratio, and they differ somewhat from the curves for the fleece of the 1948 ewe (Part III). The chief points of difference are: (i) The α range extends to a rather higher concentration (0·0027M, compared with 0·0023M); (ii) The inactivation produced in the α range is higher (a maximum of about 35 mg./g. compared with 25 mg./g.); (iii) The β mechanism operates over a wider range of concentration (extending to an upper limit of 0·0045 to 0·005M, compared with 0·0035 to 0·004M); (iv) The γ-range inactivation is suddenly arrested and is followed by considerable activation. This is entirely different from anything observed with the 1948 samples.

The isotherms obtained with the 1949 fleece (Fig. 2) illustrate almost exactly a half-way stage between the isotherms of the fleeces of the hogget, and the 1948 ewe. The α range extends to an initial concentration of 0·0025M, and the inactivation produced is also intermediate. The β range is wider than that for the 1948 samples, but shorter than the range for the hogget samples; the γ range shows considerable inactivation, but this is not arrested so rapidly as with the hogget samples, nor is the following activation so pronounced. The grease plus suint content of sample 1949.A1 is twice that of 1949.B1, but curves D and E are not separated to the extent that would be expected if the grease and suint content was the one major factor determining their position.

The isotherms for samples A1 and B1 of the 1946 fleece (Fig. 3) are very similar to

those for the 1948 samples; it therefore appears that the inactivation isotherms vary with age of sheep only in the case of the hogget and 2-year-old ewe. Whatever the age of the sheep above this figure, the inactivation produced by its fleece will not differ appreciably from that described in Part III.

Variation in surface area of fleece

Since the inactivation isotherms change in position with duration of immersion it appeared possible that they might be influenced by the availability, as well as the quantity, of grease and suint on the fibres; this availability will be related to the surface area over which a given quantity of grease and suint is spread. Again, the quantity of wetting agent undergoing adsorption will depend directly on the surface area, which should therefore represent an important factor that has not hitherto been considered in these systems. This is especially so if the surface area varies appreciably with type of fleece.

Selected samples were therefore examined under a microscope and the diameters of the various fibres in a typical cross-section of each sample were measured. From these figures the surface area of 1 g. of wool from each sample was evaluated. The range of fibre diameters and the surface areas are shown in Table II.

Table II

Wool samples	Relative no. of fibres with diameters (mm.)					Surface area, cm.²/g. of wool
	0·017	0·034	0·07	0·102	0·153	
1950 A1	92	—	2	5	—	930
1950 B2	150	—	3	1	—	1436
1950 C1	115	—	8	—	—	1242
1949 A1	98	10	—	3	1	971
1949 B1	99	40	—	1	3	889
1946 A1	95	35	6	3	4	724
1946 B1	73	56	8	9	4	661

The surface area of hogget fleece is much greater than that of the older fleeces; the area then decreases with age of sheep from which the fleece was taken, but the values vary little after the third year. Visual examination of a large number of samples suggests that, except with the hogget fleece, the surface area of back samples is less than that of corresponding flank samples, although the limited number of actual area measurements reported here do not fully illustrate this.

Correlation of results

The inactivation isotherms, which can be only partly interpreted on the basis of grease and suint contents of the wool, can be more fully explained by a combination of this effect with change in surface area of the fleece samples. The interactions of grease and suint with wetting agent will not, in themselves, be altered by variation in the surface area of the sample, but such variation will modify the extent to which the various reactions take place. These modifications are considered separately for the α, β and γ concentration-ranges.

The α range

For a given concentration of wetting agent, inactivation will increase with surface area, and this in turn will increase the upper concentration limit of the α range, since the final concentration of cetylpyridinium chloride in the solution after immersion must be greater than the critical concentration for micelles before the β mechanism can operate.

The isotherms in Figs. 1, 2 and 3 and in Part III confirm this; the highest inactivation in the α range and the highest value of the upper concentration limit are given by the hogget samples, together with the largest surface area. The inactivation produced by the 1949 samples is the next highest, whereas the remaining fleeces, 1948 and 1946, show the lowest values and have the smallest surface areas. The slight variation of inactivation in the α range produced by different samples from the same fleece will also be largely due to the differing surface areas of the samples.

The β range

In this range, increase in the area of grease in contact with the solution of wetting agent will facilitate grease removal; the more grease that is removed, the greater will be the reduction in the inactivation. The grease–cation interaction will tend to prevent the γ mechanisms from operating until a higher concentration of long-chain cations is present in solution, and

hence the upper limit of the β range will be moved to higher concentrations. For samples with small grease content, an increase in surface area will have little effect in this range, as virtually all the grease is removed in any case. The resulting exposure of the protein chains enables the cation–protein interaction to proceed with the formation of more surface-active products.

The inactivation isotherms support these conclusions. The isotherms for the 1946 samples show a narrow β range (extending to 0·0032M); for the 1949 samples the β range extends to 0·0045M, and for the hogget samples, with the highest surface areas, to 0·005M.

The γ range

Variation in the surface area of the wool sample will not affect both mechanisms operating in this range to the same extent. Flocculation is a bulk phenomenon, and increase in the area on which flocculated material can deposit will not materially assist this re-deposition. On the other hand, increase in the surface area of exposed wool-fibres will increase the protein–cation interaction, and may even cause activation of the solution.

This effect is best illustrated by the isotherms for the hogget samples in Fig. 1. At a

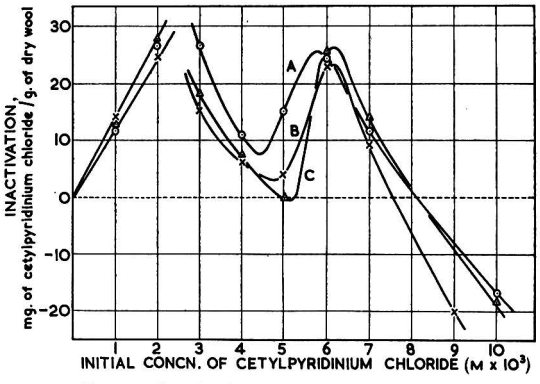


FIG. 1.—*Inactivation isotherms for hogget fleece*

Curve	Sample	Grease, %	Suint, %
A	1950.A1	8·6	5·7
B	1950.B2	5·6	10·1
C	1950.C1	11·4	3·6

cetylpyridinium chloride concentration of 0·005M flocculation occurs, and accounts for the rapid increase in inactivation; much grease has already been removed from the fibres at this stage. The flocculated grease is re-deposited as discrete particles, and does not therefore hinder the interaction between long-chain cations and wool protein in the same way as does the original grease layer. Because of the large surface area of these samples, the grease is spread thinly, and is more readily removed, so that the attack of wetting agent on the large surface area of exposed protein is sufficient at 0·006M to cause this effect to predominate, giving a peak in the curve and rapid activation of the solution.

The isotherms for the 1949 ewe (Fig. 2) show a similar effect, but the smaller surface area leads to a thicker grease layer (compared with that of the hogget fleece) than the grease content itself would imply. Within the same period of immersion, less of the protein fibres are therefore exposed; this allows the re-deposition effect to assume greater proportions and the activation produced in higher concentrations is not so pronounced.

With the 1946 sample A1, of high grease-content (curve F, Fig. 3), the small surface area results in a grease layer of such a thickness that no protein fibres are exposed at the end of the β range; the only effect observed is inactivation due to flocculation. With the hogget fleeces (Fig. 1) some wool protein was exposed in the γ range with each sample, so that the position of the isotherms was not greatly influenced by grease content. With small-area fleeces of the 1946 type, the position of the isotherm in the γ range depends upon whether or not wool protein is exposed, and is therefore much more sensitive to grease content; this is shown by comparison of curves F and G (Fig. 3) and by Fig. 3 (Part III).

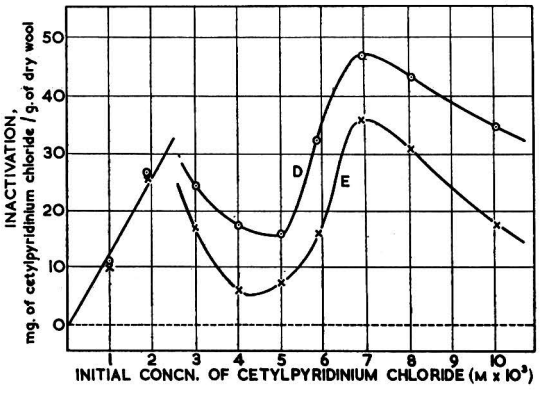


FIG. 2.—*Inactivation isotherms for fleece of* 1949 *ewes*

Curve	Sample	Grease, %	Suint, %
D	1949.A1	8·6	12·5
E	1949.B1	4·1	6·6

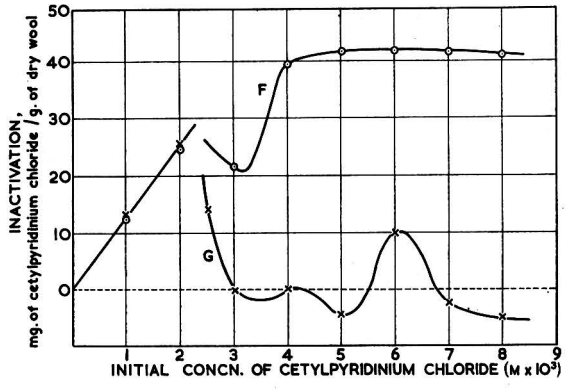


FIG. 3.—*Inactivation isotherms for fleece of* 1946 *ewes*

Curve	Sample	Grease, %	Suint, %
F	1946.A1	10·7	8·4
G	1946.B1	7·5	7·8

Conclusion

The work described in this paper has confirmed that the general principles governing inactivation of solutions of cationic wetting agents by sheep fleece, outlined in Part III, are applicable to any type of Scotch Blackface fleece, but that a quantitative treatment of inactivation must also take into account the surface area of the fleece.

Acknowledgments

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The University, Nottingham

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SOIL STERILIZATION. III.*
The Effect of Cultivation on Ammonia and Nitrate Production in a Glasshouse Soil Steam-Sterilized *in situ*

By J. N. DAVIES and O. OWEN

Ammonia and nitrate production and the nitrifying capacity of the soil, as measured by incubation with ammonium sulphate, have been periodically determined in a glasshouse soil steam-sterilized *in situ* and subsequently cultivated under commercial conditions. The soil was apparently not contaminated with nitrifying organisms to any extent before the pre-planting manurial treatment. Flooding the soil had little effect on either the soil ammonia or on the nitrifying capacity of the soil, but some nitrate was washed out. The application of a fertilizer dressing, including stable manure, three months after steaming, resulted in increased ammonia-production followed within a few weeks by renewed nitrification. The planting of tomatoes after the manurial treatment markedly accelerated the onset of nitrification. In contrast, recolonization of the steamed soil by nitrifying organisms was very slow where the soil, apart from periodical watering, was left undisturbed. Evidence is presented to indicate that the steamed soil in which the tomato plants were raised was contaminated with nitrifying organisms when the seeds were sown. The infection was then introduced into the potting soil and finally into the glasshouse border.

Introduction

It has been shown that, in the absence of any disturbance of the soil, contamination by nitrifying organisms of glasshouse soils steam-sterilized *in situ* is a slow process ; high ammonia-concentrations persist, and no conversion into nitrate occurs for several months.[1, 2] Digging a steamed soil at regular intervals was found to accelerate the onset of nitrification, presumably as a result of incorporating surface infections into the main body of the soil.[2] The present paper deals with the ammonia and nitrate changes in a glasshouse soil which, after being steamed, was flooded and treated with a fertilizer dressing before being planted with tomatoes in accordance with normal commercial practice.

Experimental methods

In this investigation an old tomato-soil was used that has been in continuous cultivation for over 30 years and has been steamed repeatedly. This soil, at the time of steaming, showed the following analysis :

Total nitrogen (Kjeldahl)	0·33%
Organic carbon (Walkley & Black)	3·07%
Potash (K_2O) (soluble in 0·5N-acetic acid)	0·13%
Phosphoric acid (P_2O_5) (soluble in 0·5N-acetic acid)	0·39%
Total carbonates (as $CaCO_3$)	1·74%
pH	7·33

A length of tomato border in the glasshouse was divided into two ; one half was steamed in the late autumn by the Hoddesdon pipe system, and the other half left unsteamed. At the time of steaming, both steamed and unsteamed plots were divided into three sub-plots by means of asbestos sheeting buried vertically in the soil to a depth of 15 inches. The pipes were buried at a depth of nine inches and steam was forced into the soil for a total time of 20 minutes. Subsequently the three pairs of steamed and unsteamed plots were treated as follows : (*a*) left undisturbed, apart from periodic watering to keep the moisture content at a reasonably constant level, (*b*) flooded with water, 46 days after being steamed, at a rate equivalent to 50,000 gal. per acre, and a fertilizer dressing dug in on the 95th day after the steaming, and (*c*) treated similarly to (*b*) but in addition planted with tomatoes on the 98th day after the steaming. The fertilizer dressing used in treatments (*b*) and (*c*) was thoroughly incorporated into the top spit of soil and comprised 4 oz. per square yard each of bone meal, hoof and horn, and sulphate of potash with the addition of stable manure (10 tons per acre). Where tomatoes were planted, the plots were subsequently watered in accordance with the plants' requirements. At the same time an approximately equal volume of water was applied to the plots, which, after being flooded, received the manurial treatment but were not planted. The glasshouse was not heated for the first 88 days after the steaming.

* Part II : *J. Sci. Fd Agric.*, 1953, **4**, 248

The ammonia- and nitrate-nitrogen contents of the various plots were determined at intervals, as described previously.[1]

At the beginning of the investigation it was considered desirable to obtain some indication of the extent of contamination of the steamed soil with nitrifying organisms throughout the course of the experiment. A preliminary laboratory study of the capacity of the unsteamed, steamed, and artificially contaminated steamed-soils to nitrify ammonium sulphate was therefore made. Samples (100 g.) of soil in cotton-wool-plugged flasks were steamed in an autoclave for 20 minutes at zero pressure (i.e. with the valve open). When cold, some samples were contaminated with 2 ml. of a soil : water infusion (1 : 10) prepared from the original unsteamed soil, and to other samples 2 ml. of distilled water were added. Treated samples were then moistened with 5 ml. of a solution of ammonium sulphate, equivalent to 7·5 mg. of nitrogen, and an equal volume of water was added to the control samples. The final moisture content of the soil corresponded to 90% of the moisture equivalent (Bouyoucos[3]). The samples were then incubated at 23·5° and duplicate samples analysed at intervals for ammonia and nitrate.

Table I shows that, in the unsteamed soil, over 90% of the added nitrogen was nitrified at the end of six days' incubation. In the steamed soil, nitrification was suppressed in the absence of deliberate contamination, but, where an unsteamed-soil infusion had been added immediately after steaming, nitrification began within a fortnight. By the 21st day approximately 40% of the added nitrogen had been nitrified. In the samples incubated without added ammonium sulphate, however, the soil ammonia was almost completely nitrified after 14 days where the soil was deliberately contaminated after being steamed (Table II). It is apparent from these results that, after three weeks, deliberate contamination of the steamed soil only partially restored its original nitrifying capacity.

Table I

Percentage recovery of added ammonium sulphate (equivalent to 7·5 mg. of nitrogen) as nitrate in unsteamed, steamed, and contaminated steamed-soil

Days after steaming	Unsteamed	Steamed	Steamed and contaminated
1	2·5	1·7	0·2
3	19·1	1·5	—
6	94·2	0·5	0·5
10	97·9	—	3·0
14	100·1	—	8·7
17	97·6	0·1	30·2
21	97·7	1·9	38·2

Table II

Ammonia and nitrate production in unsteamed, steamed, and contaminated steamed-soil incubated without added ammonium sulphate

Days after steaming	Unsteamed		Steamed		Steamed and contaminated	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N
1	0·4	94·2	4·4	91·5	4·1	95·0
3	0·9	94·9	9·1	92·2	11·1	95·4
6	1·3	97·1	11·9	93·3	14·5	95·5
10	1·3	99·1	16·0	94·5	16·6	95·7
14	0·8	100·1	18·0	94·9	12·4	102·7
17	—	101·8	18·0	93·4	0·9	107·6
21	1·7	105·6	21·7	94·5	2·7	113·9

In view of the fact that the unsteamed soil was capable of nitrifying over 90% of the added nitrogen in six days the following procedure was adopted. Immediately after the plots in the glasshouse were sampled, the soil was passed through a clean $\frac{1}{8}$-in. sieve, and 100-g. samples were weighed into conical flasks. The soil was then moistened with 5 ml. of ammonium sulphate solution (equivalent to 7·5 mg. of nitrogen), the flasks were plugged with cotton wool and incubated for six days at 23·5°. Untreated samples were moistened with 5 ml. of distilled water and incubated at the same time. It was considered that steamed soil from the glasshouse plots was effectively contaminated with nitrifying organisms if, after six days' incubation with added ammonium sulphate, over 90% of the added nitrogen was recovered as nitrate.

Ammonia and nitrate production, and the capacity of the soil to nitrify ammonium sulphate, were also studied in the propagating and potting soils in which the tomato plants were raised before being planted-out in the glasshouse border. The propagating soil consisted of five parts of virgin loam and one part of stable manure steamed together in a small steam-sterilizing unit. After being steamed the soil was stored under cover for three weeks before use. Before tomato seeds were sown in this soil in shallow trays, 6 oz. each of lime and superphosphate were well mixed with each 100 lb. of soil. The resultant mixture, at the time of seed sowing, had the following analytical figures: total nitrogen 0·39, organic carbon 3·56, phosphoric acid (P_2O_5) 0·07, potash (K_2O) 0·08, total carbonates (as $CaCO_3$) 0·30%, and pH 7·16. Seeds of tomato, variety Ailsa Craig, were sown in this mixture in early January, and 100% germination was observed after seven days. Three weeks later the young seedlings were potted-up in a mixture similar physically to that described above but having the following analytical figures: total nitrogen 0·57, organic carbon 4·96, phosphoric acid (P_2O_5) 0·09, potash (K_2O) 0·19, total carbonates (as $CaCO_3$) 0·35% and pH 6·67. The plants were finally planted-out in the glasshouse border 24 days after potting-up.

Results

Ammonia and nitrate production in the undisturbed steamed-soil are shown in Fig. 1. After the usual initial rapid rise, ammonia-nitrogen fluctuated between 30 and 45 p.p.m. up to the 110th day after steaming. Afterwards there was a slow fall in the ammonia concentration, until, on the 223rd day after steaming, it was low and similar to that in the corresponding unsteamed soil. Nitrates meanwhile showed a general tendency to increase, slowly at first and then more rapidly as the ammonia decreased. Ammonia concentrations were invariably low in the undisturbed unsteamed-plot (Table III). Nitrates increased slowly when the temperature was low and more rapidly when the glasshouse was heated.

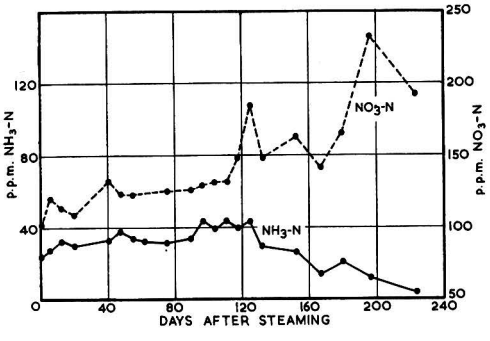

FIG. 1.—*Ammonia and nitrate production in undisturbed steamed-soil*

The changes in the steamed plot which, after being flooded, received the fertilizer treatment, are shown in Fig. 2. Fig. 3 shows the changes in the steamed plot which was planted with tomatoes. Ammonia concentrations in both plots were similar to those in the undisturbed steamed-plot up to the time of the fertilizer application. Flooding the soil with water on the 46th day after steaming washed some nitrate out of the soil, but apparently had little effect on the ammonia. In both these plots increased ammonia-production was observed after the pre-planting manurial treatment; peak ammonia values of approximately 80 p.p.m. were recorded on the 104th day (i.e. 9 days after the incorporation of the dressing). Within three weeks of the fertilizer dressing's being applied almost complete nitrification of the soil ammonia had taken place in the planted plot. In the absence of the tomato plants, nitrification proceeded considerably more slowly, and some eight or nine weeks elapsed before ammonia values similar to those in the unsteamed soil were observed.

In the unsteamed plots that received the fertilizer dressing, ammonia concentrations were low up to the time of its application, but some ammonia production was observed after it had been applied (Table III). Within a fortnight, however, this had been nitrified with the

Table III

*Changes in ammonia and nitrate nitrogen, p.p.m., in the unsteamed plots**

Time, days	Undisturbed		Receiving the fertilizer dressing but not planted		Receiving the fertilizer dressing and planted	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N
1	1·8	20·2				
6	1·3	26·0				
13	2·0	38·2				
20	1·4	40·2				
41	1·3	30·8				
46	—	—	Flooded		Flooded	
48	1·6	65·9	1·5	24·3	0·8	20·8
55	1·4	60·8	0·8	36·0	0·6	28·7
62	1·4	79·4	2·6	41·6	1·4	37·4
76	1·7	55·9	2·0	40·4	1·8	37·3
90	3·4	77·9	3·2	43·6	3·1	39·2
95	—	—	Fertilizer dressing applied			
97	6·5	51·4	28·6	37·9	33·2	44·5
98	—	—	—	—	Tomatoes planted	
104	2·4	52·8	15·9	62·4	10·5	111·2
111	4·6	64·1	7·3	96·7	3·8	122·5
125	4·3	69·9	2·6	96·7	2·0	108·4
153	4·1	104·9	2·2	139·2	2·2	108·4
181	3·0	133·5	3·0	195·9	2·5	34·3

* Composite samples from all three unsteamed plots were used to obtain the results shown for the first 41 days

formation of considerable amounts of nitrate. Subsequently nitrates increased in the unplanted plot and slowly decreased in the planted plot.

The results of the incubation experiments with ammonium sulphate are shown in Table IV. Up to the 95th day after steaming little or no response to the added nitrogen after six days' incubation was observed in soil from any of the steamed plots. In addition, ammonia in the control samples incubated without added ammonium sulphate did not disappear, but in general showed a tendency to increase, as is shown in Table V. It therefore seems unlikely that during this time the steamed soil was contaminated to any extent. After the 97th day, the response to added nitrogen, of soil from the unplanted plot that had received the manurial treatment, slowly increased, until, 37 days later, on the 132nd day after steaming, over 90% of the added nitrogen was recovered as nitrate in six days. Where tomatoes were planted, the steamed soil was apparently much more rapidly contaminated with nitrifying organisms, and, within

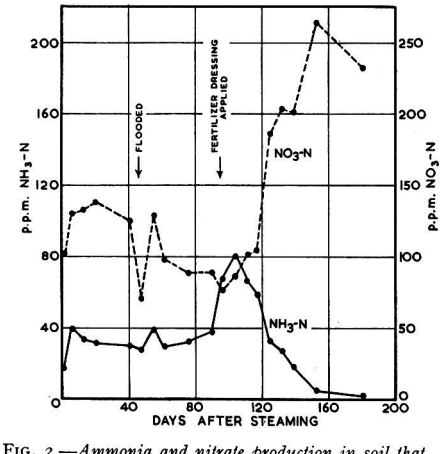

FIG. 2.—*Ammonia and nitrate production in soil that had been steamed, flooded, and treated with a fertilizer dressing*

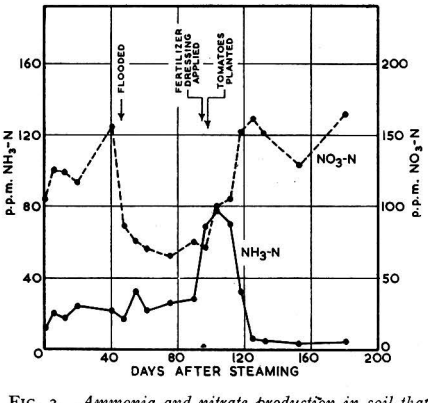

FIG. 3.—*Ammonia and nitrate production in soil that had been steamed, flooded, treated with a fertilizer dressing, and planted with tomatoes*

a fortnight of planting, over 89% of the added nitrogen was nitrified after six days' incubation. Table V also shows that, after the application of the fertilizer dressing, considerable amounts of nitrate were formed in the control samples from the plots receiving the manurial treatment and incubated without the addition of ammonium sulphate; at the same time ammonia concentrations decreased.

Table IV

Percentage recovery of added ammonium sulphate as nitrate after six days' incubation at 23·5° in soil from the steamed plots

Sampling day	Undisturbed	Receiving the fertilizer dressing but not planted	Receiving the fertilizer dressing and planted
1	< 1·0	1·4	< 1·0
6	< 1·0	< 1·0	< 1·0
13	< 1·0	< 1·0	< 1·0
20	< 1·0	1·5	< 1·0
41	1·3	4·4	1·3
48	< 1·0	< 1·0	< 1·0
55	1·2	< 1·0	< 1·0
76	< 1·0	< 1·0	3·7
90	2·4	< 1·0	1·6
97	—	< 1·0	< 1·0
104	< 1·0	3·8	< 1·0
111	4·2	22·7	89·4
118	< 1·0	26·0	94·2
125	1·2	73·0	96·5
132	9·4	92·8	89·3
153	24·5	85·5	95·3
167	88·9		
181	91·2	94·4	95·5
195	94·4		
223	92·7		

Table V

Changes in ammonia- and nitrate-nitrogen, p.p.m., in control samples taken from the steamed plots and incubated for six days at 23·5° without the addition of ammonium sulphate

Sampling day	Undisturbed		Receiving the fertilizer dressing but not planted		Receiving the fertilizer dressing and planted	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N
1	+9·0	+2·5	+11·3	−0·5	+7·3	−0·5
6	+1·4	−0·7	+2·7	+1·9	+1·7	+2·2
13	+1·4	−0·3	+4·5	−1·1	+2·8	−0·1
20	+1·6	−1·1	+4·2	−1·2	+0·4	−1·8
41	+3·4	+1·3	+2·0	+0·3	+1·4	+0·9
48	+0·7	−4·4	+4·9	+1·3	−1·9	+0·5
55	+2·8	+1·2	+5·5	+1·9	+2·2	+5·5
76	−0·6	+1·8	+4·0	+0·6	+0·2	+0·8
90	−2·7	+0·2	−1·3	+0·7	−3·6	+0·4
97	−4·1	+5·5	+14·2	+18·5	+22·7	+5·7
104	+0·5	+2·7	−52·9	+63·7	−34·1	+50·3
111	−17·0	+5·0	−62·0	+76·8	−70·5	+84·7
118	−5·7	−1·0	−63·3	+70·4	−30·7	+37·7
125	−13·7	+18·8	−32·2	+49·1	−4·9	+17·8
132	−24·9	+25·9	−26·8	+38·2	−4·1	+24·0
153	−25·5	+28·8	−3·9	+7·7	−1·9	+6·3
167	−12·1	+23·0				
181	−18·4	+22·0	−2·0	+5·2	−2·1	+4·5
195	−10·6	+12·0				
223	−2·0	+4·2				

Soil from the undisturbed steamed-plot was, in contrast, not capable of nitrifying approximately 90% of the added nitrogen in six days until the 167th day after being steamed. Nitrification in the control samples from this plot did, however, begin earlier (Table V), and after the 125th day ammonia disappeared on incubation with the concomitant formation of nitrate.

Whatever the treatment, soil from the unsteamed plots invariably nitrified between 85 and 100% of the added ammonium sulphate in six days.

Changes in the propagating and potting mixtures

Ammonia and nitrate production in the propagating soil are shown in Table VI, together with the nitrate recoveries obtained when soil samples from the seed trays were incubated with ammonium sulphate for six days. It will be seen that ammonia in this soil was high when the seeds were sown. However, the high nitrate-recovery after six days' incubation with ammonium sulphate, suggests that at this time the soil was already contaminated with nitrifying organisms.

Table VI

Ammonia- and nitrate-nitrogen in the propagating soil and the percentage recovery of added nitrogen as nitrate after six days' incubation with added ammonium sulphate

Time, days, after sowing of seed	NH_3-N, p.p.m.	NO_3-N, p.p.m.	Percentage recovery of added N as nitrate after six days' incubation
0	90·4	85·0	93·5
6	92·4	93·7	97·3
14	19·8	145·7	90·9
27	7·5	79·8	92·7

This view is supported by the subsequent rapid disappearance of the soil ammonia with the formation of nitrate. Thus 27 days after seed was sown and one day before the young seedlings were transferred to pots, the ammonia concentration was low, and the soil was apparently in an actively nitrifying condition.

When the seedlings were potted-up, ammonia in the potting soil was high at 160 p.p.m., and less than 10% of the added nitrogen was recovered as nitrate after six days' incubation (Table VII). Four days later, however, ammonia had begun to decrease, and by the 11th day it had to a large extent been nitrified. During the same period, the nitrifying capacity of the soil increased rapidly, and by the 21st day over 90% of the added nitrogen was nitrified in six days. It will be seen from these results that the potting soil, although not markedly contaminated by nitrifying organisms when the seedlings were potted-up, was actively nitrifying by the time the plants were set out in the glasshouse border.

Table VII

Ammonia- and nitrate-nitrogen in the potting soil and the percentage recovery of added nitrogen as nitrate after six days' incubation with added ammonium sulphate

Time, days, after potting	NH_3-N, p.p.m.	NO_3-N, p.p.m.	Percentage recovery of added N as nitrate after six days' incubation
0	160·4	60·2	9·2
4	142·5	65·2	34·5
11	42·3	217·0	83·2
21	4·6	80·3	96·3

Discussion

In agreement with previous work,[1, 2] high ammonia-concentrations persisted for long periods in the steamed soil that was left undisturbed. About three months after the soil had been steamed, ammonia slowly began to decrease, but a further three or four months elapsed before it completely disappeared. In contrast to previous experiments, the soil was regularly watered in order to maintain the moisture content at about 18%, and the surface soil was never allowed to dry out completely. It therefore seems likely that, in addition to any contamination by nitrifying organisms arising from the subsoil, chance surface-infections were able to multiply and spread downwards into the soil. It will be seen, however, that if this did in fact occur, the process was an extremely slow one. Warcup has recently shown that the recolonization of steamed forest-nursery soils by fungi is very slow; numbers were still very much lower than in the corresponding unsteamed soil even after 18 months.[4]

Where the soil, after being steamed, was treated in accordance with normal glasshouse practice, the onset of nitrification was accelerated. Flooding soil after it has been steamed

is a recognized practice, but the reasons for its beneficial effects are not fully understood. From the present results it apparently has little effect on ammonia concentrations or on the nitrifying capacity of steamed soil as measured with ammonium sulphate, but some nitrate was washed out of the soil by the treatment.

The incorporation of organic nitrogenous fertilizers into a steamed soil resulted in increased ammonia-production, which was followed fairly rapidly by the nitrification of the soil ammonia. Walker & Thompson[5] have reported that the addition of farmyard manure to a soil immediately after steaming resulted in increased ammonia-production in comparison with untreated steamed-soil. They found, however, that nitrification began within three weeks of steaming, whether farmyard manure had been added or not; this suggests some gross contamination of the soil. Although these workers give no details of any changes in the soils in which the tomatoes were raised before planting-out, and draw no conclusions about the source of the infection, it seems likely that it was introduced when tomatoes were planted out 12 days after the soil was steamed. In the present investigation, nitrification in steamed soil began much earlier where stable manure had been added than in its absence. When the fertilizer dressing was incorporated into the steamed soil, some surface infections may have been introduced into the main body of the soil, but it seems more probable that the stable manure was largely responsible for the contamination of the steamed soil with nitrifying organisms. It is also possible that some contamination may have arisen from the bone meal included in the pre-planting manurial treatment, since it has been shown that bone meal may undergo biological decomposition in storage.[6]

The vigorous ammonification that followed the application of organic nitrogenous fertilizers to the steamed soil shows that the low maximum ammonia-concentrations found in steamed glasshouse-soils are due more to a lack of a suitable precursor than to a moribund soil population or some unfavourable soil condition.

The most rapid nitrification of the soil ammonia in the steamed soil was observed where tomatoes were planted-out after the fertilizer dressing had been applied. Within three weeks of planting the soil ammonia had almost completely disappeared. In contrast, where the soil received the pre-planting manurial treatment but subsequently remained unplanted, some eight or nine weeks elapsed before the soil ammonia was completely nitrified. These results suggest that nitrifying organisms were introduced into the steamed soil at the time of planting. When planting, holes are made in the glasshouse border with a special dibber; the holes are of the same size and shape as the inside of the pots in which the young plants are grown. The ball of roots and soil on removal from the pot then fits closely in the hole, and is in intimate contact with the soil of the glasshouse border. It therefore seems likely that any infections present in the potting soil will spread rapidly into the surrounding soil of the glasshouse border. That the soil in the pots at the time of planting-out in the border was, in fact, actively nitrifying is shown by the changes that took place in the propagating and potting soils. Nitrifying organisms appear to have been present in the propagating soil when the seeds were sown, since at that time, although the ammonia was high, the soil was capable of nitrifying over 90% of added ammonium sulphate in six days (Table VI). By the time the seedlings were ready to be potted, ammonia concentrations were low, and it is reasonable to suppose that the transplanting of the young seedlings into the potting mixture was accompanied by the transfer of nitrifying organisms in the soil adhering to the roots. After the introduction of the seedlings into the potting mixture, this soil, which was apparently not contaminated to any extent at first, rapidly became so. Subsequently the introduction of this soil into the glasshouse border proved to be an effective source of infection, and nitrification began almost immediately.

The results of the nitrification experiments with ammonium sulphate corroborate these conclusions. Before the pre-planting manurial treatment, the nitrifying capacity of the soil from all the steamed glasshouse-plots was very low. This failure of the steamed soil to nitrify the added nitrogen does not necessarily imply the complete absence of nitrifying organisms; they may have been present but other unfavourable conditions may have prevented their proliferation. In these experiments, however, the moisture content, pH and available calcium and phosphate levels all favoured nitrification. Moreover, during the first 90 days after steaming, the ammonia in control samples from the steamed plots incubated without added ammonium sulphate did not disappear on incubation, and no significant increases in nitrate were observed (Table IV). In addition, similarly treated but unsteamed-soil samples nitrified over 90% of the added nitrogen after six days' incubation. It therefore seems likely that the steamed soil in the glasshouse was not contaminated to any extent before the manurial treatment.

After the fertilizer dressing was applied, the nitrifying capacity of the steamed soil that remained unplanted slowly increased, until, some seven weeks later, approximately 90% of

the added nitrogen was nitrified after six days' incubation. In the samples from this plot that were incubated without the addition of ammonium sulphate, nitrification of the soil ammonia was observed earlier. These results, taken in conjunction with those for the plot sampled *in situ* (Fig. 2), indicate that the soil became rapidly contaminated by nitrifying organisms after the application of the dressing, but that some time elapsed before the contamination became completely effective in the glasshouse.

Where the soil was planted with tomatoes after the manurial treatment, it was capable of nitrifying over 90% of the added nitrogen within a fortnight of planting, which suggests that contamination here was extremely effective. In contrast, soil from the undisturbed steamed-plot did not nitrify over 90% of the added nitrogen after incubation for six days until the 167th day after being steamed. In the plot itself ammonia did not completely disappear for a further six or seven weeks, which suggests that, like the unplanted steamed-plot that received the manurial treatment, contamination *in situ* was not followed by appreciable nitrification for some time.

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THE USE OF PROCAINE PENICILLIN IN THE PRODUCTION OF TABLE POULTRY UNDER PRACTICAL CONDITIONS IN THE UNITED KINGDOM

By W. F. J. CUTHBERTSON and H. GLASSER

Procaine penicillin (procaine benzylpenicillin) given from the first day after hatching to slaughter led to a total increase in output of 11 and 15·4% respectively in two trials on different strains of table cockerels. The improved yield was partly due to improved growth and partly to a reduction in cull rate. Penicillin improved food-conversion up to seven weeks of age, but this advantage was no longer evident at 15 weeks.

Introduction

In America penicillin is increasingly used in the rations of table poultry, both of turkeys and of cockerels, but so far little has been published on the use of antibiotics in the United Kingdom under farm conditions, although Coates *et al.*[1] have shown that, in the laboratory, procaine penicillin (procaine benzylpenicillin) can greatly improve the growth of young chicks, and Golden *et al.*,[2] in a practical test, showed that it increased the growth of cockerels for at least 13 weeks.

The following experiments were carried out to determine the effect of procaine penicillin on growth and cull rate of roasters reared on two different farms under conditions typical of the United Kingdom.

Experimental

First experiment (Paddock Cottage Farm)

This establishment is mainly engaged in egg production, but approximately 2000–3000 table-birds are reared each year to a weight of about 5½ lb. The chicks used in this trial were brought up in the equipment and by the methods normal to the farm.

A batch of 1107 Brown Leghorn × Light Sussex day-old cockerels was divided into two groups of about 550 birds each; they were kept in metal brooders with wire floors until three weeks of age and then transferred to coolers. At seven weeks of age the birds were put outside in night ark verandahs (small huts with wire-mesh runs raised above ground-level) and remained there until they were finally sold. At 12 weeks of age all birds in both groups were chemically caponized by implantation with hexoestrol (15 mg.). From the age of one day to eight weeks the birds were given baby-chick mash and from then on growers ration; [3] both foods were offered as dry meal. The rations used for the two groups were identical except that 5 g. of procaine penicillin in 1 lb. of oyster-shell meal was added to each ton of food consumed by one lot of cockerels. The penicillin concentrate was mixed in by hand with some meal to make a premix, which was then incorporated in the bulk of the food. Penicillin was given to this group of birds from arrival until slaughter. The other batch of chicks received rations identical except for omission of the penicillin.

Records were kept of total food consumption, number of culls and weights of the birds at seven and 15 weeks. The culls included all weak birds eliminated as well as those that died. The results of this test are shown in Table I.

Table I

Effect of procaine penicillin on growth of Brown Leghorn × Light Sussex cockerels

Treatment	No. of birds per group	Mean wt. at		Total food consumed at		Feed efficiency*		Total culls, %	
		7 weeks	15 weeks	7 weeks	15 weeks	7 weeks	15 weeks	7 weeks	15 weeks
Nil	549	1 lb. 6 oz.	4 lb. 10½ oz.	21 cwt.	91 cwt.	3·3	4·44	5·3	10·0
5 g. of procaine penicillin per ton of food	558	1 lb. 10 oz.	5 lb. 0 oz.	23 cwt.	105 cwt.	2·94	4·53	3·4	6·6

* Ratio of food consumed to live-weight gain

Second experiment

This test was carried out in the home counties at a farm where about 10,000 table-birds are produced per year. In each of three successive months 700 Rhode Island Red × Light Sussex cockerels were used to determine the effect of procaine penicillin on growth and cull rate. The birds were obtained from a commercial hatchery and divided into two groups, each of 350 birds. All chicks were reared by the normal methods except that 1 lb. of oyster-shell meal containing 5 g. of procaine penicillin was incorporated in each ton of diet fed to one of the two groups of 350 birds put on test each month.

The chicks were reared on wire floors throughout. For three weeks they were kept in metal brooders and given a baby-chick mash; they were then transferred to coolers and offered a growers ration until they were seven weeks old, when all of them were moved out to night ark verandahs and continued on the growing ration. At 12 weeks the birds were caponized by implantation with hexoestrol (15 mg.) and finally sold when they reached about 5¼–5½ lb. live weight.

Chick weights and culls were noted at three weeks and seven weeks of age. Both the live weights and the ages of the cockerels were recorded, together with the over-all number of culls when the birds were finally sold, having reached the required weight of 5¼–5½ lb. The results of these tests are shown in Tables II and III.

Discussion

These trials on the two different farms and with two strains of cockerel indicate that the use of penicillin has led to a marked reduction in culls at all ages. At the first farm about 35 and at the second about 60 extra table-birds were obtained from every 1000 chicks given 5 g. of procaine penicillin per ton of ration from the first day.

At both farms the rate of growth was increased. The penicillin-fed cockerels in the first experiment weighed, on average, 5 lb. at 15 weeks, compared with the average of 4 lb. 10½ oz. for the birds given the normal ration; thus the penicillin had brought about an increase of almost 7½% in the rate of growth. In the second experiment the growth rate of the cockerels given penicillin was 8½% higher than that of the birds without it.

Measurement of food consumption at the first farm (Table I) showed that the efficiency of its utilization was increased during the first seven weeks, but at 15 weeks there was little

Table II

The effect of procaine penicillin on the mean growth rate and culls in groups of 350 Rhode Island Red × Light Sussex cockerels from 1 day to 7 weeks of age

Experiment	Procaine penicillin per ton of diet, g.	Wt., oz., at 3 weeks	Wt., oz., at 7 weeks	Total culls at 7 weeks, %
1	0	8·72	26·0	6·55
	5	9·75	28·8	1·1
2	0	8·37	24·3	8·3
	5	9·89	27·0	0·86
3	0	7·36	24·0	8·8
	5	9·01	26·4	0·57
Means (1050 chicks)	0	8·15	24·8	7·9
	5	9·51	27·3	0·86

Table III

Effect of procaine penicillin on time of marketing and live weight of Rhode Island Red × Light Sussex cockerels (350 chicks placed on each treatment)

		Controls		Procaine penicillin, 5 g./ton of food		Mean age at disposal, weeks	
	Age sold	No. sold	Total wt., lb.	No. sold	Total wt., lb.	Control	Penicillin
Expt. I, hatched 1 July, 1952	15 weeks	—	—	103	541		
	16	56	295	181	954		
	17	112	596	23	128	17·18	15·9
	18	105	599	—	—		
	Total	273	1490	307	1623		
Expt. II, hatched 5 August, 1952	15	—	—	112	590		
	16	34	182	173	937		
	17	101	549	14	81	17·4	15·67
	18	141	780	—	—		
	Total	276	1511	299	1608		
Expt. III, hatched 9 September, 1952	15	—	—	102	530		
	16	50	260	164	891		
	17	114	591	29	170	17·25	15·7
	18	122	669	—	—		
	Total	286	1520	295	1591		
Grand total		835	4521	901	4822	17·3	15·8
Average weight per bird		5 lb. 6·3 oz.		5 lb. 5·8 oz.			

difference in the food required per lb. of live-weight gain for the penicillin-fed and the other birds.

In the first experiment the total yield of table poultry (live weight) was 2308 lb. from 549 chicks given normal rations, and the 558 birds given penicillin yielded 2605 lb. at 15 weeks, an increased yield of approximately 300 lb. or 11%. This increased yield could be attributed both to improved growth and to the decrease in culls.

At the second farm the total live-weight yield from the 1050 birds given a normal ration was 4521 lb., and the 1050 chicks given penicillin in addition yielded 4822 lb. live weight, a gain of 301 lb. The birds given penicillin reached the required weight at 15·8 weeks, whereas the birds without it were not ready until 17·3 weeks; thus feeding penicillin led to a saving of 1½ weeks (9½%) in time, besides producing 300 lb. (6·7%) increase in yield through the reduction in culls. In the three trials at the second farm the average increase in growth rate was 8·6%, and the total advantage in terms of output of poultry per unit of time and equipment amounted to 15·4%.

Statistical analysis of the results showed that the gains were highly significant and may be summarized in the statement that average growth was improved by 8·6% (fiducial range, 6·2–11·0%, $P = 0.95$) and average cull rate decreased by 63 birds per 1000 chicks (fiducial range, 2·76–9·52%, $P = 0.95$), giving a total increase in output in a given time of 15·4% (fiducial range, 11·3–19·7%, $P = 0.95$).

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EFFECT OF ADSORBED CATIONS AND FREE SALTS ON PHOSPHATE FIXATION IN SOME EGYPTIAN ALKALINE SOILS

By S. K. TOBIA and N. E. MILAD

The effect of adsorbed calcium, magnesium, potassium and sodium on the fixation of phosphate in soil was studied. The fixing power of the cations was in the order Ca > Mg > K > Na. The carbonates, sulphates and chlorides of the corresponding cations were found to affect the concentration of water-soluble phosphate. Potassium and sodium carbonates increased the concentration but all other salts tended to decrease it. The effect of such salts was more pronounced with magnesium, potassium and sodium soils and increased with increase of base-exchange capacity of the soil. In a calcium soil this effect was very small and fixation was brought about mainly by the adsorbed calcium.

Introduction

Egyptian alkaline soils, in general, suffer from a low concentration of soluble phosphate, and in determining the quality and quantity of phosphate fertilizer to be added to such soils it is important to consider the possible reactions between the added phosphate and the soil constituents. The availability or fixation of the phosphate ion may be affected by the type and proportion of ions, which may be either adsorbed on the soil complexes or in a free state. The more common salts that provide such ions are the carbonates, sulphates and chlorides of calcium, magnesium, potassium and sodium.

The effect of salts on the availability of phosphate in calcareous and alkaline soils has been studied by several investigators. Buehrer[1] found that neutral salts such as calcium sulphate and calcium chloride that have a common ion with the phosphate compound tend to decrease phosphate solubility. Singh & Nijhawan[2] found that crops taken from an alkaline soil treated with calcium chloride contained more phosphorus and calcium than those grown on a gypsum-treated soil. The results on the effect of neutral salts having no common ion with the phosphate compound were conflicting and differed with different techniques. Buehrer[1] found that sodium salts decrease the availability of phosphate whereas Lewis *et al.*[3] found the opposite to be true. Shawarbi,[4] while leaching an alkaline soil with water, found that no phosphate appeared in the first leachate but in the second leachate phosphate started to appear after removal of the greater part of the soluble salts. Tobia[5] found that when an ordinary soil (taken from Harlington, England) was treated with sodium chloride it showed a decrease in soluble phosphate. The same effect took place also on treating the same soil with calcium carbonate.

Diverse theories have been put forward on the mechanism of phosphate fixation in calcareous soils. Puri[6] stated that phosphates are fixed by soils in nature by precipitation

reactions. In a calcium soil he found that the amount of P_2O_5 fixed from the soil an equivalent amount of calcium, and with a magnesium soil fixation took place only after the addition of ammonia to form magnesium ammonium phosphate. On the other hand Barbier *et al.*[7] found that PO_4^{3-} fixation in a calcium soil was an adsorption phenomenon. Boischot *et al.*[8] also showed that under conditions ordinarily found in a soil, the phosphate ion is not precipitated by, but is adsorbed on, the surface of calcium carbonate; desorption takes place under the influence of the humate ion.

In alkali and saline soils the mechanism of phosphate fixation is still not fully understood, and the problem is complicated by the wide variation in the type and proportions of the different native-salt constituents. When a soluble phosphate solution is added to such soils it is difficult to ascertain whether the disappearance of phosphate is due to the effect of free salts in the soils or to the ions adsorbed on the soil complexes. It was the purpose of this investigation to examine the effect of each of the adsorbed cations Ca^{++}, Mg^{++}, K^+ and Na^+ on phosphate fixation in the absence of other salts, and also the effect of the carbonate, sulphate and chloride ions, taken independently, on such fixation.

Materials and methods

Two soils, A and B, were selected. Soil A was a saline calcareous clay obtained from Fayoum province near lake Karun. Soil B was an alkaline calcareous sandy loam obtained from Natron Valley. The soils showed the following analytical figures:

I. Mechanical analysis

Soil fraction	Soil A	Soil B
Coarse sand, %	2·2	4·2
Fine sand, %	2·8	43·47
Silt, %	24·83	14·88
Clay, %	44·55	11·12
Moisture, %	6·25	2·38
Carbonates, %	15·0	13·8
Loss on solution, %	2·05	2·18
Soluble salts ($Cl^- + SO_4^{--}$), %	2·17	0·32
Difference due to organic matter removed by H_2O_2, errors etc., %	0·15	7·65
Total, %	100·00	100·00

II. Analysis of water extract (1 : 5 soil : water ratio)

	Salts	Soil A	Soil B
	Carbonates, %	Nil	0·06
	Bicarbonates, %	Nil	0·25
	Chlorides, %	1·5	0·19
	Sulphates, %	0·67	0·13
III.	Base-exchange capacity	39·63	20·66 milliequiv./100 g.
	pH	7·80	9·60

Procedure

(*a*) *Preparation of soils.*—Four 100-g. portions of air-dry soil sieved through the 2-mm. sieve were transferred to 600-ml. beakers. The carbonates were decomposed by adding sufficient N-acetic acid, the soils were warmed to 40°, stirred occasionally and then set aside overnight to settle. The soil suspensions were then filtered through a No. 1 Whatman filter paper and washed with distilled water until the filtrates were free from calcium. Each portion was then leached with a normal solution of either calcium, magnesium, potassium or sodium chloride to saturate it with the corresponding cation. In this way calcium, magnesium, potassium and sodium soils were obtained from each of the two original soil samples A and B. Five litres of such solutions were found to be sufficient to saturate the heavy soil A with the necessary cation and only 2·5 litres were used for the lighter soil B. The excess of leaching solutions was removed by washing the soils with 70% alcohol and finally the soils were dried at 100–110° for 24 hours.

(*b*) *Effect of adsorbed cations on the soil phosphate.*—Portions (5 g.) of each cation-saturated soil were transferred to a 30-ml. beaker, 25 ml. of distilled water was added, the suspensions were stirred every 5 minutes for half an hour and then left for one hour to settle. The suspensions were filtered through a No. 42 Whatman filter paper. With the highly dispersed sodium soils filtration was replaced by centrifuging. The soluble phosphate was then determined in the filtrate colorimetrically by means of the molybdenum-blue method and the Unicam G.P. photoelectric colorimeter.

(*c*) *Effect of adsorbed cations on phosphate added as monopotassium phosphate.*—The previous procedure was repeated but instead of adding 25 ml. of water, only 24 ml. was added, followed by 1 ml. of a monopotassium phosphate solution containing 100 μg. of PO_4.

(*d*) *Effect of salts on fixation of phosphate added as monopotassium phosphate.*—The salts used for each soil were the carbonate, sulphate and chloride of the corresponding cation that saturates the soil. The sparingly soluble salts, calcium carbonate, magnesium carbonate and calcium sulphate, were added in powder form to the soil. The other salts were added as normal solutions so that the effect of equivalent amounts of salts could be determined and results could be compared. The treatments were carried out on 5-g. portions of each soil. Each portion was suspended in distilled water, the salt was then added followed by 1 ml. of monopotassium phosphate solution containing 100 μg. of PO_4, and the final volume was adjusted to 25 ml. The suspensions are then stirred, filtered and the phosphate determined in the filtrate as in (*a*). The amounts of salts added were as follows:

(i) Calcium soil
$CaCO_3$: 5, 10 and 15%
$CaSO_4$: 5, 10 and 15%
$CaCl_2$: 1, 2 and 3 ml. of N-solution

(ii) Magnesium soil
$MgCO_3$: 5, 10 and 15%
$MgSO_4,7H_2O$: 1, 2 and 3 ml. of N-solution
$MgCl_2,6H_2O$: 1, 2 and 3 ml. of N-solution

(iii) Potassium soil
K_2CO_3: 1, 2 and 3 ml. of N-solution
K_2SO_4: ,, ,, ,,
KCl: ,, ,, ,,

(iv) Sodium soil
Na_2CO_3: 1, 2 and 3 ml. of N-solution
Na_2SO_4: ,, ,, ,,
NaCl: ,, ,, ,,

Results

The results of analysis are summarized in Tables I, II, III and IV.

Table I

Effect of adsorbed cations on soil phosphate and on phosphate added as monopotassium phosphate

		Concn. of PO_4^{3-} in water extract, p.p.m.				
		Original soil	Calcium soil	Magnesium soil	Potassium soil	Sodium soil
Soil A	Before addition of monopotassium phosphate	0·3	0·8	1·1	9·2	12·0
	After addition: calculated	4·3	4·8	5·1	13·2	16·0
	After addition: observed	0·4	0·8	2·6	12·6	16·3
	Difference	−3·9	−4·0	−2·5	−0·6	+0·3
	% of PO_4^{3-} fixed	90·7	83·3	49·0	4·55	—
Soil B	Before addition of monopotassium phosphate	2·2	2·6	2·9	3·5	5·0
	After addition: calculated	6·2	6·6	6·9	7·5	9·0
	After addition: observed	4·6	5·0	6·4	7·3	9·1
	Difference	−1·6	−1·8	−0·5	−0·2	+0·1
	% of PO_4^{3-} fixed	25·8	27·3	7·25	2·66	—

Table II

Effect of CO_3^{--} on phosphate fixation

	CO_3^{--} added, %	Concn. of PO_4^{3-}, p.p.m. Calcium soil	Magnesium soil	CO_3^{--} added, ml. of N-soln.	Concn. of PO_4^{3-}, p.p.m. Potassium soil	Sodium soil
Soil A	0	0·8	2·6	0	12·6	16·3
	5	0·7	2·3	1	20·0	24·0
	10	0·6	2·4	2	20·4	26·0
	15	0·7	2·3	3	20·8	28·7
Soil B	0	5·0	6·4	0	7·3	9·1
	5	3·5	5·6	1	7·8	10·2
	10	3·4	5·3	2	8·0	10·4
	15	3·4	5·5	3	8·5	10·9

Discussion

(I) *Effect of adsorbed cations on phosphate fixation (Table I)*

It was found that the concentration of soil phosphate in the water extracts of soils differed with different adsorbed cations. In each soil the concentration increased in the order

Table III

Effect of sulphates on phosphate fixation

	SO_4^{--} added, %	Concn. of PO_4^{3-} p.p.m. Calcium soil	SO_4^{--} added, ml. of N-soln.	Concn. of PO_4^{3-}, p.p.m. Magnesium soil	Potassium soil	Sodium soil
Soil A	0	0·8	0	2·6	12·6	16·3
	5	0·8	1	2·2	6·1	10·0
	10	0·7	2	2·3	5·2	8·5
	15	0·8	3	2·4	5·0	7·0
Soil B	0	5·0	0	6·4	7·3	9·1
	5	4·6	1	5·8	5·0	7·5
	10	4·7	2	5·9	4·7	7·4
	15	4·6	3	6·2	4·3	7·1

Table IV

Effect of chlorides on phosphate fixation

	Chloride added, ml. of N-soln.	Concn. of PO_4^{3-}, p.p.m. Calcium soil	Magnesium soil	Potassium soil	Sodium soil
Soil A	0	0·8	2·6	12·6	16·3
	1	0·8	2·0	5·2	9·0
	2	0·8	2·2	4·9	7·0
	3	0·7	2·0	4·2	6·2
Soil B	0	5·0	6·4	7·3	9·1
	1	4·0	4·5	5·2	8·0
	2	3·6	4·7	4·7	7·8
	3	3·2	4·6	3·5	7·4

$Ca < Mg < K < Na$. The increase in concentration from the calcium to the sodium soil in the heavier soil A was much greater than in the lighter soil B; in soil A the concentration has increased up to 15 times (from 0·8–12 p.p.m.), but in soil B it was only doubled (2·6–5 p.p.m.). After the addition of 1 ml. of monopotassium phosphate solution (containing 100 μg. of PO_4) to the soil suspensions, the maximum possible concentration of phosphate should have increased by 4 p.p.m. However, the observed concentrations were less than the calculated (except in the sodium soil) owing to fixation. It is clear from the Table that the adsorbed cations differ greatly in their effect on phosphate fixation and the fixing power of these cations is in the order $Ca > Mg > K > Na$.

The great decrease in phosphate concentration in the calcium and magnesium soils cannot be explained by assuming that simple precipitation as an insoluble salt takes place. If this were so, a magnesium soil would be expected to remove substantially as much phosphate as does a calcium soil, but actually it was found that the phosphate removed by a magnesium soil was only half that removed by the calcium soil of A. Moreover, the removal of some phosphate by the potassium soil is incompatible with the precipitation theory.

The removal of phosphate can, however, be regarded as an adsorption phenomenon. The adsorbed cation on the soil complexes can retain the phosphate by secondary adsorption and a state of equilibrium is established between the adsorbed and free phosphate ions. The difference between the fixing powers of the different cations would depend on the cationic charge and its degree of hydration. Calcium and magnesium with a double positive charge can retain more phosphate ions than potassium or sodium. The marked difference, however, between the fixing powers of calcium and of magnesium in both soils can be correlated with the radii of the hydrated ions. Since the magnesium ion is more hydrated than the calcium, it will be less effective in fixing the phosphate ion. The radius of the hydrated Ca^{++} ion is 6·7 Å and that of the Mg^{++} ion is 7·7 Å, which is approximately equal to that of the Na^+ ion as determined by Nachod & Wood.[9] The small amount of fixation in the potassium soil is also in accordance with the above theory; since the potassium ion is univalent and highly hydrated it will be less effective in fixing the phosphate ion. The Na^+ ion is still more hydrated than the K^+ ion and does not cause fixation in either soil. In support of this adsorption theory, soil A, with a greater active surface than soil B, has caused more fixation. The base-exchange capacity of soil A is 39·63 milliequiv./100 g. whereas that of soil B was 20·66 milliequiv./100 g.

(II) *Effect of salts on phosphate fixation*

(*a*) *Effect of carbonates.*—From the results in Table II it is obvious that whereas the carbonates of calcium and magnesium tend to fix phosphate, those of potassium and sodium tend to release it. Increasing amounts of the alkali carbonates increase the soluble phosphate considerably, but increasing amounts of calcium and magnesium carbonates have only a slight and opposite effect. It is also clear that, in both soils, calcium carbonate has caused the fixation of more phosphate than has magnesium carbonate.

The fixation due to calcium and magnesium carbonates may be explained by assuming that the phosphate ion is adsorbed on the carbonate particles, as was previously suggested by Boischot *et al.*[8, 10] The small effect caused by increasing amounts of calcium and magnesium carbonates suggests that phosphate fixation in calcium and magnesium soils is due mainly, to the already adsorbed calcium or magnesium, respectively.

The release of phosphate by the addition of alkali carbonates in the potassium and sodium soils may be due to anionic exchange; the OH^- ion, resulting from the hydrolysis of the carbonates, replaces the phosphate ion on the soil complexes.

(*b*) *Effect of sulphates and chlorides* (*Tables III and IV*).—Sulphates and chlorides were found to reduce the amount of soluble phosphate in all instances, but the fixation in the presence of alkali sulphates or chlorides was more than that in the presence of the calcium and magnesium salts. The effect of the magnesium salts was more pronounced in the light soil B than in the heavy soil A, which suggests that when the soil has a relatively high base-exchange capacity, and consequently a bigger active surface, the effect of the salts of calcium and magnesium is very small and the main cause of phosphate fixation is the adsorbed calcium or magnesium ions. The fixation brought about by the chlorides or sulphates can also be explained by anionic exchange. Both the SO_4^{--} and the Cl^- ions can be adsorbed by the soil colloids, as was shown by Mattson.[11]

Sussman *et al.*[12] have also found that anionic exchange can proceed by equivalents in some types of resins. Since the phosphate ion has a higher energy of absorption than the SO_4^{--} or Cl^- ions, it can replace them in the soil colloids and consequently become fixed. Kunin & Myers[13] have also shown that the strength of binding of the Cl^- ion is much less than that of the phosphate ion. More fixation has taken place in soil A, which is heavier than soil B, suggesting that phosphate fixation caused by the salts was a surface phenomenon.

Conclusions

1. The concentration of water-soluble soil phosphate or of phosphate added to the soil as monopotassium phosphate is affected by the type of cation that saturates the soil complexes. The concentration increases in the order $Ca < Mg < K < Na$. Fixation probably increases with increase of positive charge on the cation and with decrease in its degree of hydration.

2. The carbonate, sulphate or chloride of calcium when added to a calcium soil will reduce the amount of soluble phosphate; the effect is more pronounced in a light than in a heavy soil.

3. In a magnesium soil, magnesium carbonate will also reduce the amount of phosphate. The sulphate or the chloride is more effective in fixing the phosphate than is the carbonate, and fixation increases with increase of the base-exchange capacity of the soil. The same is true when the sulphate or chloride of potassium or sodium is added to the corresponding soils.

4. The carbonates of potassium and sodium increase the amount of soluble phosphate.

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