and also purified human prostate phosphatase, can hydrolyse G 1-P more or less rapidly. On the other hand, Swanson (1950) found that her purified preparation of liver glucose 6-phosphatase had very little activity towards G 1-P.

In the present work, the hydrolysis of G 1-P by liver extracts was most active at pH 7·2–7·5, with a rapid decline in activity on either side of the optimum, although the shape of the pH–activity curve did not completely preclude the hydrolysis of G 1-P by non-specific alkaline phosphatases at higher pH values.

The characteristics of the hydrolysis of G 1-P by unfractionated extracts of liver differed from those of glucose 6-phosphatase but closely resembled the characteristics of liver phosphoglucomutase. These findings lend strong support to the views of Broh-Kahn & Mirsky (1948) that G 1-P undergoes a preliminary conversion into G 6-P before hydrolysis and that this conversion into G 6-P is the rate-controlling step in the over-all reaction. This view is strengthened by the finding that no fraction of liver cytoplasm hydrolysed G 1-P alone, but that combination of either microsomal or mitochondrial fraction with cell sap caused significant hydrolysis. In these cases the microsomal and, to a lesser degree, the mitochondrial fractions contained glucose 6-phosphatase activity whereas the cell sap did not. The cell sap is, however, a rich source of the enzyme phosphoglucomutase.

The ability of the nuclear fraction to hydrolyse G 1-P was not studied in the present work, but it has been found by one of us (G.T.M., unpublished observation) that isolated rat-liver nuclei cause no significant breakdown of G 1-P.

SUMMARY

1. The hydrolysis of glucose 1-phosphate (G 1-P) by unfractionated rat-liver homogenate differs from the hydrolysis of glucose 6-phosphate (G 6-P) in having a higher optimum pH, being much more strongly activated by Mg^{2+} ions and by showing inhibition by high substrate concentrations.

2. Some properties of liver phosphoglucomutase have been investigated and the enzyme was found to be similar in many respects to that isolated from other tissues.

3. The results of the present work support the view that the main route of G 1-P hydrolysis in liver is through a preliminary conversion into G 6-P, catalysed by phosphoglucomutase.

4. An examination of the abilities of the separated cytoplasmic fractions to hydrolyse G 1-P and G 6-P supports the above view.

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Studies on Sulphatases

16. A SOLUBLE PREPARATION OF ARYLSULPHATASE C OF RAT-LIVER MICROSOMES*

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The arylsulphatase activity of rat liver can be attributed to at least three distinct enzymes which can be differentiated by their relative substrate specificities, behaviour towards inhibitors and their localization within the liver cell. Two of the arylsulphatases (A and B) have very similar properties and are found mainly in the mitochondria. They are easily obtained in a soluble form by any of the procedures which are known to disrupt mitochondria, and have been termed the 'soluble' arylsulphatases. On the other hand, the remaining enzyme (arylsulphatase C), which is located almost exclusively in the microsomes, has been termed 'insoluble' since

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the methods which are effective in solubilizing the mitochondrial arylsulphatases fail to bring it into solution (Dodgson, Spencer & Thomas, 1955). Purification of the microsomal arylsulphatase has been hindered by its insoluble nature. This paper describes the attempts made to prepare a soluble extract of this enzyme.

EXPERIMENTAL

Materials. The potassium salts of *p*-acetylphenyl sulphate (APS), *p*-nitrophenyl sulphate (NPS) and 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) were prepared as before (Dodgson *et al.* 1955). Crude trypsin and Pancreatin were obtained from British Drug Houses Ltd., crystalline trypsin and Steapsin from L. Light and Co. Ltd., and Lissapol-N from Imperial Chemical Industries Ltd. The various pancreatic preparations were devoid of arylsulphatase C activity. Medical Research Council hooded rats were used.

Sodium acetate–acetic acid buffer. In many of the enzyme experiments sodium acetate–acetic acid mixtures (hereafter referred to as acetate buffer) were used at pH values where such mixtures have little buffering power. In these, pH was checked at the beginning and the end of the experiment. No detectable changes in pH were observed.

Determination of arylsulphatase C activity. Arylsulphatase C was measured by methods used previously, in which the phenols liberated from APS and NPS, after 1 hr. incubation periods at 37°, were measured spectrophotometrically (Dodgson *et al.* 1955).

The major part of the work was carried out with rat-liver preparations which still contained the 'soluble' arylsulphatases A and B. However, previous work (Dodgson *et al.* 1955) has shown that the microsomal enzyme can be effectively measured in the presence of A and B by using either 0·007 M-APS in 0·5 M-acetate buffer at pH 7·2, or 0·0025 M-NPS in the same buffer at pH 7·0, since under these conditions the activity of, and competition by, the 'soluble' enzymes is quite small. Liberated *p*-nitrophenol cannot be quantitatively recovered from fresh rat-liver suspensions (Dodgson & Spencer, 1953), and the arylsulphatase activity of such preparations was determined with APS. Recovery of *p*-nitrophenol is complete from fresh microsome preparations and from acetone-dried liver, and although in these cases choice of substrate is immaterial, NPS has generally been used since the spectrophotometric readings of the control determinations were lower than those obtained with APS.

Determination of arylsulphatases A and B. The combined activity of the A and B enzymes was approximately determined by measuring the nitrocatechol liberated during 1 hr. at 37° from 0·02 M-NCS in 0·5 M-acetate buffer, pH 6·0 (Dodgson *et al.* 1955).

Criteria of solubility. The following standard procedure was adopted for assessing the degree of solubilization of arylsulphatase C achieved by treatment of the liver preparations by various methods. A portion of the treated material was centrifuged at 25 000 *g* for 1 hr. at 0°. Subsequently, both uncentrifuged material and the supernatant prepared by centrifuging were adjusted to the assay pH before determining arylsulphatase activity with APS or NPS. The activity appearing in the supernatant was expressed as a percentage of that of the uncentrifuged material and represented the amount of arylsulphatase C in solution. In view of the large experimental error of the assay method when measuring the low enzyme C activities which were found in many experiments, solubilization was considered to have been achieved only when more than 5% of the total arylsulphatase C activity appeared in this fraction. When centrifuging indicated that solubility had been achieved, a further test was applied. The soluble extract was treated with acetone at −5° until the concentration of acetone was 80% (v/v). The precipitated material was separated, washed well with cold acetone and dried *in vacuo*. The solubility in water of the arylsulphatase C of the resultant powder was then assessed.

All values for *g* are average values.

RESULTS

Attempted solubilization of arylsulphatase C

Extraction with aqueous solutions. Suspensions of fresh rat liver (1–4%, w/v) were prepared by use of a glass homogenizer (Potter & Elvehjem, 1936). The suspension media used were water, sodium acetate–acetic acid (0·05–2 M) or sodium phosphate (0·05–0·2 M) buffers covering the pH range 4–10, and iso-, hypo- or hyper-tonic NaCl solutions. The suspensions were centrifuged immediately or after incubation for 1, 3 or 24 hr. at 37°. In no case was there more than 5% of the arylsulphatase C activity of the whole suspension present in the centrifuged supernatant.

Microsomes prepared by the method of Schneider (1948) were also subjected to the above treatments without any solubilization of the arylsulphatase occurring. The arylsulphatase of suspensions of microsomes in 0·5 M-acetate, which were adjusted to pH 3 with HCl or pH 11 with NaOH and kept at 0° for 1 hr., was considerably inactivated and still insoluble.

Alternate freezing and thawing. Alternate freezing (−70°) and thawing (37°) of fresh liver or microsome suspensions in 0·5 M-acetate, pH 7·0, progressively destroyed arylsulphatase activity and it was not possible to extract the arylsulphatase from the treated material with various buffers or saline solutions. The arylsulphatase C of freeze-dried liver suspensions was similarly insoluble.

Mechanical degradation. The use of a tissue disintegrator (Mickle, 1948) in which fresh rat-liver suspensions were shaken at 50 cyc./sec. for 30 min. with Ballotini no. 12 (English Glass Co., Ltd.) failed to release arylsulphatase C in a soluble form.

Treatment with organic solvents. It was possible that the high lipid content of microsomes (40%; Dounce, 1950) was responsible for the failure of the above methods to bring the enzyme into solution. The presence of a lipid barrier through which the aqueous media could not penetrate or, alternatively, the presence of the enzyme as a lipoprotein complex,

would explain the ineffectiveness of the aqueous-extraction procedures. Attempts were therefore made to remove lipids with organic solvents.

Rat liver was acetone-dried and washed with ether but no arylsulphatase C could be extracted from the resulting powder with any of the solvents used previously in the extraction of fresh liver preparations. The arylsulphatase C of acetone-dried powders of fresh liver prepared after subjection of the fresh tissue to alternate freezing and thawing, lyophilization or the use of the tissue disintegrator was similarly insoluble. Similar results were obtained with ethanol or dioxan in place of acetone. Extraction of fresh, freeze-dried or acetone-dried liver suspensions with *n*-butanol as described by Morton (1955) failed to bring the arylsulphatase C into a soluble form.

Surface-active agents. The effect of surface-active agents on the activity and solubility of arylsulphatase C has been considered separately (Dodgson, Rose, Spencer & Thomas, 1957) and may be summarized as follows. All haemolytic surface-active agents appeared to bring the enzyme into solution, and solubilization appeared to depend on the presence of detergent micelles in the mixture. When the detergent was removed with acetone the enzyme once more became insoluble, and it was concluded that the solubility effect was due to the formation of a soluble micelle-complex between the detergent and the arylsulphatase.

Use of pancreatic enzymes. Treatment of acetone-dried liver with crude trypsin resulted in considerable destruction of arylsulphatase C with, however, some release of activity into solution. The extent of the destructive and solubilizing effects was variable and depended on the particular trypsin and acetone-dried liver preparations used. In a typical experiment, treatment of 10 ml. of a 0·8% aqueous suspension of acetone-dried rat liver with 5 mg. of crude trypsin for 30 min. at 37° and pH 8·4 resulted in a loss of 49% of the original arylsulphatase C activity, but 36% of the remaining activity was in solution. Similar results were obtained with other crude pancreatic preparations, including Pancreatin and Steapsin, but crystalline trypsin, although causing considerable destruction of the arylsulphatase, failed to release any activity into solution.

Attempts were made to minimize the destructive effects of the pancreatic preparations and to increase the solubilizing effect by varying the amounts of the different pancreatic extracts used, the duration of the incubation period, the concentration and type of buffer and the pH and temperature at which the treatment was carried out. The results were variable and seldom repeatable, and the efficacy of the various commercial pancreatic preparations varied from batch to batch. In no case was it possible substantially to increase the solubilizing effect without also increasing the rate of destruction of arylsulphatase C. Thus in the most successful experiment with Steapsin at pH 8·0 in the absence of buffer, and incubating for 1 hr. at 4°, 70% of the arylsulphatase activity remaining after the treatment was soluble but there was an over-all loss of 60% of the original activity.

Use of pancreatic preparations in the presence of surface-active agents. In view of the high lipid content of microsomes, and the fact that crystalline trypsin failed to solubilize arylsulphatase C, the action of the crude pancreas extracts could possibly be explained on the assumption that the arylsulphatase was released from an insoluble lipid–protein complex by lipolytic enzymes, whereas proteolytic enzymes were responsible for the loss in activity. A surface-active agent was therefore used in an attempt to disperse the supposed arylsulphatase complex and thus stimulate the lipolytic action of the pancreatic preparation.

The solubility of arylsulphatase C of an aqueous suspension of acetone-dried liver was determined before and after treatment with Lissapol-N or Steapsin alone or with Lissapol-N plus Steapsin (Table 1). The combined action of Lissapol-N and Steapsin, in the absence of buffer at pH 8·0 and upon incubation for 30 min. at 37°, resulted in only 56% destruction of the arylsulphatase, as compared with 72% by Steapsin alone, and brought almost the whole of the remaining activity into solution. When this treatment was carried out at 4° over a period of 1 hr. there was a loss of only 31% of

Table 1. *Effect of treatment with Steapsin and Lissapol-N on the activity and solubility of the arylsulphatase C of acetone-dried rat liver*

Portions (10 ml.) of an aqueous suspension (0·8%) of acetone-dried rat liver were incubated for 30 min. at 37° with 1 ml. of water (control), or 1 ml. of an aqueous suspension of Steapsin (containing 0·1 g. of Steapsin), or 1 ml. of an aqueous solution of Lissapol-N (containing 0·02 g. of Lissapol-N), or 1 ml. of an aqueous mixture containing 0·1 g. of Steapsin and 0·02 g. of Lissapol-N. All suspensions or solutions were adjusted to pH 8 with N-NaOH before mixing. The arylsulphatase C activity remaining and the amount present in a soluble form as a result of these treatments were then determined as described earlier.

Acetone-dried rat liver incubated with	Arylsulphatase C activity of the treated suspensions (% of water control)	Arylsulphatase C in solution (%)
Water (control)	100	4
Lissapol-N	106	34
Steapsin	28	20
Lissapol-N + Steapsin	44	95
Lissapol-N + Steapsin*	69	91

* Incubation was carried out at 4° for 1 hr.

the arylsulphatase activity with little decrease in the percentage of the enzyme brought into solution. Similar results were obtained with Pancreatin or crude trypsin in combination with Lissapol-N. Initial treatment of rat liver with acetone was not an essential prerequisite for the liberation of arylsulphatase C by the pancreatic preparations since the arylsulphatase of fresh microsomes could be similarly solubilized.

Although the sedimentation criterion suggested that the arylsulphatase C obtained after treatment of rat liver with Lissapol-N and Steapsin was in a soluble form, the possibility existed that the enzyme was being held in solution by the detergent (cf. Dodgson *et al.* 1957). The centrifuged supernatants of acetone-dried liver or fresh microsomes after Steapsin–Lissapol-N treatment at 4° were therefore treated with acetone (final concentration of 80%, v/v) at −5° and the resultant precipitate was separated, washed well with cold acetone and dried *in vacuo*. Extraction of this material, which was now free from Lissapol-N, with water showed that in the best preparations 90% of the arylsulphatase C activity was soluble and could not be precipitated by centrifuging at 25000 *g* for 1 hr. When Lissapol-N was used in the absence of Steapsin, the arylsulphatase C of acetone-dried material prepared in this way was completely insoluble (Dodgson *et al.* 1957).

Table 1 indicates that Lissapol-N, in addition to increasing the solubilizing effect of the pancreatic preparations, also inhibited the ability of the preparations to inactivate the arylsulphatase. This point was further investigated by studying the extent of the inactivation of the arylsulphatase C of acetone-dried liver by Steapsin in the presence of increasing concentrations of Lissapol-N. The inactivation decreased as the concentration of Lissapol-N was increased (Fig. 1). Lissapol-N similarly protected the arylsulphatase C of fresh microsomes against Steapsin inactivation, but in this case the degree of protection afforded was appreciably less than that observed with the acetone-dried liver.

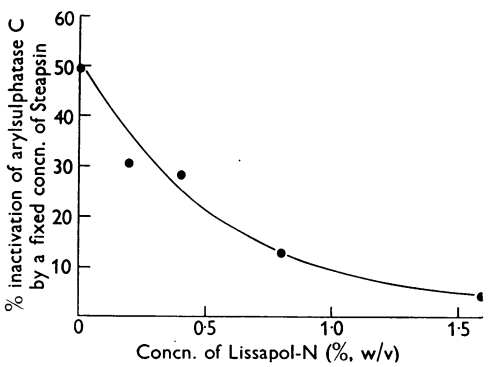

Fig. 1. Effect of Lissapol-N on the inactivation of rat-liver arylsulphatase C by Steapsin. Each reaction mixture consisted of 2·5 ml. of a 17% (w/v) aqueous suspension of acetone-dried rat liver, 0·5 ml. of a 5% (w/v) aqueous suspension of Steapsin and 3 ml. of an aqueous solution of Lissapol-N of twice the required final concentration. In the control determinations water was substituted for the Steapsin suspension. All the mixtures were adjusted to pH 8·0 with N-NaOH and maintained at 4° for 1 hr. before arylsulphatase assay.

The ability of pancreatic preparations to release arylsulphatase C into true solution was not affected by high Lissapol-N concentrations. Thus Steapsin (0·75 g.) was added to a suspension of 0·75 g. of acetone-dried liver in 75 ml. of water containing 1·4 g. of Lissapol-N, the whole being adjusted to pH 8·0 with NaOH. After incubation for 30 min. at 4° the mixture was centrifuged at 25000 *g* for 1 hr. at 0° and the supernatant treated with acetone as described above in order to precipitate the enzyme and to remove Lissapol-N. Over 90% of the total arylsulphatase C activity of the resulting powder was soluble in water.

Soluble preparations of arylsulphatase C

The preparation of arylsulphatase C free from arylsulphatases A and B was now considered. It seemed possible that this could be done by a preliminary extraction of arylsulphatases A and B from acetone-dried rat liver with water or buffer; or alternatively by preparing microsomes by differential centrifuging in isotonic sucrose, thus largely removing A and B which are located mainly in the mitochondria.

Although repeated extraction of acetone-dried liver with water or acetate buffer (0·5M, pH 7·0) removed the bulk of the arylsulphatases A and B (Dodgson *et al.* 1955) a certain amount of these enzymes (about 10% of that originally present) still remained in the washed debris. This was deduced from the fact that the washed debris contained activity towards NCS (0·02M, pH 6·0) which was completely inhibited by 0·0025M-PO_4^{3-}. The activity of arylsulphatase C is largely unaffected by this concentration of phosphate (Dodgson *et al.* 1955). Furthermore, the repeated extraction of the acetone-dried liver caused appreciable destruction of arylsulphatase C and the residual C could not be completely solubilized by the subsequent Steapsin–Lissapol-N treatment.

The elimination of arylsulphatases A and B by separation of the microsomal fraction in isotonic sucrose appeared to be no more effective than the washing of acetone-dried liver, since the microsomal fraction contained about 20% of the A and B activity of the whole liver cell (Dodgson *et al.* 1955). On the other hand, the arylsulphatase C of fresh microsomes could be completely solubilized and

microsomes were therefore preferred as starting material for the preparation of a soluble extract of arylsulphatase C. Unfortunately, treatment of microsomes with Steapsin and Lissapol-N also liberated into solution the arylsulphatases A and B, and attempts were therefore made to remove these enzymes from the microsomal fraction before solubilization of arylsulphatase C.

Removal of arylsulphatases A and B from microsomes. About 15–30 % of the arylsulphatase A and B activity of microsomes (measured with 0·02 M-NCS at pH 6·0) could be washed out by suspending the microsomes in water or 0·15 M-NaCl and centrifuging at 25 000 *g* for 1 hr. Little further activity could be removed by subsequent washings. Alternate freezing and thawing of water suspensions of microsomes or treatment of such suspensions with *n*-butanol at 37° for 30 min. with the two-phase system of Morton (1955) liberated less than 50 % of the A and B activity into solution.

A more complete removal of arylsulphatases A and B was achieved by maintaining suspensions of microsomes in water, 0·15 M-NaCl or 0·5 M-sodium acetate at a pH of 10 for 1 hr. at 4°, followed by readjustment of the pH to 7·0 with acetic acid and centrifuging at 25 000 *g* and 0° for 1 hr. Less than 10 % of the A and B activity of the fresh microsomes remained in the centrifuged debris. No solubilization of arylsulphatase C occurred during these procedures but some loss (approx. 10 %) in activity was observed. The A and B activity still remaining in association with the microsomal material could not be extracted by repetition of any of the above treatments. This procedure, in the presence of sodium acetate, was adopted as a preliminary step in the preparation of soluble arylsulphatase C.

Large-scale preparation of soluble arylsulphatase C. Wet microsomes (8 g. wet wt.) prepared by the method of Schneider (1948) from 26 g. of rat liver were suspended in 30 ml. of 0·5 M-sodium acetate at 4° (assay point 1) and the pH of the suspension was adjusted to 10 with N-NaOH. After keeping at 4° for 1 hr. (assay point 2), the pH was readjusted to 7·0 with acetic acid and the whole centrifuged at 25 000 *g* and 0° for 1 hr. The sedimented material (assay point 3), which now contained appreciably smaller amounts of arylsulphatases A and B, was suspended in 100 ml. of water containing 3 g. of Lissapol-N (assay point 4) and the whole was adjusted to pH 8·0 with N-NaOH. To this mixture was added 10 ml. of an aqueous Steapsin suspension (containing 1·8 g. of Steapsin) which had been adjusted to pH 8·0 with N-NaOH. After standing for 1 hr. at 4° (assay point 5), the mixture was readjusted to pH 7·0 with acetic acid before centrifuging for 1 hr. at 25 000 *g* and 0°. The clear supernatant solution was cooled to 0° and cold acetone was added slowly, with mechanical stirring, until the concentration of acetone was 80 % (v/v). The precipitate was allowed to flocculate and was separated by centrifuging at 2000 *g* and 0° for 1–2 min. The precipitate was immediately resuspended in dry acetone at 0° with the aid of a glass homogenizer, filtered off at the pump and washed well with dry acetone at 0°. The filter-cake was not allowed to crack before the precipitate had been thoroughly washed. This precipitation and washing procedure must be followed exactly in order to avoid the formation of gummy enzyme material. The washed material was dried *in vacuo* (assay point 6). The resultant powder (1 g.) was suspended in 60 ml. of water, by use of a glass homogenizer, and allowed to stand at 4° for 2 hr. before centrifuging at 25 000 *g* and 0° for 30 min. The clear supernatant (assay point 7) was adjusted to pH 4·5 with acetic acid and the precipitate which separated was collected by centrifuging and redissolved in 0·5 M-sodium acetate–acetic acid at pH 7·0. The precipitate dissolved completely (assay point 8). This final treatment with acetic acid was not an essential step in the actual solubilization procedure but was included since it brought about a threefold purification of the enzyme on a protein basis. At various stages (described above as assay points) samples were assayed with NPS and NCS, the appropriate experimental conditions described earlier being used. The results of these assays are recorded in Table 2.

It can be seen from Table 2 that the final preparation still contained appreciable amounts of arylsulphatases A and B, although the amounts present represent less than 0·5 % of the A and B activity present in fresh whole liver. The final preparation was stable if kept in the frozen state but rapidly lost

Table 2. *Total arylsulphatase C activity and total combined activities of arylsulphatases A and B remaining at various stages during the procedure for the solubilization of the arylsulphatase C present in 8 g. (wet wt.) of rat-liver microsomes*

Assay point (see text)	Total arylsulphatase activity present at the assay point: Arylsulphatase C (μg. of *p*-nitrophenol liberated from NPS/hr.)	Total arylsulphatase activity present at the assay point: Combined activities of arylsulphatases A and B (μg. of nitrocatechol liberated from NCS/hr.)	Ratio of NPS:NCS activities
1	20 500	60 400	1:3·0
2	18 200	44 500	1:2·5
3	15 300	14 100	1:0·9
4	22 400	15 000	1:0·7
5	10 800	13 700	1:1·1
6	6 200	2 400	1:0·4
7	4 150	2 150	1:0·5
8	3 100	1 130	1:0·4

activity at room temperature. This was presumably due to the continued presence of the crude Steapsin in the final preparation.

DISCUSSION

Clearly, more work on the preparation of soluble arylsulphatase C will be required before a completely satisfactory procedure is evolved. Two outstanding problems have not yet been solved, namely, the complete elimination of arylsulphatases A and B from the final preparation and the stabilization of this final preparation. Further work now proceeding in this laboratory is directed at solving these problems. Because of the unstable nature of the final soluble enzyme preparation it has not been possible to compare the properties of the original and the soluble enzyme with any degree of accuracy. Such a comparison would be of interest in connexion with the mechanism of arylsulphatase action. In the untreated microsomal material, enzyme action would appear to be necessarily confined to the surface of the insoluble enzyme complex and hence presumably occurs at a liquid–solid interface. It has been suggested by Bücher (1953) that insoluble particulate enzymes might act by physical reaction mechanisms not explicable by ordinary chemical-collision theory and that differences in the kinetics of soluble and insoluble enzyme systems might be observed.

The original observations of Dodgson, Spencer & Thomas (1954), which indicated the microsomal origin of arylsulphatase C, have since been confirmed by Gianetto & Viala (1955). These workers suggest that the small amount of arylsulphatase C activity which can be found associated with the nuclear and mitochondrial fractions obtained by differential centrifuging is due to contamination of these fractions by microsomes. It seems highly probable that the whole of the arylsulphatase C activity of the liver cell resides in the microsomal particles. The distribution of arylsulphatase C in the rat-liver cell thus closely resembles that of glucose 6-phosphatase (Hers, Berthet, Berthet & de Duve, 1951), and it is therefore not surprising that the latter enzyme also presents solubilization problems similar to those encountered during the present work. Although glucose 6-phosphatase has been obtained in soluble form by treatment with surface-active agents (Beaufay & de Duve, 1954; R. G. Langdon & D. R. Weakley, private communication), like arylsulphatase C, it resumed its insoluble form after removal of the detergent.

The present study of the behaviour of arylsulphatase C towards various extraction procedures when considered in conjunction with the studies of the ability of detergents to solubilize the enzyme (Dodgson *et al.* 1957) has provided little definite evidence as to the physical state of the enzyme within the cell. However, the results obtained are not incompatible with the possibility that the enzyme is a soluble protein existing within the microsomes as an insoluble lipoprotein complex. The crude pancreatic enzyme preparations used in the solubilization of the arylsulphatase contain both lipolytic and proteolytic enzymes, but it is perhaps particularly significant that crystalline trypsin, although causing appreciable inactivation of arylsulphatase C, is incapable of solubilizing the enzyme. It will be desirable to use purer enzyme preparations of known specificity to obtain more definite evidence as to the nature of the insoluble arylsulphatase complex.

The arylsulphatase C of human liver is also insoluble in nature (Dodgson, Spencer & Wynn, 1956) and may be presumed to be located in the microsomes. Attempts to solubilize this enzyme by procedures similar to the ones used in the present work have been unsuccessful (Dodgson *et al.* 1956), but whether this represents a fundamental difference between the two enzymes is not certain since it has not so far been possible to examine the human-liver enzyme within less than 48 hr. after death.

The distribution of arylsulphatases A and B in the liver cell is worthy of some comment. The original claim of Dodgson *et al.* (1955) that the bulk of the A and B activity of rat liver was associated with the mitochondria has been confirmed and extended by Viala & Gianetto (1955), who suggested that the enzyme was mainly confined to the so-called 'light mitochondria'. However, it seems certain that significant amounts of these enzymes also occur in the microsomes, and repetition of the microsome–mitochondria separation stage during the fractionation procedure of Schneider (1948) still gave a microsomal fraction containing about 15% of the total A and B activity of the liver cell. This microsomal A and B activity can be completely inhibited by 0·0025M-phosphate without appreciably affecting the activity of arylsulphatase C. About 80% of the microsomal A and B can be extracted from the microsomes by fairly simple procedures. The remainder cannot be extracted by these procedures, however, but is solubilized during the treatment of microsomes with pancreatic preparations in the presence of detergent. These findings might indicate that arylsulphatases A and B both exist in the microsomes in two different physical states. This is perhaps not surprising in view of the heterogeneity of the microsomal material (cf. Slautterback, 1952).

SUMMARY

1. Attempts have been made to obtain the arylsulphatase C of rat-liver microsomes in soluble form. The enzyme cannot be solubilized by simple extrac-

tion procedures or by methods which are known to be effective in rupturing the enveloping membranes of the particulate components of the liver cell.

2. Treatment of fresh liver microsomes, or acetone-dried whole rat liver, with crude pancreatic enzyme preparations in the presence of the non-ionic detergent Lissapol-N effectively solubilizes arylsulphatase C, although some loss of enzyme activity occurs. Similar treatment with crystalline trypsin in the presence of Lissapol-N results in considerable destruction of arylsulphatase C and is ineffective in solubilizing the enzyme.

3. The final soluble preparation of arylsulphatase C is unstable at room temperature and still contains traces of rat-liver arylsulphatases A and B.

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Studies on Sulphatases

17. THE ACTION OF SURFACE-ACTIVE AGENTS ON THE ARYLSULPHATASE C OF RAT LIVER

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Arylsulphatase C of rat-liver microsomes cannot readily be obtained in solution. Attempts to solubilize this enzyme have been reported in the preceding paper (Dodgson, Rose & Spencer, 1957), a soluble preparation finally being obtained by incubating microsomes with pancreatic preparations in the presence of the synthetic non-ionic surface-active agent, Lissapol-N.

During these investigations it was noted that a number of surface-active agents were apparently capable of solubilizing arylsulphatase C and, in view of the several interesting phenomena observed, this work has been considered separately. A preliminary account of the work has already appeared (Spencer, Dodgson, Rose & Thomas, 1955).

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EXPERIMENTAL

Acetone-dried rat liver. Fresh rat liver (50 g.) was macerated for 3 min. in a Townson and Mercer macerator with 300 ml. of acetone at 0°. The macerated material was filtered at the pump and the filter cake immediately re-macerated with a further 300 ml. of acetone at 0°. After filtering at the pump the filter cake was washed with three 300 ml. portions of cold acetone, sucked dry at the pump, and broken up and dried *in vacuo*. The dried powder was sieved (40-mesh) in order to remove connective tissue.

Assay of arylsulphatase C. The methods of assay were those employed previously (Dodgson *et al.* 1957), potassium *p*-nitrophenylsulphate (NPS) and *p*-acetylphenylsulphate (APS) being used. The activities of fresh whole-liver suspensions were measured with APS, and those of microsomes and acetone-dried powders were assayed with NPS. The surface-active agents did not hydrolyse these substrates under the conditions of assay and did not interfere with the recovery of the phenols which were liberated as a result of enzyme action.