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The Enzymic Properties of Rat-liver Nuclei

1. ESTIMATION OF THE EXTENT TO WHICH CONTAMINANT MATERIAL CONTRIBUTES TO THE ACTIVITY OBSERVED IN THE NUCLEAR FRACTION

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The isolation of nuclei from tissue homogenates by differential centrifuging has not given very clear information about the enzymic properties of the nucleus. One of the main difficulties is that often only a small percentage of the total activity of the homogenate is found in the nuclear fraction and, as many of the fractionation methods most commonly used for enzyme studies do not prevent the contamination of the nuclei with larger or smaller amounts of cytoplasmic particles, unbroken cells,

fibrous material and erythrocytes, it is very difficult to decide whether the observed activity is to be ascribed to the nuclei themselves or to the contaminants. It was therefore decided to investigate the position for two enzymes, aldolase and succinioxidase, in an attempt to establish whether the low activities reported by other workers to be present in the nuclear fractions are due to the nuclei or not.

Dounce (1948) has reported that liver nuclei prepared in dilute citric acid do not contain succinic dehydrogenase, and this enzyme was also reported to be absent from rat-liver and pig-kidney nuclei prepared by Lang & Siebert (1950). However, none

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of these workers gives recovery of enzyme after fractionation, so that it is not clear whether the absence could have been due to inactivation during the isolation procedure. It has been established by many other workers that rat-liver nuclear fractions contain succinoxidase but in lower concentration than in the whole tissue. It has also been consistently found that the bulk of the liver succinoxidase is in the mitochondrial fraction (see, for example, Schneider, 1946; Schneider & Hogeboom, 1950; Schein, Podber & Novikoff, 1951; Kensler & Langermann, 1951; Shepherd & Kalnitsky, 1951; Novikoff, Podber, Ryan & Noe, 1953).

Dounce & Beyer (1948) reported that rat-liver nuclei isolated in dilute citric acid contained aldolase at a concentration of about 40% of that of the whole tissue. Kennedy & Lehninger (1949), by using rat liver fractionated in 0.88M sucrose, found that 96% of the total activity was in the soluble fraction, 1% in the cytoplasmic particles and 3% in the nuclear fraction.

The work to be presented is an attempt to establish whether the small activities observed in both cases are due to nuclei or to contaminant material.

EXPERIMENTAL

Materials

Cytochrome c. This was prepared by the method of Keilin & Hartree (1945). Recently, a sample that had been further purified by the method of Margoliash (1954) was used. No difference in the results with the succinoxidase assay could be observed with the two samples.

Succinic acid. This was recrystallized twice from the commercial sample, with ethanol and water.

Hexose diphosphate. This was prepared as the dibarium salt by an unpublished method used in this laboratory. The concn. was measured by total P or by fructose analyses, by the method of Roe (1934). For the latter it was assumed that one mole of hexose diphosphate gave 0.525 of the colour of one mole of fructose in the method of Roe (Sibley & Lehninger, 1949). The barium hexose diphosphate was decomposed in acid solution by the addition of Na_2SO_4 and stored at -15° at pH 6.0 as a 0.1M solution. Samples were taken for aldolase assay after adjusting to pH 8.6 and 0.05M. The sodium salt had a yellow colour which was partially removed by shaking the solution at pH 6.0 with Norit decolorizing charcoal. All aldolase assays were done on the same sample of barium hexose diphosphate, kept dry at 0° . The ratio (mg. of total P – mg. of inorganic P)/mg. of fructose was 0.293 (0.291 for 100% purity) and 1.7% of the total P was inorganic P. The sodium salt was analysed chromatographically by the system of Hanes & Isherwood (1949), and it ran as a single spot except for the small amount of inorganic phosphate. Thus, apart from the pale-yellow colour, the material appeared to be of a satisfactorily high standard of purity for aldolase assays.

All other compounds used were of AnalaR grade purity, all reagents being made up in glass-distilled water.

Methods

Phosphate determinations. These were carried out by the method of Fiske & Subbarow (1925). The inorganic P determinations were adapted so that the final colour was determined in a volume of 4.0 ml. Fructose and phosphate colours were read in a Beckman DU spectrophotometer.

Total nitrogen. This was determined by a micro-Kjeldahl procedure.

Deoxyribonucleic acid. This was determined by the method of Schmidt & Thannhauser (1945) as modified by Davidson, Frazer & Hutchison (1951). The nucleic acid was estimated by its total P content.

Enzyme assays. All assays were carried out within the range in which the rate of reaction was proportional to tissue concentration. All fractions from the same liver were estimated simultaneously, as soon as the last fraction was obtained. Also, all fractions were estimated under the same conditions of pH, and salt and sucrose concentration. To obtain this it was usually sufficient to suspend all fractions finally in the same medium and use the same volume for enzyme assay. In some cases this was not possible and it was then necessary to make up compensating solutions, the addition of which brought the conditions of assay to the same in all manometer flasks or assay tubes.

(a) *Succinoxidase.* The manometric method of Schneider & Potter (1943) was used, with conventional Warburg apparatus of approximately 20 ml. gas space, calibrated by the method of Dickens (1951). The results are given in $\mu\text{l. of O}_2/\text{hr. at pH 7.4 and } 37^\circ$.

(b) *Aldolase.* The splitting of hexose diphosphate was followed by the appearance of alkali-labile phosphate (Meyerhof & Lohmann, 1934*a, b*). The reaction conditions were the same as those used by Sibley & Lehninger (1949), the reaction mixture having the following final composition: hydrazine sulphate 0.056M, aminotrihydroxymethylmethane-HCl mixture 0.04M, and sodium hexose diphosphate 0.005M. All reagents were at pH 8.60. The reaction was carried out at 37° for 15 min. and was started by the addition of enzyme. It was stopped by adding 10% (w/v) trichloroacetic acid to give a final concentration of 4.5%. The tubes were put in ice for 10 min., and then centrifuged at 2000g for 10 min. Samples of the supernatant were estimated for inorganic phosphate and for alkali-labile plus inorganic phosphate after incubation in n-NaOH at room temp. for 20 min. followed by neutralization with n-HCl . Blanks were set up by adding trichloroacetic acid before the enzyme, and it was invariably found that the latter contained a negligible amount of phosphate that was labile under these conditions of alkaline hydrolysis. Thus the alkali-labile phosphate blank from 50 mg. (wet weight) of rat liver was less than 0.35 $\mu\text{g. of P}$, whereas the same amount of tissue released 700 $\mu\text{g. of alkali-labile P}$ in the enzyme assay. Similarly, it was found that the enzyme did not have appreciable hexose diphosphatase activity under the conditions used, the amount of inorganic phosphate finally present being accounted for by that in the tissue and substrate. It was assumed that the release of 62 $\mu\text{g. of alkali-labile P}$ corresponded to the splitting of 1 $\mu\text{mole of hexose diphosphate}$. Aldolase activity is therefore expressed in $\mu\text{moles of hexose diphosphate split/hr. at pH 8.60 and } 37^\circ$. One unit of aldolase is defined as the amount that splits 1 $\mu\text{mole of hexose diphosphate/hr. under these conditions}$.

Cell fractionation. All work was done on male adult black hooded rats (body wt., 150–250 g.) and fed on a mixed stock diet. Suitable precautions were taken at all stages to keep the temperature of the fractions between 0° and 4°.

Method 1. The method used differed little from that of Hogeboom, Schneider & Striebich (1952) in which the nuclei are separated from the homogenate by three sedimentations for 10 min. each at 600 g. The liver was perfused via a polythene cannula through the portal vein and then through the inferior vena cava. The liver was forced through a small Latapie mincer and then homogenized in a Potter–Elvehjem apparatus with a Perspex head fitted into Viridia precision-bore tubing. The homogenate was filtered through one layer of plain, sterile surgical lint. Supernatants were removed with a hypodermic syringe fitted with a tapered polythene tube of such a length that when the base of the syringe is dropped over the mouth of the centrifuge tube, the end of the polythene tube lies just above the pellet. A clean removal of the supernatant is obtained with minimum disturbance of the underlying sediment. In all other respects the method was carried out as described by Hogeboom *et al.* (1952). The term homogenate is defined as the liver suspension from which some fibre, whole cells and nuclei have been removed by filtration; supernatants refers to the combination of all fractions derived from the homogenate, apart from the nuclear fraction. Nuclear fraction is the final sediment obtained by Method 1 or modifications of it. All nuclear fractions were finally suspended in 0.25 M sucrose–0.00018 M-CaCl₂.

Method 2. A medium of graded specific gravity was used (cf. Holter, Ottesen & Weber, 1953; Kuff & Schneider, 1954; Anderson, 1955). A volume (40 ml.) of a 20% (w/v) homogenate of liver was fractionated by Method 1 and the final pellet made up to 5.0 ml. in 0.25 M sucrose–0.00018 M-CaCl₂; 1 ml. was kept for analysis and the remainder layered over a medium of graded sucrose concentration. All solutions were 0.00018 M with respect to CaCl₂. A bottom layer of 5.0 ml. of 40% (w/v) sucrose was used and over this eleven layers, each of 3.0 ml., were carefully introduced with a bent-tipped pipette. The solutions added extended over the range 36–11% (w/v) of sucrose. A short centrifuging was used and the maximum care was taken to prevent disturbance of the layers. The layers were removed with a bent-tipped pipette. (In many preparations serious clumping of the tissue occurred during the centrifuging and the fractionation was spoiled. Such clumping was kept to a minimum by preparing the nuclear fraction by Method 1 as quickly as possible, and by working as near to 0° as possible.) In the fractionation described in Fig. 4 the conditions were altered somewhat in order to obtain a better definition of the nuclear peak.

RESULTS

Succinoxidase activity of nuclear fractions

The results of several fractionations by Method 1 are given in Table 1. It can be seen that only about 1.5% of the total succinoxidase was found in the nuclear fraction. The term relative concentration refers to the activity/mg. of N of the nuclear fraction divided by the activity/mg. of N of the homogenate from which it was derived. Thus 100 × relative concentration is equivalent to the more commonly used term percentage specific activity. The relative concentration for succinoxidase was low, and on some occasions nuclear fractions were obtained with much lower values (0.04–0.01 or less). It was not possible to obtain such low values consistently, however. A rough correlation was observed between the whole cell and succinoxidase content of nuclear fractions, by the following method. The number of whole cells originally present in the liver used, before cell rupture, was calculated from counts of nuclei and whole cells in the homogenate, assuming (a) that no nuclei were completely destroyed by homogenization and (b) that 30% of liver cells are binucleate (Harrison, 1953). The number of whole cells in the nuclear fraction was counted and it was found, by averaging five fractionations, that this was 1.3% of the cells originally present in the liver. This was only slightly lower than the percentage of total succinoxidase found in the nuclear fraction (Table 1) and it was likely that the difference was due to mitochondrial contamination.

Fractionation by Method 2 confirms these indications. It can be seen in Figs. 1 and 2 that there is no correlation at all between the distribution of nuclei and of the enzyme. In the first experiment (Fig. 1) compensating solutions were not used in the assay, which probably explains the poor recovery of enzyme. In the second experiment (Fig. 2) all the observed activity in the original nuclear fraction was due to two types of contamination, one sedimenting above the nuclei (presumably mitochondria, since the upper part of the centrifuge tube contained the bulk of the particulate material in the original

Table 1. *Summary of results of fractionations by Method 1*

Average values given with standard error of mean. The number of fractionations is given in brackets. HDP, Hexose diphosphate.

	Nitrogen	Succinoxidase (μ l. of O ₂ /hr.)	Aldolase (μ moles of HDP split/hr.)
Percentage of amount in homogenate found in nuclear fraction	9.0 ± 0.36 (19)	1.48 ± 0.39 (10)	12.5 ± 1.29 (37)
Percentage recovery after fractionation	100.2 ± 0.40 (19)	97.5 ± 0.86 (6)	96.0 ± 1.16 (28)
Activity/mg. of N of homogenate	—	797 ± 23.5 (10)	28.6 ± 0.99 (37)
Activity/mg. of N of nuclear fraction	—	138 ± 35 (10)	39.8 ± 3.41 (37)
Relative concn.	—	0.17 ± 0.04 (10)	1.44 ± 0.12 (37)

nuclear fraction), and one sedimenting below the nuclei (presumably unbroken liver cells, as in Fig. 1). In Fig. 2 there were not enough whole cells present in the original nuclear fraction to obtain accurate counts on all fractions, but it was observed qualitatively that most of the cells were found 5–6 cm. from the meniscus.

It was thought that sufficient evidence had been obtained to conclude that nuclei do not contribute to the succinoxidase activity of nuclear fractions obtained by Method 1.

Fig. 1. Distribution of succinoxidase, erythrocytes, nuclei and whole cells along the length of the tube after centrifuging in a medium of graded specific gravity. ■, Succinoxidase; ○, erythrocytes; ●, nuclei; □, whole cells. The concentrations per ml. of original nuclear fraction of the components were: succinoxidase, 504 μ l. of O_2 /hr.; erythrocytes, 14.8×10^6 ; nuclei, 88×10^6 ; whole cells, 1.38×10^6 ; the recoveries after fractionation were 67, 100, 114 and 109%, respectively. Nuclear fractions were layered over graded medium (11–40% of sucrose by 2.5% intervals) and centrifuged for 4 min. at 300 g.

Aldolase activity of nuclear fractions

The results of thirty-seven fractionations by Method 1 are given in Table 1. It can be seen that much more aldolase than succinoxidase was found in the nuclear fraction (12.5%) and that the enzyme was 1.44 times more concentrated in the nuclear fraction than in the homogenate. In several of the fractionations parallel assays with succinoxidase were carried out (Table 2) and on every occasion

Fig. 2. Distribution of succinoxidase and nuclei along length of tube after centrifuging in a medium of graded specific gravity. ○, Succinoxidase; ●, nuclei. The original nuclear fraction had 2.91 mg. of nitrogen, a succinoxidase activity of 425 μ l. of O_2 /hr. and 127×10^6 nuclei/ml. The recoveries after fractionation were 102, 100 and 118%, respectively. The nuclear fraction also had 0.2×10^6 whole cells/ml., but these were not sufficient to obtain accurate counts in the various fractions after centrifuging. Original nuclear fraction layered over graded medium [11–40% (w/v) by 2.5% intervals] and centrifuged for 4 min. at 300 g.

Table 2. *Parallel assays for succinoxidase and aldolase in nuclear fractions prepared by Method 1*

The fractionations have been arbitrarily arranged in order of descending succinoxidase activity.

Fractionation no.	Percentage of total activity of homogenate found in nuclear fraction		Relative concn.		Relative concn. of aldolase/ relative concn. of succinoxidase
	Succinoxidase	Aldolase	Succinoxidase	Aldolase	
1	3.0	6.7	0.28	0.63	2.25
2	2.0	9.7	0.24	1.10	4.58
3	0.89	11.6	0.13	1.68	12.9
4	0.40	17.5	0.053	2.50	47.1
5	0.27	6.0	0.039	0.88	22.6
6	0.10	7.2	0.013	0.94	72.4
7	Negligible	3.0	—	0.64	(Infinity)

both the percentage of the total enzyme and the relative concentration were higher than for succinoxidase. Also the values for aldolase bore no constant relationship to those for succinoxidase. The difference in behaviour of the two enzymes is further shown by fractionations by Method 2. In the first case (Fig. 3), a sharp correlation between nuclei (as estimated by direct count) and aldolase was

Fig. 3. Distribution of nuclei, whole cells, and aldolase after centrifuging in a medium of graded specific gravity. ○, Nuclei; □, whole cells; ●, aldolase. The concentrations per ml. of the original nuclear fraction for the various components were: nuclei, 115×10^6 ; whole cells, 0.7×10^6 ; aldolase, $220 \mu\text{moles of hexose diphosphate split/hr.}$; nitrogen, 3.14 mg. ; the recoveries after fractionation were 97.9% for aldolase and 99% for nitrogen. Original nuclear fraction layered over graded medium [11–40% (w/v) of sucrose by 2.5% intervals] and centrifuged for 4 min. at 300 g.

Fig. 4. Distribution of aldolase and deoxyribonucleic acid along the length of the tube after centrifuging in a medium of graded specific gravity. ●, Aldolase; ○, deoxyribonucleic acid. The original nuclear fraction had $0.947 \text{ mg. of deoxyribonucleic acid}$ and an aldolase activity of $115 \mu\text{moles of hexose diphosphate split/hr./ml.}$ The recoveries after fractionation were 94.4 and 100%, respectively. The volume (4 ml.) of fraction was layered and centrifuged for 6 min. at 300 g. Graded medium from 10–40% (w/v) of sucrose by 2% intervals.

observed. Fig. 4 shows that a good correlation is observed if the nuclei are estimated by their deoxyribonucleic acid content. In this experiment the nuclear fraction used for centrifuging in the graded medium had a low succinoxidase and a moderately high aldolase activity (Table 2, fractionation no. 4). There were a few whole cells in the preparation and they were mostly found at the bottom of the tube after the centrifuging.

Contribution of cell particles and erythrocytes

Succinoxidase assays (Figs. 1 and 2) in two fractions and microscopic examination of nine fractionations by Method 2 showed that the bulk of the cell particles in the original nuclear fraction were left in the upper layers of the tube after centrifuging in the graded medium. There is no evidence of a peak near the top of the tube in the distribution of aldolase (Fig. 3, and particularly Fig. 4) and so it is unlikely that mitochondria have much aldolase activity (cf. Kennedy & Lehniger, 1949). Also, it was confirmed that most of the liver aldolase, apart from that found in the nuclear fraction, is in the soluble fraction. Thus on one occasion 97% of the total aldolase of a liver homogenate was not sedimented by centrifuging for 25 min. at 2500 g followed by 30 min. at $100\,000 \text{ g}$.

Direct estimation of the aldolase activity of erythrocytes that had been washed once with the suspending medium showed that their activity per million cells was so low that it contributed only 0.067% to the total activity in a nuclear fraction obtained from a poorly-perfused liver, and only 0.00096% to the activity observed in a nuclear fraction obtained from a well-perfused liver. It was also found that a nuclear fraction obtained from a perfused lobe of a rat's liver had a greater specific activity ($45.2 \text{ units/mg. of N}$) than a nuclear fraction obtained from a lobe that had not been perfused ($30.7 \text{ units/mg. of N}$). This would be expected if the second nuclear fraction were heavily contaminated with enzymically inactive material, i.e. erythrocytes. Finally, Fig. 1 shows that some separation was obtained between nuclei and erythrocytes after centrifuging in the graded medium.

Contribution of fibrous and connective tissue

The amount present in the nuclear fraction, although variable, was usually fairly small, and it was noticed that Method 2 resulted in some separation of nuclei from this contamination, since fine, floating pieces of connective tissue appeared mainly in the upper fractions in the centrifuge tube and large pieces of fibre sedimented to the bottom. An attempt was made to estimate the aldolase activity of liver fibrous tissue directly. A rat liver was forced through the Latapie mincer (see Methods),

fitted with a plate pierced by 1 mm. diam. holes. The aldolase activity of the fibrous and connective tissue that remained attached to the plate was 16.8 units/mg. of N, whereas that of the whole liver was 24.9 units/mg. of N. Under the microscope the preparation of fibre was found to be grossly contaminated with adhering liver cells, and so its lower aldolase activity, compared with the original liver, even in the presence of so much contamination, indicates that the aldolase activity of pure fibrous material cannot be very high.

Effect of washing the nuclei

Repeated resuspension and sedimentation of the nuclear pellet showed that after three sedimentations no more enzyme appears in the supernatant (Fig. 5). To avoid the danger of sucking up nuclei while removing the supernatants, 0.8 ml. of supernatant was left overlying the nuclear pellet at each stage, so that removal of supernatant was not as efficient as in the normal Method 1. (Five, instead

Fig. 5. Effect of repeated sedimentations on distribution of aldolase between nuclei and supernatants. A volume (10.0 ml.) of a 10% (w/v) liver homogenate was centrifuged at 600 *g* for 10 min.; 9.0 ml. of the supernatant was removed and the remaining nuclear pellet (0.2 ml.) and overlying supernatant (0.8 ml.) were resuspended, made up to 10.0 ml. and centrifuged again. The process was repeated four times. All resuspensions were in 0.25M sucrose-0.00018M-CaCl₂. □, Total recovery of enzyme; ●, aldolase in sediment (nuclei plus overlying 0.8 ml. of supernatant); ○, aldolase in upper 9.0 ml. of supernatant.

of three sedimentations, were needed to obtain constant activity in the nuclear fraction, but the small fall in activity in the nuclear fraction after the third and fourth sedimentations was probably due to inactivation of the nuclear enzyme, since the total recovery of enzyme fell off at the same time to about the same extent.)

If much free soluble enzyme were left in the nuclear pellet obtained by Method 1 one would expect a small peak in activity in the top of the centrifuge tube in Method 2, since the very short centrifuging used would not sediment the free enzyme. It can be concluded that the three sedimentations (with layering) used in Method 1 adequately removed all free enzyme from the nuclear pellet.

Contribution of whole cells

The nuclear fractions averaged in Table 1 had 12.5% of the total aldolase. As stated above, in the average of five fractionations only 1.3% of the total cells originally present were found in the nuclear fraction. If all the activity in the nuclear fraction were due to whole cells they would therefore have had a specific activity eight to nine times that of the homogenate. Similarly, if the number of whole cells in the fractionation described in Fig. 3 contributed to all the activity observed in the original nuclear fraction, they would have to possess a calculated activity of about 700 units/mg. of N (assuming that one whole cell has ten times the N content of one nucleus). Such a specific activity is 25 times the average observed in thirty-seven homogenates (Table 1). If the specific activity of the whole cell were so high one would expect higher specific activities in the fractions in Method 2 nearest the bottom of the centrifuge tube. It was actually found in the fractionations described in Figs. 3 and 4 that the specific activity was nearly constant along the length of the tube, it possibly being slightly higher in the fractions that contained most of the nuclei.

The complete absence of correlation between whole cells and aldolase is shown in Fig. 3 and was observed qualitatively in the fractionation in Fig. 4. Also the presence of considerable aldolase activity in one of the fractions (Fig. 3, 2 cm. from the meniscus) that had no detectable whole cells makes it very unlikely that they contribute a large amount to nuclear aldolase.

Variability in aldolase of nuclear fraction

A striking effect observed in the course of this work was the extreme variability in the aldolase activity of isolated nuclei. On some occasions only about 3% of the total activity (e.g. Table 2, fractionation no. 7) was found in the nuclear fraction, while on others almost 30% was found.

Table 3. *Aldolase distribution in two apparently similar fractionations by Method 1*

Fractionation no.	Fraction	Percentage of total N in homogenate	Percentage of total aldolase in homogenate	Relative concn.
1	Supernatants	89.0	49.0	2.90
	Nuclear fraction	9.5	27.6	
	Recovery	98.5	76.6	
2	Supernatants	88.0	90.5	0.68
	Nuclear fraction	10.6	7.2	
	Recovery	98.6	97.7	

Table 3 shows two fractionations by method 1, apparently carried out in exactly the same way, where one nuclear fraction had about four times the activity of the other. In addition, as the recovery was poor in the first fractionation, it is possible that this nuclear fraction actually had much more than 27.6 % of the total activity. While such variations made a moderate difference to the total balance sheet of recovered enzyme, they made a very significant difference to the absolute nuclear activities. An investigation into the origin of this variability is presented in the next paper (Roodyn, 1956).

DISCUSSION

Absence of succinoxidase

The absence of succinoxidase from rat-liver nuclei is not surprising and is probably not an original observation. Indeed the fact that liver nuclei are deficient in cytochrome oxidase (Hogeboom *et al.* 1952) makes it difficult to imagine how nuclei could oxidize succinate with oxygen. The questions whether the nuclear membrane is permeable to the substrates used in the assay or whether the test conditions are suitable to demonstrate any oxidative ability of nuclei have not been investigated. The enzyme has been used in this paper entirely as a guide to methods for revealing the extent of the contribution of contaminants to activity in the nuclear fraction.

Association between aldolase and nuclei

The striking correlation between the distribution of aldolase and nuclei after centrifuging in the graded medium and the observations that the most likely forms of contamination do not contribute greatly to aldolase in the nuclear fraction make it possible to state that aldolase assays carried out on nuclear fractions prepared by the method of Hogeboom *et al.* (1952) mainly measure the activity of the nuclei present. A more detailed study of the significance of this association between nuclei and aldolase is presented in the next paper (Roodyn, 1956).

SUMMARY

1. An attempt has been made to determine whether the succinoxidase and aldolase activities of rat-liver nuclear fractions are due to the nuclei themselves or to contaminant material.

2. The succinoxidase activity of nuclear fractions prepared by the method of Hogeboom *et al.* (1952) was only 1.5 % of the total in the homogenate and was only slightly higher than the percentage of unbroken liver cells in the nuclear fraction. Centrifuging the nuclear fraction in a medium of graded sucrose concentration revealed a complete absence of correlation between the distribution of succinoxidase and of nuclei along the length of the centrifuge tube.

3. In thirty-seven fractionations by the method of Hogeboom *et al.* (1952) the nuclear fraction carried on the average 12.5 % of the total aldolase activity of the liver homogenate, the enzyme being 1.44 times as concentrated in the nuclear fraction as in the original whole tissue.

4. The aldolase activity of seven different nuclear fractions was always greater than the succinoxidase and bore no constant relation to it.

5. Evidence is presented that it is unlikely that fibrous material, erythrocytes, cytoplasmic particles and insufficient washing of the nuclei contribute significant amounts to the aldolase observed in the nuclear fraction.

6. When the nuclear fraction was centrifuged in a medium of graded sucrose concentration a close correlation was observed between the distribution of nuclei and of aldolase along the length of the centrifuge tube. No correlation was observed between the distribution of unbroken liver cells and aldolase.

7. It is concluded that the succinoxidase activity observed in the nuclear fractions was due to contaminant material, but that the aldolase was associated with the nuclei.

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The Enzymic Properties of Rat-liver Nuclei

2. FACTORS AFFECTING THE ALDOLASE ACTIVITY OF RAT-LIVER NUCLEI

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It was established in the previous paper (Roodyn, 1956) that rat-liver nuclei prepared in sucrose-calcium chloride media possess aldolase activity, but that the amount recovered in the nuclear fraction in different experiments was very variable, although apparently the same method of isolation of the nuclei had been used. During the course of an investigation into the origins of this variability certain effects were found to produce changes in the aldolase activity of the nuclei.

METHODS

Maleate buffers. These were prepared by the neutralization with N-NaOH of boiled solutions of maleic anhydride. The buffers were brought to the required ionic strength by adding NaCl.

Preparation of nuclei. Unless indicated otherwise, nuclear fractions were prepared by the method of Hogeboom, Schneider & Striebich (1952) carried out as described

previously (Method 1 of Roodyn, 1956). All other methods, materials and terms used were as described previously (Roodyn, 1956).

RESULTS

Effect of method of cell breakage

Pretreatment of the liver by forcing it through a Latapie mincer caused some fall in activity. (Unminced liver gave a nuclear fraction with aldolase activity of 52.1 units/mg. of N while minced liver from the same rat gave a nuclear fraction with an activity of 46.2 units/mg. of N.) A similar effect was observed by Hogeboom & Schneider (1952) with the diphosphopyridine nucleotide-synthesizing enzyme.

The time of homogenization in the Potter-Elvehjem apparatus was not very critical. Treatment for 6 sec. gave a nuclear fraction with a relative concentration [see Roodyn (1956) for a definition of this term] of 0.75; a further 24 sec. caused this to fall to 0.63 and the same activity was obtained with up to 4 min. homogenization of the liver.

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