MANURING OF GRASSLAND. I.—Some Effects of Heavy Dressings of Nitrogen on the Mineral Composition of Grassland Herbage*

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Results are given for the effects of heavy dressings of nitrogen, in the presence and absence of phosphorus and potassium supplements, on : dry-matter yield ; botanical composition of herbage ; contents of crude protein, phosphorus, potassium, calcium, magnesium, sodium and manganese. Brief reference is also made to the effects of the various experimental treatments on the uptake of certain other trace-constituents.

The results indicate that high yields of pasture herbage having high protein and normal mineral contents can be maintained over a period of years by the use of fertilizer nitrogen, provided adequate fertilizer dressings of other major nutrients are given. Attention is drawn to various ion-antagonism effects that may influence the mineral composition of the herbage.

In recent years pasture has acquired a steadily increasing importance as a crop in its own right. This is indeed in welcome contrast with the more or less incidental contribution that it made in early pastoral farming and, unfortunately, still makes in considerable areas of the British Isles. In the northern half of Great Britain, rotation or temporary pasture has long played an important part in the maintenance and improvement of the fertility of the arable land, and the importance of ley-farming systems is now well appreciated throughout the U.K. Even so, the term ' grassland ' still embraces land of widely varying agricultural value, ranging from rough mountain or heath, described, euphemistically in many cases, as used or capable of being used as grazing, through permanent grassland of indeterminate quality, to intensively managed and highly productive swards. It is necessary, therefore, in any consideration of the problems of the manuring of grassland, to define the category or type of grassland ; in particular, it is necessary to relate manuring to specific systems of management and utilization, to the botanical composition of the swards, to soil and climatic conditions and to other factors influencing manurial requirement.

Manuring for the establishment and maintenance of pasture

In natural grazings and permanent grassland on unploughable land, substantial increases in output can be achieved by such remedial measures as good grazing management, surface cultivation, seeding and top-dressing with fertilizers. The maximum output of such land will normally, however, be much less than that of ploughable land and it is, of course, to the improvement of the latter that first consideration should be given. Although there are areas in this country where good grassland has been maintained over indefinite periods without recourse to ploughing, most ploughable pastures can with advantage be renovated by ploughing occasionally. Two main methods of laying down land to grass are : (1) undersowing a cereal crop, or occasionally a brassica such as rape, with grass and clover seeds, and (2) direct reseeding without a nurse-crop. The former is the more widely practised method, especially for rotation pasture. Treatment varies, of course, with soil and climatic conditions and farming systems. On the principle that it is sounder theory and easier practice to begin with a good pasture than to improve a poorly established one by subsequent treatment, the general objectives will normally be : (1) to have lime applied where necessary, preferably some considerable time ahead of sowing-out to grass ; (2) to have phosphate and potash well incorporated in the seed-bed ; and (3) to have nitrogen applied either with the phosphorus and potassium in the seed-bed or, preferably, as a top-dressing to assist early growth. Although many farmers believe that heavy initial dressings of phosphate are necessary it is our experience that, where soluble or quick-acting forms are used, little is likely to be gained by applying more than the equivalent of about 80 lb. of P_2O_5 per acre in the seed-bed. It is well to remember that heavy manuring is not a substitute for good pasture-management. It has frequently been our experience, for instance, that moderate phosphate application and good management have given a much better sward than heavy phosphate application and poor management.

In the maintenance manuring of grassland a common practice on many farms is simply to apply a complete NPK dressing in spring and then to hope for the best. Separate applications of individual fertilizer-ingredients do, of course, add considerably to the cost, but apart from this, there are certain advantages in applying phosphate and potash before the beginning of spring growth and following with top-dressings of nitrogen, the amount depending on the use

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to be made of the pasture. For instance, comparatively little nitrogen will normally be needed where non-intensive grazing is practised, but where pasture is to be cut and removed from the land as hay or, by more frequent cutting, as silage or as grass for drying, the amount and frequency of top-dressing must, of course, increase.

Manuring for intensive production of herbage

The following results, from experimental work at the Hannah Dairy Research Institute on the intensive production of herbage for crop-drying, serve to emphasize the importance of adequate replacement-manuring, particularly when acreage output is increased by the application of heavy dressings of nitrogen. They also give some information on the possibility of maintaining the normal mineral composition of such herbage when heavy dressings of nitrogen are used to boost yields. Earlier results from this work, which was designed to study such questions as the yield response of grassland to nitrogen, the needs of intensively cropped grassland for other mineral supplements, and the influence of intensive management on the mineral components in plant and soil, have already been described.[1-3] It has been shown, for instance, that the nitrogen treatments increased acreage yields from 4300 lb. of dry matter and 600 lb. of crude protein to 9000 lb. of dry matter and 1900 lb. of crude protein.

The soil of the experimental area is developed on a water-sorted till derived mainly from Productive Coal and Barren Red Measures. There is a slight igneous influence in the lower layers. The top soil is a brown, sandy loam with crumb structure and few stones. Marked induration occurs between the 14- and 18-inch depths. The underlying till is a red-brown, sandy clay loam, containing many small carboniferous-sandstone, coal, shale and igneous fragments. The soil is freely drained and, at the beginning of the experiment, was slightly acid (pH just above 6), slightly low in phosphate and relatively lower in potassium than phosphorus.

In view of the very large amount of analytical work involved, it has not been possible to obtain information on the mineral composition of the herbage under all the different experimental treatments described in the earlier papers.[1-3] Results have, however, been obtained from samples representative of the no-nitrogen and one of the heavy-nitrogen treatments in the Graeco–Latin-square lay-out already described.[1-3] The herbage being tested consisted of a perennial rye-grass dominant ley, sown under oats in 1945; 24 lb. of leafy perennial rye-grass (*Lolium perenne*), 3 lb. of timothy (*Phleum pratense*) and 2 lb. of S100 white clover (*Trifolium repens*) were used. Basal dressings of lime were applied to bring the soil to a pH of about 6·5, and the treatments from which representative samples of herbage were drawn for determinations of mineral composition are as follows:

$N_0P_0K_0$: control, no mineral supplements.

$N_3P_0K_0$: nitrogen alone as 'Nitro-Chalk', 6 cwt. per acre in March and 6 cwt. per acre after each cut. The total amount of nitrogen applied was equivalent to 520 lb. of N per acre during each of the years 1947–51 inclusive.

N_0K: no nitrogen; potassium as 60% muriate equivalent in lb. of K_2O per acre to approximately 270 annually in 1947, 1948, 1949 and 1950, and 400 in 1951, i.e. 940 lb. of K_2O in the three years 1949–51 for which pasture analytical data are available. Up to 1950 the potassium was applied in two equal dressings in spring and midsummer; in 1951 it was applied in five dressings, one for each cut.

N_3K: nitrogen as in $N_3P_0K_0$ plus potassium as in N_0K.

N_0P: no nitrogen; phosphorus as 18% superphosphate, equivalent in lb. of P_2O_5 per acre to approximately 120 annually in the four years 1947–50, and 180 in 1951, i.e. 420 lb. of P_2O_5 in the three years 1949–51.

N_3P: nitrogen as in $N_3P_0K_0$, plus phosphorus as in N_0P.

N_0PK: no nitrogen; phosphorus and potassium as in N_0P and N_0K respectively.

N_3PK: nitrogen, phosphorus and potassium as in $N_3P_0K_0$, N_0P and N_0K respectively.

The herbage was cut each time it reached a height of 8–11 inches, i.e. four cuts per season in the absence of nitrogen, and five cuts per season in the plots receiving nitrogen. The experiment is being continued.

Results

Dry matter.—Reference has already been made to the striking increases in dry-matter yield obtained by the liberal use of nitrogen. In the earlier papers[1-3] it has, however, also been stressed that these responses can be obtained only when other nutrients, especially potassium, are present in the soil in adequate amounts. Table I shows for the treatments under consideration the dry-matter yields in 1949, 1950 and 1951, i.e. the last three years of a five-year period from the beginning of the experiment.

Table I

Yields of dry matter, lb./acre, in 1949, 1950 and 1951

Treatment	Dry matter, lb./acre			
	1949	1950	1951	Total in 3 years
$N_0P_0K_0$	3660	3280	3640	10,580
$N_3P_0K_0$	4250	3740	3820	11,810
N_0K	3800	3500	4380	11,680
N_3K	9210	9290	10,020	28,520
N_0P	3120	3460	3530	10,110
N_3P	5110	3840	3800	12,750
N_0PK	4660	4920	7110	16,690
N_3PK	9730	9390	10,770	29,890

In the treatments with $N_3P_0K_0$ and N_3P, yields dropped appreciably after 1949, and in 1950 and 1951 these yields were not much higher than those from the corresponding no-nitrogen treatments. On the other hand, treatments with N_3K and N_3PK gave very high yields which increased in 1951 when the heavier dressings of potassium were given. With treatment N_0K there was an appreciable yield-increase in 1951, and with N_0PK there was a steady increase in dry-matter yield over the period. As is noted below, this is associated with considerable development of white clover.

Botanical composition of experimental plots.—Broadly, treatments N_3K and N_3PK have maintained a vigorous rye-grass–timothy sward, almost devoid of clover or weeds. With treatments $N_3P_0K_0$ and N_3P there has been a marked deterioration of the sward. Clovers are again absent but, in addition, the sown grasses are being replaced by *Agrostis tenuis, Festuca rubra* and *Holcus lanatus*. Treatments $N_0P_0K_0$ and N_0P have also led to swards of poor botanical composition, but with N_0K a sward having a moderate clover-content has developed. On this soil, where potassium supply is obviously an important limiting factor, it is of interest to note that phosphate alone has been ineffective in preventing deterioration of the sward, that potassium alone has had a distinctly beneficial effect, and that phosphorus and potassium together, in the absence of nitrogen, have by 1951 led to a clover-dominant rye-grass sward. The marked clover dominance in this N_0PK treatment should be remembered when changes in the chemical composition of the herbage produced by the different manurial treatments are considered.

Crude protein.—Mean values for the percentages by weight of crude protein in the dry matter and for the yields obtained in the three-year period 1949–51 are given in Table II.

Table II

Crude protein as mean percentages in dry matter and as lb./acr yielded in 1949, 1950 and 1951

Treatment	Crude protein						
	Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$	13·4	14·2	12·9	489	464	471	1424
$N_3P_0K_0$	23·2	23·5	21·9	985	878	835	2698
N_0K	13·3	13·2	15·4	505	465	673	1643
N_3K	20·3	20·5	19·6	1873	1904	1962	5739
N_0P	12·1	13·9	13·8	379	481	487	1347
N_3P	23·1	23·6	23·1	1179	905	879	2963
N_0PK	13·7	14·6	16·6	640	720	1184	2544
N_3PK	20·9	20·6	19·7	2029	1930	2126	6085

These figures serve clearly to emphasize : (1) the markedly positive effects of nitrogen in increasing both the protein content in the dry matter and the protein yields per acre ; (2) the striking increase in yield produced by potassium in the presence of nitrogen and, in 1951, the beneficial effect of potassium even in the absence of nitrogen. It is also of interest to note that, in the presence of nitrogen, potassium causes a slight reduction in the protein content of the dry matter, presumably by promoting a greater amount of photosynthesis; but in 1951, by encouraging the growth of clover, it tended to increase the percentage crude protein in the absence of added nitrogen; (3) the virtual lack of response to phosphorus on this particular soil, even after five years' intensive cropping. In general, however, there is a positive interaction effect between phosphorus and potassium, an effect which is reflected particularly by the strong development of clover in the absence of nitrogen.

The extreme limits of variation in the percentages of crude protein in the dry matter are 9·1% in the second cut of the N_0PK treatment in 1950 and 29·8% in the last cut of the $N_3P_0K_0$ and the N_3P treatments in 1951. Discussion of seasonal variation in the yields of dry matter and crude protein is, however, beyond the scope of the present paper.

Phosphorus.—From the percentage values shown in Table III it will be seen that the various treatments have been practically without effect on the phosphorus content of the dry matter. The differences in the figures for removal in crop are thus essentially a reflection of the yield-differences due to the various treatments. Although the soil in the present experimental area is much less responsive to phosphate than to potash, the removal, for instance, of 208 lb. of P_2O_5 per acre in a period of three years in the N_3K treatment indicates that replenishment of phosphate reserves in the soil cannot be delayed indefinitely. It will, however, be of interest to see how long this delay can be.

Table III

Phosphorus contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Phosphate (as P_2O_5) in herbage						
	Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$ ..	0·85	0·86	0·80	31	28	29	88
$N_3P_0K_0$ ..	0·75	1·00	0·74	32	37	28	97
N_0K	0·76	0·76	0·75	29	26	33	88
N_3K	0·72	0·77	0·71	66	71	71	208
N_0P	0·88	0·88	0·88	28	30	31	89
N_3P	0·80	0·89	0·95	41	34	36	111
N_0PK ..	0·90	0·90	0·90	42	44	64	150
N_3PK ..	0·79	0·82	0·81	77	77	87	241

Although individual samples have had phosphorus contents appreciably divergent from the mean values in Table II, there has been no consistent or marked seasonal variation throughout the three-year period. The extreme limits of variation are from 1·59% of P_2O_5 in the fourth cut of the $N_3P_0K_0$ treatment in 1950 to 0·54% of P_2O_5 in the third cut of the N_3K treatment in 1949. Most of the values are, however, within ± 0·1 of the means.

Potassium.—Potassium is the element which, under the conditions of this particular experiment, shows the most spectacular variations with differential fertilizer treatment. Except in the $N_3P_0K_0$ and N_3K treatments in 1951, nitrogen has markedly reduced the potassium content of the dry matter, both in the absence and presence of P and K. The most striking feature of the results shown in Table IV, however, is the positive effect of potassium additions on the potassium contents of the herbage. It will be seen, for instance, that the percentage figures for potassium in the presence of added muriate are between twice and more than four times those in the herbage grown without added potassium. The figures for removal in pounds per acre reflect, in the absence of added potassium, the dominant effect of nitrogen which, by depleting soil reserves, has reduced uptake of potassium, and, in the presence of added potassium, the dominant effect of the nitrogen on yield of herbage. The total amounts of K_2O removed in the three-year period emphasize the importance of adequate replacement-manuring; the role of potassium as a limiting factor in growth is reflected especially by the low percentage and removal figures in the P_0K_0 and N_3P treatments in 1951.

There have been no regular or consistent trends in seasonal variations in potassium uptake; generally, however, seasonal variation has been less in the presence than in the absence of nitrogen. In 1949 and 1950, when potassium was applied twice in the season, there was a slight tendency for potassium content to be lower in the second and third than in the earlier and later cuts; but in 1951, when the potassium was applied in five dressings, this effect was absent.

Table IV

Potassium contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Potassium (as K_2O) in herbage						
	Means; % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$ ..	1·63	2·07	0·97	60	68	35	163
$N_3P_0K_0$ ..	0·94	1·16	0·95	40	43	36	119
N_0K	3·00	4·50	4·20	117	158	184	459
N_3K	2·58	3·93	4·32	237	365	433	1035
N_0P	1·60	2·12	2·34	50	74	83	207
N_3P	1·13	1·07	0·84	58	41	32	131
N_0PK ..	3·48	4·19	5·05	162	206	359	727
N_3PK ..	2·60	3·50	4·15	250	328	446	1024

The extreme limits of variation in potassium during the three years have been from 0·57% in the third cut of the $N_3P_0K_0$ treatment in 1951 to 5·36% in the second cut of the N_0PK treatment in the same year.

Calcium.—In 1949 and 1950 the nitrogen dressings increased appreciably the calcium contents of the herbage, both in the absence and the presence of potassium and phosphorus (Table V). The same effect was found in the absence but not in the presence of added potassium in 1951; also in 1951 the herbage was appreciably lower in calcium in the N_3PK than in the N_0PK treatment and slightly lower in the N_3K than in the N_0K treatment. It may be that this is to be associated with the effect of potassium in stimulating growth of clover in the absence of nitrogen, but it is possible that ion-antagonism effects between potassium and calcium may also be involved as a result of the increase in the potassium additions in that year. The effects of the various treatments on the removal of calcium in crop are also illustrated in Table V. The variations in both composition and yield of herbage are indicated and, in terms of total quantities removed in the three-year period, range from 87 to 336 lb. of CaO per acre in the N_0P and N_3PK treatments respectively.

Table V

Calcium contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Calcium (as CaO) in herbage						
	Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$ ..	0·94	0·89	0·90	35	30	33	98
$N_3P_0K_0$ ..	1·52	1·36	1·23	65	51	47	163
N_0K	1·00	0·86	0·99	38	30	43	111
N_3K	1·32	1·12	0·89	122	104	89	315
N_0P	0·92	0·83	0·82	29	29	29	87
N_3P	1·50	1·42	1·38	77	55	52	184
N_0PK ..	0·95	0·77	1·33	44	38	95	177
N_3PK ..	1·30	1·09	0·99	126	103	107	336

The extreme limits of variation in the percentages of calcium in the dry matter are 0·61 in the first cut from the N_0K treatment in 1950 and 1·92 in the second cut from the N_0PK treatment in 1951. In each of the three years, 1949, 1950 and 1951, there was a distinct seasonal variation in calcium content, with the values in the first and second cuts being appreciably lower than in the later cuts.

Magnesium.—In 1949 and 1950 nitrogen increased the magnesium content of the dry matter both in the absence and the presence of potassium and phosphorus, but in 1951 it was without effect on magnesium content, except in the PK treatment where the percentage figure for MgO was lower in the presence than in the absence of nitrogen (Table VI). The overall effect of applying potassium was to decrease magnesium content, except in the N_0PK treatment in 1951. Phosphorus additions had a similar but less pronounced effect in 1950 but not in the other two years. In the section of Table VI showing the amounts of magnesium removed in herbage, the most striking figure is probably that indicating the relatively low removal of only 38 lb. of MgO per acre over a period of three years in the N_0K treatment.

Table VI

Magnesium contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Magnesium (as MgO) in herbage Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$ ..	0·39	0·50	0·54	15	17	20	52
$N_3P_0K_0$ ..	0·55	0·71	0·53	23	26	20	69
N_0K	0·30	0·38	0·33	11	13	14	38
N_3K	0·43	0·45	0·33	39	42	33	114
N_0P	0·32	0·43	0·57	10	15	20	45
N_3P	0·53	0·55	0·57	27	21	22	70
N_0PK ..	0·31	0·39	0·54	15	19	38	72
N_3PK ..	0·44	0·49	0·35	43	45	38	126

The figures for magnesium in the individual cuts over the three-year period indicate that the general trend is for the magnesium content of the herbage to increase as the season advances. This is rather more marked in the presence than in the absence of nitrogen. The extreme range of variation observed is from 0·16% of MgO in the N_0P treatment in the first cut in 1949 to 1·17% in the fourth cut of the $N_3P_0K_0$ treatment in 1950.

Sodium.—The results in Table VII provide a striking illustration of the effect of potassium in decreasing sodium uptake, both in the presence and absence of nitrogen. Except in the absence of both phosphate and potash in 1951, the sodium contents of the nitrogen-treated herbage are all appreciably higher than those of the herbage grown without nitrogen. The phosphorus supplements in the absence of potassium have had the general effect of decreasing the sodium contents of the herbage in the absence of nitrogen and of increasing them in the presence of nitrogen. The negative interaction effect between potassium and sodium is also evident in the figures for sodium removed in crop. Despite yield-increases after the addition of potassium, the total amount of sodium removed, for instance during the three-year period in the N_0K treatment, was only about one-quarter of that in the control treatment. During the three-year period there were no regular or consistent trends in seasonal variations in sodium content, but there was a tendency for the sodium values to be lower in the mid-season than in the earlier and later cuts.

Table VII

Sodium contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Sodium (as Na_2O) in herbage Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$ ..	1·39	1·31	1·46	51	43	53	147
$N_3P_0K_0$ ..	1·58	1·42	0·78	67	53	30	150
N_0K	0·47	0·31	0·15	18	11	7	36
N_3K	1·29	0·83	0·38	119	77	38	234
N_0P	0·87	0·91	0·93	27	32	33	92
N_2P	1·76	1·79	1·44	90	69	55	214
N_0PK ..	0·46	0·41	0·26	22	20	19	61
N_3PK ..	1·27	0·99	0·34	123	93	37	253

Manganese and trace-elements.—Details of the manganese contents of the various samples and of the quantities removed in crop are presented in Table VIII. Nitrogen in the presence of phosphorus appears to decrease slightly the percentage of manganese in the herbage, but apart from this the various treatments have had no pronounced effects on manganese contents.

Determinations have also been made on all the samples of the contents of cobalt, nickel, molybdenum, iron, lead, zinc, vanadium, titanium, chromium, strontium, barium, silver, tin and copper. Tabulation and discussion of the results for all these elements separately are, however, beyond the scope of this paper. Generally it may be said that, with very few exceptions, the heavy dressings of nitrogen have had very little effect on the trace-element contents of the produce, and there are no obvious interaction effects between potassium and phosphorus and the various trace-elements. Copper is probably the only element providing an exception to the above generalization. The nitrogen dressings have had the overall effect of increasing the range in copper content of the herbage in parts per million from the 6 to 10 to the 9 to 16 level. This may be a factor of some importance in animal-health problems, the tentative conclusion being that nitrogen is likely to decrease rather than increase the risk of copper deficiency in a pasture diet. The trace-element results also suggest that there is a true increase in iron content of the herbage as the season advances, and there is an indication that the molybdenum content of the herbage is usually slightly lower in the presence than in the absence of added potassium (about 1 p.p.m. in the potassium treatments and 2 p.p.m. in those without potassium).

Table VIII

Manganese contents as mean percentages in dry matter and as lb./acre removed in crop

Treatment	Manganese (as MnO) in herbage						
	Means, % in dry matter			lb./acre removed			
	1949	1950	1951	1949	1950	1951	Total in 3 years
$N_0P_0K_0$	0·008	0·007	0·010	0·28	0·24	0·36	0·88
$N_3P_0K_0$	0·009	0·009	0·007	0·38	0·33	0·26	0·97
N_0K	0·006	0·007	0·008	0·25	0·26	0·37	0·88
N_3K	0·006	0·004	0·007	0·55	0·40	0·74	1·69
N_0P	0·010	0·010	0·018	0·30	0·36	0·65	1·31
N_3P	0·007	0·009	0·007	0·37	0·33	0·26	0·96
N_0PK	0·008	0·008	0·012	0·35	0·38	0·83	1·56
N_3PK	0·007	0·005	0·005	0·69	0·49	0·51	1·69

Discussion and conclusions

From the results given in the various Tables, it is clear that there are, between the main nutrients, numerous interaction effects which are reflected in the yield, the mineral composition and the botanical composition of the herbage. Heavy dressings of nitrogen increase enormously both the yield of dry matter and the percentage of crude protein in it. The results discussed are those obtained in the last three years of a five-year period and the experiment is still being continued. It is evident, however, that provided adequate fertilizer dressings of the major nutrients are given, very high yields of herbage dry matter can be maintained for some years by the use of fertilizer nitrogen. The figures in Table II show that these high yields apply to dry matter containing a high percentage of crude protein. If lower protein contents are acceptable, even greater responses in yield of dry matter to nitrogen applications can be obtained by allowing the herbage to become more mature before each cut.

In the present series of experiments on a soil limed to a normally satisfactory level, and having a moderate phosphate reserve and a relatively less satisfactory potash status, there has been a strikingly positive N : K interaction effect but, even after five years of intensive cropping, there is little apparent need for phosphate. It is to be noted, however, from the yield results that there is a distinctly positive interaction effect between phosphorus and potassium, especially in the absence of nitrogen when there has been a strong development of clover.

The need for potassium and the dependence of nitrogen response on the presence of adequate amounts of potassium and, to a much lesser extent, phosphorus are clearly reflected in both the yields and the potassium contents of the produce. It is evident, too, from the figures for the removal of major nutrients in crop, that adequate replacement-manuring becomes a factor of prime importance when yields are increased by the application of heavy dressings of nitrogen. To realize the importance of this, one has only to note, for instance, that, in plots receiving 940 lb. of K_2O and 420 lb. of P_2O_5 per acre in the three-year period, over 1000 lb. of K_2O and nearly 250 lb. of P_2O_5 have been removed.

In general, the effect of applying nitrogen has been to decrease the potassium and to increase the calcium, magnesium and sodium contents of the dry matter. The effect on the sodium content varies, however, according to the potassium level. The contents of phosphorus, manganese and trace-elements other than copper in the herbage have been largely unaffected by the addition of nitrogen. The uptake of K, Ca, Mg and Na by the pasture plants has been influenced to a large extent by the application of fertilizer potassium. Potassium supplements have given from two- to four-fold increases in the potassium contents of the herbage, but the potassium dressings have caused marked reductions in the uptake of sodium, magnesium and, to a lesser extent, calcium. In soils that are being regularly and adequately limed there is unlikely to be a shortage of calcium in the pasture; but from the point of view of stock health it may be necessary to keep a watchful eye on the magnesium content of produce grown with the aid of nitrogen in the presence of adequate potassium supplements. It has of course been well established that increasing the relative concentration of any one cation in the soil will increase its concentration in the leaf of the plant and consequently decrease that of other cations. This was shown, for instance, by Marshall[4] in the U.S.A. with bluegrass and sweet clover. In the present experiments this particular aspect of ion-antagonism is well illustrated by the depressing effect of potassium additions on sodium, magnesium and, to a lesser extent, calcium uptakes. It has been our experience, in other experiments on the possible interrelations between certain animal disorders and magnesium in the diet, that the magnesium content of ordinary pasture herbage can be readily increased by the use, for instance, of a magnesian instead of an ordinary calcium limestone. The magnesium contents are not abnormally low in any of the samples analysed in the present experiments; where heavy dressings of potassium are being applied on pasture herbage it should, however, be a normal precautionary measure to watch the effects on the magnesium and other cation contents of the produce.

Seasonal variations in the mineral composition of pasture herbage must also be remembered. Although there have been no consistent or regular seasonal variations in the present series of experiments, there have been general tendencies for potassium and sodium to be lower in the mid-season than in the earlier and later cuts, and for calcium and magnesium contents to increase as the season advances.

The trace-element contents of the pasture have been largely unaffected by the various experimental treatments. Copper is the most striking exception, in that the herbage content of this element tends to be increased by nitrogen dressings. In general it may be said that, for stock-feeding, none of the treatments have yet caused either a reduction of the amounts of desirable trace-elements, such as cobalt, or an increase in the contents of undesirable elements, such as molybdenum, in the produce.

Acknowledgments

The authors are indebted to Dr. R. L. Mitchell for the spectrographic determinations of many of the elements reported, and to Miss A. J. Preddy for other determinations of the mineral composition of the herbage samples. They also acknowledge the assistance of Dr. R. Waite, who arranged for the protein analyses to be carried out.

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MANURING OF GRASSLAND. II.*—Use of Nitrogen†

By W. F. RAYMOND

The use of nitrogen on grassland on the general farm is considered, particularly in relation to the contribution of the clovers to the sward. This is reduced both by heavy nitrogen applications and by unsuitable management. With low clover content in the sward, fertilizer nitrogen becomes the main source of nitrogen for the grass. Not only does this demand a high level of fertilizing, but flexibility in management, which is one of the main advantages from nitrogen manuring, is lost. Thus on the general farm nitrogen should be used at a level which allows an effective clover contribution to the sward : annual dressings of up to 4 cwt. of 'Nitro-Chalk' per acre can be used, with suitable management. However, a consideration of the availability of nitrogen fertilizers shows that even this level of application is possible only on a small proportion of our grasslands. It is concluded that we should now be encouraging the widespread use of medium levels of nitrogen fertilizing, allowing both an adequate clover contribution in our swards and the considerable increases in production possible with these fertilizers.

Nitrogen manuring of grassland on the general farm, rather than the intensive grassland farm, is considered, for it is from improvements in grassland output from the general farm that the greatest returns can be expected. On these farms the system of alternate husbandry, or ley farming, must be encouraged, with its two main functions of increasing production and building up soil fertility in the rotation. In both of these the clovers play an important role, and nitrogen manuring must be discussed in relation to its effect on the clovers as well as on the grasses.

Nitrogen fertilizers may be used in the ley system in many ways, and these can be grouped under headings : (i) in the initial establishment of swards, (ii) for the production of 'early bite', (iii) for the rapid growth of hay or silage aftermaths, (iv) for the production of winter foggage and (v) for the stimulation of herbage growth at special times during the summer.

Under the first heading, the sowing of 1 cwt. of nitrogenous fertilizer with seed mixtures sown without a cover crop is of importance in assuring rapid establishment, before the clovers make any significant contribution of nitrogen. The other four headings are particular uses of nitrogen for increasing or speeding up herbage production when it is most required. There is considerable information to show that 2 cwt. of sulphate of ammonia applied to an average sward in one year will give about 1000 lb. of extra dry matter as summer keep, and somewhat less as winter foggage. Particularly if the sward is being grazed, this use of nitrogen should lead to little depression of clover.

Clover in swards

It is in the use of larger amounts of nitrogen that the maintenance of clover in the sward becomes a problem. Results were quoted from the experiments of T. E. Williams, working at the Grassland Research Station, who studied the effects of increasing dressings of 'Nitro-Chalk' on the grazing output of pasture. Up to 4 cwt. of 'Nitro-Chalk' per acre considerable increases of yields of dry matter were found, but little further increase was obtained between 4 and 8 cwt. Dressings above this level again gave increased yields. Between 4 and 8 cwt. the contribution of clover to the sward decreased markedly, and the increased nitrogen manuring did little beyond replacing the nitrogen which would have been supplied by the legume. The interaction of grazing management and level of manuring was emphasized : the yield under a system of ten times grazing in the season with 12 cwt. of 'Nitro-Chalk' was the same as under six grazings with no nitrogen fertilizer.

Once the contribution of clover to the sward has been greatly reduced—and both nitrogen manuring and unsuitable management may bring this about—further considerations arise. Although the response of the remaining herbage to level of nitrogen fertilizer may be largely linear, this fertilizer is now the main source of nitrogen for the sward. To maintain production it is essential to use fertilizer. J. O. Green has studied the effects of nitrogen manuring in one year on subsequent pasture yields. Once the clover has been eliminated from the sward,

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* Part I : Preceding paper

† Read before the Agriculture Group on 20 January, 1953, in a modified form

heavier dressings of nitrogen must be used to maintain yields in the following years. This means that one of the main benefits of nitrogen manuring to the grassland farmer, namely the increased flexibility in management which it offers, is lost.

The loss, or absence, of clover from a sward may also be of importance when the sward is ploughed in for cropping in the rotation. Here, however, the evidence is less certain. It is generally assumed that a clovery sward is the best to plough in, but in fact there is little quantitative evidence available in this country. There is also little evidence on the comparison of the effects of ploughing in clover swards and nitrogen fertilized grass swards on subsequent cropping, though some experiments suggest that very high levels of nitrogen may reduce soil aggregation. Here is an important field of study in the relationship between nitrogen fertilizing and clover.

Discussions on clover versus no clover in swards generally concentrate on the question of nitrogen, but in fact clover is likely to have other important functions, especially in grazing swards. It is undoubtedly a factor in increasing the palatability of grazing and in introducing some variety into the grazing diet. Little is known of the relative merits of different swards as ruminant feeds, but it may be significant that the highest increase in sheep liveweight per acre at the Grassland Research Station has been from a pure clover sward. On the other hand, high clover content in swards is frequently given as the main cause of bloat and infertility in cattle. The system of strip grazing with the electric fence is likely to give a good measure of control of bloat. Recent evidence on infertility suggests that this may often be due more to low phosphorus in the diet than to high calcium (which was associated with high clover).

Supply of nitrogen fertilizers

The aim should be to use nitrogen manuring to increase yields, and at the same time maintain a reasonable balance (say 30% of the annual production from a sward) as clover. Under certain systems up to 4 cwt. of 'Nitro-Chalk' can be used, but much more research is required before this amount could be generally adopted.

However, the use of heavy nitrogen fertilizing on any but special grass-drying swards is likely to be largely of theoretical interest for some time to come.

A consideration of the present supply of nitrogen fertilizers shows that even applications of 4 cwt. of 'Nitro-Chalk' are not possible on more than a small proportion of our farms. The 1952 rate of delivery of nitrogen to the farms was equivalent to 24 million cwt. of 'Nitro-Chalk' per annum. The corn crop must be given high priority, as the increase in yields from nitrogen manuring is so considerable and tangible. Even at 1 cwt. per acre our corn land should take up over 8 million cwt., a third of the available supply. Potatoes, sugar beet and other arable crops, well farmed, might take a further 8 million cwt., leaving only 8 million cwt. of 'Nitro-Chalk' to go to our grasslands. This has to serve 6 million acres of leys and 13 million acres of permanent grassland. Although production of fertilizers is steadily rising, we are still far from the time when even ½ cwt. of nitrogen fertilizer per acre of grassland is available.

Agronomic studies show that the maintenance of clovers in our general swards is of importance, and is difficult to attain with high levels of nitrogen fertilizing; also the availability of nitrogen fertilizers is such that they can only be used at a high level on a small proportion of our grasslands. Dr. Stewart (preceding paper) has shown that the higher the level of nitrogen manuring, the larger are the amounts of other fertilizers that must be used at the same time. Thus an uneven distribution of nitrogen on our farms must also lead to the most uneven distribution of phosphate and potash.

The widespread use of medium levels of nitrogen fertilizing on our grasslands is advocated, so that the undoubted benefits of these fertilizers, and also the contribution that the clovers can make, can be obtained.

The use of nitrogen on pastures, besides giving increased yields and greater flexibility in management, also leads to a new outlook and interest in the grassland crop. This should be encouraged on every farm, rather than the increased use of nitrogen on a limited number of farms, for it is only in this way that an increase in grassland output can be expected.

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MANURING OF GRASSLAND. III.*—Manuring and Utilization†

By R. A. HAMILTON

The role of grassland in increasing the nation's food supplies is discussed. Fertilizer dressings for various swards are recommended, with the proviso that plans for efficient utilization must precede any step to grow more grass. Figures from cost accounts taken on some 60–70 dairy and mixed farms are quoted.

There has never been a time in the history of British agriculture when output from our grasslands was of such importance as it is today. For it is now agreed that Britain must grow more food at home—the outstanding need being for more animal products. To feed the additional animals required to provide these products, the agricultural industry must rely mainly on grass, because it is the cheapest source of feed for cattle and sheep and because much more of it can be readily produced. Improving grass production will not only provide more dairy products and more beef and mutton, but will also play an important part in helping the production of more bacon and eggs. The more grass is used to feed cattle, particularly dairy cows, the more will concentrated feeding-stuffs be available for pigs and poultry. Moreover, the higher the yields of grassland, the more will land be available to grow coarse grains and other arable crops.

It is therefore in the national interest, and in the interest of every stock farmer, that we should grow more grass and make better use of the grass we grow. This does not mean that the area under grass should be increased. Indeed, if adequate steps are taken to increase the effective yield of grassland, sufficient feed can be produced to meet any projected expansion of grass-eating livestock, even with the area under grass a million acres less than it is today. But such an objective means that the average farmer in this country will have to adopt a new approach to the manuring of grassland.

It is very difficult to dissociate the manuring of grass from its use and it will not be possible in the space available to deal in any detail with utilization. Nevertheless, it should be pointed out that, in any attempt to increase output from grassland, the importance of efficient utilization cannot be over-emphasized. It is pointless, and often harmful, to manure grassland if it is not going to be used properly. Indeed, the use to which the grass is to be put should almost invariably determine how it should be manured. Plans to make proper use of grassland, therefore, must take precedence over steps to increase production either by manuring or by other means.

It is now widely agreed that one of the major factors determining output from grassland is fertility. When fertility is low, high output from grassland is impossible.

In papers[1] read before The Fertiliser Society in 1950 and The Farmers' Club in 1952, it was demonstrated that fertilizers are still needed to obtain the level of fertility required for high output from grassland, even after allowance has been made for the contributions derived from farmyard manure and urine, and nitrogen fixation.

Substantial improvements in the use of fertilizers on grassland, coupled with good management, could, in fact, make a greater contribution to increased food production, and at lower cost, than any other method available to us. The manuring of grassland, therefore, is now a subject of considerable national importance.

The most profitable average dressings for grassland up to a certain prescribed level of grassland management are: $\frac{3}{4}$ cwt. of 'Nitro-Chalk', $\frac{2}{3}$ cwt. of superphosphate and $\frac{1}{2}$–1 cwt. of muriate of potash per acre, per annum; or other suitable fertilizers equivalent to these, e.g. basic slag instead of superphosphate. At a higher level of management and utilization, much heavier dressings, particularly of nitrogen, will be more profitable.

It must be admitted, however, that there is little experimental evidence to support this view. When Crowther & Yates[2] suggested the most profitable dressings of fertilizers, under varying conditions, for arable crops, their suggestions were based on the results of large numbers of field experiments. The views of the author on the most profitable dressings of fertilizers for grassland under average stock-farming conditions in this country are not based on the results of carefully controlled field experiments, because such data do not exist. Indeed, it is probably not practicable to design a series of field experiments that will provide the evidence

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* Part II: Preceding paper

† Read before the Agriculture Group on 20 January, 1953

needed for such recommendations. This does not mean that fertilizer experiments on grassland which have been, and are being, carried out in this country are valueless. On the contrary, they are extremely valuable—often, indeed, essential,—particularly for the purpose of providing information on specific aspects of grassland manuring. But, for the purpose of providing data on which average recommendations can be based, they do not go very far and can often be misleading.

The views of the author on the manuring of grassland are based on the experience gained during the past five or six years on a considerable number of commercial farms, where detailed records have been kept of costs and output, together with all the relevant information from field experiments available, particularly those of the I.C.I. field station at Jealott's Hill. The suggested average fertilizer dressings for grass will not, of course, apply to all grassland : some conditions may demand more and others less.

There will probably be little difference of opinion on the need for approximately the quantities of *phosphate* stated. The amounts of *potash* required, on the other hand, will depend on the type of soil and, to a considerable extent, on the use that is made of the grass. About $\frac{1}{2}$–1 cwt. of muriate of potash per acre is normally adequate for a reasonably good level of production. If, however, a large proportion of the grass is to be removed by cutting and little is returned in the form of farmyard manure and liquid manure, or if the aim is a very high level of grass production, then much heavier dressings of potash will be needed—possibly up to 2, 3 or even 4 cwt. of muriate of potash per acre.

Nitrogen is the fertilizer nutrient that, when used on grass, requires the most consideration. Its application can be discussed under two headings : time of application and type of sward. The following suggestions are offered as a general guide.

Time of application of nitrogen

The cost of grass consumed as grazing is less than half of that of grass in any other form, and it is of the utmost importance, therefore, to have good grass available as grazing over the longest period possible. The value of nitrogen in extending the grazing season is one of its most important properties, and for early grass production (' early bite '), the use of nitrogen is essential in all parts of the country. Recent information suggests that it is most profitable to use larger dressings than those hitherto recommended for this purpose. Three cwt. of ' Nitro-Chalk ' applied early would seem to be more profitable than half that quantity applied twice.

Autumn applications of nitrogen may be valuable, particularly in the milder districts on (*a*) stubbles undersown with Italian or H.1 rye-grass for late autumn or winter grazing, (*b*) undersown leys as soon as the cover crop has been cleared (this should ensure good recovery and the establishment of a full plant before the winter) and (*c*) fields intended for ' early bite '. (The effect will be to carry over some growth till spring. This will be more fibrous as ' early bite ' and as a result may make a better diet than very young lush grass.)

The application of nitrogen to fields to be cut for hay, silage or drying, 10 to 14 days before cutting, substantially increases, in nearly all cases, the crude protein content of the grass, and the increase is nearly all as digestible protein. The application has usually no effect on the yield of that cut, but the swards recover quickly and the next growth benefits. Recent experiments suggest that very high yields of high-protein grass can be obtained by this technique.

Although nitrogen is most valuable on grass in the early and late season, on most farms there are some fields that need nitrogen throughout the season. This need varies with climatic conditions, but it seems that in the south-west there is less need for nitrogen in the middle of the season than there is further north.

Type of sward and the use of nitrogen

It is sometimes said that nitrogen depresses or 'kills out' clovers. This is misleading. Nitrogen encourages grasses and, in consequence, if large quantities are applied, and the management of the sward promotes the growth of very vigorous and tall grasses, then the clovers are often suppressed or killed out because of their inability to compete. Although clovers contribute substantial amounts of nitrogen to swards, and the maximum advantage should be taken of this fact, it does not follow that grass should always be managed with a view to maintaining a high clover content.

New Zealand and Holland, the leading grassland countries in the world, have opposite views on the value of clover. In New Zealand it is considered to be of the utmost importance, and in Holland it is practically ignored, especially on the good grassland farms. Great Britain has adopted a position between these two.

The following may be a useful guide to the use of nitrogen on different types of sward:

(*a*) *H.1 rye-grass short-rotation ley.*—This type of ley is seldom worth sowing unless nitrogen is used liberally. If 5–10 cwt. per acre of 'Nitro-Chalk' is used, it will produce heavily, and early and late, and total production over the season should exceed 3 tons of dry matter to the acre—sometimes over 4 tons. Much of this is produced when other fields are bare.

With this ley it is not necessary to retain clover, but, even with such high-level production, it is often worth while to sow 2 lb. of S.100 white clover. It is surprising that it persists.

(*b*) *Timothy/meadow fescue/white clover, timothy/meadow fescue/cocksfoot/white clover, cocksfoot/white clover and similar leys.*—These leys make their most important contribution during mid-summer, when rye-grasses are not productive. A high clover content is essential and heavy dressings of nitrogen should be used only in special cases. If no nitrogen is used, however, the sward tends to become very clovery and the good grasses die out as they are not adequately fed. This may produce digestive and other troubles in stock. Moderate dressings of nitrogenous fertilizer, e.g. 'Nitro-Chalk' at 2–4 cwt. per acre, are necessary to maintain a better grass/clover balance and increase yield.

(*c*) *Cockle Park-type leys and special-purpose leys based on perennial rye-grass.*—So far as the need for nitrogen is concerned, these leys are intermediate between (*a*) and (*b*). The clover content should be maintained at a reasonable level, but the rye-grass will not be productive, or productive as early as it should be, unless fertilizer nitrogen is used. Dressings of 2–6 cwt. of 'Nitro-Chalk' can be applied, according to conditions.

(*d*) *Permanent pasture.*—Even though permanent pasture may contain some wild white clover, the bulk of it, in this country, is relatively unproductive unless manured with nitrogen. Properly manured, however, even moderate-quality permanent pasture in the high-rainfall districts can become quite productive and can provide feed at very low cost. Anything from 2–6 cwt. of 'Nitro-Chalk' yearly is essential for good production.

Records obtained for the output of grass from a number of fields over recent years suggest that nitrogen used as described above will usually increase effective grass production by 25–50% and, in some circumstances, by much more, up to double the yield obtained without the use of fertilizer nitrogen. Further, it is found that the protein content of the grass is, on the average, increased by about one-fifth, and the grazing season is normally substantially extended.

Economic aspects

Over the past few years I.C.I. has been conducting an investigation in which detailed records are kept of output and costs on some 60–70 dairy and mixed farms. An important feature on these farms has been the drive to increase output from grassland, and certain results obtained from this investigation are of some interest.

The average cost on these farms of producing starch equivalent (S.E.) from various sources is set out in Table I.

Table I

Estimated cost of starch equivalent (S.E.) from various crops in 1950 *and dairy cake*

Crop	Cost of S.E., £ per ton	Relative cost (grazing = 100)	
Section A			
Grazing (effective production)	8·5	100	Section A contains the more firmly based estimates taken from the farms on which full cost accounts are kept
Grass silage	18	212	
Hay	19	224	
Dried grass	31	365	
Oats, straw-fed	20	235	
Kale, cut	22	259	
Arable silage	33	388	
Mangolds	37	435	
Section B			
'Early bite' (effective production)	12	141	Section B is more speculative—the figures are drawn from smaller samples with a greater degree of estimation
Kale, grazed	15	176	
'Extra' barley, half straw-fed	25	294	
Fodder beet	23	271	
Section C			
Dairy cake at £35 per ton	58	682	Section C is based on dairy cake at today's price

It will be seen from Table I that: (1) As a source of S.E., grass in all its forms is outstandingly cheap compared with purchased concentrates. (2) Grazing, silage and hay are cheaper sources of S.E. than any other crops except grazed kale. (3) Grass as grazing (normal or 'early bite') is by far the cheapest source of S.E. (4) Dried grass, although a more expensive source of S.E. than other forms of conserved grass, is still only slightly more than half the cost of dairy cake. These points are further evidence for the view that well-managed grass is a low-cost feed for cattle and sheep.

What level of grass production can be achieved, in general, by the application of modern techniques? The experience of I.C.I. indicates that by applying the quantities of fertilizers already mentioned, coupled with good swards and good management, including full utilization of the grass by good stock, the output of utilized S.E. per acre on small or medium-sized farms should be about 22–25 cwt. per acre annually. This does not mean that all the farms used for these experiments have reached this level. Some have not yet done so for a variety of reasons; others, however, have achieved an average production of over 30 cwt. of utilized S.E. per acre; and one 100-acre dairy farm has reached the exceptionally high level of 33·5 cwt. of utilized S.E. per acre over all its grassland. Many instances have also been recorded where individual fields have yielded over 2 tons of S.E. per acre. To achieve this level of production, however, much heavier manuring than so far indicated is necessary. For example, the 100-acre dairy farm, referred to above, used last year: 7·6 cwt. of sulphate of ammonia, 3·3 cwt. of superphosphate and 1·2 cwt. of muriate of potash per acre of grassland. Well-managed grass is thus cheap and very high yielding compared with most other crops. This, together with the fact that most stock farms in the U.K. are small, should receive prime consideration when a manurial treatment for grassland is prescribed.

The main hope for the small or medium-sized stock farms of Britain is to raise overall outputs to the highest level economically attainable. There can be no doubt that it is, financially, in the interest of the small farmer to raise the production of his grass to the highest level that can be used efficiently. Intensive grass production is usually more expensive per acre, and often also per unit of nutrient produced, than low-level grass production, but on most small and medium-sized farms it will still provide cattle feed at a fraction of the cost of that obtainable in any other way, and will thus enable the farmer to achieve maximum profit from his holding. Records in the possession of I.C.I. show that the present cost of producing leys in the manner suggested is about £9–£10 per acre per annum.

The stock farmer's main concern is, of course, the production of the maximum quantity of salable stock products from his holding and, although he may be interested in high yields of grass, he is more interested in finding out whether he can turn that grass into salable products such as milk, meat and mutton. It is not possible to deal with this important aspect of grass production in the space available, but all our evidence shows that well-managed grass can achieve a high output of milk, meat and mutton per acre and at low cost.

Some of the farms in these experiments made by I.C.I. have achieved a net average production of milk per acre over the whole farm which exceeds 350 gallons. One farm has now reached a net average production from grass of 400 gallons per acre, with a cash value of £60 per acre, without using any foods other than grass for cows. Nine heifers on this farm, calving in autumn 1951 at an average age of 25 months, averaged 740 gallons of milk in their first lactation, during which they were fed on nothing but silage and grazing.

Production as high as this cannot be achieved without liberal fertilizer treatment. The raising of the average output of S.E. from grassland to 22–23 cwt. per acre, which is the output necessary to meet national needs, will require fertilizer dressings of the order mentioned, and good grassland management as well.

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LABORATORY TESTS ON SOME HOOF AND HORN MATERIALS USED IN HORTICULTURE. I.—Raw Samples without Preliminary Heat Treatment

By O. OWEN, G. W. WINSOR and M. I. E. LONG

Twenty-two samples of raw hoof and horn materials have been examined in the laboratory with particular reference to their nitrogenous constituents. The samples, which included hoof, horn and mixtures of these two materials, were milled in the laboratory before testing. The nitrogen content of the samples and their 'availability' in the soil, as shown by incubation for 10 weeks, indicate that hoof and horn materials constitute a reasonably homogeneous class of nitrogenous fertilizer. Considerable differences were, however, found in the initial rates of mineralization of nitrogen from the samples.

Highly significant correlations were found between the initial rates of mineralization of nitrogen and the bulk densities and ease of hydrolysis of the samples. Hoof was at first more rapidly decomposed in the soil than horn, though after incubation for 10 weeks there was little difference between the two. Soil tests made with different sieved fractions of hoof and horn showed that the nitrogen of the finest particles was most rapidly mineralized at first, but the coarsest samples ultimately showed the highest 'availability'

Among the properties desirable in a given type of fertilizer reproducibility of effect must be accounted of considerable practical importance. Recommendations for the specific use of one fertilizer in preference to another for a given purpose depend on this quality of reproducibility. The composition of inorganic fertilizers can in general readily be found by chemical analysis, and apart from gross differences in particle size, deterioration in storage or unwise combinations in mixing, there is little reason for variation from sample to sample. With organic fertilizers, however, the possibility of variation in composition is considerably greater, the materials being obtained from varying sources throughout the world and processed in different ways. Organic fertilizers are, in addition, prone to biological decomposition in storage. Very marked differences have already been shown in the nitrogenous constituents of a group of bone materials;[1] these cannot, however, be regarded as a major source of nitrogen, and further experiments were therefore made with other organic nitrogenous fertilizers. For this purpose 41 commercial samples of hoof and horn were obtained from widely different sources. Originally it had been intended to group these samples under the three headings of hoof, horn, and mixed hoof and horn. Preliminary examination of the materials soon showed, however, that heat treatment by the manufacturers greatly affected the physical properties of the products, variations due to this cause being in many ways more marked than the differences found between hoof as compared with horn. The samples were accordingly separated into two groups, only those samples which had not been subjected to heat treatment during processing being included in the present paper. These raw materials included 7 samples of hoof, 5 samples of horn and 10 of mixed hoof and horn. For the purpose of statistical correlation these 22 samples have been treated as a single population consisting of various samples of crude scleroprotein.

A brief description of the samples as received, together with their nitrogen content and the percentage of each sample fine enough to pass a 40-mesh sieve, is given in Table I. In appearance the original horn samples differed markedly from the hoof materials, the former being in thin strands of a tough and fibrous nature. The nitrogen content of the samples, determined by the Kjeldahl method, showed considerable uniformity of composition, the values ranging from 13·48 to 15·72%, with a mean of 14·78%. No significant differences in the mean nitrogen content of the hoof, horn and mixed groups of material could be demonstrated in the present samples, though the four highest values in Table I were found among samples of hoof. The values found in the sieve test show that most of the samples were in relatively coarse condition when received. Thus only 10 of the 22 samples contained more than 10% of material passing a 40-mesh sieve and only 5 samples more than 20%. After the preliminary sieve test in Table I all samples were ground in a high-speed laboratory mill fitted with a 1-mm. grid, thus greatly reducing the original gross differences in particle size.

Experimental methods and results

The following tests were made on the milled samples: (1) formation of ammonia and nitrate in the soil; (2) determination of water-soluble nitrogen; (3) hydrolysis by dilute sulphuric acid in the autoclave; (4) determination of bulk density and (5) sieve analysis. The experimental details, together with the main conclusions arising from each of the tests, are given below under their respective headings. The full numerical results of the soil tests are given in Table II, and of the remaining tests in Table III. Correlations between the results of the various tests are discussed in a later section of this paper.

Table I

Description of samples as received, together with nitrogen content and the percentages passing a 40-mesh sieve before and after milling through a 1-mm. grid

Material	Sample No.	Description	Nitrogen content, %	Percentage passing 40-mesh sieve: As received	Percentage passing 40-mesh sieve: Milled
Mixed hoof and horn	1	Chips < ¼ in. and fibrous material < 1 in.	14·82	7·4	49·1
	2	Chips < ⅜ in. ,, ,, ,, < 2 in.	15·38	0·1	45·9
	3	Chips < ¼ in.	14·37	16·6	66·7
	4	Chips < ⅛ in. and fibrous material	14·27	31·0	54·3
	5	Chips < ¼ in.	15·01	28·2	56·6
	6	Chips < ¼ in. and fibrous material	14·72	7·3	64·1
	7	Contains fibrous material < 2 in.	15·35	9·5	64·4
	8	Chips and fibrous material < 1½ in.	14·97	8·0	53·0
	38	Chips < ⅛ in. and fibrous material < ½ in.	14·24	35·9	66·9
	42	Chips < ¼ in. ,, ,, ,, < 2 in.	14·60	1·2	43·7
Hoof	17	Chips < ¾ in. dark colour	13·97	4·3	61·0
	18	Chips and shavings < 1½ in.	14·96	6·0	51·8
	19	Coarse powder and fine shavings	14·00	45·5	78·5
	20	Coarse powder < ⅛ in.	15·46	6·8	53·9
	21	Chips < ½ in.	15·64	11·2	57·7
	22	Chips < ½ in.	15·55	13·8	56·4
	23	Chips < ¼ in.	15·72	14·9	45·6
Horn	27	Long thin shreds	13·48	3·7	48·0
	28	Fine shavings < 1½ in.	14·60	2·7	50·5
	29	Very fine shavings	14·58	37·5	67·1
	30	Shavings < 1 in. width	14·82	17·7	70·2
	41	Fibrous material < 3 in.	14·61	0·3	45·0

Table II

Percentage mineralization of the nitrogen of hoof and horn samples in soil at 23·5°
Series A and B after incubation for 7 days, series C after 10 weeks

Sample No.		Per cent. mineralization of nitrogen: 7-day incubation, Series A	7-day incubation, Series B	10-week incubation, Series C
Mixed hoof and horn	1	24·0	24·0	74·9
	2	19·7	25·4	75·0
	3	32·6	30·4	73·5
	4	19·4	22·0	64·2
	5	31·2	29·6	80·6
	6	26·6	29·5	71·4
	7	19·1	21·8	71·4
	8	21·4	23·0	68·7
	38	24·4	29·0	75·3
	42	11·8	17·1	70·2
Hoof	17	31·1	30·7	75·7
	18	29·5	30·4	76·6
	19	16·2	22·2	74·3
	20	35·4	44·9	78·6
	21	37·1	35·3	72·6
	22	40·1	35·8	75·2
	23	32·0	32·4	75·5
Horn	27	8·5	20·8	72·8
	28	12·7	22.6	73·2
	29	10·9	19·1	73·9
	30	7·2	19·3	74·7
	41	6·0	17·8	72·1

(1) *Formation of ammonia and nitrate in the soil*

Two aspects of mineralization of the nitrogen of organic compounds in soil are of particular importance, these being the rate of conversion into inorganic forms and the ultimate 'availability' of the added nitrogen. The number of samples involved in the present investigation made the determination of complete nitrification curves impracticable, and two arbitrary periods of incubation were therefore chosen. To obtain information concerning rates of decomposition, samples were incubated for seven days in the soil; as a measure of the completeness of transformation from organic to inorganic forms a longer period of 10 weeks was taken. The soil used in these tests was a market-garden soil having the following analysis:

Total nitrogen (Kjeldahl)	0·28%	Organic carbon (Walkley & Black)	2·49%
Phosphoric acid (P_2O_5) (soluble in 1% citric acid)	0·28	Total carbonate (as $CaCO_3$)	0·41
Potash (K_2O) (soluble in 1% citric acid)	0·028	Initial moisture content	19·8
	pH 7·27		

For the 7-day soil tests, samples of hoof and horn were weighed into flasks in quantities equivalent to 30 mg. of nitrogen per 100 g. of moist soil. The flasks were incubated at 23·5°, all treatments being in duplicate. Soil extracts were prepared by leaching with N-sodium chloride, and ammonia and nitrate determined by successive distillations with magnesia and with Devarda's alloy. Two such series of incubation tests were made at different times, these being referred to subsequently as soil tests A and B. The soil sample used in series B was taken from the same site as that for series A; the analysis given corresponds to the A series, but chemical differences in the second batch of soil were negligible.

In series A, a considerable range of values were obtained for the percentage of the added nitrogen converted into ammonia and nitrate in seven days, the values ranging from 6 to 40%. The highest values were obtained with samples of hoof, the lowest with horn.[2] This result is in accord with the traditional belief that hoof is more readily decomposed in the soil than horn; it is almost certainly related to the tough fibrous structure of raw horn materials. In the incubation tests of series B, the soil organisms showed somewhat greater activity, the percentage mineralization of nitrogen being in general slightly higher than in series A. Differences between the results of series A and those of series B obtained one month later were most marked with the more highly resistant samples. The actual range of values for percentage mineralization in series B was thus somewhat less than in the preceding series, being from 18 to 45%. Hoof materials again proved to be the more readily decomposed and horn materials the more resistant. Differences found between the two series of tests were somewhat greater than expected, even allowing for the variation of microbiological activity of stored soils. Further examination of the relationship between percentage mineralization and period of incubation shows, however, that under the conditions of this test the rate of decomposition increases markedly after some days in the soil. The greater biochemical activity noted in series B thus shifted the comparison of samples to a somewhat different stage of their decomposition in soil. Despite such differences in the actual values the relative order of decomposition was not substantially changed and, as shown in a later section, correlation between the two series was highly satisfactory.

As a measure of the nitrogen mineralized during a longer period in the soil further samples of hoof and horn were incubated for 10 weeks at 23·5°, quantities containing 50 mg. of nitrogen being mixed with 250 g. of moist soil. The results of this test, expressed as the percentage of the added nitrogen recovered as ammonia and nitrate, are given in Table II as soil test C. The values for percentage mineralization found in this test ranged from 64 to 81%, the general mean being 73·7%. Mean values for hoof, horn and commercial mixtures of the two materials were 75·5, 73·3 and 72·5% respectively. Variation between different samples of either hoof or horn was relatively small, but greater variability was found among the mixed hoof and horn samples. Agreement between duplicates in this incubation test was good, the significant difference being 2·0% ($P = 0{\cdot}05$).

(2) *Water-soluble nitrogen*

Samples (1 g.) of hoof and horn were shaken for one hour with 100 ml. of water and filtered under reduced pressure. The residues were repeatedly washed on the filter to give a final volume of 250 ml. Total nitrogen was determined in the extracts by the Kjeldahl procedure, the values recorded being the means of duplicate extractions. The results, given in Table III, show that the soluble constituents of raw hoof and horn materials form only a small percentage of the total nitrogen of the samples. Though the actual values are small, hoof samples showed a higher solubility than horn, and both groups showed relatively little variation as compared with the less uniform results obtained with mixed hoof and horn.

(3) *Hydrolysable nitrogen*

The solubility of hoof and horn materials in water at room temperature having proved

Table III

Results of tests on 22 samples of hoof and horn

Sample No.		*Water-soluble nitrogen, %	*Hydrolysable nitrogen, %	Bulk density, g./c.c.		Sieve analysis, % passing sieves	
				Sieved	Levelled	30-mesh	60-mesh
Mixed hoof and horn	1	3·7	32·4	0·23	0·27	71·7	26·2
	2	1·0	30·5	0·24	0·24	82·0	31·1
	3	2·1	39·6	0·43	0·47	94·7	45·5
	4	1·7	32·0	0·19	0·25	87·7	38·4
	5	1·1	38·7	0·28	0·29	87·8	38·7
	6	1·4	38·5	0·39	0·40	91·1	44·8
	7	1·3	26·3	0·18	0·20	79·2	43·0
	8	1·2	38·9	0·18	0·22	85·1	34·2
	38	4·7	44·1	0·24	0·33	88·7	46·7
	42	0·8	31·5	0·13	0·16	63·9	21·7
Hoof	17	1·3	33·6	0·43	0·43	91·4	42·8
	18	1·3	31·7	0·30	0·32	85·8	35·2
	19	1·4	21·8	0·28	0·27	98·5	54·2
	20	1·3	39·4	0·51	0·48	94·0	30·0
	21	1·9	43·2	0·42	0·46	86·4	36·7
	22	1·6	42·0	0·43	0·46	89·9	41·4
	23	1·7	40·7	0·48	0·53	87·5	32·6
Horn	27	0·9	27·0	0·15	0·14	67·8	27·0
	28	0·7	27·7	0·12	0·18	80·5	29·5
	29	0·5	23·7	0·26	0·29	98·5	41·9
	30	0·4	31·1	0·26	0·32	93·0	45·8
	41	0·5	29·0	0·12	0·15	61·3	33·2

* Water-soluble nitrogen and hydrolysable nitrogen expressed as a percentage of the total nitrogen

too low to serve as a possible measure of their ease of decomposition in the soil, further tests were made in an autoclave in the presence of dilute sulphuric acid. On the basis of these preliminary trials the following arbitrary conditions were chosen to give comparative values for the ease of dissolution of hoof and horn: 0·75-g. samples of hoof and horn and 50 ml. of 0·25N-sulphuric acid were heated in an autoclave for one hour at a pressure of 15 lb./sq. in. The products were filtered under reduced pressure immediately after removal from the autoclave and were washed repeatedly on the filter to give a final volume of 250 ml. Total nitrogen was then determined in the filtrate by the Kjeldahl procedure. The amounts of nitrogen brought into solution in this test, expressed as a percentage of the total nitrogen of the samples, ranged from 22 to 44%. The significant difference ($P = 0{\cdot}05$) between any two means of duplicate hydrolyses in this test was 1·49% of the total nitrogen. With one exception, higher values were found with hoof than with horn, the means for the unmixed samples being 36·1 and 27·7% respectively.

(4) *Bulk density*

Considerable differences in the bulk densities of the various samples were readily apparent, and it was expected that these would bear some relationship to the rates of decomposition in the soil. The method first tried was that used previously for bone-meals, in which the volume occupied by a known weight of material is read after gently sieving it into a measuring cylinder. This procedure proved somewhat unsatisfactory for the lightest samples, particularly with the milled horn materials, as the upper boundary of the undisturbed sample in the cylinder was badly defined. To overcome this difficulty a simple alternative was devised in which the sample was poured into a rimless 50-ml. tube and the excess of material removed by gently drawing a flat scraper across the top. The weight of sample filling the levelled tube was then determined. A calibrated 50-ml. centrifuge tube proved highly suitable for this test. The bulk densities of the milled products as determined by both methods have been included in Table III. The two sets of determinations are very closely related, the value of the correlation coefficient r being $+ 0{\cdot}977$. Values obtained by the second method, in which the samples were levelled with a scraper, are to be preferred, in that better agreement between replicates was obtained. A wide range of values, from 0·14 to 0·53 g./c.c., was found in this test. In general, the bulk density of milled hoof exceeded that of horn, as might be expected in view of the fibrous nature of the latter. Mean values for hoof and for horn samples separately were 0·421 and 0·215 g./c.c., as compared with 0·283 g./c.c. for the mixed samples. The significant difference between mean values for any two samples was 0·014 g./c.c. (P = 0·05). The interpretation of these results is discussed below.

(5) *Sieve analysis*

Milled samples of hoof and horn were graded on sieves having nominal mesh numbers of 30, 40 and 60 per inch. Despite passage through the high-speed mill, considerable differences were found in the fineness of the resulting powders. The results obtained with the 30- and 60-mesh sieves are recorded in Table III; values obtained with the 40-mesh sieve have been included in Table I for comparison with the original samples. The percentages of the milled products passing the 30-, 40- and 60-mesh sieves ranged from 61·3 to 98·5, 43·7 to 78·5 and 21·7 to 54·2 respectively. The range of values for each sieve was less than that previously found for bone materials and, as will be shown in the next section of this paper, the results did not prove to be of major value as a guide to the rates of decomposition of the samples when incubated in soil. Results of interest were, however, obtained when different sieved fractions were tested separately in the soil.

Correlation between the results of the various tests

The chemical and physical tests described above were chosen as possible guides to the rate and extent of mineralization of hoof and horn materials in the soil. Correlation coefficients between these tests and the results of soil tests A and B are given in Table IV. The A and B series of soil tests, in which hoof and horn samples were incubated for seven days, are a measure of the initial rates of decomposition in the soil. The factor found to give the highest correlations with these soil tests was bulk density, the correlation coefficient being significant at P = 0·001. Despite this highly significant result, the existence of a positive correlation in this instance was somewhat unexpected. It might in general be expected that low bulk density would be related to high surface area and thus to relatively rapid attack by soil micro-organisms. Results in accord with this hypothesis were previously obtained with bone materials, whereas the reverse relationship is found for the present group of hoof and horn samples. With hoof and horn, however, the explanation is probably related to the amount of fibrous material present. Horn materials, in particular, contain much fibrous material which combines low bulk density with relatively high resistance to rapid biological decomposition in the soil. In this connexion it is of interest that when the materials are subjected to heat treatment before milling the fibrous nature of horn is greatly altered, the bulk density of milled horn then being at least as high as that of hoof. It thus appears that for a range of different raw hoof and horn materials the expected relationship between bulk density and relative ease of biological decomposition in the soil is modified by differences in the shape and physical nature of the particles. When the different sieved fractions of a given sample are investigated the expected relationship between bulk density and rate of decomposition can be demonstrated, low bulk density being associated with high initial rates of decomposition in the soil.

Table IV

Correlation between the 7-day soil tests and properties of raw hoof and horn materials

Tests		Correlation coeff.	Significance
Soil test A	Per cent. hydrolysable nitrogen	$r = +0·762$	P = 0·001
,, ,, ,,	Bulk density (levelled)	+ 0·878	0·001
,, ,, ,,	Per cent. passing 30-mesh sieve	+ 0·445	0·05
,, ,, ,,	Per cent. passing 60-mesh sieve	+ 0·144	—
,, ,, ,,	Soil test B	+ 0·903	0·001
Soil test B	Per cent. hydrolysable nitrogen	+ 0·715	0·001
,, ,, ,,	Bulk density (levelled)	+ 0·833	0·001
,, ,, ,,	Per cent. passing 30-mesh sieve	+ 0·460	0·05
,, ,, ,,	Per cent. passing 60-mesh sieve	+ 0·074	—
Per cent. hydrolysable nitrogen	Bulk density	+ 0·675	0·001
,, ,, ,, ,,	Per cent. passing 60-mesh sieve	+ 0·020	—

A further test found to be significantly related to the rates of decomposition of hoof and horn in the soil is the determination of hydrolysable nitrogen in the fertilizer samples. Correlations between soil tests A and B and the percentage of hydrolysable nitrogen were in both cases significant at P = 0·001. This hydrolysis test was devised after it had been found that the content of water-soluble nitrogen in the samples, which has often proved to be a useful guide to the decomposition of nitrogenous materials used as fertilizers, was in this case too low to be of practical value. Though the experimental conditions in this test were quite arbitrarily chosen, the high correlation with the soil tests suggests some analogy between the susceptibility of hoof and horn materials to biological transformation in the soil and to combined chemical and physical decomposition in the autoclave.

In an earlier section of this paper reference was made to the considerable differences in particle size of hoof and horn materials even after milling through a 1-mm. grid (Tables I and III). Experience with other fertilizers would suggest that, despite the general fineness of grinding, these differences would be reflected in the initial rates of decomposition in the soil. Table IV shows that correlations significant at the 5% level were in fact obtained between the two series of soil tests and the percentage of each sample passing a 30-mesh sieve. No such correlation was found, however, with the 60-mesh sieve. These results indicate that, although particle size can influence the tests, the percentage of very fine material in the various samples was not apparently a controlling factor in the early stages of decomposition in the soil. In this connexion it is of interest that no correlation was found between the percentage of each hoof and horn sample passing a 60-mesh sieve and their ease of hydrolysis in the autoclave as previously described.

The exact reproduction of incubation tests that depend on the rate rather than the extent of a transformation in the soil is made more difficult by variations in the biological activity of stored soils. In order to obtain reliable results, the 7-day soil-incubation tests in the present investigation were made on two separate occasions. As already described, the actual values for percentage mineralization in the soil were not exactly reproduced, but the correlation found between the two sets of values is regarded as satisfactory, the value of the correlation coefficient r being $+ 0{\cdot}903$, significant at $P = 0{\cdot}001$. The correlation coefficients obtained between either of the two series of soil tests and the other factors in Table IV are also in good agreement, and such differences as do exist do not in any way affect the conclusions reached.

From the results presented in Table IV, the most important factors correlated with rates of mineralization of the nitrogen of hoof and horn materials in the soil include the bulk density of the milled materials and their ease of hydrolysis in the autoclave. Multiple-regression equations were accordingly calculated relating these properties for both series of 7-day incubation-tests in the soil. The equation relating the results of soil test A, x, to the bulk densities, y, and percentage hydrolysis of the nitrogen of the samples, z, is: $x = 48{\cdot}7y + 0{\cdot}603z - 13{\cdot}0$. Values calculated from this equation have been plotted against the experimental values of soil test A in Fig. 1, the multiple correlation coefficient being 0·868. Of the two independent terms in this equation, bulk density, y, is the more important, but the percentage hydrolysis term, z, is significant at the 5% level. For the soil tests of series B, however, the percentage hydrolysis term, z, fails to reach significance at the 5% level in the multiple-regression equation. This is in part due to the significant correlation found between bulk density and hydrolysable nitrogen, the value of the correlation coefficient r being $+ 0{\cdot}675$.

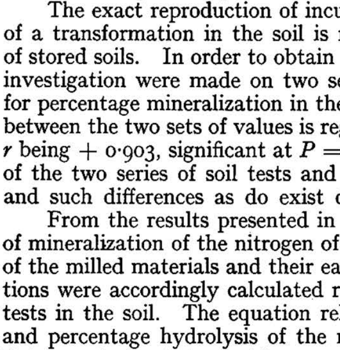

FIG. 1.—*Correlation between experimental values for 22 samples of hoof and horn in the 7-day soil tests of series A, and calculated values from the multiple-regression equation based on bulk density and percentage hydrolysable nitrogen in the milled samples*

The results given in Table IV show that the rates of decomposition of hoof and horn materials in the soil, as measured by incubation for a period of seven days, are significantly correlated with various other properties measurable in the laboratory. With the 10-week incubation tests, however, planned as a measure of the 'availability' of nitrogen in the fertilizer samples, no such correlations have as yet been found. The main feature of these results for pure samples of hoof and of horn separately is in fact the lack of variation from sample to sample. Among the mixed samples of hoof and horn the limits of variation are somewhat greater, being from 64·2 to 80·6% of the total nitrogen of the samples. Much of this variability is due to samples 4 and 5, however, these giving the lowest and highest values respectively in the whole group of 22 materials tested.

The effect of particle size upon the decomposition of hoof and horn materials in the soil

As already described, the samples used in the preceding experiments were milled through a 1-mm. grid before testing. The results of sieve analyses recorded in Table III show that this treatment, though reducing the gross differences in particle size found in the original samples, did not entirely eliminate these differences. Experiments were accordingly made to test the effect of particle size in different sieved fractions of hoof and horn

Table V

Nitrogen content (%) of sieved fractions of hoof and horn, and the bulk density of the hoof samples

Sieve range, mesh	Hoof		Horn
	Nitrogen content, %	Bulk density, g./c.c.	Nitrogen content, %
20–30	14·89	0·532	14·53
30–40	14·86	0·513	14·82
40–60	14·85	0·456	14·84
60–80	14·91	0·386	14·78
80–100	15·14	0·354	14·62
Passing 100	15·07	0·295	14·48

materials upon their decomposition in soil. For this purpose relatively coarse samples of hoof and of horn were first sieved to remove fine constituents already present, and then milled. The products were separated by sieving into six fractions, ranging from 20–30-mesh to materials passing a 100-mesh sieve. The nitrogen content of the various fractions is recorded in Table V.

Apart from differences in particle size the various sieved fractions of hoof appeared to be identical in appearance; their nitrogen content also showed little variation. Hoof thus appears to be a relatively homogeneous material which, unlike the bone materials described elsewhere,[1] can be separated into different ranges of particle size without revealing any obvious differences in composition. Horn is a less satisfactory material to grade by sieving, owing to the elongated shape of the particles, but the nitrogen content again showed relatively little variation. Determinations of the bulk density of the hoof fractions were made in triplicate by the cylinder method previously described, the mean values being included in Table V. As expected, the bulk density of the fractions decreased markedly as the particle size was reduced. The samples referred to in Table V were incubated at a concentration of 300 p.p.m. of added nitrogen in pots containing 1·5 kg. of glasshouse soil. Analyses of ammonia and nitrate were made at intervals, the increase in inorganic nitrogen above that of the control soil being expressed as a percentage of the nitrogen added. The results of these experiments are given in Table VI.

Table VI

Percentage mineralization of nitrogen from different sieved ranges of hoof and horn on incubation in soil at 23·5°

Material	Sieve range, mesh	Period of incubation, days				
		7	12	21	29	37
Hoof	20–30	33·4	70·3	78·1	82·7	82·8
	30–40	45·3	74·4	78·1	82·0	81·2
	40–60	55·5	74·8	78·1	81·2	81·4
	60–80	61·6	74·4	76·6	79·7	79·9
	80–100	59·8	70·2	71·4	75·0	75·5
	Passing 100	40·3	62·8	66·9	71·2	71·6
Horn	20–30	25·0	68·5	75·2	76·9	78·5
	30–40	30·5	69·6	72·9	75·1	76·9
	40–60	40·6	69·8	73·3	74·3	74·1
	60–80	54·5	72·1	74·1	74·5	75·9
	Passing 100	41·8	49·0	57·4	57·7	59·5

Referring first to the sieved fractions of hoof, after seven days the percentage mineralization increased as the size of the particles decreased from the 20–30-mesh to the 60–80-mesh fractions, but decreased again with the two finest fractions. By the 12th day, differences between the various fractions had been greatly reduced, though the finest fraction lagged considerably behind the others. After incubation for 21 days and at subsequent sampling dates, the percentage mineralization of the added nitrogen was found to decrease with increasing fineness of the original fractions. The results obtained with different sieved fractions of horn were essentially similar to those described for the hoof fractions. Thus at the first date of sampling the coarsest fractions lagged behind the others in percentage mineralization of nitrogen, but this order was subsequently reversed. That the initial rates of decomposition in the soil should increase with fineness of grinding is to be expected in view of the relatively greater surface area of the finer fractions; the subsequent reversal of this order was not, however, foreseen.

To obtain more detailed information, the experiment with graded fractions of milled hoof was repeated, the soils being analysed at more frequent intervals to cover the period of active decomposition in the soil. The results obtained are shown in Fig. 2. For the first 11 days of incubation the percentage of the added nitrogen mineralized at each date of sampling increased consistently with the fineness of the particles. At the 13th and 18th days mineralization of nitrogen was greatest in the middle ranges of particle size. By the 39th day the initial order was reversed, the percentage of nitrogen mineralized decreasing in order of fineness of the original sieved fractions. This relationship to particle size had not previously been encountered in this Laboratory, and a full explanation has not yet been established. It is possible, however, that the most finely ground materials, being the most rapidly attacked by the soil organisms in the early stages, cause the greatest increase in numbers of these organisms. The proportion of the added nitrogen transformed into cell-substance or relatively resistant organic products may thus be greatest for the finest particles. The differences in percentage mineralization of the various sieved fractions may thus be due to different amounts of nitrogen immobilized by the soil organisms.

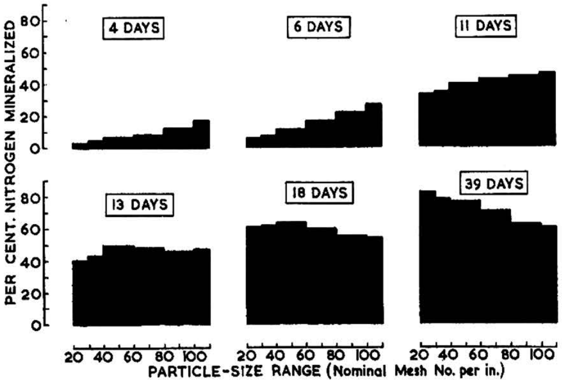

FIG. 2.—*Mineralization of the nitrogen of different sieved fractions of hoof on incubation in the soil*

Conclusions

(1) Laboratory tests on milled samples of hoof and horn have shown considerable variation in their initial rates of decomposition in the soil. These differences do not persist for long in the soil and are considerably less than those previously found for bone materials.

(2) Differences in initial rate of mineralization of nitrogen in the soil are significantly correlated with the bulk density of the samples and their ease of hydrolysis by dilute sulphuric acid in the autoclave.

(3) Hoof materials are at first decomposed in the soil more rapidly than horn.

(4) The availability of the nitrogen of hoof and horn samples as measured by incubation for 10 weeks in the soil shows relatively little variation.

(5) It is concluded that hoof and horn materials that have not been subjected to heat treatment during processing constitute a reasonably homogeneous class of nitrogenous fertilizer.

(6) The influence of particle size below 20-mesh upon the mineralization of nitrogen in the soil has been investigated under laboratory conditions. The nitrogen of the finer fractions is most rapidly mineralized at first, but this order is subsequently reversed. This result suggests that differences in particle size can have a marked effect upon the 'availability' of fertilizers as measured by incubation tests in the soil.

Acknowledgment

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LABORATORY TESTS ON SOME HOOF AND HORN MATERIALS USED IN HORTICULTURE. II.*—Materials Heat-treated during Processing

By O. OWEN, G. W. WINSOR and M. I. E. LONG

Nineteen samples of 'calcined' hoof and horn materials have been examined in the laboratory with particular reference to their nitrogenous constituents. All the samples, which included hoof, horn and mixtures of the two materials, were milled in the laboratory before testing. The nitrogen content of the samples ranged from 12·98 to 15·10%. Considerable differences were found in the initial rates of mineralization of the nitrogen of the samples in soil. Highly significant correlations were found between the initial rates of mineralization of nitrogen and the contents both of water-soluble nitrogen and of ammonia as determined by distillation with magnesia.

A rapid test, in which aqueous extracts of the hoof and horn samples were titrated with a standard solution of sodium hydroxide in the presence of formaldehyde, was found to give results approximating to the ammonia values as determined by distillation and highly correlated with the soil-incubation tests. A positive correlation was also found between the initial rates of mineralization of nitrogen and the bulk density of the samples. After incubation for 10 weeks in the soil the percentage mineralization of nitrogen in the samples ranged from 62 to 76%; no correlation could be found between these values and the other properties of the samples tested.

It is concluded that calcined hoof and horn materials constitute a reasonably homogeneous class of nitrogenous fertilizer, except where the preliminary heat treatment was insufficient to destroy the fibrous nature of the horn samples.

In Part I the results of laboratory tests made on raw hoof and horn were reported. Such materials are somewhat difficult to grind into forms suitable for use as fertilizers, and require heavy milling equipment for this purpose. Much of the hoof and horn sold for horticultural purposes does, however, receive some form of heat treatment which renders it easier to crush. The actual conditions of this preliminary treatment differ considerably from one manufacturer to another, and little information is available concerning the temperatures and periods of heating of the samples used in the present work. The terms used to describe this preliminary treatment are also diverse. Thus samples 35, 36 and 37 were described as being 'dried' at 300° F before milling, sample 33 is of 'steamed' horn and other samples are referred to as 'calcined'. For convenience in the present work the term 'calcined' will be used to denote all samples that have been subjected to any form of heat treatment during processing. Such materials are almost invariably characterized by their colour, ranging from pale-straw to dark-brown in contrast to the paler colours, frequently grey or white, of the original materials. This visual effect of calcining is accompanied by marked changes in the physical and chemical properties of the materials, as will be seen by comparison of the results here reported with those already given for 'raw' samples in Part I of the present investigation. All samples were ground before testing in a high-speed mill fitted with a 1-mm. grid; the nitrogen content of the milled products is included in Table II.

Experimental methods and results

The following tests were made on the milled samples: (1) formation of ammonia and nitrate on incubation with soil; (2) determination of water-soluble nitrogen; (3) distillation with an aqueous suspension of magnesia; (4) formol titration of aqueous extracts; (5) determination of bulk density (cylinder method); (6) sieve analysis.

The experimental details of tests 1, 2, 5 and 6 have already been given in Part I; details of the remaining tests are given with the results under their respective headings. The numerical results of the soil tests are given in Table I and of the remaining tests in Table II. Discussion of the results is at this stage limited to the range of values obtained by each of the six experimental techniques; correlations between the various tests are described in a later section.

(1) *Formation of ammonia and nitrate in the soil*

As a measure of the initial rates of decomposition in the soil, two sets of 7-day incubation tests were made, these being run at the same time as the A and B series of tests made with raw hoof and horn as described in Part I. Mean values for the percentage mineralization of nitrogen in soil tests A were 29·5, 30·9 and 24·9 respectively for the mixed hoof and horn

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* Part I: Preceding paper

Table I

Per cent. mineralization of the nitrogen of calcined hoof and horn samples in soil at 23·5°
Series A and B after incubation for 7 days, series C after 10 weeks

Sample No.		7-day incubation		10-week incubation
		Series A	Series B	Series C
Mixed hoof and horn	9	27·2	27·0	66·6
	10	26·8	36·4	70·8
	11	32·3	33·2	69·4
	12	27·0	27·5	67·4
	13	22·9	24·5	70·7
	14	32·6	32·9	68·9
	15	27·5	30·9	73·0
	16	39·3	38·0	72·3
	35	21·3	26·0	67·4
	39	38·1	40·8	71·8
Hoof	24	28·6	33·0	71·9
	25	29·8	34·8	74·5
	26	31·5	35·9	75·9
	36	33·8	36·6	69·2
Horn	31	17·7	22·0	67·0
	32	18·3	21·4	62·0
	33	33·4	39·9	67·1
	37	9·0	14·5	73·7
	40	46·3	45·1	71·2

(10 samples), hoof alone (4 samples) and horn alone (5 samples). Corresponding values for the B series of soil tests were 31·7, 35·1 and 28·6% respectively. The mean values for all samples were 28·6 and 31·6% in series A and B, the soil used in the tests of series B thus showing greater biological activity, as noted with the raw samples. The significant differences between any two samples in the 7-day soil incubation tests were 1·97 and 2·75% for the A and B series respectively. Significant differences between many of the samples were thus obtained in these tests. As the highest ultimate values for percentage mineralization of calcined hoof and horn rarely exceed 75% it is apparent from these 7-day incubation tests that calcined hoof and horn is quite rapidly decomposed in the soil when finely ground.

As a measure of the ultimate 'availability' of hoof and horn materials further samples were incubated in soil for 10 weeks at 23·5°. The results for percentage nitrified in these tests ranged from 62 to 76% of the total nitrogen added. The standard error of the sample

Table II

Results of tests made on 19 samples of calcined hoof and horn

Sample No.		Total nitrogen, %	Water-soluble nitrogen*	Ammonia-nitrogen*	Nitrogen* by formol titration	Bulk density, g./c.c.	Per cent. passing 60-mesh sieve
Mixed hoof and horn	9	13·28	13·7	2·92	2·82	0·64	76·8
	10	14·08	8·8	2·23	1·78	0·63	75·5
	11	14·29	12·2	3·61	2·62	0·62	74·9
	12	13·38	11·7	2·37	2·35	0·58	84·8
	13	14·00	7·4	1·74	1·54	0·61	60·4
	14	13·39	14·7	3·23	2·68	0·66	73·4
	15	14·94	8·1	1·64	1·74	0·58	78·2
	16	13·25	29·6	4·81	5·00	0·64	87·4
	35	13·90	4·1	1·82	1·51	0·47	66·6
	39	12·98	9·5	2·04	1·85	0·69	70·2
Hoof	24	14·95	6·0	1·67	1·49	0·57	68·9
	25	14·09	6·7	2·07	1·95	0·65	69·2
	26	15·10	7·6	2·50	1·68	0·62	75·5
	36	13·16	7·6	1·96	1·74	0·59	69·0
Horn	31	14·63	7·6	1·33	0·85	0·64	71·3
	32	14·94	11·2	1·32	1·40	0·60	83·0
	33	13·06	18·7	3·62	3·22	0·65	86·4
	37	14·80	2·6	1·54	0·51	0·28	57·8
	40	14·29	21·7	3·09	3·30	0·66	80·4

* Expressed as a percentage of the total nitrogen

means in this test was 1·25%, differences between any two sample means exceeding 3·69% being significant at $P = 0{\cdot}05$. The variations found between individual samples, though relatively small, were in many cases significant. Mean values for mixed hoof and horn, hoof alone and horn alone were 69·8, 72·9 and 68·2% respectively, the mean for all three groups together being 70·0%. Thus, in general, the availability of hoof slightly exceeds that of horn, but the difference is not very marked. Finely ground hoof and horn materials thus form a relatively homogeneous group of nitrogenous fertilizers with respect to their ultimate availability in the soil.

(2) *Water-soluble nitrogen*

In contrast with the raw samples of hoof and horn, calcined materials may contain a relatively high proportion of water-soluble nitrogen. The soluble nitrogenous matter consists of protein and its decomposition products, including some ammonia. Expressed as a percentage of the total nitrogen of the samples the water-soluble nitrogen ranged from 2·6 to 29·6. The lowest values in the group were given by samples 35 and 37, known to have been only lightly calcined; the highest solubilities were found among the horn samples and mixtures containing horn. The significant difference ($P = 0{\cdot}05$) between mean values of duplicate determinations in this test was 0·30%. The wide variation found in water-soluble-nitrogen content of the various samples is not unexpected in view of the different conditions of heat treatment employed by the manufacturers and the possibility of biological decomposition in storage. In view of this variability little significance can be attached to the mean values found for the three groups of calcined materials. It is of interest that calcining appeared to have more effect on horn than on hoof, none of the four samples of calcined hoof having a content of water-soluble nitrogen greater than 8%. It is possible, however, that hoof materials, being more readily crushed than horn, are not in general subjected to such severe preliminary heat treatment as the latter in commercial practice.

(3) *Distillation with an aqueous suspension of magnesia*

Samples (1 g.) of hoof and horn materials were distilled with 2 to 3 g. of freshly ignited magnesia and 500 ml. of water, the ammonia evolved being collected in 0·02N-acid. The ammonia found in this test will include that present in the original samples and possibly small amounts produced by slight hydrolysis. The results obtained are considered to be related to biological decomposition of the hoof and horn materials during storage. Values for ammonia-nitrogen, expressed as a percentage of the total nitrogen, ranged from 1·32 to 4·81%, the mean value being 2·40%. Highly reproducible results were obtained in this test, a difference of 0·13% between mean values for any two samples being significant at the 5% level. Mean values for mixed hoof and horn, hoof alone and horn alone were 2·64, 2·05 and 2·18% respectively. From these values it is apparent that ammonia is not in itself a major constituent of calcined hoof and horn materials; in a later section it will be shown, however, that highly significant correlations exist between these ammonia values and other properties of the samples.

(4) *Formol titration of aqueous extracts of hoof and horn materials*

Aqueous extracts were prepared by shaking 2-g. samples of hoof and horn with 150 ml. of distilled water for one hour. The suspensions were filtered and duplicate 50-ml. aliquots pipetted into conical flasks. After addition of formaldehyde to give an initial concentration of 8% the extracts were titrated with 0·02N-sodium hydroxide with phenolphthalein as indicator. The pH of the formaldehyde was first adjusted with sodium hydroxide so as to give a pale-pink colour when added to 50 ml. of distilled water. This arbitrary procedure was used as a rapid test for soluble nitrogenous constituents, including ammonia and amino-acids, that can be directly titrated in presence of formaldehyde. Highly significant correlations were found between the results so obtained and various other properties of the samples, including total water-soluble nitrogen, ammonia by distillation with magnesia, and the initial rates of decomposition of the samples in soil. The procedure itself is a rapid one, not involving distillation. The results obtained are reported in Table II in terms of nitrogen titrated, expressed as a percentage of the total nitrogen of the samples. The values ranged from 0·5 to 5% of the total nitrogen and are closely related to the ammonia liberated by distillation. These correlations are discussed in a later section of this paper.

(5) *Bulk density of the milled samples*

The bulk density of the samples was determined by sieving into a measuring cylinder and hence finding the volume occupied by a known weight of powdered material. The values obtained ranged from 0·276 to 0·685 g./c.c., the lowest value being given by horn sample No. 37, which received only mild heat treatment. The mean value for 10 samples of mixed hoof and horn was 0·610 g./c.c., for 4 samples of hoof alone 0·607 g./c.c., and for 5 samples of horn alone 0·563 g./c.c. On omitting sample 37 the mean value for 4 samples of horn was 0·635 g./c.c. The process of calcining thus markedly reduces the differences in bulk density formerly observed between raw hoof and raw horn, probably by modifying the fibrous nature of the latter and rendering it easier to crush.

(6) *Sieve analysis*

The percentage of the milled samples passing 30- and 60-mesh sieves was determined, values for the finer sieve alone being recorded in Table II. The percentages of the various samples passing the 60-mesh sieve ranged from 58 (sample 37) to 87% (sample 16). It is of interest that these same two samples gave the lowest and highest values respectively for water-soluble nitrogen, namely 2·6 and 29·6% of the total nitrogen.

Correlation between the results of the various tests

The main emphasis of the present work lies in the correlations found between the soil-incubation tests and the other chemical and physical tests. Correlation coefficients between soil tests A and B and the chemical and physical properties investigated are recorded in Table III; no significant correlations have been found with the 10-week soil-incubation tests. As an aid to interpretation of the results, correlation coefficients between results from tests other than those from the soil tests are recorded in Table IV.

Table III

Correlations between the 7-day soil-incubation tests and chemical and physical properties of the samples

Tests		Correlation coeff.	Significance
Soil test A ..	Per cent. water-soluble nitrogen	$r =$ + 0·699	$P =$ 0·001
,, ,, ,, ..	Per cent. ammonia-nitrogen	+ 0·657	0·01
,, ,, ,, ..	Per cent. nitrogen by formol titration	+ 0·740	0·001
,, ,, ,, ..	Bulk density	+ 0·692	0·01
,, ,, ,, ..	Per cent. passing 60-mesh sieve	+ 0·487	0·05
,, ,, ,, ..	Soil test B	+ 0·945	0·001
Soil test B ..	Per cent. water-soluble nitrogen	+ 0·533	0·02
,, ,, ,, ..	Per cent. ammonia-nitrogen	+ 0·558	0·02
,, ,, ,, ..	Per cent. nitrogen by formol titration	+ 0·614	0·01
,, ,, ,, ..	Bulk density	+ 0·661	0·01
,, ,, ,, ..	Per cent. passing 60-mesh sieve	+ 0·438	0·1

Table IV

Correlations between various chemical and physical tests made on 19 samples of calcined hoof and horn

Tests		Correlation coeff.	Significance
Per cent. water-soluble nitrogen ..	Per cent. ammonia-nitrogen	$r =$ + 0·858	$P =$ 0·001
,, ,, ,, ,, ,, ..	Per cent. nitrogen by formol titration	+ 0·942	0·001
,, ,, ,, ,, ,, ..	Bulk density	+ 0·500	0·05
,, ,, ,, ,, ,, ..	Per cent. passing 60-mesh sieve	+ 0·754	0·001
Per cent. ammonia-nitrogen ..	Per cent. nitrogen by formol titration	+ 0·933	0·001
,, ,, ,, ..	Bulk density	+ 0·392	0·1
Per cent. nitrogen by formol titration	Bulk density	+ 0·503	0·05
	Per cent. passing 60-mesh sieve	+ 0·698	0·001
Bulk density	Per cent. passing 60-mesh sieve	+ 0·517	0·05

As is frequently found in investigations of the mineralization of nitrogen from organic materials, the determination of water-soluble nitrogen proved to be a reliable guide to the initial rates of decomposition of calcined hoof and horn samples in the soil. The correlations found between water-soluble nitrogen and the A and B series of 7-day soil tests were significant at $P =$ 0·001 and 0·02 respectively. Analogous results with the nitrogenous constituents of bone materials have already been published. Such correlations are to be expected in that, under suitable conditions and in absence of toxic constituents, soluble nitrogenous compounds are readily decomposed in the soil. The lower significance of the correlation coefficient for soil tests B as compared with A can be attributed to the relatively greater biological activity of this batch of soil, resulting in increased decomposition of the initially insoluble constituents of the samples.

The results of the formol titration are in fact even more closely correlated with the 7-day soil tests than are the values for total water-soluble nitrogen. The ammonia determined by distillation of the samples with magnesia is also significantly correlated with the initial rates of mineralization of nitrogen in the soil, the correlations with soil tests A and B being significant at $P = 0{\cdot}01$ and 0·02 respectively. Thus all three chemical determinations related to the soluble nitrogenous constituents of the hoof and horn samples have in the present work proved reliable guides to their initial rates of decomposition in the soil. Correlations between these three series of measurements of available nitrogenous constituents are all very high, being significant at $P = 0{\cdot}001$ as shown in Table IV, and further examination of the results is of interest. The values for ammonia by distillation and the nitrogen determined by formol titration are not only highly significantly correlated ($r = +0{\cdot}933$) but are also numerically similar (Table II). The regression equations relating ammonia by distillation, x, to the results of the formol titration, y, are:

$$x = 0{\cdot}856y + 0{\cdot}59\,;\quad y = 1{\cdot}02x - 0{\cdot}33$$

both estimations of nitrogen being expressed as percentages of the total nitrogen of the samples. The mean value for ammonia-nitrogen is 2·4%, and, for nitrogen by formol titration, 2·1%. In addition to the nitrogen initially present as ammonia in the samples, the ammonia determined by distillation with magnesia will include small amounts of nitrogen derived from possible hydrolysis of other nitrogenous constituents. In the formol titration such hydrolysis does not occur, but nitrogen from any free amino-acids and some protein groups will be included. In view of the close numerical agreement between the two sets of data it is reasonable to conclude that the main nitrogenous constituent determined in both cases is ammonia, the small amounts of additional nitrogen hydrolysed during distillation being counterbalanced by the amino-nitrogen titrated in the presence of formaldehyde. In both these estimations the nitrogen determined is but a relatively small fraction of the total soluble nitrogen, but both analyses are highly correlated with the total soluble nitrogen at the 0·1% level. Whereas the total water-soluble nitrogen as a constituent of the calcined hoof and horn samples is of direct practical importance, such nitrogen being very rapidly mineralized by the soil organisms, the significance of the small amounts of ammonia-nitrogen requires a different interpretation. Numerically too small to influence the soil-incubation tests directly to any appreciable extent, the presence of ammonia is doubtless related to the degree of decomposition of the original protein materials by heat treatment or by biological processes during storage.

As previously found for milled samples of raw hoof and horn, the simple physical determination of bulk density is significantly related to the initial rates of decomposition of the samples as shown by the 7-day soil-incubation tests. For both the A and B series of soil tests the correlation with bulk density was significant at $P = 0{\cdot}01$. These correlations are not as high as those found for the raw samples however, and are not considered to be as valuable a guide to mineralization in the soil as the chemical determination of water-soluble nitrogen. If samples 35 and 37 are omitted, these being known to be only lightly calcined, the actual range of bulk densities is relatively low, being from 0·57 to 0·66 g./c.c. Thus, despite the significance of the correlations obtained in the present work, the determination of bulk density as a guide to rates of decomposition in the soil is not to be recommended for calcined hoof and horn; it can, however, indicate samples in which heat treatment has been insufficient to modify the fibrous nature of the raw protein material.

The percentages of the various samples passing a 60-mesh sieve were significantly correlated with the results of the A series of 7-day soil-incubation tests at the 5% level, but just failed to attain this level for the B series. It is concluded that physical tests are of greater value for raw samples of hoof and horn than for the calcined materials; calcining greatly modifies the fibrous nature of the original materials and thus eliminates a major physical effect. It is of interest, however, that a highly significant correlation at the 0·1% level was obtained between the percentage of the calcined materials passing the 60-mesh sieve and both the total water-soluble nitrogen and the nitrogen by formol titration. It thus appears that particle size had a greater effect on the preparation of aqueous extracts for analysis than on the decomposition of the samples in the soil.

Multiple correlations

As shown in Table III, the various tests correlated with the initial rates of mineralization of nitrogen from calcined hoof and horn include the determination of both chemical and physical properties. Of the physical tests, bulk density proved the more important, while determinations of water-soluble nitrogen, ammonia by distillation and soluble nitrogen by formol titration each gave very similar values when correlated with the 7-day soil-incubation tests. Multiple-regression equations relating the soil tests to the bulk densities and any one of the three types of nitrogen determination were accordingly calculated. Thus for the soil tests of series A:

$$A_1 = 44{\cdot}5B + 0{\cdot}562C_1 - 4{\cdot}2 \qquad (1)$$

$$A_2 = 48{\cdot}0B + 4{\cdot}18C_2 - 10{\cdot}1 \qquad (2)$$

$$A_3 = 39{\cdot}9B + 4{\cdot}41C_3 - 4{\cdot}5 \qquad (3)$$

where A is the estimated value for percentage mineralization of nitrogen in the first seven days, B is the bulk density of the milled samples, and C_1, C_2 and C_3 are the total water-soluble nitrogen, ammonia-nitrogen by distillation, and soluble nitrogen determined by formol titration respectively, the C terms all being expressed as a percentage of the total nitrogen of the samples. In each equation both regression terms are significant, the corresponding multiple-correlation coefficients being 0·785, 0·809 and 0·827 for equations 1, 2 and 3 respectively. Values for the percentage mineralization of nitrogen, calculated from equation 2 based on ammonia content and bulk density, are plotted against the experimental values in Fig. 1.

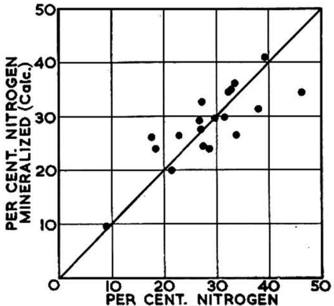

FIG. 1.—*Correlation between experimental values for 19 samples of calcined hoof and horn in the 7-day soil tests of series A, and calculated values from the multiple-regression equation based on ammonia content and sieved bulk density of the milled samples*

With the B series of soil tests the total correlation coefficients for water-soluble nitrogen, ammonia and nitrogen by formol titration (Table IV), though significant in each case, are lower than for series A; on calculating the multiple-regression equations for series B, in no case did the terms relating to the nitrogen determinations attain significance at the 5% level. A rather similar result was previously obtained for raw samples of hoof and horn in the A and B series of soil tests. Thus in the multiple-regression equations relating the 7-day soil tests with bulk density and percentage hydrolysable nitrogen in the raw samples, the last two terms attained significance for soil tests A, but only the bulk density term was significant for soil tests B. For both the raw and calcined materials the failure of the soluble nitrogen and related terms in the multiple-regression equations calculated for the B series of soil tests may be attributed to the greater biological activity of the soil used as compared with series A. As a result of this greater activity a higher proportion of initially insoluble material was decomposed, thus reducing the worth of determinations of water-soluble-nitrogen content and related properties as a guide to the extent of decomposition. A shorter period of incubation in the soil used for series B would almost certainly have yielded results more similar to those obtained after seven days in series A. It is only to be expected that the content of water-soluble nitrogen will be most closely related to the percentage mineralization of nitrogen in the soil before appreciable decomposition of initially insoluble constituents begins. Experiments with raw hoof and horn have shown that, after an initial period of relative inactivity, quite rapid mineralization of insoluble nitrogen can occur within a few days.

Comparison of tests made on raw and calcined hoof and horn

Examination of the results of the present investigation shows that, for almost every test applied to both raw and calcined samples of hoof and horn, significant though not necessarily large differences can be demonstrated between the mean values in the two groups. Mean values for both raw and calcined hoof and horn are given in Table V, together with the level of statistical significance of differences between the two means where such differences are not self-evident.

The main effects of heat treatment of the samples thus appear to include increased bulk density of the milled products, a greatly increased proportion of water-soluble nitrogen, and greater ease of milling. The initial rates of mineralization of nitrogen are slightly increased by calcining, as shown by soil tests A and B, whereas the final 'availability' of the samples is significantly but not greatly reduced (soil test C). The percentage of nitrogen in the calcined samples is less than in the raw materials, suggesting slight losses of nitrogen either during calcining or as ammonia during subsequent storage.

Table V

Mean values for 22 samples of raw hoof and horn and 19 samples of the calcined materials

Test	Mean values		Significance of difference between means
	Raw	Calcined	
Total nitrogen, %	14·78	14·03	$P = 0·01$
Water-soluble nitrogen	1·5*	11·0	
Sieved bulk density, g./c.c.	0·284	0·597	
Per cent. passing 60-mesh sieve	37·3	74·2	
Soil test A (7 days)	22·6*	28·6	0·1†
Soil test B (7 days)	26·5*	31·6	0·05
Soil test C (10 weeks)	73·7*	70·0	0·01

* Expressed as a percentage of the total nitrogen of the samples
† Only just fails to attain significance at $P = 0·05$

Direct comparisons of the mineralization of nitrogen from raw and calcined samples of hoof and horn have been made in three different soils, the results of one of these experiments being shown in Fig. 2. Both the calcined samples mineralized rapidly at first, whereas some lag occurred with the raw materials. This initial lag was particularly marked in the case of raw horn, but rapid decomposition of both the raw samples soon followed. At the end of the tests, hoof showed a higher 'availability' than horn, and the raw samples surpassed the calcined materials in this respect. From the form of the curves the difficulty of reproducing the results of short-term incubation experiments in different batches of soil, as in the A and B series of soil tests, will be readily apparent. The differences in long-term availability found between the raw and calcined materials can possibly be attributed to greater stimulation of the soil population by the latter, resulting in increased immobilization of nitrogen. Despite the marked effect of heat treatment upon the chemical and physical properties of the materials, the differences observed in rates of mineralization of nitrogen were relatively small.

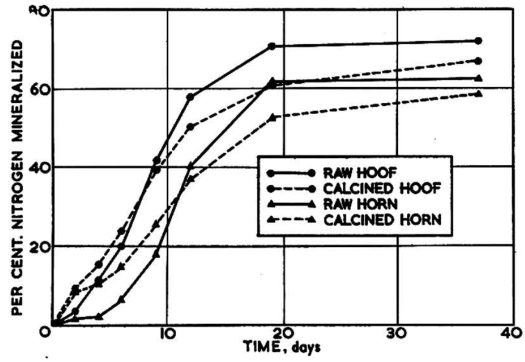

FIG. 2.—*Mineralization of the nitrogen of raw and calcined hoof and horn in soil*

Conclusions

(1) Laboratory tests made on calcined samples of hoof and horn after grinding to pass a 1-mm. sieve have shown considerable variation in their initial rates of decomposition in the soil. As for the raw samples previously described, these differences do not persist for long in the soil.

(2) Differences in initial rates of mineralization of nitrogen in the soil are significantly correlated with the bulk densities of the samples and with their contents of water-soluble nitrogen and of ammonia as determined by distillation with magnesia.

(3) Titration of aqueous extracts of calcined hoof and horn with sodium hydroxide in the presence of formaldehyde gave rapid results closely approximating to the ammonia values and significantly correlated with the initial rates of decomposition in the soil.

(4) The availability of the nitrogen of calcined hoof and horn samples, as measured by

incubation for 10 weeks in the soil, ranged from 62 to 76% of the total nitrogen. No correlation was found between these values and the other properties investigated.

(5) It is concluded that calcined hoof and horn materials form a reasonably homogeneous class of nitrogenous fertilizer, provided that the preliminary heat treatment was sufficient to destroy the fibrous nature of the original materials.

(6) Results for raw and calcined hoof and horn are compared. Preliminary heat treatment by the manufacturers appears to have a greater effect on the solubility and physical properties of the materials than on their rate and extent of decomposition in the soil.

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NON-TOXIC STABILIZERS FOR ANIMAL FATS

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The addition of citric and tartaric acids is shown to produce a noticeable increase in the induction periods of edible tallow and lard. The use of mixed glyceryl esters of citric acid and fatty acids is suggested as a convenient method of incorporating citric acid into fats.

Chemicals used for stabilizing edible fats are usually divided into (1) compounds mostly of phenolic character (e.g. gallates, substituted hydroxyanisoles), which are regarded as ' true ' antioxidants, and (2) a group comprising mainly polybasic organic and inorganic acids (e.g. maleic, citric, phosphoric), which act as metal deactivators and as synergists in conjunction with natural and artificial true antioxidants. The first group is particularly effective in animal fats, which, as a rule, are lacking in natural antioxidants, whereas the second group is considered suitable for vegetable fats but is said to produce little effect in animal fats. However, phenolic antioxidants are not permitted in most British and Western European countries, mainly on account of their possible toxicity. Experiments conducted by the health authorities in the U.S.A. have shown some of them to be harmless, but the evidence has not yet been accepted elsewhere as conclusive and, as has been recently stated by Lea,[1] ' more information would seem to be required about the toxicity of the quinonoid oxidation products into which phenolic antioxidants are converted in the course of their protective action '. For the time being, therefore, the only truly safe artificial stabilizers appear to be some members of the second group, i.e. citric, tartaric and phosphoric acids, which are found in so many natural foods that their use in the food industry is generally permitted by law. Of these three compounds it is the citric acid that seems to have found wide application as a stabilizer in refined vegetable fats, particularly in soya-bean oil,[2, 3] and its synergistic function with true antioxidants has been also studied quite extensively.[4, 5] Not much has been published on the applicability of citric and similar acids as sole stabilizing agents for animal fats since they were found by Olcott & Mattill[6] to have but little effect in lard. However, the low stability of animal fats makes even a small improvement desirable ; moreover, some of this writer's recent experiments seemed to indicate that Olcott & Mattill's results might not be conclusive. Apart from this fundamental issue there is the question of a convenient method of incorporating citric acid into fats, in which medium it is only sparingly soluble. According to Lundberg[4] its solubility in lard at 50° is probably less than 0·005%. The usual method of adding citric acid to fats, as an aqueous solution, followed by dehydration *in vacuo*, does not offer any difficulty in a vegetable-oil refinery, but is often impracticable in a tallow- or lard-rendering plant. A further difficulty arises from the observation[7] that in the presence of an aqueous phase citric acid might act as a pro-oxidant with animal fats if its concentration in that phase amounts to 2% or more. An investigation has therefore been carried out in this laboratory with the object of re-examining the stabilizing effect of citric and related acids on animal fats such as tallow and lard, and—should the results be promising—of solving the two difficulties mentioned above.

Experimental

Of the three acids in question, i.e. citric, tartaric and phosphoric, the last was discarded on account of its possible corrosive action on iron storage-tanks. The stabilizing effect of the two other acids and their sodium salts was tested by adding them in the form of an aqueous solution (0·01% on the fat basis) to samples of commercial edible beef and mutton tallow and lard, and determining the induction periods of the fats by aeration at 100° (active-oxygen method). A distinctly rancid odour was regarded as denoting the end-point of the induction period which usually coincided with peroxide values of approximately 10 millimoles of peroxide per 1000 g. of fat. Results of some of these experiments are shown in Table I. The stabilizing effect was found to decrease with the diminishing acidity of the various sodium salts, as would be expected, but in some of the samples examined was quite appreciable for free acids.

Table I

Effect of the addition of 0·01% of citric and tartaric acids and their salts on induction periods of various fats

Fat	Induction period at 100° (hours)							
	Control	Citric acid	Mono-sodium citrate	Disodium citrate	Tri-sodium citrate	Tartaric acid	Mono-sodium tartrate	Disodium tartrate
Beef I	3	12	9	5	4	12	11	4
,, II	6	18	—	—	—	17	—	—
,, III	7	13	—	—	—	14	—	—
,, IV	10	14	—	—	—	14	—	—
Mutton I	2	7	6	3	2	8	7	3
,, II	3	10	—	—	—	11	—	—
,, III	5	12	—	—	—	12	—	—
,, IV	10	15	—	—	—	15	—	—
Lard I	4	10	9	5	4	11	9	4
,, II	6	12	—	—	—	12	—	—

This encouraged the search for a method of increasing the low solubility of citric acid in fats. The use of monoalkyl and alkylene esters of citric acid, such as *iso*propyl, octyl and oleyl,[8] was considered not entirely satisfactory, since even in conjunction with solubilizing agents such as free fatty acids a dispersion, but not a solution, of these esters in fats could be achieved. An attempt was made therefore to introduce citric and tartaric acids into fat molecules by treating the former with technical monoglycerides. Success was achieved with citric acid[9] and the technique finally adopted is illustrated by the following example:

Edible beef tallow (500 g.) was heated with 18·5% of glycerol and 0·1% of sodium hydroxide as catalyst to 250° in a nitrogen atmosphere until the mixture became clear, and held at this temperature for half an hour. After cooling to 120° in nitrogen, 5 ml. of aqueous solution of citric acid (1 : 1) was added to decompose the catalyst and the free glycerol was removed by three washings with a saturated solution of sodium sulphate, maintaining the temperature just below 100°. This step proved to be essential, as otherwise glycerol reacted with the subsequently added citric acid with the formation of oil-insoluble products. To the resulting mixture of mono-, di- and tri-glycerides with an acid value of 1·9, 100 g. of powdered citric acid was added and the temperature raised to 130° while stirring and passing through nitrogen. After approximately one hour the mixture became clear with some residue (9·5 g.) left on the bottom, and after cooling was ready for use. The final product had an acid value of 89, a glassy appearance and a distinctly acid taste, and was freely soluble in alcohol and light petroleum.

By varying the amount of glycerol and citric acid a series of products with acid values ranging from 40 to 100 were obtained. Monoglycerides from various animal and vegetable fats reacted with up to 20% of citric acid, forming products that were soluble to the extent of 1–2% in the original fats. Usually, however, a fraction of this amount was found sufficient for stabilizing purposes (cf. Table II).

Table II

Stabilizing effect of mixed glyceryl esters of citric acid and fatty acids on various fats (acid value of esters, approximately 90)

Fat	Induction period at 100° (hours)		
	Control	0·1% of stabilizer	1% of stabilizer
Beef I	3	11	12
,, IV	10	14	19
Mutton I	2	7	8
,, IV	10	14	15
Lard I	4	10	10

To test the stabilizing effect of these mixed glycerides of citric acid and fatty acids in the presence of an aqueous phase, samples of beef and mutton tallow after addition of 0·2–2% of the above compounds were emulsified with 20% of water, spread on glass in a 1-mm. layer and kept at 30°. Controls were run under similar conditions and acquired a rancid odour and flavour and blanched appearance usually about twice as rapidly as the stabilized samples. Peroxide values determined periodically by Lea's 'cold' method[10] also indicated in each case a stabilizing action of the mixed glycerides. A few typical results are shown in Table III.

Table III

Stabilizing effect of mixed glyceryl esters of citric acid and fatty acids on fats emulsified with 20% of water

Fat	Peroxide values (millimoles of peroxide per 1000 g. of fat)						
	0 days	7 days			14 days		
	Control	Control	0·2% of stabilizer	2% of stabilizer	Control	0·2% of stabilizer	2% of stabilizer
Beef I	0·2	8·0	2·1	1·8	25·5	10·3	9·8
,, IV	0·3	4·2	0·5	0·5	11·9	4·5	3·0
Mutton I	0·8	12·6	3·0	3·5	31·2	13·0	11·6
,, IV	0·0	5·7	1·1	0·9	15·4	6·2	4·6

Tartaric acid was found to react with monoglycerides with difficulty. The quantity of it that would form mixed esters never exceeded 5% and the acid values of these esters were below 20. It was shown many years ago[11] that glycerides of phosphoric acid are unstable and that, on standing, they lose most of this acid with the formation of neutral esters.

Discussion

The increase in stability produced in animal fats by the addition of citric and tartaric acids was, according to Table I, on the whole greater than that observed by Olcott & Mattill.[6] Protection factors found by these workers (expressed in the form of a ratio of induction periods with and without added stabilizers) were about 1·5, whereas factors calculated from Table I range from 1·5 to 4. The likely explanation is that Olcott & Mattill used lard of high quality and that the increase in stability reported in the present work (in which other animal fats, besides lard, were used) was especially pronounced in fats with short induction periods. This, together with recent findings on metal deactivation in lard,[12] seems to indicate that, whereas citric acid can produce both a synergistic and a metal-deactivating effect,[13] the synergistic effect is not pronounced in animal fats. (No definite proof of this is available since no correlation between trace-metal content and induction periods could be detected, but it is reasonable to assume that the form in which metals are present is more important than their quantity.) Nevertheless the regular use of citric or similar acids in animal fats appears advisable if only to counteract the effects of a fortuitous metal contamination during the manufacturing process and storage. The effectiveness of citric and tartaric acids is about the same, but citric acid might be the less toxic of the two.[14] In this instance it is worth mentioning that the legal position in the U.K. concerning the use of the two acids in edible fats is not entirely clear. Their addition is certainly not directly permitted, and could even raise questions of adulteration, of not being 'of the substance and quality demanded' etc., unless declared by labelling or similar means. It is, however, unlikely that any legal objections could be raised against their use on grounds of toxicity.

To facilitate an adequate incorporation of citric acid into fats the use of mixed glycerides of this acid and fatty acids is suggested. These products form true solutions with fats and are effective stabilizers in the absence and in the presence of an aqueous phase. Although intended primarily for animal fats they might be found suitable also for vegetable fats, such as ' virgin ' olive oil, which are not subjected to a refining process.

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HARVESTING HYBRID AND INBRED SWEET-CORN SEED IN ENGLAND

By GORDON HASKELL

Sweet corn is increasingly grown for seed in England. As the seeds are unusually susceptible to fungal diseases, especially if left too long in the ear, a better understanding is required of the relationship between seed harvesting and climatic conditions. Correct harvesting is essential for the different varieties grown in England, since they react differently to English climatic conditions. A study was therefore made of growing, harvesting, drying, threshing and storing hybrid and inbred sweet-corn seed in England.

Inbred C 13 and its hybrid with Extra Early Bantam (John Innes Hybrid No. 1) were sampled between 12 September and 26 November, 1951. Harvestings on 23 October and 5 November gave the heaviest yields of hybrid, and on 5 November of inbred. Mature hybrid seeds were heavier than inbreds, the heaviest individual seed-weight of inbreds being on 8 October, and of hybrids on 23 October. Seed quality and appearance were best on 8 October for inbred and 23 October for hybrid, which could be harvested over a longer period without deterioration. Harvestings of 8 and 23 October gave most viable hybrid seeds, and inbred seeds from 24 September germinated best in the glasshouse, but inbreds from 23 October gave best germination outside. This indicates better adaptability of hybrid seeds. Seedling vigour is associated with harvesting date; inbreds were weaker and wilted more than hybrids. These results are discussed in relation to climatic conditions at the time of the experiment.

Introduction

Until a few years ago, sweet-corn seed requirements were satisfied by imports from the U.S.A., but it is now necessary to grow the seed in the U.K. This is due partly to restrictions on dollar imports and partly to increased demands in England for seed of varieties adaptable to English growing conditions.[1] Sweet corn is extremely sensitive to low soil-temperatures at germination and to effects of changes in day-length ; hence only comparatively few American varieties have proved acceptable. The open-pollinated varieties originally grown in England from imported seeds included those like Golden Bantam and Country Gentlemen ; these have been superseded in America. Stocks of these are maintained by allowing plants to interpollinate each other naturally, removing off-type plants before they shed pollen, and then bulking the seed. Generally the germination of these varieties is poor, especially if sown too early, and the plants are often late in producing ripe ears for market.

Because of the advantages of hybrid vigour for improving germination, uniformity and other properties, these American varieties have now been replaced by hybrid varieties, such as the John Innes (J.I.) Hybrids Nos. 1 and 2. These are maintained by cross-pollinating a selected variety, itself separately continued as an open-pollinated variety, with an inbred or pure-line variety. Stocks of this are produced by artificially self-pollinating individual plants from time to time, and then bulking the seed. The parental combinations for producing a commercial hybrid have been carefully chosen not only for the quality of their hybrids, but also because hybridization is comparatively easy. Both parents must overlap in flowering times, and they are themselves easily maintained separately in England. Although these hybrid varieties readily produce marketable ears, harvested shortly after seeds are pollinated and while they are still at the soft milk stage, little is yet known of their properties in grain harvesting. Moreover, owing to the sensitivity of sweet corn to variations in climate, any study of harvesting must be assessed in terms of climatic conditions.

Previous investigators in England have studied sweet corn as a vegetable crop,[1, 2] seed germination at low temperatures [3] and frit-fly attacks.[4] But in addition to growing an introduced crop successfully it is important to know whether the seeds can be produced locally, especially when economic factors restrict seed importations. So far only cross-pollination and seed-setting in small hybrid-seed plots have been investigated,[5] yet sweet-corn seed stocks have been grown in Surrey, Hertfordshire and Suffolk for several years. These include the varieties Extra Early Bantam (E.E.B.) and Canada Gold, inbred C 13 and its top-crosses with E.E.B. and Canada Gold, known as J.I. Hybrid No. 1 and No. 2 respectively. There have been no previous investigations on seed harvesting and handling in England, and these subjects are omitted from the Ministry's Advisory Leaflet.[6]

It was hoped to observe experimental seed plots for several years, but this has been impracticable. However, the 1951–52 experiments are considered worth recording since they show clearly some of the important points of sweet-corn seed production in relation to climatic conditions in England. Because the seed parents of the J.I. Hybrids behave very similarly in England,[1] these results are applicable to both varieties and their top-crosses with C 13, even though the experiments were concerned only with seed production of J.I. Hybrid No. 1, namely E.E.B. × C 13.

Materials and methods

The hybrid-seed isolation plot comprised six pairs of rows, at 2 ft. apart, of E.E.B. alternating with C 13, used as pollen parent, which was also planted at each end. There were 25 plants per row at 1 ft. apart ; the plot measured 52 ft. × 25 ft. Seeds were sown on 4 May, 1951, in soil ploughed 9 in. deep which was previously under vegetables. The plot in 1950 received superphosphate and sulphate of potash, 5 cwt. of each per acre ; no fertilizers were given in 1951.

The E.E.B. was detasselled by pulling out male inflorescences as they appeared, so all pollen came from C 13. This began shedding pollen slightly ahead of protrusion of silks on E.E.B. ears, but there was sufficient pollen because seed setting was good. There were six harvestings—on 12 and 24 September, 8 and 23 October, 5 and 26 November. The first was approximately at the stage when ears would have been taken for eating (soft milk stage), and the second somewhat after the best stage for eating (doughy stage). The last two were well after winter conditions had set in, and long after best conditions for harvesting. Fig. 1 gives the meteorological data for most of the experiment and the conditions obtaining when ears were picked.

One ear from each pair of E.E.B. and C 13 rows was taken at random, excluding those on end plants. Ears were immediately husked, placed in porous fine-canvas bags, and hung to dry in a glasshouse. Table I gives temperatures while ears were drying. The six ears from each sampling were threshed by hand, pooled and weighed on 8 December ; ears were then dry and their seeds were easily rubbed off the cobs. Weighings were made on three lots of 100 seeds per sample. Threshed seeds were stored in stout brown-paper bags in a tin at room temperature.

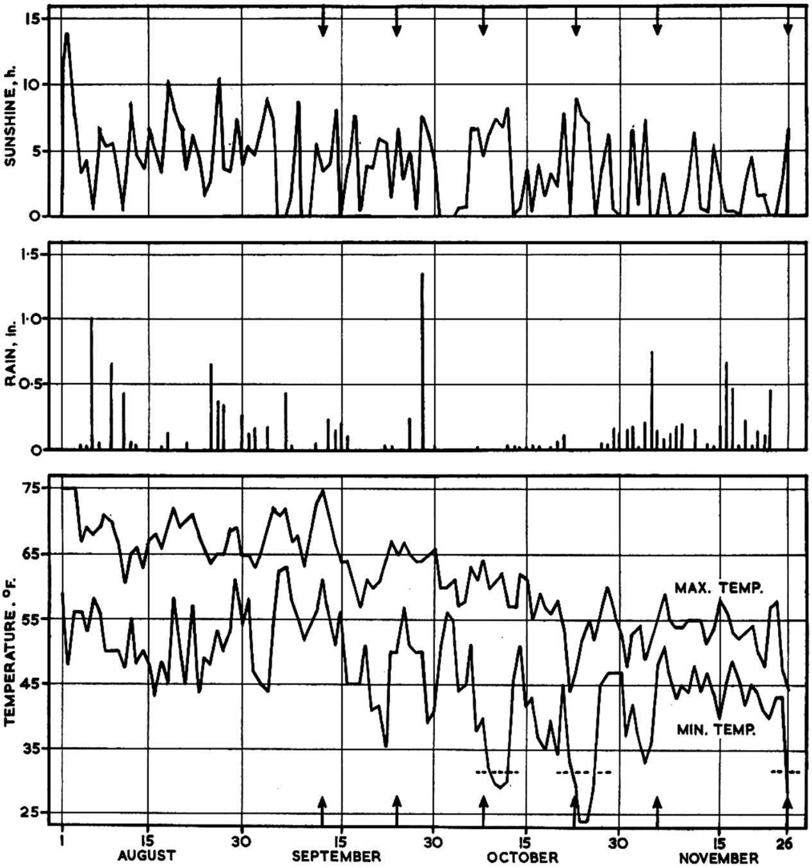

FIG. 1.—*Meteorological conditions at Hertford,* 1951, *during harvesting trials of sweet-corn hybrid and inbred seed. Arrows indicate dates when samples of ears for grain were taken*

Greenhouse germination tests in sterilized J.I. seed compost were initiated on 31 December. There were 4 random plots, each containing 6 samples for hybrid and inbred testing. Fifty seeds of each were sown and kept for 6 days at 50–56° F before transference to a warmer greenhouse at about 60° F. Records were taken of date of first emergence, total germinated and number of seedlings wilted. Wet weights were determined 30 days from sowing.

Table I

Temperature in glasshouse during drying-out of inbred and hybrid sweet-corn seed

	8 a.m. temp., ° F		Minimum temp., ° F	
Date, 1951	Mean	Range	Mean	Range
12–23 Sept.	64·3	57–72	57·8	47–69
24 Sept.–7 Oct.	66·2	56–75	61·0	52–68
8–22 Oct.	61·1	56–74	54·6	47–63
23 Oct.–4 Nov.	57·9	52–64	51·8	44–60
5–27 Nov.	55·0	47–62	51·5	44–57

Field germination tests were made from 8 May until 30 May, 1952, when emergence ceased; the meteorological conditions are given in Table II. The same experimental layout as for

Table II

Meteorological conditions during field germination tests, 8–19 May, 1952

Maximum temp., °F		Minimum temp., °F		Total rainfall, in.	Daily sunshine, h.	
Mean	Range	Mean	Range		Mean	Range
68·0	57–80	45·2	42–50	0·58	7·8	2·1–12·0

greenhouse germination tests was used, with 50 seeds per row sown singly 6 in. apart in rows 2 ft. apart. The soil had previously been ploughed to a depth of 9 in. and had received farmyard manure at 10 tons per acre. Dates of first emergence and maximum germinations were recorded. These results are presented in Table III.

Table III

Comparison of sweet-corn hybrid and inbred samples

		Harvesting date, 1951					
		12 Sept.	24 Sept.	8 Oct.	23 Oct.	5 Nov.	26 Nov.
Dried seed yield, oz. per 100 ears	Hybrid	92	154	138	229	242	175
	Inbred	113	113	150	129	175	113
Wt. of 100 seedlings, oz.	Hybrid	2·2	2·9	2·9	3·3	3·4	3·3
	Inbred	1·7	2·6	2·2	2·3	2·2	2·0
1000 seed wt., oz.	Hybrid	4·7	7·5	7·7	9·7	9·1	8·2
	Inbred	5·3	7·2	8·2	7·8	7·3	7·3
% of germinating seeds	Hybrid in field ..	86	88	92	92	66	52
	,, ,, glasshouse	73	86	94	91	69	56
	Inbred in field ..	77	84	77	85	45	37
	,, ,, glasshouse	43	92	65	58	46	40
% of wilting seedlings	Hybrid	13	2	0	2	4	5
	Inbred	36	8	8	20	9	11

Climatic conditions

Climatic conditions when seed was harvested at Hertford in 1951 are given in Fig. 1. Before the harvestings made on 12 September there were high temperatures, plenty of sunshine and some rain; these factors help ear ripening and seed maturation. Before 24 September there was still plenty of sunshine but less rain and minimum temperatures were dropping. By 8 October both maximum and minimum temperatures were dropping, sunlight was fluctuating, yet except for one heavy precipitation there was little rainfall. Frosts occurred after 8 October but sunlight was still good and there was low precipitation until 23 October. From then on, maximum and minimum temperatures continued to drop, sunlight became sparser and precipitation increased. These conditions are unfavourable to good seed production. It is therefore seen that, after 8 October, harvestings were liable to frosts, heavier rainfall and less sunshine. Hence conditions for seed harvesting at Hertford in 1951 were most favourable up to early October.

Results

(i) *Seed yield.*—Hybrid-seed yield steadily increases with later harvestings until a peak is reached by 5 November, with a rapid increase between 8 and 23 October. Inbred yield also shows a rise until 5 November. Both harvestings made on 26 November show considerably decreased yields, probably owing to seeds' being diseased and decayed. Yields are similar between 12 September and 8 October, but eventually hybrid gives higher yields than inbred.

(ii) *Seed weight.*—Individual seed weight is also important as sweet-corn seed is usually sold by weight. At first both have similar seed weights but eventually hybrid weighs more than inbred. Hybrid seed increases in seed weight till harvested on 23 October, after which it declines. The inbred reaches its peak in seed weight by 8 October, thus maturing earlier than the hybrid.

(iii) *Seed quality.*—The changes in appearance of the samples with progressive harvestings are given in Table IV. At first, seeds of hybrid and inbred are under-developed, but are in good marketable condition when harvested from 24 September to 23 October. Hybrid seed from 23 October and inbred seed from 8 October are best ; thus C 13 ripens up to 2 weeks ahead of E.E.B. or its hybrid. Seeds in the November batches are unsuitable for market, being either diseased, broken or shrivelled.

(iv) *Germination.*—Behaviour of hybrid seed is similar for both glasshouse and field conditions, germination being best from harvestings of 8 and 23 October, after which it rapidly becomes less good. Earlier harvestings germinate only slightly less satisfactorily than those from optimum dates, although seeds are much lighter. Germination of later harvestings is poorer than decrease in seed weight warrants ; other factors, such as fungal attacks, may be the cause. Glasshouse and field results for C 13 are not in such agreement. Germination under sterilized conditions is best from the harvestings of 24 September, afterwards becoming steadily poorer, but in the field comparatively high numbers of germinations occur for seed from 12 September to 23 October harvestings, after which germination becomes rapidly less satisfactory.

Table IV

Dried sweet-corn seed quality after harvesting at Hertford, 1951

Date harvested	J.I. Hybrid (E.E.B. × C 13)	Inbred C 13
12 Sept. ..	Small and shrivelled but fresh appearance ; not suitable for marketing	Seed clean but included many undeveloped ; not suitable for marketing
24 Sept. ..	Good commercial appearance	Fairly good commercial appearance, some shrivelled seed
8 Oct. ..	Good commercial appearance	Good commercial appearance but undeveloped seeds occur
23 Oct. ..	Excellent commercial appearance	Good commercial appearance, with some undeveloped seeds
5 Nov. ..	Rather poor appearance, some attacked by fungi ; still marketable	Poor appearance, diseased and discoloured pink by fungus ; not suitable for marketing
26 Nov. ..	Very poor appearance, shrivelled and discoloured black by fungus, pericarps sometimes broken ; not suitable for marketing	Diseased and discoloured pink by fungus, shrivelled and pericarps often burst ; of no commercial use

The length of time before first emergence is not related to harvesting date. In the glasshouse at about 68° F the first seedlings in all pans appear either 9 or 10 days from sowing, but in the field all have started emerging within 11 days. Inbred and hybrid seed show no differences in this respect.

(v) *Seedling vigour.*—This is important, especially when environment is not optimum for germination. Without allowing for competition effects due to variation in numbers in each pan, the seedling weights, 30 days after sowing, show that there is a steady increase in vigour of hybrid seedlings with progressive harvesting until the 5 November harvesting is reached. The most vigorous inbred seedlings are those from the 24 September harvesting ; later ones show no increased vigour. Both types of seedlings from earliest harvestings have little vigour, perhaps because of incompletely mature endosperms, suggested by low seed-weights.

Table V shows the percentage of wilting among seedlings germinated in J.I. compost. On the average, wilting occurs twice as often among inbreds than among hybrids ; this may be associated with reduced vigour or a gene for susceptibility. Hybrid seedlings from harvestings of 24 September to 23 October show little wilting, but then there is a slight increase. Wilting of inbreds is also lowest for harvestings on 24 September and 8 October but that for 23 October unexpectedly shows increased wilting. Wilting in the inbred is generally high irrespective of harvesting date.

Problems of harvesting and storage

Both hybrid and inbred varieties took over two months from the time of first sampling to reach the heaviest seed-yields, which they did simultaneously. They differed little in yield until after 8 October, when the hybrid gained considerably. The inbred increased only slightly; this was not unexpected as open-pollinated or hybrid maize usually gives higher yields than inbred. At first, while seeds were ripening, inbred and hybrid seed weights were the same, but hybrid seeds were heavier from harvestings later than 23 October. This date was therefore crucial in hybrid-seed development, being slightly later than for inbred seed. This may be due to the inbred's being earlier-maturing through selection, rather than to an inbred–hybrid relationship. Kiesselbach[7] found no differences due to influence of seed maturity on yield of crops grown from them.

Table V

Wilting of inbred and hybrid sweet-corn seedlings germinated in J.I. seed compost

Seed harvesting date	Per cent. of wilting seedlings	
	Hybrid	Inbred
12 Sept.	13	36
24 ,,	2	8
8 Oct.	0	8
23 ,,	2	20
5 Nov.	4	9
26 ,,	5	11
Mean	6	15

Harvesting is desirable when individual seed weight is at its maximum, as sweet-corn seed is sold by weight, but selling price is also correlated with appearance and much is lost in cleaning if seeds are too light, broken or diseased. Hybrid seeds had better marketable appearance than C 13 from a wider range of harvestings. This means taking precautions to catch a C 13 crop before its seeds deteriorate, especially as inbred germination is not so good as hybrid germination under English field conditions.

Sweet corn cannot be sown earlier than May, as most varieties germinate poorly in England.[1] John Innes Hybrid No. 1 is better in this respect and, as Table III shows, if harvested correctly and not sown too early 90% of fresh seed germinates; even C 13 gives a germination of over 80% if carefully harvested and dried. Harvesting time clearly affects germination, which falls off rapidly after the optimum date. Seeds from first pickings germinated better in the field but the percentages of glasshouse germinations from very late harvestings of both strains were slightly higher than those outdoors. This was perhaps due to fungi acting less detrimentally when seeds were in warmer, more sterile conditions, or to lack of rain during field germination tests (Table II). The germination characteristics of inbred C 13 varied with sowing conditions, whereas those of the hybrid were relatively stable. Hybrid maize has recently been found to be more adaptable than inbred to wider differences in environment. Hence seed tolerance is important when attempting to establish inbreds and hybrids in unsuitable surroundings.

Wilting in seedlings may be due to some defect of their root development. Possibly the high proportion of wilting seedlings from very early harvestings may result from some degree of immaturity of the embryo and a reduced amount of endosperm, making for slower root development and easier parasitic attack.

Ear samples from late milk stage to mature condition hardly affected germination in Nebraska,[7] but there was a steady increase in immediate seed-yield, as found in the present experiment. Frost injury to viability and moisture content of seeds are highly correlated; hence ears should be harvested before danger of frosts. Hybrid seeds harvested on 23 October must have been sufficiently mature to avoid frost injury. Husking the ears immediately after harvesting helps seeds to dry out more rapidly,[8] and to reduce fungal attacks during drying.

Plants should not be left unharvested longer than necessary, as several animals attack ears in the field. Rodents such as squirrels gnaw them and do considerable damage; birds, such as sparrows and pheasants, peck at the seeds, especially those at the tips. This is common when ripening yellow seeds are exposed, which may be a varietal characteristic. With Extra Early Bantam and Canada Gold it more likely results from pulling back husks to see if seeds are ready for harvesting. Birds soon spot ears with disturbed husks; these should always be carefully replaced. Ears attacked by smut, which is uncommon in England, are usually swollen, and individual seeds full of black spores may be enlarged to the size of small eggs. Such ears should be discarded to help control the disease, even though some seeds appear satisfactory.

It is considered advisable in America to dry all maize and sweet-corn seed artificially; great pains are taken over this. However, such measures have not been necessary with the seed crops that the author has obtained in England. Three harvesting methods have been used, according to the size of the crop. For large quantities, husked ears are spiked on nails projecting upwards from a wooden frame, and dried out slowly in a barn. This allows free circulation of air currents which help to evaporate moisture from the seeds; otherwise, ears remain damp and fungi spoil them. Where glasshouse space is available husked ears can be placed on trestle tables, which also allow free circulation of air. Ears are then turned every few days so that moisture evaporates evenly from them. Mice are liable to cause great damage unless controlled. For small quantities of stock seed, or for breeding experiments with pedigree material, fine-canvas bags containing ears can be hung up in the glasshouse kept at about 65° F, but having too many ears in one bag may result in spoilage by fungi. This method has the advantage that the ears are not so accessible to mice.

Maize seeds are graded by size in the U.S.A. to facilitate mechanical sowings. This is not required with sweet corn that is to be hand-sown. Flatter seeds from the more central parts of cobs are best for market. Seeds from the lower ends of ears may be removed for mechanical reasons or quality standards rather than from deleterious effects on germination. When hand-threshing, chaff is easily removed by blowing; sweet-corn seed sold commercially should be completely free from chaff and debris. The threshed and cleaned seeds can be held in sacks or in stout brown-paper bags conveniently kept in surplus army metal storage containers (19 × 12 × 7 in.), which should be stored in a cool, dry place. Seeds sprinkled with 5% DDT dust will escape attacks by moths during storage; they are viable for five or more years under these conditions.

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THE COMPOSITION OF THE BLUBBER FAT OF CRABEATER SEAL

By G. WINTER and W. NUNN

The composition of the blubber fat from a male crabeater seal has been determined. In general the composition is similar to that of the fat of other seals.

The crystallization procedure in which methyl esters are used has been improved, and the efficiency of separation is now equal to that obtainable with fatty acids. The use of a rotating-strip fractionation column has almost eliminated the formation of tarry residues in the fractional distillation.

In previous papers we have given the compositions of blubber fats from elephant seal[1, 2] and a leopard seal.[3] An important seal species of the southern hemisphere is the crabeater seal, *Lobodon carcinophagus*, which is found mainly on the antarctic pack ice. It is one of the larger seal species, although the males are considerably smaller than those of the elephant seals. Its diet is reported to consist entirely of Euphausiids. A detailed study of its natural history has been made by Bertram.[4]

Experimental

Although rarely seen at Heard Island a young male crabeater seal, 5 ft. 10 in. long, was obtained there on 13 August, 1949, by officers of the Australian National Antarctic Research Expedition. A five-pound sample of the blubber taken from the belly was stored under acetone in a sealed container until it was brought to Melbourne. The slurry obtained on passing the blubber sample through a mincer was combined with the acetone solution and the acetone removed by evaporation. Ether was then added, the solution dried over sodium sulphate and filtered. Evaporation of the ether yielded a clear yellow oil which had the following constants:

Iodine value (Wijs 1 h.)	165·3	Acid value, mg. KOH/g.	1·7
Saponification value, mg. KOH/g. ..	190·4	Unsaponifiable matter, %	0·4

The methyl esters obtained by methanolysis of the fat were separated into concentrates of highly unsaturated, medium unsaturated and saturated esters by low-temperature crystallization from acetone solution. It has already been pointed out by us[1] that there are certain advantages in working with the methyl esters rather than the fatty acids, which have to be methylated before distillation. In the crystallization procedure adopted by us initially,[1] the separation obtained was less efficient than that obtained by Hilditch & Pathak,[5] who used fatty acids. In later work[2] we improved the separation of the methyl esters, and the separation obtained by the procedure now described is similar to that obtainable with fatty acids. It was found that poor separation was due mainly to difficulty in filtration and washing of the portion that was precipitated at − 50°, which includes the major portion of the methyl oleate. At − 45°, however, the methyl oleate remains in solution and no difficulty in filtration is encountered. The methyl oleate is separated from the highly unsaturated esters at − 80°, when it crystallizes in a readily filterable form. Details of the method adopted in the analysis of the blubber fat of the crabeater seal and of the blubber fats from a number of elephant seals reported in the next paper are as follows:

The methyl esters (400 g.) dissolved in acetone (4 l.) were submitted to a preliminary crystallization at − 45°, to facilitate filtration of the precipitate after the crystallization at − 80° to which the filtrate was subsequently submitted. The combined precipitates (at − 45 and − 80°) were dissolved in acetone (100 g./l.) and cooled to − 45°. The insoluble portion from this crystallization was again dissolved in acetone (100 g./l.) and crystallized at − 20°. All crystallizations were placed overnight (approximately 16 hours) in a thermostatically controlled cabinet described by Cook,[6] except the crystallization at − 80° when the vessel was packed in solid carbon dioxide for 16 hours. The iodine values of the fractions obtained are shown in Table I.

Table I

Iodine values of fractions obtained by low-temperature crystallization

Fraction	Wt., %	Iodine val.
Soluble − 80°	45·3	248·2
Soluble − 45°	38·1	129·9
Soluble − 20°	8·4	24·6
Insoluble − 20°	8·2	2·4

Each portion was then submitted to fractional distillation at 1 mm. pressure. In previous work[1, 2] we employed a packed column of design similar to that described by Longenecker.[7] Such a column has a considerable back-pressure, causing changes in the more highly unsaturated esters that result in the formation of tarry residues. We have now replaced the packing of the column by a 3-ft. stainless-steel gauze strip rotated at approximately 2000 r.p.m. by a motor sealed to the top of the column. The gauze strip is a sliding fit in the column. With this arrangement even the most highly unsaturated esters were substantially unaffected, the saponification equivalents of the residues being either equal to or only slightly higher than those of the last fractions distilled. Typical fractionation data as obtained with this column are shown in Table II.

From the saponification equivalents and iodine values of the various fractions the composition of the fat was calculated in the manner outlined by Hilditch.[8] The results are shown in Table III.

Table II

Fractionation data of portion soluble at − 45°

Fraction No.	Wt., g.	Saponification val.	Iodine val.
491	2·12	246·4	59·8
492	3·06	256·6	69·3
493	2·94	268·1	83·6
494	4·72	278·5	93·9
495	4·22	281·9	94·9
496	3·44	282·7	96·3
497	4·64	289·5	97·1
498	7·81	295·0	105·8
499	8·12	295·8	107·7
500	8·18	296·0	113·7
501	5·31	299·8	114·3
502	4·28	304·0	155·3
503	3·57	316·8	237·2
504	3·13	332·5	308·0
Residue	3·51	336·5	301·3

Table III

Composition of crabeater-blubber fat (percentage by weight)

	IS–20	S–20	S–45	S–80	Total (esters)	Fatty acids	
						% (wt.)	% (mol.)
Myristic	1·0	2·8	0·6	0·3	4·7	4·7	5·7
Palmitic	6·0	3·2	0·9	—	10·1	10·1	10·9
Stearic	1·0	0·4	0·7	—	2·1	2·1	2·0
Unsaturated*							
C_{14}	—	0·1 (−2·0 H)	1·0 (−2·0 H)	2·1 (−2·0 H)	3·2 (−2·0 H)	3·2	3·9
C_{16}	0·1 (−2·0 H)	0·5 (−2·0 H)	6·3 (−2·0 H)	12·9 (−2·4 H)	19·8 (−2·3 H)	19·8	21·5
C_{18}	0·1 (−2·0 H)	0·7 (−2·0 H)	22·0 (−2·6 H)	7·8 (−4·4 H)	30·6 (−3·0 H)	30·3	29·7
C_{20}	—	0·7 (−6·1 H)	4·4 (−6·3 H)	13·9 (−8·6 H)	19·0 (−7·9 H)	19·2	17·4
C_{22}	—	—	2·2 (−10·0 H)	8·3 (−11·0 H)	10·5 (−10·8 H)	10·6	8·9

* Mean unsaturation shown in parentheses

Discussion

From the data recorded in Table III it can be seen that good separation of the mixed methyl esters was obtained by the modified crystallization procedure. In the least soluble portion (insoluble at − 20°) 60% of the total palmitic ester was concentrated; 60% of the myristic ester appeared in the portion soluble at − 20° (insoluble at − 45°) and 72% of the unsaturated C_{18} esters (mainly oleic) were contained in the portion soluble at − 45° (insoluble at − 80°). The most soluble portion (soluble at − 80°) contained most of the unsaturated C_{16} esters (65%), the more highly unsaturated C_{18} esters and the highly unsaturated C_{20} and C_{22} esters.

The composition of the blubber fat from this specimen of crabeater seal is in general similar to that of most other seal-blubber fats, except for the presence of somewhat larger proportions of C_{16} acids. In this respect it resembles the blubber fat of the common seal examined by Hilditch & Pathak.[9]

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THE COMPONENT FATTY ACIDS OF ELEPHANT-SEAL OIL: VARIATIONS AND RELATIONSHIP TO BLUBBER FATS OF OTHER SEALS

By G. WINTER and W. NUNN

The work reported deals with the variations in composition of seal-blubber fats in general and with that of elephant-seal blubber in particular. The iodine values of the blubber fats of elephant seal varied between 98 and 153. Although there appears to be a tendency for the iodine value to be higher during the winter months, the results of detailed analyses failed to show any correlation of the fat composition with seasonal changes or the physiological state of the animal. The compositions of the blubber fats from other seal species are similar to those observed for the elephant seal, indicating that no distinct feature of blubber-fat composition can be attributed to any one species.

The results presented show that the saturation and desaturation processes during the depot-fat metabolism are in the main confined to acids of chain length greater than C_{18}.

The distribution of the unsaturated fatty acids in seal-blubber fats is similar to that in whale-blubber fats.

In recent years the appearance in the literature of several detailed analyses of seal-blubber fats has revealed certain unexpected variations for which no satisfactory explanation has yet been given. In 1944 Burke & Jasperson[1] reported the composition of the blubber fat from a Newfoundland seal, and later Hilditch & Pathak,[2] when reporting the composition of grey-seal blubber fat, drew attention to the large differences between these two seals in the fatty acid distribution. They offered the suggestion that the difference could be due to differences in species or diet. Doubt was cast on this suggestion, however, when later Hilditch & Pathak[3] noted distinct differences in the composition of the blubber fat from two common seals, and the present authors[4, 5] reported large variations in the composition of blubber fats of elephant seals. As the Australian National Antarctic Research Expedition was studying the habits of the elephant seal and was able to supply us with fully identified specimens of blubber fat, we were prompted to investigate the problem further. We have now examined the blubber fats from a number of male and female animals, ranging from young pups to adults, taken at various seasons at Heard Island and Macquarie Island.

Experimental

After determination of the chemical constants the fats were analysed by the methods described in the preceding paper.[6] The method involves methylation, preliminary separation by low-temperature crystallization and fractional distillation through a 3-ft. rotating-strip column.

Tables I and II show the biologists' observations, together with the results of the chemical examination of the blubber fats. For comparison, results previously reported by us have been included. All compositions are reported as molecular percentages rather than percentage by weight, since this provides a clearer picture of the fatty acid distribution.

Table I

Elephant-seal oils from Macquarie Island

Specimen No.	15	16	17	20
Date killed	29/7/48	30/7/48	12/9/49	21/9/49
Description	Mature male	Mature female	Mature male	Mature female
Estimated age	2–3 years	2 years	—	—
Length	9 ft. 1 in.	7 ft. 8 in.	15 ft. 4 in.	9 ft. 1 in.
Blubber thickness, in. (belly)	—	—	4·7	4·3
Notes		Not pregnant		Pregnant
Blubber fat:	Belly	Belly	Neck	Belly
Iodine value	136·4	152·9	105·0	117·6
Sap. value	192·1	191·7	185·6	186·2
Acid value	1·5	1·1	0·5	0·4
Unsap., %	0·6	0·6	0·3	0·5
Acid composition, % (mol.)				
Lauric	0·4	0·1	—	—
Myristic	6·3	5·4	2·1	2·1
Palmitic	12·4	12·6	8·2	10·8
Stearic	2·4	2·4	3·9	3·4
Arachidic	—	—	0·6	0·2
Behenic	—	—	—	—
Unsaturated*				
C_{14}	2·1 (−2·0 H)	1·6 (−2·0 H)	0·6 (−2·0 H)	0·8 (−2·0 H)
C_{16}	15·9 (−2·1 H)	14·4 (−2·1 H)	8·2 (−2·0 H)	10·9 (−2·0 H)
C_{18}	34·6 (−2·7 H)	32·9 (−2·4 H)	39·4 (−2·1 H)	35·1 (−2·3 H)
C_{20}	17·0 (−5·4 H)	18·9 (−5·9 H)	25·3 (−3·3 H)	24·0 (−4·0 H)
C_{22}	8·9 (−10·0 H)	11·7 (−10·8 H)	11·0 (−5·7 H)	12·2 (−6·5 H)
C_{24}	—	—	0·7 (−2·0 H)	0·5 (−2·0 H)

Specimen No.	26	31	33	35
Date killed	26/10/49	13/11/49	8/1/50	22/2/50
Description	Male pup	Female pup	Male pup	Mature male
Estimated age	—	—	—	—
Length	4 ft. 9 in.	5 ft.	5 ft. 1 in.	14 ft. 2 in.
Blubber thickness, in. (belly)	2·4	2·6	1·7	3·4
Notes	Soon after leaving mother, almost completed moult	Just before leaving mother	End of starvation period	Half-way through moult
Blubber fat:	Belly	Belly	Belly	Belly
Iodine value	123·8	98·6	126·9	119·8
Sap. value	192·3	183·2	189·2	192·6
Acid value	0·6	0·6	1·1	0·5
Unsap., %	0·2	0·8	0·2	0·3
Acid composition, % (mol.)				
Lauric	—	—	—	—
Myristic		1·1	2·4	
Palmitic		8·2	11·6	
Stearic		4·6	3·4	
Arachidic		0·4	0·4	
Behenic		0·2	—	
Unsaturated*				
C_{14}	not determined	0·6 (−2·0 H)	1·2 (−2·0 H)	not determined
C_{16}		9·1 (−2·0 H)	10·0 (−2·1 H)	
C_{18}		38·4 (−2·1 H)	40·5 (−2·2 H)	
C_{20}		22·2 (−2·8 H)	20·6 (−4·4 H)	
C_{22}		12·1 (−4·9 H)	9·9 (−9·0 H)	
C_{24}		3·1 (−3·9 H)	—	

* Mean unsaturation shown in parentheses

Discussion

The results of analysis of the various samples of blubber fat of elephant seals shown in Tables I and II still further emphasize the very large variation in composition of the fat from different animals. Whereas the range of iodine values previously reported by us was 115–153, fats having iodine values of about 100 are now reported. This extends considerably the range of recorded iodine values for the blubber fats of elephant seals (98–153). An unexpected feature of these low-iodine-value fats is the relatively low content of saturated acids (15%) compared with the more highly unsaturated fats, which contain up to 22% of saturated acids. This variation is contrary to previous observations, by Hilditch & Pathak[3] and by us,[4] that the total amount of these acids in seal-blubber fats in general is fairly constant. The low-iodine-value fats are also distinguished by the presence of substantial amounts of long-chain acids (C_{20} to C_{24}) having a low degree of unsaturation. We have previously expressed some doubt[4] as to the presence of C_{24} acids in seal-blubber fats because the values reported were based only on the saponification equivalents of the residues from the fractional distillation of the highly unsaturated ester portion. There is, however, little doubt as to the presence of C_{24} acids of low degree of unsaturation in three of the fats examined (specimens No. 17, 20, and 31) because they were observed in the ester portion soluble at − 20° (insoluble at − 45°) and were contained not only in the distillation residues but also in some of the fractions.

Evidence has already been presented by us[5] that the fat composition of the blubber from various parts of the body of one animal is substantially constant. Further evidence is presented in Table III, which gives the chemical constants of the fats extracted from several parts of two elephant seals whose blubber fats showed a large difference in iodine values. The composition of the fat as determined on a portion of any elephant-seal blubber should therefore be reasonably representative of that of the whole blubber fat.

Although in general there appears to be a tendency for the total unsaturation of the blubber fat of elephant seal to be highest during the winter months, certain facts other than seasonal changes seem to have a major influence on the composition of these fats. There appears to be little correlation between fat composition and size, age or sex of the animal. It was thought that the variation in composition might possibly be connected with the starvation periods

Table II

Elephant-seal oils from Heard Island

Specimen No.	D	E	F	G	H	I
Date killed	12/12/48	12/12/48	12/12/48	19/12/48	19/12/48	19/12/48
Description	Mature female	Young male	Young female	Mature male	Male pup	Female pup
Estimated age	—	1 year	1 year	—	10 weeks	10 weeks
Length	7 ft. 6 in.	8 ft.	6 ft.	14 ft.	3 ft. 6 in.	4 ft.
Blubber thickness, in. (belly)	2·2	1·7	1·5	3·0	1·5	1·2
Notes	Not pregnant		Not pregnant			
Blubber fat:	Belly	Belly	Belly	Belly	Belly	Back
Iodine value	128·1	128·3	127·0	114·8	124·2	115·3
Sap. value	190·5	188·7	189·2	188·6	194·3	193·1
Acid value	0·8	0·6	0·7	0·8	0·7	0·9
Unsap., %	0·3	0·6	0·5	0·4	0·2	0·3
Acid composition, % (mol.)						
Lauric	—	—	—	—	—	—
Myristic	3·3			4·2	6·2	4·6
Palmitic	9·7			10·6	12·6	14·0
Stearic	4·2			2·5	2·1	2·4
Arachidic	0·5			0·3	0·3	0·2
Unsaturated*						
C_{14}	1·5 (−2·0 H)	not determined	not determined	1·5 (−2·0 H)	2·3 (−2·0 H)	1·4 (−2·0 H)
C_{16}	12·5 (−2·2 H)			11·7 (−2·1 H)	14·0 (−2·2 H)	13·3 (−2·1 H)
C_{18}	38·6 (−2·5 H)			40·1 (−2·2 H)	40·7 (−2·3 H)	44·4 (−2·2 H)
C_{20}	20·9 (−4·4 H)			16·6 (−4·0 H)	12·7 (−5·5 H)	11·7 (−4·5 H)
C_{22}	8·8 (−8·3 H)			12·5 (−7·4 H)	9·1 (−9·9 H)	8·0 (−9·6 H)

Table II (*contd.*)

Specimen No.	207	235	303	314	342	480	500
Date killed	13/7/49	27/7/49	19/9/49	26/9/49	5/10/49	9/12/49	12/12/49
Description	Mature male (young)	Mature female	Mature male	Mature female	Male pup	Young male	Female pup
Estimated age	—	—	—	—	11 days	—	—
Length	12 ft. 1 in.	8 ft. 5 in.	14 ft. 1 in.	7 ft. 9 in.	4 ft. 4 in.	8 ft. 7 in.	5 ft. 10 in.
Blubber thickness, in. (belly)	2·9	2·1	3·5	2·2	0·6	1·7	0·9
Notes		Not pregnant	One of first bulls ashore	Pregnant (male foetus)	Not moulted	Moult begun	Half moulted; at end of starvation period
Blubber fat:	Belly	Belly	Belly	Belly	Belly	Belly	Belly
Iodine value	145·8	130·7	142·6	120·1	123·0	122·3	112·8
Sap. value	190·6	190·3	190·6	190·6	194·6	189·9	193·2
Acid value	1·0	0·7	0·6	0·4	1·2	0·6	0·4
Unsap., %	0·3	0·5	0·5	0·3	0·3	0·4	0·2
Acid composition, % (mol.)							
Lauric	—	—	—	—	—	—	—
Myristic	3·1		5·9	5·8	4·8		
Palmitic	11·2		11·0	12·8	12·8		
Stearic	4·5		2·3	2·8	2·7		
Arachidic	0·7		0·3	0·4	—		
Unsaturated*							
C_{14}	1·1 (−2·0 H)	not determined	1·9 (−2·0 H)	1·6 (−2·0 H)	2·2 (−2·0 H)	not determined	not determined
C_{16}	14·0 (−2·2 H)		15·2 (−2·2 H)	11·5 (−2·1 H)	17·8 (−2·1 H)		
C_{18}	35·2 (−2·5 H)		33·3 (−2·6 H)	38·1 (−2·3 H)	40·6 (−2·3 H)		
C_{20}	20·2 (−6·1 H)		16·9 (−5·5 H)	18·1 (−5·1 H)	13·2 (−6·7 H)		
C_{22}	10·0 (−10·0 H)		13·2 (−8·8 H)	8·9 (−8·3 H)	5·9 (−10·5 H)		

* Mean unsaturation shown in parentheses

which these animals undergo as pups during the period between leaving the mother and going to sea, and as young and adult animals twice yearly during the mating and moulting seasons. We have been unable to find any such relationship. Thus, for instance, specimen No. 33, from a pup which had just reached the end of the starvation period, had a similar total unsaturation to that of specimen No. 26, from a pup which had just commenced the starvation period, whereas the total unsaturation of specimen No. 31, taken shortly before the pup was due to leave the mother, was particularly low. Again, on comparing specimens from two mature animals taken in July, 1949, at Heard Island at a time when fat deposition would have reached a high level, it will be seen that the iodine value of the fat of the female is very much lower

Table III

Constants of blubber fat taken from various portions of the body

Specimen No.	Position	Iodine val.	Saponification val.	Acid val.	Unsaponifiable matter, %
207	Neck	144·0	190·5	1·0	0·3
	Posterior dorsally	145·8	191·6	1·4	0·4
	Mid dorsally	145·5	191·3	1·3	0·3
	Mid ventral	145·8	190·6	1·0	0·3
	Under foreflippers	143·2	191·8	1·0	0·3
17	Neck	105·0	185·6	0·5	0·3
	Posterior dorsally	106·2	185·6	0·4	0·4
	Mid dorsally	105·2	186·1	0·7	0·2
	Under foreflippers	105·0	186·0	0·4	0·2

than that of the male, whereas with the two animals taken in the same months in 1948 at Macquarie Island the opposite was observed.

Assuming that the observed variations are due to metabolic changes which may or may not be influenced by external factors, the data collected may give some insight into the processes involved. Two important mechanisms are generally recognized: one involves saturation and desaturation of the fat, and the other progressive chain-lengthening and chain-shortening of the fatty acids.

The saturation and desaturation process may be studied with advantage by comparing the ' unsaturation value ' for each group of fatty acids, as shown in Table IV. The unsaturation value is defined as the product of the molar percentages of the fatty acid group and its mean unsaturation divided by 200. It represents the number of double bonds contributed to one average molecule of fat by each group of fatty acids. It will be noted that the major variations are to be found mainly in the groups of acids having a chain length of more than 20 carbon atoms and that the unsaturation values of the C_{18} acid groups remain substantially constant. This constancy in the C_{18} acid groups is remarkable since they constitute the largest group (33–44%) of acids in the fat. It seems, therefore, that the saturation and desaturation processes are confined mainly to fatty acids of chain length greater than C_{18}.

Table IV

Range of unsaturation values in the blubber fats of elephant seals

Chain length	Unsaturation value
C_{14}	0·01–0·02
C_{16}	0·08–0·19
C_{18}	0·38–0·48
C_{20}	0·26–0·62
C_{22}	0·30–0·63
C_{24}	0·01–0·06

The process governing changes in the relative proportions of fatty acids of various chain lengths does not appear to be so well defined. There is apparently no constancy in the total molar percentages of fatty acids of any particular chain length nor in the sum of the molar percentages of any two groups of fatty acids. By summing the molar percentages of alternate groups of fatty acids, that is, dividing the fatty acids into two parts, one consisting of the C_{14}, C_{18} and C_{22} acids and the other consisting of the C_{16}, C_{20} and C_{24} acids, the totals are more nearly constant (see Table V). The difference in spread of values relating to the latter groups, as compared with that of any of the groups containing acids differing by two carbon atoms, has been examined statistically by testing the variance and was found to be significant. Thus it appears that the change in proportions of the fatty acids of various chain lengths is not

Table V

Comparison of molecular percentages of acids varying by 2 and 4 carbon atoms

Specimen No.	$C_{14}+C_{16}$	$C_{16}+C_{18}$	$C_{18}+C_{20}$	$C_{20}+C_{22}$	$C_{14}+C_{18}$	$C_{14}+C_{18}+C_{22}$	$C_{16}+C_{20}+C_{24}$	$C_{18}+C_{22}$
Macquarie Island								
15 ..	36·7	65·3	54·0	25·9	45·4	54·3	45·3	45·9
16 ..	34·0	62·3	54·2	30·6	42·3	54·0	45·9	47·0
17 ..	19·1	59·7	69·2	36·9	46·0	57·0	43·0	54·3
20 ..	24·6	60·2	62·7	36·4	41·4	53·6	46·4	50·7
31 ..	19·0	60·3	65·6	34·9	44·7	57·0	43·0	55·3
33 ..	25·2	65·5	64·9	30·9	47·5	57·4	42·6	53·8
Heard Island								
D ..	27·0	65·0	64·2	30·2	47·6	56·4	43·6	51·6
G ..	28·0	64·9	59·5	29·4	48·3	60·8	39·2	55·1
H ..	35·1	69·4	55·8	22·1	51·3	60·4	39·6	39·6
I ..	33·3	74·1	58·7	19·9	52·8	60·8	39·2	54·8
207 ..	29·4	64·9	60·6	30·9	43·9	53·9	46·1	49·7
303 ..	34·0	62·8	52·8	30·4	43·4	56·6	43·4	48·8
314 ..	31·7	65·2	59·4	27·4	48·3	57·2	42·8	49·8
342 ..	37·6	73·9	56·5	19·1	50·3	56·2	43·8	49·2

restricted to one or two groups of fatty acids but is distributed throughout the whole range of chain lengths.

When the ranges of fatty acid distribution in the various specimens of blubber fat of elephant seal are compared with those in blubber fats from other seals (see Table VI), it will be seen that there is a similarity between all the seal-blubber fats. Somewhat larger amounts of C_{16} unsaturated acids are present in the blubber fats of the common seal and crabeater seal, but since there is a large difference in this respect (22·7–27·8%) between the two specimens of common seal examined by Hilditch & Pathak[3] it is doubtful whether the presence of the greater amount of C_{16} acids can be considered as a specific feature of these species. Thus, from the results of detailed analyses of seal-blubber fats that are available at present, it may be concluded that the fat composition of the blubber varies appreciably between individual animals, and no characteristic distribution pattern can be attributed to any one of the species so far examined.

Table VI

Composition of blubber fats from different seals

Fatty acids, % (mol.)	Elephant seal	Leopard seal[5]	Grey seal[2]	Newfoundland seal[1]	Common seal[3]	Crabeater seal[6]
Lauric	0–0·4	—	—	trace	—	—
Myristic ..	1·1–6·3	4·9	4·6	6·6	2·7–2·9	5·7
Palmitic ..	8·2–14·0	8·1	11·5	11·7	11·1–11·4	10·9
Stearic	2·1–4·6	1·6	2·0	1·3	2·4–4·3	1·6
Arachidic ..	0–0·7	0·2	—	0·5	0·3	—
Behenic ..	0–0·2	—	—	—	—	—
Unsaturated*						
C_{14}	0·6–2·3	2·6	2·1	2·2	2·7–3·6	3·9
	(−2·0 H)	(−2·0 H)	(−2·0 H)	(−2·0 H)	(−2·0 H)	(−2·0 H)
C_{16}	8·2–17·8	14·1	17·2	11·6	22·7–27·8	21·5
	(−2·0–2·2 H)	(−2·1 H)	(−2·2 H)	(−2·1 H)	(−2·1 H)	(−2·3 H)
C_{18}	32·9–44·4	42·2	30·9	39·5	33·2–33·7	30·0
	(−2·1–2·7 H)	(−2·2 H)	(−2·7 H)	(−2·4 H)	(−2·4–2·7 H)	(−3·2 H)
C_{20}	11·7–25·3	15·8	15·2	16·0	12·4–13·6	17·5
	(−2·8–6·7 H)	(−4·6 H)	(−5·7 H)	(−5·6 H)	(−5·9–7·2 H)	(−7·9 H)
C_{22}	5·9–13·2	10·5	15·5	9·0	10·3–12·2	8·9
	(−4·9–10·8 H)	(−9·4 H)	(−10·6 H)	(−9·3 H)	(−11·0 H)	(−10·8 H)
C_{24}	0–3·1	—	1·0	1·6	—	—
	(−2·0–3·9 H)		(−11·0 H)	(−10·9 H)		

* Mean unsaturation shown in parentheses

The composition of seal-blubber fats may usefully be compared with that of another important family of sea mammals, the Baleen whales. Few detailed analyses of whale-blubber fat are available in the literature, but Hilditch & Pathak[3] expressed the view that there exist fairly well-defined differences in the blubber-fat composition between different genera and species of this family. They also stated that a number of independent specimens of antarctic whale oil taken in different years showed closely similar fatty acid distribution. Since we presume that these samples were taken from commercially produced oil, they should represent the average blubber fat from a number of animals ; hence differences in blubber-fat composition between individual animals would not be apparent. Lund,[7] using the average iodine values and saponification values observed over a number of years, was able to show a definite relationship between these constants and the locality of the catch. No such data exist for seal-blubber fats.

In Table VII the ranges of fatty acid distribution in seal-blubber fats are compared with those of whale-blubber fats. The latter data have been compiled from the more recent literature.[8–10] Earlier analyses have not been included, since there is some doubt as to the reliability of the analytical techniques that were then available. Although it must be emphasized that the ranges for the fatty acid distribution in whale blubber are based only on three analyses, it seems that there is a close similarity in the distribution pattern of the unsaturated acids between the blubber fats of seals and whales. The two types of fat seem to differ mainly in the amount of saturated acids present, the whale-blubber fat containing approximately 30%, as compared with 15–22% of these acids in seal-blubber fat.

Table VII

Comparison of fatty acid composition of blubber fats of seal and whale

Fatty acids % (mol.)	Seal fats	Whale fats
Lauric	0–0·4	0–trace
Myristic	1·1–6·3	7·5–11·0
Palmitic	8·1–14·0	16·5–20·7
Stearic	1·3–4·6	0–2·3
Arachidic	0–0·7	0–0·5
Behenic	0–0·2	—
Unsaturated*		
C_{14}	0·6–3·9 (−2·0 H)	1·8–4·4 (−2·0 H)
C_{16}	8·2–27·8 (−2·0–2·3 H)	12·4–14·8 (−2·0–2·1 H)
C_{18}	30·0–44·4 (−2·1–3·2 H)	35·8–38·0 (−2·4–2·9 H)
C_{20}	11·7–25·3 (−2·8–7·9 H)	10·1–13·1 (−5·6–7·1 H)
C_{22}	5·9–15·5 (−4·9–11·0 H)	4·9–5·8 (−9·0–9·6 H)
C_{24}	0–3·1 (−2·0–11·0 H)	—

* Mean unsaturation shown in parentheses

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ERRATA

In the paper entitled ‘ The determination of arsenic in gelatin by means of the molybdenum-blue reaction ’ by J. E. Eastoe and B. Eastoe, *J. Sci. Fd Agric.*, 1953, **4**, 310, for ‘ 1 grain ’ in the 7th line of p. 311 read ‘ 1/100 grain ’.

In the paper entitled ‘ Flavour assessment of sugar solutions ’ by Gweneth M. Chappell, *J. Sci. Fd Agric.*, 1953, **4**, 346, for ‘ 5% ’ and ‘ 10% ’ in the 28th line of p. 350 read ‘ 25% ’ and ‘ 40% ’ respectively.

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