of enzyme in the fast peak with a corresponding increase in the concentration of the slow moving component. The splitting of the enzyme with DPNH may be completely reversed upon removal of the DPNH by dialysis. In addition to the influence of DPNH concentration, the pH of the solution appears to affect the extent of dissociation in the presence of DPNH, although the amount of dissociation of enzyme alone does not appear to be influenced by these same pH changes. No splitting of the enzyme is obtained by using *p*-chloromercuribenzoate or α-ketoglutarate, another substrate of the enzyme.

In view of the fact that Zn has been reported as a constituent of glutamic dehydrogenase[3] and of the apparent involvement of Zn in some enzymes which use DPNH as substrate[4], *o*-phenanthroline was added to the enzyme. The sedimentation diagram which resulted is shown in Fig. 1c. This enzyme has a sedimentation constant of slightly greater than half of the original material and as may be seen, the pattern is symmetrical as contrasted to Fig. 1a. The concentration of the *o*-phenanthroline used completely inhibited the enzymic reaction.

OLSON AND ANFINSEN have reported a molecular weight, from sedimentation and diffusion studies, for the enzyme of one million. Determinations of the molecular weight of *o*-phenanthroline-treated enzyme were made by the method of BALDWIN[5]. These determinations indicated that the protein may exist as a polymer of four enzyme subunits. The tetramer may be split either into dimers or monomers depending on the concentration of either *o*-phenanthroline or DPNH. As a result of splitting, the diffusion constant increases and the axial ratio decreases. The splitting caused by *o*-phenanthroline, a potent Zn binding reagent, is evidence for the role of Zn as an important component in the binding of the subunits. That both *o*-phenanthroline and DPNH cause dissociation of the enzyme may indicate the importance of Zn in the binding of DPNH.

So far as is known, glutamic dehydrogenase is the only enzyme which is affected in such a way by one of its substrates. The enzyme phosphorylase may also be dissociated into monomers, but only in the presence of *p*-chloromercuribenzoate[6]. In this case, the involvement of sulfhydryl groups is indicated in the combination of the four phosphorylase subunits.

Such a change in the molecular size and shape of glutamic dehydrogenase by a substrate for the enzyme offers a method by which further studies of the mechanism of the reaction may be made. Such investigations of the molecular and kinetic properties of the enzyme are under way in this laboratory.

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The degree of splitting of the enzyme by DPNH, but not by *o*-phenanthroline, has been found to be dependent on the specific activity of the enzyme.

A study of the synthesis of catalase in liver of tumor-bearing mice by means of radioactive iron

It is well known that the liver catalase activity is sharply lowered in tumor-bearing animals. It has not yet been ascertained, however, whether this decrease is due to an actual decrease in the enzyme concentration or to the presence of some inhibitor which interferes in its determination[1]. According to PRICE AND GREENFIELD[2], less catalase may be prepared from livers of tumor-bearing rats than from the healthy control animals. However, their technique, although accurate, is a preparative one, and therefore involves the possibility of losses; furthermore, it clearly cannot distinguish between the catalase already present at the moment of tumor implantation and that formed thereafter. In an attempt to give an answer to this problem, we studied by means of radioactive iron the synthesis of liver catalase after tumor implantation.

Male albino mice of an inbred strain (ALAL) were implanted with S.180 and divided into two groups. Respectively 5 and 10 days after the implantation, the tumor-bearing mice and the

corresponding controls of the same age and sex were injected intravenously with $FeCl_3$, containing 2.5 μg Fe labeled with ^{59}Fe, specific activity 200 μc/mg, in 0.1 ml of physiological saline. After 4 days, the animals were killed by decapitation, the livers washed in distilled water, blotted between sheets of filter paper and weighed. The weight of the tumors was also recorded. A portion (200 mg) of the liver was used for catalase-activity measurements; the rest was homogenized with quartz sand and resuspended in 3 times its weight of physiological saline. The suspension was centrifuged at 13,000 *g* for 10 min. The supernatant was shaken with an equal volume of *n*-butanol, and centrifuged again at 20,000 *g* for 10 min. The aqueous phase was dialyzed for 12 h in the cold against distilled water, centrifuged to remove a small white precipitate, and then lyophilized. This extraction procedure was also applied to normal mouse liver homogenates, after addition of ^{59}Fe *in vitro*. Very little or no radioactivity was present in the extracted proteins; the possible interference of ferritin was thus excluded (*cf.* ref.[3]). The lyophilized proteins were dissolved in enough borate buffer at pH 8.6 (μ 0.06) to give a 4% solution and their radioactivity determined in a liquid scintillation counter. The solution was then submitted to paper electrophoresis; 15 μl, corresponding to 0.6 mg lyophilized proteins, were used for each strip. Electrophoresis was run with borate buffer at pH 8.6, μ 0.06, with 5 V/cm, for 8 h. Catalase was revealed on the strips by spraying them with 0.5 *N* H_2O_2, and, after 20 min, with 2% $TiCl_3$. A white spot on a yellow background appears at the site where catalase is located.

Radioactivity, as revealed by direct counting on the most active strips and by autoradiography. was located only at the same site as catalase activity, and corresponded to the mobility of crystalline catalase[4].

TABLE I

ENZYMIC ACTIVITY AND INCORPORATION OF ^{59}Fe IN LIVER CATALASE OF TUMOR-BEARING MICE

Catalase activity was measured by the method of VON EULER AND JOSEPHSON[5] on an amount of extract corresponding to 1 mg liver (fresh weight). Radioactivity counts were made as described in the text. The values given are means ± standard errors of the means.

	Controls	*5-day tumors*	*10-day tumors*
No. of mice	22	9	12
Weight of tumors (g)	—	0.68 ± 0.09	2.95 ± 0.38
Catalase activity ($k_0 \times 10^4$)	1130 ± 51	935 ± 51	710 ± 25
^{59}Fe incorporation (counts/min/mg lyophilized protein)	504 ± 33	727 ± 81	48 ± 12

Table I shows that in animals injected 5 days after the implantation and bearing small tumors, the incorporation of ^{59}Fe (counts/min/mg lyophilized protein) was somewhat greater than in the controls. The catalase activity, on the other hand, already showed a decrease. In animals injected later and bearing larger tumors, a very striking decrease of radioactivity, much greater than the decrease of the catalase activity, was observed. The detailed results, not given in Table I, showed that the decrease of the radioactivity was greatest in animals bearing the biggest tumors.

It seems, therefore, that the action of tumors on liver catalase is a complex one and results from an impairment of the balance between synthesis and destruction of the enzyme, as anticipated by THEORELL and coworkers[3]. From a comparison between radioactivity and catalase-activity measurements, it appears that there is at the beginning an increased destruction, without alteration or perhaps with a slight increase of the synthesis; subsequently, the synthesis is sharply affected and the rate of destruction lowered. Apparently there is no need to assume the presence of an inhibitor which interferes in the activity measurements. A complete account of this work will appear elsewhere.

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