STUDIES ON THE ANTINEURITIC VITAMIN

I. ON THE USE OF ALBINO MICE AS TEST ANIMALS FOR DETERMINING THE POTENCY OF ANTINEURITIC CONCENTRATES*

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In connection with an investigation dealing with certain aspects of the nutrition of the mouse, we were confronted with the problem of estimating the nutritive value of the daily doses of yeast given as a source of the vitamin B factors. Inasmuch as the mouse requires 200 mg. of yeast daily, which amounts in some cases to one-tenth of the daily food intake, this was a factor which could not be neglected. It was decided, therefore, to prepare concentrated preparations of the antineuritic vitamin.

The next question which arose was the choice of a method for testing our concentrates. Although the procedures in which pigeons and rats are used for this purpose have been well standardized, we thought it worth while to attempt to use mice as test animals. We reasoned that the mouse, on account of its high metabolic rate and its small size, would offer certain advantages for this type of work. In the first place, much less material would be necessary for making the tests. Secondly, we expected that mice would show symptoms of vitamin B deficiency at an earlier date than rats or pigeons. Lastly, it was deemed of interest to repeat, if possible, the work of Jansen and Donath (1) on the isolation of the antineuritic vitamin from rice polishings and to corroborate their findings on another species, the mouse. The observa-

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tions of Tsukiye (2), Beard (3), and Bing and Mendel (4) have shown that mice may be used with advantage for vitamin studies.

EXPERIMENTAL

As a source of the antineuritic vitamin we used rice polishings. In the preparation of the concentrates we followed Jansen and Donath's procedure. Very few changes were made in their method of isolation. Care was taken that no operation was carried out at a higher temperature than 40°. All evaporations were made *in vacuo*. We found that the treatment of the tikitiki extract with 80 per cent alcohol is very useful (Hofmeister (5), Evans and Lepkovsky (6)). Under these conditions it is possible to eliminate nearly all the contaminating carbohydrate material. Instead of the acid clay which was employed by Jansen and Donath, we used Lloyd's reagent for the adsorption of the vitamin with excellent results.

The liberation of the vitamin from the adsorbent seems to be a controversial subject. Jansen and Donath used in their experiments barium hydroxide and reported a recovery of 80 per cent of the vitamin. Later workers observed considerable losses of the potent factor at this stage of the isolation process. We refer to the studies of Williams and his coworkers (7), Seidell (8), and Evans and Lepkovsky (6). Mukherji (9) and Van Veen (10), on the other hand, have confirmed Jansen and Donath's findings. In our own experiments the recovery was 75 per cent.

In the later stages of the isolation process we followed exactly Jansen and Donath's directions. The Lloyd's reagent extract was fractionated according to Kossel and Kutscher (11) with silver nitrate and baryta. Three fractions have been prepared: the first one was precipitated at pH 2 to 4.5, the second at pH 4.5 to 6, and the third at pH 6 to 8. Inasmuch as the second fraction\(^1\) was shown to contain more than 50 per cent of the vitamin, we used this material for the further purification. After removing the silver, the potent factor was precipitated with silicotungstic acid, according to Jansen's modification of the first method (12). The last stages of the concentration were carried out exactly as de-

\(^1\) From the first and third fractions we isolated a small amount of material in form of the flavianates, but found these to be inactive. We intend to use flavanic acid in our further work of purification.
scribed by Jansen and Donath, only that instead of platinum chloride we used cadmium chloride, as recommended by Jansen (12).

In the following diagram an outline of the purification process is given.

\[
\begin{align*}
\text{Rice polishings} & \quad 25 \text{ per cent alcohol} \\
& \quad \text{Concentrated} \\
& \quad \text{Tikitiki extract} \\
& \quad 80 \text{ per cent alcohol} \\
& \quad \text{Lloyd's reagent} \\
\end{align*}
\]

\[
\begin{align*}
\text{Activated Lloyd's reagent (A I) (90 gm. = 25 kilos rice polishings)} & \quad \text{Ba(OH)\textsubscript{2}} \\
\text{Filtrate (after removal of Ba) (A II) (700 cc. = 25 kilos rice polishings)} & \quad \text{AgNO\textsubscript{3} + Ba(OH)\textsubscript{2} at pH 4.5 to 6.5} \\
& \quad \text{HCl} \\
& \quad \text{Precipitate} \\
& \quad \text{Silicotungstic acid} \\
& \quad \text{Ba(OH)\textsubscript{2}} \\
& \quad \text{Absolute alcohol + CdCl\textsubscript{2}} \\
& \quad \text{H\textsubscript{2}S} \\
\end{align*}
\]

Impure hydrochloride (A III) (120 mg. = 25 kilos rice polishings)

**Physiological Tests**

Our experiments were carried out on albino mice bred and reared from an original stock obtained from the zoology department of this University. Young mice were separated from their mothers when they were 3 weeks old. As basal diet for the normal
controls we used the food mixture recommended by Bing and Mendel (4), consisting of purified casein\textsuperscript{2} 31 per cent, purified cornstarch 38 per cent, Crisco 24 per cent, and Osborne and Mendel's salt mixture 7 per cent.\textsuperscript{3} In addition, each mouse received separately 200 mg. of dried yeast\textsuperscript{4} and 2 drops of cod liver oil daily. In the course of our experiments we found that the cod liver oil could be mixed with the food with apparently the same results.

The basal diet for the test animals was the same as above, except for the yeast, which was substituted by the same amount of autoclaved yeast. The mice which were receiving the diet lacking the antineuritic factor showed the first symptoms of vitamin B\textsubscript{1} deficiency when they were about 25 days old. They began to lose weight, became restless, and their fur was unkempt in appearance. Between the 28th and 35th days, definite symptoms of vitamin B\textsubscript{1} avitaminosis were manifest, such as appreciable loss in weight, the arched back, and darkening of the tail. At this point we began our tests.

\textit{Evaluation of Antineuritic Concentrates}

We controlled the process of purification of our vitamin preparation by testing the potency of our concentrates at four stages during the course of the work.

The first tests were made on the tikitiki extract, prepared according to Wells (13), by extracting the rice polishings with 25 per cent alcohol and concentrating this extract to a syrup \textit{in vacuo}. In agreement with Bing and Mendel's observations it was found that 0.03 to 0.04 cc. of the tikitiki extract, given daily, was sufficient for good growth.

For the second test we chose the activated Lloyd's reagent precipitate (Preparation A1). 90 gm. of this precipitate correspond to 25 kilos of rice polishings. This preparation was fed, mixed with the autoclaved yeast, separately to the mice. 10 mg. of the substance, given daily, were sufficient to cure the animals of polyneuritis and to promote growth.

Inasmuch as several workers have reported unsuccessful at-

\textsuperscript{2} The casein was purified according to the directions of Evans and Lepkovsky (6).
\textsuperscript{3} Osborne, T. B., and Mendel, L. B., \textit{J. Biol. Chem.}, 37, 572 (1919).
\textsuperscript{4} The product from the Northwestern Yeast Company was used.
tempts to recover the vitamin from the adsorbent in as good a yield as that obtained by Jansen and Donath, it was deemed necessary to test the potency of the concentrate obtained after removal of the vitamin from the Lloyd's reagent. 700 cc. of this concentrate (Preparation A II) were obtained in the extraction of 25 kilos of rice polishings. It was found that in all cases prompt and complete cures of polyneuritis resulted from doses of 0.12 cc. of this preparation, and a marked increase in weight occurred.

The last test was carried out with the substance obtained from the cadmium chloride precipitate, after decomposing it with hydrogen sulfide. The filtrate of the cadmium sulfide was evaporated to dryness in vacuo. The residue (Preparation A III) weighed 0.12 gm., corresponding to 25 kilos of rice polishings. This substance was taken up in water, and the solution injected subcutaneously. The results obtained with this preparation are shown in Chart 1. It was found that a daily dose of 0.025 mg. of this substance was sufficient to cure the animals of polyneuritis and to promote normal growth. A few tests were carried out with lower doses of this concentrate, which showed that a daily dose of 0.010 mg. cured the mice of polyneuritis, but in no case, however, did an increase in weight occur.
DISCUSSION

The experiments reported above show that mice may be used with advantage as test animals for the evaluation of antineuritic concentrates.

We have been able to isolate from rice polishings, according to the method described by Jansen and Donath, a potent principle which, in daily doses of 0.025 mg., cures polyneuritis in mice. We believe that we are justified in assuming that the curative and growth-promoting dose of this preparation probably lies between 0.010 and 0.025 mg. Jansen and Donath reported that the substance which they obtained at this stage of the isolation process was curative for rice-birds (Munia maja) in daily doses of 0.008 mg. Considering the fact that we are dealing with two different species, we may safely assume that our preparation approaches in its purity the substance obtained by Jansen and Donath.

SUMMARY

1. A potent antineuritic concentrate has been obtained from rice polishings, with Jansen and Donath's method of isolation.
2. This preparation cures polyneuritis and promotes growth in mice in daily doses of 0.025 mg.
3. Our experiments corroborate the findings of Jansen and Donath on still another species, the mouse.
4. Our results show that mice may be used with advantage as test animals for the evaluation of antineuritic concentrates.

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