BIOCHEMICAL AND MYCOLOGICAL STUDIES ON STORED FOODS.

A Thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Science.

by

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CONTENTS.

INTRODUCTION.

The present war has given particular prominence to the supply and safe storage of both human and animal foodstuffs. Before September, 1939, Britain had become increasingly dependent upon imported feeding-stuffs for the maintenance of its livestock. Average figures given by Wright (1940a) for the years 1934-38 showed that as much as one-fifth of all animal feeding-stuffs available in the United Kingdom were of imported origin. Moreover, these imported feeding-stuffs provided between one-third and one quarter of the total available nutrients, calculated either as protein equivalent or starch equivalent. In all some 8½ million tons of feeding-stuffs were imported annually: cereals and cereal by-products formed the larger portion (four-fifths) of these imports, while the remainder was in the form of oil seed products. The British farmer had come to rely upon these imports of concentrate feeding-stuffs for the feeding of his dairy stock, particularly during the winter period when the supply of home produced feeding-stuffs was at its minimum. The use of imported concentrates enabled the production of high milk yields and, at the same time, was economical in its demands upon farm labour which, in the years just before 1939, was becoming a factor of great importance.

Complete figures for imported feeding-stuffs since 1939 are not available, but it is variously estimated that, due to the reduction in available shipping space, these imports have been reduced by 50 or 75%. In normal times the supply and demand for such imported feeding-stuffs could be adjusted to entail the holding of minimum stocks for limited storage periods. With the introduction of a rationing system under war conditions the storage of larger stocks for very much longer periods became necessary. Further, for reasons of security these stocks had to be dispersed to country stores, such as improvised warehouses, sheds and farm buildings. Many of these premises were not designed for storing feeding-stuffs and were inferior to the storage accommodation available at ports and at the place of manufacture.

Deterioration of feeding-stuffs is usually associated either with a high atmospheric humidity or with a high moisture content in the stored material. Thus damage most frequently occurs in areas that are susceptible to fogs or in buildings which have incomplete protection against the weather. Some types of feeding-stuffs are stored as loose materials in sacks, bins or in silos. In particular, the different ingredients of compound feeding-stuffs are commonly stored in this way before manufacture. Before the war many of these ingredients were of imported origin and, being ripened under warmer climatic conditions than are

typical of this country, were of low moisture content. Since the outbreak of war, however, many home-produced cereals have been used in these compound mixtures. Their moisture content being higher than that of imported cereals has meant that they were more susceptible to damage during storage. This problem has been accentuated by the limited number of grain driers available for the treatment of home produced grain.

A similar difficulty has arisen in the storage of oil cakes. Before 1939 a wide variety of oil seeds were imported into this country and, after extraction of the oil for margarine manufacture, etc., the remainder was available for sale as oil cakes of different kinds. These oil seeds were imported from many parts of the world including Africa, North and South America, India and Malaya and the Eastern Mediterranean. War-time control and priority of shipping has meant that importations of essential oil seeds are made only from those countries that are free from enemy occupation and from which transportation is quickest and most easily made. A large proportion of the oil seed imports have, therefore, consisted of palm kernels from West Africa. To obtain the maximum oil extraction from these kernels it is necessary to raise the moisture content of the ground meal to 13½-14%. The final oil cake product retains

approximately the same amount of moisture after manufacture and, although if dry conditions prevail 1½-2% will usually be lost by evaporation during the first day of storage, this amount of moisture will be retained and deterioration will be liable to occur in more humid weather.

It is common experience in commercial storage to find that feeding-stuffs are more susceptible to deterioration during the summer months when the average atmospheric temperature is higher than during the winter months. The importance of maintaining summer stored feeding-stuffs at a low moisture content is clear. Such precautions are, of course, also specially necessary in the storage of foodstuffs in tropical and sub-tropical climates.

In general, deterioration of feeding-stuffs may take one of three forms; firstly, heating which can lead to the compaction of loose materials into lumps, loss of weight resulting from an increase in respiratory processes, darkening of colour or, in extreme cases, charring and possibly spontaneous combustion; secondly, deterioration due to infestation by insects; and thirdly, deterioration caused by micro-organisms, of which moulds are the most important. The palatability of such damaged foods will be decreased along with their marketable value. Damaged foodstuffs are characterised by their loss of freshness, the presence of a musty smell, chemical breakdown of the food

constituents by enzymes in the material itself or from the damaging micro-organisms, and by the presence of injurious by-products.

The work that is presented here on the storage of feeding-stuffs follows upon that of Wright (1940b) who considered the relation of humidity and moisture content to the storage condition of artificially dried grass. These observations were extended to include a wide range of feeding-stuffs. Some of the results have now been prepared for publication by Snow et al. (1944). The particular experiments described in the present thesis were carried out with the object of studying the following items:- (1) the feasibility of including non-protein nitrogen compounds, such as urea, in cereal feeding-stuffs, and of storing these feeding-stuffs under typical farm conditions; (2) the loss of dry matter from feeding-stuffs; (3) acidity values as a measure of deterioration in feeding-stuffs; (4) the water uptake of feeding-stuff constituents; (5) the species of moulds isolated from feeding-stuffs stored at fixed humidities for periods up to 4 years; (6) the suitability of some antiseptics in the control of mould growth.

SUMMARY.

Part I. The storage of feeding-stuffs with incorporated non-protein nitrogenous compounds.

The feasibility of including ammonium bicarbonate, ammonium sulphate and urea in cereal feeding-stuff mixtures is considered. Losses amounting to between one third and one half of the ammonium bicarbonate originally incorporated in such mixtures occurred during manufacture, but the corresponding losses from the ammonium sulphate and urea mixtures were very small. The mixtures were manufactured in cubes of different sizes and were stored in various types of containers under farm conditions for 30 weeks.

Analyses were made on samples taken at intervals during the storage period to determine the losses of nitrogen from these mixtures. The losses of ammonia sustained during the manufacture of the ammonium bicarbonate cubes continued during storage irrespective of the type of cube. There was some indication that cubes stored in paper bags lost less ammonia than cubes stored in jute sacks. Losses of ammonia from the cubes containing ammonium sulphate were small. The cubes containing urea suffered no significant loss of ammonia until deterioration of the cubes occurred with the development of moulding and heating. Laboratory experiments showed that the loss of urea from small samples stored at fixed temperatures and humidity was

related to the development of these two forms of deterioration. Apparent increases of up to 6% in the total nitrogen values (calculated on a 100% dry matter basis) for some of these mixtures were recorded throughout the storage period. These were considered to be due to a loss of dry matter from these feeding-stuffs.

Part II. The storage of bran.

The apparent increases in total nitrogen found throughout the storage period of feeding-stuffs containing urea etc. (see Part I) were shown to be undoubtedly due to losses of dry matter. This was proved by analyses and weighings made on bran stored (a) in closed containers, (b) in sacks under farm storage conditions, and (c) as small 1 g. samples at controlled temperatures and humidity. Further analyses were made to estimate the chemical changes occurring with the development of moulding and heating in samples of bran. These showed that, with the onset of moulding, changes in the acidity fractions of the bran took place, the fat acidity value showing marked reduction. Where fermentation of the bran samples occurred in the closed tins, acids were produced which caused a rise in these acidity values. The development of moulding or heating also resulted in a loss of over half the ether-soluble fraction.

Experiments have been made on the respiration of bran at different moisture levels using a continuous

absorption apparatus in which the humidity of the air stream was adjusted to be at equilibrium with the respiring bran. The respiratory rate was accelerated with increased moisture content and was clearly attributable to two causes:- (1) the respiration of the bran material itself and (2) the respiration of developing micro-organisms. Bran of 13% moisture content and below was shown to have a very low respiratory rate. It will, therefore, not be liable to heating during storage.

Part III. Experiments on acidity values in stored feeding-stuffs.

A critical survey is made of the methods that have been used by various authors to assess the different acid fractions of feeding-stuffs. The degree of fineness of grinding and the time and temperature of extraction with various solvents are shown to influence the results obtained by the generally recognised methods of estimation. The fat acidity value is shown to be the fraction most susceptible to changes in the condition of stored feeding-stuffs.

Part IV. The water uptake of feeding-stuff constituents.

The water uptake of samples of glutenin, edestin, egg albumin, casein, rice starch and bran fibre and of mixtures of these substances in definite proportions has been determined over a range of fixed humidities. The shape and level of the water uptake curves is shown to be characteristic for each of the different

types of substance. Observations have also been made on the extent to which these materials exhibited hysteresis. With egg albumin the amount of water absorbed after 24 hours at a given humidity was found to be greater than that finally held when equilibrium had been obtained.

Part V. The isolation of mould types from feeding-stuffs stored at fixed humidities.

Isolations were made of mould types actively growing on a variety of feeding-stuffs stored at fixed humidities between 100 and 65% for periods up to 4 years. The factors affecting the species of moulds isolated were:- (1) the humidity of the atmosphere; (2) the period of storage; and, (3) the type and origin of the samples from which the isolates were obtained. It was evident that a critical humidity existed for each species, below which development of mould spores could not take place. At 100% R.H. an infinite variety of mould types were able to develop. In general, below this humidity members of the Mucorales and the Fungi Imperfecti were not isolated. Below 80% R.H. Penicillium spp. were not able to grow. Aspergillus spp., on the other hand, were able to develop under conditions of very restricted moisture supply. Members of the Aspergillus glaucus group were able to germinate at humidities as low as 65 to 70%. The most commonly occurring mould species on these feeding-stuffs were small ascospored types of the

Aspergillus glaucus group, particularly A. repens and A. ruber.

Part VI. The use of antiseptics in controlling moulds.

Mineral additions to palm kernel cake were shown to exert no antiseptic effect.

At 80% R.H. borax and boric acid, incorporated in samples of linseed cake at a 1% level, increased the freedom from moulding by 50%. The spraying of cubes of linseed cake with a 10% borax solution doubled their storage life. The use of borax or boric acid for this purpose is not recommended since it only exerts a mild antiseptic effect and also because of the possible toxic effect of excreted boron in the soil.

The reaction of different mould species to sulphanilamide and other drugs have been tested on agar plates. Some of these drugs were found to inhibit effectively the development of certain mould types at very low dilutions (some as low as 0.006%). The results of these agar plate experiments were applied in the treatment of stored linseed cake samples with incorporated sulphonamide drugs. The incorporation of 0.2% of a soluble derivative of sulphanilamide, commonly known as E.O.S., doubled the storage life of samples stored at 85 and 80% R.H. It is considered possible that these drugs might be of use in the control of certain micro-organisms on stored products.

PART I. THE STORAGE OF FEEDING-STUFFS WITH INCORPORATED NON-PROTEIN NITROGENOUS COMPOUNDS.

Introduction.

Owen et al. (1943) and Pearson and Smith (1943) have shown by metabolism trials and by experiments in vitro that urea can act as a partial substitute for protein in the diet of ruminants, and that in doing so it is first converted to ammonia in the rumen. In view of the possibility of a shortage of protein feeding-stuffs occurring during the present war it was considered desirable to investigate the feasibility of including such non-protein nitrogenous compounds in cereal feeding-stuffs. Three different compounds were suggested for inclusion in this way; firstly, urea, which metabolism trials had indicated as being suitable; secondly, ammonium bicarbonate which is more readily available than urea; and thirdly, ammonium sulphate which is cheap, easily manufactured, and plentiful.

As well as studying the feasibility of manufacture of these cubes, it was considered important to carry out observations on the keeping qualities of such feeds, particularly in regard to the losses of ammonia that might take place from the different mixtures. Two factors which might influence this loss were the type of cube and the type of container in which the cubes

Table 1.

Details of ingredients.

	Cereals				Ammonium Sulphate			
	cwts.	qrs.	st.	lbs.	cwts.	qrs.	st.	lbs.
Bran	9	1	1	0	3	3	0	0
Barley	14	0	0	13	5	2	1	6
Oats	14	0	0	13	5	2	1	6
Molasses	2	1	1	2	0	3	1	6
Urea	-	-	-	-	-	-	-	-
Ammonium bicarbonate	-	-	-	-	-	-	-	-
Ammonium sulphate	-	-	-	-	0	3	1	6
	40	-	-	-	16	2	0	4

	Ammonium Bicarbonate				Urea			
Bran	8	3	1	4	9	0	1	7
Barley	13	1	0	12	13	3	0	10
Oats	13	1	0	12	13	3	0	10
Molasses	2	0	1	11	2	1	0	5
Urea	-	-	-	-	0	3	0	10
Ammonium bicarbonate	2	0	1	3	-	-	-	-
Ammonium sulphate	-	-	-	-	-	-	-	-
	40	-	-	-	40	-	-	-

were stored. The feeding-stuff mixtures were therefore made up in different sizes of cubes so that different surface areas of the mixtures were exposed, and these cubes were then stored in different types of containers; some in jute sacks, others in 3-ply paper bags, and others in 5-ply bitumin lined paper bags. In addition to investigating whether these paper bag types were better able to retain ammonia, it was considered that the general use of such bags in commercial practice might be necessitated by the shortage of jute sacks resulting from the limited importation of jute from abroad in war-time.

Details of manufacture.

Four types of feeding-stuff mixtures were made up using an ordinary commercial cubing machine; the first consisted of a cereal mixture alone, the second contained ammonium bicarbonate, the third ammonium sulphate and the fourth urea. The amount of non-protein nitrogen included in the form of these different compounds was calculated to be equivalent to 30% of the total nitrogen in the ammonium bicarbonate and urea mixtures, but to only 6% in the ammonium sulphate mixture. This smaller quantity was considered advisable because of the scouring effect of ammonium sulphate when fed to dairy cattle. The details of the ingredients are shown in Table 1. The dry materials were all weighed separately before mixing but it was not possible to assess the exact amount of molasses

added to each batch because this had to be introduced during the cubing process.

The mixtures were made up in three sizes, as pellets, as 3/8" and as ½" cubes. These were obtained by using the appropriate sized die in the machine for each of the four types of mixture in turn. Thorough cleaning of the whole machinery was carried out before each type of mixture was introduced and sample bags for the storage experiment were selected from the later portion of each batch. In this way any risk of contamination from material of the previous batch was eliminated. The cubes were fed, as they came off the cooler, into jute sacks, 3 ply paper and 5 ply bitumin lined paper bags.

Triplicate sacks of each type were selected for the storage experiment. The cereal, ammonium bicarbonate and urea pellets were stored in jute sacks only (3 storage types), the 3/8" cubes and the ½" cubes of these mixtures were stored in jute, 3 ply paper and 5 ply bitumin lined paper bags (18 storage types); the ammonium sulphate mixture was made up in ½" cubes only and stored in jute sacks. This amounted to 22 storage types in all.

Methods of analysis.

The cubes were allowed to stand at the manufacturing warehouse for two days to allow excess moisture to evaporate before being transported to be

stored on the farm of the Hannah Institute. Initial analyses of all storage types were carried out on arrival at the Institute to determine moisture content (air oven method at 100°C for 3 hours), total nitrogen, non-protein nitrogen (the protein being precipitated overnight by sodium tungstate and N/6 sulphuric acid), free ammonia nitrogen and urea nitrogen. All nitrogen values are quoted in mg.N/100 g. dry matter unless otherwise stated.

Samples of the ammonium bicarbonate, ammonium sulphate and urea cubes were taken after storage for periods of 3, 7, 10, 13, 16, 21 and 30 weeks; the cereal cubes were sampled after 7, 13, 21 and 30 weeks. For the samples a representative handful of cubes was taken from each of the three sacks of the different storage types. The samples were ground in a Christy & Norris laboratory mill and determinations made corresponding to the initial analyses. Samples of the added non-protein nitrogenous compounds were analysed and all were found to be at least 99.7% pure.

Loss of nitrogen during manufacture.

From the initial analyses it was possible to calculate the loss of nitrogenous compounds that had occurred during manufacture and transit. This initial loss was considerable in the ammonium bicarbonate cubes, amounting to 32% of that incorporated in the ½" cubes transported in 5 ply bitumin lined paper bags and to

Table 2.

Temperature and relative humidity of the store.

	Period before sampling - weeks.						
	0-3	3-7	7-10	10-13	13-16	16-21	21-30
Average temp. °F	45.4	45.8	35.9	37.7	36.7	46.8	54.4
" % R.H.	76.5	83.0	79.0	81.4	74.0	70.5	60.5

as much as 47% of that in similar cubes transported in jute sacks. Most of this loss occurred when steam was passed under pressure into the cubing machine at the time of manufacture. This results in a very strong smell of free ammonia, and the consequent loss of nitrogen from these cubes is thought to be inevitable with such high concentrations of ammonium bicarbonate and this type of manufacturing process. The ammonium sulphate cubes sustained an initial loss of 11% of that incorporated into the mixture, but only a negligible amount of urea was lost during manufacture and transit.

Storage conditions.

The sacks were stored under farm conditions in a well ventilated and rat-proof store with damp-proofed walls. The relative humidity was determined daily by means of a psychrometer. Daily fluctuations in temperature were recorded by means of a thermograph and these readings were confirmed by a maximum and minimum thermometer. The daily average fluctuation was 7°F. During the 30 week period the humidity of the store showed wide variation within the range of 34-96% R.H. Over the same period the temperature varied between 29 and 68°F. Average temperature and relative humidity values for the different sampling periods are shown in Table 2.

At the time of sampling notes were made of the

condition of the cubes. These remained fresh for the first 13 weeks of storage, after which mould mycelium developed on some of the cereal and urea cubes, especially on those stored in sacks placed near the walls of the store. In spite of the damp-proofing treatment some condensation took place down the walls during periods of high humidity. These conditions favoured the development of mould growth at the top of the sacks near the walls. Where such deterioration occurred the moulded layer of cubes was, as far as possible, removed from the top of the sack before sampling. During the 13-16 weeks period there was no further development of moulding but, at the time of the 21 week sampling, it was noted that moulding had taken place (in some sacks to a marked extent) on many of the cereal, urea and ammonium sulphate cubes. The humid and warm conditions of part of the 16-21 weeks period resulted in the heating of many of the sacks of feeding-stuffs, the inside of many of the paper bag containers being thoroughly damp from water condensation. This was particularly true of the 5 ply bitumin lined bags which allowed very little ventilation. During the 21-30 week period drying-out took place with all the cubes and at the time of the final sampling (after 30 weeks) little further mould deterioration could be detected. The cubes containing ammonium bicarbonate remained in good condition throughout the 30 week storage period: it is probable that the free

Table 3.

Cereal mixture.

Period before sampling (weeks)	Moisture content %	Total N.	% Increase	N.P.N.	Free NH_3-N.
Initial	14.1	2,075		173	24
7	14.7	2,131	2.7	175	19
13	15.0	2,140	3.1	173	23
21	13.7	2,190	5.5	139	8
30	13.5	2,205	6.3	141	9

Table 4.

Relationship between moulding, heating and change in N.P.N.

Storage type	Condition of cubes and samples during period 13-30 weeks	N.P.N. After 13 weeks	N.P.N. After 30 weeks
Pellets - Jute	No moulding*, no heating, samples fresh	173	167
½" - Jute	Slight " , slight " , " musty	170	146
½" - 3 ply	Slight moulding, very moist and heated, samples musty	174	121
3/8" - Jute	Very slight moulding, very moist and heated, samples musty	174	111

* moulding refers to the surface layer of the sack only.

ammonia given off from these cubes acts as a deterrent to mould growth.

Analytical results.

(a) Cereal mixture.

There was no significant difference due to the size of cube or type of container in the values obtained for the different storage types of this mixture. Average values only of the seven types are therefore given in Table 3. These values show an apparent increase in total nitrogen, which was progressive throughout the period of storage, and amounted to a 6.3% increase after 30 weeks. Over the period 0-13 weeks there was no significant change in N.P.N. but from 13-30 weeks this dropped from 173 to 141 mg.N/100 g. dry matter. This drop is equivalent to a 1.4% decrease in total nitrogen. That this loss of N.P.N. occurs after the development of mould growth and heating is evident from a consideration of the extent of this deterioration and the consequent change in N.P.N. of the different cereal storage types (Table 4).

(b) Ammonium bicarbonate.

It was mentioned previously that between a third and a half of the ammonium bicarbonate incorporated in this mixture was lost during the manufacturing process and subsequent transit. This loss continued during the period of storage. The average analytical values for the 7 storage types are shown in Table 5, while the

Table 5.

Ammonium bicarbonate mixture.
(Average of the 7 storage types).

Period before sampling (weeks)	Moisture content %	Total N		N.P.N.		Free NH_3-N	
Initial	16.4	2,778		873		737	
3	14.2	2,713	(2.3)	746	(14.5)	592	(19.7)
7	16.7	2,741		759		602	
10	16.9	2,713		720		580	
13	17.1	2,744		719		563	
16	16.3	2,724		698		535	
21	15.6	2,738		691		532	
30	15.0	2,680	(3.5)	671	(23.8)	496	(32.7)

% loss figures in brackets.

Table 6.

Loss in N.P.N. and Free NH_3-N from Ammonium Bicarbonate Cubes.

Period before sampling (weeks)	Pellets	JUTE 3/8"	JUTE 1/2"	3-PLY 3/8"	3-PLY 1/2"	5-PLY 3/8"	5-PLY 1/2"
N. P. N.							
Initial	863	841	780	853	934	885	952
3	766 (11.2)	686 (18.4)	665 (14.7)	713 (16.4)	753 (19.4)	836 (5.5)	792 (16.8)
7	777	689	667	752	754	860	814
10	720	685	633	698	696	834	775
13	717	668	618	701	721	837	768
16	689	653	600	686	692	824	744
21	678	659	606	678	670	807	742
30	638 (26.1)	621 (26.2)	591 (24.2)	655 (23.2)	644 (31.0)	820 (7.4)	727 (23.6)
FREE NH_3-N							
Initial	721	712	641	730	805	738	812
3	630 (12.6)	543 (23.7)	495 (22.8)	552 (24.4)	596 (26.0)	684 (7.3)	642 (20.9)
7	626	543	500	588	596	703	656
10	570	528	492	561	577	694	639
13	556	513	469	547	564	676	616
16	533	488	440	515	527	655	585
21	525	498	447	529	516	637	575
30	486 (32.6)	450 (36.8)	429 (33.1)	480 (34.2)	466 (43.4)	625 (15.3)	537 (33.9)

% loss figures in brackets.

detailed losses of N.P.N. and of free ammonia-N for the different types are recorded in Table 6. The loss of ammonium bicarbonate, as represented by loss in N.P.N., amounted to as much as 23-31%* over the 30 week storage period for the different storage types. The rate of loss of ammonia during the first 3 weeks of storage was greater than that during the following 27 weeks; the average loss of ammonia from the 7 types amounted to a 15% loss of N.P.N. during the first 3 weeks and the remaining 9% loss was gradual over the following period of 27 weeks (see Fig.1). It is probable that such large amounts of ammonium bicarbonate (originally calculated to be equivalent to 30% of the total nitrogen in the mixture) cannot be retained by the cereal mixture in which they are incorporated. Large quantities of ammonia are therefore lost in manufacture and during the early part of storage, after which an equilibrium is reached and loss of ammonia is more gradual. In passing it may be noted that the continued evolution of ammonia from this mixture would detract from the palatability of these cubes when fed to livestock.

The retention of ammonium bicarbonate by the cubes in the early part of storage is considered to be

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*The low figure (7.4%) for the 3/8" cubes in 5 ply bags is regarded as exceptional. This small storage loss of nitrogen is thought to be due partly to the small loss of water vapour (and, hence, ammonia) from these cubes sustained during the first 3 weeks of storage.

FIG. 1 Rate of loss of Ammonium Bicarbonate and Ammonium Sulphate from stored cubes.

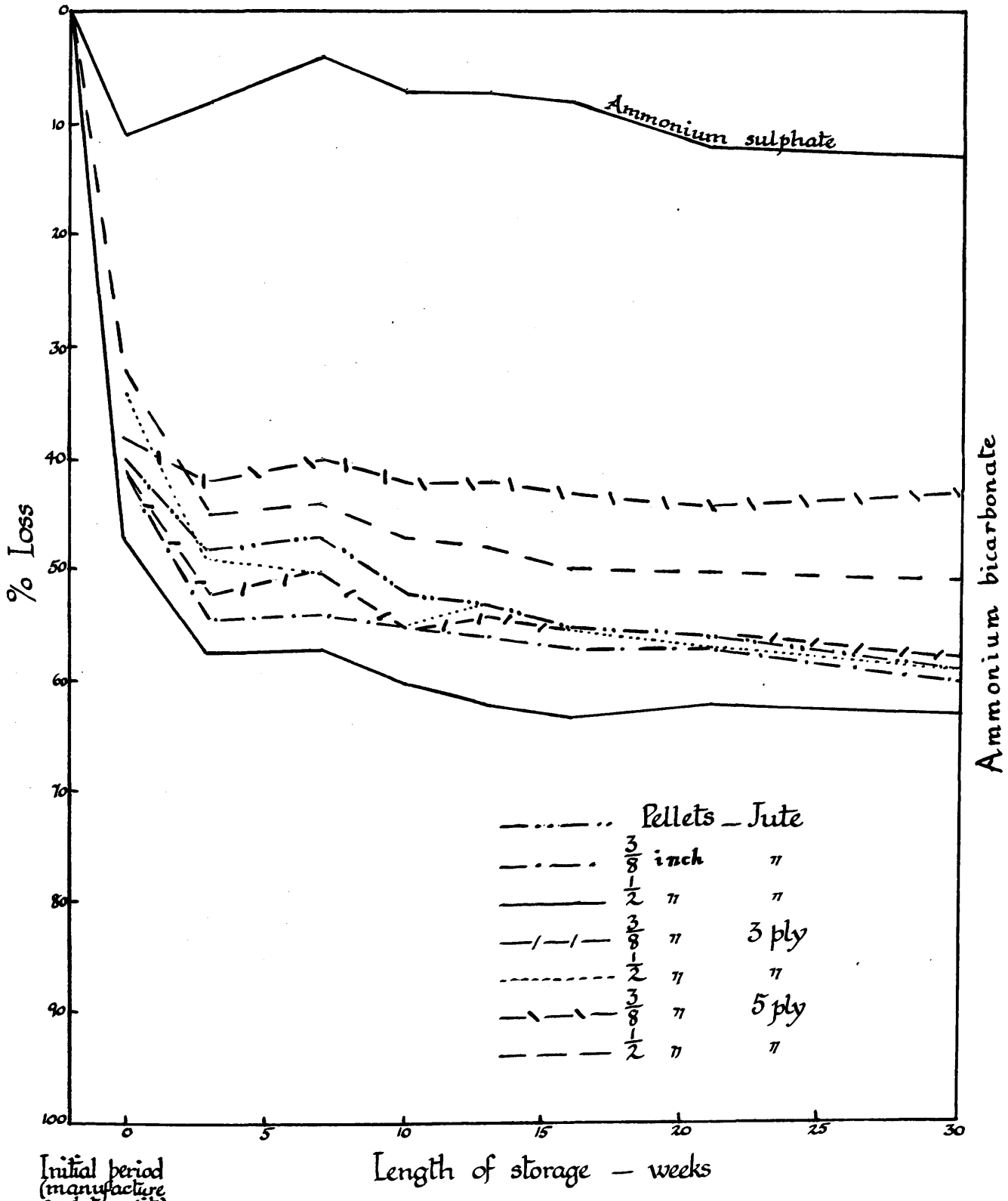


Table 7.

Relationship between loss of moisture and loss of ammonia during first 3 weeks of storage.

	Initial		After 3 weeks	
	Moisture content %	N.P.N.	% loss of moisture	% loss N.P.N.
Pellets Jute	16.5	863	7.8	11.2
1/2" "	15.6	780	12.5	14.5
3/8" "	16.3	841	17.7	18.1

Table 8.

Initial, storage and total losses of ammonium bicarbonate*.

Storage type	Loss during manufacture and transit	Storage loss	Total loss
Pellets Jute	40	20	60
3/8" "	42	19	61
1/2" "	47	16	63
Average Jute	43	18	61
3/8" 3 ply	41	17	58
1/2" "	34	25	59
Average 3 ply	38	21	59
3/8" 5 ply	38	6	44
1/2" "	32	20	52
Average 5 ply	35	13	48

* figures calculated as percentage of initial amount added.

related to the moisture content of the cubes. Thus it was found that cubes of relatively high moisture content were able to retain more ammonia and, conversely, those cubes that lost large amounts of water vapour lost correspondingly larger amounts of ammonia than those in which the moisture loss was small. This is shown by the results of the three cube types stored in jute sacks (see Table 7).

If the loss of ammonium bicarbonate during storage, which may be determined from the loss in N.P.N., be added to the initial loss (manufacture and transit) the total loss of ammonium bicarbonate sustained will be seen from Table 8 to be considerable, amounting to approximately half that originally incorporated in the mixture. There appears to be no significant difference between initial, storage, or total loss of ammonium bicarbonate and the size of the cube, but it may be generally stated that the loss of ammonia from cubes stored in 5 ply bitumin lined paper bags was less than from those stored in jute sacks during both transit and storage. This may be accounted for by the fact that this heavy type of paper bag provides the cubes with less ventilation than the jute sack. The cubes contained in 3 ply paper bags, when compared with those in jute sacks, showed only a small increased ability to retain ammonia during the period of transit.

Table 9.

Ammonium sulphate cubes.

Period before sampling (weeks)	Moisture content %	Total N.	% increase	N.P.N.	% increase or loss	Free NH_3-N	% loss
Initial	14.0	2,680		834		731	
3	13.8	2,716	1.3	857	+2.8	730	
7	15.4	2,794	4.3	889	+6.6	736	
10	16.0	2,766	3.2	871	+4.4	727	0.5
13	16.2	2,765	3.2	869	+4.2	721	1.4
16	15.2	2,812	4.9	861	+3.2	717	1.9
21	14.3	8,825	5.4	828	-0.7	696	4.8
30	13.7	2,857	6.6	819	-1.8	665	9.0

Table 10.

Urea mixture.
(Average of the 7 storage types).

Period before sampling (weeks)	Moisture content	Total N	% increase	N.P.N.	% increase or loss.
Initial	14.0	3,132		1,251	
3	13.1	3,142	0.3	1,257	+0.5
7	14.4	3,183	1.6	1,289	+3.0
10	15.0	3,161	0.9	1,259	+0.6
13	15.0	3,213	2.6	1,250	-0.1
16	14.8	3,223	2.9	1,241	-0.8
21	13.7	3,289	5.0	1,214	-3.0
30	13.4	3,300	5.4	1,214	-3.0

(c) Ammonium sulphate.

The cubes containing ammonium sulphate maintained a fairly good condition throughout the period of storage. Traces of mould growth were evident after 16 weeks storage, but there was no general mould deterioration. Losses of nitrogen from these cubes were small and markedly less than those from the cubes containing either ammonium bicarbonate or urea. There was an apparent increase in total nitrogen, progressive throughout the storage period, and amounting to a 6.6% increase after 30 weeks storage. Over the same period there was a small decrease of 1.8% in N.P.N. (equivalent to a 0.6% loss in total nitrogen). Analytical values for this mixture are recorded in Table 9 and Fig.1.

(d) Urea.

Estimations carried out for the sampling periods between 0-30 weeks gave average values for the 7 urea storage types of moisture content, total nitrogen and N.P.N. shown in Table 10. As with the cereal and ammonium sulphate cubes a progressive increase in total nitrogen appeared to occur throughout the 30 week storage period and, for the urea cubes, ultimately amounted to a 5.4% increase. A loss of 3.0% in the N.P.N. (equivalent to a 1.2% loss in total nitrogen) was observed. The amount of urea-N, free ammonia-N, and urea + free ammonia-N was estimated for each

Table 11.

Conversion of urea to Ammonia.
(Average of the 7 storage types).

Period before sampling (weeks)	Urea-N	% increase or loss	Free NH_3-N	Urea + Free NH_3-N	% increase or loss
Initial	1,043		49	1,092	
3	1,058	+1.4	43	1,101	+0.8
7	1,072	+2.8	54	1,126	+3.1
10	1,055	+1.2	60	1,114	+2.0
13	1,002	-3.9	87	1,089	-0.3
16	990	-5.1	96	1,086	-0.6
21	716	-31.4	297	1,012	-7.3
30	578	-44.6	404	982	-10.1

(A loss of 10.1% urea + free NH_3-N is equivalent to a 3.4% loss in total nitrogen.)

Table 12.

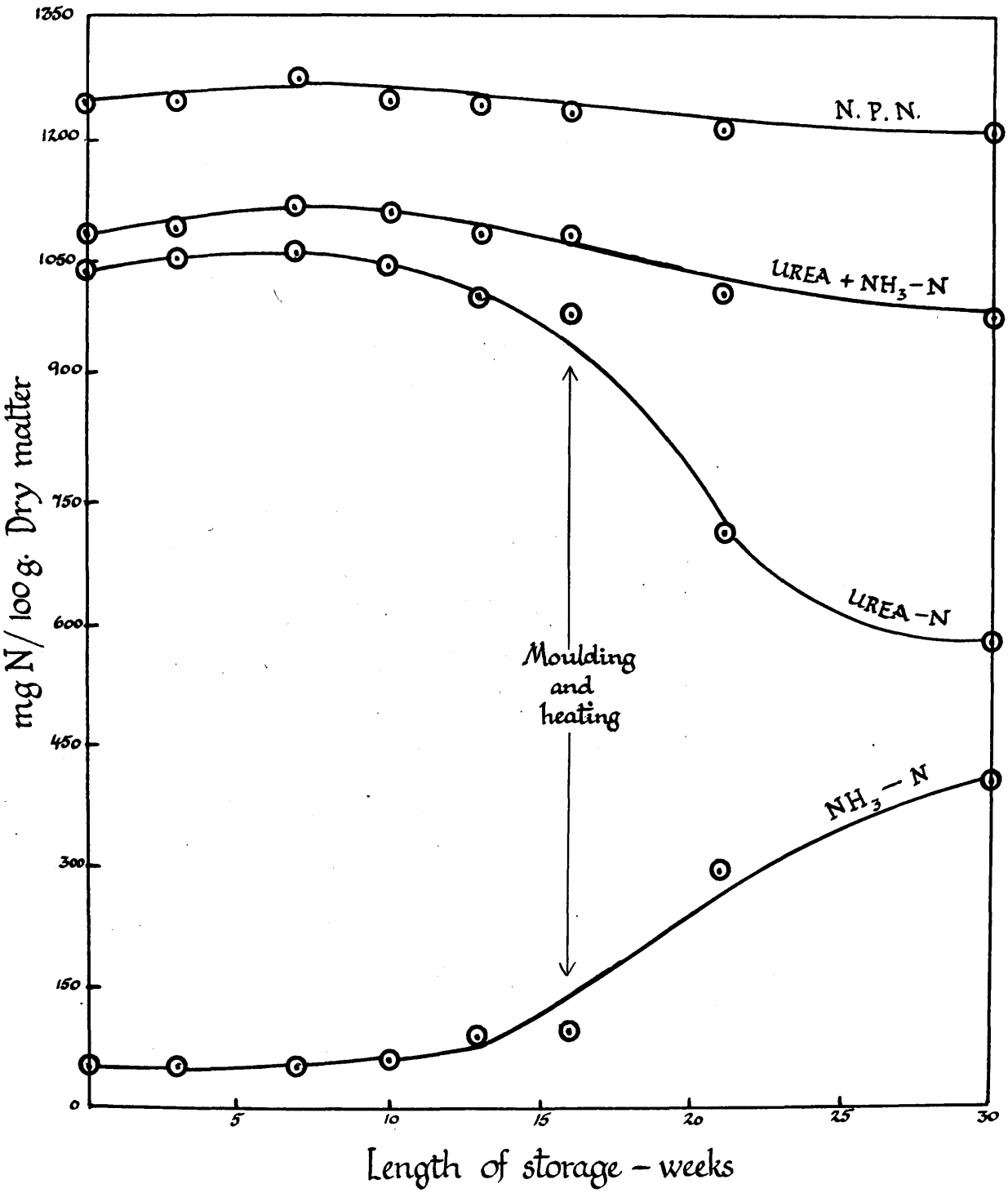
Relationship of moulding and heating to urea hydrolysis.

Storage type	Condition of cubes after 21 weeks storage	% conversion of urea after 21 weeks
Pellets, Jute	No moulding, no heating.	8.7
3/8" 5 ply	Slight moulding, no heating.	11.7
3/8" 3 ply	Badly moulded and heated.	45.9
1/2" 3 ply	Very badly moulded and heated.	52.1

storage type but average figures only for these 7 storage types are given in Table 11.

Tables 10 and 11 and Fig.2 illustrate that there was no measurable conversion of urea to ammonium salts during the first 10 weeks of storage. During the period 10-16 weeks the conversion was only slight, amounting to a 0.8% decrease in N.P.N. and a 5.1% drop in urea-N. After 21 weeks, following the extensive development of moulding and heating in many of the sacks containing the urea cubes, conversion of urea to ammonium salts was considerable and, in those types that showed the most deterioration, as much as half the total amount of urea introduced was hydrolysed (see Table 12). Slight moulding and no heating of the cubes resulted in only a small amount of urea being hydrolysed, while extensive moulding and excessive heating brought about considerable conversion of urea to ammonium salts. Moulding was delayed in the pellets stored in jute sacks and in the 3/8" cubes in 5 ply bags until the 21-30 week period, after which all storage types showed that considerable hydrolysis of urea had taken place, amounting to an average of 44.6% over the 30 weeks of storage. Some of the ammonia of the ammonium compounds formed in this way was lost, the loss being equivalent to 1.2% of the total nitrogen when measured by decrease in N.P.N. and to 3.4% of the total nitrogen if measured by drop in urea - free ammonia-N.

FIG. 2 Changes in nitrogen values of urea cubes during 30 weeks storage.



Laboratory storage of small samples of cereal feeding-stuffs containing urea.

The farm storage experiment outlined above had shown that the extent of urea hydrolysis in these cubed feeding-stuffs was determined by the temperature conditions to which these mixtures were subjected and also by the extent of mould deterioration. It was assumed that such hydrolysis was brought about by enzymes in the cereal constituents under suitable temperature conditions or by the katabolic action of enzymes produced by the developing micro-organisms. A laboratory experiment was therefore planned whereby 1 g. (dry wt.) samples of a cereal mixture containing urea were exposed in small petri dishes to an atmosphere of 70% R.H. controlled by a sulphuric acid solution of known specific gravity (Wilson, 1921). These samples were incubated at 0, 15.5, 22, 37 and 55°C and analyses were carried out at appropriate intervals to estimate N.P.N., urea-N, and urea + free ammonia-N.

The samples stored at 0°C remained perfectly fresh throughout the whole period of storage (273 days). Those stored at 15.5°C remained free from moulding for the first three months of storage but developed a fine mould mycelium after 128 days which, even at the time of the final sampling, had not developed to any great extent. Mould mycelium developed on the samples stored

Table 13.

Decreases in N.P.N. and urea-N in 1 g. samples stored at 70% R.H.

Non-protein Nitrogen.

Length of storage (days)	55°C	37°C	22°C	15.5°C	0°C
Initial	1,291	1,291	1,291	1,291	1,291
28	1,119(13.1)	1,238(4.1)	1,272(1.5)	1,311	1,302
60	956(26.0)	1,206(6.6)	1,232(4.6)	1,262(2.2)	1,299
92	864(33.1)	1,189(7.9)	1,183(8.4)	1,258(2.6)	1,270(1.6)
123	832(35.6)	1,178(8.8)	750(41.9)	1,240(4.0)	1,265(2.0)
273	598(53,7)	1,046(19.0)	593(54.1)	1,194(7.5)	1,199(7.1)

Urea-Nitrogen.

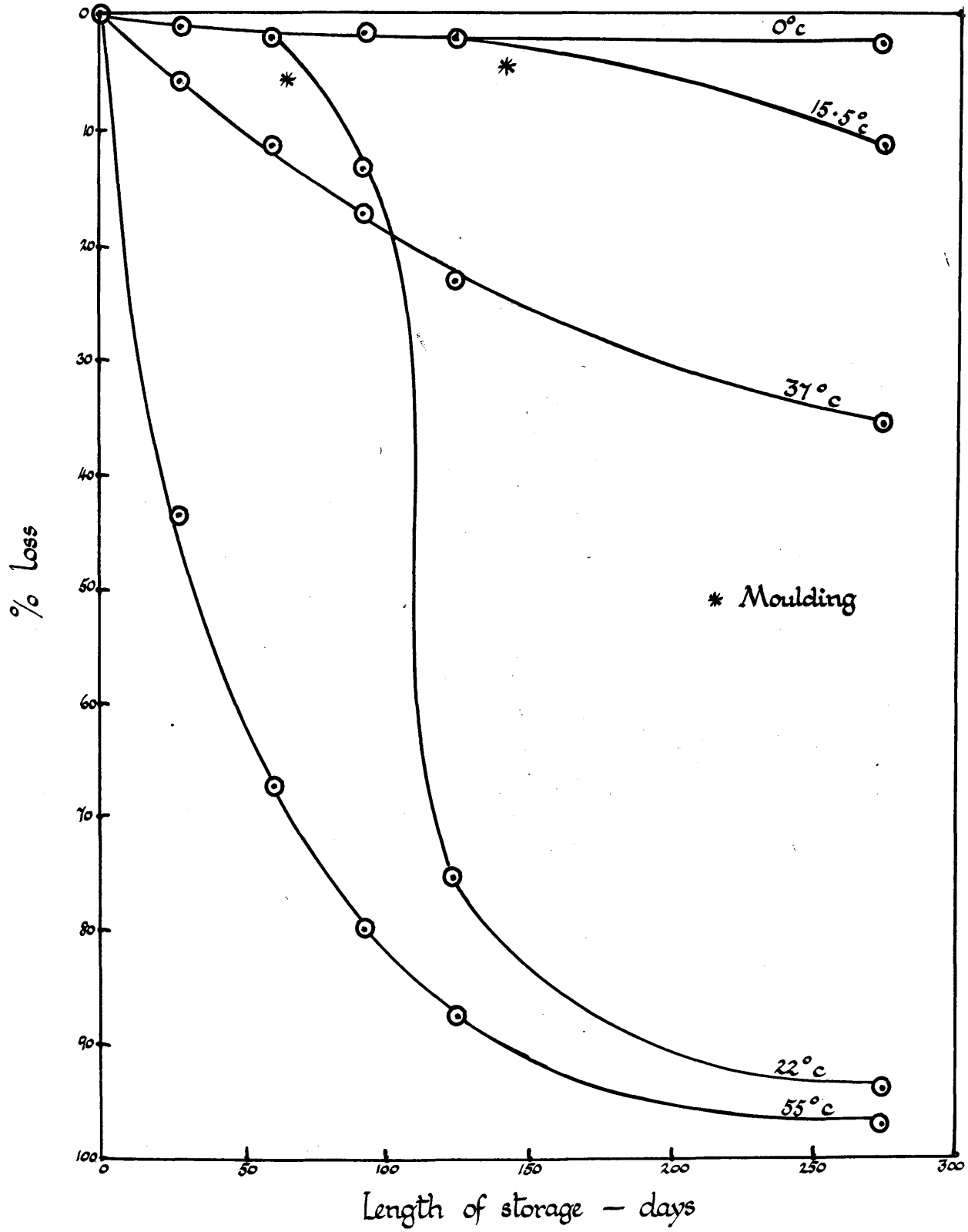
Initial	1,039	1,039	1,039	1,039	1,039
28	588(43.4)	979(5.8)	1,028(1.1)	1,046	1,041
60	340(67.3)	922(11.3)	1,019(1.9)	1,019(1.9)	1,044
92	215(79.3)	861(17.1)	898(13.6)	1,026(1.3)	1,024(1.4)
123	131(87.4)	802(22.8)	258(75.2)	1,019(1.9)	1,020(1.8)
273	30(97.1)	668(35.7)	62(94.1)	922(11.3)	1,010(2.8)

% loss figures shown in brackets.

at 22°C after 60 days of storage and, at this optimum temperature for mould growth, rapidly produced a dense network of hyphae and mould fructifications during the later period of storage. The samples stored at the two higher temperatures showed no signs of moulding within the duration of the experiment although they darkened in colour showing typical characters of heating resulting from the high temperature of incubation.

It will be seen from Table 13 and Fig.3 that those samples which were exposed to the most heating (i.e. those stored at 55°C) rapidly lost their incorporated urea, approximately one half being hydrolysed after only 28 days storage and almost the whole amount incorporated was hydrolysed at the time of the final sampling. The samples stored at 37°C did not suffer heating to as great an extent as those incubated at 55°C and, consequently, the rate of urea conversion proceeded more slowly. At the end of the experiment one third of the urea contained in these samples had been hydrolysed. With the samples stored at temperatures more typical of Britain (22° and 15.5°C) the urea conversion was small for the early part of storage but, with the onset of moulding a marked hydrolysis of urea and a consequent drop in N.P.N. took place. This was of particular note with the samples incubated at 22°C which, by the end of the experiment had lost as much urea due to mould development as had

FIG. 3 Rate of loss of urea in 1 g. samples.



those samples stored at 55°C which had experienced excessive heating. At 0°C there was no measurable conversion of urea after 60 days, while at the end of the storage experiment a conversion of only 2.8% was measured. Such a small value might be due to experimental error.

These farm storage and laboratory experiments with cereal mixtures containing urea indicate that there is only a small amount of urea hydrolysis as a result of enzyme activity within the fresh feeding-stuff mixtures stored at typical atmospheric temperatures. Therefore, if these mixtures are of good manufacture and stored under dry conditions such losses will be of little practical importance. Deterioration of the cubes either by moulding or heating in the sacks will, however, bring about a considerable conversion of urea to ammonium salts, some of the ammonia of which will be lost from the feeding-stuff.

Apparent increases in nitrogen in feeding-stuffs stored on the farm.

Throughout the storage period apparent increases in nitrogen were measured in some of the estimations. These increases are of particular note in the figures for the total nitrogen of the cereal (Table 3), urea (Table 10), and ammonium sulphate cubes (Table 9); and in those figures relating to the first 16 weeks of storage for the N.P.N., urea-N, and urea + free ammonia-N of the cubes containing urea (Tables 10 and 11),

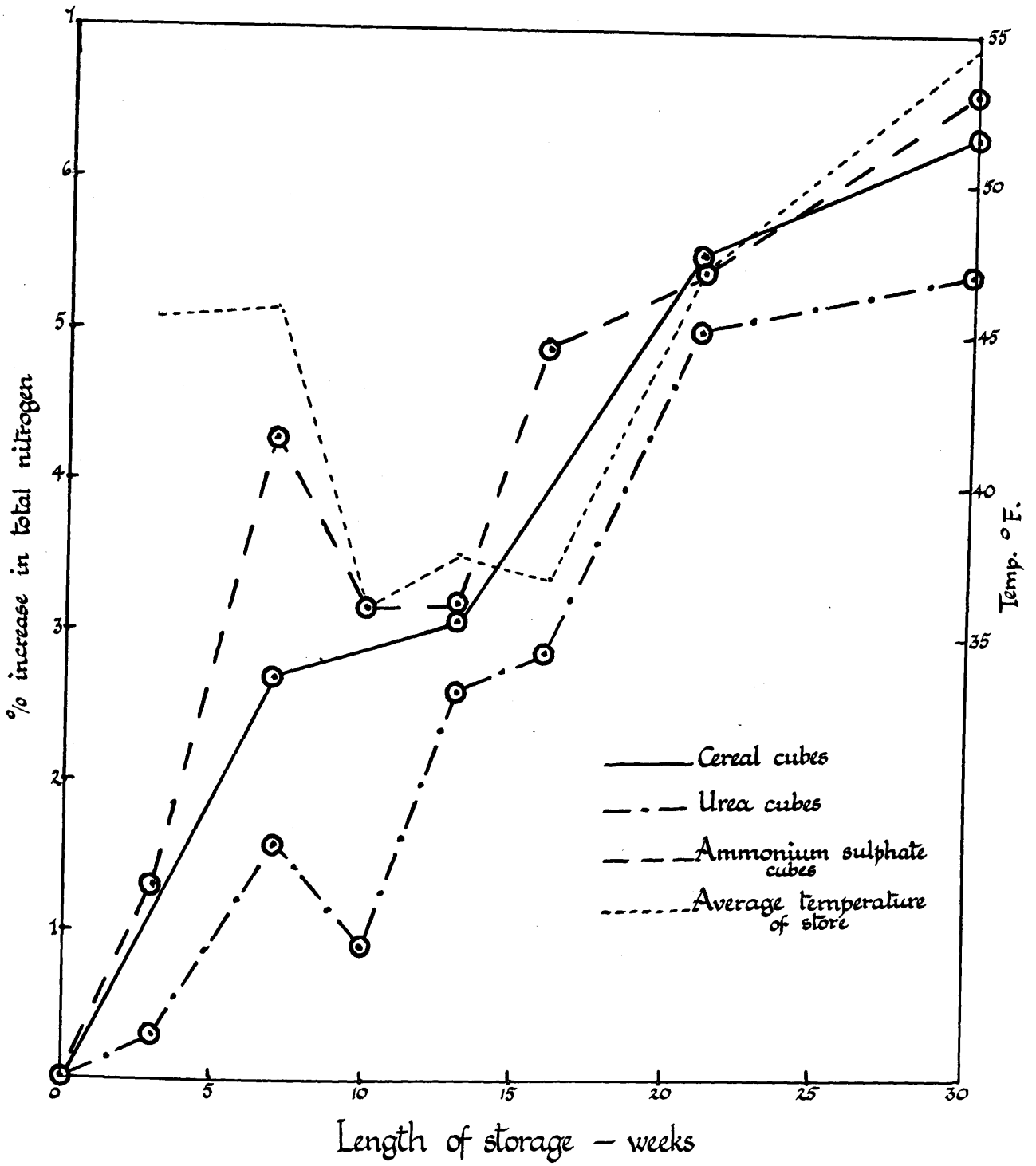
and for the N.P.N. of the ammonium sulphate cubes (Table 9). It is thought that the values for the ammonium bicarbonate cubes did not show these apparent increases in total nitrogen and N.P.N. owing to the magnitude of the ammonia loss throughout storage and particularly during the first three weeks. During the three weekly periods between sampling, however, when the relative humidity had been high and the loss of ammonia consequently small, e.g. periods 3-7 weeks and 10-13 weeks, measurable increases took place in total nitrogen, N.P.N. and free NH_3-N (see Tables 5 and 6). Also, it is assumed that the losses of nitrogen from the cubes containing ammonium sulphate and urea after 16 weeks of storage counteracted any measurement of apparent increases in the N.P.N. values (Tables 9 and 10).

Such increases in total nitrogen might be due to:- (1) fixation of atmospheric nitrogen by micro-organisms present in the stored materials; (2) deficiencies in the Kjeldahl method of estimating nitrogen; (3) a loss of dry matter from these feeding-stuffs. Fixation of atmospheric nitrogen in the quantity necessary to obtain the observed increases is not considered to be feasible. Nor does it seem likely that nitrogen exists in feeding-stuffs in forms which cannot be estimated by the Kjeldahl method, and that these forms become changed on storage. Later experiments

with bran (see Part II of this thesis) have shown that these increases in nitrogen were undoubtedly due to losses of dry matter from the feeding-stuffs. Such losses might be brought about either by the action of enzymes in the cereal constituents, by insect infestation, or by the activity of micro-organisms in the cubed mixtures. In the main such activity would effect a breakdown of some of the carbohydrate material, some of which would be lost as gaseous carbon dioxide and water. Thus the proportion of nitrogen to carbohydrate in the feeding-stuffs would increase, and analytical values calculated on a dry matter basis would show an apparent increase in nitrogen.

The apparent increases in total nitrogen were found to be progressive throughout the 30 week period as is shown by Fig.4. The graphs show a levelling of the values for total nitrogen increase between the period 7-13 weeks. During this period, from the beginning of January until the middle of February, the lowest average temperatures were recorded and averaged 36.8°F for the whole period. At such temperatures enzymatic, insect and microbial activity would be reduced and consequently losses of dry matter would be very small. The decrease in total nitrogen values for the urea and ammonium sulphate mixtures during this period (7-13 weeks), when dry matter losses would be reduced, was presumably due to the slight loss of ammonia from these cubes. The greatest

FIG. 4 Apparent increases in total nitrogen.



apparent increase in total nitrogen occurred during the period 16-21 weeks, when the heating and moulding of the sacks was most extensive. Such deterioration would increase the enzymatic and katabolic processes resulting in the most marked losses in dry matter. The levelling of the urea curve between 21 and 30 weeks has almost certainly resulted from loss of ammonia formed from urea.

Conclusion.

1. Ammonium bicarbonate, ammonium sulphate and urea were introduced into cereal feeding-stuffs which were stored under farm conditions for 30 weeks.
2. Between 44 and 63% of the ammonium bicarbonate incorporated in the cubes was lost as ammonia during manufacture and the subsequent storage period. The size of the cube did not affect the amount of ammonia lost, although there was some indication that cubes stored in 5 ply paper bags lost less ammonia than those in jute sacks.
3. Losses of nitrogen from cubes containing ammonium sulphate were very small both during manufacture and the storage period.
4. Urea is considered to be the most suitable non-protein nitrogenous compound for incorporation into such feeding-stuffs. No significant losses of ammonia were sustained from these cubes until deterioration of the cubes occurred with the development of moulding and heating.

5. Apparent increases of up to 6% in total nitrogen (calculated on a dry matter basis) were recorded for some of the mixtures throughout the storage period. These were considered to be due to losses of dry matter from the feeding-stuffs.

PART II. THE STORAGE OF BRAN.

In the experiment outlined in Part I on the farm storage of feeding-stuffs with incorporated non-protein nitrogen compounds apparent increases in total nitrogen were recorded throughout the storage period. In the main these were attributed to a loss of dry matter resulting from enzymatic and microbial activity. Such losses (estimated at about 5% of the total dry matter for these feeding-stuffs) would be of economic importance if sustained by feeding-stuffs in commercial stores. It was also evident that with any other storage experiments of this nature (e.g. estimation of carotene in dried grass meal) the accuracy of any particular analytical value determined throughout a storage period would depend on the amount of dry matter remaining constant or on the loss of dry matter being known. It was therefore decided to investigate the rate of dry matter loss by storing bran as a typical feeding-stuff under carefully controlled conditions.

(a) STORAGE OF BRAN AT DIFFERENT MOISTURE CONTENTS IN CLOSED CONTAINERS.

A quantity of bran (containing 16% moisture) was divided into three samples and the samples were damped to three levels of moisture content. Sample A initially contained 16.0% moisture, sample B 19.2% and sample C 25.4%. The samples, each of about ¼ cwt., were introduced into large tins which were then

placed in a container which provided lagging for the cans by means of an 8" surround of fine sawdust.

Representative samples from the three tins were taken at suitable intervals over a 39 week storage period. Notes were made on the condition of the bran at the times of sampling. All three samples showed mould deterioration and some fermentation during the period of storage and this deterioration developed sooner and was most extensive on the moister samples. There was also some indication of infestation with mites during the early part of storage. These samples were analysed for moisture content, total nitrogen, N.P.N., total phosphorus, titratable acidity and pH value.

Analytical results.

1. Gas analyses.

Before each sampling a sample of gas was removed from each tin and estimations of the concentration of oxygen and carbon dioxide were made using an Orsat-Fischer gas analysis apparatus. These analyses showed that during the periods between sampling the oxygen in the tins was almost completely used up, due to respiration of the moist bran and the metabolic processes of the developing micro-organisms and mites, and replaced by carbon dioxide. The fact that the carbon dioxide concentrations sometimes exceeded 20% was probably due to differential diffusion of the

Table 14.

Gas analyses from tins containing bran at different moisture contents.

Period before sampling (weeks)	% carbon dioxide			% oxygen		
	Tin A	Tin B	Tin C	Tin A	Tin B	Tin C
Initial	0	0	0	21	21	21
2	9	22	23	8	0	0
9	20	22	23	4	1	0
18	-	23.5	23.5	-	0	0.5
39	24	23	22	1	1	1

Table 15.

Increases in moisture content of bran stored in closed containers.

Period before sampling (weeks)	% moisture content		
	Tin A	Tin B	Tin C
Initial	16.0	19.2	25.4
2	16.1	19.2	25.3
9	18.0	21.0	27.9
18	20.4	24.0	31.2
39	25.3	28.7	35.4

Table 16.

Apparent increases in total nitrogen values (mg.N/100 g. dry matter) of bran stored in closed containers.

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	2,873	2,875	2,861
2	2,825	2,892 (0.6)	2,894 (1.2)
9	2,947 (2.6)	2,993 (4.1)	3,150 (10.1)
18	3,071 (6.9)	3,252 (13.1)	3,401 (18.9)
39	3,563 (24.0)	3,814 (32.7)	4,073 (42.4)

% increase figures in brackets.

various gases through the bran and also through a small cotton wool plug in the lids of the tins.

2. Moisture content.

Throughout the period of storage the moisture content of the bran in the three tins increased. This increase, amounting to as much as 10% moisture for each of the three samples of bran over the 39 weeks storage period, was presumably due to the respiration of the bran itself and also to the action of micro-organisms. The high moisture levels at which these samples were stored would result in extensive breakdown of the carbohydrate fraction of the bran to carbon dioxide and water. Increases in the concentration of carbon dioxide in the tins have been recorded in Table 14, and in moisture content in Table 15.

3. Total nitrogen.

Analytical figures for total nitrogen were reduced to a 100% dry matter basis for each of the samples of bran taken at the various sampling periods (see Table 16). On this basis all the samples of bran showed apparent increases in total nitrogen which were progressive throughout the storage period. Furthermore, the extent of these increases was related to the amount of moisture present in the different bran samples, and the most substantial increases took place after extensive mould deterioration and fermentation had occurred (i.e. during the later part of the storage period). In the sample of bran of highest moisture

Table 17.

Change in N.P.N. (mg.N/100g. dry matter) of bran stored in closed containers.

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	244	238	244
2	238	204	199
9	212	184	332
18	188	229	478
39	344	631	1,310

content (Tin C) the total nitrogen value was over 40% greater at the end of the period of storage than at the start of the experiment. It must, of course, be realised that this exceptionally high figure was only obtained after extensive mould deterioration and fermentation had occurred. Under normal conditions of storage such a high loss of dry matter would be most unlikely.

4. Non-protein nitrogen.

During the early part of storage a loss of N.P.N. was measured for all three bran samples but, with the extensive development of mould and other deterioration, the N.P.N. values increased (see Table 17). Thus with the sample containing the highest moisture content (Sample C) the N.P.N. value, though depressed after 2 weeks storage, showed an increase at all subsequent sampling periods. Sample B showed a reduced N.P.N. value until after 18 weeks of storage after which it markedly increased; sample A, which was the least moist sample and, consequently, the sample in which extensive deterioration was most delayed, showed the longest period of storage during which the N.P.N. value decreased and, by the end of the experiment, the N.P.N. value for this sample had shown the smallest rise of all three samples. This fall in N.P.N. during the early part of storage may be partly accounted for by the volatile nature of some of the non-protein nitrogen compounds and partly by the fact that, during

Table 18.

Apparemt increases in total phosphorus (mg. P./100g. dry matter) of bran stored in closed containers.

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	1,222	1,223	1,225
2	1,312 (7.4)	1,294 (5.8)	1,345 (9.8)
9	1,358 (11.1)	1,357 (11.0)	1,394 (13.8)
18	1,423 (16.4)	1,463 (19.6)	1,502 (22.6)
39	1,602 (31.1)	1,688 (38.0)	1,830 (47.2)

% increase figures in brackets.

the early stages of microbial activity, non-protein nitrogen is probably utilised by the growing micro-organisms. With their more extensive development, however, they bring about an increase in N.P.N. by the breakdown of more complex nitrogenous compounds to simpler derivatives such as ammonia.

5. Total phosphorus.

In view of the measurement of apparent increases in total nitrogen, presumably due to losses of dry matter, in the farm storage experiment with cubed feeding-stuffs containing incorporated non-protein nitrogen compounds it was considered important in this series of experiments with bran to estimate an analytical value that would not be affected by respiratory enzymes in the stored material or by the action of micro-organisms. Estimations of total phosphorus were therefore carried out on all samples using the colorimetric method of Fiske and Subbarow (1925), and a Hilger's Spekker photoelectric absorptiometer. The phosphorus content of bran forms a high proportion of the ash fraction and it is most unlikely to be lost from the feeding-stuffs during mould deterioration or fermentation. The analytical results showed that, throughout the period of storage, apparent increases in total phosphorus (calculated on a 100% dry matter basis) took place (see Table 18). These increases, moreover, were of comparable magnitude to the apparent increases in total nitrogen for the same samples.

Table 19.

Changes in total nitrogen values (calculated as a percentage of that weight of dry matter containing the initial amount of phosphorus).

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	2,873	2,875	2,861
2	2,630 (8.5)	2,732 (5.0)	2,635 (7.9)
9	2,651 (7.7)	2,697 (6.2)	2,768 (3.3)
18	2,636 (8.3)	2,717 (5.5)	2,774 (3.0)
39	2,716 (5.5)	2,763 (3.9)	2,726 (4.7)

% loss figures in brackets.

Table 20.

Changes in protein nitrogen values (calculated as a percentage of that weight of dry matter containing the initial amount of phosphorus).

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	2,629	2,637	2,617
2	2,407 (8.5)	2,540 (3.7)	2,456 (6.2)
9	2,461 (6.4)	2,531 (4.0)	2,476 (5.4)
18	2,476 (5.8)	2,528 (4.1)	2,385 (8.9)
39	2,455 (6.6)	2,306 (14.4)	1,850 (29.3)

% loss figures in brackets.

It is evident, therefore, that the calculation of analytical values on a 100% dry matter basis is liable to misinterpretation where changes in the condition of the stored feeding-stuffs are liable to occur either by normal respiratory processes of the material itself or by microbial activity. A more correct indication may be obtained of the total nitrogen value of such samples by its calculation as a percentage of that weight of dry matter containing the initial amount of phosphorus. When these values are used (See Table 19) it is seen that instead of an apparent increase in total nitrogen a loss of nitrogen was sustained by all three samples of bran during storage. If the N.P.N. values for these samples are subtracted from the total nitrogen values and the resulting figures calculated as a percentage of that weight of dry matter containing the initial amount of phosphorus, it can be seen that the losses of protein nitrogen from these samples of bran amount to 6.6, 14.4 and 29.3% respectively for the samples at the three moisture levels (see Table 20). The sample containing the highest percentage of moisture experienced the highest protein breakdown. It is possible that the figures given in Tables 19 and 20 do not represent the complete losses in total nitrogen or in protein nitrogen from the bran because these figures may include some nitrogenous compounds synthesised by the developing micro-organisms from atmospheric nitrogen.

Table 21.

Changes in titratable acidity values (cc.N.NaOH required to neutralise acids from 100 g. dry matter).

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	11.61	10.39	12.51
2	12.03	9.44	7.31
9	6.91	6.65	9.95
18	2.15	4.84	13.87
39	5.69	7.69	19.68

It has been shown by Latham (1909) that some mould species are capable of such synthesis. This may account for the losses in total nitrogen, recorded in Table 19, after 39 weeks being smaller than those after 2 weeks, for microbial activity was limited during the first two weeks but extensive during the later period of storage.

6. Titratable acidity.

An indication of the acid fractions contained in the three bran types throughout the period of storage was obtained by measuring the titratable acidity of a 67% alcoholic extract of the samples according to the method of Schulerud (1932). The suitability of various methods of acidity estimations for samples of feeding-stuffs are discussed in Part III of this thesis but the method of Schulerud (1932) was chosen for these samples because it gave a maximum acidity value representative of amino acids, free fatty acids and acid phosphates present in the material. At all three moisture content levels a marked reduction in the titratable acidity was observed with the development of mould deterioration (see Table 21). Experiments carried out at a later date with 1 g. samples of bran showed this to be due partly to the utilization of free fatty acids by the micro-organisms. The titratable acidity value will, however, also be reduced by the appearance of mould by-products many of which are alkaline in reaction. During the later period of

Table 22.

Changes in pH of bran stored in closed containers.

Period before sampling (weeks)	Tin A	Tin B	Tin C
Initial	5.77	6.07	5.87
2	5.82	6.09	6.19
9	6.25	6.71	6.80
18	7.26	6.89	6.81
39	7.42	8.15	8.03

storage where fermentation was proceeding rapidly the titratable acidity increased in all samples and was greatest for the sample of highest moisture content (Tin C).

7. pH values.

The pH value of each sample of bran was estimated at the different sampling periods according to the official method (Cereal Laboratory Methods, 1941). Bran has a high buffer value amounting, for these samples, to 13 ml. N/50 acid or alkali required to give a unit change in pH (Cereal Laboratory Methods, 1941). This will minimise any variations in pH. However, all samples showed an increased pH towards the alkaline side of neutral as moulding and general deterioration proceeded (see Table 22). This finding confirms the observations of Sharp (1924). It will be seen that these increases in pH towards the alkaline side of neutral were, for some samples, accompanied by increases in the acid fractions of the bran measured by the titratable acidity. These two facts appear to be contradictory, but it must be remembered that the pH is measured on the whole bran material while the titratable acidity is measured on an alcohol extract of the acid fractions which may not include all the mould by-products many of which are alkaline in reaction.

(b) FARM STORAGE OF BRAN IN SACKS.

A newly milled sample of fresh bran was obtained of 13.0% moisture content. The moisture content of two sacks of the bran was adjusted to 16.5% and 18.4% moisture, respectively, by adding water and thoroughly mixing the samples. Representative initial samples of each of the three types were taken and the sacks were then weighed on a weighing machine on which there was an error of ± 0.1 kg. The sacks were allowed to stand for a period of 16 weeks in the farm store, described on page 15 . At weekly intervals the sacks were re-weighed and changes in weight recorded. Daily readings of the temperature and humidity of the store were taken. Notes were made of the condition of the bran in the three sacks at the time of the weekly weighing. Type I (initially containing only 13% moisture) remained in fresh condition throughout the storage period. Type II (initially containing 16.5% moisture) developed heating after 8 weeks storage and type III (initially containing 18.4% moisture) heated after only 4 weeks storage. At the end of the storage period the samples were emptied out of the sacks and thoroughly mixed. The heating of the bran in Types II and III caused compaction of the bran into hard lumps. Representative samples of each type were taken at the beginning and at the end of the storage period and were analysed for moisture content, total nitrogen, non-protein nitrogen and total phosphorus.

Changes in weight of sacks of bran during storage.

The sack (Type I) containing bran at the lowest moisture content (initially 13%) showed the least change in weight. Fig.5 shows how the weight of this sack was influenced by the variation in humidity of the store. After 16 weeks storage its weight showed a small change from 52.2 Kg. to 53.0 Kg. This could be attributed to the rise in moisture content of the bran from 13% at the start of the experiment to 14.6% at the end. The weights of dry matter in the sack initially (44.8 Kg.) and finally (44.6 Kg.) showed a loss of dry matter of only 0.4%. This is within the experimental error of the weighings.

The sacks containing bran at the higher levels of moisture content (Types II and III), on the other hand, showed a marked loss of weight during the period of storage. This loss occurred sooner in the sack containing the moister bran of 18.4% moisture (Type III) and was of greater extent than in the sample that initially contained 16.5% moisture (Type II) - see Fig.5. The loss of weight in these two sacks took place at the the time of and after heating of the bran. The fresh weight of Type II fell from 51.2 Kg. to 49.9 Kg. and of Type III from 55.3 Kg. to 51.6 Kg. While some of this weight loss can be accounted for by loss of water vapour (the moisture content of Type II fell from 16.5% to 14.8% and of Type III from 18.4% to 15.1%) it cannot

FIG. 5 Change in weight of sacks of bran during storage.

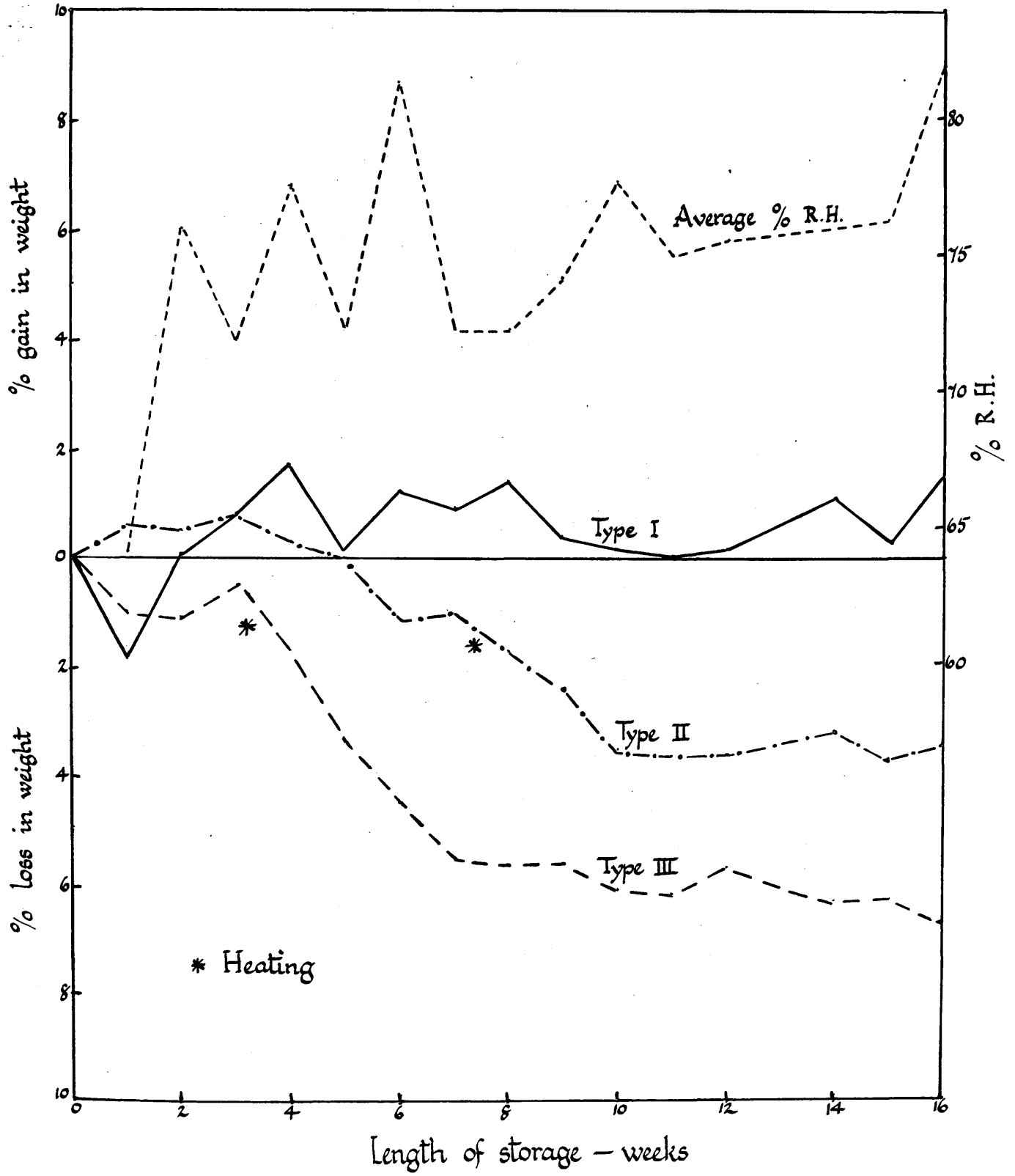


Table 23.

Analytical results of bran stored in sacks on farm.

Total Nitrogen (mg N/100 g. dry matter).

	I	II	III
Initial	2,520	2,443	2,445
After 16 weeks storage	2,523	2,528 (3.5)	2,527 (3.4)

N.P.N. (mg N/100 g. dry matter).

Initial	221	216	214
After 16 weeks storage	196	162	147

Total phosphorus (mg P./100 g. dry matter).

Initial	1,114	1,111	1,126
After 16 weeks storage	1,144 (2.7)	1,147 (3.2)	1,162 (3.2)

% increase figures in brackets.

be completely accounted for in this way. The weights of dry matter contained in the sack Type II fell from 43.2 Kg. to 42.5 Kg. - a dry matter loss of 1.5%, while that contained in the sack Type III fell from 45.1 Kg. to 43.7 Kg. - a dry matter loss of 3.0%. It would appear, therefore, that the increased respiration of the bran at these two higher moisture levels resulted in heating and consequent loss of dry matter.

Analytical results.

Results of estimations made to determine total nitrogen, N.P.N., and total phosphorus in samples taken at the start and at the end of the storage period are given in Table 23. These figures are in agreement with those for the samples of bran stored in closed containers described previously. All three types showed a loss in N.P.N. after 16 weeks storage. Types II and III showed an apparent increase in both total nitrogen and total phosphorus of about 3%. These apparent increases can be attributed to the losses of dry matter which occurred when the two sacks heated. As shown above these losses were also recorded by weighing.

(c) LABORATORY STORAGE OF 1 g. SAMPLES OF BRAN AT CONTROLLED HUMIDITY AND TEMPERATURE.

The chemical changes in the composition of bran occurring during storage deterioration were also investigated under the more carefully controlled conditions of humidity and temperature which are

Table 24.

Chemical analyses of 1 g. samples of bran after 4 weeks storage at 85% R.H.

Temperature of storage °C	Total Nitrogen (mg N/100g. dry matter)	N.P.N.	Total Phosphorus (mg P/100g. dry matter)	% Ash	% Ether extract
0	2,440	220	1,057	5.54	3.94
25	2,435	106	1,060	5.46	1.89
50	2,348	143	1,056	5.45	1.86

Table 25.

Changes in acidity of 1 g. samples of bran after 4 weeks storage at 85% R.H.

Temperature of storage °C	A. Titratable acidity (67% alcohol extract)	B. Fat acidity (benzene extract)	Amino acid + phosphate acidity (by subtraction of B from A).
0	7.65	3.23	4.42
25	6.13	1.79	4.34
50	6.83	2.23	4.64

possible with small samples of feeding-stuffs in the laboratory. 1 g. (dry wt.) samples of bran were weighed into small petri dishes which were then exposed in desiccators to an atmosphere of 85% R.H. controlled by sulphuric acid solutions using the data of Wilson, 1921. The desiccators were kept at three temperatures, 0°C, 25°C, and 50°C. After 4 weeks storage the samples at 0°C showed no sign of deterioration; those at 25°C had developed extensive mould mycelium and fructification; and those at 50°C showed a darkening of colour due to the heating effect of this storage temperature and also showed some deterioration by moulds which were resistant to this temperature. At the end of the storage period the samples were analysed in duplicate for moisture content, total nitrogen, N.P.N., total phosphorus, ether extract, ash, titratable acidity, fat acidity and pH. In this way it was possible to obtain some indication of the chemical changes that occurred with extensive moulding (the samples at 25°C) and with heating and some moulding (the samples at 50°C). The results are recorded in Tables 24 and 25.

Analytical results.

(1) Moisture content.

The moisture content of these samples of bran was fixed by the equilibrium reached with the controlled humidity of the atmosphere to which they were exposed. The samples were weighed at the end of the storage

period and from these weights it was possible to calculate the amount of water held at the different temperatures. At 0°C this amounted to 17.28% moisture, at 25°C, 15.38% moisture and at 50°C only 12.03% moisture. Oven drying these samples for three hours gave 17.27%, 18.62% and 16.76% moisture respectively. The discrepancy in the figures for the 25°C and the 50°C samples may be accounted for by the fact that the moisture content figures for the moulded and heated samples include errors due to losses of dry matter during storage and of volatile matter during the moisture content estimation at 100°C. There was no loss of dry matter from the samples stored at 0°C, but a 3.8% loss was recorded for the samples at 25°C and a 5.4% loss for the samples at 50°C.

2. Total nitrogen.

There was no significant change in total nitrogen for the samples stored at 0°C. and at 25°C. over the 4 week storage period, even though the samples at 25°C had suffered mould deterioration. The heated samples stored at 50°C showed a 3.8% loss in total nitrogen. It is of interest to note that if these total nitrogen values had been calculated as a percentage of the dry weight present in the damaged samples after the storage period they would have shown apparent increases in total nitrogen of 3.8% and 7.6% respectively for the 25°C and 50°C samples.

3. Non-protein nitrogen.

The damaged samples of bran showed marked losses of N.P.N., the moulded sample (25°C) showing a reduction to less than one half of the N.P.N. present in the undamaged samples stored at 0°C.

4. Total phosphorus.

There was no significant change during storage in the total phosphorus content of the bran samples, irrespective of any deterioration that took place. It may be noted, however, that had the values for the 25°C and the 50°C samples been calculated as a percentage of the dry matter present in the damaged samples after the storage period, they would have shown apparent increases in total phosphorus amounting to 3.7% and 4.7% respectively.

5. Ether extract.

The development of mould and heating deterioration resulted in a loss of over 50% of the ether-soluble fraction of the bran stored at 25°C and 50°C. The fat is presumably broken down to free fatty acids and used as a source of energy by the developing mould species.

6. Ash.

There was no significant change in the ash content of the samples during the storage period.

7. Acidity values.

It was mentioned on page 35 that acidity in feeding-stuffs is due mainly to three fractions (a) free fatty acids, (b) amino acids, and (c) acid

phosphates. An indication of the total acidity (all three fractions) was obtained by estimating the titratable acidity after extracting these bran samples with 67% alcohol,(Schulerud, 1932), while the amounts of free fatty acids were estimated by the rapid fat acidity method (benzene extract) of Zeleny (1938). The results for these acidity estimations are given in Table 25 and show that there was a marked reduction in the titratable acidity for the damaged samples (stored at 25°C and 50°C). This reduction was even more marked with the figures for the free fatty acid fraction which showed a decrease of almost 50% for the moulded samples stored at 25°C. The utilization of this fraction by developing mould species enables the fat acidity to provide an index of the condition and extent of deterioration of such stored feeding-stuffs. Some indication of the amounts of amino acids and acid phosphates in these samples may be obtained by subtracting the values for fat acidity from those of the titratable acidity (which accounts for all three acid fractions). Table 25 shows that the values for amino acid + phosphate acidity remain constant for the variously damaged samples which suggests that during the early stages of deterioration these two acid fractions are but little affected by moulding or heating.

(d) THE RESPIRATION OF BRAN AT DIFFERENT MOISTURE CONTENTS.

Survey of literature.

The respiration and the development of heating in stored feeding-stuffs has been attributed to two causes; firstly to the respiration of the plant cells still active in the stored feeds themselves, and secondly to the respiratory activity of moulds and other micro-organisms developing on these materials.

The relation of moisture content to the rate of respiration and heating of spring wheat was pointed out by Bailey (1917) whose observations of practical storage conditions showed that wheat of 14.5% moisture would not heat, but that at 15.5% and above heating was liable to occur. Bailey and Gurja (1918, 1920) made laboratory observations on the rate of respiration of wheat of 12 to 17% moisture and the effect of the type, size, and condition of the grain on the respiration rate. Bailey (1921) made similar observations with shelled corn and Smith and Bartz (1932) undertook similar experiments with oats, corn and mixed feeds. Bailey (1940) extended this work to include studies on a variety of cereals and flaxseed, The results recorded by these authors are in general agreement in so far as they confirm the observation that the chief factor controlling the rate of respiration and the heating of feeding-stuffs is the moisture content of the material stored; as the moisture

content was increased so the respiratory rate was accelerated and heating was more liable to occur.

James et al. (1928) attributed heating in corn meal mainly to bacteria and to thermophilic and thermogenic moulds. Gilman and Barron (1930) found that the rise in temperature of mould inoculated cereals at 18% and 20% moisture was greater than that of sterile cereals. Using toluene and carbon tetrachloride Larmour et al. (1935) differentiated between heating due to the respiration of wheat grains themselves and that due to the development of micro-organisms.

This study of the literature failed to reveal any observations on the respiration of bran which, compared with other feeding-stuffs, was known to be very susceptible to heating during storage. It was decided (a) to investigate the effect of the moisture content on the rate of respiration of bran; (b) to make observations on the extent to which developing micro-organisms increased the respiration rate of the bran material itself; and (c) to obtain some indication of the level of moisture content at which bran could be stored without the danger of heating resulting from a high respiration rate.

Experimental.

An apparatus, similar to that described by Hatfield (1931) for observations on the water uptake of samples of wood and on the respiration of wood-

destroying fungi was employed. By its use a continuous stream of carbon dioxide - free air at a fixed humidity could be passed over a weighed quantity of bran of given moisture content. The carbon dioxide respired by the bran was absorbed in standardised N/10 barium hydroxide. Each bran sample was contained in a Dewar vacuum vessel and the temperature of the sample was recorded throughout the respiration period. The air stream was regulated by means of an aspirator which enabled 750 ml. of carbon dioxide - free air to be passed through the apparatus in an hour. Data given by Spencer (1926) in the International Critical Tables for fixed humidities controlled by saturated salt solutions were used, careful note being made of the temperature of the various solutions during the experiment. The use of these solutions enabled the passage of an air-stream at the humidity that was in equilibrium with the moisture content of the bran sample under trial. The moisture content-relative humidity equilibrium curve for bran given by Snow et al. (1944) provided the data for the adjustment of these values. The employment of a continuous air-stream obviated the danger, pointed out by Bailey and Gurja (1919), of reducing the rate of respiration by allowing the accumulation of carbon dioxide in the bran sample under trial. The use of saturated salt solutions to control the humidity of the air-stream in relation to the moisture content of the bran sample was an

improvement on the technique of Larmour et al. (1935) who carried out observations on wheat of various moisture contents with a continuous absorption apparatus, irrespective of changes in the moisture content of the samples during the experiment.

Bran was introduced into the Dewar flask in weighed amounts equivalent to 40 g. of dry matter. The moisture content of the sample was estimated before and after each total period of respiration, which lasted 4-5 days. For the samples of low moisture content, where the respiratory rate was slow, a single estimation was made of the carbon dioxide respired over this whole period. But for samples of high moisture content, which respired rapidly, estimations were made over periods of 5 or 24 hours on each day of the whole respiration period.

After absorption of the respired carbon dioxide in barium hydroxide the excess alkali was titrated with N/10 hydrochloric acid using thymol phthalein as an indicator. No attempt was made to filter off the barium carbonate, formed during the respiratory period, before titration with hydrochloric acid because the presence of carbonate in the titration solution has been shown by Martin and Green (1933) not to affect the result, provided that hydrochloric acid not exceeding this strength is used. The suggestion of Truog (1915) that the solution be well shaken during the titration to prevent local concentrations of acid was also adopted.

Table 26.

Rate of respiration of bran at different moisture levels.

% R.H. of air-stream	Sat. salt solution	% Moisture content		Rate of respiration*
		Before respiration	After respiration	
64)	$NaNO_2$	12.71	12.83	1
64)		12.95	12.96	20
72.6	$NH_4Cl+KNO_3$	14.97	14.98	39
84	KBr	18.87	18.85	76
88	$BaCl_2 2H_2O$	23.69	23.69	624+
90	$ZnSO_4 7H_2O$	27.34	28.04	916+

* mg.CO_2/100 g. dry matter/24 hours.

\+ figure obtained for the first day of respiration period.

Results.

It can be seen from Table 26 that the rate of respiration of bran was directly related to its moisture content. The moisture content of the samples, controlled by the humidity of the air-stream, remained constant over the period of the experiment. Below 13% moisture the rate of respiration was very slow but, as the moisture content increased, so the respiratory rate was accelerated. At 15% moisture content it was double that at 13%, and at 18.9% almost four times that at 13% moisture. At moisture contents within this range (12.7-18.9%) the carbon dioxide evolved was mainly attributable to the respiration of the bran itself because, at the end of the respiration period, the samples showed no signs of mould development. At moisture contents above 20%, however, the rate of respiration was markedly accelerated above that at 18.9% moisture. It was evident that the carbon dioxide evolved at these high moisture content levels was due, not only to the respiration of the bran itself, but also to the development of micro-organisms on the feeding-stuff. The respiration rates for these samples increased throughout the 4-5 day period. Thus the rate of respiration for the 23.7% moisture sample was 624 mg.CO_2 on the first day, on the second it had risen to 1,019 mg. CO_2 and on the fifth day to 6,443 mg. CO_2/100 g. dry matter/24 hours. The sample at 27.3% moisture content showed an even greater rise from

916 mg. CO_2 on the first day, to 1,636 mg. CO_2 on the second day, to 11,860 mg. CO_2 on the third day and eventually to as much as 29,210 mg. CO_2/100 g. dry matter/24 hours on the fourth day. The figures for the second to fourth day are of even greater magnitude than the figure given by Swanson (1935) for the respiration of 35.5% moisture wheat (1,370 mg. CO_2/100 g. dry matter/24 hours). It is probable that bran has a higher respiration rate than wheat owing to the nature of the bran material, which comprises the outer seed coat and the germ of the wheat grain. These portions of the seed are more active than the inert starch grains of the whole kernels. However, increases such as those recorded above for bran of over 20% moisture can only be attributed to the development of moulds and other micro-organisms. Such deterioration was observed microscopically at the end of the respiration experiment.

Conclusion.

In regard to the practical storage of bran it is evident from these experiments that bran stored at a moisture content of less than 13% will have a very low respiratory rate and therefore will not be liable to heating*. Bran of 15-19% moisture content respires at a much faster rate, and the length of time during

*This finding is based on experiments with fresh bran, free from insect infestation. It may be noted that a sample of bran of 12.8% moisture which was infested with mites had a respiratory rate of as much as 288 mg. CO_2/100 g. dry matter/24 hours.

which such material can be stored with freedom from heating will be limited. Samples of bran containing over 20% moisture will show very rapid deterioration resulting from a high respiration rate and microbiological damage.

PART III. EXPERIMENTS ON ACIDITY VALUES IN STORED FEEDING-STUFFS.

Survey of literature.

The various forms of acidic compounds occurring in cereals and cereal products together with methods of their estimation have been summarised by Zeleny and Coleman (1938). Acidity in such feeding-stuffs may be due to (a) acid phosphates, (b) free fatty acids, (c) amino acids. These fractions have been variously estimated by extraction with water, alcohol of different strengths, benzene or ether. Anomalous results have been obtained by different workers because they employed different methods which extracted the acid fractions in different proportions.

The water soluble acidity (Methods of Analysis, 1940), is open to criticism because it does not account for either free fatty acids or amino acids and also because it includes acid phosphates produced as breakdown products from the phytin in the feeding-stuffs during the period of extraction. The Greek or Balland method which involves extraction with 85% alcohol (Cereal Laboratory Methods, 1941) gives low results because acid phosphates are not completely soluble in this solvent. Schulerud (1932) found that both phosphates and fatty acids were easily soluble in 67% alcohol and that this concentration gave the highest acidity value. Zeleny and Coleman (1938) considered

Table 27.

Effect of fineness of grinding on acidity values of bran.

	Water soluble acidity	Fat acidity*	Titratable acidity (60% alcohol)
Bran (finely ground	9.30	8.71	13.01
(coarsely ground	9.22	7.14	11.65

* Rapid method of Zeleny (1938).

that the fat acidity fraction gave the best indication of the condition of cereals since only this fraction increased significantly during the early stages of deterioration. They put forward a method for the estimation of the three separate acid fractions.

Criticism of methods of acid extraction from cereal products.

The methods surveyed above have been used in this present work for the estimation of acidity in bran and in some other feeding-stuffs. The reliability of these methods has been investigated, particularly in regard to variations in the degree of fineness of grinding of the material as well as in the length of time and conditions of the extraction. All results are expressed as ml. of N.KOH (or NaOH) required to neutralise the acids from 100 g. dry matter.

(1) Effect of fineness of grinding.

A sample of bran which had been stored under farm conditions for some months was used in its original coarsely ground state and acidity extraction results compared with those of the same material ground finely. The figures given in Table 27 show that extraction with 60% alcohol gave a higher acidity value than extraction with either water or benzene. The finer ground sample gave higher fat acidity and titratable acidity values than the coarser sample.

(2) Effect of time of extraction.

Zeleny (1938) suggested that the rapid extraction

Table 28.

Fat acidity values of bran.

Period of shaking (hours)	Coarse	Medium	Fine
½	6.29	6.96	6.63
1	6.79	-	7.20
2	7.17	-	7.40
4	7.75	-	7.93
Petroleum extract	8.30	8.75	8.56

of free fatty acids should be carried out on a mechanical shaking device for 30 minutes. Comparable results are stated to have been obtained by shaking by hand for 45 minutes. It was found in the present experiments, however, that an increase in the fat acidity value was obtained for periods of mechanical shaking (revolving wheel type of shaker) up to 4 hours.

A fresh sample of bran was used for fat acidity estimations which were made on (i) the bran in its original coarsely ground state, (ii) the bran medium ground on a coffee mill, and (iii) the bran finely ground on a Christy and Norris laboratory mill.

The rapid method of fat acidity determination (Zeleny, 1938) was used but shaking was carried out mechanically for periods up to 4 hours. Results obtained by this method were compared with those obtained by soxhlet extraction for 16 hours with light petroleum (B.P.40-60°) and are shown in Table 28.

The fat acidity value increased with the length of the shaking period and had not reached its maximum even after 4 hours. It was also noted that the figures obtained after the longest period of shaking were still less than those obtained by soxhlet extraction for 16 hours. For bran and other feeding-stuffs of high free fatty acid content it will be necessary, therefore, to use the soxhlet extraction method in order to obtain the maximum fat acidity

Table 29.

Effect of temperature on fat acidity extracts of bran.

Sample	Soxhlet extract (light petroleum)	Rapid benzene extract	
		Cold	Hot
A	2.95	2.49	3.05
B	0.99	1.01	1.33
C	0.72	0.83	1.09
D	1.84	1.91	2.49

value. Where the fat acidity value is low, as in moulded samples of bran and other feeding-stuffs and in whole cereals and flour samples, such marked differences will not be observed in the fat acidity values obtained by the two methods (see Table 29). Where the rapid method for the estimation of fat acidity is used, care must be exercised in the standardisation of the time of shaking. Such care does not seem to be of so great importance in the titratable acidity extracts with 60 or 70% alcohol, since comparable results were obtained for the same sample of bran using both these strengths of alcohol and shaking for periods of ½ or 1 hour. Zeleny (1938) has emphasised, however, that the concentration of alcohol in the solution titrated is of greater significance than the concentration of alcohol used for extraction. He has suggested extracting with 60-70% alcohol and then increasing the strength to 85% before titration to obtain the maximum value for the amino acid fraction. Results obtained in the present series of experiments confirm this finding.

The figures obtained in this experiment (Table 28) agreed with those obtained in the previous series (Table 27) in regard to the finer ground samples which gave higher fat acidity values than the coarsely ground samples. This increased value was more marked for the medium ground sample than for the finer ground sample. This finding is probably accounted for by the

medium sample being ground 48 hours before the finer sample, although the fat acidity extracts were made at the same time for each sample. It has been pointed out (Cereal Laboratory Methods, 1941), that fat acidity determinations should, where possible, be carried out immediately after grinding because the figures obtained are known to increase rapidly after such treatment.

(3) Temperature of extraction.

The rapid method of fat acidity estimations (Zeleny, 1938) was applied to four different samples of bran, three of which had shown extensive mould deterioration, using benzene at laboratory temperature (17°C) and at 47°C. The bran samples were all dried to contain 1% moisture before the estimations were made. The hot benzene extraction gave a higher value than the cold extraction (see Table 29). Soxhlet extraction with light petroleum for 16 hours gave results more comparable with the cold benzene rapid extraction.

The Change in acidity values of bran stored at different moisture content.

An estimation of the three separate acid fractions for these four samples was made according to the method of Zeleny and Coleman (1938). Sample A contained 16% moisture and had been stored in the ice-chest for 10 months, samples B-D were originally damped to 16, 19 and 25% moisture and stored in closed tins for the same period, after which their moisture

Table 30.

Acidity fractions of damaged bran.

Sample	1 Fat acidity (Soxhlet extract)	2 Amino acid acidity	3 Phosphate acidity	Total acidity (sum of 1, 2 and 3)	Titratable acidity (67% alcohol)
A	2.95	2.79	3.28	9.02	6.01
B	0.99	3.89	2.82	7.70	5.61
C	0.72	3.45	3.63	7.80	5.84
D	1.84	13.28	14.56	29.68	22.21

contents had increased to 25, 29 and 35% respectively. Considerable mould deterioration and some fermentation had taken place in three of these samples (B-D) of bran. All four samples were dried at 100°C until they contained only 1% moisture before acidity estimations were made.

If the values for the three different acid fractions be added together all four samples show a higher figure for total acidity than that obtained from the extraction with 67% alcohol (see Table 30). This indicates that, for these samples, either the method of Zeleny and Coleman (1938) does not ensure complete separation of the phosphate and amino acid fractions by the adjustment of the concentration of alcohol in the solution titrated, or that the acid fractions are not completely extracted by 67% alcohol.

These results do show, however, that the onset of moulding serves to reduce the free fatty acid content of the bran. This fraction is presumably utilised as a source of energy by the moulds as they develop. The fat acidity value does, therefore, not only serve as an index of the age and quality of a feeding-stuff before mould deterioration (Zeleny and Coleman, 1938), but is also an indication of the onset of mould growth. The amino acid and acid phosphate fractions did not show marked differences until extensive moulding and fermentation had caused marked breakdown of proteins, and other bran

constituents as in sample D (Table 30).

Conclusion.

1. The fat acidity fraction has been shown to be a valuable index of the condition of stored feeding-stuffs both before and after the development of mould deterioration.
2. The rapid method of fat acidity estimation (Zeleny, 1938) was found to be unsuitable for application to samples of bran since it was affected by the degree of fineness of grinding, the time and temperature of the extraction and the period during which the sample remained ground before analysis. Soxhlet extraction with light petroleum is suggested as the most reliable method for the estimation of the free fatty acids in these samples.

PART IV. THE WATER-UPTAKE OF FEEDING-STUFFS CONSTITUENTS.

It has long been recognised that humidity and moisture content are the primary factors controlling the condition of stored products, in particular their freedom from moulding. Snow et al. (1944) recorded the minimum humidities at which moulds could develop on feeding-stuffs and laid down safe limits of moisture contents for different feeding-stuffs corresponding to their water-uptake at these humidities. These authors also showed (loc. cit.) that the degree and character of the water uptake of feeding-stuffs could be correlated with the relative amounts of starch, protein and fibre which they contained. For this purpose the water-uptake of these purified feeding-stuff constituents had to be studied. Details of these experiments are given below.

Rice starch, two plant proteins (glutenin and edestin), two animal proteins (egg albumin and casein) and purified fibre obtained from bran* were used for the water uptake experiments. 0.4 g. (dry weight) samples of these materials and of mixtures of these materials in definite proportions were weighed into small glass petri dishes. The samples were then dried

*Extracted according to the method of crude fibre estimation. (Methods of Analysis, 1941).

Table 31.

Water uptake of starch, protein and fibre samples (g.water/100 g. equilibrium weight).

% R.H.	Rice starch		Glutenin		Bran fibre	
	a	b	a	b	a	b
10	5.80	6.12	2.69	3.86	2.11	2.20
20	7.21	8.00	3.93	5.39	2.70	3.30
30	8.89	9.26	5.66	7.47	3.67	3.97
40	9.80	10.78	6.71	8.73	4.27	5.08
50	11.22	12.16	7.95	10.04	5.40	5.82
60	12.46	13.40	9.77	11.53	6.25	7.36
65	13.13	14.50	10.32	11.91	7.08	8.13
67	13.73	14.43	10.75	12.17	7.57	8.17
70	14.14	14.99	11.15	12.36	7.89	8.66
72	14.37	15.40	11.62	12.65	8.05	8.83
75	14.80	15.62	11.90	12.90	8.51	9.27
80	15.47	16.70	13.40	13.46	9.29	10.05
85	16.46		15.04		10.49	
90	19.9		18.8		14.1	
100	26.8		45.0		27.8	

a = Hydration value. b = Dehydration value.

in a desiccator at 0% R.H. until no further loss in weight was measurable. They were transferred to a range of humidities from 0 to 100% (using the data of Wilson, 1921) and stored at 25°C. The dishes were weighed after 1, 4, 7 and 14 days and the moisture uptake at each humidity was calculated. From these equilibrium values the hydration curve for each sample was obtained.

Hysteresis effect.

After the completion of the weighings to obtain the hydration equilibrium values of the materials each sample was exposed for 24 hours to the humidity immediately above that at which it had reached equilibrium in the range of humidities used. Thus the sample that had attained equilibrium at 10% R.H. was exposed to 20% R.H. for 24 hours, the sample at 20% R.H. to 30% R.H. and so on. After this period the samples were returned to their original desiccators and re-weighed after periods of 3 and 7 days. In this way it was possible to calculate dehydration equilibrium values for the different materials. Some indication of the extent to which these substances exhibit the phenomenon of hysteresis could thus be obtained. The differences in hydration and dehydration equilibrium values for rice starch, glutenin and bran fibre are recorded in Table 31.

It was apparent that different dehydration values could be obtained for these samples according to the

Table 32.

Water uptake at equilibrium of rice starch and bran fibre showing hysteresis effect.
(g.water/100 g. equilibrium weight).

% R.H.	Starch			Fibre		
	a	b	c	a	b	c
10	5.80	6.12	6.84	2.11	2.20	2.59
20	7.21	8.00	8.16	2.70	3.30	3.53
30	8.89	9.26	9.89	3.67	3.97	4.53
40	9.80	10.78	10.82	4.27	5.08	5.25
50	11.22	12.16	12.32	5.40	5.82	6.42

a = Hydration value.

b = Dehydration value at given R.H. after exposure to the R.H. immediately above it for 24 hours.

c = Dehydration value at given R.H. after exposure to 85% R.H. for 24 hours.

humidity at which they were exposed during the 24 hour period. Thus if the samples were all exposed to 85% R.H. for 24 hours and dehydration values were obtained by calculating their weights at equilibrium with the original humidities to which they were exposed, higher dehydration values were obtained than by using the technique described in the previous paragraph. Both dehydration values were higher than the hydration values (see Table 32).

If the water uptake figures at the various humidities, given in Tables 31, 33 and 34, be represented graphically typical hydration and dehydration curves for these materials can be obtained. Such curves for glutenin are given in Fig.6. The difference between these two curves indicates the hysteresis effect. In general this amounted to about 0.5% moisture for all the materials except glutenin, which showed differences averaging 1.5% between the hydration and dehydration values.

Water uptake of starch, protein and fibre samples.

If the values given in Table 31 be graphically represented typical hydration curves for the purified materials (rice starch, glutenin and bran fibre) can be obtained and are shown, for the range 40 to 100% R.H., as continuous lines in Fig.7. It will be seen that the curves are of two general shapes. The starch gives a straight line relationship from below 40% R.H. to 85% R.H.

FIG. 6 Water uptake curves of Glutenin.

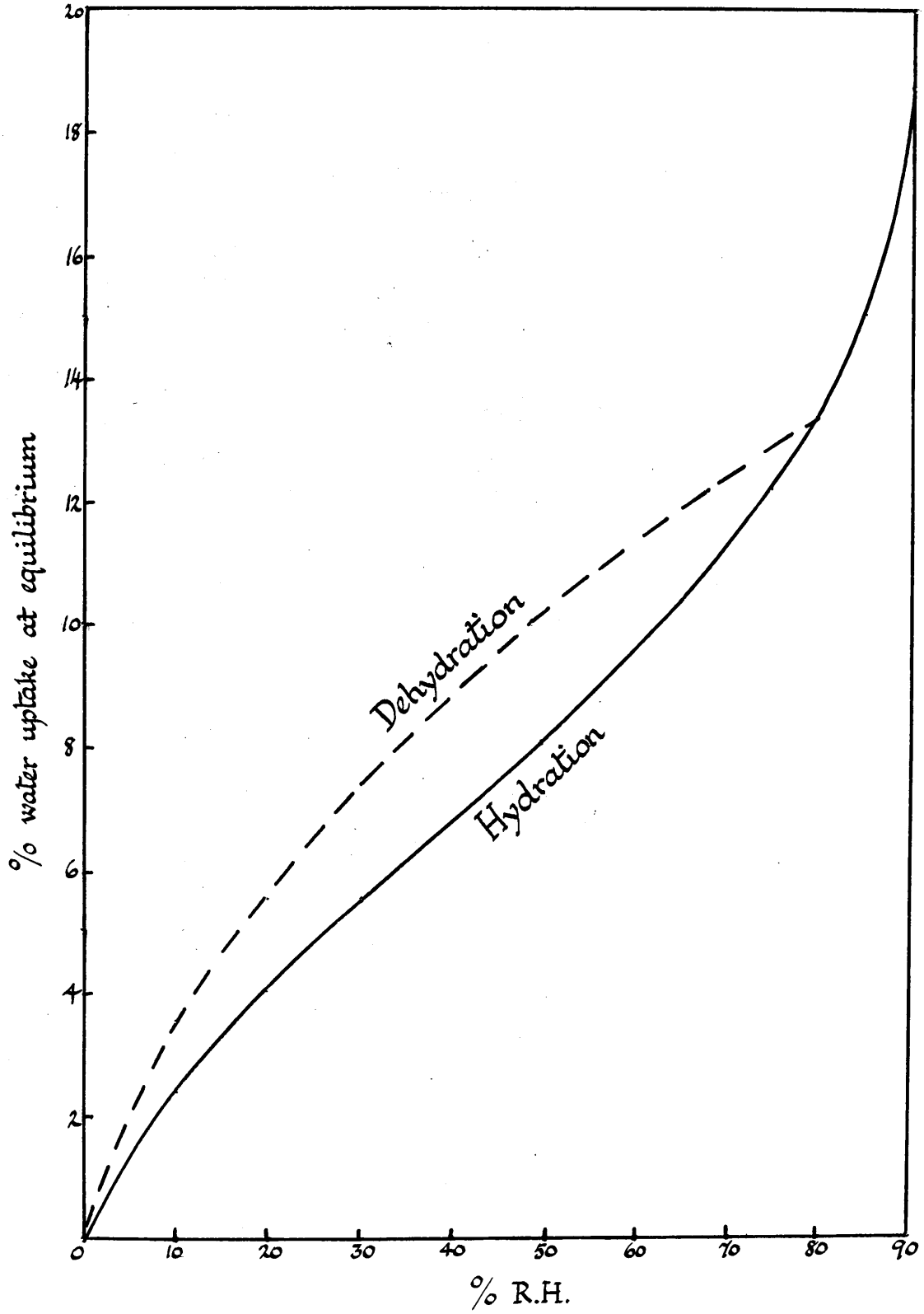


FIG. 7 Water uptake curves of starch, protein and fibre mixtures.

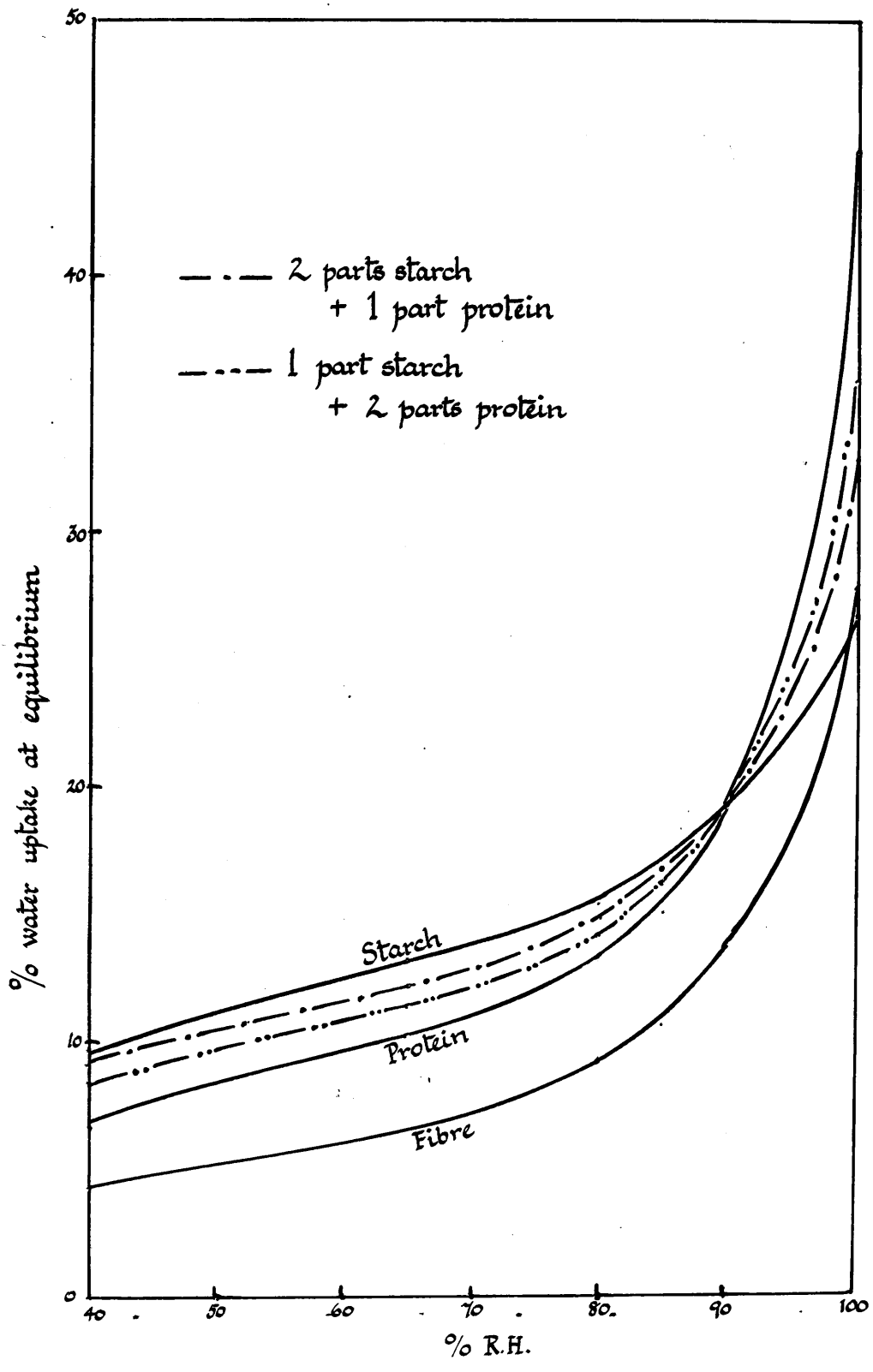


Table 33.

Water uptake (hydration values) of mixtures of starch, protein and fibre samples). (g.water/100g.equilibrium weight).

% R.H.	2 parts starch + 1 part protein	1 part starch + 2 parts protein	2 parts protein + 1 part fibre	2 parts starch + 1 part fibre	1 part starch + 2 parts fibre
40	9.31	8.39	6.07	8.42	6.51
50	10.36	9.37	7.54	9.61	7.63
60	11.60	10.79	8.70	10.69	8.48
70	13.20	12.90	10.55	12.20	10.39
75	13.80	13.01	11.31	13.06	10.81
80	14.81	14.24	12.33	14.07	11.82
85	16.45	16.24	14.14	15.05	12.96
90	19.4	19.5	17.4	17.8	16.3
100	33.0	36.1	33.2	30.2	29.2

Table 34.

Water uptake of proteins. (g.water/100 g. equilibrium weight).

% R.H.	Edestin		Casein		Egg albumin	
	a	b	a	b	a	b
10	3.81	4.27	3.47	4.38	3.65	4.04
20	5.20	5.49	4.66	5.73	5.16	5.36
30	6.63	6.88	6.83	7.89	6.56	6.80
40	7.71	8.14	8.18	9.49	7.95	8.07
50	8.73	9.13	9.85	11.25	9.67	10.16
60	10.02	10.39	11.21	12.81	12.24	12.69
70	11.58	11.82	13.15	14.31	15.71	16.56
75	12.74	12.78	14.25	14.76	17.68	18.54
80	14.19	14.21	15.93	15.85	20.67	21.45
85	15.97		18.35		25.10	
90	19.3		25.8		36.0	
100	48.6		45.0		63.0	

a = hydration value. b = dehydration value.

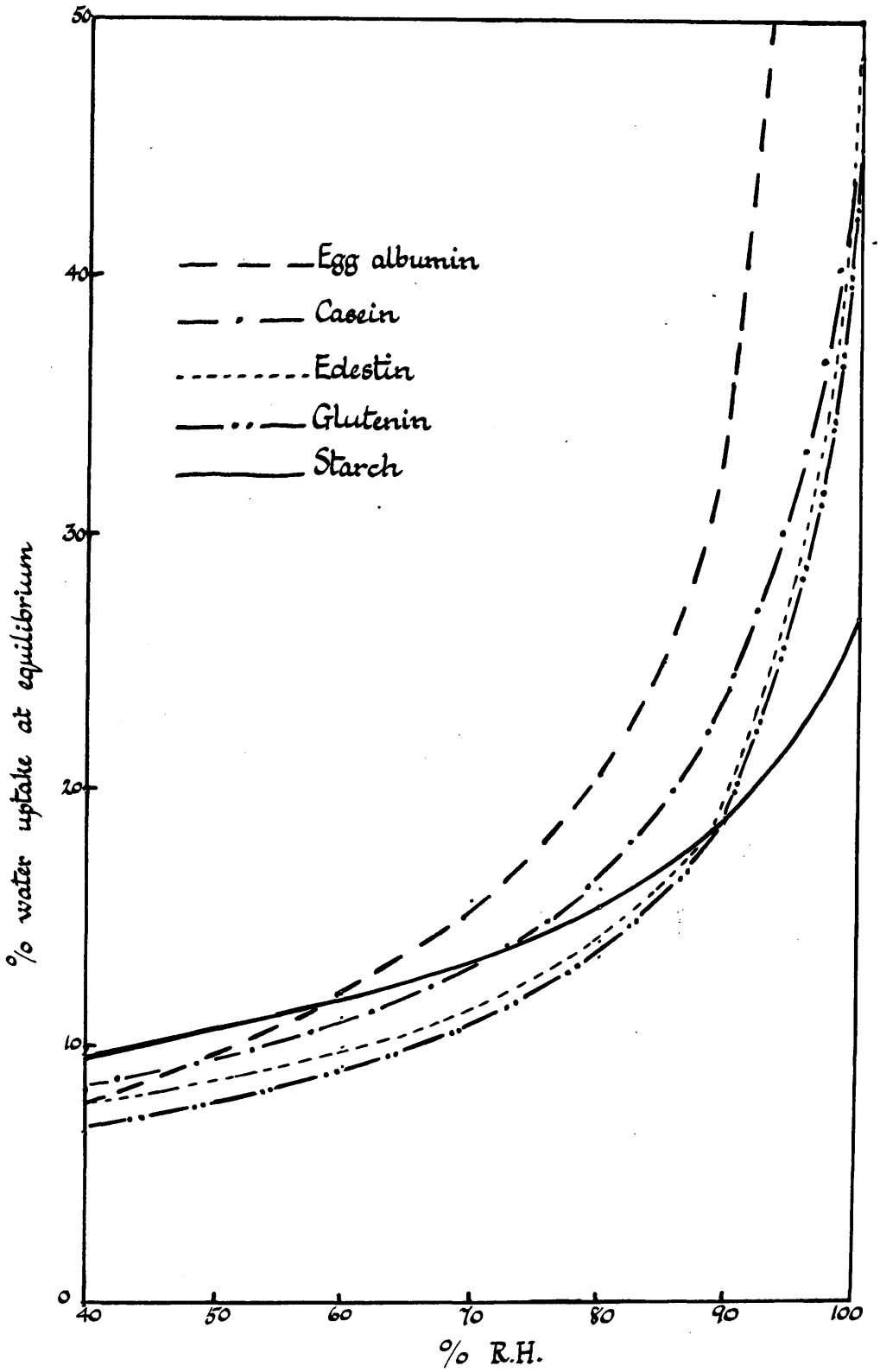
Over this range the starch curve lies well above the protein curve. Above 85% R.H. the slope of the starch curve sharpens but the water uptake at 90 to 100% R.H. only reaches about 27%. The protein curve, on the other hand, gives a relatively steep slope at humidities above 75%, and while it starts well below the starch curve at 40% R.H., it crosses it at about 90% R.H. and ultimately gives the exceptionally high water uptake of 40 to 50% at humidities between 90 and 100%.

It may be noted that this steep type of curve appears to be a general characteristic of proteins since experiments carried out with edestin and two animal proteins (casein and egg albumin) gave similar results, though with a far more marked rise in water uptake at high humidities. The hydration and dehydration equilibrium values for these proteins are given in Table 34 and their hydration curves are compared with that of rice starch in Fig.8.

The curve of the purified fibre is similar in shape to that of the starch, but the level of water uptake is exceptionally low throughout practically the whole range of humidities.

If these results be applied to the findings of Snow et al. (1944) with regard to the water uptake of feeding-stuffs, it would appear that the shape of the water uptake curve of a feeding-stuff will depend primarily on the relative proportions of soluble

FIG. 8 Hydration curves of proteins and starch.



carbohydrate and protein which it contains. The level will depend on the absolute quantities of these two constituents present in the feed. The only effect likely to be caused by the presence of a high proportion of fibre will be to depress the general level without materially altering the shape. From the point of view of water uptake the fat fraction of any feeding-stuff may be considered inert, while the constituents of the ash (unless these are of an exceptionally hygroscopic nature) are also unlikely to affect the water uptake materially.

These facts are clearly illustrated in Fig.7 where the curves of two composite mixtures have been graphed as broken lines using the values recorded in Table 33. It will be seen that the slopes of these two curves are governed by the starch-protein ratio. Thus at low humidities both mixtures give curves below the pure starch but above the pure protein: at high humidities the position is reversed, the mixtures giving curves well above the pure starch but well below the pure protein. The four curves, pure protein, 2 parts protein + 1 part starch, 2 parts starch + 1 part protein, and pure starch show, in fact, the same crossing over phenomenon which was found to be typical of the water uptakes of the intact feeding-stuffs studied by Snow et al. (1944).

"Excess absorption" phenomenon.

These thin layered samples rapidly reached

Table 35.

Rate of water uptake of proteins, starch and fibre.
(g.water/100 g. equilibrium weight).

% R.H.	Egg albumin		Glutenin		Rice starch		Fibre	
	a	b	a	b	a	b	a	b
10	3.88	3.65	2.57	2.69	5.80	5.80	2.15	2.11
20	5.39	5.16	3.84	3.93	7.15	7.21	2.74	2.70
30	6.89	6.56	5.52	5.66	8.89	8.89	3.62	3.67
40	8.38	7.95	6.54	6.71	9.80	9.80	4.26	4.27
50	10.32	9.67	7.73	7.95	11.28	11.22	5.40	5.40
60	12.62	12.24	9.75	9.77	12.40	12.46	6.25	6.25
65	13.79	13.63	10.10	10.32	12.99	13.13	6.94	7.08
67	14.62	14.60	10.75	10.75	13.64	13.73	7.52	7.57
70	15.89	15.71	11.03	11.15	14.01	14.14	7.65	7.89
72	17.15	16.71	11.63	11.62	14.33	14.37	8.05	8.05
75	18.02	17.68	11.60	11.90	14.78	14.80	8.53	8.51
80	21.38	20.67	13.25	13.40	15.41	15.47	9.31	9.29
85	25.46	25.10	14.81	15.04	16.47	16.46	10.48	10.49

a = water uptake after 1 day.

b = water uptake at equilibrium.

equilibrium with the humidity to which they were exposed, equilibrium usually being established within 24 hours. For the egg albumin sample, however, and for mixtures of this material with rice starch, the water uptake after 1 day's exposure was greater than that measured on subsequent days and at equilibrium. These increased values for egg albumin above the equilibrium figures are shown in Table 35 and may be compared with the figures obtained for the water uptakes of glutenin, rice starch and bran fibre which, after 1 day's exposure, did not show any "excess absorption" of water above that held at equilibrium. The explanation of this phenomenon is not known. It is, however, of interest to note that similar observations were made with the water uptake of spray dried milk powder by Supplee (1926) and Gane (1941). Experiments to confirm these observations showed that the water uptake of this substance at 50% R.H. was 6.81, 6.26, 4.99 and, at equilibrium, 4.55 g.water/100g. material after 1, 4, 7 and 11 days exposure respectively. The difference in the water uptake after one day and that held at equilibrium was very much greater than the corresponding difference in values recorded for egg albumin. Gane (1941) has suggested that this instability could be attributed to a conversion of anhydrous lactose present in the dried milk powder to the monohydrate form. This explanation cannot, of course, apply to egg albumin.

Conclusion.

1. Observations have been made on the water uptake of glutenin, edestin, egg albumin, casein, starch and bran fibre and of mixtures of these substances in definite proportions, when exposed to a range of humidities.
2. Hydration and dehydration values have been determined and, from these figures, some indication has been obtained of the extent to which these substances exhibit hysteresis.
3. The shape and level of the water uptake curves have been shown to be characteristic for each of the different types of substance, and the results obtained have been applied to the findings of Snow et al. (1944), with regard to the water uptake of feeding-stuffs.
4. The water uptake of egg albumin after 24 hours at a given humidity was found to be in excess of that finally held at equilibrium.

PART V. THE ISOLATION OF MOULD TYPES FROM FEEDING-STUFFS STORED AT FIXED HUMIDITIES.

Survey of literature.

A survey of the literature revealed that little detailed work had been done on the isolation and identification of mould species from stored feeding-stuffs. It was commonplace to find authors who referred to the occurrence of mould types on stored products in the very general terms of Aspergillus spp. and Penicillium spp.. McHargue (1920), in describing the change in acidity values in corn meal during storage, mentioned the occurrence of Aspergillus glaucus and one or two other mould types on corn of 15% moisture. Thom and Le Fevre (1921) found that corn of 13 to 15% moisture commonly supported the growth of Aspergillus repens. When the moisture was as high as 16% Aspergillus flavus could develop. Between 18 and 20% many different mould species were able to grow. Similar observations were made by Koehler (1938) who gave a critical moisture content of 14.3% for the development of Aspergillus repens on shelled corn. A species of Penicillium, causing "blue-eye" disease, grew on corn of 16% moisture. Some other species were isolated by this author from samples of 18.4% and 23.8% moisture content. A study of the moulds developing on cocoa beans was made by Bunting (1930) who found that most of the types occurring could be assigned to the

Mucorales or the Aspergilli. Aspergillus chevalieri, and possibly Aspergillus sydowi, had the lowest moisture requirements of all the types studied and were able to grow on cocoa beans of 8 to 9.5% moisture (equivalent to between 82 and 87% R.H.).

A more comprehensive account of the types of mould fungi occurring on stored products can be obtained from the work of Smith (1928, 1931) and Galloway (1930, 1935) on those species causing mildew on textiles. These authors describe a number of species of moulds that commonly cause staining on yarns and cloths, and they state that the Aspergilli, especially members of the Aspergillus glaucus group, have the smallest moisture requirement of all the types studied. In particular, Galloway (1935) studied the germination of the spores of fungi isolated from textiles when exposed on viscose sheeting soaked in dilute wort to given humidities. Spores of members of the Aspergillus glaucus group, Aspergillus versicolor and Aspergillus candidus, were able to germinate at 75-80% R.H.; other Aspergillus spp. required 80-90% R.H.; most Penicillium spp. could germinate at 85% R.H., but members of the Mucorales and the Fungi Imperfecti groups required humidities of over 90%. It must be pointed out, however, that these observations on spore germination only covered a period of 2 weeks.

Methods of isolation of mould species.

The development of mould types on feeding-stuffs stored at humidities as low as 65% was described by Snow et al. (1944). Isolations of the mould species growing on feeding-stuff samples used in these experiments were made. Many of these samples had been stored for as long as 4 years at fixed humidities. The isolations were made by transferring a small piece of growing mycelium on to the centre of a petri dish containing beer-wort agar with the aid of a sterile needle. For those samples of feeding-stuffs that had supported the development of mould fructifications a single spore head was transferred with the sterile needle point on to an agar plate. In this way it was possible to obtain isolates of the mould species that were actively growing on the feeding-stuffs stored at the various humidities. This method was considered to be preferable to any "plating-out" technique which would, of course, have allowed the appearance on the plates of colonies from dormant mould spores present on the feeding-stuff but unable to grow at the particular humidity of storage. The petri dishes were incubated at 25°C and fresh transfers were made of the developing colonies on to beer-wort or Czapek-Dox agar (20% or 40% sucrose) contained in petri dishes or as slopes.

Careful notes were kept of the feeding-stuff from

which the isolate was made and of the humidity which had allowed the development of the mould species. The list of mould types isolated from these feeding-stuffs given in Tables 36 and 37 cannot be regarded as complete. Indeed, the complete isolation of the large number of mould species that are able to develop on feeding-stuffs stored at 90 to 100% R.H. would prove never-ending. Particular attention was therefore paid to the isolation of those species whose moisture requirements were low (i.e. those species which could develop at humidities of 85% or less). It is evident that a study of these mould types appertains more to the conditions that are likely to be found in the practical storage of feeding-stuffs. The various species of moulds were identified with the use of the keys provided for the Aspergilli by Thom and Church (1926) and Thom and Raper (1941) and for the Penicillia by Thom (1930). "Industrial Mycology" by G. Smith was valuable as a general guide.

Thanks are due to H.A. Dade, Esq., of the Imperial Mycological Institute, Kew, for his help in the confirmation of some of the species of Aspergillus isolated and to George Smith, Esq., of the London School of Hygiene and Tropical Medicine, for help in the identification of some of the species of Penicillium.

Factors affecting the mould species isolated.

1. Humidity of storage.

The isolation of mould species from feeding-

Table 36.

Minimum humidities below which mould species were not isolated.

% R.H.	
65%	Aspergillus echinulatus (Delacr.)
67%	Aspergillus repens (Corda) Saccardo.
70%	Aspergillus ruber (Spieckermann and Bremer).
	Aspergillus candidus Link.
75%	Aspergillus penicilloides series.
	Paecilomyces varioti Banier.
	Penicillium sartoryi Thom.
80%	Aspergillus chevalieri (Mangin).
	Aspergillus amstelodami (Mangin).
85%	Aspergillus versicolor (Vuillemin) Tiraboschi.
	Aspergillus sydowi (Banier and Sartory).
90%	Aspergillus niger series.
	Penicillium luteum series.
	Penicillium puberulum Banier.
	Penicillium chrysogenum Thom.
	Penicillium spinulosum Thom.
	Sporotrichum sp.
	Mucor spinosus van Tieghem.
100%	Trichoderma sp.
	Alternaria tenuis Nees.
	Rhizopus nigricans Ehrenberg.
	Verticillium cinnabarinum (Corda).

stuffs stored at fixed humidities showed that, while a large number of types were able to develop at humidities of 90% and above, the number of different species isolated from humidities below this figure was limited. It was evident that a critical humidity existed for each species, below which the development of mould spores could not take place. Thus, in general, members of the Mucorales and the Fungi Imperfecti were only isolated from humidities of 90% and above, Penicillium spp. were not able to grow below 80% R.H., while Aspergillus spp. were able to develop under conditions of very restricted moisture supply (members of the Aspergillus glaucus group being able to germinate at humidities as low as 65 to 70%). A list of critical humidities, below which the various mould species were not isolated, is shown in Table 36. This list shows that the species most resistant to dry conditions were members of the Aspergillus glaucus group (A. echinulatus, A. repens and A. ruber) which were able to develop at humidities of 70% and below. Other members of the Aspergillus glaucus group that were isolated (A. chevalieri and A. amstelodami) required a minimum humidity of 80% for their development. Most species of Penicillium grew extensively at 90% R.H. and some species were able to develop at 80-85% R.H. One species which was placed in the Monoverticillata-ramigena group and identified as near to P. sartoryi Thom

was isolated from 75% R.H.

2. Time of making isolations.

The length of the storage period of these feeding-stuffs samples markedly affected the types of moulds that were isolated at some humidity levels. At humidities of 80% and below the number of species that were able to develop was limited, and these often required long latent periods before spore germination could take place. Thus at 65% R.H. a latent period of over 2 years was required before spores of Aspergillus echinulatus were able to germinate on locust beans (Snow et al. 1944). At humidities above 80%, however, a succession of mould types was able to develop on many of the samples. For instance, at 100% R.H., members of the Mucorales quickly established themselves within 2 or 3 days and provided the dominant mould type present. After a week, however, members of the Aspergillus glaucus group and Penicillium spp. had grown rapidly on these samples and provided the dominant types at the expense of the Mucorales. Slower growing types, e.g. Aspergillus candidus, did not appear on samples stored at 70 to 100% R.H. until after many weeks of storage. Some members of the Fungi Imperfecti, e.g. Sporotrichum sp. were not evident in the early period of storage but later provided the dominant mould type on many of the samples (see Plate 1).

It was evident that those species which were well

adapted to growing on these feeding-stuff materials, e .g. members of the Aspergillus glaucus group and, at 85-100% R.H., Penicillium spp., developed at the expense of other species that were slower growing or less well adapted. Probably such competition was also influenced by the production of mould by-products from species that were quickly established which, by their staling effect, prevented the development of slower growing species.

3. Type and origin of samples.

The infection of feeding-stuffs with mould spores may take place (a) at the time when the original plant which provides the raw material for the feeding-stuffs is growing in the field, (b) during the manufacturing process, or (c) during transit or storage of either the raw material or the processed feeding-stuff. Galloway (1935) showed that the origin of some species of mould fungi (e.g. Aspergillus niger) found on manufactured cotton goods could be traced back to the cotton field where they caused diseases of the boll. In a similar way it is probable that some mould species isolated from the feeding-stuffs used in these experiments could be traced back to the country from which the original plant constituents came. This is particularly true of imported oil seed cakes manufactured in tropical or sub-tropical countries. Thus Aspergillus chevalieri is known to be a common contaminant of samples from such parts (Bunting, 1930).

Table 37.

Variety of feeding-stuffs from which mould species isolated.

ASPERGILLUS GLAUCUS GROUP.

Aspergillus repens (Corda) Saccardo. (67-100% R.H.)

Bone meal, bran, dried grass, ground-nut cake, linseed cake, locust beans, milk powder, oats, scotch beans.

Aspergillus ruber (Spieckermann and Bremer). (70-100% R.H.)

Bran, ground-nut cake, linseed cake, locust beans, scotch beans.

Aspergillus chevalieri (Mangin). (80-100% R.H.)

Linseed cake, palm kernel cake.

Aspergillus amstelodami (Mangin). (80-100% R.H.)

Linseed cake.

Aspergillus echinulatus (Delacr.). (65% R.H. only).

Locust beans.

OTHER ASPERGILLI.

Aspergillus candidus Link (70-100% R.H.)

Linseed cake, oats, bran, locust beans

Aspergillus penicilloides series (75-100% R.H.)

Bone meal, oats.

Aspergillus versicolor (Vuillemin) Tiraboschi. (85-100% R.H.)

Linseed cake, oats.

Aspergillus sydowi (Banier and Sartory). (85-100% R.H.)

Linseed cake.

Aspergillus niger series (90-100% R.H.)

PENICILLIA.

Paecilomyces varioti Banier. (75-100% R.H.)

Palm kernel cake.

Monoverticillata.

Penicillium sartoryi Thom. (75-100% R.H.)

Linseed cake, oats.

Penicillium spinulosum Thom. (90-100% R.H.)

Linseed cake, palm kernel cake.

Asymmetrica.

Penicillium luteum series. (90-100% R.H.)

Palm kernel cake.

Penicillium puberulum Banier. (90-100% R.H.)

Linseed cake, palm kernel cake.

Penicillium chrysogenum Thom.

Linseed cake, palm kernel cake.

OTHER SPECIES.

Mucor spinosus van Tieghem. (90-100% R.H.)

Linseed cake, palm kernel cake.

Rhizopus nigricans Ehrenberg. (100% R.H. only)

Linseed cake.

Trichoderma sp. (100% R.H. only)

Palm kernel cake.

Sporotrichum sp. (90-100% R.H.)

Linseed cake.

Alternaria tenuis Nees. (100% R.H. only)

Linseed cake.

Verticillium cinnabarium (Corda). (100% R.H. only)

Bran, linseed cake.

Those feeding-stuffs which are manufactured in this country from oil seeds or other raw materials of imported origin will doubtless be partially sterilised by heat treatment during the processing. The extent to which mould spores are destroyed will depend upon the temperature and duration of the cooking process. Subsequent mould infestation will, however, readily take place from the machinery, workers' clothes, sacks and from the atmosphere of the places of storage.

It is possible that different feeding-stuffs, providing different types and quantities of nutrients, may affect the developing mould flora. The importance of this factor is hard to assess because of the difficulty of differentiating its effect from those of humidity, the place of origin and the conditions of storage, three factors which appear to be of greater importance. It can be noted, however, that several isolates of the same mould species were often obtained from a particular feeding-stuff sample stored at a range of humidities. This was true of Paecilomyces varioti which was isolated from palm kernel cake stored at 75, 85, 95 and 100% R.H. This species can, therefore, be said to be either typical of this feeding material or typical of the conditions under which it was manufactured and stored.

Frequency of occurrence of different species.

Table 37 gives details of the origin of the isolates of mould species from feeding-stuffs

stored at fixed humidities. Members of the Aspergillus glaucus group form the majority of these isolates. Only one isolate was made of a large ascospored type (A. echinulatus*) although the small ascospored types were of frequent occurrence. The rarity of these large-spored types has been noted by Smith (1931) for cotton goods and also by Thom and Raper (1941). These last authors show that these types are low temperature organisms and they suggest that more isolations of them might be made if lower incubation temperatures were employed.

Of the small ascospored types (6μ or less in long axis) of this group A. amstelodami was easily distinguished by the roughened faces of the spores. The isolates of this species were very constant and colonies were of a dark blue green colour in the conidial areas, brightly speckled with the yellow of the perithecia.

Several strains of A. chevalieri were isolated from different feeding-stuffs. The ascospores of all these strains were very typical with their crestlike ridges, giving rise to the recognisable pulley shape. The colony character of these strains was, however, very variable. In general at an incubation temperature of 25°C the colonies were largely perithecial, the

*Average measurements of the ascospores of this type were 8.8μ by 6.4μ. These are somewhat smaller than the figures given by Thom and Raper (1941) for this species, but the roughened ridges and faces of the ascospores justified its inclusion in this group of the large ascospored types.

yellow colour predominating over the green shades of the conidia, although different strains of this species showed different ratios and different forms of zonation of the perithecial and conidial areas. Thus seven different strains of this species gave colonies measuring 7, 11, 14, 20, 26, 33 and 38 mm. when grown on duplicate plates of beer-wort agar incubated at 25°C for 8 days. In addition, the amount of red colour produced in the medium (given as a character for this species by Thom and Raper (1941) and by other authors) varied with the different strains. Slow growing strains produced abundant red colouration in the medium while the faster growing types were often quite colourless in reverse.

The most commonly isolated mould species from these feeding-stuffs, which were also members of the A. glaucus group, were those types whose ascospores showed no roughenings or crests on their faces. These were assigned to the A. repens or the A. ruber groups. Ascospores of A. repens were identified as being lenticular and without any well marked furrow. Those of A. ruber, on the other hand, showed a well marked broad shallow furrow. For some strains of these two species the definiteness of the furrow did not provide a reliable index for identification; some ascospores from a particular strain of A. ruber were without any furrow, while others showed only a trace of a furrow. More attention was therefore paid, for these strains,

to colony characteristics. Strains of A. ruber could be differentiated by the ruby red pigment produced in the medium while A. repens gave a dirty grey to black colouration in the medium as the colonies developed.

Several strains of A. repens were isolated, the majority of them being closely allied in growth characteristics to the type strain, but some showed much slower rates of growth and a sparser development of mycelium and conidia on beer-wort agar plates than the normal strain. Variation was much more marked in the strains of A. ruber isolated from these feeding-stuffs. The majority of the types were mainly perithecial when grown as colonies on beer-wort agar at 25°C, but the extent of red or orange-red colouration of the mycelium and the perithecia varied for the different strains. One strain isolated was predominantly conidial, perithecia being produced only with difficulty, but the ascospores showed the characteristic broad shallow furrow of the A. ruber type. Other strains were isolated whose colonies showed bright emerald conidia intermixed with red perithecia.

The frequency of occurrence of small ascospored types on such a wide variety of feeding-stuffs and, in particular, A. repens, which was isolated from humidities ranging from 67 to 100% R.H., suggests that they are the species best adapted to growth on these materials in which the supply of moisture is

limited. Mould damage to feeding-stuffs in commercial stores will, in the main, be due to these types: it is only under conditions of prolonged high humidity (above 85% R.H.) that other Aspergilli (e.g. A. sydowi and A. versicolor), Penicillia and other mould species will be able to establish themselves.

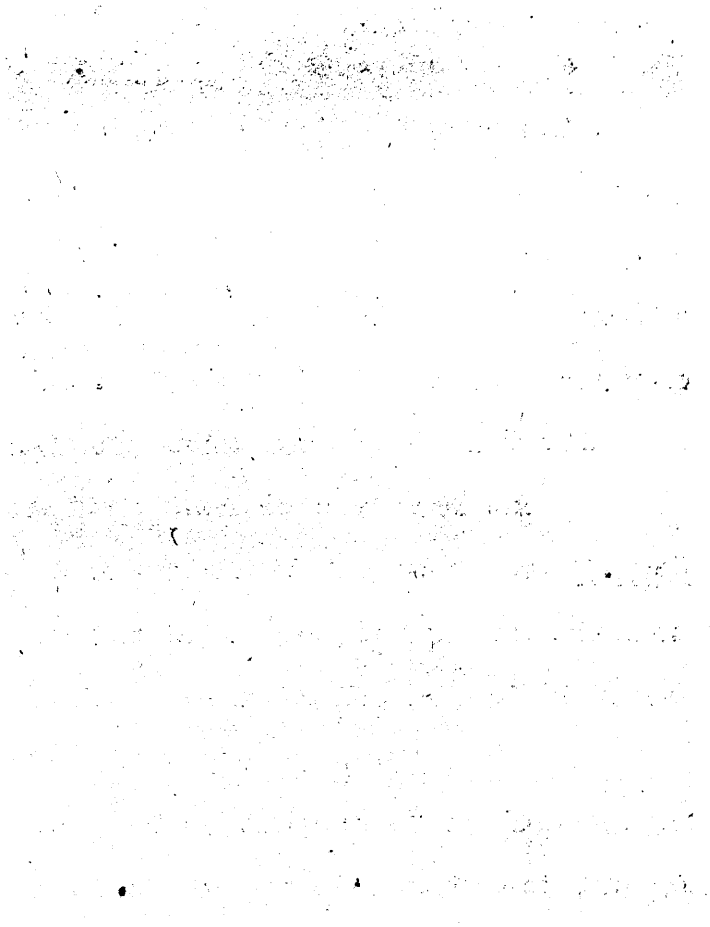


Table 38.

% composition of palm kernel cake.

	With minerals	Without minerals	
Moisture	10.51	10.16	
Protein	16.16	16.04	
Ether extract	12.02	11.29	
Fibre	9.71	9.65	
Ash	4.71	3.66	
Soluble carbohydrate (by subtraction)	46.89	49.20	
			Difference
% Calcium	0.523	0.256	
% Calcium (expressed as $CaCO_3$)	1.309	0.640	0.67%
% Chloride	0.462	0.188	
% Chloride (expressed as NaCl)	0.76	0.31	0.45%

Table 39.

Rate of mould development on palm kernel cake.

	Days to moulding		Days to fructification	
% R.H.	With minerals	Without minerals	With minerals	Without minerals
100	2	2	3	3
95	2	2	3	3
90	3	3	4	4
85	5	5	7	7
80	12	12	20	20
75	34	29	52	52
70	86	77	*	*
60	*	*	*	*

* Mould mycelium or mould fructification had not developed on these samples after 256 days.

PART VI. THE USE OF ANTISEPTICS IN CONTROLLING MOULDS.

(a) Mineral additions to palm kernel cake.

Additions of salt and ground limestone are sometimes made to oil cakes and compound feeding-stuffs to improve their mineral balance when fed to livestock. Some observations on cakes stored under commercial conditions had suggested that cakes with the added minerals showed less deterioration during storage than cakes without this addition. A laboratory experiment was therefore planned to establish whether these additions did affect the keeping quality of oil cakes.

Two samples of palm kernel cake, manufactured from the same batch of kernels, were obtained. It was intended that one sample should have approximately 1% added sodium chloride and 1% added calcium carbonate, while the other should have no mineral additions. After grinding the samples were analysed and were found to have the composition shown in Table 38. The analytical figures show that the mineral additions to the cakes were somewhat less than that originally intended and amounted to approximately 0.7% $CaCO_3$ and 0.5% NaCl.

Small samples of these two cakes were exposed to a range of humidities from 100-60% at 25°C and observations were made as to the number of days before the development of mould mycelium and mould

fructification (Table 39). Mould development took place just as readily on the samples with added minerals as on those without. This indicates that mineral addition in these proportions had little or no preservative action.

(b) The use of borax and boric acid as preservatives for oil cakes.

Preservatives are commonly used for the treatment of many materials, including foodstuffs, which require to be stored for long periods. In view of the increased period of storage of feeding-stuffs (often under unfavourable conditions) necessitated by the present war, it was suggested that a suitable antiseptic might be usefully employed to lengthen the storage life of such materials. The majority of antiseptics used in the control of moulds, some of which are reviewed by Morris (1926), are of a toxic nature and cannot, therefore, be used for food preservation. Of the less toxic compounds borax and boric acid were suggested as possible substances for incorporation in feeding-stuffs. These compounds are commonly used in the dressing of meat carcasses and hams as a preservative against deterioration in storage. Incorporation of borax and boric acid at a 1% level was suggested. Morris (1926) showed that 0.3% boric acid controlled the development of some mould species but that others

Table 40.

Rate of mould growth on linseed cake samples with incorporated borax or boric acid.

	% R.H.					
	100	95	90	85	80	70
	Days to development of mould mycelium					
Linseed cake only	4	5	6	8	15	284
Linseed cake + 1% borax	4	5	6	9	23	348
Linseed cake + 1% boric acid	4	5	6	10	24	*
	Days to development of asexual fructifications (conidia)					
Linseed cake only	5	6	7	12	24	491
Linseed cake + 1% borax	5	6	8	13	33	652
Linseed cake + 1% boric acid	5	6	8	14	37	*
	Days to development of perithecia					
Linseed cake only	11	10	13	17	54	*
Linseed cake + 1% borax	11	13	13	23	69	*
Linseed cake + 1% boric acid	13	13	15	31	95	*

* More than 652 days.

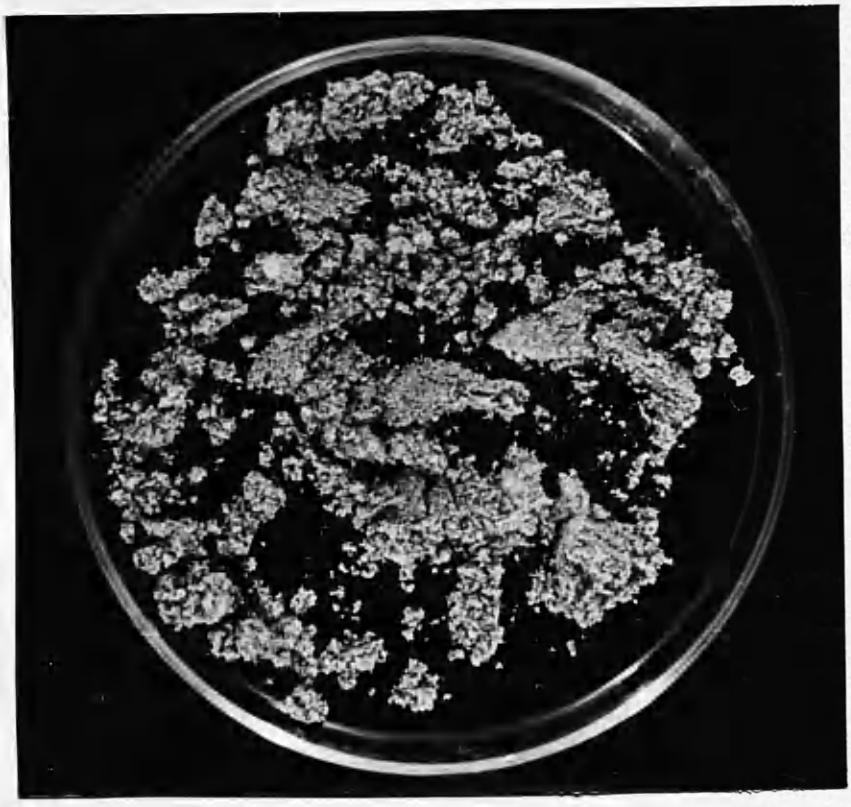
were unaffected by 1% boric acid. 3% boric acid was suggested for use in size pastes. Brenchley (1914) held that fungi were very indifferent to boric acid. A laboratory storage experiment was therefore planned to investigate the possible antiseptic effect of borax or boric acid (i) when incorporated into ground meals and (ii) when sprayed on to blocks of oil cakes.

(i) Incorporation of borax and boric acid in ground linseed cake.

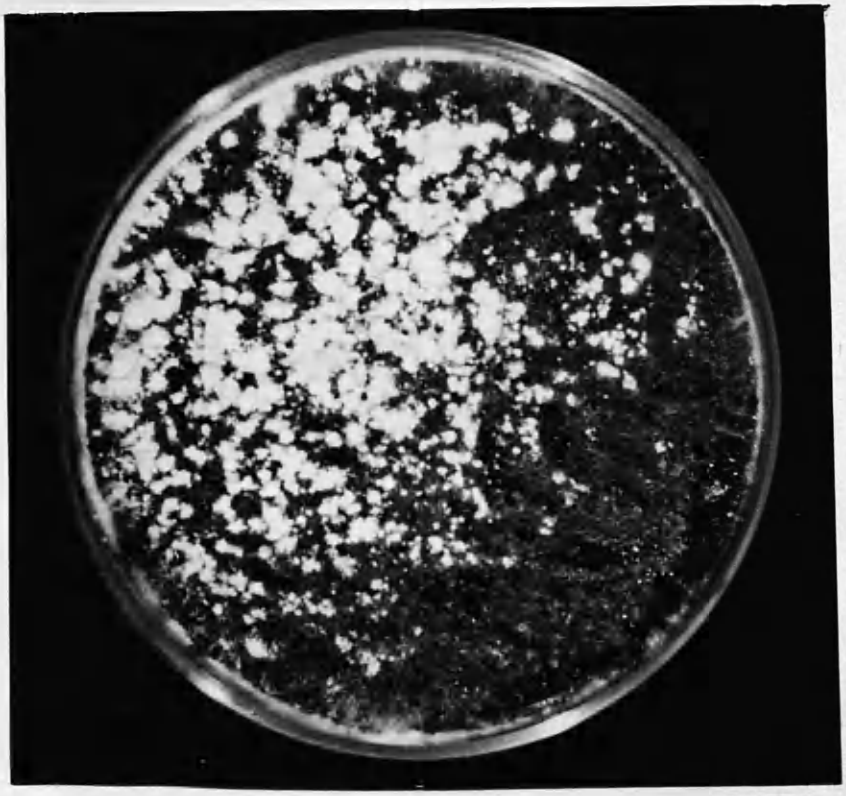
Borax and boric acid were introduced into samples of ground linseed cake. The quantities of borax and boric acid were accurately weighed to represent 1% of the mixture and were thoroughly incorporated into the meal by grinding in a pestle and mortar. Small samples of the three types of mixture (linseed cake only, linseed cake + 1% borax, and linseed cake + 1% boric acid) were stored at humidities of 100 to 70% at laboratory temperature. The data of Wilson (1921) were employed for maintaining the fixed humidity levels. Observations were made to determine the number of days before the development of mould mycelium and also before the appearance of asexual fructifications (conidia). In addition, the time taken for the development of the characteristic yellow perithecia of members of the Aspergillus glaucus group on each sample was noted. These results are given in Table 40.

At humidities of 100 to 90% the addition of borax

or boric acid did not significantly affect the time of moulding or the time before asexual fructifications appeared. At 85% R.H. these substances effected a small delay in the time of appearance of mould mycelium and of asexual fructifications. At 80% R.H. the addition of 1% borax or boric acid increased the period of freedom from mould mycelium or asexual fructifications by roughly 50%. In the presence of borax or boric acid mould growth tended to be sparser at all humidities. Some indication of the rate of this growth can be obtained from the figures for the development of perithecia on these samples. These show that borax and boric acid had a delaying effect on the appearance of this stage of mould growth at storage humidities between 100 to 80%. At 85 and 80% R.H. borax increased the time before the appearance of perithecia by roughly 30%, and boric acid increased the time by roughly 80%. On the other hand, certain species of moulds (e.g. Aspergillus niger and some Penicillium spp.), which have an especially damaging effect on feeding-stuffs stored at high humidities, appeared to thrive better on samples containing borax or boric acid than on the control samples. Presumably this is due to the partial control of other mould types by boron concentrations which allow such resistant species to develop. Plate 1 shows that borax or boric acid exerted some control over the mould types developing on samples stored at 90% R.H.

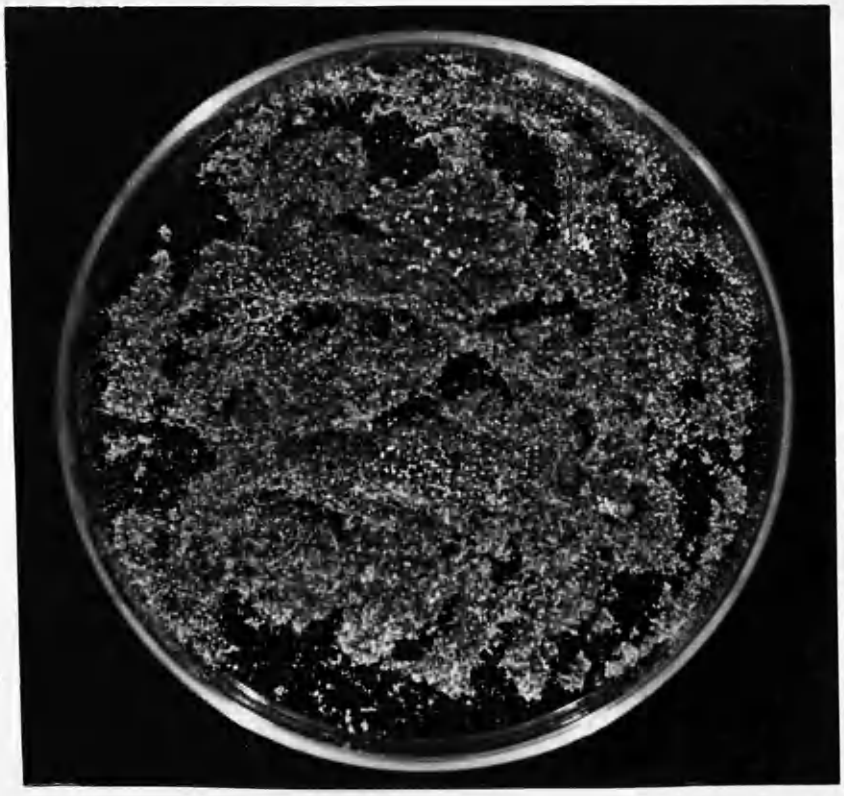


(a) Control sample with no moulding.

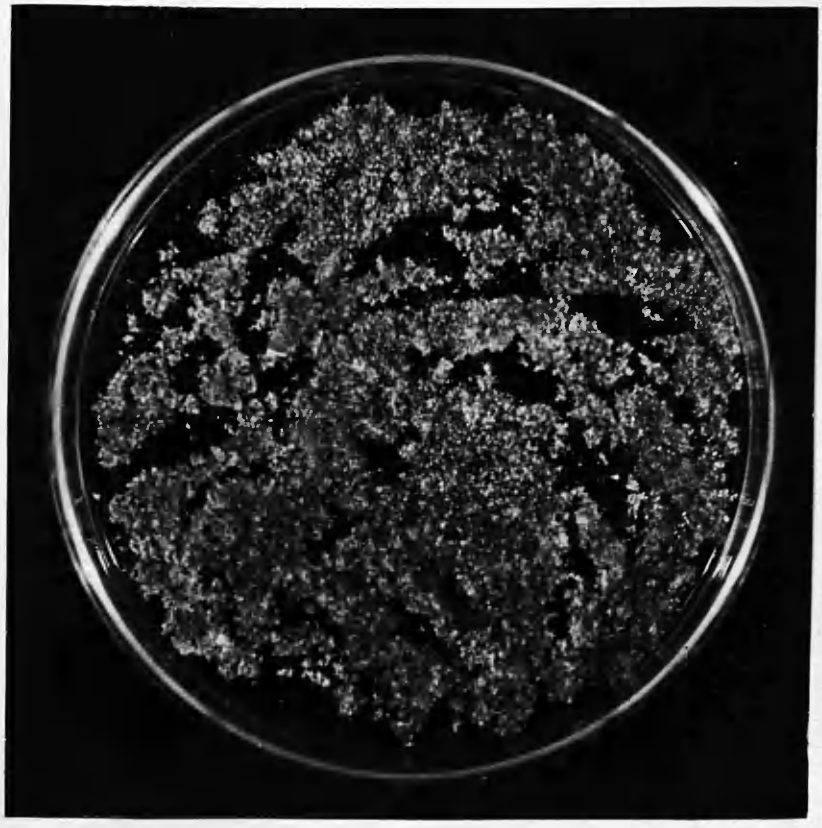


(b) Linseed cake only.

Plate 1.



(c) Linseed cake + 1% borax.



(d) Linseed cake + 1% boric acid.

Linseed cake samples with incorporated borax and boric acid stored at 90% R.H. for 20 months.

Thus, after 20 months storage, extensive growth of Sporotrichum sp. had developed on the sample with no incorporated borax or boric acid; on the sample with 1% borax there was no development of Sporotrichum sp. but a few small white patches of Aspergillus candidus were evident; while on the third sample 1% boric acid inhibited the development of both these mould types.

Samples of linseed cake with increased amounts of borax (above the 1% level) were stored at 90% R.H.: these samples contained 2, 5 and 10% borax respectively. No significant difference was found for those samples with either 1 or 2% borax. At the 5% level, however, the period of freedom from moulding was doubled at 90% R.H., but this concentration of borax only served to limit the mould types developing to Penicillium spp., which eventually thrived particularly well. Incorporation of as much as 10% borax inhibited the development of mould growth on linseed cake stored at 90% R.H. for over 652 days. Such a high concentration of borax would, of course, not be feasible in practice.

(ii) Spray treatment of oil cakes with a 10% borax solution.

Small 1" cubes of ground-nut cake and cottonseed cake were stored at 100-70% R.H. at laboratory temperature. The cakes were treated in four ways, (1) untreated control, (2) thorough spraying with a fine mist of methyl alcohol, (3) cursory spraying with a 10% borax solution in methyl alcohol, (4) thorough

Table 41.

Days to development of mould mycelium on oil cakes sprayed with 10% borax solution.

	% R.H.			
	100	90	80	70
Ground-nut cake untreated	5	8	21	85
" " " + cursory borax spray	7	9	25	158
" " " + methyl alcohol	7	11	26	101
" " " + heavy borax spray	11	16	60	246
Cottonseed cake untreated	5	10	25	151
" " " + cursory borax spray	8	11	28	158
" " " + methyl alcohol	7	12	29	151
" " " + heavy borax spray	14	20	60	318

spraying with this borax solution so that the faces of the cakes became very wet.

Observations were made on the rate of mould development on the variously treated cakes. These are recorded in Table 41. Thorough spraying with 10% borax solution approximately doubled the fresh storage life of these cakes stored at each humidity from 100 to 70% R.H. Cursory spraying with 10% borax solution (as would probably be carried out in commercial practice) or a thorough spraying with methyl alcohol had only a small delaying effect on the development of moulding at these humidities.

Summarising the results of the two laboratory storage experiments just described it can be stated that, while at 80% R.H. 1% borax or boric acid incorporated in ground meals increased the period of freedom from moulding by 50%, and while the heavy spraying of oil cakes with a 10% borax solution approximately doubled their storage life, neither treatment was completely effective in preventing the development of mould deterioration. It is concluded that borax or boric acid, whether incorporated in ground meals or applied as a spray on the surface of oil cakes, has only a mild antiseptic value.

When considering the advisability of using borax or boric acid as a preservative for these oil cakes it is of great importance to take into account the possible action of excreted boron on soil fertility.

Owen (1944) has shown by metabolism trials with dairy cows, whose production rations included oil cake containing 1% borax, that 98% of the ingested borate was excreted in the faeces and urine. The borate content of the combined excreta was about 500 p.p.m. Five tons of such excreta would go to the making of some 10 tons of farmyard manure. A dressing of 10 tons per acre of such manure would therefore represent an application of over 5 lb. of borax. While traces of boron are needed for the healthy development of most plants this element is extremely toxic if present in large amounts. Thus the toxic level for cereals and grassland is generally considered to be 5-10 lb. of borax per acre (Willis, 1936), although this figure will be subject to some variation according to the boron status of different soil types. Moreover, on certain types of soil the toxic effect of boron is cumulative, and it is practically impossible to eliminate it from such soils once it has been applied. While a single dressing of 10 tons of boronated farmyard manure would probably not be harmful, repeated dressings at this level would, however, involve the risk of permanent damage to the land. The use of borax or boric acid as an antiseptic for oil cakes cannot, therefore, be recommended. It is considered wiser, for successful storage, to keep the moisture content of the oil cake low (preferably below 12%), to allow "sweating" of the newly

manufactured cakes to be completed in a dry and well ventilated atmosphere, and to take all practicable precautions to reduce the dampness, humidity and temperature variations of the place of storage.

(c) The use of sulphanilamide and other drugs in the control of moulds.

Drugs of the sulphonamide group have been used extensively in therapeutic practice for the control of bacterial diseases. While numerous publications have given accounts of experiments concerning the effect of these drugs on many bacteria and hypotheses have been put forward as to their mode of action, a study of the literature failed to reveal any detailed experiments on the effect of these drugs on mould fungi. Tatum and Beadle (1942) described experiments in which the inhibiting effect of sulphanilamide on Neurospora crassa was overcome by an excess of p-aminobenzoic acid. Brian (1944) in a letter to "Nature", stated that sulphanilamide had a delaying action on the germination and growth of Penicillium digitatum Sacc. and on two other mould species. This delaying effect was eliminated by very small concentrations of p-aminobenzoic acid as was found for bacteria by Woods (1940).

(i) The growth of moulds on agar plates in the presence of added drugs.

Investigations were made to determine the action of sulphonamide drugs on typical mould species isolated from feeding-stuffs. The strains of moulds

used were those described in Part V of this thesis. In the first experiment sulphanilamide, propamidine and phenamidine* were tested for their fungistatic properties. The following three Aspergillus spp. and two Penicillium spp. were used:-

Type A. Aspergillus repens (Corda) Saccardo
- isolated from linseed cake stored at 80% R.H.

Type B. Aspergillus chevalieri (Mangin).
- isolated from palm kernel cake stored at 85% R.H.

Type C. Aspergillus versicolor (Vuillermin) Tiraboschi.
- isolated from linseed cake stored at 90% R.H.

Type D. Penicillium chrysogenum Thom.
- isolated from linseed cake stored at 90% R.H.

Type E. Penicillium spinulosum Thom.
- isolated from linseed cake stored at 90% R.H.

The moulds were grown on beer-wort agar, the three drugs being introduced in concentrations of 0 (control), 1 part in 16,000, 1/8,000, 1/4,000 and 1/2,000. These amounts were equivalent to 0%, 0.006%,

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*The complete chemical names for these drugs are:

Sulphanilamide - p-aminobenzenesulphonamide.

Propamidine - 4:4'-diamidino-diphenoxy propane di-(β-hydroxyethane-sulphonate).

Phenamidine - 4:4'-diamino-diphenyl ether di-(β-hydroxyethane-sulphonate).

Table 42.

Details of media containing drugs.

	Control 0	Concentration of drug. 1/16,000	1/8,000	1/4,000	1/2,000
Beerwort	112 ml.	112 ml.	112 ml.	112 ml.	112 ml.
Drug solution (0.1%)	0 ml	14 ml.	28 ml.	56 ml.	112 ml.
Water	112 ml.	98 ml.	84 ml.	56 ml.	0 ml.
Agar	5 g.	5 g.	5 g.	5 g.	5 g.
Citric acid	3.2 g.	3.2 g.	3.2 g.	3.2 g.	3.2 g.
Potassium citrate	1.8 g.	1.8 g.	1.8 g.	1.8 g.	1.8 g.

Table 43.

Regularity of growth of triplicate colonies - Type A.

Drug	Concentration	Diameter of colonies (mm.) after 8 days
Control	0	22, 23, 24.
Sulphanilamide	1/16,000	17, 17.
	1/8,000	12, 12, 12.
	1/4,000	4, 6.
	1/2,000	2, 2, 2.
Propamidine	1/16,000	14, 15, 15.
	1/8,000	12, 12, 13.
	1/4,000	8, 8, 9.
	1/2,000	-, -, -
Phenamidine	1/16,000	20, 20, 21.
	1/8,000	18, 20.
	1/4,000	18, 18, 18.
	1/2,000	14, 13, 14.

0.012%, 0.025% and 0.05% respectively. This was achieved by adding the appropriate volume of a 0.1% solution of each drug to the different media whose composition is shown in Table 42. The citric acid potassium citrate buffer fixed the pH of the medium at 3.5.

A spore suspension of each of the five mould species was made up with sterile water and a drop of the suspension was introduced with the aid of a platinum wire loop on to the centre of each plate. Triplicate plates were used for the five concentrations of each of the three drugs. Observations on the rates of growth of the five mould species and their reaction to different concentrations of the drugs were carried out by measuring the diameters of the mould colonies after incubation at 25°C for 4, 6 and 8 days. Close agreement in the rate of growth was shown between the triplicate plates of each mould type at the various drug concentrations. Typical measurements are shown in Table 43, where it is seen that the triplicate colonies seldom showed a variation of more than 1 mm. in diameter.

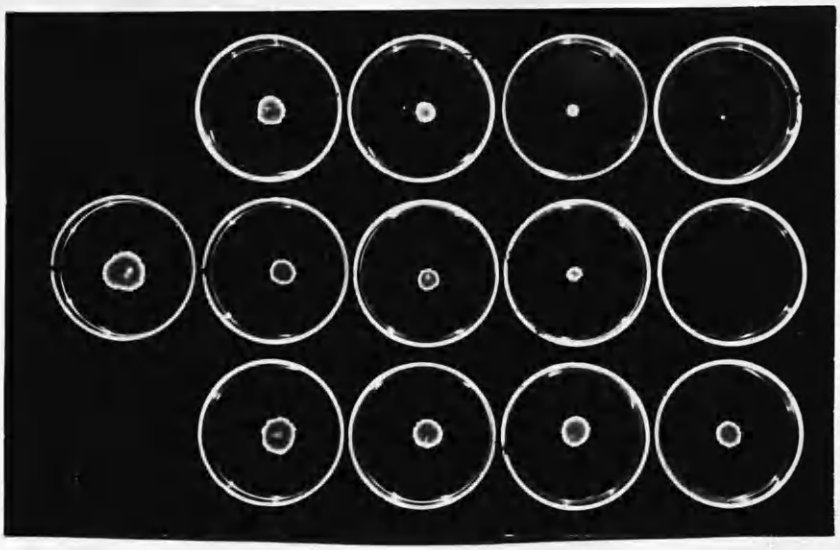
Fungistatic effect of the drugs.

The rates of growth of the five mould species at the different drug dilutions for an eight-day period are shown in Table 44. Plate 2 shows the effect of the drugs on the size of the normal colonies after 8 days.

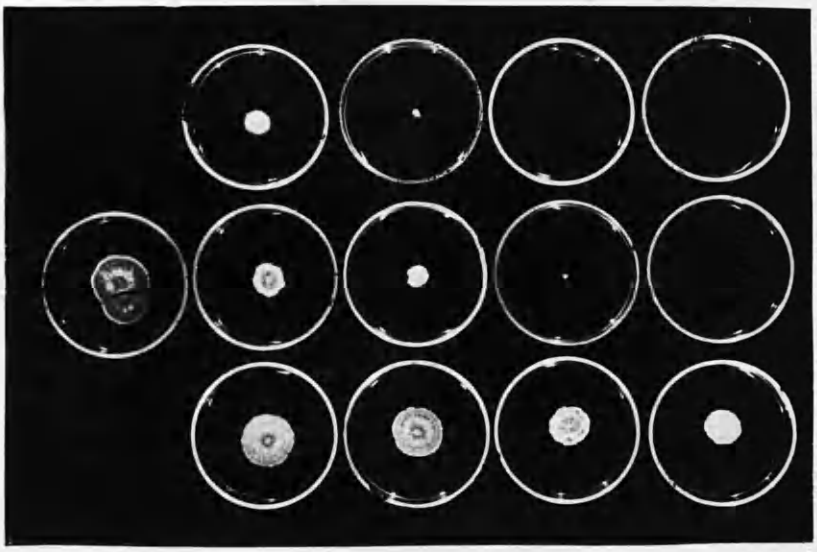
Table 44.

Fungistatic Effect of Sulphonamide Drugs.

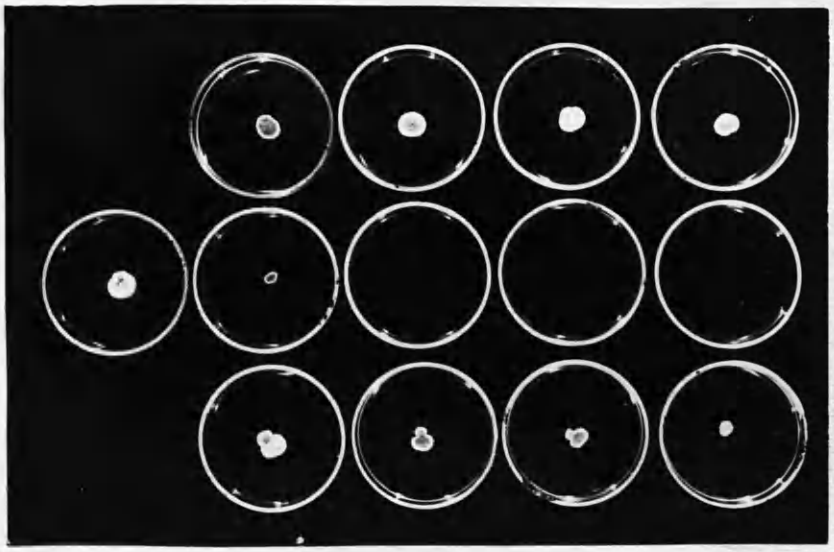
Drug	Concentration	Diameter of colonies (mm) after three periods (days) 4	6	8
Type A. Aspergillus repens.				
Control	0	8	15	23
Sulphanilamide	1/16,000	6	12	17
	1/8,000	3	7	12
	1/4,000	2	2	5
	1/2,000	2	2	2
Propamidine	1/16,000	7	11	15
	1/8,000	4	9	12
	1/4,000	-	4	8
	1/2,000	-	-	-
Phenamidine	1/16,000	8	15	20
	1/8,000	8	14	19
	1/4,000	8	13	18
	1/2,000	4	10	14
Type B. Aspergillus chevalieri.				
Control	0	13	25	35
Sulphanilamide	1/16,000	3	6	14
	1/8,000	-	3	8
	1/4,000	-	-	-
	1/2,000	-	-	-
Propamidine	1/16,000	10	15	22
	1/8,000	3	10	14
	1/4,000	-	-	3
	1/2,000	-	-	-
Phenamidine	1/16,000	13	23	32
	1/8,000	13	21	31
	1/4,000	12	18	24
	1/2,000	10	15	22
Type C. Aspergillus versicolor.				
Control	0	7	12	15
Sulphanilamide	1/16,000	8	13	16
	1/8,000	7	13	17
	1/4,000	7	12	16
	1/2,000	7	12	14
Propamidine	1/16,000	5	6	8
	1/8,000	-	-	-
	1/4,000	-	-	-
	1/2,000	-	-	-
Phenamidine	1/16,000	6	12	15
	1/8,000	6	10	12
	1/4,000	6	9	10
	1/2,000	7	7	8
Type D. Penicillium chrysogenum.				
Control	0	25	37	50
Sulphanilamide	1/16,000		35	45
	1/8,000		35	44
	1/4,000		34	44
	1/2,000		34	45
Propamidine	1/16,000		37	48
	1/8,000		35	42
	1/4,000		37	45
	1/2,000		36	44
Phenamidine	1/16,000		36	44
	1/8,000		36	42
	1/4,000		36	43
	1/2,000		36	43
Type E. Penicillium spinulosum.				
Control	0	5	11	15
Sulphanilamide	1/16,000	5	11	15
	1/8,000	5	11	15
	1/4,000	5	10	14
	1/2,000	3	9	13
Propamidine	1/16,000	-	-	-
	1/8,000	-	-	-
	1/4,000	-	-	-
	1/2,000	-	-	-
Phenamidine	1/16,000	5	11	13
	1/8,000	5	10	11
	1/4,000	3	8	10
	1/2,000	-	-	2



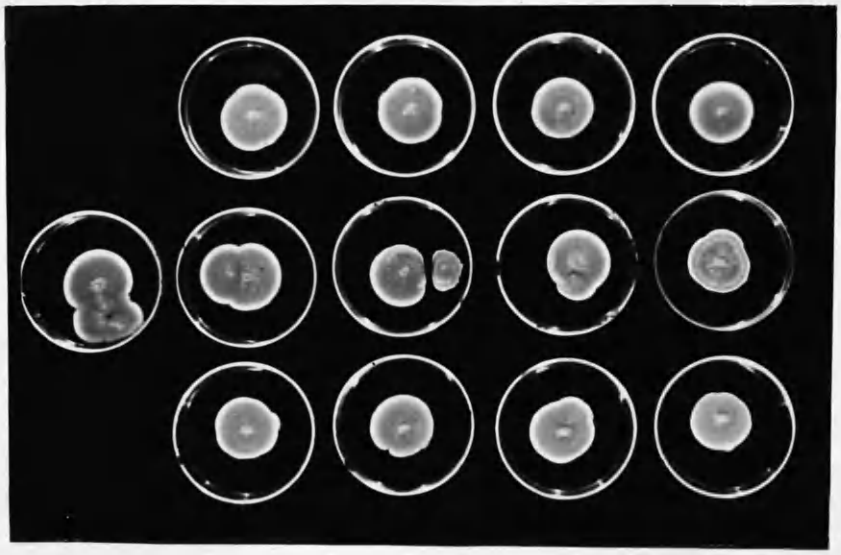
Type A - Aspergillus repens.



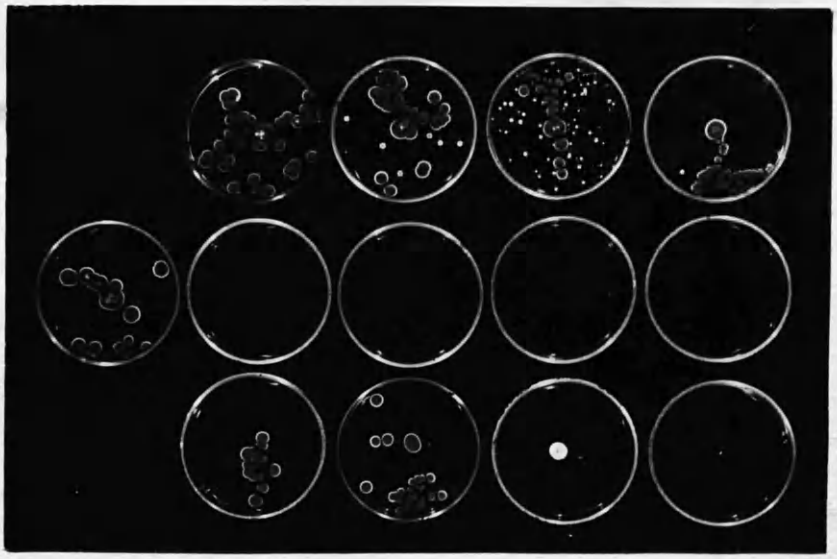
Type B - Aspergillus chevalieri.



Type C - Aspergillus versicolor.



Type D - Penicillium chrysogenum.



Type E - Penicillium spinulosum.

Top row - Sulphanilamide.
Middle row - Propamidine.
Bottom row - Phenamidine.

In dilutions, from left to right, of 0 (control), 1/16,000, 1/8,000, 1/4,000 and 1/2,000.

Plate 2. Inhibiting effect of sulphanilamide and other drugs on moulds growing on agar plates.

The five mould types were found to be specific in their reaction to the three drugs. Type D was not significantly affected in its growth rate by any of the drug dilutions used in this experiment: colonies of this mould species grew at a similar rate and to similar size on all the different media. Subsequent experiments on the effect of these sulphonamide drugs on Penicillium spp. showed that, in general, higher concentrations than 1/2,000 were required before any reduction in growth rate was noted and that, while concentrations of 1/1,000 or greater of a soluble derivative of sulphanilamide, commonly known as E.O.S.*, reduced the rate of growth, the drugs used were not successful in completely inhibiting development even at concentrations as high as 1/125. Plate 3 illustrates the reduction in size and buckling of a colony of Penicillium chrysogenum grown on agar containing 1/1000 E.O.S.

The most marked fungistatic action was shown by Propamidine which prevented the development of type E at all the dilutions studied. This drug inhibited the growth of type C at concentrations of 1/8,000 and above, and also types A and B at 1/2,000. For the lower concentrations of this drug the growth rate of types A, B and C were reduced as compared with the control

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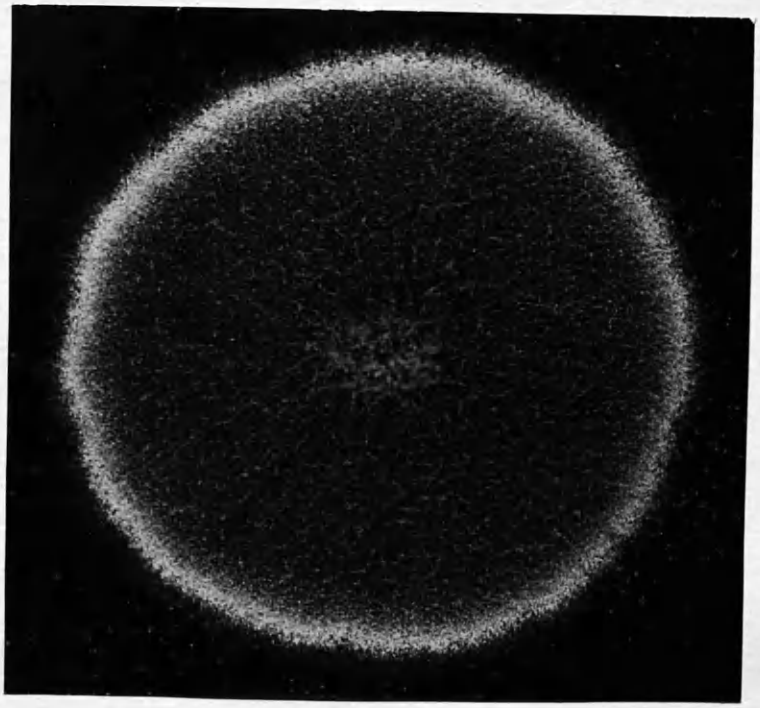
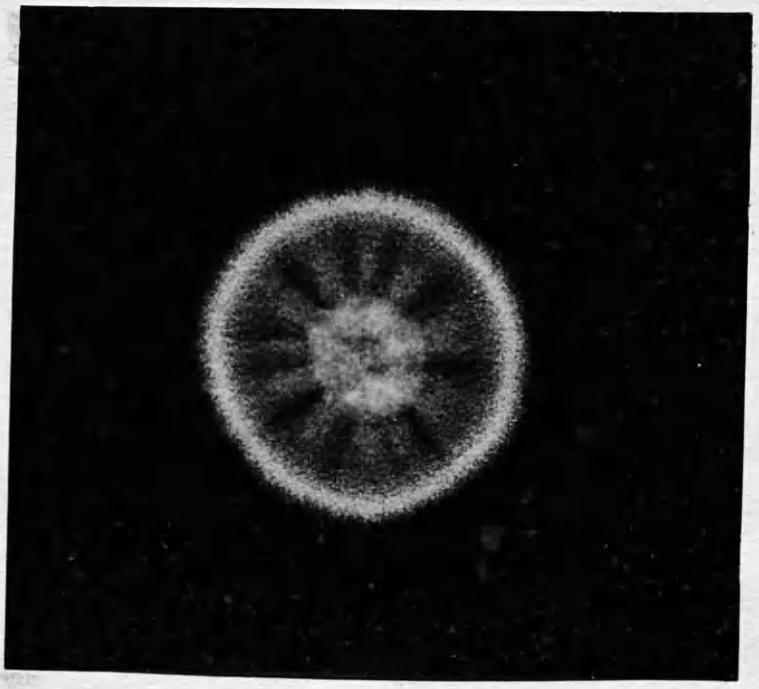
*Sodium p-sulphonamidophenylamino ethane -∝- sulphonate.



Control



1/1000 E.O.S.

Plate 3. Effect of 1/1000 E.O.S. on the growth of Penicillium chrysogenum (6 day colonies).(x4).

(see Table 44).

Sulphanilamide at the concentrations used had no significant fungistatic action on types C, D and E. Indeed, the rate of growth and the production of asexual fructifications. (conidia) of type C was very slightly stimulated by the presence of the drugs in these small concentrations. It is possible that for some mould species small dilutions of these drugs have a stimulating effect similar to that described by Brenchley (1914) and other authors for very low concentrations of toxic heavy metals and other trace elements. Sulphanilamide reduced the growth rate of type A in relation to the concentration of the drug present. In addition, this drug inhibited type B at concentrations of 1/8,000 and above and only permitted slow development of this species at the more dilute concentrations.

Phenamidine had the weakest fungistatic action of the three drugs used in this trial. All five species were able to develop to some extent at all the drug concentrations used. There was some indication that this drug slowed down the rate of growth of some of the mould species, although at the two weakest dilutions this was hardly significant. The effect of this drug was most marked with type E, which after 8 days at a concentration of 1/4,000, was only able to produce a round colony of white mycelium and no

conidial fructifications, while at 1/2,000 the colonies were only of 2 mm. diameter as compared with 15 mm. for the control (see Plate 2).

More extensive experiments were carried out on the effect of different sulphonamide drugs on a wide variety of mould strains isolated from feeding-stuffs and grown on agar plates. Some 50 different strains have been tested and, in addition to the three drugs mentioned previously, the fungistatic action of E.O.S., sulphapyridine, sulphamezathine and sulphaguanidine have been assessed. Results of these experiments confirmed the findings of the first series which have already been described. They also showed that the most effective drugs for the control of members of the Aspergillus glaucus group (the species which occur most frequently on stored feeding-stuffs) were sulphanilamide, E.O.S., and propamidine. Thus propamidine at all the dilutions used (1/16,000 and below) inhibited the development of nearly all the tested strains of species of this group. Approximately half of the E.O.S. molecule consists of the sulphanilamide radical. It is therefore of interest to note that sulphanilamide and E.O.S. were effective in preventing the growth of members of the A. glaucus group on agar plates at dilutions of 1/8,000 and 1/4,000 respectively. From these observations it seemed probable, therefore, that these drugs could be

used with advantage for the prevention of moulds on feeding-stuffs.

(ii) The storage of linseed cake with added drugs.

A storage experiment was undertaken with a fresh sample of linseed cake, using a technique similar to that described on page 79 for the use of borax and boric acid in oil cakes. For this experiment the two most soluble* and effective drugs were chosen. E.O.S., propamidine and a mixture of these two drugs were incorporated in separate samples of ground linseed cake at concentrations of 1/500, 1/2,000 and 1/8,000 for the single drugs, and of 1/1,000, 1/4,000, and 1/16,000 for each of the mixed drugs. This was done by adding the appropriate volume of a 0.4% solution of each drug to weighed amounts of the ground linseed cake and mixing thoroughly to ensure that the drugs were distributed throughout the samples. The mixtures were then dried in an oven providing a warm air current at 50°C. The meals were re-ground and small samples of each type were exposed to relative humidities ranging between 100 and 70%.

Observations were made to determine the number of days required for the development of mould mycelium and mould fructification. These results are recorded

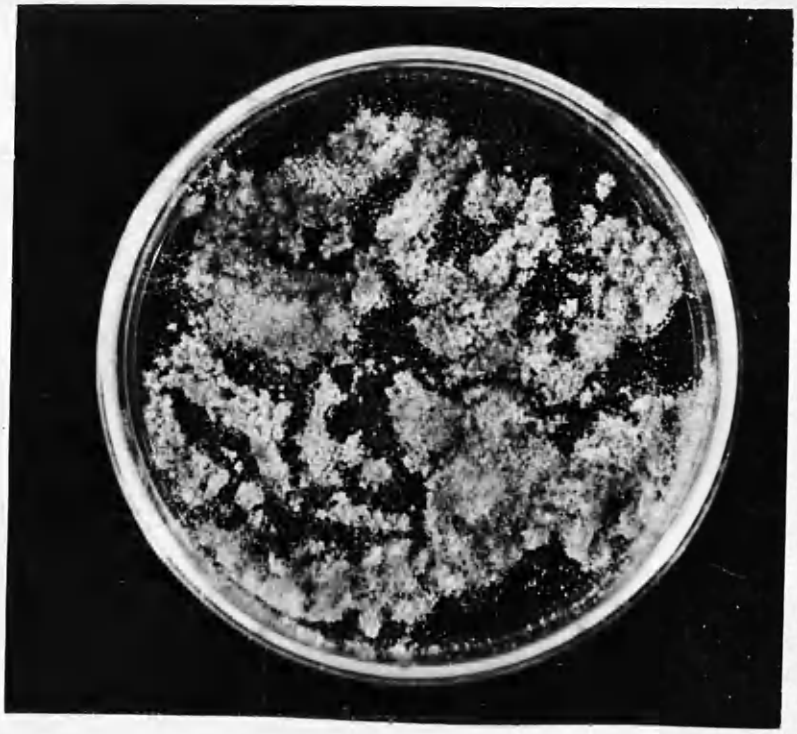
*Drugs of the sulphonamide and related groups are, in the main, only very slightly soluble in water. Their sulphonate derivatives, however, are very soluble and still retain the operative part of the molecule.

Table 45.

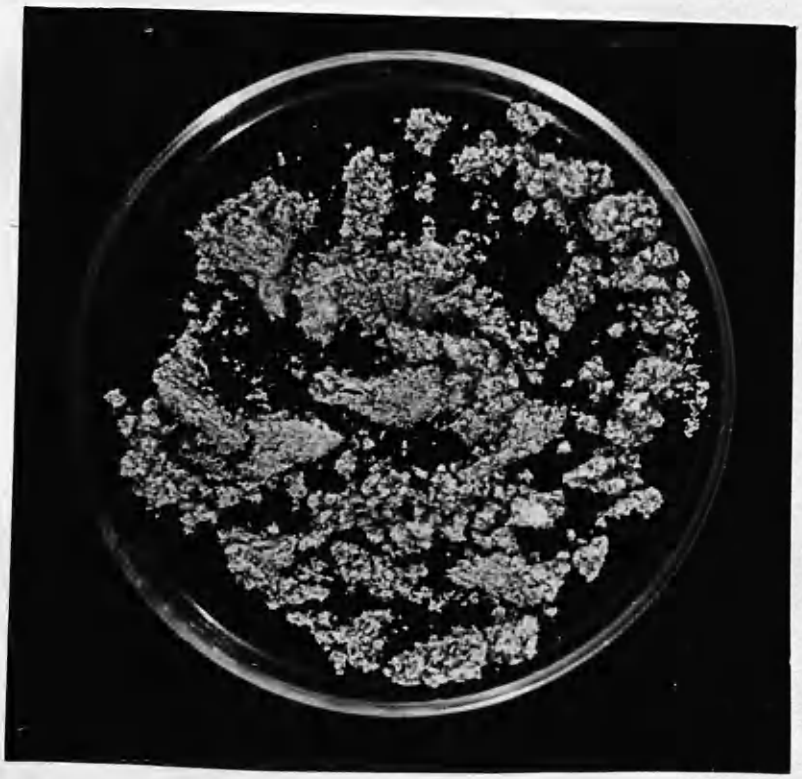
Rate of mould growth on linseed cake samples with incorporated drugs.

		% R.H.			
		100	90	85	80
		Days to development of mould mycelium			
Linseed cake only		2	4	7	20
E.O.S.	1/8,000	2	4	7	19
	1/2,000	3	4	9	25
	1/500	3	5	15	45
Propamidine	1/8,000	2	4	7	20
	1/2,000	2	4	8	25
	1/500	3	4	9	25
Mixed drugs	1/8,000	3	4	8	22
	1/2,000	3	5	9	32
	1/500	3	5	13	34
		Days to development of mould fructification			
Linseed cake only		3	5	10	25
E.O.S.	1/8,000	3	5	9	25
	1/2,000	4	5	13	34
	1/500	4	6	18	58
Propamidine	1/8,000	3	5	11	25
	1/2,000	3	5	11	34
	1/500	4	5	14	33
Mixed drugs	1/8,000	4	5	11	26
	1/2,000	4	6	13	34
	1/500	4	7	16	38

in Table 45. They show that the presence of the drugs, particularly at the higher concentrations, had an inhibitory effect on the development of moulds on these samples. At the more dilute concentrations the drugs did not have such marked antiseptic effect on the growth of moulds as would have been expected from the trials previously made on the agar plates. It is probable that on the plates better contact existed between the drug dissolved in the agar medium and the mould spores than was possible with the dry feeding-stuffs. The inhibitory effect was most marked for the samples containing E.O.S. which, when introduced at a concentration of 1/500, more than doubled the fresh storage life of these samples stored at 85 and 80% R.H. This may be compared with corresponding figures found for 1% borax and boric acid (see page 79), which showed no significant antiseptic effect at 85% R.H., and at 80% R.H., only lengthened the storage life of linseed cake samples by 50%. The fungistatic effect of 1/500 E.O.S. is shown in Plate 4 where the linseed cake sample with the incorporated drug is shown to be free from mould deterioration after 8 days storage at 90% R.H. while the control sample without added E.O.S. shows extensive moulding. In addition to this delay in the germination of moulds on samples containing E.O.S., their subsequent growth was very much sparser in the presence of the drugs. Propamidine was less effective than E.O.S. when introduced in this feeding-stuff.



Linseed cake only - general moulding.



Linseed cake + 1/500 E.O.S. - free from moulding.

Plate 4. Antiseptic effect of 1/500 E.O.S. incorporated in linseed cake samples stored at 90% R.H. for 8 days.

This fact is surprising since this drug had a greater fungistatic effect on the mould types used in the agar plate experiments. It is possible that E.O.S. provides a better general antiseptic for a variety of developing mould species than does propamidine. Certainly microscopical examination of the samples showed that the presence of the drugs determined the types of moulds developing. Thus on the samples containing high proportions of these drugs Penicillium spp. were able to become dominant over members of the Aspergillus glaucus group (particularly A. repens, A. ruber and A. amstelodami) which were the species commonly found on the control sample and on those containing the smaller drug concentrations. It was pointed out previously that these drugs have but little fungistatic effect on Penicillium spp.

As an additional experiment small 1" cubes of linseed cake were treated with solutions of these two drugs in water at concentrations of 1/250, 1/1,000 and 1/4,000. The cubes were thoroughly dried before exposure to the range of fixed humidities and observations made on their rates of moulding. Results obtained for these cubes showed that the drugs had a similar antiseptic effect as they had with the ground samples. The drugs, particularly E.O.S., increased the period of freedom from moulding and also reduced the subsequent rate and spread of mould growth. Plate

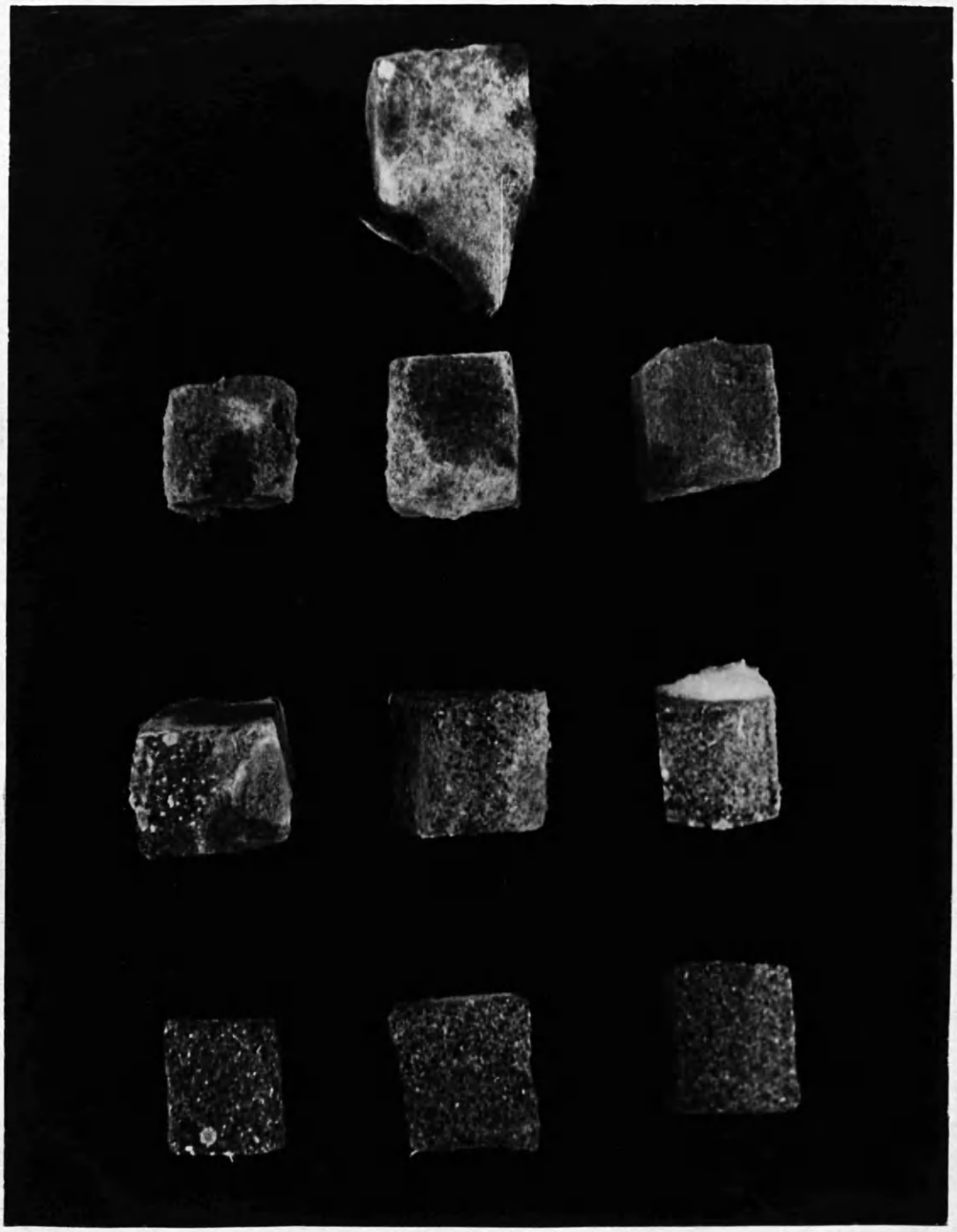
5 illustrates the antiseptic effect of the drugs when sprayed on to cubes of linseed cake.

Conclusion.

These experiments have demonstrated the feasibility of including sulphanilamide and other drugs in feeding-stuffs of this type and have shown that the storage life of ground linseed cake can be more than doubled at 85% and 80% R.H. by the incorporation of 1 part in 500 (0.2%) of E.O.S. Smaller concentrations of this drug will have a correspondingly smaller antiseptic effect.

No ill-effect could result from feeding cakes with 0.2% incorporated E.O.S.. The normal therapeutic dose of E.O.S. given orally in the treatment of mastitis in dairy cows is 4 oz. per day for 14 days (McEwan et al. 1941). If a dairy cow was to consume 5 lb. of cake per day, containing 0.2% E.O.S., the cow would have a daily intake of 1/25th of the normal therapeutic dose, which is far below the toxic level. By spraying a solution of E.O.S. on to the outside of feeding-stuff cakes the amount of E.O.S. ingested could, of course, be greatly reduced. It is equally unlikely that any toxic effects would result from using propamidine.

It is obvious that trials in the use of these drugs for extending the storage life of feeding-stuffs would have to be made on a larger commercial scale before their general adoption could be recommended. The initial experiments which have just been described



Row 1 Single control cube.) General mould
Row 2 Cubes sprayed with 1/4000 drug solution.) development
Row 3 " " " 1/1000 " ")
Row 4 " " " 1/250 " " - Free from moulding.

Right hand column - E.O.S.
Centre column - Propamidine.
Left-hand column - E.O.S. + propamidine.

Plate 5. Antiseptic effect of E.O.S. and propamidine when sprayed on to cubes of linseed cake stored at 100% R.H. for 18 days.

indicate, however, that the drugs have a marked antiseptic value even when present in very small concentrations (0.2%). These concentrations are very much smaller than those of other preservatives that have been suggested for foodstuffs (e.g. borax, which requires to be present in more than 10 times the concentration of E.O.S. to give an equivalent antiseptic effect).

The incorporation of such small concentrations (o.2%) of these drugs in oil cakes would result in a daily intake of 1/25th of the normal therapeutic dose for dairy cows. It is unlikely, therefore, that they would have harmful effects when fed to livestock. The drugs may also have some useful application to the preservation of other foodstuffs, particularly where the moisture content of the product is higher than in feeding-stuffs, when the drugs at very low concentrations might be used for effective microbial control.

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