CIII. THE HEAT-STABILITY OF THE (ANTI-DERMATITIS, "ANTI-PELLAGRA") WATER-SOLUBLE VITAMIN B₂. II.

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THE following experiments form a continuation of those recently published by Chick and Roscoe [1930] which indicated that vitamin B₂ in yeast or yeast extracts, though relatively stable to temperatures at 100–120° in acid solution, was sensitive to these temperatures if the reaction were alkaline. Thus, at p_H 5.0–3.0 there was a 50 % loss in vitamin B₂ content after heating for 4–5 hours at 123°; at p_H 8.3–10.0 a loss of between 75 and 100 %. When heated for 2 hours at 90–100° in faintly acid solution no loss in potency was detected; at p_H 8.3, on the other hand, about one-half was lost and even when maintained for 10 days at room temperature (summer) in a weakly alkaline solution there was a loss of about one-third of the original strength. Vitamin B₂ was estimated by the method previously described [Chick and Roscoe, 1928].

These conclusions are not in accord with those of Reader [1929, 1930], who found vitamin B₂ able to withstand heating for 1 hour at 120° at p_H 9.0, or of Narayanan and Drummond [1930], who state that no appreciable destruction of this vitamin takes place when yeast products are heated for 1–3 hours at 110–120° in a solution containing 10–15 % barium hydroxide.

This divergence is of more than usual interest, for the stability of vitamin B₂ to heat and alkali is important in the chain of arguments indicating the existence of Reader's third water-soluble B vitamin. This new factor is stated to differ from vitamin B₂ in being sensitive to heat and to alkali.

CRITERION USED AND METHODS EMPLOYED IN ESTIMATION OF VITAMIN B₂.

In the experiments of Reader and of Narayanan and Drummond, as well as in our own, the criterion used in the detection and assay of vitamin B₂ was the power to promote or restore growth in young rats receiving diets complete in every other respect. It is obvious that a more satisfactory criterion than growth, because more specific, would be the cure of the dermatitis (? rat-pellagra) which develops in young rats after several weeks of vitamin B₂ deficiency. Such experiments are by nature unsuited for quantitative work owing to the irregularity of these symptoms as regards their nature, time of onset and degree of severity. Nevertheless, it was felt that they might provide

useful confirmation of previous work [see Chick and Roscoe, 1930, p. 111] and in this hope the following experiments have been carried out. The results have confirmed our previous findings in demonstrating that the curative property for ratdermatitis, present in yeast extract equally with the power to restore growth of rats on diets lacking only vitamin B₂, is sensitive to high temperatures in alkaline solution.

Technique of the curative tests.

The increase in body weight of young growing rats is extremely sensitive to deprivation of vitamin B₂. When these animals are placed on diets from which this vitamin is absent, increase in weight usually ceases within a few days and it is only in exceptional circumstances (as for example in the condition known as refection [Fridericia *et al.*, 1927; Roscoe, 1927]) that an approach to even subnormal growth is manifested. The onset of the characteristic dermatitis is, however, more irregular. Sometimes it is marked after 6-8 weeks of deprivation; on the other hand, it may take 20 or more weeks to develop. The distribution, character and severity of the skin affection also vary greatly [Chick and Roscoe, 1928]. The curative tests described below were carried out on a collection of "negative" control rats belonging to different litters which had been maintained for various periods on the standard "-B₂" diet for the express purpose of studying these skin lesions. As will be seen from the details set out in the accompanying table, these varied considerably, but in selecting animals for the tests care was taken that, as far as was possible, the doses of the materials compared were administered to animals whose condition was similar.

The symptoms usually present when treatment began were the following. Sunken eyes with blood-stained secretion and inflamed eyelids, inflamed skin, bare of fur, surrounding the eyes giving a characteristic "spectacled" appearance. The fur was thin and poor, especially over the head, neck and chest; in severe cases the bald patches often developed into raw areas spreading round the axilla and down the forearm. The wrists of the forepaws were almost invariably stained with the bloody discharges from eyes and nostrils, as the result of incessant rubbing. The fur of the lower part of the abdomen was also stained reddish brown with what appeared to be blood from the urine. These stains, however, unlike those on the wrists, have repeatedly failed to give the characteristic tests for blood or blood-pigments.

The stains in both places are alike the first symptoms to disappear, often within a day or two after treatment with an adequate curative dose. Next in order, the eyes usually recover their normal prominent position and the skin begins to assume a healthy texture and appearance. Lastly, new fur makes its appearance, 2-3 weeks being needed before the coat may be described as normal.

A set of skin appearances differing somewhat from the above was frequently encountered in our earlier work, but now rarely occurs [Chick and Roscoe, 1928, p. 795]. These include (1) inflammation of the tips of the ears, which

become cheesy in texture and, during the cure, shed their dead epidermis in flakes, (2) an oedematous inflammation of the digits of the paws, which become bright red in colour. On one occasion these symptoms made their appearance while cure of a rat (564 ♀) with general dermatitis was being attempted with an inadequate dose of material containing vitamin B₂, suggesting the possibility that a small, though inadequate, amount of vitamin B₂ in the diet was necessary for demonstration of these more florid skin symptoms of vitamin B₂ deficiency [see also Sherman and Sandels, 1929].

It is worth noting that these particular symptoms of vitamin B₂ deficiency have been less frequent since the use of more highly purified caseinogen in the basal diet, although the failure to grow and the development of a generalised dermatitis have been more constant. The suggestion that these florid symptoms are induced by a less complete deprivation of vitamin B₂ is confirmed by the fact that, on one occasion, a rat (456 ♀) exhibiting these symptoms, but otherwise in good condition, was slowly cured when treated with a yeast extract which had been autoclaved at 120° for 4 hours in alkaline solution, this being the only instance where material so treated has, in our experience, exhibited curative properties for the dermatitis caused by vitamin B₂ deficiency.

An analogous instance is probably that of rats receiving diets almost, but not entirely, deprived of the antineuritic vitamin B₁. In the course of several weeks such rats will often develop the characteristic paralysis due to vitamin B₁ deficiency, whereas those suffering complete deprivation usually die in 3-4 weeks from collapse without manifestation of any nervous affection [Hofmeister, 1922, 1, 2].

Vitamin B₂ material used in the present tests.

A 0.01 % acetic acid extract was prepared from washed brewer's yeast, sample XXIV, similar to that made from yeast XII, used in the experiments of Series B (Table II) described in the previous paper [Chick and Roscoe, 1930]. One portion (*a*) of the extract was acidified to p_H 2.5, and a second portion (*b*) made alkaline by addition of sodium hydroxide to p_H 10.3, the hydrogen ion determinations being made with a hydrogen electrode. After heating for 4 hours at 119° the p_H of (*b*) (which had fallen to 9.6) was adjusted to about 3.0 for storage. That of (*a*) remained at 2.5. Both solutions were adjusted to their original volume and in the following tests of the curative power of the two solutions all doses are reckoned as the equivalents of the original yeast (dry weight).

No tests were made with the unheated material, as it was felt that no accurate quantitative results could be expected from such curative tests and that it would be sufficient to determine the relative curative value of the materials heated in acid and alkaline solution respectively, comparing the results obtained with those of the previous work when growth was the criterion. Ten tests were made, six rats being treated with material (*a*) and four with (*b*).

RESULTS OF THE CURATIVE TESTS.

Yeast extract autoclaved in acid solution.

Of six rats treated with doses of this material, three received a daily dose equivalent to 0.5 g. yeast. They all showed marked improvement in a week, and normal appearance of skin and fur was attained in 2½ to 3 weeks (rats 645, 574, 624). Simultaneous with the improvement in the skin condition there was immediate resumption of growth, the average weekly increase in body weight being 10–20 g.

With smaller doses of the same material the result was not so dramatic. Rat 589, which had a severely affected skin after 16 weeks' deprivation of vitamin B₂, received the equivalent of 0.33 g. yeast daily for 4½ weeks. At the end of this time the body weight had increased by 38 g. (from 66 to 104 g.), and the skin and fur, though much improved, were not quite restored to a normal condition. Rats 664 and 564 received the equivalent of 0.16 g. yeast daily. This proved inadequate for complete cures, though the skin condition was improved and growth was resumed.

Yeast extract autoclaved in alkaline solution.

Four rats received this material in doses equivalent to 0.5–1.0 g. yeast daily, for periods of from 3 to 5 weeks; they showed no improvement and in three cases the skin lesions became definitely worse. For example, rat 599, with only a moderate skin affection, received the equivalent of 1.0 g. yeast for 23 days, during which time the symptoms became progressively more severe. Bare, desquamating patches of skin developed round the eyes, and on the neck and shoulders bald areas appeared, which later became raw and inflamed. Diarrhoea also occurred and there was a slight loss in weight (10 g.).

Rat 565 received a dose equivalent to 0.5 g. yeast daily for 21 days during which the skin symptoms became steadily worse, and there was slight loss of weight. Increasing the dose to the equivalent of 1.0 g. yeast was of no use, but, when this was replaced by extract autoclaved in acid solution equivalent to 0.5 g. yeast, a swift improvement took place. In 10 days the skin had assumed a healthy appearance, new fur was growing, the blood-stained discharges from nose and eyes had discontinued and the diarrhoea, which had developed during the preceding period, also disappeared. There was an increase in weight of 27 g. in the 10 days.

Rats 616 and 635 received the material treated in alkaline solution in the equivalent of 0.5 and 1.0 g. yeast respectively. Rat 616 became definitely worse during the 27 days of its treatment and lost 9 g. in weight; rat 635 on the larger dose maintained weight and continued with symptoms unchanged for 5 weeks. The subsequent cure of these two rats took place with material kindly provided by Dr Reader. This material was a solution of marmite autoclaved at 120° for 1 hour at p_H 9.0 and was found by Dr Reader to be effective in curing the characteristic dermatitis due to deficiency of vitamin B₂, but

*Heat-stability of vitamin B₂ in yeast extract (XXIV). Autoclaved at 119° for 4 hours.
Curative tests. Vitamin B₁ provided as Peters's concentrate.*

<i>p_H</i> of extract when heated	Dose, expressed as equivalent of dry yeast g.	Rat no. and sex	Time on " - B ₂ " diet. Weeks	Symptoms: general condition	Body weight g.	Average weekly increase in body weight during treat- ment g.	Result of treatment
2.5	0.5	574 ♀	16	Eyes badly affected, bloody discharge from nose and eyes. Fur very poor on chest	72	16	Rapid cure. 1st week, eyes and skin became healthy and new fur began. Skin and eyes normal in 2½ weeks
2.5	0.5	624 ♀	12	Skin symptoms severe. Eyes sunken, secreting, "spectacled." Bloody discharge also from nose. Fur very poor, thin and "stringy" over face and neck. Abdomen stained	47	14	Rapid cure. 1st week. Eyes nearly normal, posture also. 2nd week. All symptoms disappeared. 3rd week. Normal fur
2.5	0.5	645 ♀	6	Skin symptoms slight but animal very weak and apparently moribund. Abdomen stained	42	21	Rapid cure. Coat appeared normal in 9 days
2.5	0.33	589 ♀	16	Skin symptoms severe. Eyes "spectacled"; left nearly closed. Fur poor and stringy on face and neck. Bloody discharge from nose and eyes. Abdomen stained	66	9	Slow cure. Eyes improved and stains on fur from discharges disappeared in 3 weeks. New fur growing and coat becoming normal in 4½ weeks
2.5	0.16	604 ♀	12	Eyes "spectacled." Paws blood-stained. Fur poor and "stringy" over face and neck. Abdomen stained. Condition miserable	55	6	Very slow cure. Symptoms arrested in 1st week. Stains on abdomen disappeared in 3rd week, skin and eyes not normal after 4 weeks
2.5	0.16	564 ♀	21	Skin symptoms severe. Eyes "spectacled," nostrils inflamed, bloody discharge. Fur "stringy," bald patches round ears, raw patches round anus and vagina. Abdomen stained. Condition miserable	50	7	Marked improvement in 1 week, ears and forepaws desquamating. Skin round vagina dry and healthy

10-3-9-6	1-0	599 ♂	17	Symptoms slight. Eyes sunken, "spectacled." Bloody discharge from nose. Fur thin on face. Abdomen stained	81	Lost 10 g. in 23 days	No cure. Symptoms became worse in 2-3 weeks. Bare, raw, desquamating patches developed round eyes and on left side of neck and shoulder. Skin round nose and lips became swollen and cracked, area of inflamed skin developed round anus. Animal very miserable. Killed
10-3-9-6	1-0	635 ♂	10	Skin symptoms severe. Eyes sunken, "spectacled." Bloody discharge from nose. Fur "stringy," bald patches round ears. Nose tip inflamed	49	2-5	No cure. Condition remained almost unchanged except for onset of diarrhoea during 5 weeks' treatment. Treated with Dr Reader's preparation of marmite. Marked improvement in 1 week. In 3 weeks coat almost normal, animal in normal health, increase in weight 72 g.
10-3-9-6	0-5 later 1-0	565 ♂	15	Skin symptoms not severe. Eyes sunken and inflamed. Bloody discharge from nose. Diarrhoea	88	Lost 15 g. in 22 days	No cure. Condition became worse. In 3 weeks raw, bald patches were spreading from the axillae down the forearms. Mouth sore and inflamed. Condition of animal miserable. Treated with acid-autoclaved material equivalent to 0-5 g. yeast. Rapid cure. In 10 days nose healing; new fur growing everywhere, increase in weight 27 g.
10-3-9-6	0-5	616 ♂	13	Skin symptoms slight and incipient. General condition fair	56	Lost 9 g. in 27 days	No cure. Condition rapidly grew worse. In 3 weeks inflamed, bare patch of skin developed under chin, nostrils inflamed, fur "stringy." Treated with Dr Reader's preparation of marmite. Marked improvement in 1st week, in 3 weeks nostrils healed, coat almost normal; increase in weight 56 g.

incapable of restoring growth on basal diets lacking in vitamin B₂ and provided with abundant vitamin B₁. In our experiments, not only were the skin lesions rapidly healed but immediate vigorous growth took place. The weight of rat 635, on the dose prescribed by Dr Reader, was increased by 72 g. in 3 weeks and that of rat 616, on about half this dose, by 56 g. in the same time.

This divergence would be explained if our preparation of vitamin B₁ (Peters's concentrate prepared from brewer's yeast) contained Dr Reader's third factor. We are now examining this preparation for evidence of any factor other than the antineuritic vitamin B₁ and are endeavouring to separate it, if present. The results of these researches will be published shortly. At the present time we can only report that we have failed to obtain satisfactory cures of dermatitis unaccompanied by increase in weight. Another possible explanation of the results obtained with Dr Reader's solution is that the material was originally so rich in vitamin B₂ that the residue, after exposure to 120° for 1 hour in alkaline solution, still contained sufficient in the doses given to cure the dermatitis and to stimulate growth in the rats of our experiment.

SUMMARY.

1. An extract made from washed brewer's yeast (XXIV) by boiling in dilute acetic acid, after being heated in acid solution (p_H 2.5) for 4 hours at 119° (solution *a*), retained its power to cure the skin affection in rats due to deprivation of vitamin B₂ swiftly and completely in doses equivalent to 0.5 g. yeast (dry wt.) and more slowly and less completely in doses equivalent to 0.16–0.33 g. yeast.

2. When the same extract was exposed to the same treatment in alkaline solution (p_H 10.3–9.6) (solution *b*), no curative properties could be demonstrated in doses equivalent to 0.5 g. or 1.0 g. yeast and in three out of four cases the skin affection became rapidly worse.

3. Growth of the rats was parallel with the changes in the condition of the skin; when treated with solution (*a*) growth was restored, with solution (*b*) body weight remained stationary or decreased.

In conclusion we wish to thank Dr Reader for providing the material used for the cure of rats 565 and 616, and Messrs Watney, Coombe, Reid & Co. for providing the brewer's yeast from which our extract was made.

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