

the reduction of absorption at 6700 Å. U. it seems that the change at this wavelength will be smaller the greater the amount of trimethyl thionine present as an impurity. With a mixture of 10% trimethyl thionine and 90% methylene blue the change at 6700 Å. U. ought to be about 21% instead of 22%. In one experiment of this type no definite difference was discovered; however, this was not surprising as the difference of 1% is of the order of magnitude of the experimental error. It seems in any case that the presence of trimethyl thionine should reduce the sensitivity of the methylene blue solution to a certain extent though not enough to account for all the difference we have noticed with solutions made up at different times or from different samples of methylene blue.

### 8151 C

#### Requirement of the Flour Beetle (*Tribolium confusum* Duval) for Vitamins in the B Group.

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Insects offer certain possibilities for vitamin assay. If the vitamin requirements of certain insects were known it should be possible to assay vitamins with much less time and labor than with rats or similar animals. A suitable insect for nutritional research is the flour beetle, *Tribolium confusum* Duval.

When raised on whole wheat flour at 32° and 70% humidity, the larvae transform to the pupal stage in an average time of 16 days. Sweetman and Palmer<sup>1</sup> found that on a diet of purified food materials devoid of all vitamins the insects did not pupate. The addition of a small amount of material containing the vitamin B complex enabled the larvae to pupate in a normal time.

The renewed interest in methods for assay of the vitamins in B group brought about by the recent advances in the chemistry of these substances prompts us to report briefly experiments conducted by us in 1931-32. In this study an attempt was made to determine which members of the vitamin B complex are necessary for *Tribolium*. A basal diet of casein 15, crisco 3, Osborne-Mendel salt mixture 4, dextrin to 100 was used. Various vitamin supplements

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<sup>1</sup> Sweetman, M. D., and Palmer, L. S., *J. Biol. Chem.*, 1928, **77**, 33.

were added to 5 gm. quantities of the basal diet, and the food was placed in a vial with 20 two-hour-old larvae. The vials were kept in a temperature cabinet at 32°C. and 70% humidity and the average time of pupation recorded. A vitamin B<sub>1</sub> concentrate prepared from rice polishings was used. The average time required for pupation with the various supplements to the basal diet were: vitamin B<sub>1</sub> concentrate equivalent to 50% rice polish no pupation, 5% autoclaved yeast no pupation, 1% autoclaved yeast plus vitamin B<sub>1</sub> concentrate equivalent to 2% rice polish 19.2 days. This indicates that *Tribolium* requires a heat stable factor in addition to vitamin B<sub>1</sub>. It was found that this heat stable factor was destroyed by autoclaving at pH 13 for 4 hours.

With 1% autoclaved yeast to supply the heat stable factor the larvae responded in a quantitative manner to the addition of the vitamin B<sub>1</sub> contained in small amounts of rice polish as follows: with 0.1% rice polish average time of pupation was 20.3 days; 0.05% rice polish, 23.8 days; 0.025% rice polish, 40.2 days. With 1% rice polish as the sole addition to the basal diet the average time of pupation was 98 days.

## 8152 C

### Positive Flocculation Tests in Rabbits Inoculated with Flocculate from Human Syphilitic Serum.

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In an effort to determine the nature of flocculate obtained from human syphilitic serum, by means of flocculation tests for syphilis, an attempt was first made to produce precipitins in rabbits by intravenous inoculations with such flocculate. Attempts in dissolving the flocculate were at the time unsuccessful. A rabbit was therefore inoculated with flocculate suspended in sterile saline. After 5 intravenous injections the rabbit serum was tested for precipitin formation and found negative in various dilutions. On the other hand, the serum from that rabbit, when added to 2 plus positive syphilitic serum, changed the positiveness to a strong 4 plus reaction.

An attempt was then made to produce in rabbits, by inoculation