IN VITRO STUDIES OF RHEUMATOID SYNOVIUM. PRELIMINARY METABOLIC COMPARISON BETWEEN SYNOVIAL MEMBRANE AND VILLI.

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SOME of the earliest pathological changes which occur in rheumatoid arthritis are proliferative in nature. As the synovial membrane proliferates in response to the disease stimulus more villi are formed and give rise to the florid histological picture. In view of this we felt that the metabolic requirements of villi and membrane would differ quantitatively. We have compared the carbohydrate katabolism of these tissues, since this probably supplies the readily available energy for tissue proliferation.

We have also compared the *in vitro* effects of hydrocortisone (free alcohol) on the two types of tissue. This we felt to be of interest in view of the profound effects of the hormone when administered *in vivo*.

METHODS.

The tissue was obtained at operation from cases of rheumatoid arthritis undergoing synovectomy or arthrodesis.

Immediately after excision the synovial tissue was placed in ice-cold oxygenated Krebs Ringer phosphate solution (Umbreit, Burris and Stauffer, 1951). As these studies were to be made on a membrane and its villous processes, it was unnecessary to use the classical techniques of tissue slice or mince. A homogenate technique was unsuitable, since the intact cell appears essential for hydrocortisone action (Sutherland and Cori, 1951 ; Miller, 1954). Therefore the chilled tissue was dissected, to remove the membrane from the underlying tissue and to separate the villi if present. The free villi, or membrane strips (5 × 2 mm.), were then washed with fresh ice-cold oxygenated Krebs Ringer phosphate solution. By preparing the tissue as described and storing at 4° in the same oxygenated medium, with the addition of 1 mg./ml. glucose, it usually retained its activity for up to 36 hr. with no significant loss. This was especially true of the villi, whose large surface area allowed free gaseous interchange (Bywaters, 1937).

After re-suspension and thorough mixing, random samples were taken, quickly dried on filter paper, and placed in ice-cold Warburg flasks. The flasks contained 3·5 ml. of oxygenated Krebs Ringer phosphate, with appropriate amounts of substrate and hormone in solution. The hydrocortisone (free alcohol, Merck) was solubilised by the method of Hechter (Miller, 1954). After the addition of the tissue and immediately before incubation, 0·5 ml. of the suspending fluid was removed for zero time estimations on the various substrates, or metabolites, in each flask. The flasks were then attached to the manometers, placed in the bath at 37°, and gassed with 100 per cent oxygen for 10 min. After gassing, a further 10 min. equilibration was allowed. The flasks were then incubated for 1 hr., removed and chilled in an ice bath.

The tissue was separated from the supporting fluid by filtration through glass wool and washed with water. It was then placed in 30 per cent KOH and heated at 100° for 30 min. After solubilisation, aliquots were taken for estimation of total tissue nitrogen by the micro-Kjeldahl method (Hawk, Oser and Summerson, 1952) and glycogen by the method of Walaas and Walaas (1950). The protein in the filtrate was precipitated with zinc and barium, and aliquots taken for the estimation of glucose (Nelson, 1944) and lactic acid, (Barker and Summerson, 1941).

The results of the estimations, including manometric data, were expressed on a tissue nitrogen basis. This gave a more reliable figure than that obtained by the usual method of wet or dry weight reference. The tissue desoxyribonucleic acid content (Schmidt and Tann-

hauser, 1945) was estimated in some cases in an endeavour to assess the variation in cellular number. The results of the total tissue nitrogen paralleled those of the desoxyribonucleic acid values. As the nitrogen estimation was less laborious it was chosen as a standard of reference.

RESULTS.

The Table shows the rate of O_2 uptake (Q O_2 (N), *i.e.*, μl. O_2/mg. tissue N/hr.), glucose utilisation and lactic acid production by a number of different tissue samples. Considerable difference in all three values can be seen between the two types of tissue.

TABLE.—*Metabolic Activities of Rheumatoid Villi and Synovial Membrane* in vitro.

	Villi.	Membrane.
Q O_2 (N)	24·7 (25)	4·2 (30)
μM. glucose utilised	1·32 (9)	0·52 (10)
μM. lactic acid produced	0·71 (12)	0·11 (9)

Figures are mean values of numbers of observations given in brackets. All are expressed per mg. tissue nitrogen per hr.

In the absence of added substrate during incubation, villous tissue showed a marked decrease in endogenous glycogen. In the membrane the tissue glycogen was too small to allow accurate measurement and no comparison of the rates of utilisation could be made.

The action of hydrocortisone on the O_2 uptake of the tissue is shown in Fig. 1. In the presence of glucose the inhibitory effect of the hormone on both types of tissue is diminished.

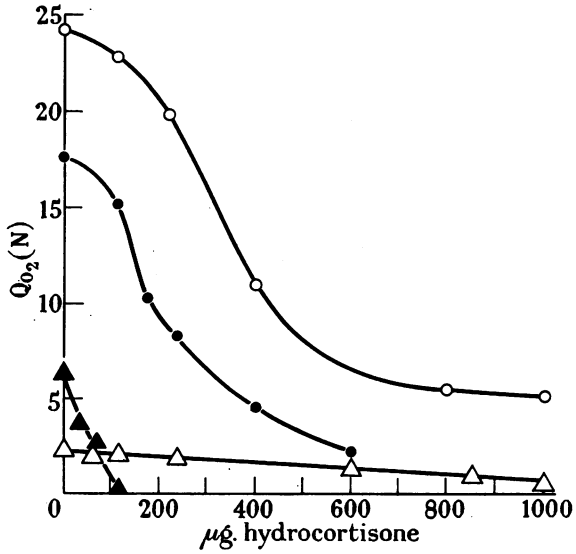

FIG. 1.—The inhibition of oxygen uptake of villi and membrane by hydrocortisone. ○ Villi with glucose substrate. ● Villi with no substrate. △ Membrane with glucose substrate. ▲ Membrane with no substrate.

Fig. 2 and 3 show the inhibition of glucose utilisation and lactate production by varying hormone concentrations.

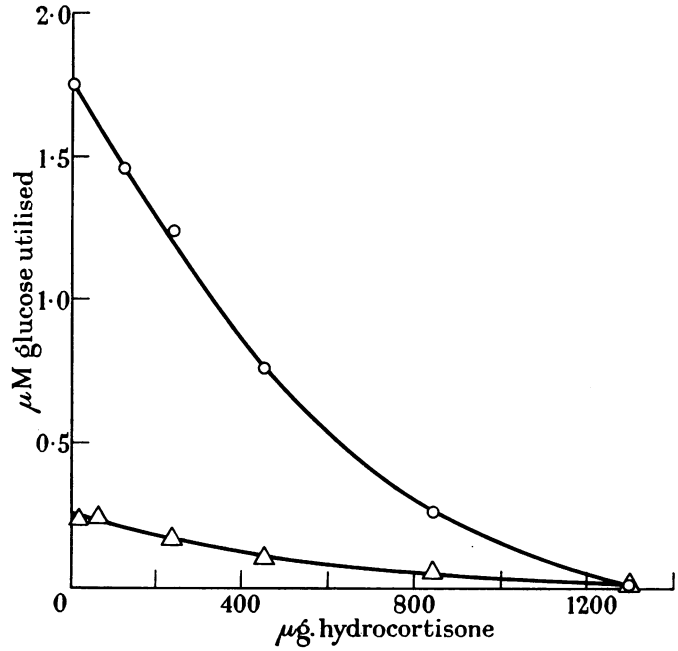

FIG. 2.—Inhibition of glucose utilisation by hydrocortisone. ○ Villi. △ Membrane.

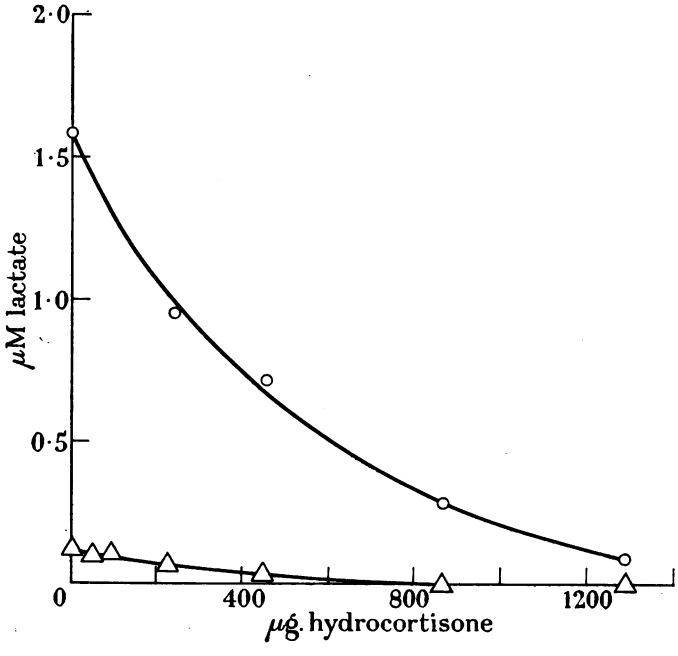

FIG. 3.—Inhibition of lactate production by hydrocortisone. ○ Villi. △ Membrane.

DISCUSSION.

The results show that villous tissue is more active metabolically than membrane. Thus the former has a higher O_2 consumption and utilises more glucose. It is interesting that the patient-to-patient variation in metabolic activity is relatively small when membrane and villous tissue are considered separately. Though these variations are large in comparison with those obtained from homogeneous normal tissues, it is unlikely that such uniformity would be obtained in view of the heterogeneity of the cellular components, and the different phases of the disease process. Experiments performed using a mince of the whole synovium showed wide variations between individual patients. These variations are presumably due mainly to different proportions of membranous and villous tissue.

It is of interest that Miller (1954), using rat thymus lymphocytes, found that hydrocortisone increased aerobic glycolysis and inhibited oxygen uptake. In view of the subsynovial aggregations of lymphocytes in rheumatoid arthritis, similar findings were a possibility. Using the same range of hormone concentrations we have been able to confirm the respiratory inhibition by hydrocortisone, but not an increase in aerobic glycolysis.

Further studies are in progress concerning the action of cortico-steroids on rheumatoid synovium *in vitro*, with special reference to the mechanism of glycolytic inhibition and possible effect on the tricarboxylic acid cycle.

SUMMARY.

A comparison of the *in vitro* metabolic activity of rheumatoid synovial membrane and villi has been made, with reference to oxygen uptake, glucose utilisation and lactate production. Under these conditions villi showed greater metabolic activity.

The *in vitro* effect of hydrocortisone on these tissues has been demonstrated as it affects carbohydrate katabolism. A similar response to the hormone was given by both types of tissue.

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