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The Indophenol Oxidase Content of Normal and Scorbutic Guinea Pig's Tissues.

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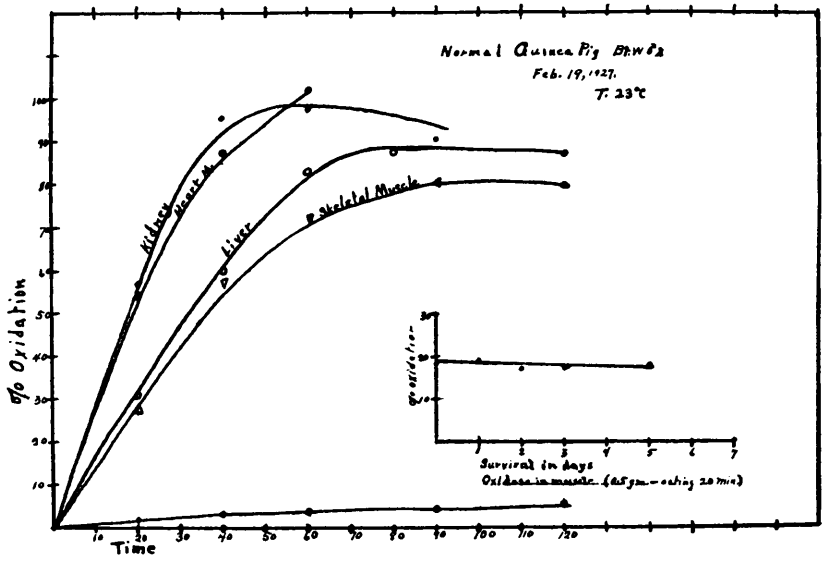
The method employed in these investigations was that reported by one of us.[1] Kidney, liver, heart and skeletal muscles were used. The last two named tissues were practically free from blood. Small amounts of connective tissues were always present, but were eliminated when possible from the test material, as they have practically no oxidizing power. The velocity of the oxidation, as indicated by the quantity of indophenol formed, was determined for tissue previously minced without sand, and tissue ground with an equal volume of quartz sand for exactly 10 minutes. In neither case were the particles of tissue more than 0.5 mm. in thickness. Determinations were made at a temperature of approximately 23° C.

The curves in the accompanying figure represent the velocity of oxidation with tissue ground with sand, and differ from those obtained from minced tissue in being steeper, and running to completion earlier. These curves have been corrected for spontaneous oxidation according to the method described.[1] In either case there is a marked difference in the power of the various tissues studied to



form indophenol. In no instance did the rate of oxidation and the time show an exact linear relationship, although in some individual experiments, particularly skeletal muscle minced without sand, an exact linear proportionality existed. In all of our experiments the curves of oxidation were almost exactly linear for a time. The deviation, which represented a diminution in the oxidation velocity, became progressively greater, until finally an equilibrium was reached. This state was not always maintained, but frequently dropped, as in the case of kidney shown in the figure. This suggests that a reductase may also be present. These observations lead us to agree with Vernon[2] that there is an excess of substrate present during the initial stages of the reaction so that an optimal contact between tissue and substrate is insured. Above this optimal there is no acceleration of the oxidation process, below it the reaction is retarded and comes quite rapidly to an equilibrium which usually lies below 100 per cent.

The oxidation of guinea pig's tissues is exceptionally stable under certain conditions. When the tissues are left in the body their power to form indophenol is scarcely altered after 5 days. An exact curve for skeletal muscle is shown in the inserted plat of the figure. Similar results were obtained for the other tissues. Alcohol and formalin inactivate the oxidase; but when the tissues are left in the body, readings may safely be made for 1 or 2 days after death.

In the comparison of the oxidizing power of normal and scorbutic animals, the readings were taken in duplicate or triplicate when the amount of tissue permitted, and were made for a period of 20 minutes only. The average error in these tests from identical material of any single animal was not more than 2 or 3 per cent. These experiments showed a marked diminution in the indophenol oxidase content of the tissues from the scorbutic animals. In those showing the most severe symptoms this reduction may be as much as 20 to 30 per cent. In general this is proportional to the severity of the disease. Quite wide variations were found between the same tissue from different animals, both normal and scorbutic; but since typical velocity curves were always obtained when any one tissue was studied and, on the other hand, duplicate or triplicate readings were made with not more than an error of 3 per cent, these variations must be interpreted by assuming that the oxidase content of these tissues differs quantitatively from animal to animal.

In the most severe cases of scurvy the body temperature was decidedly subnormal. These animals further showed a definite inability to maintain even these low temperatures when exposed to

−10° C., while in the controls the heat regulating mechanism was entirely adequate. This is a preliminary report.

[1] Dye, J. L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 640.
[2] Vernon, H. M., *J. Physiol.*, 1911, xlii, 402.

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Action of the Bacteriophage on a Low Temperature Bacterium.

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The demonstration of the existence of a bacteriophage active at low temperatures, on a bacterium which grows well at temperatures of 0.5° C. to 4° C., is of interest in connection with the work of Koser,[1] who reported a bacteriophage active at 55° C., on a thermophilic bacillus. Since studies are in progress in this laboratory on psychrophilic bacteria from those used by Greenfield and Elder,[2] it seemed of interest to us to determine whether organisms capable of growth at low temperatures, the psychrophiles, would also be susceptible to the action of a lytic principle or bacteriophage.

The active lytic principle against this organism was obtained from raw sewage by employing the method of alternate feeding and filtration.[3] The incubation temperature during the experiment was 4° C. The lytic filtrate was found to be very active, as shown by the complete clearing of broth tubes in two days, and large lysed areas on agar slants previously inoculated with the culture. The organism which was used was a true psychrophile, since it grew well at around 4° C. There was also fairly good growth at 20° C. but no growth at all at 37° C. All of the experiments reported in this paper were carried out at 4° C. An electrical refrigerator was used for maintaining a constant low temperature. This work is being continued in connection with the studies on low temperature microorganisms.

This is a preliminary report.

[1] Koser, S. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 109.
[2] Greenfield, R. E., Elder, A. L., McMurray, R. E., *J. Ind. and Eng. Chem.*, 1926, xviii, 1276.
[3] d'Herelle, F., The Bacteriophage and its Behavior. English translation by G. H. Smith. Williams and Wilkins, Baltimore, 1926.