CCV. A QUANTITATIVE COMPARISON OF THE CURATIVE ACTIVITY OF TORULIN (VITAMIN B_1) UPON THE ADULT PIGEON AND THE ADULT WHITE RAT.

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The standard method of testing for vitamin B_1 in this laboratory has been a curative and protective test upon pigeons, as laid down by Kinnersley, Peters and Reader [1928]. It is known from the work of Chick and Roscoe [1927] that torulin preparations made by our methods are highly curative to rats suffering from polyneuritis, and that they can be used as a growth factor by the growing rat. This has been confirmed by Reader [1930] as an incident in the elaboration of a technique for testing for Reader's vitamin B_4. In spite of the growth-promoting effect of these preparations, there is as yet no strict proof that the vitamin B_1 under consideration in our concentrates is the same factor for the pigeon and the rat. We felt that if it could be shown that the curative and protective effect for the rat in several different preparations ran parallel to that for pigeons, it would constitute a proof that the factor concerned was essentially the same for both animals. Accordingly we have compared the vitamin B_1 (torulin) preparations from this point of view. A preliminary account of the experiments appeared in the Harben lectures [Peters, 1929, 1930], stating that approximately the same dose was required to cure and protect the rat as in the case of the pigeon. About the time of publication of these lectures, a paper by Smith [1930] appeared, in which a curative test is employed. The author uses as a standard the minimum dose of a preparation required to cure a rat suffering from polyneuritis. In an accompanying paper, Reader [1930] deals with the question of the symptoms in these tests. A rough comparison of Smith's tables with our results suggests that our method with rats gives rather more regular answers, but comparative tests would have to be made to determine the relative merits of the two methods.

Experimental.

Pigeon tests. These have been made by the method previously described. The objections put forward by Smith to the pigeon-cure test have been dealt with in our previous communication [1928]. The first objection that spon-

taneous cures occur, we have dealt with in full. His second objection is as follows: "Cures effected in polyneuritic pigeons by the administration of the antineuritic substance have often been noted to be little or not at all sustained, and instances have been known in which birds were totally refractory to treatment during subsequent attacks." We are not quite certain that we understand what is here considered. Out of a very large experience of these birds (amounting to some 1000 birds a year for the last 6 years), we have come across few instances (certainly nor more than 1 %) in which a genuine vitamin B_1 extract would not cure a bird suffering from apparently genuine symptoms. Such cases are evidently not suffering from vitamin B_1 deficiency, and that is why they do not react to the tests. It is unfortunate that there is at present no means of easily distinguishing such cases. Many other physiological disturbances will induce head retraction in pigeons, as for example an overdose of insulin. Such instances have been collected elsewhere [Peters, 1929, 1930]. It conveys a false idea of the facts to think that enough of these cases appear under controlled feeding conditions to disturb the tests seriously. Further, it must be recognised that it is unlikely even that the rat will not be occasionally abnormal. Polyneuritic symptoms in the rat resemble rather closely those of an overdose of insulin in the mammal. The important point in using a complex symptom in animals as a means of testing is to eliminate as far as possible similar disturbances due to other causes. This we know to be a truism, but it is often overlooked. Smith's third objection as to the temporary cures produced by other substances is not a serious one in our opinion. It is in the main eliminated by the preliminary dosing, and if necessary it can be eliminated completely by the method recently described by Gulland and Peters [1929]. In our experience the only one of these reagents mentioned which we have ever found to cure is histamine. The fact that any test dose of histamine which we have given of late has not induced cure has led us to suppose that the histamine cure is due to occasional traces of impurities in commercial samples.

The difficulties to be found in applying the pigeon curative and preventive test are those inherent in all biological tests, namely the innate biological variation of the animals. In the case of the pigeon, the absence of a standard strain probably makes this wider than for an inbred strain of rat. To give some concrete idea of this, in Table I, out of the 20 tests upon a given dose of the average value of 4·4 days, 10 % of the bird tests gave values under half the average, and 10 % values approximately twice the average. With preparation B, a similar kind of result was obtained. We do not think that the values shown by Smith in his Table I give any better agreement than this, and doubt whether for many preparations the tests can be made with a less variation. We would record it as our impression however that we have had in our hands preparations which have given much more consistent results than these; at present we do not pretend to understand this. C, for instance (Table I), has been tested indirectly many times, and has seldom given less

than 3 or more than 5 days. The results obtained for the rats, by a precisely similar method of testing, form valuable independent evidence of the validity of our pigeon methods.

Rat tests. Adult albino rats (bred in this department) of approximately maximum weight (males 250 g., females 170 g.) were fed on a diet of caseinogen (20 parts), rice starch (70 parts), agar-agar (2 parts), salt mixture (5 parts), cod-liver oil (3 parts) and alkaline-autoclaved yeast extract (6 parts). This last item was supplied as a source of vitamin B_2 [*cf.* Reader, 1930]. In the course of 21–24 days the rats were found to decrease in weight to about 100–120 g. Convulsions then occurred rather suddenly accompanied by partial paralysis, especially of the hind legs. The feet are always splayed out, and often the head is held on one side. The animal may live for a few hours in this condition, but frequently death occurs in about 30 mins. It is advisable to give the test dose of torulin (vitamin B_1) as soon after the onset of symptoms as possible, as the throat often becomes paralysed, so making dosing by mouth impossible. The test doses were always given by mouth from a pipette. A marked improvement may often be noticed within 20 minutes of the time of dosing the animal and always in 2–3 hours, especially if not less than a three-day dose is given. The second onset of convulsions occurs on the 2nd, 3rd or *n*th day according to the size of dose given. Convulsions and paralysis of the hind limbs appear to be the true polyneuritic symptoms and are cured by vitamin B_1 in its purest form. The symptoms due to lack of vitamin B_1 are usually accompanied by those due to deficiency of vitamin B_4, such as swollen paws, ataxia, etc. The latter are not removed by dosing with vitamin B_1 and can be used for the assay of vitamin B_4 [see Reader, 1930].

The range of variation of the rat curative test for vitamin B_1 may be judged from the figures for a given preparation (preparation B in Table I) where 12 rats were used; the maximum value obtained was 6, minimum 4, and the average (for 12) 4·5.

Results. We have collected in Table I the results of tests upon pigeons and rats of different preparations, varying in activity from one with a pigeon-day dose of 0·025 mg. to one of 1·2 mg.

Table I.

	Dose of vitamin B_1 prep. cc.	No. of day doses		Activity mg.
		Pigeons	Rats	
(A)	1·0	4·4 (20)	4 (3)	1·2
(B)	0·1	2·9 (37)	4·5 (12)	0·13
	0·15	—	6 (2)	0·13
(C)	0·10	3·8 (8)	3·5 (5)	0·10
(D)	0·08	5·0 (3)	4 (6)	0·25

The activity is expressed in mg. organic solids per daily pigeon dose.

In the above table it will be seen that the agreement is reasonably close except in the case of preparation B. In this the preparation appears to be

1·5 times more active for the rat than for the pigeon. When it is remembered that there is no reason why there should be any correspondence between these figures apart from identity of the factors concerned and that biological tests are subject to rather wide variations unless very large groups of animals are taken, we think that the figures leave no doubt in the mind that we are dealing with the same factor in the rat and the pigeon.

SUMMARY.

Parallel tests upon pigeons and rats by a curative and protective method with torulin (vitamin B_1) preparations of different degrees of purity indicate that the B_1 factor for the two animals is identical, and show that the dose of vitamin B_1 required by each animal is approximately the same.

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