result from a change of physical structure of microsomes in electrolyte solutions. After exposure to non-ionic detergents the time-course became linear for 20–30 min.

4. The microsomal adenosine triphosphatase was inhibited by phospholipase C from *Clostridium welchii* which removes phosphorylcholine from lecithin. This effect was prevented by antisera to *C. welchii* and by omitting Ca^{2+} ions from the medium. Neither phospholipase A nor neuraminidase significantly altered the adenosine-triphosphatase activity.

5. These findings indicate that structural factors are important in determining the behaviour of the Na^+ ion-activated adenosine triphosphatase.

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Constituents of a Microsomal Fraction from the Mammalian Brain: their Solubilization, especially by Detergents

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Many characteristics of neural systems have been ascribed to membrane phenomena, and, on mechanical disruption of cerebral tissues, their cellular membranes appear in microsomal fractions (Toschi, 1959; Hanzon & Toschi, 1959; Wherrett &

McIlwain, 1962). These fractions, like the central nervous system itself, are rich in cholesterol and phospholipids, and also carry appreciable quantities of glycolipids and proteins including specific enzymes such as cholinesterase (Toschi, 1959) and

the Na^+ ion-plus- K^+ ion-activated adenosine triphosphatase (Schwartz, Bachelard & McIlwain, 1962). Certain of these components, though themselves water-soluble (e.g. gangliosides and cholinesterase), remain firmly attached to microsomal particles in most aqueous systems. Initial stages in the biochemical examination of such components, as is exemplified by the investigation of the Na^+ ion-plus- K^+ ion-activated adenosine triphosphatase by Swanson, Bradford & McIlwain (1964), thus consist of solubilizing or dispersing the microsomes, and procedures for this have now been examined. These procedures include, especially, the use of detergents, and during the study the opportunity has been taken to examine the nature of the interaction between these agents and the microsomal fraction.

The cationic detergent cetyltrimethylammonium bromide and the anionic detergent sodium deoxycholate are the ionic surface-active agents whose solubilizing effects were examined initially in the investigation, and digitonin (a steroid glycoside of the saponin class) was a non-ionic agent subsequently examined. The disrupting effects of ultrasonic irradiation on the microsome fraction have also been investigated.

EXPERIMENTAL

Preparation and treatment of microsomal fractions

Tissue dispersion and fractionation. The dispersion and fractionation of guinea-pig cerebral grey matter was carried out as described by Schwartz *et al.* (1962). The microsomal pellet was suspended in 0.32M-sucrose at a concentration such that the yield of microsomal material from 1 g. wet wt. of material was contained in 5–6 ml. of sucrose solution. This gave a microsomal protein concentration of 2.0–2.5 mg./ml.

Storage. The fractions were kept overnight in a deep-freeze at -20° in the 0.32M-sucrose in which they were prepared and treatments were carried out the following day.

Treatments with detergents. Fractions were treated at 0° , for periods ranging from 10 to 60 min. The agents were added in aqueous 0.32M-sucrose to microsomal material from approx. 1 g. of grey matter suspended in 6 ml. of 0.32M-sucrose; 6 ml. of sucrose was added to the microsomal suspension used for the control experiment. At the end of the treatment period the suspensions were centrifuged in the no. 40 head of the Spinco model L preparative ultracentrifuge at 105000g for 60 min. at 0° . The supernatants were pipetted off, leaving 0.1–0.2 ml. in contact with the pellets, which were then resuspended in 0.32M-sucrose in an ice-cold Teflon homogenizer (A. H. Thomas Co., Philadelphia, Pa., U.S.A.) with a clearance of 0.13–0.18 mm.

Treatment by ultrasonic irradiation. These treatments were carried out at 0° with a titanium probe 19 mm. at the tip, attached to an ultrasonic disintegrator which operated at 18–20 kcy./sec. (Measuring and Scientific Equipment Ltd., Birmingham). The tip of the probe was immersed to

2 mm. below the surface of the microsomal suspension contained in a thick-walled glass vessel (10.5 cm. $\times$ 2.5 cm. diam.) which was cooled in an ice bath. The material was treated by ultrasonics for 2–5 min. periods, followed by 4 min. cooling periods. The treated suspension was centrifuged and the pellet and supernatant fractions were obtained as described above.

Reagents and analyses

Reagents. Sodium deoxycholate was supplied by L. Light and Co. Ltd., Colnbrook, Bucks., cetyltrimethylammonium bromide (Cetavlon) was from Imperial Chemical Industries Ltd., Wilmslow, Cheshire, and digitonin was a sample of analytical grade supplied by British Drug Houses Ltd., Poole, Dorset.

Determination of protein. This was by the method of Lowry, Rosebrough, Farr & Randall (1951). Crystalline bovine plasma albumin was used as standard. When sucrose was present in the samples analysed it was included in similar concentrations in the albumin standards, as it diminished colour formation. When detergents were present in the samples they were included in the control samples in similar concentrations; they did not appreciably influence the colour formation in these standards.

Determination of phospholipid phosphorus. Samples (1 ml.) were pipetted into 1 ml. of 1.3N- HClO_4 and the mixtures were allowed to stand for 15 min. in the cold before being centrifuged at 1800g for 15 min. The supernatants were decanted off and the pellets, consisting of the precipitated tissue sample, were drained. The pellets were extracted twice by resuspension in 5 ml. of 0.65N- HClO_4 , standing in the cold for 15 min. and centrifuging at 1800g for 15 min. The pellets were drained after each extraction. The residues from this treatment were shaken for 30 sec. with two 5 ml. volumes of chloroform-methanol (2:1, v/v) and kept for 15 min. at room temperature before being centrifuged at 1800g for 15 min. The clear supernatant was pipetted off, leaving 0.1–0.2 ml. in contact with the pellets. The combined chloroform-methanol extracts were made up to a volume of 10 ml. and 2 ml. duplicate samples were evaporated to dryness at 60° in a stream of air. The residue was digested with 10N- HClO_4 , and inorganic phosphate was determined by the method of Martin & Doty (1949). The presence of the detergents did not interfere with the assay, and recoveries of phospholipid phosphorus were always about 100%.

Determination of cholesterol. This determination was carried out as described by Schwartz *et al.* (1962). The presence of detergents in the samples did not interfere with the assay except for digitonin, which caused low recoveries of cholesterol. The assay was modified to include the removal of the digitonin from the lipid extract. The chloroform-methanol extract was partitioned against 0.2 vol. of aq. 0.1M-KCl and washed by shaking with 0.2 vol. of 'upper phase mixture' of composition 0.1M-KCl-methanol-chloroform (47:48:3, by vol.). The lower phase was evaporated to dryness and the residue was redissolved in 1 ml. of chloroform-methanol (2:1, v/v); 10 ml. of diethyl ether was added and the mixture was kept for 10 min. in the cold. The resulting white suspension was centrifuged at 10° at 1500g for 15 min., and the clear supernatant was removed with a pipette and evaporated to dryness *in vacuo*. The residue was dissolved in 1 ml. of light petroleum (b.p. 40 – 60°), and 3 ml. of acetone was added followed by

0.5 ml. of ethanolic saturated $MgCl_2$. The remainder of the determination was as described by Schwartz *et al.* (1962).

Assay of adenosine triphosphatase. This employed the procedure of Swanson *et al.* (1964).

Thin-layer chromatography of lipids. Chromatographic plates were prepared with Kiesel-Gel G (E. Merck, Darmstadt, Germany) by using the apparatus of Stahl [Camlab (Glass) Ltd., Cambridge]. Chromatography was carried out with the solvent systems described by Wagner, Hörhammer & Wolff (1961). Lipids were identified by the spray reagents employed by these workers, and by chromatography of known lipid markers in parallel with the lipid extracts. Samples of the pellet and supernatant fractions were evaporated to dryness at 60° in a steam of air. The residues were extracted with chloroform-methanol (2:1, v/v), and the extracts were filtered and partitioned against 0.2 vol. of 0.1M-KCl. After brief centrifugation to effect separation of phases, the upper phase was discarded, and the lower chloroform-rich phase was stored at -20° for chromatography. Samples of extracts from supernatant and pellet fractions were chromatographed side by side, and differences in lipid composition were judged visually.

RESULTS

Composition of the microsomal fraction

The amounts of certain constituents of the microsomal fraction have been compared with the amounts in the whole dispersion of cerebral cortex (Table 1). These values are in close agreement with the results obtained by Schwartz *et al.* (1962) for material prepared by the same method. These workers have also observed low concentrations of RNA and succinate dehydrogenase in the microsomal fraction, suggesting that microsomes so prepared are relatively free from ribosomes and from contaminating mitochondria. The percentage of the total phospholipid of the tissue found in this fraction was similar to that of cholesterol. The amount of extractable *N*-acetylneuraminic acid contributed by the gangliosides was similar to that observed by Wherrett & McIlwain (1962).

Assuming that 1 mg. of phospholipid phosphorus was derived from 25 mg. of phospholipid, it is also apparent from Table 1 that protein, phospholipid and cholesterol together make up over 80% of the dry weight of the microsomal fraction.

Effects of agents on microsomal fraction

Treatment with sodium deoxycholate and Cetavlon. After exposure of the microsomal suspension to concentrations of sodium deoxycholate up to 2.4 mM for 10 min. at 0°, only 1-5% of the lipid and protein was solubilized. At three times this concentration of sodium deoxycholate, however, about two-thirds of the protein and one-half of the cholesterol and phospholipid was released, together with one-third of the total Na^+ ion-stimulated adenosine-triphosphatase activity (Fig. 1).

Table 1. *Composition of the microsomal fraction*

Dispersions of guinea-pig cerebral cortex were fractionated by centrifugation, as described in the text. The values quoted are means ($\pm$ s.d. when appropriate) of the numbers of observations in parentheses.								
	Dry wt. (mg./g. of tissue)	Protein (mg./g. of tissue)	Phospholipid P (μ g./g. of tissue)	Cholesterol (mg./g. of tissue)	RNA (μ g./g. of tissue)	Succinate- dehydrogenase activity (μ moles of CO ₂ /g. of tissue/hr.)	Ganglioside N-acetyl- neuraminic acid (μ g./g. of tissue)	
Original dispersion of cerebral cortex	—	96.8 $\pm$ 1.7 (5)	1585 $\pm$ 80.5 (5)	14.3 $\pm$ 0.8 (4)	2404 $\pm$ 239 (4)	530 (2)	752*	
Microsomal fraction	29.6 (2)	14.3 $\pm$ 0.4 (5)	290 $\pm$ 30.0 (5)	2.8 $\pm$ 0.4 (4)	265 $\pm$ 17 (4)	8.2 (2)	300 (3)†	

* Determined by Bial's orcinol method (Wherrett & McIlwain, 1962).
† Determined by thiobarbituric acid method (Swanson *et al.* 1964).

* Determined by Bial's orcinol method (Wherrett & McIlwain, 1962).
† Determined by thiobarbituric acid method (Swanson *et al.* 1964).

Microsomes were exposed to Cetavlon for 10 min. at 0°. This detergent was ineffective in solubilizing at 0.7 mM, but at 2.7 mM it released considerable proportions of both protein and lipid from the microsomes (Fig. 1*b*). Although about one-half of the total Na⁺ ion-stimulated adenosine-triphosphatase activity was recovered in the supernatant after exposure to 2.7 mM-Cetavlon, the recovery of the original activity was decreased to 13% compared with 90% recovery after treatment with 0.7 mM-Cetavlon, where little solubilization of enzyme or microsomal constituents had occurred.

The percentage release of cholesterol and phospholipids was similar with both of these agents, but 10% more of the protein was solubilized than of either phospholipid or cholesterol, thus leaving the pellet slightly enriched in lipid.

On a molar basis, Cetavlon solubilized more effectively than sodium deoxycholate in the con-

centration range examined. For example, at a concentration of 2.7 mM, Cetavlon solubilized approximately two-thirds of the protein, cholesterol and phospholipid, whereas under the same conditions of treatment sodium deoxycholate solubilized only about one-fifth of these microsomal constituents.

Chromatography of the lipid extracts on thin layers of silicic acid indicated that lipids were present in the pellet and supernatant fractions in the same relative concentrations as in the original microsomes, showing that there had been no preferential solubilization of the individual lipids.

Treatment with digitonin. It was possible with digitonin to make use of the well-established interaction between digitonin and 3 α -hydroxy steroids which, with cholesterol, produces a 1:1 molecular complex. Concentrations of the agent were, therefore, selected which included a 1:1 molar ratio between the cholesterol present in the microsomal sample and the added digitonin. The amount of microsomal protein released into the supernatant increased up to 20% of the total as the digitonin concentration was increased from zero to 0.8 mM, at which concentration the digitonin was present in equimolar concentration with microsomal cholesterol (Fig. 2). A further 30% of the total protein was released at concentrations from 1.2 to 5.6 mM, and in all treatments there was a greater release of

Fig. 1. Solubilization of microsomal constituents by increasing concentrations of (a) deoxycholate and (b) Cetavlon. Microsomal suspensions in 0.32M-sucrose equivalent to 1 g. of tissue and containing 2.26 mg. of microsomal protein/ml. were treated for 10 min. at 0° with equal volumes of the surface-active agent in 0.32M-sucrose. After treatment, the mixture was fractionated as described in the text. Protein (○), cholesterol (△), phospholipid phosphorus (●) and Na⁺ ion-stimulated adenosine triphosphatase (□) were determined in the pellet and supernatant fractions.

Fig. 2. Solubilization of microsomal constituents by increasing concentrations of digitonin. Microsomal suspensions in 0.32M-sucrose equivalent to 1 g. of tissue were treated for 60 min. at 0° with equal volumes of digitonin in 0.32M-sucrose. After centrifugal fractionation, as described in the text, protein (○), cholesterol (△), phospholipid phosphorus (●) and Na⁺ ion-stimulated adenosine triphosphatase (□) were determined in the pellet and supernatant fractions. The point at which the cholesterol in the sample was equimolar with the added digitonin is indicated with an arrow.

Table 2. *Stimulation of adenosine-triphosphatase activity by digitonin*

Microsomal suspensions in 0.32M-sucrose from approx. 1 g. of tissue were treated at 0° for 60 min. with equal volumes of digitonin solutions in 0.32M-sucrose. After treatment the mixtures were centrifuged at 104000g for 60 min. to yield supernatant and pellet fractions. Mean values were obtained from two experiments.

Concn. of digitonin (mM)	Adenosine triphosphatase				
	Total recovered activity (μ moles of inorganic phosphate liberated/g. of tissue/hr.)	Specific activity (μ moles of inorganic phosphate liberated/mg. of protein/hr.)		Total activity in supernatant (%)	Phospholipid P in supernatant fraction (%)
		Pellet	Supernatant		
0	461	40	0	0	0
1.6	673	70	16	5	1
2.0	681	64	16.5	7	2
2.4	578	51	19	10	5
2.8	467	43	27	29	31
3.2	460	41	30	41	45

protein than of total lipids into the supernatant. All phospholipid present in the microsomal samples was recovered in the pellet fractions after treatment with digitonin at concentrations of 2.0 mM and below, but 35 % remained unsedimented after exposure to a concentration of 2.8 mM. The pattern of release of the Na^+ ion-stimulated adenosine-triphosphatase activity between concentrations of 2.0 and 2.8 mM resembled that of the phospholipid rather than that of the protein, showing a marked increase from 12 to 48 % of the total in this range.

The Na^+ ion-stimulated hydrolysis of ATP by this enzyme was considerably accelerated (Table 2) by exposure to concentrations of digitonin up to 2.0 mM; beyond 2.0 mM this stimulating effect of digitonin was greatly diminished, and the enzyme, together with the phospholipids, began to be solubilized (Swanson *et al.* 1964).

The cholesterol was almost all (94 % of the total) recovered in the pellet fraction after treatment of the microsomal fraction with concentrations of digitonin up to 2.4 mM, but 15 % was solubilized at concentrations greater than 2.8 mM, when phospholipids began to appear in the supernatant fractions (Fig. 2).

To ascertain whether the action of the digitonin had gone to completion, the time-course of the release of protein and phospholipids into the supernatant fractions was followed after admixture of equal volumes of microsomal suspensions containing 2.2 mg. of microsomal protein/ml. and 0.5 % digitonin solutions. The action of digitonin was rapid, the same degrees of solubilization of protein (30 %) and phospholipid (5 %) occurring when the mixtures were fractionated directly after addition of digitonin at 0° as after 60 min. treatment at 0°. However, any effect of the digitonin during the 60 min. period required for centrifugation was not excluded.

Thin-layer chromatography of lipid extracts from the supernatant and pellet fractions showed that no individual lipid component had been preferentially solubilized, and confirmed the presence of most of the cholesterol in the pellet fractions.

Treatment by ultrasonic irradiation. Microsomal suspensions exhibited considerable optical clearing during ultrasonic treatment. Little difference could be detected between the degrees of solubilization of the total proteins and lipids brought about by irradiation for 20 and 40 min. However, the microsomal lipids, particularly the phospholipids, were in each case released to a slightly greater extent (5–10 %) than the protein component (Table 3). Saunders, Perrin & Gammack (1962) found that the dispersion of purified lecithin preparations was also complete after 20 min., as judged by the change in extinction. The ultrasonic irradiation had a deleterious effect on the activity of the adenosine triphosphatase; its activity in the absence of Na^+ ions was diminished more than its Na^+ ion-stimulated activity.

Lipid extracts from pellet and supernatant fractions, when chromatographed in parallel, were judged visually to contain the same lipid components in similar concentrations.

DISCUSSION

Microsomal fractions from rat brain have been shown to consist of two main components, membrane structures and RNA granules (Toschi, 1959; Hanzon & Toschi, 1959). The microsomal fraction used in the present investigation was chosen to contain only 12 % of the total RNA of the original dispersion. It presumably consists largely of the membrane structures from the endoplasmic reticulum and plasma membranes of the original cells of the tissue (Hanzon & Toschi, 1959).

Table 3. *Effect of ultrasonic irradiation on microsomes*

Samples of microsomal suspension in 0.32 M-sucrose equivalent to approx. 3 g. of grey matter and containing 2.5 mg. of microsomal protein/ml. were exposed at 0° to ultrasonic irradiation for the times indicated. The treated suspensions were centrifuged at 105000g for 60 min. to yield a clear supernatant fraction and a pellet. Samples of supernatant and pellet, which was resuspended in 0.32 M-sucrose, were taken for analysis. Mean values were obtained from three experiments. The values for the percentage of adenosine-triphosphatase activity recovered in the supernatant were obtained by dividing the amount of enzyme activity in the supernatant by the total recovered activity, in the presence of 100 mM-NaCl.

Period of treatment (min.)	Adenosine-triphosphatase activity (μ moles of inorganic phosphate liberated/g. of tissue/hr.)						Recovery of constituents in supernatant fraction (%)				
	Untreated microsomes			Pellet		Supernatant		Adenosine triphosphatase	Protein	Cholesterol	Phospho-lipid
	Without NaCl	With NaCl (100 mM)	Without NaCl	With NaCl (100 mM)	Without NaCl	With NaCl (100 mM)					
20	309	472	93	292	33	70	19	41	42	45	
40	309	472	41	152	38	59	28	40	48	59	

Release of microsomal protein. The degree of protein release brought about by the surface-active agents studied in the present work, and by the polyoxyethylene ethers examined in the preceding paper (Swanson *et al.* 1964), was consistently 10–15 % greater than the release of lipid. This may be due to the existence of a fraction of microsomal protein which is readily released by low concentrations of the detergents, or may simply result from the inherently greater water-solubility of proteins.

The preferential release of protein was especially marked at low concentrations of digitonin (0.08–2.0 mM), where 30–40 % of the total microsomal protein was released without solubilization of lipid. Part of the protein may be largely anchored to membrane by association of its hydrophobic side chains, through van der Waals forces, with the cholesterol in the lipid phase of the membrane. Such a mode of association of lipid with protein was postulated as occurring in serum lipoproteins (Salem, 1962), and studies of the interaction of proteins with cholesterol monolayers (Doty & Schulman, 1949; Eley & Hedge, 1956) have indicated involvement of van der Waals forces in a similar way. The special effect of digitonin in releasing 20 % of the microsomal protein, when present in a 1:1 molecular ratio with the membrane cholesterol, could be pictured as a displacement of these cholesterol–protein linkages by the successfully competing digitonin. The further 10–20 % of the protein which was released at 2.0 mM, when none of the lipid was solubilized, could correspond to the loosely bound protein fraction released by other treatments.

Release of adenosine triphosphatase and lipid. The solubilization of protein by sodium deoxycholate and Cetavlon was always accompanied by solubilization of lipid and adenosine triphosphatase. The correlation between release of adenosine triphosphatase and phospholipid, on treatment with digitonin in the concentration range 2.0–2.8 mM, suggests that the enzyme protein may, in some way, be associated with phospholipid and does not form part of the cholesterol-bound protein fraction proposed above. The inhibiting effect of phospholipase C on the adenosine-triphosphatase activity further supports such an association (Swanson *et al.* 1964). Examination of the activities of the Na^+ ion-stimulated adenosine triphosphatase/mg. of protein after digitonin treatment revealed that the values in the supernatants were consistently lower (50 %) than those for the adenosine triphosphatase remaining in the pellet fraction. When a correction was made for the amount of protein released at lower concentrations of digitonin, at which neither phospholipid nor appreciable enzyme activity was released, then the specific activity of the enzyme in

the supernatant approximated to that in the pellets. Thus solubilization of phospholipid was accompanied by parallel solubilization of adenosine triphosphatase, whose specific activity on a protein basis was similar to that remaining in the pellet.

The observed decrease of the stimulating effect of digitonin on the activity of the adenosine triphosphatase which occurred at concentrations causing solubilization of the enzyme and phospholipid could indicate that the stimulating effect requires, between the enzyme and other membrane components, certain spatial relations that are present in the intact membrane and displaced on solubilization. Studies with Lubrol W (Swanson *et al.* 1964) also revealed that this agent effected maximal stimulation of the adenosine triphosphatase under conditions where the degree of solubilization of the microsomes was small.

Mechanism of solubilization. Certain features of the solubilization of microsomes by detergents suggest that the mechanism involved may be similar to that postulated as occurring in the lysis of erythrocytes by detergents (Rideal & Taylor, 1956, 1958). In this mechanism, haemolysis was pictured as resulting from breakdown of a lipoprotein-detergent complex formed by penetration of the detergent into the erythrocyte membrane. Analogous penetration of microsomal membranes by detergents is suggested by the marked effects of the detergents on the activity of the Na^+ ion-stimulated adenosine triphosphatase even at concentrations of these agents too low to bring about solubilization (Swanson *et al.* 1964). At higher concentrations where solubilization occurs, the microsomal constituents would then be incorporated into the detergent micelle to form mixed-micelle structures. It is well established that at the higher concentrations both sodium deoxycholate and Cetavlon form micelles (McBain & Hutchinson, 1955). Digitonin also forms micelles in aqueous solutions, as shown by measurements made in the analytical ultracentrifuge, a method that has been used in characterizing other micelle systems (Gammack, 1963). This observation is contrary to the report of Ruyssen & Loos (1947) that saponins, including digitonin, exist as single molecules in water, even at relatively high concentrations.

Detergents are most likely to interact directly with the microsomal constituents, and to cause their reassociation and reorientation, both among themselves and with the detergent micelles in the bulk phase. Ultrasonic irradiation is likely to decrease microsomal particle size by mechanical disruption as a result of cavitation of the liquid, as proposed in the process of dispersion of whole cells (Hughes & Nyborg, 1962) and of isolated lipids (Saunders *et al.* 1962). Ultrasonic irradiation of

turbid sols of lecithin resulted in clearing of the sols owing to breakdown of the myelinic structures to micelles with a particle weight of about 5×10^6 . The observation that ultrasonic treatment did not result in the enrichment of the pellet fraction in lipid components, which occurred as a result of treatment with each of the detergents, may partially reflect the fundamental differences existing between the two disrupting mechanisms.

The inactivation of the adenosine triphosphatase by ultrasonic irradiation may be due to a change in the tertiary structure of the protein as a result of the shear forces in the liquid, or to local increases in temperature. However, free radicals known to be produced during ultrasonic irradiation of aqueous systems (Weissler, 1959) may also be important in inactivating the enzyme. For example, oxidation of the protein thiol groups, shown to be important for maintaining activity of a Na^+ ion-stimulated adenosine triphosphatase from ox brain (Skou, 1963), could be responsible for the diminished activity.

The choice of method for solubilizing cerebral microsomes has been made on the basis of its efficacy in removing lipids and proteins into aqueous solution, while maintaining the activity of a solubilized microsomal enzyme. The apparent non-specific removal of lipids by the various dispersion processes suggests that the microsomal particle size is decreased, and that the size and density of the smaller units vary with the agent used. This will determine their rate of sedimentation in the selected centrifugal field. The presence of an array of different lipid molecules in lipoprotein structures favours their release as mixed-lipid complexes with detergents. Specific affinities of individual detergents for particular lipids are probably of secondary importance except with digitonin. The selection of a dispersing agent that does not cause inactivation of a particular microsomal enzyme, as exemplified in the preceding paper (Swanson *et al.* 1964), may limit the choice of detergent. The distinction observed between the effects of different agents on enzyme activity has, however, provided some indication of the molecular environment of the Na^+ ion-stimulated adenosine triphosphatase.

SUMMARY

1. The effects of an anionic, a cationic and a non-ionic detergent, and of ultrasonic irradiation, on the composition of a microsome fraction from guinea-pig cerebral cortex were examined.

2. A proportion of the protein, phospholipid, cholesterol and Na^+ ion-stimulated adenosine triphosphatase remained unsedimented in a centrifugal field of $6.3 \pm 10^6 \text{ g} \cdot \text{min.}$ after treatment at 0° with anionic sodium deoxycholate at concentra-

tions above 2.5 mM and of cationic cetyltrimethylammonium bromide at concentrations above 0.7 mM.

3. Digitonin, a non-ionic detergent, at a concentration of 2.0 mM solubilized about one-third of the microsomal protein without releasing lipid and adenosine-triphosphatase activity. At 2.8 mM, lipid and adenosine triphosphatase were released. Digitonin at concentrations 0.01–2.0 mM stimulated the activity of this enzyme in the presence of Na^+ ions; this effect was diminished at 2.8 mM, when the enzyme and lipid were solubilized.

4. Digitonin at all concentrations examined failed to release appreciable quantities of cholesterol into solution, owing presumably to the formation of insoluble complexes which were sedimented. The relative proportions of different lipids solubilized by all other agents were, however, similar to those present in the original microsomes.

5. About one-half of the microsomal constituents was solubilized after ultrasonic irradiation. Three-quarters of the Na^+ ion-stimulated adenosine-triphosphatase activity was recovered after 20 min. treatment, and only one-half was recovered after 40 min. treatment. Recoveries of the activity in the absence of Na^+ ions were one-half of those measured in the presence of Na^+ ions.

6. The choice of solubilizing agent in relation to these properties and to the maintenance of the activity of the adenosine triphosphatase is discussed.

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Glycolytic Enzymes in Human Brain

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Histochemical studies of animal brain have elucidated the sites of activity of enzymes associated with the different metabolic routes, and reliable data are now available for the comparison of glycolytic enzyme activities in the different regions (Breckenridge & Crawford, 1961; Naidoo, 1962; Pope, 1960; Robins & Lowe, 1961; Adams, Davison & Gregson, 1963). Few reports have

appeared on the activity of a particular enzyme in human brain or of a specific enzyme associated with a disease (Friede, 1961; Friede & Fleming, 1962; Lazarus, Wallace & Volk, 1962; Hess & Pope, 1961; Aronson, Saifer, Perle & Volk, 1961). In the present investigation, enzymes concerned with glucose metabolism in the human brain and cord have been studied to determine the rate at which