accelerates the phosphorolysis of citrulline by mammalian liver (Krebs, Eggleston & Knivett, 1955; Krebs *et al.* 1958).

Related observations. In a brief note Kopper (1956) reported that washed cells of *E. coli*, *Proteus vulgaris* and twelve other un-named species can reduce tetrazolium salts in the presence of various nucleosides but cannot do so on addition of free ribose or ribose phosphates. It is likely that this behaviour is related to the phenomena described in this paper.

SUMMARY

1. Washed intact cells of *Escherichia coli* strain N.C.I.B. 8571, grown semi-anaerobically in a glucose medium, are able to degrade anaerobically D-ribose added in the form of purine and pyrimidine nucleotides or nucleosides. The products formed include carbon dioxide, hydrogen, ethanol, acetic acid and succinic acid.

2. Free ribose and ribose 5-phosphate are not degraded by intact cells grown in a glucose medium, but are fermented by disintegrated cell material obtained by supersonic vibration.

3. Intact cells grown in a medium containing ribose instead of glucose rapidly ferment ribose and 5-phosphate, as well as the ribose moiety of purine and pyrimidine nucleotides.

4. Washed cells grown in a glucose medium acquire the ability to ferment ribose if they are incubated for a few hours in the presence of oxygen, ribose and a source of nitrogen.

5. The observations are in accord with the assumption that the penetration of ribose and ribose 5-phosphate into the cell is mediated by a specific permease.

6. Washed cells also acquire the ability to ferment ribose 5-phosphate (but not ribose) when treated with cetyltrimethylammonium bromide or lysozyme plus ethylenediaminetetra-acetic acid.

These agents presumably cause structural modifications in the cell wall.

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Cholinesterase, Succinic Dehydrogenase, Nucleic Acids, Esterase and Glutathione Reductase in Sub-cellular Fractions from Rat Brain

By W. N. ALDRIDGE AND M. K. JOHNSON

Unit for Research in Toxicology, Medical Research Council Laboratories, Carshalton, Surrey

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Differential centrifuging of homogenates of tissues in sucrose solution has indicated, particularly for liver, that enzymes are localized in particles of various sizes as well as in the soluble fraction. Fractions from liver homogenates have been extensively studied and various enzymes may now be used as markers so that the distribution of other

enzymes may be compared with them (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955).

Initially in this study our aim was to determine the distribution of cholinesterase in nervous tissue, but experiments showed that although a large proportion of the cholinesterase could be sedimented with the microsomal fraction there was also a

substantial amount in the mitochondrial fractions. This paper describes attempts to decide whether cholinesterase is entirely a microsomal enzyme by using the distribution of other enzymes for comparison. While this work was being carried out, Toschi (1959) gave a preliminary account of results of a study of cholinesterase in the microsomal fraction of brain.

METHODS

Special chemicals and reagents. The following chemicals were obtained from the sources indicated: oxidized glutathione (L. Light and Co. Ltd.); bovine serum albumin (fraction V, The Armour Laboratories); phenyl butyrate (British Drug Houses Ltd.); acetylcholine chloride (ACh) (Roche Products Ltd.); reduced triphosphopyridine nucleotide (TPNH) (Sigma Chemical Co., St Louis, Mo., U.S.A.); gelatin (bacteriological) (G. T. Gurr Ltd., London); ethylenediaminetetra-acetic acid (EDTA) (Hopkin and Williams Ltd.); eserine sulphate (Burroughs Wellcome Ltd.). Butyrylcholine perchlorate (BuCh) was prepared as described previously (Aldridge, 1953). Calf-thymus deoxyribonucleic acid (DNA) and yeast ribonucleic acid (RNA) were kindly supplied by Mr C. J. Threlfall, mipafox (*N,N*-diisopropylphosphorodiamidic fluoride) by Dr G. S. Hartley (Fisons Pest Control Ltd.) and 62C47 [1:5-bis-(*p*-trimethylammoniumphenyl)pentan-3-one di-iodide] by Dr F. C. Copp (The Wellcome Foundation Ltd.).

Fractionation procedure. After decapitation of rats the brains were removed, rinsed in ice-cold 0.3M-sucrose, rough-dried and weighed. Two brains were homogenized in 15 ml. of ice-cold 0.3M-sucrose by using a smooth glass tube and Perspex pestle with an all-round clearance of 0.005 in., as previously described (Aldridge, 1957). The homogenate was diluted to 25 ml. with 0.3M-sucrose. The speed and time of centrifuging were varied for different fractions. The letters N, M₁ and M₂, P and S respectively are used to designate the nuclear, two mitochondrial, microsomal and supernatant fractions. The force and time of centrifuging is indicated by 'a-fraction-b', where 'a' is 10⁻³ g, 'fraction' is N, M₁, M₂, P or S, and 'b' is time in min. As an example, 7M₁15 indicates a mitochondrial fraction sedimented from a suspension from which nuclei have previously been removed; the sedimentation force being 7000 g and the time 15 min. All separations were carried out at 0–2° with a Measuring and Scientific Equipment Ltd. refrigerated Angle 13 centrifuge or the Spinco model L centrifuge, and g values quoted refer to the bottom of the centrifuge tubes. With the exception of the microsomal fraction, all fractions were washed once and the wash fluid from each fraction was used to wash the next. The wash fluid and supernatant from the last mitochondrial fraction were combined and the microsomal fraction was then separated from this mixture. With the standard fractionation procedure the following mean recoveries of protein (five experiments, $\pm$ s.d.) were obtained: fraction 1N10, 15.6 $\pm$ 1.8%; 7M₁15, 31.7 $\pm$ 2.0%; 13M₂15, 8.9 $\pm$ 0.4%; 145P60, 16.3 $\pm$ 1.1%; S, 25.8 $\pm$ 0.5%; total recovery 98.3 $\pm$ 1.7%. The mean protein content of rat brain is 88.6 $\pm$ 5.9 mg. of protein (as albumin)/g. wet wt. of brain.

Determination of cholinesterase. Tissue suspensions (0.5 ml.) in 0.3M-sucrose were assayed at 37° manometrically in a medium containing (in final concn.) NaCl

(0.13M), MgCl₂ (35 mM), NaHCO₃ (31 mM), and equilibrated with CO₂ + N₂ (5:95) (Aldridge, 1953). True cholinesterase and pseudocholinesterase were determined from the ratio of the rates of hydrolysis of acetylcholine (13.8 mM) and *n*-butyrylcholine (0.03M) by using the substrate ratios for the enzymes of rat brain determined by Davison (1953). Cholinesterase activity bore a linear relation to the amount of fraction taken, and no increase in activity was found after disruption of particles by repeated freezing and thawing. In experiments with inhibitors, mipafox was incubated with the enzyme for 30 min. at 37° (Aldridge, 1953), whereas inhibition by 62C47 was determined without prior incubation (Bayliss & Todrick, 1956).

Determination of succinic dehydrogenase. The manometric method of Quastel & Wheatley (1938) was used. During other studies on liver mitochondria an iso-osmotic medium (Aldridge, 1957) supplemented with NaHCO₃ and ferricyanide was used, but it became clear that, to obtain maximum and consistent activity, particles must be damaged and a hypotonic medium was used. Reproducible results were obtained when a tissue suspension in water (1 ml.) was assayed at 37° in a medium containing (in final concn.) potassium phosphate (12.5 mM), MgCl₂ (3.5 mM), EDTA (0.6 mM), potassium succinate (22.5 mM), NaHCO₃ (25 mM) and K₃Fe(CN)₆ (12.5 mM). The phosphate, MgCl₂, EDTA and succinate were adjusted to pH 7.4 with KOH before the addition of NaHCO₃. Ferricyanide was in the side arm and the tissue in the centre compartment during gassing with CO₂ + N₂ (5:95). After gassing for 5 min. and equilibration for 5 min., ferricyanide was tipped in from the side arm and readings were taken at 15, 20, 25, 30, and 35 min. Enzyme activity was calculated from the best straight line through these readings (Aldridge, Berry & Davies, 1949).

Determination of glutathione reductase. Tissue suspensions (0.4 ml.) in water were twice frozen and thawed, and assayed in a medium (2.5 ml.) containing potassium phosphate (pH 7.4; 0.05M), EDTA (0.33 mM), oxidized glutathione (0.3 mM) and TPNH (0.083 mM) (McIlwain & Tresize, 1957). The rate of change of extinction at 340 m μ at room temperature (22°) was determined graphically from readings in 1 cm. cells with a Unicam SP. 500 spectrophotometer. The rate of oxidation of TPNH in the presence and absence of glutathione was determined, the difference being glutathione reductase activity. Oxidation of TPNH in the absence of glutathione was significant for the mitochondrial fraction (7M₁15), being 30–40% of the glutathione reductase activity.

Determination of esterase activity. Tissue suspensions (0.5 ml.) in 0.3M-sucrose were assayed at 37° in a medium containing (in final concn.) NaHCO₃ (13 mM) and gelatin (1%, w/v) (Aldridge, 1954). Phenyl butyrate was added as an emulsion (70 μ l./flask) in the NaHCO₃-gelatin medium. Tissue suspensions were tipped in from the side arm after gassing and equilibration with CO₂ + N₂ (5:95). With this procedure the output of CO₂ was not quite linear with time. Reduction of the substrate concentration to 23 μ l./flask and the addition of bovine serum albumin (1.5%, w/v) and MgCl₂ (35 mM) rectified this but made no difference to the relative activities of different fractions from brain or to homogenate activity.

Determination of nucleic acids. The method of Logan, Mannell & Rossiter (1952) was used except that 8% (w/v) perchloric acid was used instead of 10% (w/v)

trichloroacetic acid for the original precipitation, and 3% perchloric acid instead of 5% trichloroacetic acid for extraction of nucleic acid: final readings were at 266 m μ . All materials were kept ice-cold throughout procedures before the final acid extraction. Extraction of albumin gave low extinctions at 266 m μ , and the sum of extinctions obtained on separate extraction of albumin and RNA equalled that obtained from mixtures of these materials. DNA used as standard was a preparation from calf thymus (Gulland, Jordan & Threlfall, 1947), and the RNA was a commercial preparation from yeast purified by repeated solution in alkali and reprecipitation with acid. At 266 m μ both these preparations had a molar extinction coefficient (based on P content) of 9930.

Determination of protein. Protein was precipitated with 8% (w/v) perchloric acid. The centrifuged pellet was rinsed with 5 ml. of ethanol-ether (55:45) and resuspended in 10 ml. of ethanol-ether at room temperature for 30 min. After centrifuging, the defatted pellet was dissolved in 1 ml. of 3% (w/v) NaOH with warming and the proteins determined by the method of Robinson & Hogden (1940) as modified by Aldridge (1957). Opalescence in the final coloured solution was avoided by this removal of lipid. The results have been expressed as milligrams of albumin.

RESULTS

Our early studies on cholinesterase showed that it was diffusely distributed among the fractions we were preparing. We therefore switched to the study of some probable mitochondrial and nuclear markers to assess the extent of mutual contamination of fractions. These results are reported first, and on them we based our modified centrifuging conditions for further cholinesterase study. Finally we studied distribution of esterase and glutathione reductase for comparison with cholinesterase.

Succinic dehydrogenase. Previously (Aldridge, 1957) it was shown that a homogenizer with an all-round clearance of 0.01 in., although suitable for the isolation of liver mitochondria, was unsuitable for brain since a large proportion of the succinic dehydrogenase sedimented at 1000 g and was presumably associated with unbroken cells. Experiments were therefore carried out to check that the homogenizer with an all-round clearance of 0.005 in. was breaking the majority of cells. Further work was done to select centrifugal speeds and time such that succinic dehydrogenase could be obtained in two mitochondrial fractions. This was important, since in our initial experiments some cholinesterase sedimented in the mitochondrial fraction and we wished to compare the specific activities of succinic dehydrogenase and cholinesterase of two mitochondrial fractions. After sedimentation at 1000 g for 10 min., only 10% of the succinic dehydrogenase was found in the nuclear fraction (Table 1). Decrease of the homogenizer clearance from 0.005 to 0.002 in. did not appreciably lower the succinic dehydrogenase content of the nuclear fraction

(Table 2). Most of the succinic dehydrogenase had sedimented after 13 000 g for 15 min. As our standard procedure therefore we have used 1000 g for 10 min. for the nuclear fraction, 7000 g and 13 000 g for 15 min. to give two successive mitochondrial fractions, and a final 145 000 g for 1 hr. for the microsomal fraction. No attempt was made to subfractionate the microsomal fraction. The distribution of succinic dehydrogenase with this technique is shown in Table 2.

Nucleic acids. DNA is accepted as a suitable marker for nuclei (Davidson, 1957). In view of the large range of nuclear size in brain (Ham, 1953) it was desirable to have a quantitative measure of nuclear contamination of other fractions. With the

Table 1. *Sedimentation of succinic dehydrogenase from homogenates of rat brain by different centrifugal speeds and times*

Fraction	Percentage of total homogenate activity	
	Sedimented	Not sedimented
0.7N10	10.4	—
1.0N10	11.7, 9.6, 7.1	—
1.3N10	16.1	—
4M ₁ 15	—	18.4
5M ₁ 15	—	15.2, 11.5
7M ₁ 15	—	9.1
9M ₁ 15	—	6.5
10M ₁ 15	—	3.5, 5.9
13M ₁ 15	—	3.3, 5.4

Table 2. *Comparative distribution of succinic dehydrogenase and cholinesterase in fractions from homogenates of rat brain*

Two homogenates (homogenizer, all-round clearance 0.005 in.) of two rat brains each were combined and then divided in two. One (Exp. 2) was rehomogenized (homogenizer, clearance 0.002 in.). SD, succinic dehydrogenase; ChE, cholinesterase.

Fraction	Recovery of homogenate activity in fraction (%)			
	Expt. 1		Expt. 2	
	SD	ChE	SD	ChE
1N10	7.7	8.7	6.1	7.5
7M ₁ 15	70.4	26.8	65.7	24.4
13M ₂ 15	13.6	15.3	11.5	14.1
145P60	5.8	37.2	4.6	45.3
S	2.4	10.3	0.2	12.1
Total	99.9	98.3	88.1	93.4
Homogenate activity (μ l. of CO ₂ /g. of brain/hr.)	13 800	12 200	15 600	11 200

Table 3. *Distribution of nucleic acids in fractions from homogenates of rat brain*

Values are the mean of results from two experiments (except for the supernatant, which is one experiment only). The values for specific activity ($\mu\text{g. of nucleic acid P/mg. of protein}$) were calculated with the protein distribution given in the Methods section. The mean nucleic acid content of the brains used was: total nucleic acid, 225; DNA, 135; RNA, 90 $\mu\text{g. of nucleic acid P/g. wet wt.}$

Fraction	Total nucleic acid (%)	DNA		RNA	
		(%)	($\mu\text{g. of P/mg. of protein}$)	(%)	($\mu\text{g. of P/mg. of protein}$)
1N10	62.9	77.2	7.6	41.6	2.8
7M ₁ 15	9.6	8.4	0.4	11.5	0.4
13M ₂ 15	3.5	1.7*	0.3*	6.4	0.7
145P60	18.2	1.8*	0.2*	42.2	2.7
S	3.9	2.7*	0.2*	5.9*	0.2*
Homogenate	100	100	1.5	100	1.0
Total of fractions	102.3	90.8	—	106.4	—

* Results were based on extinctions of <0.10 and therefore of low accuracy.

Table 4. *Distribution of cholinesterase in fractions from homogenates of rat brain*

Results are given as mean $\pm$ s.d. of seven experiments for percentage total cholinesterase (ChE), and of four experiments for units/mg. of protein. The values for true cholinesterase and pseudocholinesterase were obtained from a single experiment. Substrates are as indicated and interference corrections are described in the Methods section. 1 unit of ChE activity is 1 $\mu\text{l. of CO}_2/\text{hr.}$ Total ChE activity (ACh) of brain was $11\,700 \pm 1200$, true ChE (ACh) 11 100 and pseudo ChE (BuCh) 1530 units/g. wet wt.

Fraction	Total ChE (ACh)		True ChE (ACh)		Pseudo ChE (BuCh)	
	(%)	(Units/mg. of protein)	(%)	(Units/mg. of protein)	(%)	(Units/mg. of protein)
1N10	9.1 $\pm$ 0.8	78 $\pm$ 11	6	55	36	47
7M ₁ 15	26.9 $\pm$ 1.6	113 $\pm$ 9	27	116	19	11
13M ₂ 15	14.0 $\pm$ 1.2	216 $\pm$ 18	16	226	7	14
145P60	38.2 $\pm$ 4.3	316 $\pm$ 28	38	305	24	26
S	13.6 $\pm$ 2.3	70 $\pm$ 13	9	44	27	19
Homogenate	100	136 $\pm$ 9	100	133	100	18
Total of fractions	101.5 $\pm$ 2.9	—	96	—	113	—

Fig. 1. Histogram of the specific activities of enzymes and RNA in sub-cellular fractions of rat brain. ■, Succinic dehydrogenase; ▨, cholinesterase; ▩, esterase; □, RNA. Specific activities of homogenate were taken as 100 for comparison, and (per mg. of protein) were: succinic dehydrogenase, 165 $\mu\text{l. of CO}_2/\text{hr.}$; cholinesterase, 136 $\mu\text{l. of CO}_2/\text{hr.}$; esterase, 220 $\mu\text{l. of CO}_2/\text{hr.}$; RNA, 1.0 $\mu\text{g. (P)}$.

standard fractionation procedure, DNA is predominantly located in the nuclear fraction with a small contamination of the first mitochondrial fraction but with insignificant amounts in other fractions (Table 3). RNA is located both in the nuclear and microsomal fraction (Table 3 and Fig. 1). These distributions of nucleic acids are very similar to those obtained by Brody & Bain (1952).

Cholinesterases. The initial experiments showed that most of the cholinesterase was not sedimented in the nuclear or mitochondrial fractions. Upon high-speed centrifuging 35–40 % of the cholinesterase sedimented in the microsomal fraction (Table 4).

There was, however, 27 % of the cholinesterase activity in the heavy mitochondrial fraction (7 M₁15, 105 000 g. min.) and 14 % in the light mitochondria (13 M₂15, 195 000 g. min.). This is considerably more than is obtained by microsomal contamination of liver mitochondria. For instance, de Duve *et al.* (1955) recovered 2.7 % of the liver glucose 6-phosphatase in their heavy (33 000 g. min.) and 7 % in their light mitochondrial fractions (250 000 g. min.). Since brain has a very low glucose

6-phosphatase activity (Dixon & Webb, 1958) this enzyme cannot be used there as a microsomal marker. The specific activities of cholinesterase in brain fractions (Table 4 and Fig. 1) show that the distribution is consistent with a large microsomal contamination of the mitochondrial fractions. RNA is also concentrated in the microsomes but the proportion found in the mitochondrial fractions is much less than with cholinesterase (Table 3 and Fig. 1).

Use of acetylcholine and *n*-butyrylcholine to determine true cholinesterase and pseudocholinesterase in the various fractions reveals a marked difference in the distribution of the two enzymes (Table 4). The specific activity of pseudocholinesterase is highest in the nuclear fraction, though it is present in the other fractions. The pseudocholinesterase of the nuclear fraction was inhibited by 6 μ M-mipafox and unaffected by 0.1 mM-62C47, in agreement with results on whole rat brain obtained by Davison (1953) and Bayliss & Todrick (1956), respectively. The ratio of rates of hydrolysis of sub-

strates by this enzyme (butyrylcholine:acetylcholine) was found to be 141:100, in excellent agreement with Davison (1953). Since 90% of the cholinesterase activity is due to true cholinesterase the distribution of this enzyme is not appreciably different from that of the total cholinesterase activity.

Esterase. With phenyl butyrate as a substrate the distribution of esterase in rat-brain fractions was examined. The distribution is fairly similar to that of cholinesterase, the only difference being that a lower percentage of esterase is always found in the microsomal fraction (Table 5 and Fig. 1).

Glutathione reductase. In view of the unequal distribution of RNA, cholinesterase and esterase, other enzymes were examined. McIlwain & Tresize (1957) demonstrated the high rate of oxidation of reduced TPNH by oxidized glutathione in cerebral tissues, and the results in Table 6 show that the glutathione reductase was present mainly in the mitochondrial fraction and in the supernatant. Washing with buffered electrolyte solution (pH about 7.5) containing glycylglycine, potassium chloride and magnesium chloride did not remove the activity from the mitochondrial fraction. These results are different from those for liver: Rall & Lehninger (1952) found that after centrifuging liver homogenate at 20 000 *g* for 20 min., 90% of the glutathione reductase was present in the supernatant fraction.

Table 5. *Distribution of esterase in fractions from homogenates of rat brain*

1 unit of activity is 1 μ l. of CO₂/hr. Results are the mean $\pm$ S.D. of three experiments. The mean activity of brain is 20 000 $\pm$ 1750 units/g. wet wt. In one experiment it was shown that the esterase in each of the fractions was not inhibited by 20 μ M-eserine.

Fraction	Activity	
	(%)	(Units/mg. of protein)
1N10	13.1 $\pm$ 1.1	171 $\pm$ 28
7M ₁ 15	29.1 $\pm$ 0.3	199 $\pm$ 32
13M ₂ 15	7.7 $\pm$ 0.5	202 $\pm$ 25
145P60	26.8 $\pm$ 3.5	406 $\pm$ 55
S	14.5 $\pm$ 1.1	127 $\pm$ 23
Homogenate	100	220 $\pm$ 28
Total of fractions	91.2 $\pm$ 4.1	—

DISCUSSION

It is necessary to establish that we have chosen suitable markers for mitochondria and nuclei.

Although most of the evidence for the use of succinic dehydrogenase as a marker enzyme for mitochondria has been obtained with liver (Schneider & Hogeboom, 1956; de Duve *et al.* 1955; Kuff, Hogeboom & Dalton, 1956), mitochondria from other tissues such as heart, skeletal muscle and kidney also contain succinic dehydrogenase. There is no reason from other work (Abood, Gerard, Banks & Tschirgi, 1952; Brody & Bain, 1952) or from the present study to believe that brain mitochondria differ in this respect. On the basis that succinic dehydrogenase is exclusively located in the mitochondria, the nuclear fraction contains 7–10% of either unbroken cells or mitochondria.

The distribution of DNA is accepted as a marker for nuclei (Davidson, 1957). The distribution of DNA obtained in this study is essentially the same as that found by Abood *et al.* (1952) and Brody & Bain (1952), and indicates a contamination of our first mitochondrial fraction (7 M₁15) with 10–15% of the nuclear DNA. This is confirmed by histological examination.

Having chosen our fractionation procedure, it

Table 6. *Distribution of glutathione reductase in fractions from homogenates of rat brain*

1 unit of glutathione reductase activity is a change in extinction at 340 m μ of 1.0/min., measured in 1 cm. cells at room temperature (22°), the final volume being 2.5 ml. Activity of brain is 1.82 units/g. wet wt.

Fraction	Activity	
	(%)	(Units/100 mg. of protein)
1N10	11.9	1.56
7M ₁ 15	28.3	1.84
13M ₂ 15	7.1	1.64
145P60	9.5	1.20
S	43.9	3.50
Homogenate	100	2.05
Total of fractions	100.7	—

became obvious that cholinesterase was present in highest quantity and specific activity in the microsomal fraction but was also present to a considerable extent in the mitochondrial fraction. The specific activity increased from fraction 7 $M_{1.15}$ through 13 $M_{1.15}$ and was maximal in the microsomal fraction (Fig. 1).

When we compare the distribution of cholinesterase with that of esterase and RNA, both of which we find in the microsomal fraction to a considerable extent, there is no clear correlation. We showed that esterase is distinct from cholinesterase in that the former activity in whole homogenate and in microsomes was not affected by 20 μ M- eserine, whereas the cholinesterase of these fractions was about 95 % inhibited. The distribution of esterase is similar to that of cholinesterase, but in our opinion is sufficiently different not to be considered identical (Fig. 1): whether this is a real difference in distribution or a technical artifact must remain in doubt because the recovery of esterase from the fractions was persistently lower (91 %) than that of cholinesterase (101 %). The possibility must also be considered that the hydrolysis of phenyl butyrate is due to more than one enzyme (Myers, 1956). The distribution of RNA is bimodal, highest concentrations being found in the nuclear and microsomal fractions (Table 3; cf. Aboud *et al.* 1952; Brody & Bain, 1952). From the contamination data deduced from DNA-distribution studies, it may be assumed that some RNA found in the heavy mitochondrial fraction originated in the nuclei. Even if this were not so, the percentage of 'microsomal' RNA in the mitochondrial fractions is considerably less than that of cholinesterase and the distributions are clearly different (Fig. 1).

In work carried out concurrently with ours it has been shown that microsomal fractions from rat brain (Hanzon & Toschi, 1959; Toschi, 1959) contain both cholinesterase and RNA. The cholinesterase may be partially separated from RNA by centrifuging and by various treatments such as with deoxycholate, some detergents or ribonuclease. Toschi has correlated these findings with electron micrographs of the fractions and considers that cholinesterase is concentrated in a 'heavy' fraction whereas RNA is associated with electron-dense granules which are concentrated in a 'light' fraction. Frontali & Toschi (1958) have reported a similar association of cholinesterase with membranous structures present in sub-cellular fractions from electric tissue of *Torpedo*.

Glutathione reductase was examined in the hope of finding a similarity to cholinesterase distribution, but its two peaks of activity appear in the mitochondrial and supernatant fractions with little in the microsomes (Table 6). The presence of some enzyme in a particulate fraction probably explains

the activation which was reported to be obtained by treating brain homogenates with hypotonic solutions (McIlwain & Tresize, 1957).

A comparison of the distribution of enzymes in liver with those in brain reflects the different complexity of organization in these two tissues. Liver contains a preponderant mass of cells of similar size, whereas brain contains a great variety from large neurons down to microglia, and it is not therefore surprising that, with brain, less clear-cut conclusions can be reached. It may be that the present homogenization and centrifuging procedures will never be suited to separation of brain-cell components, and the failure to obtain brain mitochondria capable of sustained oxidative phosphorylation (Aldridge, 1957) may also reflect the inadequacy of this technique. However, in liver, RNA distribution is bimodal (Hogeboom & Schneider, 1955), as in brain, and there is some indication that microsomal diphosphopyridine nucleotide-cytochrome *c* reductase may be separated into different ranges of particle size (Kuff *et al.* 1956). Esterase has also been found mainly in the microsomal fraction from liver (Omachi, Barnum & Glick, 1948; Novikoff, Podber, Ryan & Noe, 1953). The small amount of cholinesterase present in rat liver was also mainly present in the microsomal fraction (Underhay, Holt, Beaufay & de Duve, 1956). Similar distributions are reported for glucose 6-phosphatase, esterase and cholinesterase (Underhay *et al.* 1956), about 60-70 % being found in rat-liver microsomes, and the distribution of RNA has been reported to be similar to that of esterase (Novikoff *et al.* 1953). It is obviously undecided whether RNA and microsomal enzymes are located in the cell on different structures, or whether the differences in distribution are artifacts produced by the homogenization procedure.

The finding of so large a proportion as 36 % of total pseudocholinesterase in the nuclear fraction is worthy of comment. This is a higher proportion than can be explained by the presence of unbroken cells. As previously pointed out, the maximum amount of unbroken cells must be 7-10 % of the total, assuming the general distribution of succinic dehydrogenase throughout the cellular matter of brain (Burgen & Chipman, 1951; Shimizu & Morikawa, 1957). Some evidence has been given on the other hand that pseudocholinesterase is present mainly in the glial cells and especially fibrous astrocytes (Koelle, 1950, 1954; Cavanagh, Thompson & Webster, 1954). It is therefore possible that many of the unbroken cells are glial cells; histological examination has been made but no definite conclusions were drawn. The original suggestion from histochemical work (Koelle, 1950) that the nuclei or the nuclear membrane in glial cells contain pseudocholinesterase in high concentrations has not

been substantiated (Koelle, 1954; Ravin, Zacks & Seligman, 1953; Crevier & Bélanger, 1955). The possible diffusion of pseudocholinesterase from the cytoplasm to the nuclei after sectioning (Koelle, 1951) indicates that much caution must be used before assuming that the nuclei contain pseudocholinesterase. However, other workers have found cholinesterase in the nuclear fraction from liver (Underhay *et al.* 1956), and nuclei isolated from rat liver by the method of Dounce (1950) contained one-quarter of the pseudocholinesterase (Koelle, 1951).

A snare inherent in all fractionation studies is the possibility of the absorption of soluble material on to particulate matter as happens with arginase (Rosenthal, Gottlieb, Gorry & Vars, 1956). In that case it was shown that the enzyme was readily freed from the nuclear and microsomal fractions by simple washing with iso-osmotic electrolyte solution. We therefore washed fractions of brain with buffered iso-osmotic solutions containing potassium chloride and magnesium chloride: pseudocholinesterase was not removed from nuclear or microsomal fractions, nor were true cholinesterase and glutathione reductase removed from mitochondrial fractions.

SUMMARY

1. Homogenates of rat brain in 0.3M-sucrose have been separated by centrifuging into a nuclear fraction, two mitochondrial fractions, a microsomal and a supernatant fraction.

2. Succinic dehydrogenase and deoxyribonucleic acid have been used to assess the contamination of fractions by mitochondria and nuclei, respectively.

3. Of the true cholinesterase, 38 % is found in the microsomal fraction with a specific activity 2.6 times that of the homogenate. It was not possible to obtain other fractions free from cholinesterase.

4. The distribution of ribonucleic acid and cholinesterase is different.

5. Esterase (substrate, phenyl butyrate) has a distribution similar to but not identical with that of cholinesterase.

6. The nuclear fraction contains 36 % of the pseudocholinesterase, the microsomal fraction 24 % and the supernatant fraction 27 %.

7. Of the glutathione reductase, 35 % is present in the mitochondrial and 44 % in the supernatant fractions.

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