

# CCLV. EXPERIMENTS WITH PIGS ON A PELLAGRA-PRODUCING DIET

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WHILE the rat has been used in recent years with great success in nutritional studies, the results obtained have proved to be misleading in the investigation of pellagra. Goldberger and his co-workers discovered that rats ceased to thrive, became ill and developed a symmetrical dermatitis when deprived of the heat-stable portion of the B vitamin complex contained in yeast and concluded that this disease was probably the analogue of pellagra [Goldberger, Wheeler *et al.* 1926; Goldberger & Lillie, 1926]. Autoclaved dried yeast cured and prevented this rat disease and also canine "black tongue", the experimental disorder produced in dogs by feeding on diets consisting of 80% maize with addition of ample protein in the form of purified casein. For this reason the aetiology of this canine disease was considered by Goldberger and his school to be the same as that of human pellagra [Goldberger, Wheeler *et al.* 1926; 1928, 1, 2; Goldberger & Wheeler, 1928]. Spies [1934] has since shown that human pellagra can be cured by autoclaved dried yeast.

Goldberger and his colleagues found the preventive factor or factors against canine black tongue and rat dermatitis to be present in an (autoclaved) aqueous extract of yeast and to be adsorbed on fuller's earth (at pH about 5.0). Goldberger's pellagra-preventive, or "P-P" solid, was prepared in this manner [Goldberger & Lillie, 1926; Goldberger, Wheeler *et al.* 1928, 1].

Further investigation has, however, shown that this symmetrical dermatitis of rats has not the same causation as human pellagra or canine black tongue. The constituent of the heat-stable vitamin B<sub>2</sub> complex, which has been found curative and preventive of this type of rat dermatitis (often known as "rat pellagra"), has been found present in whole maize and other cereals and to a less extent in the highly milled grains [Birch *et al.* 1935; Copping, 1936, 2; Dann, 1936]. It has been called vitamin B<sub>6</sub> by György and his colleagues.

Lactoflavin, another constituent of the heat-stable vitamin B<sub>2</sub> complex, is also water-soluble and adsorbed on fuller's earth. It is present in relatively large amounts in yeast, liver, milk and meat, the foodstuffs which have been found to have the greatest protective and curative effects in human pellagra and canine black tongue. Although necessary for health and growth of rats, it has proved to be neither curative nor preventive of this rat dermatitis; in fact the disorder has been found to be developed more regularly when pure lactoflavin is added to the experimental diet [György, 1934; Chick *et al.* 1935; Copping, 1936, 1]. Further, treatment with pure lactoflavin has been without success in human pellagra [Dann, 1936] and in experimental canine black tongue [Birch *et al.* 1935; Koehn & Elvehjem, 1936; Sebrell, Hunt & Onstott, 1937].

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It therefore seems certain that, for the prevention and cure of pellagra and black tongue, some other heat-stable constituent of the vitamin B complex than lactoflavin or "vitamin B<sub>6</sub>" is necessary [see Birch *et al.* 1935].

Many recent investigations have revealed that at least two heat-stable dietary factors, in addition to lactoflavin, are required to demonstrate the full physiological activity of the vitamin B<sub>2</sub> complex. Edgar & Macrae [1937] have separated two such factors from autoclaved yeast extract. One of these factors is adsorbed on fuller's earth together with lactoflavin and can be eluted with weak alkali; the second is contained in the filtrate after repeated adsorption of the original yeast extract with fuller's earth. A mixture of these two factors and lactoflavin manifested the full activity of the original autoclaved yeast extract for the growth of rats. Edgar & Macrae considered that the first was probably identical with György's "vitamin B<sub>6</sub>" [György, 1935; Birch & György, 1936].

Fuller's earth has also been used for separation of the vitamin B<sub>2</sub> constituents other than flavin from extracts of liver and rice bran [Richardson & Hogan, 1936; Elvehjem & Koehn, 1935; Lepkovsky *et al.* 1936; Jukes, 1937].

The filtrate from liver extract, after treatment with fuller's earth, has been found curative of canine black tongue [Koehn & Elvehjem, 1936] and also of human pellagra [Fouts *et al.* 1936]. Sebrell, Onstott & Hunt [1937] also cured black tongue with material prepared by a similar method from rice bran by Lepkovsky and Jukes. The dogs died ultimately unless riboflavin was given in addition. It was concluded that riboflavin is an essential constituent in the dietary of dogs. These results are perplexing in view of the experience of Goldberger and his colleagues, who found that the substance preventing and curing black tongue was adsorbed by fuller's earth from yeast extracts.

It seemed therefore desirable to make a study of pellagra- and black tongue-producing diets with a fresh experimental animal and the results here presented are a first instalment from experiments with pigs on diets consisting largely of maize, modelled on those used by Goldberger. Rats and mice have proved unhelpful as these animals thrive on such diets [Rhoads & Miller, 1935; Birch *et al.* 1935; Chick, unpublished experiments, see also p. 2075]. The pig was chosen as being omnivorous and easy to feed and having digestive arrangements similar to those of the dog and man.

#### *Nutritive defects of maize*

It has long been recognized that the pig cannot be reared upon maize alone, although, if suitably supplemented with minerals and various foodstuffs containing a high proportion of protein, e.g. tankage, fishmeal, skim milk, this cereal forms an excellent staple food. Hart & McCollum [1914] successfully reared pigs on the products of the maize kernel with an added salt mixture. The maize meal was supplemented by "maize gluten feed" (a mixture of gluten and bran usually containing about 25% of crude protein) so that the diet contained 15% total protein. When a suitable salt mixture was added the animals thrived. Waters and his colleagues [1915], in the course of an extensive series of experiments (1909-14) on the rearing of pigs, supplemented maize with blood albumin, total milk proteins, casein and lactalbumin, respectively. In all cases when sufficient of these supplements was given, the pigs did well. Shrewsbury *et al.* [1932] also found that casein supplemented a ration containing 84% of yellow maize when 6.7% was added, but 3% was insufficient.

In Goldberger's diets, with which he produced black tongue in dogs, the large proportion of maize was also supplemented by extra minerals and by casein to the extent of 10-15% in the diet, but the casein was purified by "leaching for a

week in daily changes of acidulated water". Birch *et al.* [1935] produced the disease in dogs on a synthetic diet containing 20% purified casein and Harris [1937], using Goldberger's diets, observed nutritive disorder in monkeys. Miller & Rhoads [1935] fed pigs on a diet similar to Goldberger's "black tongue" diet No. 123, with the primary object of studying the anaemia produced. The pigs failed to grow after the first few weeks on this food. Most of those which lived long enough appear to have developed achlorhydria and to have become anaemic; the anaemia in about half the cases was of the megalocytic type. Some of the pigs had ulcers in the mouth.

Dunlop [1935] reared 3 pigs on a diet containing maize meal 60%, barley meal 24%, soya bean meal 16% and a salt mixture. In two cases the growth slackened after 11 weeks but could be restored immediately when yeast to the extent of 1.5% was added to the diet.

Deficiency of protein or minerals cannot therefore explain the nutritive failure of these maize diets for dogs, monkeys and pigs, and the above workers agree with Goldberger and his colleagues in concluding that the diets are short of some other factor, or factors, needed for their nutrition and presumably also for that of man.

#### *General technique of the experiments*

The following experiments were made on 15 weanling pigs obtained from 2 litters. The diets used were modelled on the Goldberger No. 123 "black tongue" diet<sup>1</sup> [Goldberger, Wheeler *et al.* 1928, 1] but a salt mixture<sup>2</sup> more appropriate for pigs, containing iron, was used, the sucrose and cotton-seed oil were omitted, the proportion of maize was increased to about 80% and that of casein reduced. The casein used was "Glaxo ashless, extracted"<sup>3</sup> casein, purified by extraction first with dilute acetic acid and subsequently with hot alcohol.

The cereals were finely ground and fresh supplies obtained every few weeks, the diets were mixed freshly every few days; the food was given uncooked. The diets were mixed with water to a creamy consistency; in winter, when the weather was cold, less water was given. The pigs were fed twice daily, about 9 a.m. and 6 p.m.

The different groups of 2-4 pigs receiving the same diet were kept in separate stalls, but as a rule the individual animals were not isolated. It was not therefore possible to estimate individual food intake but the amount fed to the group was recorded and a rough estimate made of the uneaten food.

The stalls were formed by subdivision of a long barn with wooden partitions into separate rooms 15 ft. square. They communicated by doors into runs which were open to the air and separated from one another by low fences of sheep wire. The flooring of both stalls and runs was of concrete; no bedding was provided but a heavy wooden platform was placed in the corner of each stall to keep the animals off the cold concrete at night. During the summer the pigs spent most of the day in the open; in the winter during cold weather they were shut up at night.

*Exp. 1.* Nine litter-mates (sire, "Chivers large white"; dam, cross between a black boar and a large white sow). The litter of 12 pigs was born 10 April 1936

<sup>1</sup> White maize meal 67 parts, cow peas 8.1, casein (purified by leaching for 1 week in daily changes of acidulated water) 10, sucrose 5.3, with cotton-seed oil 5, cod liver oil 2.5 and additional salts 2.1.

<sup>2</sup> Salt mixture: CaCO<sub>3</sub> 2 parts, bone ash 2, NaCl 1, Fe<sub>2</sub>O<sub>3</sub> 0.08.

<sup>3</sup> Described by the manufacturers as a carefully prepared casein of the "self-soured" type, exhaustively washed with dilute acetic acid and subsequently extracted thoroughly with hot alcohol.

and weaned on 17 June; the 3 smallest were discarded and the remainder placed on experiment 30 June, at 11½ weeks old and of body weight ranging from 34 to 46 lb. Group 1 (4 pigs) received the unsupplemented maize diet containing 83% white maize meal. In the diet of group 2 (2 pigs), 8% of yeast was given and the maize meal reduced to 76%. In the diet of group 3 (3 pigs), the maize meal was substituted by a mixture of ground wheat and barley. The total digestible protein in the diets of these three groups was calculated as 10.8 to 12.1% and the nutrient ratio,  $\frac{\text{calories as protein}}{\text{calories as fat and carbohydrate}}$ , was 1:6.5 to 1:5.8.

*Exp. 2.* Groups 1-5. Six female litter-mates (sire and dam as for Exp. 1) being the largest chosen from 13 at weaning. They were placed on experiment 19 September 1936, 3 weeks after weaning, at 12 weeks old and of body weight ranging from 44 to 52 lb. In this experiment the original maize diet was modified by increasing the casein to 7%. This increased the total digestible protein in the diet of group 4 to 12.6% and the nutrient ratio to 1:5.5.

The composition of all the diets in Exps. 1 and 2 and the analyses of the materials used are set out in Tables I and II.

Table I. *Analysis of materials used*

	H <sub>2</sub> O %	N %	Protein % (N × 6.25)
Whole maize	12.6	1.36	8.5
Whole wheat	15.8	1.79	11.2
Whole barley	13.9	1.36	8.5
Pea meal	13.3	3.47	21.7
Yeast (average)	10.7	6.34	39.6
Autoclaved yeast extract		0.67-0.70	
			(N × 6.34)
Dried "whey"*	—	3.6	23.8
Casein "light white"	10.8	11.7	74.0
Casein "Glaxo ashless, extracted"	2.1	13.5	85.4

\* Dried residue from manufacture of lactose.

Table II. *Composition of diets. Amounts calculated on air-dry material*

Group	1	2	3	4	5
Whole white maize, ground	83	76	—	83	83
Whole wheat, ground	—	—	20	—	—
Whole barley, ground	—	—	63	—	—
Pea meal	11	10	11	11	11
Casein, "Glaxo ashless, extracted"	4.4	2.2	5.3	7.0	—
Casein "light white"	—	—	—	—	7.0
Yeast, dry	—	8	—	—	—
Cod liver oil	3	3	3	3	3
Salt mixture	2.5	2.5	2.5	2.5	2.5
	103.9	101.7	104.8	106.5	106.5
Computed digestible protein %	10.8	11.1	12.1	12.6	11.8
Computed digestible fat %	6.1	6.0	4.5	6.0	5.9
Computed digestible carbohydrate %	58.8	57.1	60.8	57.4	57.0
Estimated nutrient ratio	1:6.5	1:6.2	1:5.8	1:5.5	1:5.9

### Results

*Group 1. Maize diet containing 4.4% casein (4 pigs).* The pigs (2 ♂, 2 ♀) put on weight at a subnormal rate (average 3.5 lb. weekly) for 5-6 weeks, when 3 of the 4 developed diarrhoea, the appetite failed and growth was checked. One pig, No. 5, died at the end of the 7th week. Two pigs, Nos. 4 and 9, were less

acutely ill, they had diarrhoea continually for 3 or 4 weeks, during the latter part of which period they ate little or no food and suffered great loss of weight; they were killed in a moribund condition at the end of the 10th week to provide suitable autopsy material.

The remaining animal (No. 6) also ceased to grow about the 7th week and had intermittent diarrhoea 4-5 weeks later than the others. It was never as seriously ill as they and continued to consume enough food to maintain its weight. At the 13th week 4% yeast was added to the diet and the casein reduced to 2.2%. The animal at first refused its food, the body weight fell and the diarrhoea ceased during the starvation. After 8 days the pig ate greedily and then put on 50 lb. in 8 weeks, nearly 1 lb. daily. The yeast was then reduced to 2%, but nevertheless the rate of growth was maintained and 37 lb. were gained in the next 3 weeks.

*Group 2. Maize diet with yeast 8% (2 pigs).* Pigs 2 ♀ and 8 ♂ showed uninterrupted good growth and health. At the period when the pigs in groups 1 and 3 were suffering from diarrhoea they showed no symptoms or slackening of their fine growth. After 10 weeks they weighed 121 and 123 lb. respectively. The yeast was then removed from the diet and the maize increased to 83% but growth continued steadily for a further period of about 8 weeks at a rate of 8 lb. weekly weight increase, until No. 8 weighed 178 and No. 2 195 lb. During this time, however, the appearance of the pigs deteriorated, the healthy pink bloom on the skin disappeared and the colour changed to a dirty yellow. Pig No. 2 developed intermittent diarrhoea and vomiting and finally began to lose weight. Both animals recovered and resumed growth when 11% of a dried whey product was given in their diet.

*Group 3. Wheat and barley diet (3 pigs).* These pigs grew with an average weight increase of 5 lb. weekly for 5-6 weeks, when they also developed diarrhoea. Growth was checked in pigs 1 ♂ and 7 ♀ for 2 and 5 weeks, respectively, but only very slightly in pig 3 ♀. After this there was spontaneous recovery and resumption of normal growth in all 3 animals. After 16 weeks on the diet, pig 3 weighed 155 lb. and pigs 1 and 7, 119 and 118 lb., respectively.

*Groups 4 and 5. Maize diet containing 7% casein (6 pigs).* Group 4 (3 pigs) had "Glaxo ashless, extracted" casein and group 5 (3 pigs) unpurified "light white" casein. These animals did better during the first 2-3 weeks than the corresponding pigs in group 1, doubtless owing to the higher proportion of protein in the diet, 12.2 instead of 10.8%. Any beneficial effect of the extra protein was, however, soon obliterated and the average weight increase over the first 6 weeks of the 3 pigs in group 4 (Nos. 11, 14 and 15) was 3.6 lb. weekly, not higher than that of the pigs in group 1 (3.5 lb. weekly) who received a similar diet with less protein. The added nutritive value of the unpurified "light white" casein in group 5 (pigs 10, 12 and 13) was made apparent in their better growth, the average weekly increase over the first 6 weeks being 5 lb. These pigs had a better appetite than those in group 4 and were rosy and healthy looking in comparison with the latter, which soon became very thin, with a grey and dirty looking skin.

After the first 5-6 weeks these growth rates slackened in both groups. Two pigs in group 4 (Nos. 11 and 14) developed severe diarrhoea with rapid loss of weight and died. The third pig in this group, No. 15, which was in better condition, made quick recovery with resumption of growth when it received autoclaved yeast extract in amount corresponding<sup>1</sup> to about 8% (later 5%) dry yeast in the diet.

<sup>1</sup> Reckoned on the amount of yeast (dry weight) from which the extract was made.

Table III

(The 9 pigs in groups 1, 2, 3 and the 6 pigs in groups 4 and 5 were litter-mates)

Group	Diet	Pig no.	Av. weekly wt. increase during first 6 weeks lb.	Av. daily food intake during first 6 weeks lb.	Onset of diarrhoea, weeks on diet	History after first 6 weeks	Cure
1	Maize (4.4% casein)	4 ♀	3.2	—	6	Lost 11 lb. in next 3½ weeks; very ill, killed at 10 weeks	—
		5 ♂	5.0	—	5	Died at 7 weeks	—
		6 ♀	3.0	—	10	Wt. stationary for 2 weeks, slow fall for 3 weeks	Rapid, with 4% yeast in diet
		9 ♂	3.0	1.6*	6	Lost 17 lb. in 7th to 10th week; very ill, killed at 10th week	—
2	(a) Maize (2.2% casein) + yeast 8%	8 ♂	6.0	—	—	Continued in fine health and growth for 10 weeks and for 7 weeks after removal of yeast	—
		2 ♀	6.1	—	—	—	—
3	(b) Yeast removed	8 ♂	6.2†	2.6	—	No diarrhoea, but wt. increase slackened when No. 2 had diarrhoea	Dried whey product, 11% in diet
		2 ♀	9.2†	—	—	Diarrhoea and vomiting with fluctuating wt. for 6 weeks	"
		1 ♂	5.2	—	6	Diarrhoea for 2 weeks, then recovery	Spontaneous
		3 ♀ 7 ♀	5.9 4.8	—	6 5½	Diarrhoea for 2 weeks, then recovery Diarrhoea for 5 weeks with stationary wt., followed by complete recovery	" "
4	Maize (7% casein)	11 ♀	2.0	—	5	Rapid loss of wt., died at 6 weeks	† Rapid, with autoclaved yeast extract ≡ 5-6% dry yeast in diet
		14 ♀	4.5	—	6	Rapid loss of wt., died at 8 weeks	"
		15 ♀	4.2	—	—	Wt. curve flattened from 4th to 7th week	"
5	1 as 4, but casein unpurified	10 ♀	3.6	—	7	Diarrhoea from 7th to 9th week	† Rapid, with 4% dry yeast in diet
		12 ♀	4.7	—	9	Severe wt. loss from 7th week, diarrhoea from 9th week; vomiting with diarrhoea at 13th week while receiving lactoflavin 2 mg. daily	"
		13 ♀	4.4	—	13	Continued to grow at low rate throughout; diarrhoea at 13th week, while receiving lactoflavin	"
		Av. 5.0	—				

\* First 5 weeks only.

† During next 8 weeks.

† Continued to grow well for 5 weeks after discontinuing yeast extract.

The pigs which received the unpurified "light white" casein took longer to demonstrate the nutritive defects of the diet. Nos. 10 and 12 developed diarrhoea

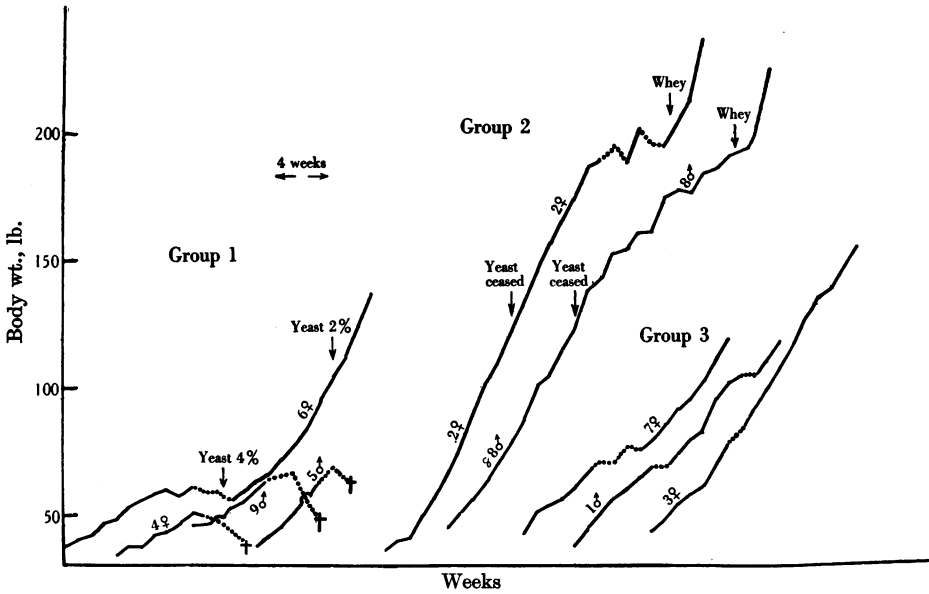


Fig. 1. Weight curves of pigs in groups 1, 2 and 3 in Exp. 1. . . . signifies diarrhoea. † signifies death. Group 1, maize diet unsupplemented. Group 2, maize diet + 8% yeast. Group 3, barley and wheat diet.

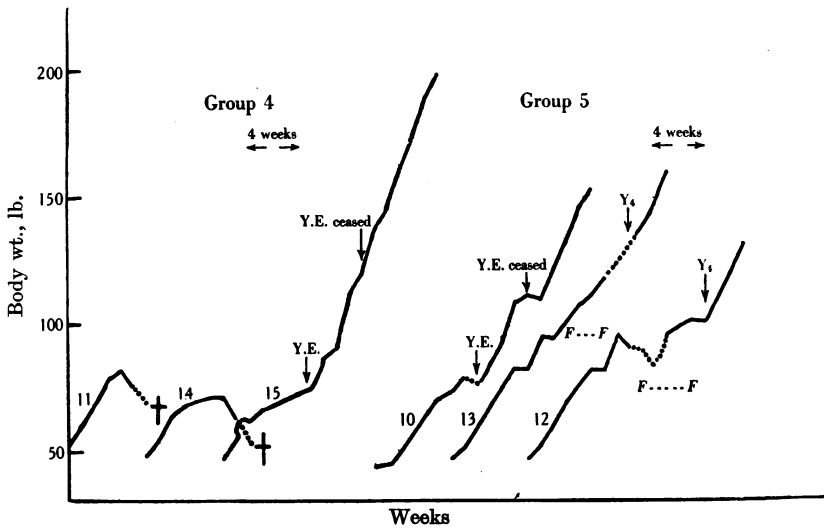


Fig. 2. Weight curves of pigs in group 4, maize diet with 7% purified casein (pigs 11, 14, 15), and group 5, maize diet with 7% unpurified casein (pigs 10, 12, 13). . . . signifies diarrhoea. † signifies death. Y.E. signifies yeast extract. Y<sub>4</sub> signifies 4% dry yeast in diet. F...F<sub>2</sub> signifies lactoflavin 2 mg. daily.

and vomiting with loss of weight after 7 and 9 weeks respectively and No. 13 only after 13 weeks. Nos. 12 and 10 recovered promptly when given respectively

4% yeast in the diet or the nominal equivalent<sup>1</sup> of 8% yeast in the form of an autoclaved yeast extract. This was later reduced to the equivalent of about 5% yeast.

The details of the dietary observations on the 15 pigs in groups 1-5 are set out in Table III and the growth curves are given in Figs. 1 and 2.

#### *Blood changes*

Examinations were made about every 10 days of the blood of all the animals in Exp. 1. These will be recorded elsewhere by Messrs Parry and Birch who, however, permit us to say that all those on the maize diet unsupplemented by yeast had a progressively increasing simple anaemia. By the time they died, or were sufficiently ill to need immediate treatment, the average fall in the number of blood cells was 24% and in the haemoglobin titration 28%.

#### DISCUSSION

The results of Exps. 1 and 2 may be summarized as follows.

The pigs were not able to thrive on the unsupplemented maize diet. After 5-6 weeks there was loss of appetite and slackening of the growth rate and 9 out of 10 pigs soon began to suffer from looseness, increasing to severe diarrhoea during the next few days. With the onset of looseness the consumption of food fell and coincident with the diarrhoea there was rapid loss of weight. Except when the diet was changed, the pigs died in periods varying from 1 to 4 weeks after onset of the diarrhoea.

When the maize in the diet was replaced by a mixture of wheat and barley, as in group 3, the pigs suffered from looseness and mild diarrhoea at the same time as their litter-mates in the adjoining pen. This checked their development but after 2-5 weeks there was spontaneous recovery with resumption of growth.

There was no indication of unusual sensitiveness to light, although the pigs spent most of the day in the open air during the summer. The pigs on the unsupplemented maize diet, however, had unhealthy, scurfy skins, with scabby patches, especially on the backs of the ears, which bled readily if scratched or handled. The scabs separated and the skin became healthy in a week or 10 days after administration of yeast.

#### *The significance of the diarrhoea and changes found at autopsy*

As, sooner or later, both dogs and monkeys subsisting on Goldberger's pellagra diet suffer from diarrhoea, and diarrhoea is a common symptom in pellagra, we were not surprised when after 5 to 6 weeks on the diet our pigs began to scour. Some features of the outbreak were, however, disquieting. In Exp. 1 diarrhoea first occurred in 3 pigs on the maize-diet that were still growing at a moderate rate—4-7 lb. a week—and before their appetite and growth rate had begun to decline. Further, diarrhoea occurred simultaneously in the 3 pigs in the neighbouring pen on the barley and wheat diet. These were growing fairly well before the diarrhoea occurred.

It seemed that the experiment was complicated by an epizootic of pig-paratyphoid and this impression was supported by the results of the post-mortem examinations of the 5 pigs on the maize diet which succumbed. In every animal there was a diffuse cellular inflammation of the mucous membrane of the caecum and large colon which were covered with a fibrinous adherent membrane, a condition characteristic in pigs dying of infectious enteritis or pig paratyphoid.

<sup>1</sup> Reckoned on the amount of yeast (dry weight) from which the extract was made.



The following is an epitome of observations made at autopsy:

The mucous membrane of the whole of the caecum and upper half of the large intestine was thickened and sodden and the surface covered by an adherent layer of cheesy material about 2 mm. thick. When the animal had died rapidly this layer was stained yellow or green by bile; when life had been prolonged for 10 days to a fortnight it was necrosed and of a dirty greenish grey colour and in parts separated from the deeper layers, leaving large raw surfaces. The serious pathological changes were confined to the large intestine and began at the ileocaecal valve. There was neither stomatitis nor glossitis in any of the pigs, but in one that survived for several months there were a few small ulcers with well-defined margins in the nasopharynx and on the buccal surface of the epiglottis. Similar ulcers in these situations have been described as occurring in pig-paratyphoid. The other organs appeared to be healthy with the exception of the lymphatic glands on the lymph channels draining the inflamed and necrotic mucous membrane of the caecum and colon. These were swollen and intensely congested and sometimes haemorrhagic.

Caseous, or necrotic, enteritis, as it is called by veterinary pathologists, is usually attributed to infection by one or other of the varieties of *B. suispestifer* and the condition can be produced by feeding young pigs with cultures of this organism [Biester *et al.* 1927].

In the present instance, however, notwithstanding the expert co-operation of Mr M. O. J. McCarthy, Bacteriologist to the Institute of Animal Pathology, neither *B. suispestifer* nor any other recognized pathogenic organism could be isolated from the tissues or intestinal contents of the animals. Attempts to obtain cultures from the blood, liver and spleen were negative. Bacterial cultures were obtained from the ileo-colic lymph glands but the only organisms cultivated were such as are found in the healthy intestine.

Our failure to discover pathogenic organisms does not exclude the possibility that such were present. Invasion of the mucous membrane by microbes from the gut undoubtedly contributed to the production of the lesions seen at the autopsies, but the results of Exps. 1 and 2 indicate that a nutritive defect in the diet of the animals was the predisposing cause of their illness, since those which received the same diet made adequate by the addition of yeast did not have enteritis. The part played by the nutritional deficiency was not less important, for those which developed diarrhoea on the uncorrected diet and had become seriously ill. These were cured by the addition of yeast or yeast extract to their food, provided that they had not ceased to eat. Indeed the nutritional defect would appear to be prepotent in the chain of causation for the process was reversed and healing occurred when the defect was made good.

We take the observations to indicate that something is missing from the basal diet, or present in insufficient amount, which is necessary to enable the mechanisms in the mucous lining of the intestine, which resist invasion by microbes from the gut, to function successfully. These local mechanisms, as far as they are known, reside in living cells and it may well be that, when there is lack of some substance essential for the metabolism of these cells, their powers to resist invasion are embarrassed or lost. Pathogenic organisms then gain entry more easily and other organisms, which are below the threshold of pathogenicity for a healthy mucous membrane, are able to penetrate and invade the tissues.

#### *Effect of yeast*

The nutritional defects of the diet were corrected by addition of yeast or yeast extract. The 2 pigs in group 2, whose diet contained 8% dry yeast, remained completely healthy. At the time when their litter-mates in groups 1 and 3 were suffering from diarrhoea, they showed no signs of it and continued to grow uninterruptedly with an average weight increase of about 8 lb. weekly

over the first 10 weeks. After the yeast was removed from the diet, the same rate of growth persisted for a further period of 8 weeks, during which the pigs continued to increase in weight by more than 1 lb. daily. Afterwards the growth slackened and one of the 2 pigs developed diarrhoea and vomiting; this ceased and growth and health were restored when 11% of a dried whey product was given in the diet. The proportion of dried yeast, 8%, given in the first period of this experiment was evidently far more than was necessary; in the case of 3 pigs (Nos. 6, 12, 13) 4% yeast was found to be ample to cure the diarrhoea and change the loss of weight into a gain of as much as 10 lb. weekly. The cures were surprisingly rapid (see Table III and Figs. 1 and 2).

#### *Effect of additional protein*

There was the possibility that some of the benefit to young swine of adding yeast to the diet or of replacing the maize by other cereals might be due to provision of better protein, seeing that about 1/3 of the proteins of the maize kernel consists of zein, which is lacking in the essential amino-acids tryptophan and lysine.

Other investigations have indicated however that, in whole maize, the defects of the zein are largely made good by the glutelins and globulins which are also present [Hart & McCollum, 1914; Fixsen & Jackson, 1932; Fixsen *et al.* 1934]. And further, in the present work, when the casein in the diet was increased to 7%, as in group 4, the pigs sickened and died in the same way as those receiving diets containing less (4.4%) casein.

The sample of dried yeast used contained 6.7% nitrogen. The nitrogen of yeast is known to possess a nutritional value in supplementing the protein of cereals [Kon & Markuze, 1931]. That the beneficial effect of the yeast was due to the protein it provided is, however, disproved by the fact that equally good results were obtained with a yeast extract from which the coagulable protein of the yeast had been separated by boiling in slightly acid solution. In two cases, pigs 15 and 10, rapid cures occurred when this yeast extract, after autoclaving, was given, in amount nominally equivalent<sup>1</sup> to 6% yeast in the diet, without any other change in the diet.

#### *General conclusions*

It must therefore be concluded that, as in the case of black tongue in dogs, the beneficial effect of the yeast is due to some heat-stable water-soluble dietary factor. There is evidence that this substance, required to supplement the maize diet, was also present to a small extent in the sample of less purified casein, the "light white" casein of which 7% was included in the diet of group 5. The 3 pigs in this group grew better than their 3 litter-mates (group 4) which received a similar diet made with 7% purified casein; during the first 6 weeks the average weekly weight increases on the two diets were 5 and 3.6 lb. respectively (see Fig. 2). The time taken for diminution of growth rate and occurrence of diarrhoea was also longer. The 3 pigs receiving unpurified casein had diarrhoea after 7, 9 and 13 weeks respectively, whereas 2 of the 3 pigs receiving the purified casein were dead within this period.

It is not surprising that dried whey should have been found a satisfactory supplement to the black tongue diet, as it is the experience in pig husbandry that a combination of maize with skim milk as source of extra "protein" forms a satisfactory diet for rearing young pigs.

<sup>1</sup> Calculated on the amount of dried yeast from which the extract was made.

The substance which can correct the defects of the maize diet is thus contained in the heat-stable portion of the vitamin B complex, hitherto known as "vitamin B<sub>2</sub>". Our experiments afford little help towards the identification of the active material with any one of the separate heat-stable dietary factors in yeast extract which are described above on p. 2066. In the trials with lactoflavin on pigs 12 and 13 (see Table III) there was no evidence of any advantage from the administration of 2 mg. daily. These experiments are now being repeated.

In view of our results, the experiments of Waters and his colleagues [1914] and those of Hart & McCollum [1914] require comment. The former were carried out from 1909 to 1915, at a time when the importance of accessory food factors was unrecognized. The famous paper of McCollum & Davis, reporting the first discovery of the fat soluble vitamin A, was published in 1915. In Hart & McCollum's experiments the pigs thrived on a diet composed of maize meal and maize gluten feed supplemented by salts. This occurred, presumably, firstly because the maize was the yellow variety (private communication from Dr McCollum) and thus contained vitamin A and secondly, because the large proportion of maize bran contained in the "gluten feed" provided an adequate amount of water-soluble vitamins. The pigs studied by Waters and his colleagues must also, presumably, have received yellow maize and derived sufficient of the other necessary accessories from the protein concentrates given as supplements. In their experiments the maize meal was adequately supplemented during experiments lasting 6 months by the casein removed from milk by precipitation with acid or by the lactalbumin precipitated by boiling the filtrate. Since, in our experiments, a supplement of 7% purified casein was unavailing, we conclude that, in addition to extra protein, a further dietary factor is required to supplement maize diets for young pigs and is adsorbed from milk on casein and on lactalbumin prepared in the above manner.

#### EXPERIMENTS WITH RATS

A whole series of diets consisting largely of maize, with addition of other grains, legumes, casein and fats (the composition being based on diets associated with human pellagra), found to be productive of black tongue in dogs by Goldberger, Wheeler *et al.* [1928, 1, 2], have been tested on rats. One of these diets, No. 114, was found to be adequate for rats by Rhoads & Miller [1935]. Another, No. 123, modified by the omission of sucrose, was investigated by Birch *et al.* [1935]. The rats at first thrived on the diet and remained free from skin lesions, but eventually the growth rates became subnormal.

The maize diet used in the present work was also tested on young rats and the results of three experiments are given in Table IV. In the first experiment, a litter of 9 young rats received the diet from time of weaning in unlimited amount; 3 rats received the diet without supplement; 3 rats received in addition a daily ration of autoclaved yeast extract in amount corresponding with 0.5 to 0.75 dried yeast and 3 rats received 14 $\gamma$  daily of pure lactoflavin. No difference could be detected in the behaviour of the rats in the three groups; growth was satisfactory in all. After 8 weeks on the diet the females were mated with males in the same group. Successful pregnancies occurred in every case and litters were born, though the young were not reared. The animals appeared healthy and normal and, except for some scaliness of the tails, the coats were good.

It is evident that either the rat is not sensitive to a deficiency in the diet of the substance required for the rearing of dogs and pigs, or that its requirements

Table IV. *Effect of "black tongue" maize diets on growth of young rats*

Exp.	Diet	Rat no.	Initial wt. g.	Average weekly wt. increase g. (2nd to 8th week on diet)	Final wt. g. (after 12 weeks on diet)	Effect of mating		
1	B.T. maize diet <i>ad. lib.</i>	85 ♂	45	22.1	236	—		
		86 ♀	45	18.5	200	—		
		87 ♀	44	14.2	144	Litter of 8		
		88 ♂	46	20.0	218	—		
		89 ♀	52	15.5	188	Litter of 10		
		90 ♀	42	12.0	150	Litter of 9		
		91 ♂	46	22.8	228	—		
		92 ♀	46	14.0	142	Litter of 9		
		93 ♀	47	16.8	152	Litter of 7		
					(2nd to 10th week on diet)	(after 12 weeks on diet)		
2	B.T. maize diet (7 g. daily) + synthetic - B <sub>2</sub> diet made with purified casein <i>ad. lib.</i>	111 ♂	45	15.4	202	—		
		112 ♀	40	13.1	168	—		
		113 ♀	45	13.2	168	—		
		114 ♂	47	16.1	197	—		
		115 ♀	45	12.0	154	—		
		116 ♂	43	17.0	189	—		
		117 ♀	45	15.5	183	—		
					(1st to 9th week on diet)	(after 9 weeks on diet)		
					9.8	131†	—	
					9.4	127†	—	
3	B.T. maize diet made with bolted degerminated maize (8 g. daily) + synthetic - B <sub>2</sub> diet <i>ad. lib.</i>	119 ♂	43	9.8	131†	—		
		120 ♀	42	9.4	127†	—		
		121 ♀	42	12.9	158	—		
		122 ♀	39	12.8	154	—		
		123 ♀	42	11.9	149	—		
		125 ♀	38	15.8	180	—		
					(1st to 9th week on diet)	(after 9 weeks on diet)		
					9.8	131†	—	
					9.4	127†	—	
					12.9	158	—	

\* Given in doses corresponding to 0.5-1.0 g. dry yeast daily, according to the weight of the rat.

† Signs of lactoflavin deficiency.

are relatively small. Accordingly, in a second experiment, the diet was fed in limited amount, 7 g. daily (which was about one-half of the average daily intake of the animals in Exp. 1), in order to see whether under these conditions any deficiencies in the diet might be made manifest. To provide the extra calories needed to satisfy the appetite, the animals received an additional ration of the usual synthetic vitamin B<sub>2</sub>-deficient diet employed in this laboratory. Of 8 litter-mates, 3 control rats received the diets without supplement, 2 rats received a supplement of autoclaved yeast extract in amount corresponding with 0.3–0.5 g. dry yeast daily, and 2 rats received 14 $\gamma$  lactoflavin daily. All the rats grew at a lower rate than in Exp. 1, as was to be expected, but again there was no significant difference between the animals in the different groups.

The experiments of Hart & McCollum [1914] (see above pp. 2066, 2075) suggest that the bran of maize may contain relatively more of the dietary factor in which the whole grain is deficient. It was therefore possible that a deficiency in our maize diet might be manifested in the case of rats if the whole maize were replaced by bolted maize, which consists largely of maize endosperm after separation of the bran and germ. The sample of bolted maize was obtained from Alabama, U.S.A., by the kindness of Dr Tom Spies, from a district in which pellagra is endemic. When the diet was thus modified and the ration limited to 8 g. daily per rat, there was definitely subnormal growth and evidence of lack of lactoflavin [see Chick *et al.* 1935; Copping, 1936, 1]. Both defects were corrected when a small daily ration of yeast extract was added. A diet, prepared with "straight-run" wheaten flour instead of maize flour, afforded a similar but less marked result (see Table IV, Exp. 3).

The results of these experiments fully confirm those of previous workers and it must be concluded that the rat is less sensitive to the nutritive defect in those diets which cause "black tongue" in dogs and the disorder described above in pigs.

#### SUMMARY

1. Young weanling pigs were fed on a diet consisting of whole ground white maize 83, peameal 11, purified casein 4.4, cod liver oil 3 and a salt mixture of 2.5 parts. The diet was similar to those with which Goldberger and his colleagues produced "black tongue" in dogs and was modelled on diets associated with the occurrence of human pellagra. It contained 10.8% protein and had a nutritive ratio of 1 : 6.5. The animals became ill with severe diarrhoea in periods varying from 5 to 10 weeks and died unless the diet were changed; they showed a progressive simple anaemia. There was no indication of unusual sensitiveness to light, although in summer the animals spent most of the day in the open air. Post-mortem examination showed a condition of necrotic enteritis of the caecum and large intestine.

2. The defects of the diet were not corrected by raising the proportion of casein to 7%, by which the total protein was increased to 12.6% and the nutritive ratio to 1 : 5.5.

3. The pigs could be satisfactorily reared on the diet if 4 or 8% dried yeast were included, the proportion of casein being suitably lowered to keep the protein level at 11.1% and the nutritive ratio at 1 : 6.2. Notwithstanding this wide nutritive ratio these pigs made uninterrupted growth with an average weight increase of 1.25 lb. daily.

4. Inclusion of this proportion of dried yeast or addition of a corresponding amount of an autoclaved aqueous yeast extract effected dramatic cures of animals which had become seriously ill on the unmodified diet.

5. The administration of 2 mg. pure lactoflavin daily did not produce any effect in the two cases tried.

6. Substitution of a mixture of whole wheat and whole barley for the maize rendered the diet just satisfactory.

7. The above "black tongue" diet without addition of yeast, on which the pigs sickened and died, was found satisfactory for rearing young rats.

8. The conclusions drawn are that the nutritive failure of the above maize diets for rearing young pigs is not due to defects in the amount or quality of the protein, but to a deficiency in some constituent of the heat-stable vitamin B<sub>2</sub> complex other than lactoflavin, and that a corresponding amount of a mixture of wheat and barley contains just sufficient of it to support satisfactory growth. Whether the missing substance is Goldberger's pellagra-preventive factor is not ascertained by these experiments, but there is no reason to suppose otherwise.

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