LIX. THE RELATION OF VITAMIN B_1 TO THE GROWTH-PROMOTING FACTOR[1] FOR A *STREPTOTHRIX*.

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In this paper it is proposed to consider together the relevant facts from the two parallel researches of the preceding papers. A preliminary account of this work has already appeared elsewhere [see Peskett, 1926]. Though the work is clearly related to the bios problem [see the review by Tanner, 1925, and Eddy, Kerr and Williams, 1924], it is probable that a different substance is involved in these effects. The most extensive investigation of the relation between the water-soluble vitamin and bacterial growth factors is that by Hosoya and Kuroya [1923]. They concluded that bacteria could be divided into two main groups. In one group, containing the *Streptococcus haemolyticus* and *Pneumococcus*, an unknown water-soluble substance was absolutely necessary for growth in a synthetic medium. For the other, which included most bacteria tested (including the *Meningococcus*), addition of the growth factor was not absolutely necessary. The purest sample of antineuritic vitamin used by these workers was Tsukiye's vitamin B (from rice), which from their figures would appear to have had an activity in our sense of about 1·0 mg. per day. It was shown that the activity of the preparation in curing pigeons (vitamin B_1 effect) was readily lost by the preparations upon heating to 140° for 2 hours in neutral solution. On the other hand, the yeast growth stimulant in the preparation, bios, withstood a similar temperature in *N* NaOH solution. The *Streptococcus* factor occupied an intermediate position. It was destroyed by heating for 2 hours in *N*/40 NaOH solution. Among the other interesting points, it was found that a strain of *Meningococcus* could be grown upon Fraenkel's solution containing glucose and *d*-glutamic acid or *l*-histidine hydrochloride; this medium did not contain the growth factor for *Streptococcus*. Further, it was shown that a strain of *B. coli* which did not need this growth-promoting factor could synthesise it; an alcoholic extract of *B. coli* was capable of stimulating the growth of *Streptococcus*. *B. coli* grown upon a vitamin-free medium contained enough vitamin B_1 to cure pigeons, therefore the organism could synthesise vitamin B_1. This latter result was contrary to that of Braddon and Cooper [1914].

[1] The nomenclature recently recommended by the Accessory Food Factors Committee of the Medical Research Council (1928) is adopted throughout.

The results communicated here are concerned with an intensive study by parallel tests of two factors, the vitamin B_1 curative for pigeons, and a bacterial growth stimulant for *Streptothrix corallinus* [Reader, 1926].

Experimental.

The organism studied has been grown upon a synthetic medium with addition of vitamin concentrates, previously standardised upon the pigeon, and the methods of testing are fully described in the previous papers. It must be emphasised that the growth obtained upon this medium by the addition of the best vitamin B_1 concentrates (with an addition of as little as 4×10^{-5} mg. %) has been quite as vigorous as that upon undiluted broth medium.

Table I shows that, after a certain stage in the fractionation is reached, treatment with alkali removes the curative property but does not depress the growth-promoting effect for *S. corallinus*. At an earlier stage the factor for *S. corallinus* is less stable to alkali.

Table I. *Action of alkali.*

Exp.	Material	Activity* in mg.	Percentage decrease in activity: *Streptothrix* %	Percentage decrease in activity: Pigeon %	Normality NaOH	Treatment and time heated at 100° mins.
1	Filtrate from Hg	50·0	100	—	0·1	60
2	Stock H	3·0	60	80	0·1	60 (oxygen passing)
3	„	3·0	Nil	Nil	0·1	10 („ „)
4	XXV	0·15–0·20	Nil	—	0·1	10 („ „)
5	„	0·15–0·20	Nil	50	0·1	10 („ „) (marmite added)
6	XXX–XXXIII	0·09	Nil	100	0·5	60

* Activity = daily dose in mg. per pigeon.

It will be noticed that 0·1 *N* NaOH is on the whole not destructive to vitamin B_1 in 10 minutes in the active preparation. In 1 hour complete inactivation takes place, whereas the growth factor is left untouched unless certain impurities are present.

The most active preparation tested (0·06 mg. curative) was not inactivated for *S. corallinus* by extensive alkali treatment.

These experiments definitely prove that the curative factor for the pigeon is not identical in all respects with the growth-promoting factor for *S. corallinus*. It is, however, interesting to note that the most active vitamin B_1 preparations still promote growth of *S. corallinus*. This effect is also quantitatively the same per pigeon dose as that of the preparations which are 100 or more times less pure. This suggests, as has occurred to others, that the same "nucleus" might be involved in each case, some slight change making the factor unavailable for the pigeon, though not for the bacillus. Admittedly, the only final evidence upon this point can be obtained by way of the pure substances. We have, however, tried to obtain indirect evidence by following step by step in the fractionations the two activities. In order to save space

we shall content ourselves with giving certain illustrative examples in the accompanying table (Table II), confining these to different types of fractionation.

Table II.

A. Silver precipitation from final alcohol fractionation (0·15 mg.).

Experiment	Total dosage S.T.	Total dosage P.T.
1. Original	1400	1400
2. 1st Ag ppt.	350	350
3. 2nd Ag ppt.	250	160
4. Filtrate	900	630
5. 1st Ag reppt. from (2)	200	350
6. Filtrate from (5)	100	

B. H_2PtCl_6 fractionation (XXX–XXXIII)

1. 1st H_2PtCl_6 ppt.	400–500	678
2. Filtrate	250	200
3. Reppt. H_2PtCl_6	200	140
4. Filtrate	50	—

C. Alcohol fractionation.

1. Original	600–800	600–800
2. 75 % alc. ppt.	4	Nil
3. Filtrate from (2)	600	600
4. 90 % alc. ppt.	550	550
5. Filtrate	24	Nil

Table II shows how closely the distribution of the two factors is followed in the fractions. Three cases of discrepancy have come to light. The so-called "anomalous" case has been discussed in the preceding communication. After a certain stage in the purification, it does not occur. The case of alkali has received mention in Table I. In certain cases of treatment with phosphotungstic acid, it was occasionally found that activity for the bacillus could be regenerated without a corresponding activity for the pigeon. This seems to be a parallel to the case of alkali previously mentioned, as the reverse has never occurred. We have attributed it to the combined action of alkali and phosphotungstic acid. Apart from these two results, not more than 10 % among the 133 parallel tests carried out during the last 18 months have been outside the limits of the experimental error of the test. These we cannot explain. We have excluded from this computation tests which gave "anomalous" results and those in which there was evidence of the toxic action of metals. There have also not been included a number of tests made upon material at intermediate stages of fractionation, which form a valuable body of indirect evidence as to the close parallelism of the two factors. Quite often it has been possible to predict the distribution of vitamin activity by the aid of the growth test. So if alkali treatment is avoided, the *S. corallinus* test has value in testing for vitamin B_1 after a certain stage. We feel that this is the strongest evidence which can be produced at present as to the close similarity of the two factors.

Discussion.

Our results confirm those of the Japanese workers in the following respects. A yeast vitamin B_1 preparation, just as their rice vitamin B_1 preparation, has bacterial growth-promoting power upon a *Streptothrix*; this organism therefore behaves similarly to their *Streptococcus haemolyticus*. The vitamin B_1 effect is readily destroyed by alkali, whereas the bacterial factor is less sensitive. In this respect the results differ, because our preparations which reached an activity considerably higher than those of the Japanese workers could be heated for 1 hour at 100° in $N/2$ NaOH solution without losing their growth-promoting effect.

It is interesting at this stage to combine the various data as to the action of what may be termed the "charcoal" concentrates. In addition to the facts presented in this paper, certain preliminary work has already been published by Peskett [1926]. He showed that the fraction of activity 0·08 mg. had bios activity. This remained upon heating with alkali, an operation which largely destroyed the vitamin B_1 effect. Further evidence as to the duality of bios and vitamin B_1 was obtained by Hawking [1927] and Peskett [1927]. *Saccharomyces cerevisiae* can synthesise vitamin B_1 from a medium containing small amounts of bios. In so far as the thermostable factor for rat growth, vitamin B_2, is not present in the charcoal concentrates, neither bios nor the *S. corallinus* factor can be identical with vitamin B_2.

Mention may also be made of the work of Heaton [1926], who has shown that the same concentrate of activity 0·08 mg. has growth-promoting effect upon epithelial tissue (in tissue cultures). Hence the thermostable vitamin B_2 does not seem to be required even for this growth, which is puzzling in view of its relation to the pellagra-like condition in rats.

In view of the results obtained by the Japanese workers it is interesting to note that two of us found that a strain of *Meningococcus* would grow upon a broth medium which had been completely freed from the *Streptothrix* factor and from vitamin B_1 by treatment with charcoal. Doubtless this depended upon the presence of one of the amino-acids mentioned by them. However, it was possible to show in extension of their results for *B. coli* that, after the growth of *Meningococcus*, there was present in the medium a growth-promoting factor for *S. corallinus*. Unfortunately, owing to the toxicity of the medium after the growth of *Meningococcus*, it has not been possible to show whether vitamin B_1 for the pigeon is also present. The facts for this organism are therefore also in accordance with the theory that those organisms which can grow upon a medium free from the factor for *S. corallinus* are able to synthesise it. It is clear that there is complexity among the lower organisms as regards their requirements in these factors [see Mueller, 1922], and that much work must be done before such a theory can be made general.

The work upon the relation of the curative factor in the charcoal concentrates to other factors is summarised in Table III.

Table III.

Stage of concentration	Activity mg.	Vitamin B: Curative B_1	Vitamin B: Thermostable* B_2	Bios† yeast growth-promoting	*Streptothrix* growth-promoting	Epithelial tissue‡ growth-promoting
Aqueous yeast extract	50 approx.	+	+	+	+§	+
Charcoal concentrates	0·5–1·0	+	Nil	+	+	+
Ag-baryta (previous exps.)	0·08	+	—	+	+	+
Phosphotungstic acid	0·1	+	—	—	+	—
H_2PtCl_6 in alcohol	0·027–0·06	+	—	—	+	—
Treatment with alkali	0·027–0·06	Nil	—	—	+	—
Treatment with alkali	0·08	Nil	—	+	+	—

* From Chick and Roscoe [1927].
† From Peskett [1926].
‡ From Heaton [1926].
§ Anomalous.
\+ Present.
— Not tested.

Conclusions.

1. Parallel tests upon the pigeon and *Streptothrix corallinus* have been made during the course of numerous fractionations of yeast concentrates.

2. Using the pigeon dose as a standard, the most active vitamin B_1 preparations (0·027 mg.) have the same relative growth-promoting powers as preparations 100 times less pure.

3. Sufficient treatment with alkali always inactivates the curative properties, but the purer extracts after such treatment still retain growth-promoting activity. From this it is concluded that vitamin B_1 and the bacterial growth-promoting factor are not identical.

4. Similarity in constitution is suggested by the fact that during varying types of fractionation (alcoholic, metallic precipitation, etc.) the two properties fractionate in parallel.

5. Since the *Streptothrix* factor is present in the charcoal concentrates, it is not identical with vitamin B_2.

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Note by R. A. Peters.

In a paper upon the vitamin requirements of certain yeasts and bacteria, Funk and Dubin [1921] make the following statement: "Peters believed at first that protozoa can live and divide on purely inorganic material, but he found subsequently that they become smaller and smaller and appeared to live at the expense of their own protoplasm: he thinks therefore that addition of vitamins is necessary for proper growth." I do not know upon what statement of mine this is based, and cannot find any words in my published work upon the question which could be interpreted to mean this. The matter is at present under investigation.

REFERENCES.

Braddon and Cooper (1914). *J. Hyg.* **14**, 331.
Chick and Roscoe (1927). *Biochem. J.* **21**, 698.
Eddy, Kerr and Williams (1924). *J. Amer. Chem. Soc.* **46**, 2846.
Funk and Dubin (1921). *J. Biol. Chem.* **48**, 437.
Hawking (1927). *Biochem. J.* **21**, 728.
Heaton (1926). *J. Path. Bact.* **29**, 293.
Hosoya and Kuroya (1923). *Imp. Govt. Inst. Infect. Dis. Tokyo Scientific Reports*, **2**, 233.
Mueller (1922). *J. Bact.* **7**, 309.
Peskett (1926). *Rep. Brit. Assoc.* 397.
—— (1927). *Biochem. J.* **21**, 1102.
Reader (1926). *J. Path. Bact.* **29**, 1.
Tanner (1925). *Chem. Rev.* **1**, 397.