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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1. Murray, H. C. and Morgan, A. F., *J. Biol. Chem.*, 1946, v163, 401.

2. Banerjee, S. and Ghosh, N. C., *J. Biol. Chem.*, 1947, v168, 207.

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

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

to increased activity of adenosine triphosphatase.

*Experimental.* Young guinea pigs (550-650 g) were paired as to sex and weight. They were pair-fed and maintained on a commercial rabbit food.† In addition to the diet all animals received a carotene-in-oil supplement sufficient to supply 1 mg of carotene daily. The normal animals were given a solution containing 10 mg of ascorbic acid 3 times weekly.

Animals kept on this diet without ascorbic acid supplementation exhibited a decreased appetite in 19-24 days. At this stage scurvy was present as evidenced by extensive hemorrhages of the fascia of the musculature particularly the legs. A rapid weight loss accompanied the loss of appetite. A few days after inanition became apparent in the deficient member of the pair, both animals were fasted 24 hours, anesthetized with sodium amytal (sodium iso-amyl ethyl barbiturate) and killed by exsanguination. Liver and kidneys were removed and placed immediately in ice cold Ringer's solution. After chilling, the liver was cut in small pieces, 10 g weighed and homogenized in a test tube (using a power driven pestle as developed by Potter and Elvehjem)(5) with 1.2 ml of 0.1 M phosphate buffer pH 7.7 per g of tissue. Both kidneys were cut up, weighed, and prepared as was the liver with the exception that 2.4 volumes of phosphate buffer were added for each gram of tissue. Liver homogenates were centrifuged for 10 minutes and kidney for 5 at approximately 2000 revolutions per minute‡ and the supernatant decanted for use. Microscopic examination of the extract showed some cell fragments. Meyerhof and Geliazkowa(6) pointed out that the enzymic extracts are less complex systems than homogenates and give more uniform results, therefore extracts were used. All extracts and flasks were chilled until placed in water bath. The technique of Colowick, Welch and Cori(7) and Colowick, Kalckar and Cori(8) was used for the determination of phosphorylation and respiration. Respiration determinations were made in air at 37°C in a Warburg respirometer on duplicate samples. Substrates (Table I) were added to the side arm of the respiration flasks; 1 ml of tissue extract and 0.1 ml of 4% sodium fluoride were placed in the body of the flask and 0.2 ml, 20% NaOH and filter paper in center well. Samples were equilibrated for 10 minutes, the vessels closed, substrates dumped from the side arm and readings taken every 15 minutes. Liver samples were incubated for 60 minutes but kidney was run only 30 minutes since respiration was so rapid readings could not be taken after this time. $Q_{O_2}$ was calculated using the dry weight of 1 ml of extract corrected for the salt content of the buffer. After incubation samples were made to a volume of 30 ml with distilled water, 1 ml of this made to 10 with 10% trichloracetic acid, centrifuged, and inorganic phosphate determined on the supernatant by the method of Fiske and Subbarow(9). Blanks were secured by determining the phosphate in 1 ml of the original extract diluted and treated as above. Phosphorylation was calculated by the difference between blank and incubated sample. Adenosine triphosphatase was determined by the method of Dubois and Potter(10) modified by the use of a borate-KCl buffer in place of diethyl barbiturate. One gram of liver was homogenized with 9 ml of water; 1 ml of this homogenate made to 10 m with water. The diluted homogenate (0.4 ml) was placed in the side arm of a Warburg vessel; 0.3 ml of 0.03 M borate-KCl buffer (pH 7.7), 0.1 ml of 0.04 M calcium chloride and 0.6 ml of

† Centennial Rabbit Grain Pellets.

5. Potter, V. R. and Elvehjem, C. A., *J. Biol. Chem.*, 1936, v114, 495.

‡ Radius of centrifuge head to center of fluid 7 cm; horizontal head used.

6. Meyerhof, O. and Geliazkowa, N., *Arch. Biochem.*, 1947, v12, 405.

7. Colowick, S. P., Welch, M. S. and Cori, C. F., *J. Biol. Chem.*, 1940, v133, 359.

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10. Dubois, K. P. and Potter, V. R., *J. Biol. Chem.*, 1943, v150, 185.

TABLE I. Phosphorylation, Oxidation, Phosphorylase, Adenosine Triphosphatase and Aldolase Determinations in Tissues of Normal and Scorbutic Guinea Pigs.

*Phosphorylation and oxidation*—1 ml extract, 2.9 micromoles adenylic acid, 1.2 micromoles cozymase, 60 micromoles glucose, 30 micromoles glutamic acid, 60 micromoles succinic acid, 10 micromoles $MgSO_4$, 95 micromoles NaF. Total vol. 1.9 ml.

| Animals | No. | Phosphate esterified, mg: Liver | Phosphate esterified, mg: Kidney | $Q_{O_2}$ Liver | $Q_{O_2}$ Kidney |
|---|---|---|---|---|---|
| Normal | 9 | .56 | .73 | 1.37 | 8.77 |
| Deficient | 10 | .16 | .59 | 2.40 | 8.57 |

Adenosine triphosphatase determinations

| Animals | No. | Phosphate liberated from liver extract, mg |
|---|---|---|
| Normal | 10 | .015 |
| Deficient | 9 | .021 |

Phosphorylase determinations

1 ml extract, 2.9 micromoles adenylic acid, 30 micromoles glucose-1-$PO_4$ (.88 mg acid labile $PO_4$), 4 micromoles glycogen. Total vol. 2 ml.

| Animals | No. | Phosphate liberated, mg: Liver total* | 7 min. | Kidney total | 7 min. | Muscle total | Glycogen* formed, mg: Liver | Glycogen* formed, mg: Kidney |
|---|---|---|---|---|---|---|---|---|
| Normal | 8 | .80 | | .71 | | .25 | | |
| Deficient | 8 | .53 | | .59 | | .33 | | |
| Normal | 10 | .59 | .18 | .57 | .29 | | 24.1 | 28.8 |
| Deficient | 10 | .59 | .22 | .45 | .18 | | 30.9 | 25.3 |

Aldolase determinations

1 ml extract, 0.012 micromoles fructose-1, 6-diphosphate, 1 ml 0.1 M glycine buffer, 0.25 ml hydrazine sulfate.

| Animals | No. | Colorimeter readings: Liver | Kidney | Muscle |
|---|---|---|---|---|
| Normal | 5 | 58 | 71 | 71 |
| Deficient | 5 | 65 | 70 | 69 |

* Theoretical yield phosphate 1.33 mg, glycogen 26 mg.

adenosine triphosphate (0.7 micro-mole containing 0.04 mg acid labile P) was added to the body. Flasks were equilibrated for 10 minutes in a water bath at 37°C after which homogenate was dumped into the contents in the body and the whole incubated for 15 minutes. The flasks were removed and 0.6 ml of 10% trichloracetic acid added; the contents removed and centrifuged. Phosphate was determined on the supernatant fluid.

Phosphorylase activity in liver, kidney and muscle was determined according to the method of Cori, Cori and Green(11) by measuring phosphate liberated and glycogen formed according to the reaction

$$\text{glucose-1-PO}_4 \xrightarrow{\text{phosphorylase}} \text{glycogen} + \text{PO}_4$$

11. Cori, C. F., Cori, G. T. and Green, A. A., *J. Biol. Chem.*, 1943, v151, 39.

Liver and kidney extracts were prepared as were those used to measure phosphorylation with the substitution of a 0.015 M cysteine for the phosphate buffer. Muscle was weighed and ground in sand with 2.4 volumes of the buffer. Tissue extract was added to the side arm and substrate (Table I) placed in the flask. Flasks were equilibrated for 10 minutes in the water bath, dumped and incubated for one hour with free access to air at 37°C. Samples were then diluted as for phosphorylation measurements and inorganic phosphate determined.

Since glucose-1-$PO_4$ can be converted to glucose-6-$PO_4$ or glycogen, another series of animals were run and glycogen as well as phosphate and 7 minute phosphate determined on incubated samples. Glycogen was determined by the method of Good, Kramer

and Somogyi(12). Aldolase activity in liver, kidney and muscle from normal and scorbutic animals was also investigated. Aldolase catalyzes the reaction

fructose-1,6-diphosphate $\xrightarrow{\text{aldolase}}$ glyceraldehyde-3-$PO_4$

The method of Sibley and Lehninger(13) was used. Liver, kidney and muscle were ground with two times their weight of Ringer's solution. One ml of the trichloracetic acid extract resulting from the procedure was diluted to 10 ml with distilled water and 1 ml used for colorimeter readings.

*Results and Discussion.* Results of the analytical work are shown in Table I. When total phosphorylation was measured, the liver tissue of the normal animal showed a markedly greater ability to phosphorylate substrates than tissue from the deficient animal. The difference in kidney tissue was much less marked. Since crude extracts were employed the activity of several enzymes must have been measured. Colowick, Welch and Cori(7) state that the product of aerobic phosphorylation of glucose in liver extracts containing sodium fluoride was mainly fructose diphosphate. In some instances these authors using kidney extracts found a considerable proportion of the product of glucose phosphorylation to be phosphoglyceric acid. They state that succinic acid oxidation caused phosphorylation of glucose; this means that the dehydrogenation of succinic acid to fumaric is connected with the transfer of inorganic phosphate to glucose.

The oxygen consumption of liver tissue in animals of both groups was relatively constant but kidney gave variable results. Oxygen consumption of liver increases noticeably in scurvy but there is no change in kidney. These results are in agreement with the findings of Stotz, Harrer, Schultze and King(14).

Adenosine triphosphatase activity of liver tissue was higher in the deficient animal as measured by the liberation of phosphate from adenosine triphosphate. This may indicate hyperactivity of this enzyme resulting in reduced amounts of this compound.

Phosphorylase measurements in the first group of normal animals gave higher results for both liver and kidney extracts. This was not true of muscle extracts. In the second series the results for liver were the same but a higher figure was again obtained in kidney extracts from normal animals. Glycogen formation in liver and kidney extracts from the two groups of animals was about the same. Apparently the inability of the scorbutic animal to form glycogen as efficiently as the normal cannot be due entirely to reduced phosphorylase activity.

There appeared to be no difference in aldolase activity in liver, kidney and muscle tissue from the two groups of animals.

*Summary.* Liver tissue of normal guinea pigs showed a markedly greater ability to phosphorylate substrates than tissue from ascorbic acid deficient animals. The difference in kidney tissue was less marked. The oxygen consumption of liver tissue increases noticeably in scurvy but there is no change in kidney. Adenosine triphosphatase activity of liver tissue was higher in the deficient animal as measured by the liberation of phosphate from adenosine triphosphate. Phosphorylase measurements in one group of normal animals gave higher results for both kidney and liver extracts. This was not true of muscle extracts. In the second series the results for liver were the same but a higher figure was again obtained for kidney extracts from normal animals. Aldolase activity did not seem to be lowered in scurvy.

12. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

13. Sibley, J. A. and Lehninger, A. L., *J. Biol. Chem.*, 1949, v177, 859.

14. Stotz, E., Harrer, C. J., Schultze, M. O., and King, C. G., *J. Biol. Chem.*, 1937, v120, 129.

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