THE ARGINASE ACTIVITY OF ISOLATED CELL NUCLEI

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Arginase appears to be one of the first enzymes which was studied in cell nuclei isolated on a relatively large scale by physicochemical methods. This enzyme was measured in isolated nuclei of liver cells by Behrens (1) who reported that the arginase activity per dry weight of isolated nuclei was about equal to the arginase activity of cytoplasm per dry weight in the case of mammalian liver. It was reported later from this laboratory (2) that the activity of arginase per dry weight of nuclei isolated by a different method from rat liver was 40 to 50 per cent of its activity per dry weight of whole rat liver. Recently the method of isolating nuclei of rat liver which was previously used in this laboratory has been improved, chiefly by better control of the pH and by employing a low temperature throughout the isolation procedure (3). It was discovered that the arginase activity of a sample of nuclei isolated from normal rat liver by the improved method was considerably higher than previously reported, and therefore it appeared desirable to reinvestigate the question of the activity of this enzyme in isolated liver cell nuclei. Moreover, it has recently been possible to extend the improved method of isolating liver cell nuclei to kidney and pancreas, and therefore it has been possible to extend the work on arginase to nuclei isolated from these two tissues. The chief results of the new investigation are (a) that the activity of arginase per dry weight of nuclei isolated from rat liver cells is slightly higher than the corresponding activity per dry weight of whole rat liver; (b) that the arginase activity of nuclei isolated from dog, lamb, rat, or chicken kidney is negligible, although arginase activity can be measured in the whole kidney of all of these species; and (c) that the arginase activity of whole chicken liver and whole sheep pancreas, as well as of nuclei isolated from these tissues, is extremely low or zero. Experiments also have been carried out to determine whether the arginase of isolated liver cell nuclei is an integral part of the nucleus, or whether it is

1 The method described in this paper for isolating cell nuclei from dog or lamb kidney is essentially the same as the improved method for isolating cell nuclei from rat liver (3). A few layers of fine cheese-cloth can be substituted for the flannel used in straining the kidney preparation, and the material need be passed through this cheese-cloth only once. Also, one sedimentation is sufficient for removing whole cells and fiber from the liver cell nuclei.
merely acquired by adsorption or chemical combination from the cytoplasmic arginase during the process of isolating the nuclei. It has been concluded from this part of the work that in all probability the arginase is an integral part of the nucleus.

**EXPERIMENTAL**

*Preparation of Isolated Nuclei of Rat Liver Cells*—The method described elsewhere¹ (3) was employed.

*Preparation of Isolated Nuclei of Kidney (Dog, Lamb, Rat, and Chicken)*—Most of the medulla of the kidneys is removed and as much fibrous tissue as possible is removed and discarded. The kidneys are then cleaned of debris and are frozen in the freezing compartment of an electric refrigerator. 50 gm. of frozen cleaned kidney are cut into small pieces and blended in the Waring blender² for 10 seconds with 100 ml. of ice-cold distilled water. 4.2 ml. of 0.1 M citric acid are added during the next 10 seconds of blending. The suspension is filtered immediately through one layer of fine cheesecloth; the residue on the cloth is washed with 25 ml. of ice-cold distilled water. The filtrate is then refiltered through one layer of fine flannel. During the filtration the suspension must be kept cold either by using an ice bath or by working in a cold room. The filtering mixture may be agitated with a stirring rod but the residue should not be squeezed out at any time. The total time of filtration should not exceed 15 minutes. The filtered material is blended for 5 minutes. During this time 125 gm. of ice are added. The final temperature should not exceed 5° and the pH should be between 5.8 and 6.0. Then the suspension is filtered through two layers of fine cheesecloth and placed in two 250 ml. centrifuge tubes and centrifuged at approximately 2000 R.P.M. for 20 minutes. The supernatant is decanted up to the loosely packed sediment and discarded. Now the sediments of the two cups are combined and thoroughly stirred with enough ice-cold distilled water to make a total volume of approximately 200 ml. The suspension is then centrifuged in one cup for 15 minutes at approximately 1700 R.P.M. The supernatant is decanted from the packed material and discarded. The washings with 200 ml. of ice-cold distilled water are repeated twice and the suspensions are centrifuged at approximately 1400 and 1000 R.P.M. for 10 and 7 minutes respectively. After the last washing, the sediment consists of only nuclei, fiber, and some very fine particles. The suspension is made up to 100 ml. with ice water and sedimented in a sediment-

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² The speed of the Waring blender is fairly critical. The old style blender which draws 2 amperes of current operates at the correct speed, but the new style blender which draws 3 amperes must be operated with a rheostat in series which reduces the operating voltage to about 95 volts, in order to avoid disintegrating the nuclei themselves.
ing apparatus (Fig. 1) for 2 hours in the cold room. At the end of this time the top 95 ml. are separated from the 5 ml. at the bottom and centrifuged for 5 minutes at approximately 1000 r.p.m. The nuclei are suspended in a small amount of ice-cold water and examined under the microscope.

![Fig. 1. Sedimentation apparatus. 1, 100 ml. graduated cylinder with bottom removed, cemented to a ground glass plate; 2, lower ground glass plate with circular hole of the same diameter as the hole in the upper plate, cemented to a graduated 50 ml. syringe; 3, upper ground glass plate with a circular hole of the diameter of the graduated cylinder, displaceable laterally in order to imprison the sedimented material; 4, 50 ml. graduated syringe with bottom removed, cemented to the lower ground glass plate and to be mounted in some suitable supporting frame; 5, plunger of the syringe for adjusting the volume of heavy sediment to be removed.](http://www.jbc.org/)

Should the preparation still be contaminated with fiber, the sedimentation procedure must be repeated, this time with only 50 ml. of suspension, allowing the material to undergo sedimentation for only 1 hour (in the cold room). If any fine particles remain after sedimentation, they are removed by centrifuging with 200 ml. of ice-cold water at 700 to 1000 r.p.m. This pro-
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The procedure unfortunately may also cause some loss of smaller nuclei. The final sediment, consisting of isolated nuclei, should be suspended in a small amount of ice-cold redistilled water and placed in the refrigerator.

A photograph of dog kidney cell nuclei prepared as described above is shown in Fig. 2.

Preparation of Isolated Nuclei from Chicken Liver—These were prepared with the use of the Waring blender in the same manner as for the preparation of other liver nuclei. The nuclei were very free from cytoplasm and quite small in size.

Preparation of Isolated Cell Nuclei from Sheep Pancreas—Essentially the same method was used as is described above for the preparation of nuclei from dog and sheep kidney.

Method of Determining Arginase—The procedure previously employed in this laboratory (2) was used, with slight modification of washing the dixanthyl urea only once with 50 per cent acetic acid and once with water, instead of twice with 50 per cent acetic acid and twice with water. Also, in most cases a substrate concentration of 3 per cent arginine carbonate was used instead of 1 per cent arginine carbonate. The relationship between the mg. of dixanthyl urea produced by a given amount of liver homogenate or of nuclei with 3 per cent substrate and that produced by using 1 per cent substrate is essentially linear, as is shown in Fig. 3. All weights of dixanthyl urea obtained with 3 per cent substrate were converted by the curve in
Fig. 3 to weights of dixanthyl urea that would have been obtained with 1 per cent substrate, and the values of $K$ per gm. for arginase which appear in Tables I, II, and IV were calculated on the basis of 1 per cent substrate, in order to obtain results which are comparable to those previously published (2). Although the use of 3 per cent arginine carbonate gives somewhat greater amounts of dixanthyl urea than does 1 per cent arginine carbonate, we do not think the waste of substrate is justified and recommend the use of 1 per cent substrate for this reason. In order to change the kinetics of the reaction from first order to zero order, which is always a desirable procedure in enzyme work, it would be necessary to employ substrate concentrations of 5 per cent or higher.

In determining the arginase activity of isolated rat liver nuclei, 0.1 ml. of a suspension containing about 8 to 10 mg. of nuclei (dry weight) per ml. was added to the arginine carbonate. In determining the activity of whole rat liver, 0.2 to 0.3 gm. of liver was homogenized in a ground glass homogenizer of the design shown in Fig. 4, with 5 ml. of water. 0.1 ml. of the suspension which contained 12 to 15 mg. (dry weight) per ml. was added to the arginine carbonate solution. The arginine carbonate was prepared by adjusting arginine monohydrochloride to pH 9.0 with sodium carbonate.

In working with tissues other than liver, more concentrated suspensions were used and in some cases (nuclei of kidney cells, pancreas cells, and chicken liver) the reaction was allowed to proceed for 1 to 3 hours. In cases in which MnSO$_4$ was used as an activator, this was added as 0.1 ml. of a 1 per cent solution.

It should be pointed out that in all cases in which nuclei were isolated
from rat liver five to six livers were pooled to give 50 gm. of material from which the nuclei were obtained. Thus each value for the $K$ per gm. of isolated nuclei of rat liver cells in a way represents an average value for five or six livers. In working with rat kidney, a much larger number than six kidneys was used. With dog kidney, however, the kidneys of only one animal were used. In the case of lamb and chicken, two pairs of kidneys were used.

Approximately equal numbers of male and female rats were used in determining the values of $K$ per gm. of the whole liver homogenate and in making a given sample of nuclei, but in general no attention was paid to the sex of the animal in a given determination.

**Analysis of Isolated Nuclei from Liver Cells for Metals**—A sample of rat liver cell nuclei of 86 mg. of dry weight was asched in the muffle furnace and the ash, which weighed 1 mg., was digested with a small amount of concentrated $\text{H}_2\text{SO}_4$. Most of the $\text{H}_2\text{SO}_4$ was then gradually replaced by HCl with evaporations nearly to dryness, and finally the residue was taken up in 2 ml. of distilled water. A small amount of material which remained undissolved and which probably contained a high percentage of silica was discarded, and the supernatant solution was analyzed spectrographically for the metals by Dr. L. T. Stedman of the Atomic Energy

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**Fig. 4. Ground glass homogenizer.** 1, Pyrex glass homogenizer tube; 2, bottom of homogenizer tube, ground to fit the pestle; 3, short glass rod held in the chuck of the stirring motor; 4, rubber or Tygon connecting tubing; 5, Pyrex glass rod; 6, rubber or Tygon connecting tubing; 7, pestle ground to fit the bottom of the homogenizer tube, with grooves on side and bottom.
Project (see Table III). The values in Table III are calculated on the basis of gm. of the element per 100 gm. of nuclei (dry weight).

Results of Determination of Arginase Activities of Various Whole Tissues and Isolated Cell Nuclei—Table I includes a number of determinations of arginase in whole tissue homogenates from rat and hen liver and dog, lamb, rat, and hen kidney. The results of determinations of the arginase activities of cell nuclei isolated from these tissues also are indicated. The distribution of the values for arginase activity in normal rat liver homogenate, determined on fifteen different liver samples without the addition of manganese, is shown in Fig. 5.

<table>
<thead>
<tr>
<th>Type of material studied</th>
<th>Normal rat liver</th>
<th>Regenerating rat liver</th>
<th>Hen liver</th>
<th>Rat kidney</th>
<th>Dog kidney</th>
<th>Lamb kidney</th>
<th>Hen kidney</th>
<th>Sheep pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole tissue</td>
<td>4.9 (15)</td>
<td>3.7 (1)</td>
<td>0</td>
<td>0.09</td>
<td>Trace</td>
<td>0.020</td>
<td>0.41</td>
<td>0</td>
</tr>
<tr>
<td>Isolated cell nuclei</td>
<td>7.6† (6)</td>
<td>5.4‡ (1)</td>
<td>10.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>9.2</td>
<td>8.9</td>
<td>7.4</td>
<td>8.6 Average</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $K = \frac{1}{t} \log_{10} \frac{A}{A - x}$, where $t = \text{reaction time in minutes}, A = \text{mg. of dixanthyl urea that would be formed from complete hydrolysis of substrate},$ and $x = \text{mg. of dixanthyl urea found in experiment}.$

† The figures in parentheses denote the number of experiments.
‡ MnSO₄ added as an activator; all other results are without the addition of MnSO₄.

The data in Table II show that the arginase of whole liver is not fully activated with manganese, since the addition of manganous sulfate increases the activity very markedly. In most of these experiments 0.1 ml. of 1.0 per cent MnSO₄ was added to the 1 ml. of arginine carbonate used for substrate, prior to addition of the whole tissue or nuclei.

The addition of manganous sulfate to isolated liver cell nuclei on the other hand does not increase their arginase activity at all. This is true even when the nuclei are allowed to remain in dilute MnSO₄ solution before adding them to the substrate. It seems likely that the arginase of the cell nuclei is quite well liberated from the nuclear residue at pH 9.0 in arginine car-
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bicarbonate; therefore it appears probable that there is sufficient metal in the isolated nuclei to activate the enzyme completely, although this is not true for whole liver homogenate.

If the assumption is made that isolated cell nuclei are about 70 per cent water, it can be calculated from data given in Table II that the manganese concentration in isolated liver cell nuclei is approximately $6 \times 10^{-5}$ M.

![Fig. 5. Distribution of values for arginase activity of normal rat liver homogenates not activated with MnSO4, expressed as values of K per gm. of homogenate (dry weight).](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Effect of Manganese Sulfate in Activating Arginase in Whole Rat Liver Homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>The values listed in each row across were obtained on the same liver homogenate.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Without MnSO4</th>
<th>With MnSO4</th>
<th>Per cent activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$ per gm.</td>
<td>$K$ per gm.</td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>6.4</td>
<td>24</td>
</tr>
<tr>
<td>4.4</td>
<td>6.7</td>
<td>52</td>
</tr>
<tr>
<td>4.3</td>
<td>6.9</td>
<td>63</td>
</tr>
<tr>
<td>3.4</td>
<td>5.5</td>
<td>64</td>
</tr>
<tr>
<td>3.0</td>
<td>4.1</td>
<td>35</td>
</tr>
<tr>
<td>3.8</td>
<td>7.5</td>
<td>96</td>
</tr>
</tbody>
</table>

This concentration of manganese might be sufficient for activation of all of the arginase in the nuclei even if none of the other metals which are listed in Table III as being present in the nuclei contribute anything to the metal activation of arginase.

The arginase activity of isolated rat liver cell nuclei decays rapidly for a time and then remains at approximately a constant activity when the nuclei are kept at $+3^\circ$ in the ice box, suspended in distilled water. Decay curves
for three samples of nuclei are shown in Fig. 6. These curves show that the nuclei must be freshly made if maximal arginase activity is to be obtained, and also that manganese does not affect the decay curve appreciably. The values listed in Table I for the arginase activities of isolated cell nuclei are for freshly prepared nuclei, not more than 6 hours old if the time is counted from the moment the frozen liver is added to the Waring blender. It is assumed that the arginase in the cell nuclei does not decay appreciably before the isolation of the nuclei while the liver is being frozen, since this appears to be true for the whole tissue.

The results given in Table I show that the average value for $K$ per gm. of cell nuclei isolated from normal rat liver is 8.6, while the average value of $K$ per gm. of whole normal rat liver homogenate, after activation with

**Table III**

<table>
<thead>
<tr>
<th>Element</th>
<th>Per cent in rat liver cell nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>$2.5 \times 10^{-2}$</td>
</tr>
<tr>
<td>Al</td>
<td>$1.7 \times 10^{-2}$</td>
</tr>
<tr>
<td>Fe</td>
<td>$1.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>Mg</td>
<td>$7.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Mn</td>
<td>$1.4 \times 10^{-2}$</td>
</tr>
<tr>
<td>Zn</td>
<td>$1.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>Ca</td>
<td>$2.3 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

![Graph](http://www.jbc.org/)

**Fig. 6.** Decay of arginase in isolated cell nuclei preparations. ▽, Sample 1, without MnSO$_4$; ○, Sample 2, without MnSO$_4$; □, Sample 2, with MnSO$_4$; △, Sample 3, without MnSO$_4$. 
MnSO₄ is 7.6. This latter value was calculated by applying the average per cent activation caused by MnSO₄ in the six experiments shown in Table II to the average value of the arginase K per gm. for whole rat liver homogenate given in Table I and calculated from the fifteen determinations on different liver samples for each determination. (This average value is 4.9; \((4.9 \times 0.56) + 4.9 = 7.6\).) The value of K per gm. of 7.6, thus obtained, is higher than the average value of 6.2 obtained directly from the data in Table II, but the values of K per gm. without adding MnSO₄, which are given in Table II, yield an average value which is considerably lower than that obtained by averaging all of the fifteen determinations.

Briefly then, the average K per gm. of arginase in cell nuclei of rat liver is about 113 per cent of the value of completely activated whole rat liver homogenate; the values of K per gm. for dog, lamb, rat, and chicken kidney are very much lower, and the value of K per gm. of cell nuclei isolated from the above tissues is negligibly small compared to that of rat liver or cell nuclei of rat liver.

No attempt was made to determine whether the trace of precipitate obtained with cell nuclei of the latter tissues was due to a very small but real arginase activity or whether it was an artifact due, for example, to bacterial action. We can state that K per gm. of these nuclei was at the most not greater than 0.002, or 10 per cent of the average value for lamb kidney. The time of incubation required to get enough urea to give any visible dioxanethylurea precipitate was so long that bacterial growth must have occurred in at least some cases. The values for the whole kidney homogenates represent real values for arginase activity, as anyone acquainted with the literature on the determination of arginase will no doubt concede, but we do not claim that our method of determination gives very accurate results in the case of kidney homogenates in which the arginase activity is quite low.

Experiments Relating to Possible Adsorption of Cytoplasmic Arginase by Cell Nuclei—A quantity of nuclei isolated from dog kidney and calculated to weigh 18.6 mg., if dried, was centrifuged from an aqueous suspension and was resuspended in 2 ml. of partially purified, soluble beef liver arginase solution having a monomolecular reaction velocity constant K of 2.2 per ml. (This represents a fairly strong arginase solution.) The arginase solution, which was water-clear and nearly colorless, and which contained a small amount of MnSO₄ as activator, had been prepared previously from beef liver.

The nuclei were recovered by centrifugation from the arginase solution after having been suspended in it for half an hour and were washed six times with distilled water. The K per gm. (dry weight) of the recovered nuclei was 0.11. By referring to Table I it can be seen that the nuclei had gained
an appreciable amount of arginase activity, but on a dry weight basis their activity was only 1.3 per cent of the activity of nuclei isolated from rat liver cells.

This experiment was repeated, with more dilute beef liver arginase, in order to duplicate more closely the arginase activity of the supernatant of rat liver homogenate used for isolation of cell nuclei. A quantity of nuclei from lamb kidney calculated to weigh 4 mg., if dried, was suspended for half an hour in 1 ml. of arginase solution having a monomolecular reaction velocity constant $K$ of 0.03 per ml. The nuclei were recovered by centrifugation, washed six times with distilled water, and the arginase concentration was determined. The $K$ per gm. of nuclei, on a dry weight basis, was 0.28, which indicates that the nuclei had an activity of only 3.3 per cent that of cell nuclei of rat liver, on a dry weight basis.

Another experiment was carried out in which the arginase was added to the homogenizing material in the Waring blender 1 minute before homogenization was stopped. In this case the arginase concentration was adjusted so as to be of the same order of magnitude as the concentration of arginase in the clear supernatant from the rat liver homogenate after centrifuging off both the nuclei and the insoluble cell granules in a high speed centrifuge. This procedure was adopted because it was found that much of the cytoplasmic arginase in the case of liver remained bound to cytoplasmic granules at the pH of the isolation of nuclei (6.0). This bound portion of the arginase therefore was discarded when the nuclei were freed from the last contaminating granules.

After adding the arginase to the suspension of lamb kidney in the Waring blender, the lamb kidney nuclei were isolated as usual, and their arginase activity was measured. Again appreciable arginase had combined with the nuclei; its concentration in the nuclei amounted to $K$ per gm. of 0.52. This is, however, only 6 per cent of the arginase activity of nuclei isolated from rat liver. Finally the experiment described last was repeated exactly, except that chicken liver was used instead of lamb kidney. In this case no detectable amount of arginase appeared in the isolated nuclei.

The results of the experiments on the possibility of adsorption of arginase by cell nuclei from the cytoplasmic arginase are summarized in Table IV. If cell nuclei of rat liver behave similarly to cell nuclei of dog and lamb kidney and chicken liver, it is unlikely that a transfer of dissolved enzyme from the cytoplasmic material to the nuclei during isolation of the nuclei could be of sufficient magnitude to affect the quantity of arginase in the nuclei appreciably. It also appears unlikely that lamb arginase would behave in a significantly different manner towards lamb cell nuclei than would beef arginase. If crystalline arginase should become available in the
future, however, it might be worth while to carry out an experiment on "adsorption" of arginase by nuclei with the homologous enzyme.

Possible Adsorption of Arginase by Cell Granules—It may be of interest to those working on isolated cell granules to know that the amount of added arginase taken up or adsorbed by the cytoplasmic granules was negligible in the experiment in which the diluted beef liver arginase was added to the homogenized lamb kidney in the Waring blender. In this work a sample of the granules (all sizes taken together) was obtained by centrifugation in the cold at 18,000 R.P.M. The supernatant solution, which was perfectly clear, contained practically all of the added arginase.

TABLE IV
Adsorption of Arginase by Cell Nuclei

<table>
<thead>
<tr>
<th>Isolated cell nuclei placed in arginase solution</th>
<th>Arginase solution added to cell homogenate in Waring blender; nuclei then isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell nuclei of dog kidney with strong arginase solution, K per ml. = 2.2</td>
<td>Cell nuclei of dog kidney with diluted arginase solution, K per ml. = 0.03</td>
</tr>
<tr>
<td>Arginase activity, K per gm. dry weight</td>
<td>Arginase activity, % of activity of rat liver nuclei</td>
</tr>
<tr>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td>1.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

DISCUSSION

It has been demonstrated in the foregoing paragraphs that the arginase content of the nuclei of rat liver cells is in all probability as high or higher in concentration than it is in the cytoplasm. The studies on whole liver can be taken to approximate cytoplasm, since the amount of nuclear material in a whole liver homogenate cannot be more than 10 per cent on a dry weight basis, as can be deduced from the studies of Marshak (4). One of the simplest possible explanations of this finding is that the enzyme arginase is synthesized in the cell nucleus and then passes out into the cytoplasm. It appears to us that the arginase located in the liver cell nucleus is not likely to have any functional significance peculiar to the cell nucleus, since this enzyme appears to fulfill a special rôle in metabolism in the formation of urea which in animals is limited to the liver. Furthermore, the substrates for urea formation must to a large extent come into the liver cells from the circulation, and thus they have to reach the cytoplasm before penetrating to the nucleus. It is entirely possible that the concentration of arginine in the nucleus may for this reason be very low compared to its concentration in cytoplasm where presumably the bulk of the urea formation is occurring.
For this reason the nuclear arginase may have little or no substrate to act upon.

It has also been demonstrated that the arginase activity of nuclei isolated from kidney cells is negligible, although arginase activity is easily demonstrable in whole kidney tissue.

It has been suggested by Kochakian (5) that the arginase of kidney is in reality a transamidinase which is responsible for the formation of glycocyamine and which accidentally possesses weak arginase activity. The fact that liver arginase is enormously higher in activity than kidney arginase lends strength to this idea. It is hard to imagine that such a small amount of arginase as occurs in the kidney can have any functional significance unless it is concentrated in a limited number of cells. If indeed the so called kidney arginase is not a true arginase, there is no reason a priori to predict its presence in kidney cell nuclei, since even in the case of liver we have encountered enzymes such as choline oxidase (6) and succinic dehydrogenase (2) which are present in very low concentration or absent in the cell nuclei.

The chief findings of interest in this work are, therefore, that the enzyme arginase appears to be a real constituent of nuclei of rat liver cells, and that it is present there in as high or higher concentration than in the cytoplasm. The latter finding indicates the possibility that arginase may be synthesized in the cell nucleus, particularly when one realizes that there may be little or no substrate for the enzyme to work upon in the nucleus.

SUMMARY

1. Cell nuclei have been isolated by an improved method from normal rat liver, normal chicken liver, normal dog, lamb, rat, and chicken kidney, and normal sheep pancreas, and the arginase activities of the cell nuclei isolated from these tissues have been compared.

2. The arginase activity per dry weight of cell nuclei isolated from normal rat liver is now found to be about 113 per cent of the activity of normal whole rat liver homogenate when the latter is activated by the addition of MnSO₄. MnSO₄ does not increase the arginase activity of nuclei isolated from normal rat liver.

Recent repetition of work on succinic dehydrogenase with cell nuclei of rat liver isolated by the improved method (3) has confirmed our earlier results (2) that the activity of this enzyme is negligible in the nuclei compared to its activity in the whole liver homogenate. Both the Warburg technique and the methylene blue technique again were used. As before (2) cytochrome oxidase was found to be present in very appreciable amount, although a careful reinvestigation of its quantitative activity has not yet been made. We wish to thank Frederick G. Smith for estimating the cytochrome oxidase activity of the isolated nuclei by means of a new colorimetric method. It thus appears to be possible to use the absence of succinic dehydrogenase as one indication of freedom from cytoplasm of the isolated cell nuclei.
3. The arginase activities of cell nuclei isolated from the kidney of dog, lamb, rat, and chicken are extremely small or zero, although by the method employed the presence of arginase could easily be shown in the whole homogenates of these tissues, all of which have low arginase activities compared to normal rat liver homogenates.

4. Experiments designed to detect a possible transfer of arginase from the soluble cytoplasmic fraction to the nuclei during the process of isolation of the nuclei indicate that any such transfer would probably be too small in magnitude to affect appreciably the arginase activity of the isolated nuclei. The same can be said, incidentally, of a possible transfer of dissolved arginase to the cytoplasmic granules.

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