QUANTITATIVE CHANGES IN THE SUBSTRATE-DEHYDROGENASE SYSTEM OF DROSOPHILA PUPAE DURING METAMORPHOSIS

THE rate of oxygen consumption in the pupae of holometabolous insects during metamorphosis can be expressed by a U-shaped curve. It is first high, then drops rapidly, while in the second half of metamorphosis it gradually increases again, so that before hatching of the imago, the rate often reaches or even surpasses the initial value. This was also shown to be true for *Drosophila* pupae.[1,2,3,4,5] Various theories have been put forward to account for this phenomenon.[6,7] In 1938 I suggested that the fact may be due to quantitative changes in the amount or activity of the oxygen-transferring enzyme system (Warburg-Keilin system, *i.e.*, "Atmungsferment" and cytochromes). This possibility was inferred from the effect of carbon monoxide on the oxygen consumption of *Drosophila* pupae in different stages.[8] Recently Schwan,[9] although criticizing my considerations, reaches the same conclusion. (His criticism, which seems to be insufficiently founded, should be dealt with elsewhere.)

The question still remained, if simultaneously with the changes in the oxygen-transferring system, other catalysts of the oxidation mechanism, especially the substrate-dehydrogenase system (Wieland, Thunberg)[10] will show similar quantitative alterations. In order to clear up this point, experiments were undertaken with four different stages of *Drosophila melanogaster* pupae, using the Thunberg methylene blue technique.[10] The age of the pupae was 5 to 10 hours (stage I), 25 to 30 hours (stage II), 50 to 60 hours (stage III) and 75 to 85 hours (stage IV), respectively, when reared at constant 25° C. temperature. (At this temperature the duration of the whole metamorphosis is 90 to 100 hours.) The stages were selected according to Wolsky.[5]

Preliminary experiments showed that the best results are obtained with 0.02 per cent. methylene blue solution (1 per cent. trunk solution, diluted with physiological saline, pH 6.8). From this 0.2 ccm was brought together with ten pupae in a Thunberg vacuum tube. The pupae were crushed in the methylene blue, then the tubes evacuated and put in a water bath, which was kept at a constant temperature of $20 \pm 0.1°$ C. The time was noted at which complete decoloration of the methylene blue set in.

The results revealed great differences as regards amount or activity of the dehydrogenase system in the four stages. With stage I pupae the average time required for complete decoloration was 28.0 minutes ($\pm$ 2.6 standard error, 7 experiments). In stage II the average was 67.4 min. ($\pm$ 7.2, 9 experiments), in stage III 66.1 min. ($\pm$ 6.3, 7 experiments) and finally in stage IV 25.6 min. ($\pm$ 2.4, 8 experiments). As these figures show, the differences between stage I and II, *i.e.*, 39.4 min. ($\pm$ 7.7 standard error) and between stages III and IV (40.5 ± 6.7), are statistically significant. It is interesting to note that in stages II and III the decoloration time is more than twice the time observed in stages I and IV. The data for oxygen consumption are, according to Wolsky,[5] 4.66 cmm per hour per 1 mg dry weight in stage I, 2.23 cmm in stage II, 2.32 cmm in stage III and 5.05 cmm in stage IV. These data, when compared with the results of the decoloration experiments, show a striking similarity as regards the magnitude of differences. Thus the graphic representation of the results of the decoloration experiments gives a U-shaped curve, which is very similar to that obtained for oxygen consumption during metamorphosis (see Fig. 1).

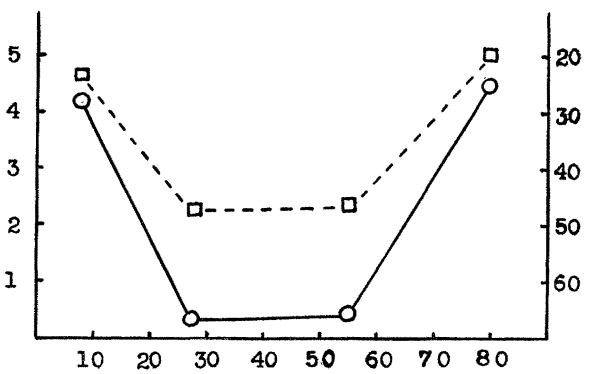

FIG. 1. Graph showing changes in oxygen consumption (squares, connected with broken line) and in substrate-dehydrogenase activity (circles, connected with full line) during metamorphosis. Abscissa: pupal age in hours. Ordinate (left): oxygen consumption in cmm per hour, per 1 mg dry weight. Ordinate (right): time in minutes, necessary to decolorize 0.2 ccm 0.02 per cent. methylene blue, containing 10 pupae.

The results can not be due to exhaustion of hydrogen-donator substances in the pupae in stages II and III. The addition of potassium succinate (0.4 per cent.), as extra donator, to the reaction mixture does not alter the results, and the differences between the four stages

[1] J. H. Bodine and P. R. Orr, *Biol. Bull. Woods Hole*, 48: 1, 1925.
[2] M. R. Clare, *Biol. Bull. Woods Hole*, 49: 440, 1925.
[3] D. F. Poulson, *Zeits. vergl. Physiol.*, 22: 466, 1935.
[4] Th. Dobzhansky and D. F. Poulson, *Zeits. vergl. Physiol.*, 22: 473, 1935.
[5] A. Wolsky, *Jour. Exp. Biol.*, 15: 225, 1938.
[6] D. M. Needham, *Biol. Rev.*, 4: 305, 1929.
[7] V. B. Wigglesworth, "Insect Physiology." London: Methuen, 1934.
[8] A. Wolsky, *Jour. Exp. Biol.*, 15: 232–233, 1938.
[9] H. Schwan, *Ark. Zool.*, 32: 1, 1940.
[10] T. Thunberg, *Quart. Rev. Biol.*, 5: 318, 1930.

remain unchanged. This means that the dehydrogenase system is saturated with substrate during the whole period of metamorphosis. From the experiments reported here, it is clear that the substrate dehydrogenase system of *Drosophila* pupae undergoes quantitative changes during metamorphosis, which run parallel with those observed earlier in the oxygen-transferring system and which are manifested in the oxygen consumption of the pupae in different stages.

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OZONIZATION OF o-XYLENE AND 1,2,4-TRIMETHYLBENZENE[1]

LEVINE and Cole[2] found that *o*-xylene on ozonization affords products evidently arising from both of the two possible Kekulé forms of the hydrocarbon, but they presented no data concerning the yields of the three substances which they isolated. A reinvestigation of this reaction in our laboratory from the analytical point of view has been completed and will be described in detail in a paper which is being prepared for publication in the *Recueil des Travaux Chimiques des Pays-Bas*. As noted in a preliminary report of some of the experiments,[3] our method of following the course of the reaction consists in converting the products of ozonization into the oximes and determining the composition of the oxime mixture by a special analytical method.

If each of the two Kekulé forms contributes 50 per cent. to the structure of *o*-xylene, there should be formed 1 mole of dimethylglyoxal, 2 moles of methylglyoxal and 3 moles of glyoxal from 2 moles of *o*-xylene. We have transformed these decomposition products into the corresponding oximes and obtained the total oxime mixture in yields of from 20 to 25 per cent. of the theoretical amount calculated on *o*-xylene. The above theoretical ratio of the free carbonyl compounds would correspond to an oxime mixture of the following composition: dimethylglyoxime, 20 per cent.; methylglyoxime, 35 per cent.; glyoxime, 44 per cent. As a mean of six ozonization experiments, we found the ratio: dimethylglyoxime, 20.7 per cent.; methylglyoxal, 34.2 per cent.; glyoxime, 44 per cent. The accordance with the theoretical values seems better than it actually is, because the separate experiments show deviations of from 3 to 7 per cent. from the theoretical values. Considering the experimental difficulties, the accordance between experiment and theory is satisfying.

We have also investigated the ozonization of 1,2,4-trimethylbenzene. In this case, if the two resonating Kekulé forms each contribute 50 per cent. to the structure of the hydrocarbon, 2 moles of 1,2,4-trimethylbenzene should provide 1 mole of dimethylglyoxal, 4 moles of methylglyoxal and 1 mole of glyoxal, and the composition of the mixture of oximes should be: dimethylglyoxime, 18.9 per cent.; methylglyoxime, 66.7 per cent.; glyoxime, 14.4 per cent. As a mean of two ozonization experiments, we found the following percentages: dimethylglyoxime, 17.9 per cent.; methylglyoxime, 66.2 per cent.; glyoxime, 14.2 per cent. The accordance with the theoretical ratio is very good. In this case the quantity of oximes recovered amounted to 15 per cent. of the theoretical yield.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PRESERVATION OF BIOLOGICAL SPECIMENS WITH ISOBUTYL METHACRYLATE POLYMER

DURING the last few years several articles have been published describing various methods of preserving biological material by the methacrylate resins. Dr. J. H. Hibben[1] described a method of allowing the plastic to polymerize around the object to be preserved. Dr. H. G. Knight[2] called attention to the expense and difficulties of this method and Professor E. C. Cole[3] mentioned the possibility of imbedding objects in a solution of methyl methacrylate polymer dissolved in chloroform, but stated that he did not get satisfactory results.

Some months ago while attempting to preserve the color patterns of *Chorthippus longicornis* for genetic studies, the writer tried dipping the grasshoppers in a solution of isobutyl methacrylate polymer dissolved in toluene. The grasshoppers were first injected with various preservatives, pinned and then dipped in a solution containing 10 gm of the polymer to 100 cc of toluene, and allowed to dry. By repeated dippings

[1] This communication is constructed from data sent to me by Professor J. P. Wibaut in a letter of February 24, 1941, with the request that I arrange for its publication in SCIENCE. Professor Wibaut states, "I would appreciate very much if our results could be made available to American scientists in this way, as it may take some time before our complete paper will be published and even then it may not be available to the chemists in your country."—L. F. Fieser, Harvard University.

[2] A. A. Levine and A. G. Cole, *Jour. Am. Chem. Soc.*, 54: 338, 1932.

[3] J. P. Wibaut and P. W. Haayman, *Nature*, 144: 290, 1939.

[1] J. H. Hibben, SCIENCE, 86: 247–248, 1937.

[2] H. G. Knight, SCIENCE, 86: 333–334, 1937.

[3] E. C. Cole, SCIENCE, 87: 396–398, 1938.