presickle stage. Rouleaux formation was uniformly absent in those cases showing sickling.

It has been established that sickling of susceptible erythrocytes will take place only when the hemoglobin in the cells is in the reduced state (*6, 7, 10*), and the reduction of hemoglobin, however brought about, is the basis underlying all tests for sickling. In the commonly used sealed preparations (*1, 5, 9*), the metabolism of the nucleated blood cells slowly effects the reduction and produces sickling. In the bacteriological test (*11*) the high metabolism of the bacteria brings about more rapid reduction. The use of such agents as BAL, ascorbic acid, etc., depends upon their active reducing properties.

Carbon dioxide, in the technique described, plays a dual role in bringing about hemoglobin reduction. The gas displaces a large part of the air in the flask, thus producing a relatively oxygen-poor environment. In addition, the acid effect of the carbonic acid formed by the reaction of the gas with the blood decreases the affinity of the hemoglobin for oxygen (*2*).

There is little doubt that partial reoxygenation occurs during the transfer of the drop of blood from the flask to the slide. The percentage of cells that are sickled in the final preparation is, therefore, probably less than in the "flask blood" before transfer. It is, of course, possible to prevent this change by fixation of the cells in the flask by the addition of a saline formalin solution before taking a drop of blood for the preparation of the slide. This refinement was not felt to be needed in this test, however, because sickling is easily demonstrated without this step and because a qualitative rather than a quantitative result is all that is necessary.

The rapidity with which sickling can be demonstrated with the carbon dioxide test makes this method of great use in establishing an immediate diagnosis of sickle cell disease or in ruling it out, particularly in emergency situations. A negative reaction conclusively eliminates the possibility of sickle cell disease, whereas a positive reaction indicates that sickle cell disease is to be considered in the differential diagnosis. The simplicity of the test makes the procedure routinely practicable.

The dependability of the test is demonstrated by the absence of false positive and false negative reactions in the series of cases studied.

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To What Extent Is Oxygen Uptake of the Intact Embryo Related to That of Its Homogenate?[1]

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Recently, much attention has been given to analyses of the functions of constituent parts of living tissues, using homogenate and centrifuge techniques. That minute parts of cells can be isolated and concentrated rather easily was demonstrated clearly by Bensley and Hoerr (*1*). Further improvements in techniques and especially the use of high speed centrifugation have made possible detailed quantitative studies on the composition and functions of constituent parts of the living cell (*3–5*). Most of these investigations, however, have dealt largely with vertebrate tissues, and few if any seem to have been concerned with intact organisms and their homogenates. Considerable data are now available on the developmental history of the embryo of the grasshopper *Melanoplus differentialis*. It seemed of interest, therefore, to use such invertebrate material in further studying functions of cell constituents by homogenate and centrifuge techniques (*2*). Many features of this material make it especially desirable for such work. The embryos, free from yolk and in all stages of development, are easily obtained in large numbers (*2*). The egg is of a cleidoic type and hence is quite independent of the external environment for its food supply; it develops quite readily at room temperatures (25° C). Individual embryos, as well as morphologically and physiologically similar ones, are readily obtained, and from them homogenates are easily made. During the course of its development at 25° C, the embryo goes into a mitotically blocked or diapause state in which metabolic and other cellular activities reach a true basal rate (*2*). After removal of this developmental block, mitosis and other cellular activities are again resumed. A study has been made of the oxygen uptake of embryos, both intact and homogenized, and the results show rather striking properties of this material, as well as some differences from vertebrate tissues similarly treated.

Embryos of known age and temperature history were dissected free of yolk, as previously pointed out (*2*). A phosphate buffered Ringer's solution (pH 6.8) was used as suspension medium. Oxygen determinations were carried out at 25° C in Warburg manometers, using respiration flasks of 5-ml capacity. Intact embryos (100), as well as homogenates made from others of the same group, were run simultaneously. A glass type of homogenizer, as described by Potter and Elvehjem (*6*), and powered by an electric motor, was employed.

[1] Aided by a grant from the National Institutes of Health. Acknowledgment is gratefully made to K. H. Lu and Etta Andrews for technical assistance in carrying out these experiments.

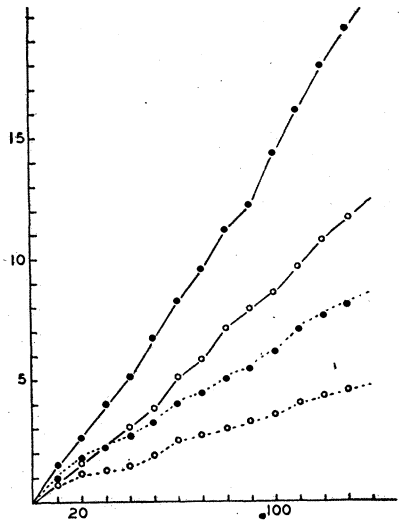

FIG. 1. Oxygen uptake of active and blocked intact embryos and homogenates; ordinates, mm³ O_2/100 embryos or equivalent; abscissas, time in min. Solid circles represent active embryos; open circles, blocked embryos; solid lines, intact embryos; broken lines, homogenates.

Results from many experiments seem similar, and in Fig. 1 are shown graphically typical curves for morphologically similar embryos and homogenates made from them. During diapause or block, oxygen uptake is always much lower than for active, nonblocked embryos. As a matter of fact, one judges the degree of block by the extent to which oxygen uptake is lowered in comparison with that of actively developing embryos. An inspection of Fig. 1 shows that oxygen uptake of intact embryos is always higher than that of the homogenates made from them. Oxygen uptake of homogenates for blocked embryos is correspondingly lower than that for homogenates of the active ones. The relative difference in oxygen uptake of embryos and homogenates, both for the active and blocked conditions, is surprisingly constant and has been found consistent in all experiments thus far carried out. Approximately 65% of the total respiration in both active and blocked cells is due to what one might term "physical structure or intactness," whereas approximately 35% is due to the basic chemical components of the system. A striking point is that a more or less linear relation between oxygen uptake and time for both intact embryos and homogenates is found (see Fig. 1). Dilution of the homogenates up to twenty times gives a straight line over a 2-hr period, showing that oxygen uptake is proportional to cellular concentration (Fig. 2).

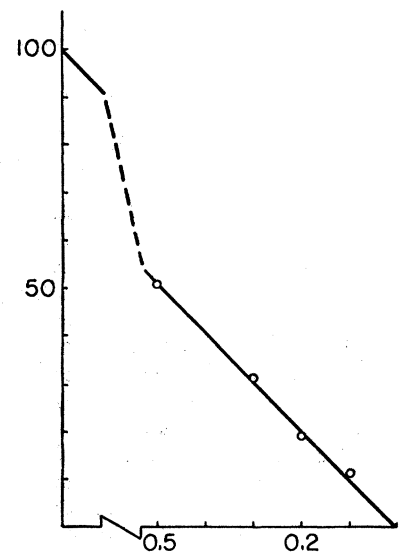

FIG. 2. Effect of dilution on oxygen uptake rate of homogenates, using original concentration (100 embryos/ml) as 100%; ordinates, % of normal oxygen uptake rate; abscissas, dilution of original homogenate.

Inasmuch as homogenates of active and blocked embryos show characteristic oxygen uptake, such systems when further analyzed should show physicochemical differences in the two physiological states of the cell. Further data on these points will be presented elsewhere.

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Effect of Zinc Deficiency on the Synthesis of Tryptophan by *Neurospora* Extracts[1]

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It has been reported that zinc is required directly for the synthesis of tryptophan and indirectly for the synthesis of auxins in tomato plants (*2*, *5*). It has also been demonstrated that tryptophan can be synthesized from indole and serine by wild-type *Neurospora* (*4*), as well as by cell-free extracts of the same organism (*6*). The present study with *Neurospora* indicates that cell-free extracts of zinc-deficient mycelia affect unfavorably the formation of tryptophan from indole and serine.

Cell-free enzyme extracts were prepared from the mats of *Neurospora crassa* (5297A) grown for 5 days in Fries basal medium, and from those grown on the same medium lacking zinc. The growth of the latter was $\frac{1}{3}$–$\frac{1}{2}$ that of the controls. The mycelial mats were washed with triple-distilled water, frozen, homogenized in three times their weight of 0.1M phosphate buffer at pH 7.5 and centrifuged. Five-tenths ml of the supernatant (10 mg dry

[1] Contribution No. 2 of The McCollum-Pratt Institute.