When rats are deprived of water-soluble vitamin B complex they quickly succumb with a variety of pathological manifestations. If the rats are adequately supplied with vitamin B, they usually survive much longer but many, sooner or later, develop a type of dermatitis.

The character of this dermatitis led the late Dr. Goldberger and his coworkers (1) to identify it with pellagra and to postulate the existence, in the complex we called water-soluble vitamin B, of a pellagra-preventive substance (P-P). In the years immediately following Dr. Goldberger's suggestion there accumulated evidence of the existence of a new vitamin which the English designate as vitamin B, and which in American literature is more commonly designated as vitamin G, also evidence supporting Goldberger's contention that this new vitamin was the pellagra-preventive factor.

At the present writing, however, the view that vitamin B, (G) is the significant factor concerned in pellagra prevention is being challenged. Aykroyd (2), Hunt and Wilder (3), Bliss (4), and Sure, Smith, and Kik (5), have recently published papers bearing on this point.

During the past 3 years our laboratory has been especially concerned with the chemical fractionation of the vitamin B complex. To satisfactorily assay the fractions it became necessary to use both
pigeons and rats as test animals and to take special precautions in
the purification of the basal diets of these animals, especially the
protein components. We also sought as an aid in B1 assay a source
of vitamin B2 (G) which should be completely free of B1. The results
of our studies of autoclaved yeast as a B2 source have already ap-
peared (6). In spite of the development of a B1—free autoclaved
yeast as a source of vitamin B2, variations in animal behavior still
developed.

We have sought for the cause of these variations in the methods
used for purifying casein or other components of the basal diets or
by utilizing other admitted sources of B1 and B2. Out of the many
series of tests involved we wish to report here a series that leads us
in common with the investigators already cited (2-5), to question
whether the B2-free diet resulting in rat dermatitis owes its action
to lack of a factor which is identical with that preventive of human
pellagra.

For our study of this series we enlisted the cooperation of Dr.
James Denton, who has been identified with the study of the his-
tology of human pellagra and black tongue of dogs (7, 8). Denton
observed rarefaction of the corium, dilation of the vessels, and con-
siderable reaction about the vessels in early cases of pellagra.1 In all
cases this was precedent to the later skin changes. Similar changes
were found to take place in “black tongue.” There is a corresponding
fragmentation of the fibrillar material contiguous to the basal layer
with very little change at this time in the epithelium itself.

His viewpoint may be stated as follows: “The distinctive lesions of
pellagra and those of black tongue of dogs appear to have their origin
in a failure on the part of the organism to maintain the specialized
supporting tissues of epithelium in various situations.” (8).

Dr. Denton separated our specimens into two distinct types of
dermatitis. One he found to show the characteristic corium changes
of human pellagra. The other showed the type of dermatitis that
seems to always develop sooner or later on deficiency of vitamin B2
but these animals failed to show the corium histology of early human

1 For purposes of comparison with the photomicrographs to be discussed
particular reference is made to the following photomicrographs: J. Trop. Med.,
1925, 5, 182, Fig. 1; Am. J. Path. 1928, 4, Plate 80.
pellagric skin. We present in evidence of these differences photomicrographs of sections made for us by Dr. Denton together with data on the diets associated with the observed effects. (See Figs. 1 to 6.)

Experiment 1.—For skin see Fig. 2; for growth curve see Chart 1.

Diet: Basal.—18 per cent raw egg white protein (commercial frozen); 68 per cent purified corn-starch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and Mendel salt mixture.

Vitamin B₁.—Supplied by 20 mg. daily of fullers' earth activated by the method of Williams and Waterman (9).

Vitamin B₂ (G).—Was supplied by feeding daily 0.5 gm. Liebig beef extract per rat. That this dosage provided B₂ adequate for growth effect is shown by the growth of this animal (Chart 1). After 60 to 70 days on this diet two of the three rats on the diet developed a dermatitis, viz. sore mouth, extremely red and sore paws, loss of hair on legs and chest and around joints, and to a lesser extent on the back. Nose and mouth were very sore, the eyelids and genitals inflamed, and autopsy revealed enlargement of the testes. Section of the skin of the paw of Rat 4325 is shown and reveals corium changes similar to those in human and dog pellagra. We have then in this exhibit a corium degeneration closely simulating human pellagra in spite of a dosage of vitamin B₂ in beef extract sufficient to produce excellent growth. The dermatitis of the other two rats in this series failed to clear up when the egg white protein was supplemented by 1 gm. of alkali-extracted casein daily or when 0.5 gm. daily of neutral autoclaved yeast was substituted for the beef extract.

Experiment 2.—For skin see Fig. 3; for growth curve see Chart 1.

Diet: Basal.—18 per cent extracted casein; 68 per cent purified corn-starch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and Mendel salt mixture.

Vitamin B₁.—Supplied by 0.15 mg. daily of Jansen-Donath fraction 16 prepared by the method of Williams, Waterman, and Gurin (10).

Vitamin B₂.—Was supplied as a water diffusate of beef extract. After 35 days this rat, in spite of fair growth (1.1 gm. daily), developed sore nose, sore reddened area about the mouth, bleeding at the joints, loss of hair around the eyes, and crinkled ears. The other three rats in this series also grew well but did not develop dermatitis. It must also be said that a large number of other rats have been reared on this same diet with fair growth and without dermatitis when the B₂ has been supplied by whole beef extract instead of the water diffusate. Since, however, only one of the four on the diffusate developed the skin lesions it is doubtful whether the use of the diffusate rather than whole extract as B₂ source could have been the significant factor in this result.

The section is from the skin of the chest. Like Rat 4325 it shows a true degeneration of the superficial supporting tissues in the corium. The lesion was not infected and resembled an early skin lesion of a human pellagrin. Again we had
obtained a corium degeneration and dermatitis similar to human pellagra on a
diet which produced good growth and was supposedly therefore adequate in B2.

Experiment 3.—For skin see Fig. 4.

Diet: Basal.—18 per cent acid-extracted casein; 68 per cent purified corn-
starch; 10 per cent cottonseed oil; 4 to 5 drops daily of cod liver oil; 4 per cent
Osborne and Mendel salt mixture.

Vitamin B1.—Supplied as for Rat 4630 by 0.15 mg. daily of Jansen-Donath
fraction 16.

Vitamin B2.—No source given.

Two rats in this series developed, after 70 to 80 days, extreme soreness of nose
and around the lower part of mouth, dermatitis of the paws, loss of hair about the
joints and around the eyes, and crinkled ears. Superficially these symptoms re-
sembled closely those of Rats 4325 and 4630. Death occurred in two animals
within 2 weeks after onset of symptoms. The remaining animal held apparently
normal as to skin for 20 weeks, after which nose and throat soreness developed but
became no worse, and the animal was killed after 27 weeks on the diet (4342).

The skin section of Rat 4343 from this series is shown. It exhibits simple
atrophic thinning of the epidermis with squamae on the surface, but with no such
significant corium changes as are shown by Rats 4325 and 4630. The lesions differ
markedly from those of the human pellagrin.

That this rat lacked growth-promoting vitamin B2 was evidenced by his failure
to grow.

Experiment 4.—For skin see Figs. 5 and 6.

Diet: Basal.—18 per cent acid-extracted casein; 68 per cent purified corn-
starch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and
Mendel salt mixture.

Vitamin B1.—Supplied by Jansen-Donath fraction 16, 0.15 mg. daily.

Vitamin B2.—No source given.

Dermatitic symptoms similar in external appearance to those of Rats 4343,
4325, and 4630 appeared in three of the four rats on the diet in about 50 days.
The rats were killed on the 85th day on the diet. No growth resulted with this
series, demonstrating lack of growth-promoting vitamin B2.

Like Rat 4343 the skin sections show no corium changes comparable to the early lesions of the human pellagrin or to those of black tongue. Some scarring of the superficial corium is observed.

Summary of Cases

The outstanding feature of these cases is the production in rats of
two histologically different types of dermatitis; the appearance in one

* In all cases care was taken when selecting skin for sectioning to avoid areas
where secondary infection might have occurred.
CHART 1. Growth curves of rats used in the tests reported. See pages 423 to 424.

P signifies pellagra symptoms.
D signifies dermatitis symptoms.
TABLE I

Dietary Factors Involved in the Rat Series Reported in This Article

<table>
<thead>
<tr>
<th>Rats</th>
<th>4325</th>
<th>4630</th>
<th>4343</th>
<th>5143 and 5146</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>18% egg white</td>
<td>18% acid-extracted casein</td>
<td>18% acid-extracted casein</td>
<td>18% acid-extracted casein</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>68% corn-starch</td>
<td>68% corn-starch</td>
<td>68% corn-starch</td>
<td>68% corn-starch</td>
</tr>
<tr>
<td>Fat</td>
<td>8% butter</td>
<td>8% butter</td>
<td>10% cotton-seed oil</td>
<td>8% butter</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>2% cod liver oil</td>
<td>2% cod liver oil</td>
<td>4 drops cod liver oil daily</td>
<td>2% cod liver oil</td>
</tr>
<tr>
<td>Salts</td>
<td>4% O &amp; M mixture</td>
<td>4% O &amp; M mixture</td>
<td>4% O &amp; M mixture</td>
<td>4% O &amp; M mixture</td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt; source</td>
<td>Activated fuller's earth</td>
<td>0.15 mg. J-D 16</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;(G) source</td>
<td>Liebig beef extract (0.5 gm.) and autoclaved yeast</td>
<td>Water difusate beef extract</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td>Good</td>
<td>Good</td>
<td>Maintenance only</td>
<td>Maintenance only</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>Dermatitis allied to human pellagra according to Denton</td>
<td>Dermatitis simulating human pellagra</td>
<td>Dermatitis lacking corium picture of human pellagra</td>
<td>Dermatitis lacking corium changes of human pellagra</td>
</tr>
</tbody>
</table>

Acid-extracted casein was prepared as follows: 10 kilos of casein (Merck technical) was stirred into 50 liters of 20 per cent methyl alcohol solution made 0.05 per cent acid with glacial acetic acid. The mixture was stirred 24 hours by an electrically driven stirrer, allowed to stand 4 to 5 hours, and the supernatant liquid siphoned off. An equal volume of solvent was again added and procedure repeated three more times, a total of four extractions involving 96 hours of continuous stirring. The casein was then filtered on Buchner funnels, washed by stirring 20 to 30 minutes with 2 to 2½ gallons of 95 per cent ethyl alcohol, filtered, air dried, and baked at 90-100°C. for 10 hours.

The corn-starch used in the first experiments was extracted by stirring 5 hours with a large volume of 65 per cent methyl alcohol at 50°C. Three extractions of at least 5 hours each were made, the starch being filtered after each extraction, washed finally with 95 per cent ethyl alcohol, and dried in a vacuum oven at 40–50°C, until all alcohol was removed. Later this step was abandoned as no effect on the results was observable from the use of the corn-starch as purchased.

The O & M salt mixture is the classic salt mixture of Osborne and Mendel.
group of a type markedly similar to that observed in human pellagrins in spite of a diet rich in classic vitamin B2 sources as evidenced by growth curves, and a quite different pathological picture in the other group which was deprived of vitamin B2 source entirely.

Others have observed dermatitis in the presence of vitamin B2 in the diet and its failure to appear in cases where B2 was omitted. Our data supply we believe new histological evidence that vitamin B2 deficiency or adequacy as measured by growth effect is not alone sufficient to explain the presence or absence of true pellagra.

**DISCUSSION**

The view that vitamin B2(G) may not be the pellagra-preventing vitamin has been suggested by other investigators.

In a detailed study of the distribution of vitamin B2 in foodstuffs, Aykroyd and Roscoe (11) in 1929 advanced evidence strongly supporting the idea of its identity with Goldberger's pellagra-preventive substance. But, in 1930, Aykroyd (2) from his studies of B2 distribution in cereals and especially in corn (maize) is led to observe: "At present the association, if any, of vitamin B2 and pellagra is obscure."

Very recently Bliss (4) notes that Goldberger's view of vitamin B2 as the pellagra-preventive substance has: "made less of an impression upon clinicians and laboratory workers in the South who are in actual contact with the disease than might be inferred from its ready acceptance in the standard texts today."

He goes on to develop the theory that the pellagra-preventive factor is not a vitamin but instead something which supplies iron deficiency. He reports a study of the response of 51 human pellagra cases to iron therapy and the cure of black tongue by intravenous iron treatment.

Sure, Smith, and Kik (5) have reported severe dermatitis accompanied by remarkable growth and have suggested that: "the growth-promoting and antidermatitis factors associated with the so-called antipellagric vitamin G are not synonymous." They have supplemented this report by several others in one of which they describe an anemia in vitamin G dermatitis which developed in spite of abundance of ferric citrate in the diet.

The activated fullers' earth used in one series was prepared by the method of Williams and Waterman and shown by growth tests to be practically B2-free (9). Later we used a fraction of high vitamin B1 concentration obtained in Williams, Waterman, and Gurin's (10) duplication of the Jansen-Donath procedure. It represents Jansen and Donath's PtCl2 precipitate after removal of the platinum.

The egg white we used was commercial frozen raw egg white. Solids were determined and the egg white fed to provide 18 per cent egg protein in the diet.
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All of these observations as well as our own experience indicate that we cannot accept the vitamin B₂ or G, as at present defined, as identical with Goldberger's pellagra-preventive substance. Whether it is a factor in the syndrome of pellagra, and what other factors are involved, are matters demanding more experimental research and comparison of results. Whether Denton's criterion for differentiating between true pellagra and non-pellagrous dermatitis is accepted or not is also immaterial, but it is at least of interest as an attempt to define sharply the pathology of pellagra.

In Table I is given the summary of the dietary factors involved in the rat series presented in this article together with data on the method of purification of the ingredients employed. Other workers may find these data of value in comparing our tests with their own. Growth curves of the rats are shown on Chart 1.

A comparison of the dietary ingredients of Rats 4325 and 4630 with that of Rats 4343, 5143, and 5146 makes it improbable that the difference in effects could have been due to either the source of vitamin B₁ used, or to the protein, fat, carbohydrate, or salt sources used. Neither do any of the diets appear to be lacking in iron.

SUMMARY

1. Isolated cases of a dermatitis resembling histologically that of human pellagra have occurred in rats supplied with sufficient vitamin B₂ (G) in the form of beef extract or neutral autoclaved yeast to produce good growth.

2. Other rats on basal diets containing similarly prepared nutrients but deprived of any known source of vitamin B₂ (G) develop a dermatitis similar in appearance to that described by other workers, but this skin effect differs in histological picture from that found in human pellagra or in black tongue of dogs. These rats showed growth failure which supports the view that they lacked growth-promoting vitamin B₂ (G).

3. It is suggested that dermatitis in rats may be of diverse type; one resulting from vitamin B₂ (G) deficiency quite different histologically from human pellagra, and one closely allied to human pellagra and black tongue in dogs due to lack of some at present unidentified factor.
BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 40

Fig. 1. Section of the skin from foreleg of a normal rat for comparison with the coria of the pathological specimens. Note especially the intact fibrillar collagen bundles in this section.

Fig. 2. Skin from cheek of Rat 4325.

Fig. 3. Skin from cheek of Rat 4360.

PLATE 41

Fig. 4. Skin from forearm of Rat 4343.

Fig. 5. Skin from forearm of Rat 5143.

Fig. 6. Skin from forearm of Rat 5148.

Giemsa stain used. Magnification = 110.
(Gurin and Eddy: Rat dermatitis and pellagra)