A Study of $d$-Amino Acid Oxidase, Uricase, and Choline Oxidase in the Livers and in Isolated Liver Cell Nuclei of Rats Bearing Transplanted Tumors*

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Since the report of Brahn (1) that very low catalase values are found in the livers of human beings dead of various forms of cancer, many papers have appeared on the effect of a growing tumor upon enzyme systems in liver tissue. Greenstein (4) has shown that in rats bearing transplanted hepatoma 31 or Jensen sarcoma, or in mice bearing various transplanted tumors, the activity of the liver catalase is notably decreased, but the activity of xanthine dehydrogenase and of acid and alkaline phosphatase is normal. Greenstein also has shown that arginase is decreased in the livers of tumor-bearing rats but not in the livers of tumor-bearing mice. Shack (8) has reported that the $d$-amino acid oxidase is not significantly low in the livers of rats bearing transplanted hepatoma 31, although the concentration of riboflavin has been found low in the livers of rats bearing transplanted tumors. However, no attempt has been made to determine the apoenzyme and coenzyme of $d$-amino acid oxidase separately.

In this work we have investigated apoenzyme and coenzyme of $d$-amino acid oxidase separately and have found that the presence of subcutaneous transplants of hepatoma 31 causes significant lowering of both $d$-amino acid oxidase apoenzyme and coenzyme in whole liver tissue of rats bearing this tumor and in nuclei isolated from the liver cells. We have also investigated the enzymes uricase and choline oxidase in the livers of rats bearing transplanted tumors and in nuclei isolated from the cells of these livers. No work on the latter two enzymes has previously been reported in this connection.

EXPERIMENTAL

We have used both Osborne-Mendel rats bearing hepatoma 31 transplants and Wistar rats bearing carcinosarcoma 256 transplants in our experiments. All the rats were maintained on a fox chow diet fed ad libitum.

The enzyme systems have been studied both in the whole liver tissue and in the isolated nuclei of the liver cells.

Preparation of whole tissue suspension.—All the rats were killed by decapitation and the blood was drained from them as completely as possible. The blood remaining on the livers was rinsed off with a small amount of saline. The livers were cut into small pieces and were ground with 0.9 per cent saline in a glass homogenizer into a homogeneous, cell-free suspension. The suspension was freed from fiber by passing through cheese cloth. The time from killing the rats to starting the oxygen consumption measurements was limited to 30 minutes. For dry weight determinations, 1 cc. of the suspension was dried in a weighed crucible to constant weight at 105°C in an electric oven.

Preparation of isolated cell nuclei from rat livers.—The livers were removed immediately from the rats after decapitation and frozen. The nuclei of the liver cells were prepared according to the method of Dounce (3).

Preparation of coenzyme of $d$-amino acid oxidase.—The coenzyme of $d$-amino acid oxidase was prepared from bakers' yeast according to the method of Warburg and Christian (10).

Determination of $d$-amino acid oxidase apoenzyme.—The $d$-amino acid oxidase was determined by oxygen consumption measurements, using a Warburg apparatus. The method of Klein (5) was followed except that air was used instead of pure oxygen. The total volume of the solutions in the vessels was 2 cc. in all experiments. The oxygen uptake of the whole liver tissue or of the isolated liver cell nuclei caused by the oxidation of $dl$-alanine was recorded at 10 or 15 minute intervals during a period of 1 hour. $dl$-Alanine was used as substrate for all the determinations, since the oxidation of $l$-alanine under the conditions of the experiment has been shown by Krebs to be negligible (6). The substrate and the coenzyme of $d$-amino acid

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oxidase, if any was added, were both dissolved in pyrophosphate buffer at pH 8.3. The controls without the substrate were carried out under the same conditions at the same time. In order to study the effect of coenzyme on the oxidation of dl-alanine in the whole liver tissue and in isolated liver cell nuclei, a sufficient amount of the above preparation was added to insure maximum enzyme activity.

Determination of uricase.—The uricase activity was determined by the method of Davidson (2) as modified by Elvehjem and his co-workers (9). The determinations were carried out in a Warburg apparatus at 37° C., and the oxygen uptake caused by the oxidation of uric acid was recorded at 10 or 15 minute intervals for 1 hour. The uricase activity was expressed as the oxygen uptake per hour per mgm. of dried tissue.

In order to confirm the presence of uricase, the poisoning effect of KCN was also tested.

Since zinc ions, thought by some to be necessary for uricase action (9), might be removed during the preparation of nuclei, zinc ions were added to the suspensions of nuclei in concentrations employed by Wachtel, Hove, Elvehjem, and Hart (9), but no activating effect was found.

Determination of choline oxidase.—The choline oxidase was determined by the method of Mann and Quastel (7). The oxygen uptake caused by the oxidation of choline hydrochloride by the whole liver tissue or by the isolated liver cell nuclei was measured in a Warburg apparatus at 10 or 15 minute intervals for a period of 1 hour, and the activity was expressed as the oxygen uptake per hour per mgm. of dried tissue.

Number of determinations carried out for each average value of enzyme activity reported.—A total of 5 to 6 animals was used in each experiment, which was performed in duplicate or triplicate. Any results that were not in agreement within 3 per cent were discarded. The liver and the tumor cell nuclei were prepared from 100 gm. of liver or tumor. Three to four experiments were done in each case.

RESULTS

d-Amino acid oxidase apoenzyme in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—It has been found that d-amino acid oxidase apoenzyme is low in the whole liver tissue of Osborne-Mendel rats bearing subcutaneous transplants of hepatoma 31. The oxygen uptake caused by the oxidation of dl-alanine in the livers of Osborne-Mendel rats bearing hepatoma 31 transplants varies from 0.16 to 0.82 cu. mm. per hour per mgm. of dried tissue, depending upon the size of the transplanted tumor, with an average value of 0.51 cu. mm. The larger the transplanted tumor, the less is the concentration of the enzyme in the livers of the tumor-bearing rats. The average value of 0.51 cu. mm. is only 22 per cent of the average value for normal livers of this strain of rat.

On addition of the coenzyme of d-amino acid oxidase to a whole liver suspension from an Osborne-Mendel rat bearing a large subcutaneous transplant of hepatoma 31, the oxygen uptake attributable to the oxidation of dl-alanine rose from 0.16 to 0.75 cu. mm. per hour per mgm. of dried tissue, which is still only 32 per cent of the value of normal liver. Similar results were obtained with several rats bearing transplants of hepatoma 31 of about the same size.

![Graph showing d-amino acid oxidase activity](image)

FIG. 1.—d-Amino acid oxidase in whole liver tissue and in isolated liver cell nuclei of Osborne-Mendel rats bearing transplanted hepatoma 31, and of normal Osborne-Mendel rats. Solid circle = Whole liver tissue of tumor-bearing rats (upper curve represents the average values; lower curve, the values obtained from rats in serious condition). Cross = Whole liver tissue of tumor-bearing rats plus coenzyme of d-amino acid oxidase. Triangle = Liver cell nuclei of tumor-bearing rats. Square = Liver cell nuclei of tumor-bearing rats plus coenzyme of d-amino acid oxidase. Circle with dot = Whole liver tissue of normal rats. Triangle with dot = Liver cell nuclei of normal rats. Square with dot = Liver cell nuclei of normal rats plus coenzyme of d-amino acid oxidase.

The average value for oxygen uptake attributable to the oxidation of dl-alanine in whole liver suspensions from Wistar rats bearing subcutaneous transplants of Walker carcinosarcoma 256, was 1.01 cu. mm. per hour per mgm. of dried tissue, which is about 41 per cent of the average value of normal liver. This value was increased to 1.21 cu. mm. by the addition of the coenzyme of d-amino acid oxidase.

In nuclei isolated from the liver cells of Osborne-Mendel rats bearing transplanted hepatoma 31, the increase in oxygen uptake caused by the oxidation of dl-alanine without added coenzyme was nearly the same as that of the corresponding whole liver tissue, as shown in Fig. 1. However, the oxygen uptake for
the isolated nuclei rose from 0.61 to 1.84 cu. mm. per hour per mgm. of dried tissue when coenzyme was added, but this value is still only 45 per cent of the value of normal liver cell nuclei under the same conditions.

In the nuclei isolated from the livers of Wistar rats bearing Walker carcinosarcoma 256 transplants, the increase in oxygen uptake caused by the oxidation of dl-alanine was very slight. However, it increased from 0 to 0.54 cu. mm. per hour per mgm. of dried tissue after the addition of the coenzyme of d-amino acid oxidase, a value which is 49 per cent of that of the normal liver cell nuclei. The results of these determinations are shown in Fig. 2.

Uricase in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—The whole liver tissue and the isolated nuclei of the liver cells of Osborne-Mendel rats bearing transplanted hepatoma 31 contained a lower concentration of uricase than that of normal livers and nuclei from normal liver cells. The oxygen consumption caused by the oxidation of uric acid in whole liver tissue of Osborne-Mendel rats bearing hepatoma 31 transplants varied from 1.26 to 1.66 cu. mm. per hour per mgm. of dried tissue, with an average value of about 1.46 cu. mm. This value is 51 per cent of the average value for normal livers. The average value for the oxygen consumption caused by the oxidation of uric acid in the isolated nuclei of the liver cells was 1.93 cu. mm. per hour per mgm. of dried tissue, which is about 46 per cent of the value of normal liver cell nuclei. The results of these determinations are shown in Fig. 3.

The livers of Wistar rats bearing Walker carcinosarcoma 256 transplants and nuclei isolated from them contained almost the same concentration of uricase as that of normal livers and their nuclei, as shown in Fig. 4. The oxygen uptake caused by the oxidation
of uric acid in whole liver tissue of Walker tumor-bearing rats was 2.90 cu. mm. per hour per mgm. of dried tissue, and that of nuclei isolated from these livers 4.28 cu. mm.

Choline oxidase in whole liver tissue and in isolated nuclei of liver cells of rats bearing transplanted tumors.—The average value for the oxygen uptake of whole liver tissue of Osborne-Mendel rats bearing hepatoma 31 transplants caused by the oxidation of choline was 1.67 cu. mm. per hour per mgm. of dried tissue, which is 69 per cent of the value of normal livers. Choline oxidase in whole liver tissue of Wistar rats bearing carcinosarcoma 256 transplants was almost the same as that of normal Wistar rat liver. The oxygen uptake caused by the oxidation of choline in such livers was 1.81 cu. mm. There was no detectable amount of choline oxidase in the nuclei isolated from the livers of rats bearing transplanted tumors, but neither was there any in normal rat liver cell nuclei. The results of the determinations of choline oxidase are shown in Fig. 5.

**DISCUSSION**

We have shown that a tumor of liver origin (hepatoma 31) can influence the liver by depleting an apoenzyme more than its coenzyme, since the addition of coenzyme of d-amino acid oxidase to the livers of rats bearing subcutaneous transplants of hepatoma 31, and to nuclei isolated from them, does not increase the oxidation of dl-alanine to the normal value. Therefore the tumor certainly causes depletion of the apoenzyme of d-amino acid oxidase, although the coenzyme also appears to be lowered somewhat. Shack (8), as has been mentioned previously, already had shown that the d-amino acid oxidase content of livers of rats bearing subcutaneous transplants of hepatoma 31 is low, but he did not investigate the apoenzyme and coenzyme separately.

From the fact that there is no inhibitor for liver catalase in tumor, or in the livers of tumor-bearing animals, and the fact that hemoglobin as well as liver catalase is low in animals bearing large tumors, Greenstein (11) has concluded that the lowering of these two substances in tumor-bearing animals is caused by an inability of the animal to synthesize the porphyrin ring system present in the prosthetic groups of both hemoglobin and catalase. However, the work reported in this paper indicates a likelihood that at least part of the difficulty lies in the inability of the animal to synthesize the protein components. Tumors in rapid growth might hinder synthesis of the protein components of enzymes by appropriating amino acids, although this is a matter of speculation at the present time.

The Walker carcinosarcoma 256 of Wistar rats is not of liver origin, and it is of considerable interest that this tumor does not appear to influence d-amino acid oxidase, uricase, or choline oxidase in the livers of animals bearing it.

**SUMMARY**

1. An investigation has been made of the concentrations of d-amino acid oxidase, uricase, and choline oxidase in livers of rats bearing transplants of hepatoma 31 and carcinosarcoma 256, and in nuclei isolated from the liver cells.

2. A tumor of liver origin (hepatoma 31) caused depletion of the apoenzyme of d-amino acid oxidase more than its coenzyme in the livers of animals bearing this tumor. Hepatoma 31 also caused lowering of uricase and choline oxidase in the livers of tumor-bearing animals.

3. Walker rat carcinosarcoma 256 did not cause appreciable lowering of d-amino acid oxidase, uricase, or choline oxidase in the livers of animals bearing this tumor.

4. It has been found that hepatoma 31 also lowered the activity of d-amino acid oxidase and uricase in isolated nuclei of liver cells of rats bearing this tumor.

5. No choline oxidase activity has been detected in the isolated liver nuclei of rats bearing transplanted tumors. This is true also of nuclei isolated from cells of normal rat liver.

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