aspects of the deficiency, namely, the high mortality of the newly born[1]; (*b*) in our purified casein, which seems to be solely responsible for the lesions observed, the orotic acid content is much decreased[7].

We have therefore now investigated the influence of orotic acid upon the body-weight curve of rats lacking the animal protein factor.

Rats of the Sprague–Dawley strain, devoid of the animal protein factor, being the sole survivors of the first generation, were fed from weaning (21 days of age) on our deficient diet, which was supplemented with 1,000γ of orotic acid (manufactured by Biochemical Inc., Co., Cleveland, Ohio) per 100 gm. of food. The results obtained are shown in Fig. 1.

The body-weight values for rats fed on a normal Randoin and Causeret diet containing crude casein, and supplemented with 1,000γ orotic acid per 100 gm. of diet, are not included, since orotic acid failed to show any influence in these conditions.

From the above results, it appears evident that orotic acid may be one of the growth-factors for rats deficient in animal protein factors, but not for normal rats. This suggests a possible relationship between orotic acid and the animal protein factors of casein.

Further work is in progress in our laboratory in order to ascertain the role of orotic acid in the numerous lesions induced in rats by the deficiency of casein animal protein factors.

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## A New Method for the Microbiological Assay of Thiamin (Vitamin $B_1$)

The purpose of this communication is to present a few experimental results obtained during the course of some investigations carried out to determine the nutritional requirements of a fungus, *Tuberculina persicina* (Ditm.) Sacc., isolated in 1949 by Dr. B. d'Oliveira from æcidia of *Puccinia rubigo-vera tritici*, and since maintained in the fungus collection of the Plant Pathology Department of Estação Agronómica Nacional, Sacavém, Portugal. These results seemed to be of special significance, and may eventually lead to the development of a new method of bio-assay of thiamin (vitamin $B_1$).

Several vitamins were tested as growth-promoting factors for *T. persicina*, namely, thiamin, biotin, riboflavin, pyridoxin, nicotinic acid, inositol and pantothenic acid (this latter in the form of its calcium salt). The vitamins were added aseptically to a synthetic basal medium (autoclaved for 20 min. at 107° C.) of the following composition: $KH_2PO_4$, 1·5 gm.; $MgSO_4.7H_2O$, 0·5 gm.; $KNO_3$, 2·0 gm.; dextrose ('Difco'), 15·0 gm.; agar ('Difco'), 15·0 gm.; distilled water, 1,000·0 ml. The final *p*H was adjusted to 5·2. Purified agar (according to Robbins and Ma's technique[1]) was consistently used throughout the experiments to avoid possible sources of error. After incubation for twenty days at 20° C., it was noticed that the plates to which thiamin had been added not only showed good growth of the fungus colony but also displayed an unusual pigmentation of the medium, whereas those lacking this factor remained colourless, even when all the others were present.

In further experiments, devised to ascertain the optimum levels of thiamin concentration for pigment production, liquid cultures were used preferentially; series of flasks were thus prepared by adding various amounts of vitamin $B_1$, within the range $5 \times 10^{-4}$ γ/l. to $5 \times 10^{4}$ γ/l. to the basal medium without agar. The cultures were incubated for thirty days, at the end of which time very striking differences in the coloration of the liquids were observed. When measured spectrophotometrically, these differences seemed to be directly related to the amount of vitamin $B_1$ present in the original culture medium. There was almost no pigment formation in the liquids of the control flasks, nor in those in which the thiamin concentration was lower than $5 \times 10^{-3}$ γ/l. Above $5 \times 10^{2}$ γ/l. of thiamin, there was a marked decrease in pigment formation, as may be seen from Table 1.

Table 1

| Thiamin concentration (γ/l.) | Optical density at λ = 3700 A. | Thiamin concentration (γ/l.) | Optical density at λ = 3700 A. |
|---|---|---|---|
| 0 | 0·083 | 5 | 0·900 |
| $5 \times 10^{-4}$ | 0·083 | $5 \times 10$ | 1·300 |
| $5 \times 10^{-3}$ | 0·082 | $5 \times 10^{2}$ | 0·280* |
| $5 \times 10^{-2}$ | 0·203 | $5 \times 10^{3}$ | 0·250* |
| $5 \times 10^{-1}$ | 0·430 | $5 \times 10^{4}$ | 0·178* |

* Culture medium diluted to 1 : 10 to permit a good reading.

Further experiments are being carried out to determine the influence of the different sources of carbon and nitrogen on pigment production by *T. persicina* in the presence of thiamin. Results will be discussed in a future publication.

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## The Duplication Mechanism of Deoxyribonucleic Acid

Recent investigations have shown it to be more and more probable that genes are deoxyribonucleic acid molecules. Furthermore, Watson and Crick[1] have given strong arguments for assuming that these molecules consist of two polynucleotide chains wound helically in a large number of turns around a common axis and tied together by hydrogen bonds between pairs of corresponding purines and pyrimidines, so that adenine can pair only with thymine and guanine only with cytosine. In order to explain the duplication process of the deoxyribonucleic acid molecule, Watson and Crick have suggested that the two polynucleotide chains may be separated and each chain catalyses