CXXXI. SOME FACTORS INVOLVED IN THE EXPERIMENTAL PRODUCTION OF PELLAGRA IN RATS.

I. PRELIMINARY.

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OWING to the work of Goldberger and his collaborators it has been shown without doubt that pellagra is a deficiency disease, curable and preventable by the administration of a pellagra-preventive factor contained, notably, in yeast. This P-P factor of Goldberger so far has not been separated from the growth-promoting component of the vitamin B complex.

That a pellagra-like condition, where in fact the nature of the dermatitis agrees remarkably closely with that occurring in human pellagra, can be produced in rats has been firmly established. But the conditions necessary for the production of pellagra in rats would appear to be less understood. Some workers have failed to produce the classical skin lesions by feeding diets apparently lacking in the pellagra-preventive factor [*e.g.* Evans and Burr, 1928] and it appears probable that, though the absence of the vitamin is the main factor in the causation of the disease, yet there may be other contributory causes, in the absence of which the disease is less likely to occur. The case against the "poor-protein" theory has been fully discussed by Aykroyd [1930] and need not be entered into here.

A diet sometimes used for producing pellagra in rats is one consisting largely of maize, which, though sufficiently rich in vitamin B₁ to prevent symptoms of polyneuritis, is poor in the pellagra-preventive factor. The shortest time taken for skin lesions to appear on this diet is about 7 weeks [Goldberger and Lillie, 1926] but the rats may remain free for much longer.

The preliminary work reported in this paper has been the outcome of an attempt to establish a routine for the experimental production of pellagra on purified synthetic diets containing caseinogen, by carefully controlling the dosage of the vitamin B complex after the rats have just been "run out" of these factors.

EXPERIMENTAL.

The basal diet in use in this laboratory is as follows:

Caseinogen (Glaxo, vitamin-free)	...	...	...	23 %
Cane sugar...	...	...	...	17
Rice starch	...	...	...	40
Palm kernel oil	...	...	...	15
Salt mixture (McCollum and Davis)	...	...	...	5

During the course of all the experiments described in this paper, cod-liver oil was given daily to each rat in 2-3 drop doses. Marmite was given in the form of an extract prepared as follows. A weighed quantity of marmite is shaken with 80-85 % alcohol. The alcohol is distilled off *in vacuo* and the fluid placed on a water-bath for about an hour. When cold it is filtered and made up to volume so that 1 cc. of the extract represents 1 g. of the original marmite.

A group of 31 rats (pure bred black and white piebalds) was placed on the above diet. Initial weights varied from 45 to 55 g. Rats nos. 1-8, and 9-15 were litter mates, and rats nos. 16-31 were mixed litters. The rats were kept on the vitamin B-free diet till the onset of polyneuritic symptoms, which occurred in 15-20 days. The animals were then divided into five groups.

Group A. Four rats received 0.75 cc. marmite daily. Growth was normal and their condition remained excellent throughout the experimental period (2 months). (Fig. 1, Curve A.)

Group B. Six rats received 0.5 cc. marmite daily. Growth was good, but five developed mild skin lesions on the mouth at about the 26th day of dosing. (Fig. 1, Curve B.)

Group C. Four rats were given 0.5 cc. marmite incorporated into the daily ration. Some food was left each day, so that the intake of vitamin was rather less than in the case of rats in group B.

Group D. Fourteen rats received 0.25 cc. marmite extract daily. Of these, nine developed severe skin lesions in 17-25 days, and five remained free. The weights of those which developed pellagra were fairly good. (Fig. 1, Curve C.) Those with no pellagra were not quite so good.

Fig. 1. Curve (A). Rat receiving 0.75 cc. marmite extract daily. Curve (B). Rat receiving 0.50 cc. marmite extract daily. Curve (C). Rat receiving 0.25 cc. marmite extract daily.

↓ indicates beginning of dosage. × indicates onset of skin lesions.

Group E. Three rats were given only 0.125 cc. marmite extract daily. Growth was stationary, polyneuritis becoming severe by the 28th day. The dose of marmite extract was then increased to 0.25 cc., which rapidly removed the symptoms of polyneuritis. Skin lesions then appeared after 11 more days (Fig. 2).

Fig. 2. Growth curves of rats receiving 0.125 cc. marmite extract daily.

From these results it is apparent that 0.5 cc. or somewhat less of marmite extract is sufficient to secure good, though somewhat irregular, growth and as little as 0.25 cc. will protect against polyneuritis for a comparatively long period, but that 0.75 cc. is required to prevent the onset of skin lesions. It should be pointed out that pellagra occurred even when growth was good. In group E, where growth was at a standstill and where polyneuritis developed, there was no pellagra, although the usual time limit for the production of skin lesions was allowed to elapse. When, however, the dose of marmite extract was increased so that growth was resumed, pellagra developed in 11 days. The mild skin lesions of the rats receiving 0.5 cc. marmite extract did not appear to get progressively worse, and after lowering the dose to 0.25 cc. the condition did not alter appreciably during the course of 3 weeks. It is to be expected that the previous higher dosage would afford some protection. Two rats in group C were rapidly cured by substituting crude marmite in equivalent doses for marmite extract. One other rat was cured by giving 0.75 cc. doses of autoclaved marmite extract, vitamin B₁ being supplied as wheat germ extract, while the fourth failed to respond to dosages of 0.5 cc. The more severe lesions occurring in group D were also cured by 0.75 cc. autoclaved marmite, but improvement was slower than that following the administration of unautoclaved marmite. 2 cc. doses of wheat germ extract alone led to no improvement.

SEASONAL VARIATION.

When an attempt was made by the method described to induce pellagra in a group of 6 rats during the months of Dec.-Feb. only two developed lesions after 10-11 weeks. The experiment was discontinued and this method of producing pellagra regarded as precarious. After good results had been obtained later in the spring however it was decided to try again during the winter months.

Group F. The experiment was carried out as described above, using 10 rats. Growth was normal in the case of those rats receiving 0.75 cc. marmite extract daily, while 0.5 cc. allowed of good growth and also appeared to afford protection from pellagra (whereas later in the year, as has been shown, slight lesions occur with this dosage). On 0.25 cc. of marmite extract growth was slow but continuous. The animals remained free from skin lesions for 3 months (as against only 3 weeks at other times in the year), but at the end of this time the typical lesions developed to a marked degree (Fig. 3, Curve B). No

Fig. 3. (A). Growth curve of rat on sugar-free diet. (B). Growth curve of rat on 17 % cane sugar diet during winter months showing delayed onset of pellagra.

doubt the two which developed lesions in the earlier winter experiment were the forerunners of others.

Sugar-free diet.

Group G. A group of rats was placed on the following modified sugar-free diet:

Caseinogen (Glaxo, vitamin-free)	...	...	...	20 %
Rice starch	...	...	...	60
Palm kernel oil	...	...	...	15
Salt mixture (McCollum and Davis)	...	...	...	5

Here the carbohydrate content of the diet was in much the same percentage as before but consisted entirely of rice starch. The caseinogen was slightly reduced in amount. The rats were "run out" of vitamin B complex as before, and then given varying doses of marmite extract. There was a marked and surprising difference between the rats of this group receiving 0.25 cc. of the extract, and those described above. No skin lesions whatever developed but there was decided polynuritis. The animals were "humped," had greasy fur, but were clean, whereas those with skin lesions were always very dirty.

Growth was practically stationary in the rats not receiving sugar whereas in those which developed pellagra on a sugar-containing diet growth was fairly good, even though the amount of vitamin B complex (as contained in 0.25 cc. marmite extract) was the same in both groups. After 21 weeks on the sugar-free diet the surviving rats were given the usual sugar-containing synthetic diet, the amount of marmite extract being kept as before (0.25 cc.). The polyneuritic symptoms remained much the same, but pellagra now also developed in about 11 days (Fig. 3, Curve *A*). It is therefore evident that polyneuritis and pellagra can exist together, but this point is discussed in detail later.

In view of the difference shown by the rats receiving 17 % sugar and those on the sugar-free diet, further experiments were undertaken to test the effect, if any, of giving increased amounts of sugar.

29 % sugar diet.

Group H. Four rats were fed on a 29 % sugar diet (vitamin B-free) for 21 days. They were then given 0.25 cc. marmite extract daily. The results were very similar to those obtained with rats which received the 17 % sugar diet, apart from the fact that diarrhoea was present at the commencement of the experiment. Skin lesions were present on 2 out of 4 after the 5th week of dosing. One died with no skin lesions.

57 % sugar diet.

Group I. Five rats were placed on a 57 % sugar diet (vitamin B-free) for 21 days, after which they were given 0.25 cc. marmite extract daily. After 6–7 weeks on this diet, they looked almost the same size as at the commencement of the experiment. Skin lesions appeared in 5–6 weeks after beginning dosage with marmite. When on a sugar-free diet, the ears, paws and tails of the animals looked very anaemic. Those on the 57 % sugar diet were strikingly different even before skin lesions appeared; the paws and tails being hyperaemic. This however was not so noticeable later and the weights improved somewhat. Four out of five had skin lesions.

It will be seen by the above experiments that the onset of the skin lesions was not hastened by the administration of a higher percentage of sugar to the diet, although on the 57 % sugar diet the general condition was not good. In this latter case also pellagra occurred in the absence of growth, whereas on the 17 % sugar diet pellagra occurred when growth was moderately good. These results are summarised in Table I.

Pellagra symptoms.

The question remains as to whether the skin lesions in these rats indicate true pellagra or not. A description of the symptoms shows however the closest resemblance to those described by Goldberger and Lillie [1926] and Chick and Roscoe [1927]. The front paws first became shiny and red and the nose

Table I.

Group	Carbohydrate in basal diet	Dose of vitamin B complex cc.	No. of rats fed	No. of rats with polyneuritis	No. of rats with pellagra	Days before lesions appeared	Remarks
A	40 % rice starch 17 % cane sugar	0.75	4	0	0	—	Normal growth. No lesions
B	Same as A	0.5	6	0	5	26 days	Growth good
C	Same as A	0.5 cc. mixed in daily ration of 2 rats	4	0	4	26 days	Growth good
D	Same as A	0.25	14	0	9	17-25 days	Fairly good growth
E	Same as A	0.125	3	3	3	30 days on 0.25 cc. 11 days on 0.125 cc.	Growth stationary on 0.125 cc. Resumed growth and pellagra on 0.25 cc.
F	Same as A	(a) 0.75	1	0	0	—	Normal growth. No lesions
		(b) 0.5	1	0	0	—	Good growth. No lesions
		(c) 0.25	6	0	5	21 weeks (Nov.-Apr.)	Subnormal growth. One death
		(d) 0.125	2	2	1	—	No growth on 0.125 cc. Resumed growth on 0.25 cc. One death
G	60 % rice starch No cane sugar	(a) 0.75	1	0	0	—	—
		(b) 0.5	1	0	0	—	—
		(c) 0.25	8	8 (3 deaths)	4/5 surviving	21 weeks on sugar-free. 11 days with sugar (Nov.-Apr.)	Subnormal growth
		(d) 0.125	2	2 (both died)	0	—	No growth
H	28 % rice starch 29 % cane sugar	0.25	4	0	2	35 days	Subnormal growth
I	57 % cane sugar No rice starch	0.25	5	0	4	35-40 days	No growth at first, improving later

and mouth looked sore. Later the ears became waxy or cheesy looking. The corners of the mouth became crusted and there was also an incrustation of the skin on each side of the nose and towards the eyes, giving the "butterfly" effect as described by Goldberger and Lillie. In severe cases the root of the tail gets sore and scaly. In all cases when the lesions are at their height the paws become cracked and scaly, giving the shoe and glove effect of human pellagra. The dermatitis was in all cases bilaterally symmetrical. The coats become discoloured and ill-kept, but there was no definite loss of fur.

DISCUSSION.

The well-known seasonal incidence of human pellagra is apparently also characteristic of the rat disease. Not only are the lesions easiest to produce in the spring months, but it seems practically impossible to induce them during December, January and February. In their studies on human pellagra Goldberger, Wheeler and Sydenstricker [1920] found that no cases developed the disease during these months. The seasonal immunity of rats may account for the failure of workers when attempting to produce pellagra on diets known to be deficient in the P-P factor.

The most interesting and surprising result from the work described is the almost unfailing occurrence of skin lesions on rats receiving cane sugar as source of part of their carbohydrate when having border-line doses of vitamin B

complex. Repeated attempts in this laboratory failed to produce pellagra on diets free from sugar (though the time of year was favourable). Aykroyd and Roscoe [1929] produced pellagra in rats on a diet containing no sugar, and in other respects very similar to the sugar-free diet used during this work, but it is interesting to find that the diet was cooked for 3 hours—a process which would to some extent dextrinise the starch. It is difficult to explain the different results obtained in the presence or absence of sugar. If the sugar itself is detrimental one would expect quicker or severer symptoms when the percentage in the diet is increased, but such did not occur in the few experiments so far tried. There was however a temporary hyperaemic disorder in the case of rats receiving a 57 % level of cane sugar. The carbohydrate percentage in the sugar-free diet is 60 and this causes no disturbance whatever. The possibility of rice starch being protective must be considered. It is interesting to note that maize when used for human consumption is nearly always stated to be accompanied by either cane sugar, syrup or molasses. Blosser [1915] states that removal of cane sugar, syrup, etc., from the diet of pellagrins is in many cases sufficient to bring about complete cure. Rice-eating communities are strangely free from pellagra, but as the rice eaten by these people would be cooked, whereas that fed to rats in the present work was raw, the situation is hardly comparable from this point of view.

Kon and Watchorn [1928] showed that pellagra can be cured by transferring the rat to a diet containing a high percentage of potato starch. This is presumably due to the occurrence of refection. Refection may occur with rice starch in the food but has so far not appeared in this laboratory and was not the cause of the failure of rats on the high starch (sugar-free) diet to develop pellagra. Another very surprising result from the work described is the frequent absence of pellagra when growth has failed and polyneuritis develops later. If the B_2 factor of the vitamin B complex is identical with Goldberger's P-P factor, one would expect a failure of growth to be a constant accompaniment of the onset of pellagra symptoms, but this was not the case, as will be seen by Fig. 1, Curve *B*.

The rats which developed pellagra on the daily dose of 0.25 cc. marmite extract were practically all growing. Growth of course was far from optimal but it never quite ceased. On the other hand, cessation of growth was almost invariably accompanied by lack of pellagra symptoms (except in the case of those receiving all their carbohydrate as sugar). This was very strikingly confirmed by reducing the amount of marmite extract to 0.125 cc. daily. If pellagra occurs in 17–25 days in a large majority of cases where the daily dose of marmite is 0.25 cc. one would expect at least equally rapidly occurring lesions on the smaller dose. It was surprising to find that such was not the case. On a diet entirely free from the vitamin B complex, no pellagra appears for the simple reason that the animals succumb to polyneuritis before the lapse of the time interval necessary for the occurrence of pellagra. In the case of rats on 0.125 cc. marmite extract some severe cases of polyneuritis

developed and the animals died. But it was unexpected to find that, though some of the rats had such mild polyneuritis that they lived for a month, their weight being stationary or declining, not the least sign of pellagrous skin lesions was ever noticed, although this interval has been shown to be sufficiently long for the appearance of the first signs of pellagra. In the case of those animals on the sugar-free diet survival with polyneuritic symptoms was indeed much longer—as long as 21 weeks having elapsed with no symptoms of pellagrous skin lesions. Again, it must be emphasised that the time of year was favourable for the appearance of pellagra, this being illustrated by a group of rats on a cane sugar diet at the same time, the majority of which developed skin lesions. Further, when the group of rats on sugar-free diet was placed on a sugar-containing diet they rapidly developed pellagra.

It is possible that those rats receiving the higher dose of marmite extract (0.25 cc. daily) which did not gain weight were also suffering from mild polyneuritis, as this dose is obviously on the border line of that necessary to prevent polyneuritic symptoms with certainty. The idea that growth itself is a contributory factor to the development of pellagra is hardly tenable nor would the suggestion that the growth-promoting factor of the vitamin B complex and the P-P factor are not identical explain the non-occurrence of pellagra in the rats receiving the smaller dose of marmite. The possibility is left that the actual occurrence of polyneuritis is inhibitory to the development of pellagra, though, as we have seen, if the animals can be kept alive for a sufficient length of time (*e.g.* 21 weeks in the case of those on the sugar-free diet) it is possible to induce pellagrous symptoms in the presence of polyneuritis, thus showing that the condition of polyneuritis does not necessarily prevent pellagra. If the suggestion should prove permissible that beriberi inhibits to some extent the development of pellagra it may explain the infrequency of the latter disease amongst rice-eating communities where beriberi is rife.

SUMMARY.

1. On a synthetic diet containing both rice starch and cane sugar pellagra could be induced in 17–25 days if the rats were first “run out” of the vitamin B complex and then given a minimum daily dose of marmite, sufficient to prevent polyneuritis.
2. In the absence of cane sugar from the diet pellagra did not appear, but all the rats developed polyneuritis.
3. Increased amounts of cane sugar in the diet did not increase the severity of the symptoms.
4. Rats suffering from polyneuritis developed pellagra after the marmite dose was raised sufficiently to protect them from polyneuritis, and in the case of those on a sugar-free diet, when sugar was added.
5. Rats were shown to have a seasonal immunity from pellagra comparable to that occurring in the human disease.

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