LVII. *STREPTOTHRIX CORALLINUS* IN THE ESTIMATION OF VITAMIN B₁.

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THIS work was undertaken as an attempt to find a substitute for the laborious technique involved in the pigeon tests.

Williams [1919] first described a method of testing for the antineuritic vitamin which was based on the assumption that the yeast cell is capable of multiplication only in the presence of this factor. The test was, however, later discarded when Funk and Dubin [1920] demonstrated that the yeast stimulant (bios) was not identical, but merely associated, with the antineuritic factor.

EXPERIMENTAL.

When testing the more active torulin concentrates (0.1 mg. per day pigeon dose), a very considerable difference in growth was noted between flasks containing 1/20 and 1/40 dose, or again between those containing 1/400 and 1/800 dose in 20 cc. medium. It was then found that, given a fraction, say 1/1000 part, of a torulin concentrate, it was possible to predict the number of doses to be found in the whole. Hence we developed the following technique. It has been found capable, with certain limitations, of replacing the pigeon tests in following the fractionation of the vitamin.

Technique of the test.

- Materials.* 1. Synthetic medium prepared as described by Reader [1927].
2. *Streptothrix corallinus* [Reader, 1926]. A young culture should be used.
3. A torulin concentrate of which the pigeon dosage is known. A dilution is made up in absolute alcohol of such a strength that 0.1 cc. = 1/20 pigeon dose. This preparation is kept permanently as a standard for comparison with the samples under test.
4. Conical flasks of 50 cc. capacity. These must be scrupulously cleaned between each estimation, using hot chromic-sulphuric acid mixture and large quantities of distilled water. Both the flasks and pipettes needed should be reserved for use in the test only.

Setting up. The experimental flasks are set up as follows.

1. Medium 20 cc. minus the volume of the test fluid, which is carefully washed into the flask. (Dilution 1/20.)

2. Medium 19 cc. + 1 cc. carried over from Flask 1. (Dilution 1/400.)

3. Medium 19 cc. + 1 cc. carried over from Flask 2. (Dilution 1/8000.)

Standard flasks. Nine dilutions of the standard torulin are put up for comparison with the above. A dilution is first made by taking 0.75 cc. of the standard preparation, evaporating to dryness on the water-bath, and washing into 30 cc. of the medium. 0.1 cc. of the standard contains 1/20 pigeon dose. This amount is now contained in 4 cc. of the dilution A.

The nine flasks are now prepared as follows:

Flask	Medium		Standard dilution A	Actual dilution (of pigeon dose)
1	4 cc.	+	16 cc.	4/400
2	12 cc.	+	8 cc.	2/400
3	16 cc.	+	4 cc.	1/400
4	18 cc.	+	2 cc.	1/200
5	19 cc.	+	1 cc. from 1	1/2000
6	19 cc.	+	1 cc. from 2	1/4000
7	19 cc.	+	1 cc. from 3	1/8000
8	19 cc.	+	1 cc. from 4	1/16000
9	19 cc.	+	1 cc. from 5	1/40000

It will be seen that the dilutions in standard flasks nos. 3 and 7 correspond respectively with those in test flasks nos. 2 and 3, the remaining flasks containing multiples or submultiples of these quantities.

All flasks are steamed for half an hour on three successive days, and subsequently incubated for 24 hours at 31° to test for sterility. Each flask is then inoculated by loop from a suspension of *S. corallinus*. This is most conveniently taken from a young culture (2 to 6 days) grown on a medium corresponding to that in standard flask no. 9. The amount of growth is estimated at the end of 48 hours' incubation at 31°.

Reading the test. The amount of growth in test flask no. 2 is compared with that in the standard flasks nos. 1-5, and that in test flask no. 3 with standards 5-9. Readings in two dilutions are thus obtained over a range from 4 times to 1/5 of the strength of the standard concentration. Readings outside this range, obtainable on one flask only, should not be relied upon.

It has unfortunately not been possible to produce a uniform suspension of the organism. Nephelometric or other accurate methods of comparing growth cannot therefore be used, and only a very approximate estimate can be made. Experience has shown, however, that the average readings in the two flasks give a result of an accuracy comparable with that of the pigeon test.

The flasks are read both before and after shaking. Flask no. 1 is not used for the comparison of growth. It serves the purpose of detecting inhibitory agents, and certain stimulating substances other than the growth factor to which reference is made below.

Example. Test fluid = 0.65 cc. from a batch of 65 cc. (1/100 part).

Flask no. 1. Good.

Flask no. 2 = Standard flasks nos. 3-4.

Flask no. 3 = Standard flasks nos. 7-8.

Test fluid is therefore < 1 and $> 0.5 = 0.75$ of the standard (*i.e.* unit dose) strength. The whole batch of 65 cc. therefore contains $0.75 \times 100 = 75$ doses.

Exceptional readings. Departures occur from this type of result; these are found to fall into two groups.

1. *Inhibition.* Certain reagents used in the extraction process, particularly mercury, silver and platinum salts, markedly inhibit the growth of the organism. Warning of this effect is always given by the poor growth in flask no. 1. Should the effect be still apparent in the higher dilutions, the estimation must be disregarded, or an approximation obtained from flask no. 3.

2. *Anomalous result.* A more serious source of error is the occurrence of an abnormally rapid falling off of growth as the dilution is increased.

Example. Test fluid = 1 cc. from a batch of 500 cc.

Flask no. 1. Very good.

Flask no. 2 = Standard 1 (standard $\times 4$).

Flask no. 3 = Standard 8-9 (standard $\times \frac{1}{2}$ to $\frac{1}{3}$).

On the flask no. 2 reading therefore the batch would be calculated to contain 2000 doses, but, on the flask no. 3 reading, less than 200 doses. The pigeon test may, in these cases, give results approximating to the higher or lower or to an intermediate value of the *S. corallinus* readings. No information of value can therefore be obtained from results of this type. They occur, fortunately, only in the early stages of the yeast extraction, before charcoal adsorption. The value of the test in the later stages is therefore not impaired.

These anomalous results suggest that some other stimulating factor is present in the crude extracts. The nature of this substance is at present under investigation by one of us (Vera Reader).

The practical value of the test is discussed in a later paper [Peters, Kinnersley, Orr-Ewing, and Reader, 1928], where tables showing the parallel *Streptothrix* and pigeon tests will be found.

SUMMARY.

A method of obtaining quantitative growth-promoting tests has been developed, which is applicable to the testing of antineuritic concentrates after the charcoal adsorption stage. A method is given of eliminating erroneous results due to the presence of inhibitory agents such as metals.

Certain anomalous results are obtained with material tested before the charcoal stage.

REFERENCES.

- Funk and Dubin (1920). *J. Biol. Chem.* **44**, 487.
Peters, Kinnersley, Orr-Ewing and Reader (1928). *Biochem. J.* **22**, 445.
Reader (1926). *J. Path. Bact.* **29**, 1.
— (1927). *Biochem. J.* **21**, 901.
Williams (1919). *J. Biol. Chem.* **38**, 465.