Effect of Beef Spleen Extract on the Respiration of Normal Mouse Tissues During Storage.* (18276)

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Previous communications(1,2) have dealt with the use of beef spleen extract(3) in the treatment of basal cell carcinoma and its mode of action(4,5). Earlier work from this laboratory(6), a study of the effect of beef spleen extract on the oxygen uptake of normal mouse liver as compared with that of dbrB carcinoma during storage at 4°C over a period of 12 days, suggested that the tumor was more vulnerable than the liver. In order to learn whether normal tissues differ among themselves and from tumorous tissue in their response, storage experiments were extended to the following normal tissues: brain, connective tissue, heart, kidney, liver and lung. The possible effects of bacterial contamination and of tissue breakdown on oxygen consumption during storage have been considered.

Experimental. Seventy-six female dba mice, 3 months old, were used as in the earlier work(6). Under light anesthesia and aseptic conditions the brain, connective tissue, heart, kidney, liver and lung were removed and macerated by passing through a fine mesh screen with a porcelain pestle. The pulp was suspended in twice its volume of Tyrode's solution and the suspension divided into 2 portions. To one was added one-third its volume of Tyrode's solution and to the other a similar volume of beef spleen extract. In the current experiments the beef spleen extract(3) contained 115 mg of solids per ml, the final concentration for storage being 30 mg per ml, and that during the period of measurement of respiration, 10 mg per ml. Respiration measurements were made in duplicate in Warburg respirometers at 37.5°C. The inner well of each respirometer flask contained 0.2 ml of 2 N sodium hydroxide and the outer well 2.5 ml of Ringer-phosphate-glucose solution (pH 7.2) and 0.5 ml of tissue suspension in Tyrode's solution or beef spleen extract. Preliminary experiments showed that the extract did not absorb oxygen. Readings were made after 60, 120, 180 minutes. The suspensions were then dried over-night in weighed crucibles in a drying oven at 70°C. The calculated weight of the solid content of the physiological solution was subtracted from the dry weight of the tissue. The cubic mm of oxygen per hour per mg of dry weight (Qo_2) of the first and second 60-minute intervals were calculated but only the total cubic mm of oxygen per 3 hours per mg of dry weight of tissue are tabulated. The values in the table represent the average of duplicate determinations which agreed within 10%.

With the foregoing technique respiration rates were determined at 48-hour intervals for each of the 6 tissues in beef spleen extract as well as in Tyrode's solution throughout the 18-day period of storage at 4°C. An initial determination of the oxygen consumption of the fresh tissue suspended in beef spleen extract and in Tyrode's solution was made within 30 minutes after its aseptic removal from the animal. These data are presented in the column of Table I designated as 0 day.

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TABLE I. Oxygen Consumption (cu mm O_2 per mg Dry Wt) Over 3 Hr Interval of Normal dba Mouse Tissues Stored 18 Days at 4°C in Tyrode's Solution and in Beef Spleen Extract.

	Lung		Heart		Kidney		Liver		Brain		Connective Tissue	
Days	Tyrode	Spleen	Tyrode	Spleen	Tyrode	Spleen	Tyrode	Spleen	Tyrode	Spleen	Tyrode	Spleen
0	2.03	0.68	2.50	1.73	6.23	8.04	3.09	2.56	12.53	3.64	.62	.53
2	2.31	1.99	1.37	2.17	2.88	6.99	2.24	2.37	4.28	3.00	.50	.84
4	1.03	1.18	1.18	0.42	2.93	5.72	3.21	2.95	4.95	1.42	.39	.22
6	0.92	0.88	0.78	0.44	3.05	4.18	2.05	1.57	1.68	1.04	.22	.12
8	0.49	0.58	0.28	0.34	1.23	2.25	1.10	1.36	1.63	1.03	.26	.10
10	0.66	0.48	1.26	3.98	1.37	2.08	1.15	1.27	1.10	0.90	.07	.10
12	0.62	4.48	1.65	7.08	2.53	2.08	1.31	2.00	1.42	0.88	1.02	.19
14	1.44	7.37	7.04	12.94	4.12	2.59	1.17	1.20	3.31	2.48	1.20	.19
16	2.53	11.63	7.20	22.07	6.64	3.53	0.95	1.04	6.36	3.53	3.51	.48
18	9.17	17.81			4.73	7.08	1.32	1.24	10.63	5.51	3.33	2.51

Microscopic examination of the tissue suspensions was made at 48-hour intervals, just a few minutes prior to making the respiration tests, for evidence of bacterial contamination. Loeffler's methylene blue and gentian violet were used for the stained preparations. Blocks of tissue stored simultaneously were fixed at the same time in 10 per cent formalin, embedded in paraffin, sectioned and stained with Harris' modification of Delafield's hematoxylin and eosin for histologic examination.

Results. In general, the respiration rate of all the normal tissues decreased on storage in both Tyrode's solution and spleen extract from the second to the eighth, tenth, or twelfth day. With the exception of liver, all tissues in both storage media thereafter exhibited an unexpected rise in respiratory rate (Table I). Examination of stained and unstained preparations of the stored tissues at the time of respiration tests for the presence of bacterial contamination revealed no changes in the types or relative numbers of organisms from those initially present and observed in the fresh aseptically removed tissues; an occasional member of the salmonella group was observed. Histologically there was no evidence of necrosis or cellular fragmentation during storage for this period. In a further attempt to determine whether tissue autolysis might have occurred during storage, with the production of oxidizable amino acids, the amino nitrogen was determined by the Van Slyke procedure at intervals during storage of the six tissues. Increases of only 0.3% to 5.4% were found, which were too small to account for the observed increase in oxygen uptake.

The immediate effect of beef spleen extract on the fresh unstored tissues, as shown in the tabulated data for 0 day, was marked depression of the amount of oxygen consumed by brain, lung, and heart tissue, a very slight decrease for liver and connective tissue, and an increased oxygen uptake by kidney. During the latter half of the storage interval oxygen consumption by lung and heart was greater in spleen extract than in Tyrode's solution. This was not noted in the case of kidney, brain and connective tissue which tended to have a lower respiration in the extract than in Tyrode's solution during this period. Liver tended to maintain a more or less steady respiration during the latter half of the storage period and it was relatively unaffected by the presence of beef spleen extract in confirmation of the earlier findings(6).

Discussion. The respiratory response of normal tissues to storage at 4°C for periods as long as 18 days in beef spleen extract varied. The values obtained for respiration of dbrB adenocarcinoma grown in dba mice under similar experimental conditions(6) fell within the extremes of the limits recorded in the present paper for the normal dba tissues. This is consonant with the observations of Greenstein(7) that the enzyme activities of

7. Greenstein, J. P., Biochemistry of Cancer, Academic Press, Inc., New York, 1947, pp. 200-201.

tumors generally lie within the range for normal tissues, and likewise confirms the lack of specificity of spleen extract in its action on tumor tissues as reported by Fardon *et al.*(4).

The steady increase in respiration during the latter portion of storage in either Tyrode's solution or in beef spleen extract cannot be attributed to bacterial contamination nor, so far as the results of histologic examination and the determination of amino nitrogen show, to the autolysis of the stored tissues. It is possible that other oxidizable substrates may be released during storage.

Summary. By means of Warburg respirometers the oxygen consumption of brain, connective tissue, heart, kidney, liver and lung from normal dba mice was measured at 48-hour intervals during a period of 18 days of storage at 4°C, in Tyrode's solution and in beef spleen extract. The respiration of all six of the normal tissues, whether stored in Tyrode's solution or in beef spleen extract, decreased during the initial 8- to 10-day period, while the same tissues, with the exception of liver, were characterized by a progressive increase in respiration during the 12- to 18-day period of storage. This increase could not be correlated with bacterial contamination or with tissue autolysis as revealed by histologic observation and amino nitrogen determination. The immediate effect of the extract was to decrease the oxygen consumption of brain, lung and heart, and, to a lower degree, of liver and connective tissue, while that of kidney was increased. The effect of beef spleen extract on dbrB adenocarcinoma in dba mice fell between the extremes of the effects on normal dba mouse tissues.

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Activity of Some Enzymes Involved in Carbohydrate Metabolism in Normal and Scorbutic Guinea Pigs.* (18277)

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Previous work has shown that an ascorbic acid deficiency in guinea pigs causes a disturbance in carbohydrate metabolism manifested by a decrease in liver and body glycogen storage, high blood sugar levels, and lowered absorption after glucose ingestion(1,2). When deficient animals were treated with adrenal cortical extract, insulin and adrenaline no improvement resulted(3). This suggests the possibility that the disturbance may be enzymatic rather than hormonal in origin.

The following study was designed to investigate the activity of several enzymes involved in carbohydrate metabolism in both normal and deficient animals. Phosphorylation and oxidation in kidney and liver extracts were first measured. Ochoa(4) found that even in the presence of fluoride, adenosine triphosphatase interfered with the phosphorylation of glucose by adenosine triphosphate and with the transfer of phosphate from phosphocreatine to glucose. This suggests the possibility that the lowered utilization of glucose in the scorbutic animal may be due to a disturbance in phosphorylation due

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3. Murray, H. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 351.

4. Ochoa, S., *J. Biol. Chem.*, 1943, v151, 493.