

culture was submitted to vigorous shaking for 30 min., after which it was maintained for 24 hr. at 37° C. Such cultures can easily be transplanted continuously in liquid hypertonic subculture as a normal bacterial suspension. Fig. 1 shows the increase of dry substance plotted against time for one of our cultures suspended in a hypertonic medium, and for a Tulasne culture (films)<sup>2</sup>. The phase of rapid growth in our culture occurs between 18 and 48 hr., whereas in the film cultures the growth takes much longer and reaches its maximum on the fifth day. As regards the morphological aspect, our cultures in hypertonic liquid medium contained large, swollen bodies, 8–12 $\mu$  in size, as well as small and elementary bodies described by many authors. We have not been able to ascertain the presence of large bodies (20 $\mu$ ) which existed in the original cultures.

The different morphological forms of our cultures were separated by differential centrifugation followed by washings with 0.3 M saccharose solution (except where washing with 9 per mille sodium chloride is specified—Fig. 2). The respiration of the different fractions was studied in a Krebs medium with a Warburg apparatus, and the values have been referred to deoxyribonucleic acid (Fig. 3).

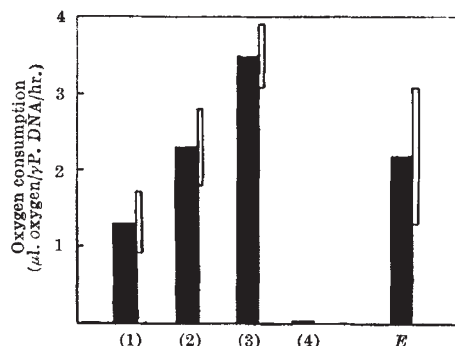


Fig. 3. Oxygen consumption of the various fractions of L forms. Values for large bodies (1), medium bodies (2), small bodies (3), elementary bodies (4) and total culture (E)

The results lead us to think that the small bodies of fraction 3, the metabolism of which seems to be most intense, correspond to a state of active proliferation. It appeared also that the oxygen consumption referred to deoxyribonucleic acid was practically nil for fraction 4 (elementary body). It seems to have lost its enzymatic apparatus for the aerobic degradation of glucose.

Supposing that the L forms are representative of a transition from one state to another—that of viruses—the elementary bodies would form a preliminary stage characterized by an already considerable reduction of the enzymatic apparatus of these microorganisms.

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## BIOLOGY

### Changes in Lymphocytes during Antibody Production

DURING the course of an investigation<sup>1</sup> into the function and life-cycle of lymphocytes, a number of physical techniques were employed to determine the identity of cells isolated from various sources, for example, lymph glands, thoracic duct, thymus, spleen, blood, etc. The average cytoplasmic refractive index of small lymphocytes obtained from the above sites had been determined with a fair degree of certainty. It was noted that in chronically infected rats (rat pneumonia) the small lymphocytes, whatever their source, showed a significantly raised cytoplasmic refractive index. The subcutaneous injection of a particulate antigen (T.A.B.) produced a similar rise preceding but paralleling the rise in the antibody titre of the serum and thoracic duct lymph. We suggest that the refractive index change in the cytoplasm of the small lymphocyte is due to increased protein concentration connected with the mechanism of production of antibodies. A detailed study of this phenomenon is in progress.

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<sup>1</sup> Keohane, K. W., and Metcalf, W. K., *Quart. J. Exp. Physiol.*, **43**, 408 (1958).

### Zone Electrophoresis of Rous Sarcoma Virus

THE value of zone electrophoresis in the study of animal viruses has been demonstrated by previous workers<sup>1,2</sup>. By this method new criteria for the classification of animal viruses can be obtained. In addition, the study of electrophoretic inhomogeneity of viruses is of special interest. Thus it could be shown for poliovirus type I that the virus activity is shared by at least two components with different electrophoretic mobilities<sup>2</sup>.

Zone electrophoresis might also be of value for the purification of viruses. In this report, preliminary results of electrophoresis of a cancer virus are given.

The supernatant of Rous sarcoma tissue cultures was chosen as a source of virus. Chicken tissue cultures were infected with Rous sarcoma virus and passaged serially by trypsinization for about two months until almost all cells showed the characteristics of Rous sarcoma cells and the titre of Rous virus in the supernatant was at least 5 log plaque-forming units per ml. The supernatants were then collected and treated by two cycles of centrifugation, one for 10 min. at 5,500g and a second for 60 min. at 54,000g. In some experiments, hyaluronidase in a final concentration of 7 units/ml. and trypsin in a final concentration of 0.025 per cent were allowed to act on the supernatant for 20 min. at 37° C. before centrifugation. The final pellet was taken up in 0.3 ml. veronal buffer pH 8.2 and mixed with an equal amount of 25 per cent cane sugar solution in buffer before introduction into the electrophoresis column. A detailed description of the electrophoresis apparatus and of the electrophoresis technique is given in previous papers<sup>1,2</sup>. Veronal buffer of pH 8.2 containing 47.6 gm. sodium diethylbarbiturate and 11.5 ml. N/6 hydrochloric acid in 4.3 l. of distilled water was used. Electrophoresis was allowed to proceed at room temperature for 3 hr., with a voltage gradient of 3.5 V./cm. and a current of 20 amp.