CXIV. EXPERIMENTS WITH PIGS ON A PELLAGRA-PRODUCING DIET. II

By HARRIETTE CHICK, THOMAS FOTHERINGHAM MACRAE, ARCHER JOHN PORTER MARTIN and CHARLES JAMES MARTIN

From the Institute for Animal Pathology, Cambridge, and the Division of Nutrition, Lister Institute, London

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In a previous paper [Birch *et al.* 1937] a description was given of a disease developing in pigs fed on a modification of Goldberger's No. 123 black tongue producing diet [Goldberger *et al.* 1928]. The diet consisted chiefly of maize supplemented with purified casein, cod liver oil and salts. The disorder, which was characterized by loss of appetite, anaemia, dermatitis and diarrhoea, proved fatal unless the diet was changed, but was prevented or cured when the pigs received in addition 4% of dried yeast or a corresponding amount of an autoclaved aqueous extract of yeast. In the present paper is recorded an attempt to discover the active substance in the yeast extract which was responsible.

A watery extract of yeast, after boiling at *p*H 5 to separate the proteins and subsequent autoclaving at 120° for 5 hr., was known to contain one substance of biological importance, riboflavin[1] [Kuhn *et al.* 1933], the identity of which had been disclosed, and two others which had been discovered only by their activity, viz. Goldberger's P-P (pellagra-preventive) factor [Goldberger *et al.* 1926] and György's "vitamin B_6" [György, 1935; Birch *et al.* 1935]. From the work of Edgar & Macrae [1937] an autoclaved aqueous yeast extract would appear to contain at least one other dietary factor necessary for rats.

Vitamin B_6 was shown by György to be present abundantly in cereals, including maize, so that it was not likely to be the dietary essential missing in our diets.

We had not advanced far towards the achievement of our object when Elvehjem *et al.* [1937] announced the discovery that nicotinic acid and nicotinamide cured dogs of black tongue. We were at first surprised, as one of us in the previous year had failed to discover from experiments on rats receiving a diet of purified casein, rice-starch and cotton seed oil supplemented by cod liver oil, pure vitamin B_1 and pure riboflavin, that either Warburg's codehydrogenase II, nicotinic acid or nicotinamide had any nutritive significance for these animals [Macrae & Edgar, 1937]. However, the importance of the statement and our respect for its authors led us to modify our programme and try the effect of nicotinic acid on some depleted pigs. The dramatic cures we obtained have been described in a recent number of this *Journal* [Chick *et al.* 1938] and the results of further experiments with nicotinic acid are given in this paper. The cure of canine black tongue by nicotinic acid has since been confirmed by Street & Cowgill [1937].

[1] The term "riboflavin", which is now generally used among workers in U.S.A., is here adopted in place of "lactoflavin", the name by which this substance has usually been known in Europe. The term lactoflavin recalls the fact that the material was separated in the pure state from whey, whereas the term riboflavin has the advantage of signifying the nature of the carbohydrate side-chain present in the molecule.

Methods

The basal diet given to the pigs, ground whole white maize 77·5, purified (Glaxo extracted) casein 6·5, ground peas 10·5, cod liver oil 3, and salt mixture 2·5 %, was described in detail in a previous paper [Birch *et al.* 1937]. The general management of the animals was also the same as previously, except that in the present (1937) experiments the young pigs after weaning were usually kept on the basal diet for 2–4 weeks before administration of the various supplements. This was done to shorten the periods of observation by depleting the animals' reserves thus reducing the cost of feeding them.

The animals were not given any bedding except in one series of experiments undertaken during the winter, in which the young pigs were supplied with straw. The effect of this upon the result is discussed later.

Supplements investigated

Riboflavin. From experiments on rats, which are extremely sensitive to deficiency of riboflavin and yet thrive on the above maize diet [Birch *et al.* 1937], it seemed improbable that this could be the missing nutrient. Preliminary tests made during the previous year had indicated that, although riboflavin might be essential in the diet of the pig, it alone was incapable of rectifying the deficiency in the maize diet. Further experiments described below confirm this conclusion.

Fractions of yeast extract obtained by employing fuller's earth. Fractionation with fuller's earth has proved a fertile method in the hands of Goldberger [Goldberger *et al.* 1928] and has since been used with success by others. An autoclaved watery extract of yeast was twice treated with 5 % of its weight of fuller's earth at *p*H 1·3. The filtrate, the washed clay and an eluate of the latter with weak alkali, were each used to supplement the basal diet; the eluate was freed from riboflavin. The precise method of preparation of the different fractions was that described by Edgar & Macrae [1937].

Extraction with alcohol. Another method employed was extraction of dried yeast with 95 % alcohol in the cold, instead of with boiling water. This was achieved by slow percolation of 135 l. of 95 % alcohol through 6·5 kg. dried yeast in a cylinder about 3 ft. high and 6 in. in diameter. The rate of flow was about 1 l. per hour. By this exhaustive treatment 14 % of the solids of the yeast were removed, as compared with 23 % by extraction with boiling water. The alcohol was evaporated from the extract, the residue extracted with hot water and cooled. The insoluble lipoids and fatty material were discarded. About 80 % of the substances extracted with 95 % acid alcohol went into the watery solution. It contained some riboflavin but most of this was left in the extracted yeast, indicating that in yeast it is present in the combined form. Euler & Adler [1934] had also found that most of the flavin contained in yeast was in the bound state.

Results of experiments

The observations were made on 19 weanling pigs and the results are shown graphically in Figs. 1 and 2. Six pigs received the maize diet without supplement and of these all except one behaved as described previously. In 3–8 weeks there occurred failure in appetite, slackening in growth rate, development of a scurfy coat and diarrhoea. Of these animals three (Nos. 16, 25 and 32) died, while two (Nos. 18 and 26) recovered on treatment with nicotinic acid; the history of the

two last has been recorded [Chick *et al.* 1938]. One pig (No. 19) grew and developed normally and is referred to again below. The appearances at post-mortem examination and the bacteriological investigation of those that died are described later.

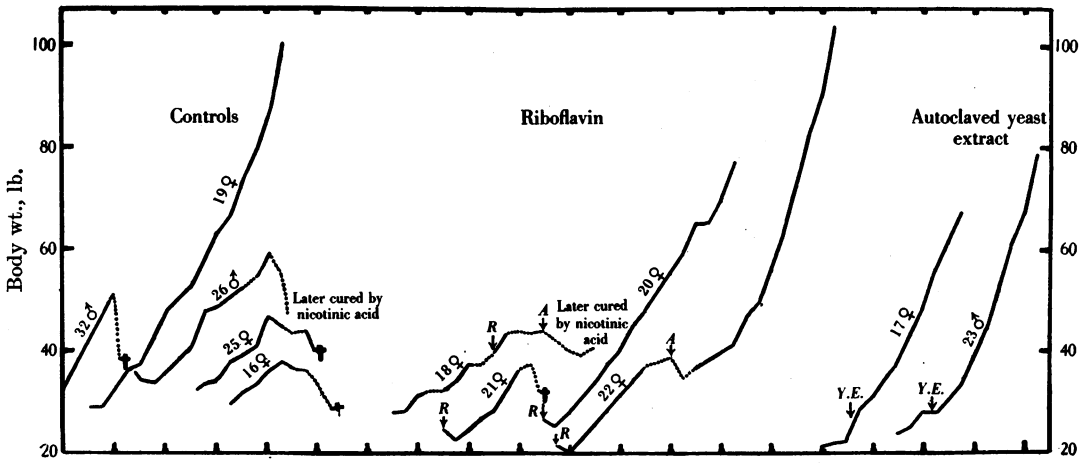

Fig. 1. Growth curves of pigs receiving the maize diet unsupplemented and supplemented by the daily additions indicated on the curves: $R = 2$ mg. riboflavin. *Y.E.* = 50 ml. autoclaved yeast extract corresponding to about 25 g. yeast, dry weight. *A* = Alcoholic extract of dried yeast. ··· Signifies diarrhoea. + Signifies death. Divisions on the abscissa indicate periods of 4 weeks.

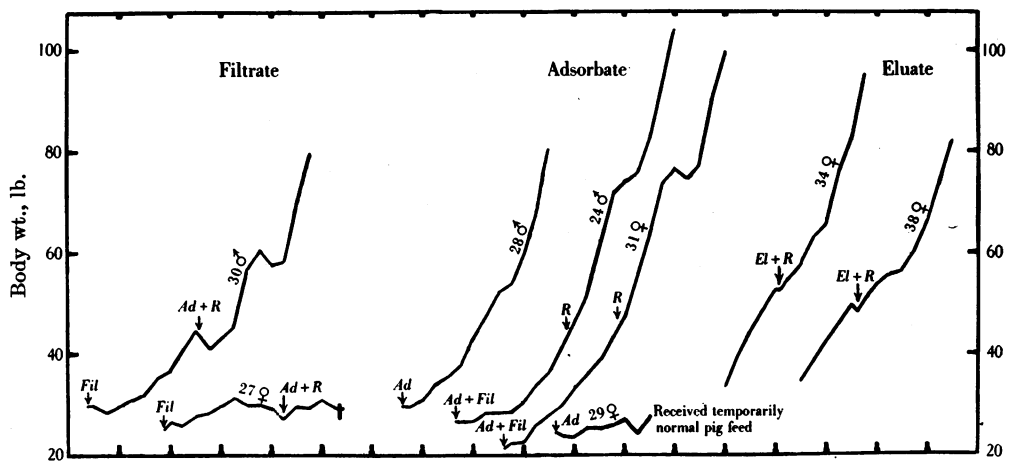

Fig. 2. Growth curves of pigs receiving the maize diet supplemented by daily additions of fractions separated from autoclaved yeast extract as indicated on the curves: *Fil* = fuller's earth filtrate corresponding to 80 ml. autoclaved yeast extract. *Ad* = fuller's earth adsorbate corresponding to about 100 ml. autoclaved yeast extract. *El* = eluate from the fuller's earth adsorbate corresponding to about 160 ml. autoclaved yeast extract. *R* indicates the addition of 2 mg. daily of riboflavin.
··· Signifies diarrhoea. + Signifies death. Divisions on the abscissa indicate periods of 4 weeks.

Riboflavin. Four pigs (Nos. 18, 20, 21 and 22) were given riboflavin in a daily dose of 2 mg. dissolved in 20 ml. water. Three pigs received it from the beginning

of the observation and one after growth had ceased on the basal diet and diarrhoea was present. These pigs behaved like the controls on the unsupplemented maize diet; one (No. 21) died, another (No. 18) remained stationary in weight and continued to have diarrhoea, and a third (No. 22) made no progress and was finally cured by administration of the alcoholic extract of yeast. The fourth pig (No. 20) remained healthy and developed normally.

The exceptional behaviour of Nos. 19 and 20 requires explanation. Of a total of 13 pigs on the basal diet studied up to the present, 12 have sickened and died unless the diet was changed; one only, No. 19 described above, throve on the basal diet. Of 6 pigs receiving riboflavin in addition, 5 have failed to show any protection or benefit and again one (No. 20) grew well and remained healthy. It was noticed that these two aberrant animals were in the habit of eating faeces. We are inclined to attribute their wellbeing to this circumstance, for, from the experiments of Hughes [1932], it is now clear that *Bact. coli* can synthesize nicotinic acid or nicotinamide, so that we assume that these 2 pigs obtained this needed material by their coprophagous addiction.

Yeast extract and fractions separated from it. The unfractionated autoclaved yeast extract, in a daily dose of 50 ml. (corresponding to about 25 g. yeast dry wt. and containing 5 g. extracted yeast solids), was given to 2 pigs (Nos. 17 and 23). These developed normally (see Fig. 1).

Two pigs (Nos. 27 and 30) received the filtrate after treatment with fuller's earth in daily doses of 80 ml., corresponding to 80 ml. of the yeast extract from which it was made. These pigs did no better than those receiving the unsupplemented basal diet. One (No. 27) increased only 5 lb. in weight in 6 weeks and during the next 3 weeks declined in weight; after 8 weeks its appetite failed, it had diarrhoea and lost 5 lb. It was then given the adsorbate in addition; a temporary improvement followed, but it died at the 13th week. The other (No. 30) increased in weight by 15 lb. in 8½ weeks, and then began to lose weight and also was given adsorbate from the 9th week; it made a good recovery (see Fig. 2).

Four pigs (Nos. 24, 28, 29 and 31) received the fuller's earth containing the adsorbate from about 100 ml. of yeast extract daily. One (No. 29) did badly and failed to grow, but the other 3 pigs, of which 2 (Nos. 24 and 31) received the filtrate fraction in addition, grew and developed fairly well. Their rate of growth was, however, poor considering the amounts of the adsorbate given, suggesting that the active material was not eluted completely in the alimentary tract.

Two pigs (Nos. 34 and 38) received the eluate from the fuller's earth adsorbate in a daily dose of 80 ml., corresponding to 160 ml. of the original yeast extract, together with 2 mg. riboflavin. As 50 ml. daily of the yeast extract were found adequate in the cases of pigs 17 and 23, ample provision would seem to have been made for losses during the various procedures involved in preparing the eluate. These pigs had previously received the unsupplemented diet for 4 weeks, and their reserves were presumably exhausted, for they had started to lose weight and a litter-mate had died with severe diarrhoea. On receiving the eluate they immediately recovered from the setback to their growth and subsequently grew at a rate of 5·5–10 lb. per week and developed into nice healthy pigs (see Fig. 2).

Alcoholic extract of dried yeast. This was given to 2 pigs (Nos. 22 and 18) which had ceased to grow and were suffering from general dermatitis with loss of hair; they had severe diarrhoea and refused to eat. These pigs had previously been treated with riboflavin without effect. Pig No. 22 received two intramuscular injections, with an interval of 4 days, of 10 ml. of a watery solution containing 2·5 g. of that portion of the yeast solids which had been extracted by alcohol and was soluble in water. There was an immediate beneficial effect on the

appetite after the first injection and soon after the second the diarrhoea abated. Thereafter the alcoholic extract was given with the food, in daily doses containing 0·65 g. solids. In the course of a few weeks the loose hair was shed, the skin became healthy and a new coat was grown and in 5 weeks the pig attained a weight increase of 8 lb. weekly. Thereafter, the weekly gain in weight steadily increased until during the last week of the observation, 13 weeks after the beginning of the treatment, it reached 13·5 lb. (see Fig. 1). Pig No. 18 received much smaller doses, 4 injections totalling 3·2 g. solids being given in the course of $3\frac{1}{2}$ weeks. An amount containing 0·5 g. solids was also added to its food daily, but as it ate little it is doubtful how much of this was consumed. These smaller doses failed to effect a cure and the dermatitis became progressively worse. After each injection there was a temporary return of the appetite and an intermission of the diarrhoea. The animal just maintained its weight, but a recurrence of all the symptoms soon occurred. It was subsequently cured with nicotinic acid.

Effect of nicotinic acid. It was at this stage of our experiments that the cure of black tongue in dogs with nicotinamide and nicotinic acid mentioned above was reported by Elvehjem *et al.* [1937]. Nicotinic acid was accordingly administered to two animals in the above series, Nos. 18 and 26, which were severely ill. The nicotinic acid was at first injected intramuscularly in doses of 100 mg.; after a few days when appetite was restored by this treatment, it was given with the food in doses of 60 mg. daily. Dramatic cures occurred. The history of these two pigs has been described in a separate paper [Chick *et al.* 1938].

Another pig (No. 29) had been used for a previous experiment and had recovered on a normal diet. It was again placed upon the basal maize diet and after 12 days 60 mg. of nicotinic acid were mixed with its daily ration. It was kept under observation for 2 months during which time it remained in good health and manifested a steady acceleration in its rate of growth. When the weekly gain in weight reached 10 lb. it was disposed of.

As soon as could be arranged, a further experiment was started to ascertain the requirements of the pig for nicotinic acid by adding it to the basal diet from weaning. By the time this experiment was under way the winter was advanced and the weather cold. It was found necessary to provide bedding for the young animals as we had no means of warming their quarters. They were supplied with wheat-straw in which to bury themselves, although we were alive to the possibility that this might frustrate the experiment if they should eat much of it.

The basal diet was simplified by the omission of the 10·5 % of ground peas. This was done in view of the fact that peas have been shown by Schulze & Winterstein [1910] to contain trigonelline, the betaine of *N*-methyl nicotinic acid. As, according to Ackermann [1912], when nicotinic acid is fed to dogs, it is in part excreted as trigonelline in the urine, it seemed not improbable that the animal body might accomplish the reverse change.

A litter of 10 weanling pigs was divided into 3 groups. One group of 3 pigs received the basal diet alone; in a second group 3 pigs each received 25 mg. and one pig 50 mg., of nicotinic acid daily; in the third group of 3 pigs a supplement of 50 ml. of yeast extract was given daily to each. The basal diet at first consisted of 69·5 % ground maize and 25 % maize gluten (containing 49 % total crude protein and 29 % zein) with 3 % cod liver oil and 2·5 % salt mixture. The gluten was used to raise the level of protein to that suitable for young pigs and thus save the cost of the purified casein. It was a failure. The pigs did not grow at all, although the proportion of protein in the diet was 15·8 % and the nutrient ratio 1:4·2. After 2 weeks the gluten was discarded and 10 % purified casein added, the maize being increased to 84·5 %. After this adjustment the diet contained

14·1 % protein and had a nutrient ratio of 1:4·8. During the next week after this alteration the average gain in weight was 3½ lb.

The experiment was continued for 9 weeks. All the pigs in the 3 groups remained healthy and grew consistently well; the 3 curves in Fig. 3 show the average rate of growth of the pigs in each group. The heaviest pigs had been chosen for the group receiving the unsupplemented diet and this advantage was maintained throughout.

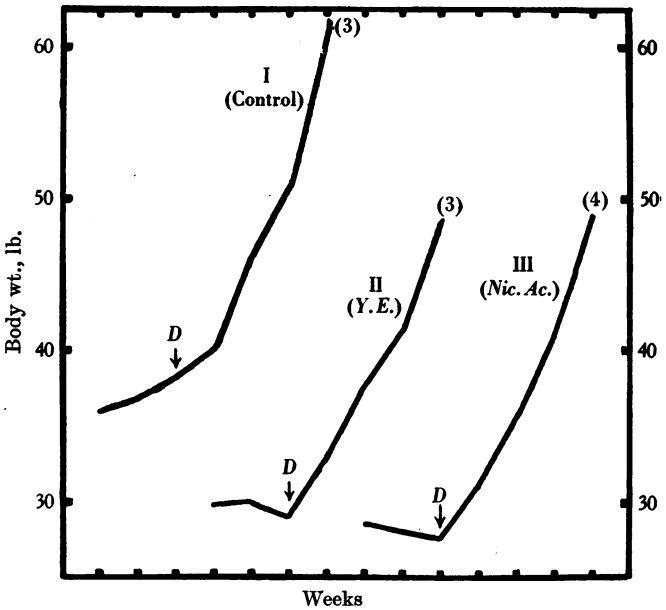

Fig. 3. Average growth curves of 3 groups of pigs bedded on wheaten straw receiving the whole maize + maize gluten diet and, from the points indicated by *D*, the whole maize + casein (10%) diet. Curve I, pigs received no added supplement; Curve II, pigs received each 50 ml. daily autoclaved yeast extract (*Y.E.*) corresponding to about 25 g. yeast, dry weight; Curve III, pigs received daily 25–50 mg. nicotinic acid (*Nic. Ac.*). The figures in brackets indicate the number of pigs from which the growth curves are derived.

That the pigs consumed the straw was obvious from their excrement. Those receiving the unsupplemented basal diet appeared to have consumed it in largest amount and their faeces resembled horse-dung.

As, with the exception of one animal which was coprophagous, all of the 13 pigs receiving the basal diet in our previous experiments during 1936 and 1937 manifested cessation or diminution of growth, developed diarrhoea after 4–6 weeks and died if their diet was not supplemented with yeast extract or nicotinic acid, we can only conclude that the consumption of wheat straw provided a sufficient additional amount of nicotinamide or some related compound of pyridine from which it could be derived. We have not yet succeeded in obtaining chemical evidence for this conclusion.

Summary of pathological and bacteriological observations made at autopsies

Five pigs (Nos. 16, 25, 32, 21 and 27) died. The first three had been on the basal diet for 8, 9 and 4 weeks respectively. No. 21 had received a supplement of 2 mg. of riboflavin daily; it died in 6½ weeks. No. 27 had been given the basal

diet together with the filtrate after treatment of a yeast extract with fuller's earth.

The autopsies were always made within a couple of hours of death. All the pigs were emaciated. They all had rough, scurfy coats, with more or less severe dermatitis, particularly on the ears. There was little subcutaneous or mesenteric fat. The blood appeared watery and was generally uncoagulated. No significant abnormalities were observed in the mouth, pharynx, larynx or trachea. The lungs were healthy, except those of No. 32, in which in the lower part of the right lung there were numerous fibrotic pneumonic patches; these contained the embryos of *Ascaris*. The hearts were flabby, their walls being thin.

Nothing abnormal was found in the abdominal organs except in the alimentary canal. In pigs 16 and 21 there were capillary haemorrhages in the mucous lining over the greater curvature of the stomach and duodenum and again at the lower end of the ileum. With the above exceptions the small intestine appeared normal until the ileo-caecal valve was approached, when more or less extensive congestion and swelling of the mucous membrane were apparent, accompanied in some cases with shallow ulcers of irregular outline and considerable extent.

In every case there were serious lesions in the large intestine. These varied from shallow serpigenous ulcers totalling a few square inches in extent, in proximity to the ileo-caecal valve, to a diffuse necrotic enteritis extending over the whole mucous surface of the caecum and upper two-thirds of the colon. Intermediate degrees of severity occurred in which the coagulative necrosis was scattered in patches of a few square centimetres in area over the caecum and upper colon, the intervening mucous membrane appearing healthy. Whether the lesions were discrete or generalized, the microscopical appearances were similar. The mucous membrane down to the muscularis mucosae was converted into a coagulated mass of necrotic tissue 1–2 mm. thick, enclosing food particles and stained by bile. This necrosed layer swarmed with microbes including *B. fusiformis* and spirochaetes.

The lymph glands on the lymphatics draining the affected parts were swollen, oedematous and often haemorrhagic.

Bacterial culture was attempted in every case from the heart blood, spleen and liver, but the culture tubes remained sterile. Cultures were, however, generally obtained from the swollen lymph glands but there was no consistency in the type of organism cultivated. The microbes isolated included various kinds of cocci, anaerobes and coliform organisms; amongst the last there was a predominance of *B. acidi lactici*. No bacteria of recognized pathogenicity were recovered.

This second series of pathological and bacteriological observations supports the conclusions arrived at from our previous experience [Birch *et al.* 1937], that the primary cause of the intestinal lesions was nutritional and that the nutritional defect had led to diminished power to resist invasion of the mucous membrane of the large gut by intestinal bacteria of low pathogenicity.

It is now clear, from the results of Elvehjem and his colleagues and from those described in this paper, that the nutritional deficiency which causes the symptoms observed in pigs is identical with that which was discovered by Goldberger to produce black tongue in dogs, a disease characterized by stomatitis and glossitis proceeding to necrosis. The question why the lessened resistance to bacterial invasion should be manifested principally in the large intestine of pigs and in the mouth of dogs, remains unanswered.

Experiments on rats with diets consisting largely of maize

A few observations were made with rats in order to see whether any deficiency of nicotinic acid in maize could also be manifested with this animal. Macrae & Edgar [1937] had shown previously that neither nicotinic acid nor nicotinic acid amide could replace any of the essential dietary factors for the rat which were separated by them from yeast extract, but since their eluate fraction from yeast was found to contain nicotinic acid amide, there was no proof that this compound might not be a necessary nutrient. Cook, Clarke & Light [1937] also found that these pyridine derivatives could not replace corresponding fractions separated from rice polishings in the nutrition of the rat.

As the actual maize, peameal and casein diet given to the pigs had been found to be adequate for growth and reproduction of rats [Birch *et al.* 1937], a series of diets was composed in which the peameal was omitted and the extra protein required to provide a total of about 15% was supplied by additional casein and, in one case, by maize gluten (Diet *a*). The results of these experiments and the details of the diets used are set out in Table I.

All the rats received extra vitamin B_1 as 5–10γ daily of the pure crystalline hydrochloride, and vitamins A and D as a daily ration (0·08–0·1 ml.) of cod liver oil, all these being administered separately to each animal. In each observation one group of rats received the basal diet without addition, while groups of their litter-mates received daily supplements respectively of autoclaved yeast extract corresponding to 0·5–0·75 g. of yeast (dry wt.), of nicotinic acid or nicotinamide (1–1·5 mg.), and of riboflavin (15γ) together with nicotinic acid or nicotinamide.

When the diet contained protein largely derived from maize gluten, even when it was fed *ad lib.*, the deficiency in quality of the proteins was manifested, as was the case with the pigs. In all groups the growth was subnormal (Exp. 1, Table I). With the diet containing 88% maize and 9% casein, on the other hand, even when the intake was limited to 10 g. daily, and the extra calories required to satisfy the diet were supplied by a synthetic "B-free" diet of purified casein, rice starch, cotton seed oil, lard and salt mixture, all groups showed satisfactory growth and addition of the autoclaved yeast extract made no difference (Exp. 2). The proportion of whole maize was therefore reduced from 77·5 to 50%, that of purified casein was increased to 11%, rice starch was added to 35% and the intake was limited to 10 and 8 g. daily, in Exps. 3 and 4 respectively. Here the beneficial effect of additions of autoclaved yeast extract or of riboflavin was at once apparent. In Exp. 3, the average weekly weight increase during 6 weeks of control rats on the basal diet was 15·2 g., but was 20·1 and 17·8 g. for those rats receiving autoclaved yeast extract and riboflavin respectively; while for those receiving nicotinamide the figure, 15·1 g., was no higher than for the controls. In Exp. 4, where the intake of the basal diet was limited to 8 g. daily, the effect of adding yeast extract was even greater. An average weekly weight increase on the basal diet of 12 g. was increased to 22·6 and 16·1 g. respectively when autoclaved yeast extract and riboflavin were given. In this experiment one of the two male rats receiving nicotinamide (No. 162) showed an improved rate of growth, the average weight increase being 16·2 g. weekly, while the weekly weight increase of the second, 10·2 g., was no higher than that of rats receiving the unsupplemented basal diet.

From the above experiments and those previously recorded [Birch *et al.* 1937] it must be concluded either that nicotinic acid or nicotinamide are not essential dietary constituents for the rat, or that the requirement of this animal for

Table I. *Effect of maize diets, nicotinic acid and nicotinamide on the growth of young rats*

Each rat received extra vitamin B_1 as 5–10 γ daily of the synthetic hydrochloride and vitamins A and D as 0·08–0·1 g. cod liver oil. The autoclaved yeast extract was given in doses corresponding to 0·5–0·75 g. dry yeast daily, and the nicotinic acid and nicotinamide in doses of 1–1·5 mg. daily, according to the weight of the rat. These doses and the riboflavin were administered separately to each rat.

Exp.	Litter	Diet	Supplement	Rat no.	Initial weight g.	Final weight after 6 weeks on diet g.	Av. weekly increase during 6 weeks g.
1	1	(*a*) Whole maize, 77 Maize gluten, 21 Salt mixture, 2·5	Unsupplemented	133 ♂	55	140	14·2
				134 ♀	50	134	14·0
		Given *ad lib.*	Autocl. yeast extract	135 ♂	52	161	17·0
				136 ♀	49	144	15·8
			Nicotinic acid	137 ♂	50	133	13·8
				138 ♀	48	124	12·7
			Nicotinic acid + riboflavin, 15 γ	139 ♂	53	140	14·5
				140 ♀	51	133	13·7
2	2	(*b*) Whole maize, 88 Purified casein, 9 Salt mixture, 2·5	Unsupplemented	141 ♂	51	177	21·0
				142 ♀	45	135	15·0
			Autocl. yeast extract	143 ♀	47	162	19·2
				144 ♀	45	159	19·0
		Intake limited to 10 g. daily + "$-B_2$" synthetic diet given *ad lib.*	Nicotinic acid	145 ♀	51	166	19·2
				146 ♀	46	143	19·5*
			Nicotinic acid + riboflavin, 15 γ	147 ♂	47	184	22·8
				148 ♀	43	145	17·0
3	3	(*c*) Whole maize, 50 Rice starch, 35 Purified casein, 11 Salt mixture, 2·5	Unsupplemented	149 ♂	49	142	15·5
				150 ♀	50	140	15·0
			Autocl. yeast extract	151 ♀	45	152	17·8
				152 ♀	47	182	22·5
		Intake limited to 10 g. daily + "$-B_2$" synthetic diet given *ad lib.*	Nicotinamide	153 ♂	51	147	16·0
				154 ♀	49	135	14·3
			Nicotinamide + riboflavin, 15 γ	155 ♀	48	155	17·8
				156 ♀	48	155	17·8
	4	(*c*) As in Exp. 3, but intake limited to 8 g. daily + "$-B_2$" synthetic diet given *ad lib.*	Unsupplemented	157 ♂	47	121	12·3
				158 ♀	42	112	11·7
			Autocl. yeast extract	159 ♂	43	200	26·2
				160 ♀	40	154	19·0
			Nicotinamide	161 ♂	43	104	10·2
				162 ♂	46	143	16·2
			Nicotinamide + riboflavin, 15 γ	163 ♂	45	149	17·3
				164 ♀	42	132	15·0

* Omitting the record for 2 weeks when the animal was evidently sick.

these pyridine compounds is very small compared with that of the pig. Diets containing large amounts of whole maize, if supplemented by additional protein of good quality such as casein, by a suitable salt mixture and by vitamins A and D, have been found to sustain growth and reproduction. When the amount of whole maize consumed was replaced by maize endosperm, a deficiency of riboflavin became apparent; but only when, in addition, the ration of the maize diet was limited and the appetite was satisfied with synthetic diet presumed to be free from nicotinamide was there a somewhat doubtful response in one experiment to a daily dose of this substance.

It is not known whether the simpler materials, rice starch and purified casein, included in the synthetic diets used for nutritional work for rats, are free from these pyridine derivatives. It may be that small residual amounts present may suffice to supply the requirements of the rat. The question could only be settled with the use of basal diets known to be entirely free from nicotinic acid derivatives.

Discussion

In the light of the dramatic results obtained with nicotinic acid, it is clear that this, or nicotinamide, is for pigs the only substance missing in the maize diets fed. The same appears to be true for human beings subsisting on diets consisting too exclusively of maize, from the reports accumulating of the curative value of nicotinic acid in human pellagra [Fouts *et al.* 1937; Smith *et al.* 1937; Hassan, 1937; Spies, 1938]. The dog, which develops black tongue on similar maize diets and is also cured by nicotinic acid, thus falls into line with the pig and with man.

The rat and mouse, on the other hand, will thrive on maize diets which cause sickness and death in the pig and the dog. If nicotinic acid or nicotinamide is an essential nutrient for the rat, the amounts required must be relatively small and attempts to demonstrate the need for these pyridine compounds, even as a supplement to diets of purified materials, have been unsuccessful.

The experiments showing that the substance required to supplement maize diets and contained in an autoclaved yeast extract can be extracted with 95% alcohol and be adsorbed on fuller's earth from an acid solution, are consistent with the conclusion that nicotinic acid or nicotinamide is the material lacking. The filtrate, after treatment of the yeast extract with fuller's earth had no supplementary activity for the maize diet, whereas the eluate obtained from the fuller's earth by $N/10$ alkali, and shown by Macrae & Edgar [1937] to contain a considerable amount of nicotinamide, was potent. The fuller's earth containing the adsorbate would also have contained nicotinamide, but its elution in the alimentary tract of the pig appears to be irregular and incomplete.

Riboflavin was not apparently deficient in the maize diets used, since addition of extra amounts had no beneficial effect for pigs or rats. Seeing that the diet was adequate for rearing rats, which are very sensitive to deficiency of riboflavin, this substance would appear to be present even in some abundance. Riboflavin may be assumed to be a necessary nutrient for the pig but the above experiments give no indication of the requirements. Sebrell and his co-workers [Sebrell *et al.* 1937, 1, 2; Sebrell & Onstott, 1938] showed that riboflavin was without effect in curing or preventing black tongue in dogs, but was a dietary essential for this animal, although more than 6 months on the particular diet used were necessary for the demonstration of the deficiency.

Summary

1. The dietary essential required to correct a diet consisting of maize 77·5, purified casein 6·5, ground peas 10·5, cod liver oil 3 and salt mixture 2·5 parts, and to render it wholesome for growth and development of young pigs, is contained in the adsorbate at *p*H 1·3 on fuller's earth from an autoclaved yeast extract, but does not appear to be satisfactorily eluted in the alimentary tract. It is also present in the eluate obtained by treatment of this adsorbate with dilute baryta and in an extract of dried yeast made with 95% alcohol.

2. Observations published in another paper and extended in this show that the diet can be rendered suitable for rearing pigs by addition of a small daily

ration (60 mg.) of nicotinic acid. Macrae & Edgar [1937] showed that the eluate fraction from autoclaved yeast extract, described under 1 above, contains a considerable quantity of nicotinamide.

3. Young pigs were satisfactorily reared on the maize diet when they were bedded on wheaten straw, of which they ate large amounts. The conclusion is drawn that this straw contained nicotinic acid or some substance from which they derived it.

4. Pure riboflavin, 2 mg. daily, was without effect in preventing or curing the symptoms developed on the maize diet, but the experiments do not show whether riboflavin is an essential dietary constituent for the pig or afford any indication of the requirement.

5. Young rats could thrive on maize diets of similar composition to those on which young pigs sickened and died. When the intake was limited and the necessary calories and protein were made good with purified constituents, deficiency of riboflavin was manifested, but no consistent advantage was obtained by giving nicotinic acid or nicotinamide. If one or other of these pyridine compounds is essential for the rat, the amount required must be very small.

6. The results of the autopsies of the five pigs that died and the bacteriological observations made at the time, confirmed those described in the previous paper. They support the conclusion then drawn that the ulceration and necrosis of the large gut are primarily due to the nutritional defect and that the bacterial invasion is a secondary phenomenon.

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