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Studies of Drugs in Tissue Metabolism. I. Method for Measuring Metabolism of Tissue in Serum.

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The action of compounds of pharmacological importance on the oxidative systems of tissue cells has received widespread attention, particularly through the work of Jowett and Quastel[1] and of Krahl and Clowes.[2] The usual procedure is to determine the metabolism of excised tissue suspended in a balanced salt solution. However, since it might be expected that the respiration of tissue would proceed more normally if the tissue were suspended in a naturally occurring physiological fluid, our attention was first directed to the development of a simple, reproducible method of measuring tissue respiration in serum. A description of this method provides the content of the present paper. Rat liver was chosen as the standard tissue whose respiration was measured, and human blood serum as the nutrient fluid medium in which the tissue was suspended.

Experimental. Indirect methods for determining the respiration of tissue in serum are already available.[3, 4] However, they have the disadvantage of being complicated and requiring more than the usual equipment for the determination of O_2 consumption by the direct Warburg technic.

The direct measurement of oxygen consumption of tissue in serum has been achieved by (a) alternately acidifying and evacuating serum until its CO_2 content has been reduced to about 0.5 mM per liter (2% of original value); (b) readjusting its pH to about 7.2-7.3; (c) using this CO_2-free serum as the fluid medium in which the tissue is immersed; (d) measuring the O_2 consumption of the tissue manometrically in the Erlenmeyer type Warburg chamber, with absorption of CO_2 by alkali. In this method, the pH of the fluid medium does not change outside the physiological limits, due to the buffering action exerted by the plasma proteins. Use of serum in the direct Warburg method without removal of CO_2 and subsequent adjustment of pH is not feasible due to the high pH which develops as the CO_2 is absorbed by the alkali.

1 Jowett, M., and Quastel, J. H., *Biochem. J.*, 1937, **31**, 565.

2 Krahl, M. E., and Clowes, G. H. E., Jr., *J. Cell. Comp. Physiol.*, 1938, **11**, 21.

3 Dickens, F., and Simer, F., *Abderhalden's Handb.*, 1933, **4**, 435.

4 Dixon, M., *Biochem. J.*, 1937, **31**, 924.

Details of Technic. Approximately 25 cc samples of blood were drawn from fasting normal human subjects, under sterile conditions, and transferred to a tube containing approximately 5 cc of mineral oil. Both the tube and the mineral oil were previously sterilized by autoclaving for 15 minutes under 20 lb pressure. The blood was centrifuged and the serum transferred to a sterile 1000 cc separatory funnel containing mineral oil. (The presence of the oil was found to be necessary to eliminate frothing during evacuation.)

It was found by repeated trials that the addition of 0.25 cc of 0.1 N HCl per cc of serum, followed by evacuation for 30 minutes at a pressure of 4-10 mm Hg, reduced the CO_2 content of the serum to about 0.5 mM per liter, with a final pH of approximately 7.25. Determinations with the glass electrode showed that the serum pH was between 4 and 5 immediately after addition of the acid. The subsequent evacuation removed the CO_2 and restored the pH to normal.

Serum treated in this way is ready for use as a nutrient medium. Dixon[4] recommended the removal of species antibodies by heating to 56°C for 2½ hours. Human serum can be heated in this manner without obtaining the jelling which occurs in certain species. However, since it was found, in a series of 24 observations with heated and unheated human serum, that there was a negligible difference in the O_2 consumption of rat liver, the results to be reported in the present studies were obtained on unheated serum.

Slices of rat liver were prepared according to Warburg's procedure[5] and placed immediately in CO_2-free, phosphate Ringer's solution prepared according to Dickens and Simer.[3] Slices approximately 0.3 mm thick, totalling from 70 to 100 mg in weight, were then transferred to the Erlenmeyer type Warburg vessels containing 2.6 cc of the CO_2-free serum, and 0.4 cc of 20% KOH in the central cup. The vessels were attached to the manometers, and filled with oxygen. All the above procedures were carried out in a constant temperature room maintained at 38°.

The vessels were shaken in a water bath maintained at 38°, and after temperature equilibrium had been established, the manometers were read at 10-minute intervals for one hour. The tissue was then removed, dried at 110° for 24 hours and the dry weight determined. The oxygen consumption, Q_{O_2}, was calculated in terms of cubic millimeters per milligram of dry weight per hour. It was found that the rate of respiration of the liver in serum was uniform up to 100 minutes, which is longer than one finds for liver in Ringer's solution.

[5] Warburg, O., *Biochem. Z.*, 1924, **152**, 51.

TABLE I.
Oxygen Consumption of Rat Liver in Human Serum.

Rat No.	Initial serum CO_2 content mM/L	Initial serum pH	Final serum pH	Q_{O_2} cmm/mg/hr
1	.40	7.26	7.56	—12.5
		7.26	7.46	—12.7
2	.50	7.25	7.38	—11.7
		7.25	7.36	—11.4
3	.53	7.28	7.54	—11.8
		7.28	7.52	—11.9
		7.28	7.56	—11.8
		7.28	7.73	—12.5
		7.28	7.72	—12.6
		7.28	7.72	—12.1
4	.52	7.10	7.57	—11.3
		7.10	7.60	—11.7
5	.50	7.12	7.67	—14.7
		7.12	7.69	—14.5
6	.24	7.18	7.32	—12.5
		7.18	7.34	—12.2
		7.18	7.34	—12.4
7	—	7.28	7.43	—12.6
		7.28	7.45	—13.0
		7.28	7.42	—12.7
		7.28	7.43	—12.7
Mean	.48	7.23	7.51	—12.5 ± 0.6

Results. Determination of liver metabolism. Table I shows the results obtained in 21 determinations on the livers of 7 different rats. The mean value of Q_{O_2} was 12.5 ± 0.6; minimum 11.3, maximum 14.7. This is 25% higher than the value of 10 usually found in our laboratory for the Q_{O_2} of normal rat liver in Ringer's solution. The average difference in the results obtained on any one rat was only about 3%. The pH of the serum measured with the glass electrode was from 0.2 to 0.5 higher at the end of the experiment than at the beginning, but was still within the normal pH range. This increase in pH occurred whether or not tissue was present during the incubation.

It was concluded from this series of experiments that the present method of studying liver metabolism in serum was suitable for studying the effect of adding different materials to the serum. It also seemed to be a suitable method for determining whether serum as drawn contains toxic materials.