the mixing chamber before the reagents are emptied into the reaction vessel. The accuracy of mixing exceeds the limit of resolution of the spectrophotometer.

3. The reaction vessel consists of a cylindrical quartz tube held in position with unstretched nylon bushings in a brass block adjacent to the cuvette housing unit. The flow of material is controlled by a needle valve at the exit of the reaction-vessel housing unit. Approximately 6 ml. of fluid is required to flush out the reaction vessel.

4. Temperature control is maintained with a water jacket round the four syringes and with cooling channels within the reaction-vessel housing unit. Steady-state temperature is reached in the syringes in a maximum of 6 min.

5. The signal from the density-scale slide-wire potentiometer of the spectrophotometer is fed through a potential divider into a Brown strip-chart recorder. As the operator tracks the changes in optical density of the reaction mixture the percentage transmission is recorded continuously. By means of a mixing switch the temperature of the reaction vessel measured with a thermopile is recorded at intervals during the reaction time.

6. The percentage error (standard deviation) of the velocity constant of decomposition of hydrogen peroxide by bacterial catalase measured with this apparatus is less than $\pm 2\%$.

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Studies on Sulphatases

12. THE ARYLSULPHATASES OF HUMAN TISSUES*

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The arylsulphatase activity of ox and rat livers is due to three distinct enzymes, which may be readily separated and which possess widely different substrate affinities (Dodgson, Spencer & Thomas, 1955b; Roy, 1953, 1954). Two enzymes, A and B, are present in the water-soluble material of acetone-dried liver and are referred to as the 'soluble' arylsulphatases. The 'insoluble' arylsulphatase is localized in the microsomes of the liver cell and has only been obtained in a soluble form by the use of haemolytic surface-active agents or by the action of crude trypsin preparations (Dodgson, Rose, Spencer & Thomas, 1955a). Both rabbit and mouse livers possessed enzymes similarly divided into 'soluble' and 'insoluble' arylsulphatases (Dodgson *et al.* 1955b), but no investigation was made into the homogeneity of the 'soluble' enzyme.

As the initial part of a study of human sulphatases the presence of two 'soluble' and one 'insoluble' arylsulphatases in human liver has been demonstrated and their characteristics were investigated. Other tissues have been examined for the presence of these three enzymes.

EXPERIMENTAL

Enzyme preparation and assay. Post-mortem samples of normal livers and other tissues, obtained within 24 hr. after death, were frozen at -15° . Little arylsulphatase activity was lost over several weeks at this temperature. Alternatively, acetone-dried powders were prepared.

The activity of fresh and acetone-dried tissue suspensions against potassium *p*-acetylphenyl sulphate (APS), potassium *p*-nitrophenyl sulphate (NPS) and dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) was measured by spectrophotometric estimation of the respective liberated phenols (Dodgson *et al.* 1955b). Incubation was for 1 hr. at 37.5° in the presence of 0.5 M

* Part 11: Dodgson, Spencer & Williams (1955c).

sodium acetate-acetic acid buffer adjusted to the appropriate pH, and the concentration of enzyme suspension was such that the spectrophotometric readings corresponded to an extinction E [$\log_{10}(I_0/I)$] of less than 1. The actual concentration required depended on the substrate used, the particular liver specimen and the nature of the enzyme preparation, and was determined by preliminary experiment.

Sodium acetate-acetic acid buffers were used throughout.

Detergents. The following were commercial samples: Liissapol-N (I.C.I. Ltd.), Triton WR-1339 (Rohm and Haas Co. Ltd.), sodium dodecyl sulphate (Light and Co.), Empicol (Marchon Products Ltd.), Vykamol and glyceryl monostearate (Croda Ltd.), cetylpyridinium bromide and cetyltrimethylammonium bromide (B.D.H. Ltd.), Teepol 530 (Shell Ltd.).

RESULTS

'Soluble' and 'insoluble' arylsulphatases

The substrate concentration-activity curves obtained with aqueous suspensions of acetone-dried liver acting on APS, NPS and NCS (Fig. 1) showed

Fig. 1. Substrate concentration (S)-activity curves for acetone-dried human liver. Incubation was for 1 hr. at 37.5° in the presence of $0.5M$ acetate. $\bullet$, APS, pH 6.9; $\blacksquare$, NPS, pH 7.3; $\blacktriangle$, NCS, pH 6.0. The curves are not quantitatively related to each other.

marked inhibition of activity by excess substrate in all cases but gave no indication of the presence of more than one arylsulphatase. The pH-activity curves (Fig. 2) also appeared to be those of a single enzyme. However, the presence of two distinct types of arylsulphatases in human liver was demonstrated by separating the soluble and insoluble components of acetone-dried liver by centrifuging an incubated water suspension of the powder, as previously described for the rat (Dodgson *et al.* 1955b). Assay of the whole powder and the separated supernatant and debris against the three substrates under the respective optimum conditions derived from Figs. 1 and 2 showed that the bulk of the original NCS activity, but only a small fraction of the APS and NPS activities, was present in the

supernatant (Table 1). On the other hand, the debris retained almost all the APS and NPS activity. Confirmation of the separation of the two types of enzyme by this procedure was obtained from the Michaelis curves of the two fractions (Fig. 3). The debris curves for APS and NPS were

Fig. 2. pH-activity curves for acetone-dried human liver. $\bullet$, APS ($0.005M$); $\blacksquare$, NPS ($0.008M$); $\blacktriangle$, NCS ($0.008M$). Enzyme activity was measured over 1 hr. at 37.5° in the presence of $0.5M$ acetate adjusted to the required pH. The curves are not quantitatively related to each other.

Fig. 3. Substrate concentration-activity curves for the debris ($\circ$) and supernatant ($\square$) fractions obtained by centrifuging after the extraction of acetone-dried human liver with water for 1 hr. at 37.5° . The curves are not quantitatively related to each other and were constructed by using APS at pH 6.7, NPS at pH 7.3 and NCS at pH 6.0.

almost identical with those obtained with whole liver and showed the presence of an enzyme ('insoluble') having a high affinity and activity (Table 1) towards these two substrates. The supernatant enzyme fraction ('soluble') had a low affinity and activity for APS and NPS but was very active towards NCS (Table 1). The separation of the two types of enzyme was not shown clearly by the NCS curves, since there was no marked difference between whole acetone-dried liver (Fig. 1), supernatant and debris. However, in a later section it is shown that the NCS activity of the debris is mainly due to residual 'soluble' type enzyme and therefore little distinction between the Michaelis curves of supernatant and debris could be expected.

Separation of two arylsulphatases from the 'soluble' enzyme fraction

The presence of two distinct arylsulphatases in the soluble material obtained by incubation (1 hr. at 37°) and centrifuging (20 000 *g* for 20 min.) of a 10% (w/v) water suspension of acetone-dried human liver was demonstrated by paper electrophoresis. Two well-defined bands of enzyme activity were separated on horizontally held Whatman no. 100 paper over 16 hr. at 220 v in the presence of 0.1 M acetate, pH 5.0, and at an ambient temperature of 4°. The enzymes were located by visual assessment of the nitrocatechol colour obtained after incubation of 1 cm. strips of the paper with 1 ml. of 0.016 M NCS (pH 5.6) for 1 hr., followed by the addition of 2 ml. of *N*-NaOH. The results are shown in Fig. 4.

Fig. 4. Electrophoretic movement of the arylsulphatases of a 10% (w/v) aqueous extract of acetone-dried human liver. Enzyme activity was visually assessed following incubation for 1 hr. with (1), 0.016 M NCS, pH 5.6; (2), 0.006 M NCS, pH 5.0 (optimum conditions for enzyme A); (3), 0.02 M NCS, pH 6.1 (optimum conditions for enzyme B). The activities shown are approximately related to each other. Electrophoresis of 0.05 ml. of the extract was in 0.1 M acetate, pH 5.0 for 16 hr. at 220 v, using Whatman no. 100 paper.

Table 1. Fractionation of an acetone-dried human liver

		Suspension of whole acetone-dried powder			'Insoluble' fraction			'Soluble' fraction			Recovery ('insoluble' and 'soluble') fraction		
		APS	NPS	NCS	APS	NPS	NCS	APS	NPS	NCS	APS	NPS	NCS
Activity (μg. of phenol liberated)		20 400	26 400	9 450	21 000	25 400	2 000	100	150	8 900	21 100	25 550	10 900
Percentage of activity of whole powder		100	100	100	102.8	96.3	21.2	0.5	0.6	94.2	103.3	96.9	115.4
Ratio of activities (moles of phenol liberated)		0.88:1:0.32			0.94:1:0.08			0.76:1:53.2			—		

The activity of each fraction arising from 1 g. of acetone-dried liver was measured in 0.5 M acetate buffer with 0.005 M APS, pH 6.9; 0.008 M NPS, pH 7.3 and 0.008 M NCS, pH 6.0, over 1 hr. at 37°. The APS and NPS activities of the 'soluble' fractions were near the lower limits of the sensitivity of the methods and the APS:NPS:NCS ratios quoted for this fraction are therefore only approximate.

Alternate freezing and thawing released into solution the major part of the NCS activity of fresh human liver, and an extract was prepared by thrice subjecting a 10% (w/v) water suspension of liver to this treatment, centrifuging at 20 000 *g* for 20 min. and concentrating by freeze-drying. On electrophoresis the extract showed two bands of arylsulphatase activity, having mobilities identical with those given by extracts of acetone-dried liver, showing that the separation of two 'soluble' enzymes is not an artifact of the acetone treatment.

From consideration of their kinetic behaviour the human 'soluble' enzymes could be termed A and B (see Fig. 4) in the same way as the corresponding enzymes of rat liver. Measurement under its own optimum conditions of the activity of each human enzyme, when eluted from the same electrophoresis paper, showed that there was approximately as much A as B, although individual livers showed some variation. This situation is different from that in the rat, where the ratio A:B is about 1:100. The NPS curves (Fig. 7) for enzyme A show that this

Fig. 5. Substrate concentration-activity curves, using NCS, for human arylsulphatases A and B. Incubation was for 1 hr. at 37.5° in the presence of 0.5M acetate adjusted to the appropriate optimum pH (see Fig. 6). x, A and B separated electrophoretically from aqueous extracts of acetone-dried liver; Δ, A and B separated by acetone fractionation of aqueous extracts of acetone-dried liver; O, A and B separated electrophoretically from aqueous extracts of acetone-dried kidney.

The results of the paper electrophoresis suggested a means of obtaining a sharp separation of the two 'soluble' enzymes for a study of their characteristics. The soluble material of a water-suspension of acetone-dried liver, prepared as previously described, was concentrated ten-fold by freeze-drying and 0.05 ml. portions were run on Whatman no. 100 papers (5 cm. in width) as described above. After localization of the enzymes on a small strip of one of the papers, sufficient amounts of the enzymes could be eluted with distilled water at 4° from the relevant portions of three such papers to allow the determination of pH-activity or substrate concentration-activity curves. The curves which were obtained (Figs. 5, 6) showed that the two enzymes had different characteristics. They were, however, more similar to those of the corresponding enzymes of rat liver (Dodgson *et al.* 1955*b*) than to those of ox liver (Roy, 1953, 1954).

Fig. 6. pH-activity curves, using NCS as substrate, for human arylsulphatases A and B. The appropriate optimum concentrations of NCS (see Fig. 5) were used, and incubation was for 1 hr. at 37.5° in the presence of 0.5M acetate. x, liver A and B separated electrophoretically; Δ, liver A and B separated by acetone fractionation; O, kidney A and B separated electrophoretically.

Fig. 7. Substrate concentration-activity (at pH 6.4) and pH-activity (at 0.008M NPS) curves for arylsulphatase A prepared electrophoretically from aqueous extracts of acetone-dried human liver. Incubation was for 1 hr. at 37.5° in the presence of 0.5M acetate.

enzyme has a low affinity for this substrate, and that its kinetic behaviour differs markedly from that of the 'insoluble' arylsulphatase (Figs. 2, 3). The NPS activity of enzyme B was almost negligible, and its kinetic properties towards this substrate could not be determined with accuracy.

A separation of the two enzymes of the soluble material of liver by acetone fractionation between the limits 0–40% (enzyme B) and 50–60% (enzyme A) as previously described for the rat (Dodgson *et al.* 1955*b*) gave fractions which had the same electrophoretic mobilities as the A and B enzymes described above. The kinetic behaviour was much the same as that of the electrophoretically prepared enzymes (Figs. 5, 6), except that enzyme B prepared by acetone fractionation had a lower pH optimum than that prepared electrophoretically. This suggested that the former was contaminated with enzyme A, although paper electrophoresis indicated that enzymes A and B prepared by acetone fractionation were largely uncontaminated with each other. However, appreciable enzyme loss occurs during the electrophoresis and small amounts of contaminating enzyme might be completely destroyed.

The 'insoluble' enzyme

From Table 1 and Figs. 1–3 it is clear that the activity of acetone-dried human liver against 0.005*M* APS at pH 6.9 and 0.008*M* NPS at pH 7.3 is essentially a measure of the 'insoluble' enzyme, since the activity and competitive power of the 'soluble' enzymes is negligible under these conditions.

Table 2. *Effects of phosphate and fluoride on the activities of enzymes A, B and the 'insoluble' enzyme*

Activities were measured under the appropriate optimum conditions for each enzyme (see Figs. 3, 5 and 6).

Inhibitor (0.025 <i>M</i>)	Percentage inhibition			
	'Insoluble'		A	B
	NPS	NCS	NCS	NCS
NaH ₂ PO ₄	25	87	90	93
NaF	11	89	34	75

As with the rat (Dodgson *et al.* 1955*b*), the once-washed debris of water-extracted acetone-dried liver was contaminated with residual 'soluble' enzymes, since further washings removed NCS activity in progressively decreasing amounts but did not affect the NPS activity. However, a debris which had been washed five times still possessed considerable NCS activity that was impossible to bring into solution. The major part of this activity

appeared to be due to 'soluble' enzymes, since it was strongly inhibited by 0.025*M* phosphate and fluoride, both of which only slightly affect the NPS activities but which are known to inhibit strongly the 'soluble' enzymes A and B (Table 2). A complete separation of the 'insoluble' enzyme from the residual 'soluble' enzymes could not be made, since it was not possible to obtain the debris enzyme in solution.

Attempted solubilization of the 'insoluble' enzyme. It has previously been reported (Dodgson *et al.* 1955*b*) that the rat 'insoluble' enzyme cannot be brought into solution by normal methods. This also applies to the human 'insoluble' enzyme. Unsuccessful attempts were made to solubilize the enzyme of acetone-dried human liver and of liver treated in the following way.

The liver (80 g.) was macerated in 300 ml. of 0.5*M* acetate, pH 7.2, and centrifuged at 2200 *g* for 15 min. The cloudy supernatant solution was thrice alternately frozen and thawed. The suspended particles were aggregated by adjusting the pH to 5.0 (Dodgson, Spencer & Thomas, 1954), when they could be collected by centrifuging at 2200 *g* for 30 min. The precipitate was washed by resuspending in 0.5*M* acetate, pH 5.0, recentrifuged and subsequently freeze-dried. Incubation of these preparations with acetate buffers of varying pH, saline solutions and glycerol, or treatment with acetone or *n*-butanol (cf. Morton, 1954) did not solubilize the enzyme. Treatment with the surface-active agents Empicol, Triton WR-1339, cetylpyridinium bromide, sodium dodecyl sulphate, Teepol-530, cetyltrimethylammonium bromide, glyceryl monostearate and Vykamol was no more effective and, with the exception of Triton, strongly inhibited enzyme activity. Lissapol-N appeared to release a small amount of the enzyme into solution but was strongly inhibitory. Incubation with papain or crystalline trypsin (Light and Co.) failed to solubilize the enzyme, but incubation with a crude preparation of trypsin (B.D.H. Ltd.) released small amounts into solution.

Distribution of the three enzymes in other tissues

A number of other tissues were examined for the presence of the three enzymes. A primary division into 'soluble' and 'insoluble' enzymes was first made as follows: suspensions of the acetone-dried powders of the tissues in 0.5*M* acetate, pH 6.5, were incubated at 37° for 1 hr. The concentration of the incubation mixture used depended on the enzyme activities of the tissues as determined by preliminary experiments but, generally speaking, varied between 0.25 and 1.5% (w/v). The incubated suspension was assayed against NCS and NPS by using the respective optimum conditions obtained for acetone-dried liver (Figs. 1, 2). A

portion (15 ml.) of the suspension was centrifuged at 22 000 *g* for 30 min. at 0°. The supernatant was separated and the debris washed with a further 15 ml. of 0.5M acetate, pH 6.5. After centrifuging as before, the two supernatants were combined and the debris was suspended in 15 ml. of 0.5M acetate, pH 6.5. Both debris and supernatant fractions were adjusted to the appropriate pH and were then assayed against NPS and NCS as described above. As explained above, the results obtained with NPS are largely indicative of the activity of the 'insoluble' enzyme, whereas the NCS results give an approximate measure of the two 'soluble' enzymes.

By the use of paper electrophoresis it has also been possible to show the presence of two 'soluble'-type enzymes in all the tissues examined. Water suspensions (10 %, w/v) of the acetone-dried tissues were incubated for 1 hr. at 37° and the supernatant obtained after centrifuging at 20 000 *g* for 30 min. was concentrated by freeze-drying. The concentrates, together with a reference liver concentrate, were then subjected to paper electrophoresis under the conditions described previously. Enzyme activity was located after incubating 1 cm. strips of the paper with NCS, using two sets of experimental conditions, namely 0.006M NCS in 1.0M

Table 3. Fractionation of acetone-dried human tissues to show the presence of 'insoluble' and 'soluble' type arylsulphatases

The activity of each fraction, arising from 1 g. of acetone-dried tissue, was measured in 0.5M acetate buffer with 0.008M NPS (pH 7.3), and 0.008M NCS (pH 6.0) over 1 hr. at 37°. The NPS activities of the soluble fractions were in some cases near the lower limits of the sensitivity of the method, and the NPS:NCS ratios quoted for these fractions are therefore only approximate. Ranges of activities are given in parentheses.

Tissue	No. of samples	Activity of 1 g. of whole acetone-dried tissue (μg. of phenol liberated)		Percentage of activity of whole tissue				Ratio of activities NPS:NCS (moles of phenol liberated)		
				‘Insoluble’ fraction		‘Soluble’ fraction		Whole tissue	‘Insoluble’ fraction	‘Soluble’ fraction
		NPS	NCS	NPS	NCS					
Liver	4	35 550 (26 400–46 100)	13 260 (9 450–16 700)	105	26	4	103	1:0.4	1:0.1	1:11
Pancreas	3	16 600 (15 000–18 200)	8 000 (6 700–9 000)	78	11	14	101	1:0.5	1:0.1	1:55
Kidney	4	13 700 (4 480–20 100)	22 750 (11 050–33 600)	96	13	4	108	1:1.6	1:0.2	1:49
Lung	3	1 150 (730–1 790)	4 780 (2 520–6 350)	97	16	13	123	1:3.4	1:0.6	1:34
Brain	4	510 (320–720)	2 900 (2 570–3 450)	73	19	13	120	1:4.1	1:0.9	1:176
Heart	3	100 (60–120)	2 100 (1 600–2 800)	77	22	18	123	1:22	1:6	1:150
Large intestine	1	950	390	94	15	7	98	1:2.3	1:0.4	1:30
Small intestine	1	2 500	4 200	56	28	14	116	1:1.7	1:0.3	1:14
Spleen	4	3 500 (2 700–6 000)	3 900 (2 200–5 500)	Fractionation was not practicable						

Table 3 shows that all the tissues examined contained both types of arylsulphatases. Since none of the tissues can be described as perfectly fresh, and in view of the small number of samples examined, no absolute quantitative significance can be placed on the results. However, the pancreas, kidney and liver do appear to be the richest sources of both types of enzyme. Moreover, the figures indicate that the ratio of the amounts of the 'insoluble' and 'soluble' enzymes in the liver as measured by the activity towards NPS and NCS (1:0.4) is greater than that observed for most other organs, and is very different from the ratio obtained for the comparable two enzyme fractions of rat liver (1:19.5; see Dodgson *et al.* 1955*b*).

acetate, pH 5.0 (optimum conditions for enzyme A), and 0.02M NCS in 1.0M acetate, pH 6.1 (optimum conditions for B). In all the tissues examined, two enzymes, whose electrophoretic mobilities and behaviour towards the two sets of experimental conditions corresponded to enzymes A and B of human liver, were present (cf. Fig. 4). The two enzymes were not artifacts resulting from the acetone treatment, since in the selected cases of liver, spleen and heart it was possible to show the presence of the same two enzymes by electrophoresis of concentrated aqueous extracts of fresh tissue homogenates. With kidney, the pH-activity and substrate concentration-activity curves for the two electrophoretically separated

enzymes (Figs. 5, 6) are similar to those obtained for liver enzymes A and B (Figs. 5, 6), but the kinetics of the enzymes of other tissues has not been investigated. It has been possible to show that the slight discrepancies which occur in the pH-activity and substrate concentration-activity curves for the various preparations of enzyme A (Figs. 5, 6) are the result of kinetic anomalies which this enzyme exhibits and which it is hoped to comment on in a later paper.

DISCUSSION

As in the case of the rat and the ox livers, the arylsulphatase activity of the human liver can be attributed to three distinct enzymes possessing very different substrate affinities. Two of these enzymes (A and B) can be readily solubilized and it is presumed that, as in the rat, they are largely associated with the mitochondrial particles of the liver cell. The remaining enzyme has not yet been obtained in soluble form and is probably microsomal in origin (cf. Dodgson *et al.* 1954, 1955*b*). Mouse and rabbit livers possess at least two of these enzymes (Dodgson *et al.* 1955*b*), and it is likely that this multiplicity of arylsulphatases is common to all mammalian species. The optimum conditions of pH and substrate concentration for the three human liver enzymes are listed in Table 4,

closely examined for the presence of an enzyme with kinetic properties similar to those of ox-liver enzyme B. No such enzyme has yet been detected. On the other hand, there is no doubt about the comparable nature of ox-, rat- and human-liver enzyme A since all show the kinetic anomalies which were first noted for the ox enzyme (Roy, 1953).

The 'insoluble' enzyme is very similar to the analogous rat enzyme though it appears to be even more tightly bound to the cellular particles. Dodgson *et al.* (1955*a*) have noted that the rat 'insoluble' enzyme can be solubilized by haemolytic surface-active agents but, with the exception of Lissapol-N, no such effect was noted with the human enzyme. In view of the insoluble nature of the enzyme it is not surprising that Dodgson & Spencer (1954) failed to detect any significant arylsulphatase activity in human urine and serum when NPS or APS was used as substrate. Urine and serum contain an arylsulphatase which is active towards NCS, and investigations now in progress in this Laboratory are directed towards establishing the nature of the enzymes which are responsible for this activity.

The separate assay of the three enzymes in human tissues will obviously be difficult. By using APS as substrate a reasonable measure of the activity of the 'insoluble' enzyme can be obtained.

Table 4. Optimum conditions of pH and substrate concentration (*S*) for the 'insoluble' and 'soluble' (A and B) enzymes of rat, human and ox liver

Species		'Insoluble'		A		B		Reference
		APS	NPS	NPS	NCS	NPS	NCS	
Rat	pH	7.2	7.0	5.8	5.0	6.2	6.0	Dodgson <i>et al.</i> (1955 <i>b</i>)
	<i>S</i>	0.007	0.006	0.12	0.008	0.12	0.02	
Human	pH	6.9	7.3	6.2	5.0	—	6.1	—
	<i>S</i>	0.005	0.008	0.07	0.006	—	0.02	
Ox	pH	—	—	—	4.9	—	5.7	Roy (1953, 1954)
	<i>S</i>	—	0.008*	0.06*	0.003	0.06*	0.06	

* Determined at pH 7.0.

and may be compared with those obtained for the comparable enzymes of ox and rat livers. With one notable exception, the kinetic characteristics of the enzymes from the different species were similar. The affinity of ox-liver enzyme B for NCS is considerably less than the corresponding affinities of rat- and human-liver enzyme, B and indeed, the optimum substrate concentration for ox-liver enzyme B could not be determined since enzyme activity was still increasing linearly when the concentration of NCS was 0.06 M, the practical limit of the solubility of NCS for enzyme experiment (Roy, 1954). During the course of the present work the soluble fractions of rat and human livers have been

Dodgson & Spencer (1953) have noted in fresh rat tissues that *p*-nitrophenol is metabolized to varying extents; caution is therefore necessary when NPS is used as a substrate. Similarly, the use of NCS to measure enzymes A and B is also limited because of the ability of certain fresh tissues to metabolize liberated nitrocatechol (Dodgson & Spencer, 1953). Further problems are posed by the kinetic anomalies of enzyme A and the presence in fresh tissues of an endogenous inhibitor which Maengwyn-Davies & Friedenwald (1954) have shown to be phosphate in the rabbit. Apart from these considerations it is obviously difficult to obtain more than an approximate estimate of the separate

enzyme activity when two or more enzymes of similar substrate specificities are present. More accurate estimates of the respective amounts of A and B await the development of methods for the quantitative separation of the two enzymes, or alternatively the establishment of conditions for reducing the activity of one of the enzymes to zero without affecting the activity of the other.

Some evidence has been presented (Dodgson *et al.* 1954) which suggested that the 'insoluble' enzyme of rat liver was not concerned with the synthesis of arylsulphates, but these observations were not extended to enzymes A and B. It seems likely that the three enzymes, which are so widely distributed in various animal tissues, may well have some important general function rather than a more specific function (e.g. detoxication) which is confined to relatively few organs.

SUMMARY

1. Human liver and a number of other tissues have been shown to possess three different arylsulphatases.

2. Two of these enzymes were readily solubilized and were comparable to enzymes A and B of rat liver. The other enzyme could not be obtained in

soluble form and had properties which were very similar to those of the microsomal arylsulphatase of rat liver.

3. The three enzymes have been separated from each other and their characteristics investigated.

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Studies on the Acetylation of Human Fibrinogen

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The effects of substitution and other modifications on the biological and physical properties of fibrinogen have been widely examined in recent years. Jaques (1943) investigated the chemical changes on iodination and showed that the aromatic residues are involved in the reaction. Laki & Mihalyi (1949) also studied the effect of iodination, and found that under the conditions of their experiments fibrinogen became incoagulable by thrombin. Physicochemical studies on the polymerization of iodinated fibrinogen by Laki & Steiner (1952) demonstrated the formation of intermediate polymers differing from those formed by untreated fibrinogen, and also a change in the pH optima of the various stages of association. The action of tyrosinase on the components of the clotting system was studied by Sizer & Wagley (1951) and Krugelis & Sizer (1954); a progressive increase in the clotting time of fibrinogen occurred with the period of enzyme action. Oxidation by gaseous oxygen increases the

clotting time of plasma (Palos, 1949). Formaldehyde and potassium permanganate have occasionally been used to stop the thrombin-fibrinogen reaction, but the properties of the modified protein have not been investigated.

In this investigation, human fibrinogen has been acetylated under exceptionally mild conditions with *NS*-diacetylthioethanolamine, which reacts only with free amino groups (Baddiley, Kekwick & Thain, 1952), and the properties of the modified protein, its reaction with thrombin and the behaviour of mixtures of the acetylated and untreated fibrinogen in the thrombin-fibrinogen reaction have been examined.

MATERIALS AND METHODS

Fibrinogen. This protein, prepared by the method of Kekwick, Mackay, Nance & Record (1955), had clottable N values between 97 and 98%, and was essentially free of plasminogen and plasmin.