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Liberation of Vitamin B₂ (Vitamin G) Adsorbed on Acid Earth by the Action of Pancreatin.

By

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In contrast to the ready adsorbability of vitamin B₂ (=Vitamin G) by a number of adsorbents considerable difficulty was experienced by previous workers in their attempts to release the adsorbed vitamin B₂⁽¹⁾. As far as we are aware no claim has been made of a successful elution, except by Kuhn, György and Wagner-Jauregg,⁽²⁾ who used pyridin as eluent. It seems to us, however, that the results of their animal tests are not as convincing as might be desired*.

Recently we found that vitamin B₂ adsorbed by acid earth from liver extract can be liberated through the action of pancreatin. In the present paper we shall first give an account of our experiments on the adsorption of vitamin B₂ by acid earth, and then proceed to describe our method of liberating the adsorbed vitamin B₂.

Adsorption of Vitamin B₂ by Acid Earth.

The liver extract used in this experiment was prepared as follows: 1 kg of fresh beef liver was mashed and thoroughly extracted with 5 litres of 60 % alcohol containing 50 c.c. of 25% sulphuric acid. Extract was separated by filtration and sufficient sulphuric acid added to bring the reaction of the extract to pH 3~4.

To this extract was added white acid earth in the ratio of 20 g of acid earth to 1 litre of extract, and the mixture was maintained at the temperature of 30°C for 2 hours with occasional stirring. The acid earth was then re-

* We hope to take up this question in our later experiment.

covered by filtration and was repeatedly washed with acidified water, with alcohol, and then with ether.

The vitamin B₂ potency of the acid earth treated in this manner was tested in accordance with the usual method. Young albino rats were first maintained on a synthetic basal diet, which was deficient in vitamin B₂, and after a period of two weeks on this diet supplementary feeding of the acid earth adsorbate was commenced and the growth response of the rats noted. The composition of the vitamin B₂ deficient diet was as follows:—

Polished rice powder	75 g
Fish protein	10 g

Chart 1—Growth curves of 5 rats showing the growth response to adsorbate on acid earth of liver extract ("activated" acid earth).

Chart 2—Growth curves of 2 rats showing the lack of vitamin B₂ action of the filtrate after the removal of adsorbate. Also included in the chart are growth curves of 3 controls on vitamin B₂ deficient diet.

Butter	10 g
McCollum's salt mixture	5 g
Oryzanin solution	5 c.c.

Chart 1 represents growth curves of 7 rats on which the effect of adsorbate was tested. In these tests 20 g of the adsorbate ("activated" acid earth) was added to 100 g of basal diet, and all the rats responded very promptly by the typical sharp increase in their body weight.

Chart 2 shows the growth curves of 2 rats on which was tested the effect of the non-adsorbable fraction after the removal of acid earth. This fraction was added to 100 g of basal diet in amounts corresponding to the yield from 1 litre of extract.

The total lack of growth response to the supplementary feeding demonstrates that the vitamin B₂ in the original extract has been entirely removed by adsorption on acid earth.

Growth curves of 3 control rats maintained on the basal diet alone throughout are also included in Chart 2.

Liberation of Adsorbed Vitamin B₂ by Pancreatin Solution.

To 1 per cent solution of commercial pancreatin preparation in 0.1 per cent aqueous solution of sodium-bicarbonate was added the "activated" acid earth in the proportion of 10 per cent, the reaction of the mixture being brought to pH 7.6 by adding sodium bicarbonate. The mixture was kept at 32°C for twenty-four hours under toluol, with frequent stirring, after which the sediment (extracted acid earth) was removed by filtration. The sediment was washed with water, and the wash water was combined with the filtrate, and the whole was concentrated into a suitable volume at a low temperature under reduced pressure in the atmosphere of CO₂ gas. The pancreatin contained in the concentrate was inactivated by heating at 85°C for 20 minutes.

The vitamin B potency of this substance, as well as of the sediment or extracted acid earth, were tested in the same manner as in the preceding experiment.

Chart 3 shows the growth curves of 6 rats on which was tested the effect of different amounts of the substance liberated by the action of pancreatin. The extraordinarily active growth response induced by the supplementary feeding of this eluted substance is remarkable, and it is obvious that the amount recoverable from 5 g of the "activated" acid earth is quite adequate as the source of vitamin B₂.

The extracted acid earth after the removal of eluate was washed repeatedly with distilled water and was tested as to its vitamin B₂ potency in order to determine the extent to which the adsorbed vitamin B₂ was liberated

by the treatment with pancreatin. 20 g of the washed and dried acid earth was added to 100 g of the vitamin B₂ deficient basal diet.

As may be seen from Chart 4, the extracted acid earth still contained some vitamin B₂, showing that the liberation was by no means complete.

Chart 3—Growth curves of 6 rats showing the vitamin B₂ action of pancreatin eluate in amounts corresponding to yields from 5~20 g of activated acid earth.

Chart 4—Growth curves of 3 rats on which was tested the effect of extracted acid earth, i.e., the sediment after the removal of the eluate. Growth curves of 3 controls are also included.

Chart 5—A comparison of the vitamin B₂ action between 20 g of acid earth after the removal of eluate ("extracted acid earth") and eluate from 10 g of activated earth.

Chart 6—Growth curves of 3 rats showing the absence of vitamin B_2 action in pancreatin itself. In addition a single control on vitamin B_2 deficient diet, and growth curves of 2 rats showing a weak response to sodium bicarbonate eluate are also included.

Chart 7—Growth curves of 2 rats showing the lack of growth response to pepsin eluate. Growth curves of 3 vitamin B_2 deficient controls are also included.

The growth response of the rats to a 20 g supplement of extracted acid earth was of about the same magnitude as that which follows a supplement of eluate in amounts corresponding to yields from 10 g or 5 g of "activated" acid earth.

It may be regarded as probable that more than two-thirds the total amount of adsorbed vitamin B₂ was liberated by our method using pancreatin.

An additional experiment showed that this supposition is correct, since, in a parallel test, 20 g of extracted acid earth was found to be slightly less potent than the eluate from 10 g of activated earth (Chart 5).

For control to the above, tests were made also for the possible vitamin B₂ potency of pancreatin itself. 2 g of the same commercial sample of pancreatin as used in the elution experiment was added to 100 g of the basal diet. As shown in Chart 6, pancreatin itself has no growth promoting action.

Needless to say that no satisfactory elution of vitamin B₂ took place when the activated acid earth was treated with 0.1% solution of sodium bicarbonate without pancreatin, although a small amount of the vitamin seemed to dissolve out into the solution. See Chart 6.

The possibility of the elution of adsorbed vitamin B₂ by pepsin solution was also examined in passing. The procedure of the manipulation was the same as in the case of pancreatin solution, which was simply replaced by one per cent solution of commercial pepsin in 0.15 per cent hydrochloric acid. As may be seen from Chart 7, no satisfactory liberation of vitamin B₂ was attained by the action of pepsin solution.

Chart 8—Growth curve of the rat developing pellagra-like symptoms on vitamin B₂ deficient diet, showing the curative effect of our eluated vitamin B₂.

Curative Action of Eluate on the so-called Pellagra-like Condition.

It is well known that vitamin B₂ deficiency produces in certain proportions of the rats pathological conditions somewhat similar to pellagra in man, and that a timely administration of vitamin B₂ brings about the disappearance of these symptoms.

Among our series of vitamin B₂ deficient rats we happened to encounter a single case with definitely recognizable symptoms and we had the opportunity of testing the curative action of our eluated vitamin B₂.

Chart 8 gives the growth curve

of the rat in question, which was fed on the same vitamin B₂ deficient diet as before. It developed marked pellagra-like symptoms during the seventh week and the end of the eighth week we began adding our eluate, in amounts corresponding to the yield from 20 g of activated acid earth, to 100 g of the basal diet. The growth response of the rat was typical.

Figure 1 illustrates the condition of the rat one day before the beginning of the vitamin B₂ supplement, showing dermatitis over the chest and under the arm, and also alopecia around the eye.

Figure 2 represents the same rat 14 days after the beginning of vitamin B₂ supplement, showing a complete recovery from dermatitis.

Figure 1—Rat A, 1 day before the commencement of eluted vitamin B₂ feeding. Note the extreme mal-nutrition and typical dermatitis.

Figure 2—The same rat, after 14 days of eluted vitamin B₂ feeding, showing a complete disappearance of dermatitis.

Comments.

From the physiological point of view, inasmuch as the vitamin B₂ adsorbed on acid earth is utilized by the animal, it is self-evident that the elution of vitamin B₂ must take place somewhere in the alimentary canal. Our present experiments show that this elution must occur in the intestine rather than in the stomach, since the elution takes place *in vitro* under the influence of pancreatin, but not through the action of pepsin.

Another point of interest in our experimental results is that vitamin B₂

is much better utilized by the animal when supplied in the form of eluate than when given adsorbed on acid earth. This difference in the rate of utilization suggests that no small amount of vitamin B₂ is excreted along with the acid earth which carries it. Our method of liberating vitamin B₂ from the adsorbent therefore offers the advantage, not only of doing away with the ingestion of bulky adsorbent, but also of facilitating the utilization by the animals of the vitamin B₂ administered to them.

Conclusion.

Vitamin B₂ in liver extract is completely absorbable by acid earth, but from the experience of previous workers, it may be expected to be difficult to eluate it by ordinary means.

In the foregoing experiments we found that the adsorbed vitamin B₂ can be satisfactorily released by the action of pancreatin. Moreover, it was found that the vitamin B₂ thus released is more readily utilized by the animal organism than the same vitamin adsorbed on acid earth.

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Isolation of Vitamin C from Vegetables and the Relations between Vitamin C and Ascorbic Acid*.

By

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In recent years no chapter but that of C in the vitamin research has made so wonderful a development.

Szent-György⁽¹⁾ first isolated in 1928 from suprarenal cortex a reducing crystalline substance consisting of an elementary composition of C₆H₈O₆, but,