THE *d*-AMINO ACID OXIDASE, URICASE, AND CHOLINE OXIDASE IN NORMAL RAT LIVER AND IN NUCLEI OF NORMAL RAT LIVER CELLS

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Although many enzyme systems in animal liver have been recently investigated by different workers, relatively little work has been done on the enzyme systems in isolated nuclei. Boell, Chambers, Glancy, and Stern (1) have demonstrated the cytochrome oxidase activity of nuclei prepared from egg cells of *Arbacia punctulata* by the method of Harvey (2) and Harvey (3). Behrens and his associates (4) succeeded in preparing nuclei from hepatic cells and were able to demonstrate the existence of arginase and a very small amount of lipase in their preparations. Recently Dounce (5) used a new method to prepare nuclei of rat liver in such a way that the enzymes and proteins are probably not damaged, and several enzymes were reported to be present in these nuclei. In this paper three more enzymes have been studied in nuclei of rat liver cells, prepared by the method of Dounce (5), and the concentrations of these enzymes in isolated nuclei have been compared with the corresponding concentrations in whole liver tissue. The enzymes in question are *d*-amino acid oxidase, uricase, and choline oxidase.

EXPERIMENTAL

In this work, both Osborne-Mendel and Wistar rats were used, and all the rats were maintained on a fox chow diet fed *ad libitum*.

In order to compare the enzyme systems in nuclei with those in the whole tissue, the enzyme systems were studied both in whole liver tissue and in isolated nuclei of the liver cells.

Preparation of Suspensions of Whole Tissue—The animals were killed by decapitation and the blood was drained from them as completely as possible. The blood remaining on the livers was washed off with saline and the livers were cut into small pieces. The small pieces were ground with 0.9 per cent saline in a glass homogenizer into a homogeneous, cell-free suspension. The suspension was passed through cheese-cloth to remove fiber. The time from killing the animals to starting the determination of oxygen consumption was limited to 30 minutes. For determinations of dry weight,

1 cc. of the suspension was dried in a weighed crucible to constant weight at 105° in an electric oven.

Preparation of Isolated Cell Nuclei from Rat Livers—The nuclei of rat liver cells were prepared by Dounce's method (5) at pH 6.0 to 6.2.

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 (6).

Determination of d-Amino Acid Oxidase Apoenzyme—The *d*-amino acid oxidase apoenzyme was determined by use of a Warburg apparatus. The procedure of Klein (7) 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 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 experiment has been shown by Krebs (8) to be negligible. The controls without the amino acid were carried out under the same conditions at the same time. The substrate and coenzyme, if any was added, were both dissolved in pyrophosphate buffer at pH 8.3.

Addition of coenzyme did not increase the activity of preparations of whole liver, but preparations of isolated nuclei were found to be deficient in coenzyme. Therefore in all experiments with isolated nuclei, the above preparation of coenzyme from bakers' yeast was added in sufficient amount to assure maximum enzyme activity.

Determination of Uricase—This enzyme was determined by the method of Davidson (9) as modified by Elvehjem and his associates (10). The determinations were carried out at 37°, and the oxygen uptake was measured at 10 or 15 minute intervals for 1 hour. The results are expressed as the oxygen uptake per hour per mg. of dried tissue.

The inhibition of uricase by KCN has been used to confirm the presence of the enzyme.

Since the metal zinc, thought by some to be necessary for uricase action (10), might be removed during the process of preparing nuclei, zinc ions were added to the suspension of nuclei in concentrations employed by Wachtel, Hove, Elvehjem, and Hart (10), but no activating effect was found.

Determination of Choline Oxidase—The choline oxidase in whole liver and isolated liver nuclei was determined by use of a Warburg apparatus, according to the method of Mann and Quastel (11). The oxygen uptake caused by the oxidation of 1 mg. of choline hydrochloride by brei of normal rat liver or by isolated liver nuclei was measured at 10 or 15 minute intervals for a period of 1 hour.

Number of Determinations Carried Out for Each Average Value of Enzyme Activity Reported in This Paper—A total of five or six animals was used in

each experiment, which was performed in duplicate or triplicate. Any results which were not in agreement within 3 per cent were discarded. The nuclei of liver and tumor cells were prepared from 100 gm. of liver or tumor. Three to four experiments were done in each case.

Results

d-Amino Acid Oxidase in Whole Liver Tissue and in Isolated Nuclei of Liver Cells—The average increase in oxygen uptake in livers of normal Osborne-Mendel rats, caused by the oxidation of *dl*-alanine, is 2.32 c.mm. per hour per mg. of dried tissue, and that in Wistar rats is 2.20 c.mm. The nuclei isolated from liver cells of normal Osborne-Mendel rats contained *d*-amino acid oxidase, as shown by the oxygen uptake of the nuclei in the presence of *dl*-alanine. The oxygen uptake caused by the oxidation of *dl*-alanine in liver nuclei of normal Osborne-Mendel rats is 1.67 c.mm. per hour per mg. of dried tissue, which is 78 per cent of the total oxidation of *dl*-alanine in whole liver tissue, and that of the nuclei isolated from Wistar rats is 1.10 c.mm., which is 50 per cent of the total oxidation of *dl*-alanine in the whole liver tissue.

The oxygen uptake caused by the oxidation of *dl*-alanine in isolated nuclei of liver cells of normal Osborne-Mendel rats was raised from 1.67 to 4.05 c.mm. per hour per mg. of dried tissue by the addition of sufficient coenzyme preparation to insure maximum enzyme activity, while that of the whole liver tissue was raised only to an insignificant degree (from 2.32 to 2.48 c.mm.). Similar results were also observed with nuclei of liver cells of normal Wistar rats.

The above results indicate that the apoenzyme of *d*-amino acid oxidase is slightly higher in activity in isolated nuclei than in whole liver suspensions, while the coenzyme is considerably deficient in the nuclei.

Uricase in Whole Liver Tissue and in Isolated Nuclei of Liver Cells—The oxygen uptake caused by the oxidation of uric acid in liver tissue in normal Osborne-Mendel rats is 2.85 c.mm. per hour per mg. of dried tissue, while that in Wistar rats is 3.03 c.mm.

The uricase activity in the liver nuclei of normal Osborne-Mendel rats is 4.16 c.mm. per hour per mg. of dried tissue, while that of the nuclei isolated from liver cells of normal Wistar rats is 3.94 c.mm. Thus the value for uricase activity per dry weight of isolated nuclei is higher than that of the whole liver tissue. It is about 46 per cent higher in liver nuclei of Osborne-Mendel rats and about 30 per cent higher in Wistar rats.

Choline Oxidase in Whole Liver Tissue and in Isolated Nuclei of Liver Cells—The oxygen uptake caused by the oxidation of choline hydrochloride in the liver tissue of normal Osborne-Mendel rats is 2.42 c.mm. per hour per mg. of dried tissue, and that of Wistar rats is 1.89 c.mm.

In the nuclei isolated from liver cells of rats of both Osborne-Mendel and

Wistar strains no significant activity of choline oxidase could be detected. This might be due to washing out of a coenzyme during the process of isolating the nuclei. However, the addition of the boiled aqueous extracts of whole liver did not promote the oxidation of choline in nuclei; so that it seems more probable that the enzyme itself is lacking in the nuclei.

DISCUSSION

From the results of recent work (1–5) it now seems clear that a number of enzymes are present in cell nuclei. In this paper we have shown that both *d*-amino acid oxidase and uricase are present in isolated nuclei from liver cells of normal rats. The concentration of the apoenzyme of *d*-amino acid oxidase in whole rat liver and in nuclei isolated from the liver cells is probably almost the same, although the nuclei appear to have a lower coenzyme concentration. This might be an indication that during the preparation of nuclei some of the coenzyme has been removed, but it is also possible that the cell nuclei normally are low in coenzyme, since this coenzyme is rather firmly bound to protein, at least in kidney preparations, and therefore should not be easily washed out at the pH range employed in preparing the nuclei.

We cannot explain, for the time being, why the isolated nuclei of liver cells of normal rats show a higher activity of uricase per dry weight than whole liver tissue.

Although choline oxidase was found in whole liver tissue, it was absent in samples of isolated nuclei. Since the addition of the boiled aqueous extracts of whole liver did not increase the activity of choline oxidase in isolated nuclei, it is not likely that a coenzyme or activator of this enzyme system is removed during the process of isolating the nuclei. Therefore it is probable that choline oxidase is lacking in nuclei of rat liver cells. The possibility that the enzyme is completely destroyed during preparation of of the nuclei appears remote.

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SUMMARY

1. An investigation has been made of the concentrations of *d*-amino acid oxidase, uricase, and choline oxidase in normal rat liver and in nuclei isolated from liver cells of normal rats.

2. It has been found that both *d*-amino acid oxidase and uricase are

present in nuclei of liver cells of normal rats. The concentration of uricase in nuclei is somewhat higher than that in whole tissue, while the concentration of *d*-amino acid oxidase apoenzyme is about the same in isolated nuclei and whole liver tissue.

3. The coenzyme of *d*-amino acid oxidase appears to be very deficient in isolated nuclei, whereas sufficient is present in whole tissue for optimal activity of the enzyme.

4. Choline oxidase was not found in isolated nuclei of liver cells. The addition of boiled aqueous extracts of whole liver did not promote the oxidation of choline in the isolated nuclei. This enzyme is present in whole liver tissue however.

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