only by a direct conversion of the fatty acid carbon.

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ENZYMES OF THE CITRIC ACID CYCLE IN TUMORS[1]

Sir:

As part of an investigation of the occurrence of the citric acid cycle in tumors, assays were made of various enzymes concerned in this process. The present report demonstrates the presence, in three transplanted mouse tumors, of the three enzymes directly involved in the metabolism of the tricarboxylic acids, *viz.*, "condensing enzyme," aconitase and isocitric dehydrogenase. The "condensing enzyme" is of particular interest because it is responsible for the initial reaction of the cycle, the formation of citrate by condensation of "active acetate" with oxalacetate.[2,3]

The data in the table indicate that the enzyme is present in the three tumors in amounts comparable with normal tissue. The other two enzymes were present also in significant amounts. Extension of these studies to other tumors and other enzymes of the cycle is under way.

TABLE I

ASSAY OF TUMORS FOR ENZYMES

	Condensing enzyme[a] μM. Citrate per 10 minutes per 100 mg. acetone powder	Aconitase[b] Units per mg. dry wt. of tissue	Isocitric dehydrogenase[c] Units per mg. acetone powder
Mouse liver	1.53[d]	33	10.8
Rhabdomyosarcoma	1.45	2.2–5.0[e]	6.4
Hepatoma	2.90	8.3	14.8
Mammary adenocarcinoma	3.30	3.1–5.9[e]	16.0

[a] For condensing enzyme the solution contained: 1 *M* KH_2PO_4 buffer, *p*H 7, 0.025 ml., 0.08 *M* $MgCl_2$, 0.05 ml., 0.2 *M* cysteine (neutralized) 0.05 ml., 0.14 *M* oxalacetic acid, 0.05 ml., 0.1 *M* synthetic acetyl phosphate, 0.10 ml., extract of *E. Coli* 4157 (from washed, lyophilized cells) 0.20 ml. To this was added 0.50 ml. of a 1–5 extract of an acetone powder of the tissue, and after incubation for ten minutes at 40°, the suspension was deproteinized and citrate determined by the method of Natelson, *et al.*[4] [b] Aconitase was determined by an unpublished method of Racker using citrate as substrate and measuring formation of *cis*-aconitate by increase in absorption of light at 240 mμ, using a Beckman spectrophotometer. The assay was made in a quartz absorption cell containing phosphate buffer, *p*H 7.4, 0.05 *M*, citrate, 0.03 *M*, and tissue extract in a total volume of 3 ml. The extract was prepared by homogenizing the tissue with 20 volumes of 0.1 *M* phosphate buffer, *p*H 7.4, and centrifuging off the residue. A unit of enzyme activity is the amount producing a change in optical density at 240 mμ of 0.001 per minute at 25°. [c] Isocitric dehydrogenase was determined on extracts of acetone powders by the optical method of Mehler, *et al.*,[5] a unit of activity being defined as the amount required to produce a change in optical density of 0.01 per minute at 25°. [d] Citrate formation in complete system without tissue was 0.21 μM. Citrate content of all tissues was <0.05 μM. [e] Three determinations on different tumors.

It might be assumed from these results that the failure of oxalacetate to be oxidized by tumor homogenates, observed by Potter and LePage,[6] may be due to loss of some necessary factor in homogenization rather than to an inability of the intact tissue to oxidize oxalacetate. We emphasize, however, that the data reported apply only to the tumors studied and generalizations are as yet unwarranted.

(1) Aided by grants from the American Cancer Society, recommended by the Committee on Growth; and the National Cancer Institute, U. S. Public Health Service. The aid of Drs. Stern and Ochoa in supplying details of the condensing enzyme assay and providing the *E. coli* and acetyl phosphate is acknowledged with deep appreciation.

(2) Stern and Ochoa, *Fed. Proceedings*, **9**, 234 (1950).

(3) Chou, Novelli, Stadtman and Lipmann, *ibid.*, **9**, 160 (1950).

(4) Natelson, Pinous and Lugovoy, *J. Biol. Chem.*, **175**, 845 (1948).

(5) Mehler, Kornberg, Grisolia and Ochoa, *ibid.*, **174**, 961 (1948).

(6) Potter and LePage, *ibid.*, **177**, 237 (1949).

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CATALYTIC DECOMPOSITION OF HYDROGEN PEROXIDE OVER SUPPORTED OXIDES OF MANGANESE

Sir:

The following results are presented in the form of a communication because they apparently offer a clue to a mechanism of heterogeneous catalysis.

Hydrogen peroxide was decomposed over a series of catalysts consisting of oxides of manganese supported on high area γ-alumina. These catalyst samples were prepared by impregnation of alumina with manganous nitrate solution followed by drying and ignition at 200°. The samples differed in percentage of manganese present, and were identical with several samples for which preparation, analytical data, and magnetic measurements have previously been reported.[1]

In each run the weight of catalyst chosen was such that the weight of manganese present was 4.60 mg. The quantity of hydrogen peroxide chosen was 2.00 cc. of 1.14 *M* solution added to the catalyst suspended in 20 cc. of distilled water. The reaction mixture was vigorously stirred, and the rate of oxygen evolution was measured on a flow-meter.

Catalytic activity results are shown in Fig. 1 where the rates of oxygen evolved are plotted against manganese concentration in the several samples.

(1) Selwood, Moore, Ellis and Wethington, THIS JOURNAL, **71**, 698 (1949).

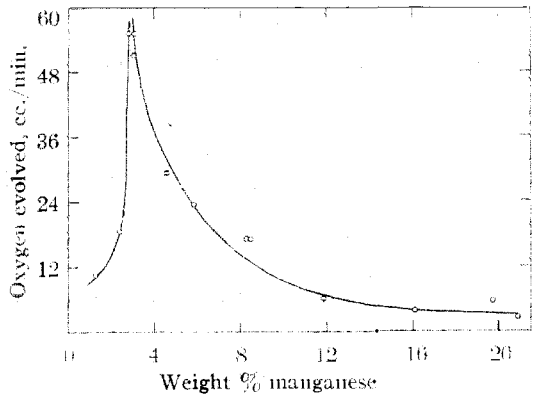

Fig. 1.—Catalytic activity of supported manganese oxides as a function of manganese concentration.

The interest in these results becomes obvious when they are considered in relation to the structural information previously reported. This supported oxide system is one in which the valence inductivity is very strong. At higher concentrations virtually all the manganese is in a +4 oxidation state; at low concentrations it is all in a +3 state. Figure 2 shows catalytic activity

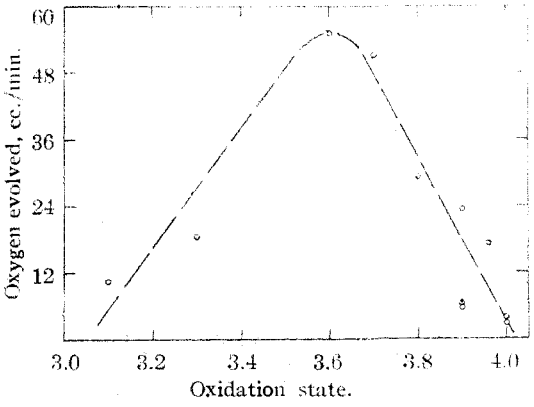

Fig. 2.—Catalytic activity of supported manganese oxides as a function of the oxidation state of the manganese.

plotted against average oxidation state. The oxidation states were found by direct quantitative analysis for active oxygen.

It is significant that proton relaxation measurements made on these catalyst samples by the method previously described[2] show no peak of activity, but merely the expected decreasing relaxation time with decreasing concentration. There is a well-defined bend in the relaxation time in the neighborhood of 4% manganese. This parallels the changing effective magnetic moment as the oxidation state changes from +4 to +3.

These catalytic data show the normal increase of activity as the accessibility of the manganese ions increases with increasing dispersion on the support.[3] The sharp decrease in activity below about 3.0% magnanese suggests that a minimum of two adjacent manganese ions is a necessary condition for activity in this system. The fact that the valence inductivity effect becomes strong at precisely this concentration of manganese gives a large number of Mn^{+3}–Mn^{+4} ion pairs. Thus a possible mechanism for the decomposition of hydrogen peroxide may involve the acceptance of an electron from the Mn^{+3}, to form hydroxide ion and hydroxyl radical, followed by donation of an electron by the hydroxide ion to the Mn^{+4} ion. The final stage is transfer of the electron back to the manganese ion which initially carried the $^{+3}$ charge.

Work is in progress on the activity of manganese oxides on aluminas of different specific area[4] and activity[5] and on other supports.

(2) Spooner and Selwood, *ibid.*, **71**, 2184 (1949).
(3) Selwood and Schroyer, Preprint, Faraday Society Discussion on Heterogeneous Catalysis, University of Liverpool, April 12, 14 (1950).
(4) Fischens and Selwood, THIS JOURNAL, **69**, 2698 (1947).
(5) Rodier and Rodier, *Compt. rend.*, **230**, 93 (1950).

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BOOK REVIEWS

Hematin Compounds and Bile Pigments. Their Constitution, Metabolism, and Function. By R. LEMBERG, Institute of Medical Research, Royal North Shore Hospital, Sydney, Australia, and J. W. LEGGE, Department of Biochemistry, University of Melbourne, Australia. Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y., 1949. xxv + 749 pp. 16.5 × 23.5 cm. Price, $15.00.

This excellent monograph, dealing with a field in which so many hundreds of investigators have worked, is a highly significant contribution to the scientific literature. It will take its place beside the monograph by Fischer, Orth and Stern as an authoritative reference work in the field. Its scope is broader than the earlier monograph and will appeal especially to biochemists, physiologists and students of the life sciences. Organic and physical chemists, particularly those interested in protein chemistry, will find in it numerous biochemical applications of their specialties and the biological background for a fuller appreciation of the significance of the work which has been done in this basic field. The authors make the point that hemoglobin alone is probably the most extensively studied biological product. This emphasis of investigators indicates the general importance of the subject matter and makes the previous absence of a suitable broad summation the more surprising.

After a brief introduction, the monograph opens with a discussion of the methods of investigation used in the