THE VITAMIN B₁ AND B₂ G CONTENT OF LIVER EXTRACT AND BREWERS' YEAST CONCENTRATE

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Recent developments in the study of specific therapy in pernicious anemia have led to the conception that two factors are responsible for the production of the anti-anemic substance which is normally stored in the liver, and which is responsible for the therapeutic benefits obtained from the administration of liver or liver extract. This view, as set forth by Castle (1) and now generally accepted, supposes (a) an intrinsic factor present in the gastric juice of normal persons but absent in the gastric juice of patients with pernicious anemia, and (b) an extrinsic factor present in certain articles of diet such as beef muscle. It has been shown that if normal human gastric juice is incubated and then fed to a patient with pernicious anemia no remission of the disease occurs. If beef muscle alone is fed to such patients no remission ensues. However, if normal human gastric juice and beef muscle are incubated together and then fed, a remission of the disease is brought about.

Little is known of the nature of the intrinsic factor, but Strauss and Castle (2) have tentatively identified the extrinsic factor as vitamin B₂ G. This conclusion is based on experiments in which human gastric juice and a brewers' yeast concentrate, vegex, were incubated together, and fed to patients with pernicious anemia. Remissions of the disease occurred under such treatment. Such remissions were obtained even when the brewers' yeast concentrate had been autoclaved at 15 pounds pressure for 5 hours prior to its incubation with normal human gastric juice. Since brewers' yeast concentrates, such as vegex, are supposed to contain vitamins B₁ and B₂ G (3, 4) and since the substance in vegex proved to be heat-stable, these workers concluded that the extrinsic factor was not vitamin B₁, which is heat-labile, but that it was probably vitamin B₂ G.
Since the experiments mentioned obviously did not involve the absolute identification of the extrinsic factor as vitamin B$_1$ G, it seemed important to approach the question from another angle; namely, to determine both qualitatively and quantitatively the vitamin B$_1$ and B$_2$ G content of this particular brewers' yeast concentrate, since it is known that brewers' yeast concentrates vary in their content of these vitamins (3, 4). As a corollary to this study, determinations were also made of the amounts of these two portions of the vitamin B complex in liver extract.

To ascertain the presence in foodstuffs of the vitamin B complex or the individual components making up that complex a standardized procedure is followed. Young rats of the usual laboratory strains weighing between 40 and 50 gm. are used as test animals. They are fed a basal ration which contains all the components required for normal growth except the vitamin in question. When growth no longer occurs on this diet the vitamin reserve of the animal body is considered to be exhausted and the substance to be tested for vitamin content is added to the ration. If growth then takes place at a normal rate for a sufficiently long period of time evidence of the presence of the vitamin in question is considered to be at hand. Unfortunately, different rates of growth have been considered normal by different investigators. Scheunert and Schelbling (3) were satisfied with a weekly gain of 3.0 gm. when testing brewers' yeast concentrates for the presence of vitamin B complex. Later Quinn, Whalen, and Hartley (4) used the same rate of growth in tests of the same material for the components of the vitamin B complex, vitamins B$_1$ and B$_2$ G. Both groups of workers concluded that the content of vitamin B complex in brewers' yeast varied considerably. Furthermore, it was thought that the content of antineuritic vitamin B$_1$ was more variable than was the content of the thermostable vitamin B$_2$ G. In comprehensive and detailed experiments Chick and Roscoe (5) demonstrated that the rate of growth (3.0 gm. weekly) obtained in the experiments described above did not even approach the normal growth rate for the experimental animals employed. They proposed that a diet should be considered adequate only when an average gain of from 10.0 to 12.0 gm. weekly occurs. Levene (6) extended the observations of Chick and Roscoe by studying the normal rate of growth of rats on adequate diets. He found that the average normal monthly gain in white rats of 50.0 gm. weight was 52.0 gm. However, it was shown that the rate of growth varied somewhat at different seasons. During July and August the average monthly gain was only 29.0 gm., whereas in September and October it was 72.0 gm.

The presence of the vitamin B complex in fresh liver and liver extract has been known for some time (7, 8). Review of the published work indicates that inadequate information is at hand as to the content in these materials of the individual components of the vitamin B complex, vitamins B$_1$ and B$_2$ G. Guha (9-11)
was the first to test liver extract for its content of vitamin B2 G. A basal diet was
used which contained vitamin B1 but which was deficient in vitamin B2 G. A
normal rate of growth was obtained (10.0 to 12.0 gm. weekly) when 40.0 to 60.0
mg. of liver extract, Lilly No. 343, an amount derived from 1 to 1.5 gm. of whole
liver, was added to the diet. If the material containing vitamin B1 was omitted
from the diet a normal rate of growth was not obtained, even if as much as 120.0
mg. of liver extract was administered daily. From these studies it was concluded
that liver extract was deficient in vitamin B1 but contained a considerable amount
of vitamin B2 G.

Gilroy (12) ascertained the presence of the vitamin B complex in a variety of
commercial liver extract preparations. He used rats maintained on a diet deficient
in the vitamin B complex. Adequate growth was considered to be about 10.0
gm. gain per week for rats of 40.0 to 50.0 gm. in weight. Normal growth was ob-
tained when he added daily to the basal ration an amount of liver extract equiva-
 lent to 16.0 gm. of fresh liver. If only half that amount of liver extract was em-
ployed, subnormal growth was obtained. No attempt was made to determine
which of the two components, vitamin B1 or B2 G, was deficient. From the
studies mentioned it has been assumed that liver extract is rich in vitamin B2 G
but deficient in vitamin B1. West (13) has prepared a substance from liver ex-
tract which produces typical remissions in patients with pernicious anemia.
This material has been shown by rat tests to be lacking in vitamin B2 G content.

Methods

The rats used in these experiments were all of the Wistar strain. They were
supplied through the Wistar Institute, or by Dr. H. M. Evans. Immediately
after weaning, all the animals were placed on a standard basal diet which was
vitamin B-free. It was of the following composition as recommended by Rose et
al. (14):

- Casein (extracted free from vitamin B) ....................... 18
- Salt mixture
  - NaCl .............................................. 3
  - CaCO3 .......................................... 1
- Butter fat ........................................ 8
- Corn-starch ..................................... 48
- Cod liver oil ................................... 2

In order to determine the presence of vitamin B1 in the substances studied it was
necessary to use a diet adequate in vitamin B2 G. This was supplied by adding to
the basal ration twenty parts of bakers' yeast (Fleischmann) neutralized and auto-
claved at 15 pounds pressure for 6 hours.

The vitamin B1 used in these experiments was the preparation adopted by the
League of Nations Health Organization, as described in the memorandum on
the International Standard Preparation of Antineuritic Vitamin (B1) (15). It is
commonly known as activated Java clay. This preparation given daily in 15.0
mg. amounts contained sufficient vitamin B₁ to support adequate growth in rats of 40.0 to 50.0 gm. when vitamin B₂ G was present in the diet. Control experiments showed that the activated Java clay was devoid of vitamin B₂ G. Tests for the absence of vitamin B₁ and B₂ G in the basal ration were always made.

After weaning the rats were maintained on the basal vitamin B-free diet for a period of 2 weeks to insure the complete depletion of the body store of vitamin B₁ and B₂ G (15). They were placed in groups of five in round wire cages with raised bottoms to prevent coprophagy. They were fed once a day and the animals were allowed to eat as much as they desired. The animals were weighed weekly. At the beginning of the experiments the weights of the rats ranged from 40.0 to 50.0 gm.

The liver extract studied was the preparation made by Eli Lilly and Company; both the injectable and the powdered forms were used. The liver extract in the powdered form was dissolved in water and mixed with a small amount of food in a dish. The form prepared for parenteral use, when given by mouth, was mixed with food and given in the same way. All the rats cleared their dishes before more food was given. The animals receiving liver extract parenterally were given daily intraperitoneal injections.

The vegex was given in a 50 per cent aqueous solution. The amount administered daily in these experiments varied from 50.0 to 250.0 mg. When the daily doses were small, the test material was given individually with a pipette. Daily doses of 150.0 to 250.0 mg. were too large to be taken at one time. Accordingly, these amounts were given with the food and the animals watched until the dishes were empty.

Experiment I.—Rats which had been kept for 2 weeks on the basal diet were divided into four groups. The basal diet was continued in all the rats. Those of the first group received in addition daily doses of 100 mg. of powdered liver extract, Lilly No. 343, an amount derived from 2.5 gm. of fresh whole liver. The diet of the rats of the second group was supplemented by the same dosage of liver extract and in addition 15.0 mg. of activated Java clay was given daily as a source of vitamin B₁. The animals of the third group received the basal diet plus the same amount of liver extract and in addition autoclaved bakers' yeast to supply vitamin B₂ G. The diet of the animals of the fourth group was supplemented by autoclaved bakers' yeast but contained no liver extract.

Group I.—Fig. 1, a shows the growth curves of four rats in the first group. These animals received the basal diet plus 100 mg. of liver extract daily. None
of these showed any appreciable gain in weight and all were dead by the end of
the 2nd week. The results indicate that 100 mg. of powdered liver extract, Lilly
343, administered daily, do not contain sufficient vitamin B complex to support a
normal growth rate in rats weighing 40 to 50 gm.

Group II.—Fig. 1, b shows the growth curves of four animals in the second
group. These animals received the basal diet, supplemented by 100 mg. of liver
extract daily and in addition 15 mg. of activated Java clay as a source of vitamin
B1. These curves are similar to those shown in Fig. 1, a. There was no appreci-
ciable gain in weight and all the animals died during the 2nd week. Since these
animals received a sufficient amount of vitamin B1 the results indicate that 100 mg.
of powdered liver extract, administered daily, do not contain an adequate amount
of vitamin B1 to support normal growth.

Group III.—Fig. 1, c presents the growth curves of animals of the third group.
The animals of this group received the basal diet, supplemented by 100 mg. of
liver extract daily and in addition an adequate amount of autoclaved bakers’
yeast to supply vitamin B2 G. Three of the four rats whose growth curves are
presented maintained an average gain of over 10 gm. per week for a period of 3
weeks. The fourth animal died of intercurrent disease after gaining at the normal
rate for the 1st week. These results demonstrate that 100 mg. of powdered liver
extract when administered daily contain sufficient vitamin B1 to maintain a normal
rate of growth in rats weighing from 40 to 50 gm., provided that the animals
receive an adequate supply of vitamin B2 G in the diet.

Group IV.—Fig. 1, d presents the growth curves of the rats of the fourth group.
These animals received the basal diet to which no liver extract was added. An
adequate supply of vitamin B2 G was supplied in the form of autoclaved bakers’
yeast. This experiment served as a control of the vitamin B1 content of the basal
diet, supplemented by autoclaved yeast. Of these animals, two lived for 3 weeks,
one gained 10 gm. in 2 weeks, the other 5 gm. in the same time. Three of the rats
died during the 2nd or 3rd week without showing appreciable growth gains.
These results indicate that the basal diet plus autoclaved bakers’ yeast was defi-
cient in vitamin B1.

To summarize, the experimental results indicate that 100 mg. of
powdered liver extract, Lilly No. 343, given daily to rats on a vitamin
B-deficient diet contain an adequate amount of vitamin B1 to maintain
a normal growth rate, but that this amount of liver extract under
similar conditions does not contain an adequate amount of vitamin
B2 G.

The Vitamin B1 and B2 G Content of Liver Extract (Lilly) in the Form
Prepared for Parenteral Injection When Given Orally

Experiment II.—Rats which had been kept for 2 weeks on the basal
diet were divided into three groups. The animals of the first group
Liver extract *343, 100 mg., p.o. + basal diet.

Liver extract *343, 100 mg., p.o. + vitamin B₁, 15 mg., I.S. + basal diet.

Liver extract *343, 100 mg., p.o. + B₂ diet.

Liver extract *343, 100 mg., p.o. + vitamin B₁, 15 mg., I.S. + basal diet.

Parenteral liver extract, 0.5 cc., p.o. + basal diet.

Parenteral liver extract, 0.5 cc., p.o. + vitamin B₁, 15 mg., I.S. + basal diet.

Parenteral liver extract, 0.5 cc., p.o. + B₂ diet.

Fig. 1

Fig. 2
were given 0.5 cc. of injectable liver extract by mouth daily in addition to the basal diet. This amount of liver extract was equivalent to 2.5 gm. of fresh whole liver. The animals of the second group received the same basal diet and the same amount of liver extract. In addition each animal received 15 mg. of activated Java clay as a source of vitamin B_1. The animals of the third group received the basal diet and the same amount of liver extract. In addition an adequate amount of autoclaved bakers' yeast to supply vitamin B_2 G was supplied.

Group I.—In Fig. 2, a are presented the growth curves of the rats of the first group. These animals received the basal diet plus 0.5 cc. of liver extract in the form prepared for parenteral use. This was administered daily by mouth. By the end of the 2nd week two of the animals had died. At this time the diet of the two remaining animals was supplemented by autoclaved bakers' yeast to supply vitamin B_2 G. Hence, from this time on the diet was the same as that supplied to the animals of the third group. One of these rats died during the 3rd week. The other rat gained 33 gm. in 3 weeks after the addition of vitamin B_2 G. This was an average gain of 11 gm. per week. The results in the case of the first two animals and in the last two, before the diet was supplemented by vitamin B_2 G, indicate that 0.5 cc. of liver extract prepared for parenteral use but administered orally does not contain sufficient vitamin B complex to support normal growth. The results in the case of the second two animals after the diet was supplemented by vitamin B_2 G will be mentioned when considering the animals of the third group.

Group II.—In Fig. 2, b are presented the growth curves of the animals of the second group. These rats received the basal diet plus the same amount of liver extract as did the animals of the first group. In addition, 15 mg. of activated Java clay were given daily to each rat as a source of vitamin B_1. Of this group one rat gained an average of 10 gm. per week for the first 3 weeks. The second rat gained an average of 5 gm. per week, while the third averaged 7 gm. per week. These animals therefore gained in weight but not at a normal rate. At the end of the 3 week period, autoclaved bakers' yeast to supply vitamin B_2 was added to the diet. In the next 2 weeks each of the rats gained considerably over 10 gm. a week, a normal rate of growth. These results indicate that 0.5 cc. of liver extract in the form prepared for parenteral use when given daily by mouth does not contain an adequate amount of vitamin B_2 G to support normal growth, even though a sufficient amount of vitamin B_1 was added to the diet. However, when vitamin B_2 G was added, normal growth occurred.

Group III.—In Fig. 2, c are presented the growth curves of the rats of the third group. The animals of this group received the same amount of liver extract as did those of the first and second groups. In addition, these rats received the basal diet supplemented by an adequate amount of autoclaved bakers' yeast to
VITAMIN B₁ AND B₂ G

FIG. 3
supply vitamin B₂ G. Of this group, one rat gained in weight an average of 21 gm. per week. Two of the rats gained 13 gm. per week, while the fourth rat gained 8 gm. per week. Three of these animals therefore maintained a normal rate of growth. Also, one of the rats in Group I which received after the 2nd week a supplement of vitamin B₂ G in its diet gained 10 gm. a week, a normal rate of growth. These results indicate that 0.5 cc. of liver extract in the form prepared for parenteral use, when given daily by mouth, contains an adequate amount of vitamin B₁ to maintain a normal rate of growth, provided an adequate supply of vitamin B₂ G is present in the diet.

To summarize, this series of tests indicates that 0.5 cc. of liver extract in the form prepared for parenteral injection, when given daily by mouth to rats kept on a vitamin B-deficient diet, contains an adequate amount of vitamin B₁ to maintain a normal growth rate. Moreover, it appears that this amount of liver extract tested in the same way does not contain an adequate amount of vitamin B₂ G.

The Vitamin B₁ and B₂ Content of Liver Extract (Lilly), the Form Prepared for Parenteral Injection, When Given Parenterally

Experiment III.—Rats which had been kept for 2 weeks on the basal diet were divided into three groups. The animals of the first group received the basal diet and in addition, 0.5 cc. of liver extract daily by intraperitoneal injection. This amount of liver extract was derived from 2.5 gm. of fresh whole liver. The animals of the second group received the basal diet and the same amount of liver extract administered in the same way and in addition, 15.0 mg. of activated Java clay administered daily to each rat to supply vitamin B₁. The rats of the third group received the basal diet and daily injections of liver extract administered as to the animals of the other two groups and in addition, an adequate amount of autoclaved bakers’ yeast to supply vitamin B₂ G.

Group I.—In Fig. 3, a are presented the growth curves of the animals of the first group. These rats were kept on the basal diet and received daily intraperitoneal injections of 0.5 cc. of liver extract. All of these rats lost weight and three of them died during the 2nd week. The two remaining rats at the beginning of the 3rd week were given daily 15 mg. of activated Java clay as a source of vitamin B₁. Thus, these two rats received the same diet as those of the second group. Both of these rats died during the 4th week. These results indicate that 0.5 cc. of liver extract in the form prepared for parenteral use and administered by intraperi-
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toneal injection does not contain sufficient vitamin B complex to support normal growth. The results obtained after the addition of vitamin B1 to the diet will be mentioned in the discussion of Group II.

Group II.—In Fig. 3, b are presented the growth curves of the animals of the second group. These rats received daily injections of liver extract, similar to those of the first group. The diet of the animals was the basal diet supplemented by 15 mg. of activated Java clay administered daily to supply vitamin B1. Of the animals of this group, one rat died during the 3rd week, having shown no appreciable gain. A second rat averaged 3 gm. gain in weight per week, a third, 7 gm., and the fourth, 4 gm. Thus, none of these animals maintained normal growth over a period of 3 weeks. At the beginning of the 4th week the diet of the three remaining rats was supplemented with an adequate amount of autoclaved bakers' yeast to supply vitamin B2 G. During the next 2 weeks one rat gained 8 gm. a week, another 10 gm., and a third, 22 gm. Hence, these rats maintained normal growth after the addition of vitamin B2 G to the diet. These results and those of the two rats in the first group to which vitamin B1 was given indicate that 0.5 cc. of liver extract in the form prepared for parenteral use when administered daily by intraperitoneal injection does not contain an adequate amount of vitamin B2 G to support normal growth, even though a sufficient amount of vitamin B1 was added to the diet. The results which followed the addition of vitamin B2 to the diet will be mentioned in the third group.

Group III.—In Fig. 3, c are presented the growth curves of the animals of the third group. The rats of this group received the basal diet and injections of liver extract similar to those of the two preceding groups. In addition, the diet was supplemented by an adequate amount of autoclaved bakers' yeast to supply vitamin B2 G. Of the animals of this group, one rat made an average weekly gain of 8 gm., another of 6 gm., a third of 5 gm., and the fourth rat of 3 gm. Thus, none of these animals grew at a normal rate. The animals of the second group of this series maintained normal growth after the addition of vitamin B2 G to the diet. However, these rats were receiving daily 15 mg. of activated Java clay as a source of vitamin B1. The results of the animals of the third group of Experiment II indicate that 0.5 cc. of liver extract in the form prepared for parenteral use, when administered by mouth, contains sufficient vitamin B1 to support normal growth. However, the animals of this, the third group of Experiment III, did not grow at a normal rate when the liver extract was given by intraperitoneal injections. It therefore seems clear that the vitamin B1 present in the liver extract was not as effective in supporting growth when given parenterally as when a similar amount of the same preparation is given by mouth.

To summarize, this series of tests indicates that 0.5 cc. of liver extract in the form prepared for parenteral use when administered daily by intraperitoneal injection to rats kept on a vitamin B-deficient diet does not contain an adequate amount of vitamin B2 G to support
normal growth. Moreover, it appears that the amount of vitamin B₁ present in liver extract of this form is not so effective in supporting normal growth when given by intraperitoneal injection as it is when given by mouth.

The Vitamin B₁ and B₂ G Content of Vegex

Experiment IV.—Rats which had been kept for 2 weeks on the basal diet were divided into five groups. The animals of the first group received the basal diet and in addition, 50 mg. of vegex administered daily to each rat. The animals of the second group received a similar amount of vegex as those of the first group. In addition, they received the basal diet supplemented by 15 mg. of activated Java clay daily as a source of vitamin B₁. The animals of the third group received a similar daily amount of vegex as those of the first and second groups. In addition, these animals received the basal diet supplemented by an adequate amount of autoclaved bakers’ yeast to supply vitamin B₂ G. The animals of the fourth group received the basal diet and in addition, 150 mg. of vegex administered daily to each rat. The animals of the fifth group received the basal diet and in addition, 250 mg. of vegex administered daily to each rat.

Group I.—In Fig. 3, d are presented the growth curves of the rats of the first group. These animals received the basal diet supplemented by 50 mg. of vegex administered daily to each rat. During the first 3 weeks the greatest average individual gain in weight was 4 gm. The other animals of this group averaged 2 to 3 gm. growth gain per week. At the end of the 3rd week the diet was supplemented with 15 mg. of activated Java clay as a source of vitamin B₁. The growth gains following this addition were negligible. Hence, after the 3rd week these rats received the same diet as those of Group II. The results obtained after this addition will be mentioned in the discussion of the second group. The results obtained during the first 3 weeks of this experiment indicate that 50 mg. of vegex given daily to rats kept on the basal diet do not contain sufficient vitamin B complex to support normal growth.

Group II.—In Fig. 3, e are presented the growth curves of the animals of the second group. These rats were fed the basal diet supplemented by 50 mg. of vegex daily, and in addition, 15 mg. of activated Java clay as a source of vitamin B₁. During the 3 week period the greatest average weekly gain for any of the rats was 4 gm. per week. The others averaged 2 to 3 gm. per week. Similarly, the average weekly gain of the rats of the first group after vitamin B₁ was added to the diet was negligible. Thus, a normal rate of growth was not maintained by any of these
Fig. 4
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rats. These results indicate that 50 mg. of vegex administered daily to rats kept on the basal diet, supplemented by an adequate amount of vitamin B1, do not contain a sufficient amount of vitamin B2 G to support normal growth.

Group III.—In Fig. 4, a are presented the growth curves of the animals of the third group. These rats were fed the basal diet supplemented by 50 mg. of vegex daily, and in addition, an adequate amount of autoclaved bakers' yeast as a source of vitamin B2 G. All of these rats for a period of 5 weeks made an average gain in weight of over 10 gm. per week; one rat averaged 19 gm. per week. A normal rate of growth occurred in all instances. These results indicate that 50 mg. of vegex administered daily to rats kept on the basal diet supplemented by an adequate amount of vitamin B2 G contain sufficient vitamin B1 to support normal growth.

Group IV.—In Fig. 4, b are presented the growth curves of the rats of the fourth group. These animals received the basal diet supplemented by 150 mg. of vegex administered daily. None of these animals grew at a normal rate for the first 3 weeks. One rat averaged 7 gm., the others, 4 to 6 gm. per week. At the end of the 3 week period the diet was supplemented by an adequate amount of autoclaved bakers' yeast to supply vitamin B2 G. Following this addition of vitamin B2 G to the diet, four of the five rats gained over 10 gm. per week, thus maintaining a normal rate of growth. These results indicate that 150 mg. of vegex given daily to rats maintained on the basal diet do not contain an adequate amount of the vitamin B complex to support normal growth. However, a normal rate of growth is obtained under similar conditions when a sufficient amount of vitamin B2 G is added to the diet. Hence, it appears that 150 mg. of vegex, under the conditions described, is adequate in its content of vitamin B1 but deficient in its content of vitamin B2 G to support normal growth.

Group V.—In Fig. 4, c are presented the growth curves of the rats of the fifth group. These animals received the basal diet, supplemented by 250 mg. of vegex administered daily. Of these rats, only one gained at a normal rate for the first 3 weeks. The other four rats averaged 3 to 6 gm. gain in weight per week. At the end of this 3 week period the diet was supplemented by an adequate amount of autoclaved bakers' yeast to supply vitamin B2 G. After this addition of vitamin B2 G to the diet three of the five rats gained at a normal rate. These results indicate that 250 mg. of vegex administered daily to rats kept on the basal diet do not contain sufficient vitamin B complex to support normal growth. However, a normal rate of growth is obtained under similar conditions, provided vitamin B2 G is added to the diet. Hence, 250 mg. of vegex under the conditions stated is adequate in its content of vitamin B1 but deficient in its content of vitamin B2 G.

To summarize, these results indicate that 50, 150, and 250 mg. of vegex administered daily to rats on a vitamin B-deficient diet contain an adequate amount of vitamin B1 to maintain a normal rate of growth, but that these amounts of vegex under similar conditions do not contain an adequate amount of vitamin B2 G.
DISCUSSION

The rat growth curves presented in this communication do not include those of all the experimental animals. The ones presented were selected as typical. Relatively large numbers of rats were used in all the experiments and the results were entirely consistent with those presented. Furthermore, the experiments were repeated as a whole. The results obtained in the second group of experiments were entirely in keeping with those obtained in the first.

From the experiments presented, it can be concluded that 100.0 mg. of powdered liver extract, Lilly No. 343, and 0.5 cc. of the form prepared for parenteral use, when given daily by mouth to rats of 40.0 to 50.0 gm. weight, on a basal vitamin B-free diet, do not contain enough vitamin B complex to support normal growth. Furthermore, when vitamin B1 is added to the diet by the daily administration of 15.0 mg. activated Java clay, an amount more than sufficient to give adequate growth to such rats if B2 G in an adequate amount were present in the diet, there is no appreciable growth gain. However, when autoclaved bakers' yeast as a source of vitamin B2 G is substituted for the vitamin B1, a normal rate of growth occurs. Hence, it seems clear that these two forms of liver extract when given by mouth under the conditions described contain adequate vitamin B1 to support normal growth, but are deficient in vitamin B2 G. This is not in accordance with the results reported by Guha (9–11) who obtained normal growth of rats under similar conditions with 40.0 to 60.0 mg. daily of the same liver extract preparation. Guha used rats fed a diet containing a vitamin B1 concentrate. He stated that this concentrate contained some vitamin B2 G, but not enough to support growth. It is possible that the vitamin B1 concentrate used by Guha might have contained enough vitamin B2 G to support normal growth when added to the small amount present in liver extract. Guha did not attempt to assay the liver extract for vitamin B1 as he did not obtain growth with as much as 120.0 mg. when the vitamin B1 concentrate was omitted from the diet. He concluded that the reason for the failure to obtain growth was the low vitamin B1 content of liver extract. Our experiments demonstrate that the failure with 100.0 mg. of liver extract is due to its low vitamin B2 G content.

The experiments in which liver extract was given by intraperitoneal
injection demonstrate that liver extract in the form prepared for
parenteral use does not contain a sufficient amount of the vitamin B
complex to support normal growth of rats under the experimental
conditions observed. The amount of liver extract injected was the
same as was tested by the oral route in other experiments. It may
be recalled that normal growth was not obtained in the case of rats
which received daily intraperitoneal injections of liver extract and a
diet supplemented by autoclaved bakers' yeast as a source of vitamin
B$_{12}$. Since the same preparation of liver extract was shown to con-
tain an adequate amount of vitamin B$_{1}$ when given by mouth in the
same dosage, it appears that the vitamin B$_{1}$ present in this form of
liver extract is not as effective when given by intraperitoneal injection
as it is when administered orally.

The experiments employing vegex, a brewers' yeast concentrate, as
a source of water-soluble vitamin indicate that it contains a large
amount of vitamin B$_{1}$ but a relatively small amount of vitamin B$_{2}$ G.
Subnormal growth was obtained when 50, 150, and 250 mg. of vegex
were given daily to rats on a basal vitamin B-free diet. However,
when vitamin B$_{2}$ G was added to the diet this growth was normal.

Strauss and Castle (2) in the treatment of pernicious anemia used 12
gm. of vegex with 150 cc. of normal human gastric juice in an incu-
bated digest. This amount, given daily over a period of 10 days,
produced typical remissions in patients with pernicious anemia. 50
mg. of vegex, given daily to a rat of 50 gm. weight is comparable to a
daily dosage of 50 gm. in a person weighing 50 kilos, if calculated by
weight. Since 50 mg. of vegex were shown to be inadequate in vitamin
B$_{2}$ G to support growth of rats, and since a much smaller amount
administered to human beings on a weight for weight basis is adequate
in content of the extrinsic anti-anemic factor, evidence is at hand that
the growth-promoting factor and the extrinsic anti-anemic factor are
dissimilar. Although the inability to support growth in rats appears
to be evidence of the inadequate content of vitamin B$_{2}$ G in a par-
ticular foodstuff, it should be borne in mind that the term vitamin
B$_{2}$ G is a general one referring to a thermostable accessory food factor.
The commonly accepted criterion for the presence of this factor in
foodstuffs is the ability of the material in question to promote normal
growth in rats. The absence of this quality in a substance such as
vegex, known to possess another biological property, the ability to promote hematopoiesis, indicates the necessity of further study of the various components of the heat-stable accessory food factor, vitamin B₂ G.

CONCLUSIONS

1. Liver extract powder, No. 343 Lilly, and the same material prepared for parenteral use, when administered daily by mouth in amounts derived from 2.5 gm. of fresh whole liver, to rats weighing from 40 to 50 gm., contain sufficient vitamin B₁ to support normal growth, provided the animals receive in addition an adequate amount of vitamin B₂ G. Moreover, liver extract in the forms mentioned, administered in the same amounts, does not contain sufficient vitamin B₂ G to maintain normal growth of similar rate when all other necessary constituents of the diet are provided.

2. Liver extract (Lilly) in the form prepared for parenteral use, when administered daily by intraperitoneal injections, in amounts derived from 2.5 gm. of fresh whole liver, to rats under standard experimental conditions, does not contain sufficient vitamin B₂ G to maintain normal growth. Furthermore, the amount of vitamin B₁ present in liver extract in this form is not as effective in supporting normal growth when given by intraperitoneal injection as it is when given by mouth.

3. Vegex, when administered daily in amounts of 50, 150, and 250 mg. to rats of 40 to 50 gm. in weight contains sufficient vitamin B₁ to maintain normal growth of the rats, provided the animals receive in addition an adequate amount of vitamin B₂ G. However, vegex in the same amounts does not contain sufficient vitamin B₂ G to support normal growth of similar rats when all other necessary constituents of the diet are provided.

4. These experiments indicate that the extrinsic, anti-anemic factor of Castle and the thermostable growth-promoting food constituent, commonly known as vitamin B₂ G, are not identical.

BIBLIOGRAPHY