The Sulfotransferases of Guinea Pig Liver

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SUMMARY

A method is described for the separation of the sulfotransferases of guinea pig liver. A phenol sulfotransferase has been obtained free from any other sulfurylating activity so far sought. Androstenolone sulfotransferase and estrone sulfotransferase have been partially separated, and it is suggested that both these enzymes also sulfurylate *p*-nitrophenol and 2-naphthylamine. Deoxycorticosterone sulfate is almost certainly formed by another sulfotransferase.

INTRODUCTION

It has long been known from studies *in vivo* that most animals can synthesize many different types of sulfate ester (1). In the past it has usually been assumed that such compounds are end products of metabolism, but it has now become obvious that, at least among the steroids, sulfate esters play an important role in normal metabolic interconversions (2, 3). Also, regardless of their importance under strictly physiological conditions, sulfate esters undoubtedly play a considerable part in the metabolism of many drugs and so have some importance in pharmacology (4, 5).

The formation of sulfate esters involves the transfer of the sulfuryl group from adenosine 3′-phosphate 5′-sulfatophosphate (PAPS) to the appropriate acceptor in a reaction catlyzed by a sulfotransferase: for example, the transfer to *p*-nitrophenol is catalyzed by phenol sulfotransferase (3′-phosphoadenylylsulfate-phenol sulfotransferase; EC 2.8.2.1.). Although the sulfotransferases have been quite extensively studied in crude preparations, none of them has been purified and there is still considerable doubt as to their specificity and their number in, for instance, mammalian liver, which is by far their most studied, although not necessarily their most important, source.

Nose and Lipmann (6) partially separated the sulfotransferases of rat liver that were responsible for the formation of androstenolone sulfate (dehydroepiandrosterone sulfate), estrone sulfate, and *p*-nitrophenyl sulfate, but they did not further characterize these enzymes. Also from rat liver, Carroll and Spencer (7) separated two groups of sulfotransferases, one of which catalyzed the formation of androstenolone sulfate and *p*-nitrophenyl sulfate, and the other of alkyl sulfates and aryl sulfamates. Although details of this latter work have not appeared it is obvious that a considerable purification of the enzymes has been achieved. On the other hand, they were unable to obtain any separation of the various sulfotransferase activities in rabbit liver.

The present investigation has been concerned with the sulfotransferases of guinea pig liver. This species was chosen because of the recent suggestion by Roy (8) that in it the synthesis of 2-naphthyl sulfamate was brought about by an allosteric enzyme which was inhibited by very low concentrations of 17-oxosteroids, an observation of obvious interest in view of the current ideas on the role of such allosteric effects in control mechanisms. The sulfotransferases present in guinea pig liver have been par-

tially purified and to some extent separated, and it is clear that the specificities of these enzymes are rather different from what has been believed in the past (4).

MATERIALS AND METHODS

Preparation of PAPS. The crude PAPS-containing solution was prepared with an extract of rat liver as a source of the sulfate activating enzymes, as previously described (9), except that the concentration of ATP was increased to 0.014 M and the time of incubation to 2 hr. After precipitation of the proteins from the reaction mixture with ethanol the solution was concentrated to about 15 ml in a rotary evaporator at a bath temperature of 37°. The pH of the concentrate was adjusted to 7.2 and, after filtration, it was applied to a column (18 × 1.5 cm) of Dowex 1 × 10 (Cl^- form). The column was washed with water and then with 0.5 M NaCl until the absorbance at 260 mμ had fallen below 0.03, whereupon the PAPS was eluted with about 700 ml of 1 *M* NaCl (10). The fractions containing PAPS were combined and stirred for 10 minutes with acid-washed Norit-A charcoal (1 g/3 μmoles PAPS, based on the absorbance at 260 mμ) to absorb the nucleotide. After it had been washed with water to remove Cl^- ions, PAPS was eluted from the charcoal with about 1 liter of aqueous ethanol (50% v/v) containing 1% of 1 M NH_4OH, and the eluate was concentrated in a rotary evaporator as before to about 25 ml. The pH of the solution was adjusted to 7.2 and, after filtration, its volume was adjusted with water to make the PAPS concentration 0.33 mM, based on the absorbance of the solution at 260 mμ.

The usual yield of PAPS was about 1.5 μmoles per gram liver, amounting to about 2.5% of the ATP used in the incubation mixture. An analysis (11) of a typical preparation gave: adenosine, 1.00; labile PO_4^{3-}, 0.98; total PO_4^{3-}, 1.95; transferable SO_4^{2-}, 0.82.

Determination of sulfotransferase activities. The method was based on that previously described by Roy (12) for the determination of steroid sulfates but differed in using PAPS as the sulfuryl donor. The reaction mixtures had the compositions given in Table 1 and after incubation of these at 37° for the appropriate time the reaction was stopped by the addition of 1 ml of methylene blue reagent (13) followed by 5 ml of chloroform. After vigorous shaking the mixture was briefly centrifuged, the chloroform layer was separated and dried over Na_2SO_4; its absorbance at 650 mμ was measured against the appropriate blank in a Unicam S.P. 600 spectrophtometer. The amounts of sulfate ester produced were determined from suitable calibration curves.

The recoveries of the various sulfate esters added to the appropriate reaction mixture containing 0.6 mg protein per milliliter were as follows: 23.8 and 47.5 μmmoles

TABLE 1

Experimental conditions used for the assay of the sulfotransferases

The concentrations given in the table are the final ones in the reaction mixture, which had a volume of 1 ml. The *p*-nitrophenol and the 2-naphthylamine (as hydrochloride) were added in aqueous solution while the steroids (androstenolone or estrone) were added in 0.1 ml of a 1 mM solution in propylene glycol.

	Phenol sulfotransferase	Steroid sulfotransferase		
Substrate	*p*-Nitrophenol	Steroid	*p*-Nitrophenol	2-Naphthylamine
Conc. (mM)	1.0	0.1	1.0	2.0
PAPS conc. (mM)	0.1	0.1	0.1	0.1
Buffer type	Acetate, pH 5.8	Tris, pH 7.5	Tris, pH 7.5	Tris, pH 8.2
conc. (M)	0.1	0.1	0.1	0.1
Mg^{2+} conc. (mM)	0	2 mM excess over the EDTA in the enzyme		
Reaction time (min)	30	30	30	60

p-nitrophenyl sulfate, 100% recovery; 8.6 and 25.3 μmmoles androstenolone sulfate, 97% recovery; 68.4 and 135 μmmoles 2-naphthyl sulfamate, 98% and 100% recovery. Obviously for the present purpose these recoveries can all be regarded as quantitative.

Preparation of the sulfotransferases. The mixture of sulfotransferases was obtained as previously described (9) by precipitating a 0.15 M KCl extract of guinea pig liver between 1.5 and 2.3 M $(NH_4)_2SO_4$ and dissolving the precipitate in water (0.5 ml per gram liver). Usually about 150 g liver was taken for a preparation. The solution, which contained both the sulfate activating enzymes and sulfotransferases, was dialyzed in the cold for 3 days against repeated changes of 20 volumes of water to destroy the former, after which the pH was adjusted to 5.0 with 0.5 N acetic acid. The resulting precipitate was centrifuged off and dissolved in 30 ml of 0.1 M Tris-acetic acid buffer, pH 7.5, and the pH was readjusted to this figure with N Na_2CO_3. After it had stood for a few hours the insoluble material was centrifuged off and discarded.

The supernatant was applied to a column (50 × 5 cm) of Sephadex G-200 in 0.1 M Tris-HCl buffer, pH 7.5, which was developed at room temperature with the same buffer. The sulfotransferases were eluted in a single sharp peak after about 600 ml of buffer had passed through the column. The fractions containing the sulfotransferases were combined and made 0.01 M in EDTA by adding the requisite amount of 5% solution of EDTA previously adjusted to pH 7.5 with NaOH. This solution was concentrated by ultrafiltration (14) to about 5 ml and exhaustively dialyzed against a 0.01 M EDTA buffer, pH 7.5 (0.01 mole disodium EDTA in water adjusted to pH 7.5 with NaOH and made up to 1 liter).

The sulfotransferases were then partially separated by chromatography at 4° on a column (30 × 1.5 cm) of DEAE-Sephadex A-50 equilibrated with the above buffer, elution being carried out with a linear gradient formed from 100 ml of the equilibrating buffer and 100 ml of a similarly prepared buffer containing 0.01 M EDTA, 0.05 M Tris, and 0.3 M sodium acetate, pH 7.5. Fractions of 4 ml were collected. Under these conditions the steroid sulfotransferases, the arylamine sulfotransferase, and part of the phenol sulfotransferase activities were eluted between the 6th and the 18th tubes. The further fractionation of those enzymes, referred to as the steroid sulfotransferases, is considered below. After about 100 ml of the gradient had passed through the column the remaining buffer was removed and the elution restarted with a buffer, pH 7.5, containing 0.03 M EDTA, 0.05 M Tris and 0.3 M sodium acetate. The major part of the phenol sulfotransferase activity was eluted around the 12th tube.

TABLE 2

Purification of the sulfotransferases from guinea pig liver

The table gives the results of a preparation from 170 g of guinea pig liver. The total activities (columns 1) are expressed as micromoles of sulfate ester formed in 30 min under the assay conditions in Table 1. The specific activities (columns 2) are based on the protein content of the solutions determined by their absorbance at 280 mμ, assuming the $E_{1\,cm}^{1\%}$ of protein to be 10.

	Phenol sulfotransferase		Androstenolone sulfotransferase		Estrone sulfotransferase	
Stage	(1)	(2)	(1)	(2)	(1)	(2)
KCl extract	92	5.1	76	4.2	27	1.5
$(NH_4)_2SO_4$ ppt.	57	7.6	69	9.1	27	3.6
pH 5 ppt.	53	19	37	14	14	5.0
G-200 eluate	42	87	29	62	11	24
DEAE eluate	[a]11	86	16	85	7.9	105

[a] Only this value refers to the synthesis of *p*-nitrophenyl sulfate by the phenol sulfotransferase alone; the others refer to the synthesis by this and by the steroid sulfotransferases.

The two sulfotransferase preparations were concentrated by ultrafiltration to about 5 ml and dialyzed against 0.03 M EDTA, pH 7.5. Under these conditions the enzymes retained their activity for many

months when stored at —30° and for several weeks when stored at 5°. These concentrated enzyme solutions were diluted with water, usually about 5 times, immediately before use.

The course of a typical preparation of the sulfotransferases is shown in Table 2.

RESULTS

Phenol Sulfotransferase

As pointed out above, enzymes capable of transferring the sulfuryl group from PAPS to *p*-nitrophenol were eluted from DEAE-Sephadex in two distinct fractions, a major fraction referred to as phenol sulfotransferase and a minor fraction associated with the steroid sulfotransferases. Both fractions formed *p*-nitrophenyl sulfate at a rate directly proportional to the amount of enzyme present but showed zero-order kinetics for only a limited time, 10 min and 30 min for the phenol sulfotransferase and steroid sulfotransferase, respectively. For kinetic studies of these enzymes incubation times of 10 min were therefore used. A striking difference between the two was their different requirements for Mg^{2+} ions: those apparently were not required for the synthesis of *p*-nitrophenyl sulfate by phenol sulfotransferase but were required by steroid sulfotransferase. This is clearly shown by the following tabulation which gives the relative rates of formation of *p*-nitrophenyl sulfate by the two fractions with and without Mg^{2+} ions.

Fraction	Phenol sulfotransferase	Steroid sulfotransferase
With 2 mM excess Mg^+	100	100
Without Mg^+	101	60

The two fractions also differed in their pH optima (Fig. 1): phenol sulfotransferase had a pH optimum of 5.8 while steroid sulfotransferase had a very flat pH optimum between 7 and 7.5. Both fractions showed optimum activity at 1.0 mM *p*-nitrophenol and had not reached an optimum by 0.2 mM PAPS. The effect of variations in substrate concentrations on the rate of synthesis of *p*-nitrophenyl sulfate by the steroid sulfotransferase fraction was not investigated further because, as is discussed below, more than one sulfotransferase was involved. Figures 2 and 3 show the effects of varying *p*-nitrophenol and PAPS concentrations on the activity of the phenol sulfotransferase. Analysis of the results by the method of Wilkinson (15) gave mean K_m values, with 95% confidence limits, of 0.080 ± 0.015 mM *p*-nitrophenol and 0.030 ± 0.005 mM PAPS. The two

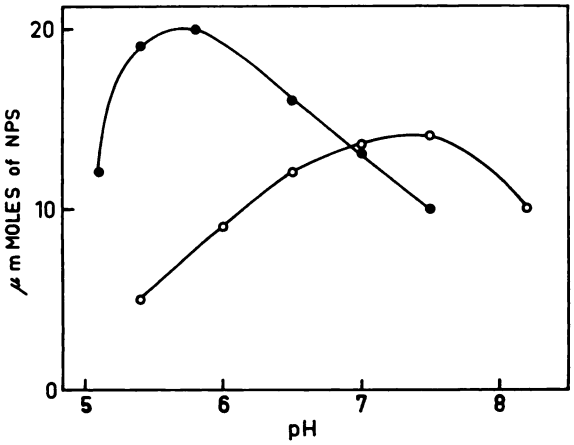

FIG. 1. *The effect of pH on the synthesis of p-nitrophenyl sulfate (NPS) by phenol sulfotransferase (●) and unfractionated steroid sulfotransferase (○) under the general conditions of assay given in Table 1*

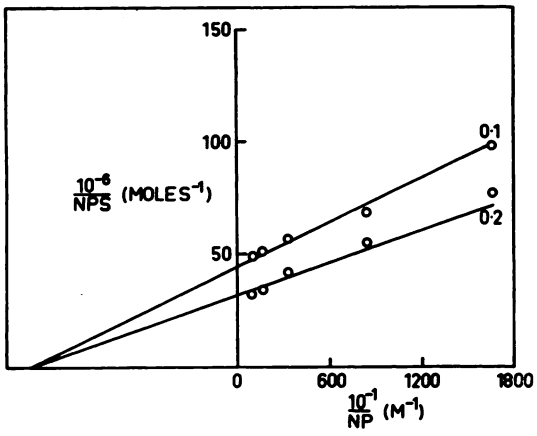

FIG. 2. *The effect of varying concentrations of p-nitrophenol (NP) on the synthesis of NPS by phenol sulfotransferase*

General conditions as in Table 1, but with the PAPS concentration (mM) specified on the figure.

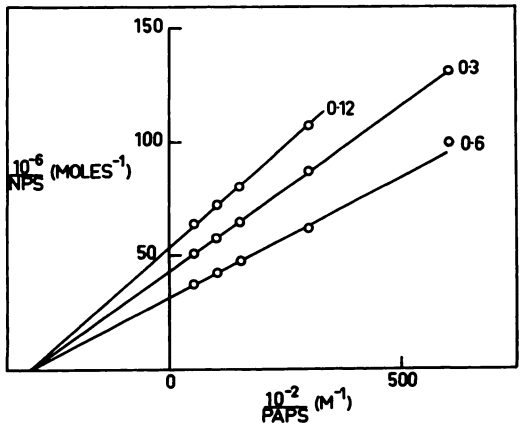

FIG. 3. *The effect of varying concentrations of PAPS on the synthesis of NPS by phenol sulfotransferase*

General conditions as in Table 1, but with the nitrophenol concentrations (mM) specified on the figure.

values were obviously independent as variation in the concentration of one substrate had no effect on the K_m of the other.

Phenol sulfotransferase could not sulfurylate any other type of potential sulfate acceptor which was tested. A preparation capable of catalyzing the formation of 44 μmmoles of *p*-nitrophenyl sulfate in 10 min formed no detectable amounts (less than 0.2 μmmole) of androstenolone sulfate, estrone sulfate, testosterone sulfate, and 2-naphthyl sulfamate, even on prolonged incubation. On the other hand, m-aminophenol, 1-naphthol, and 2-naphthol were sulfurylated at rates comparable to *p*-nitrophenol. Phenol itself reacted slowly.

Steroid Sulfotransferases

This fraction, which was eluted from the DEAE-Sephadex column with a sodium acetate gradient, could sulfurylate androstenolone, estrone, *p*-nitrophenol, and 2-naphthylamine. However, even on one passage through DEAE-Sephadex some separation of the various activities was obtained, as shown on the top section of Fig. 4. Two fractions, representing the peaks of the

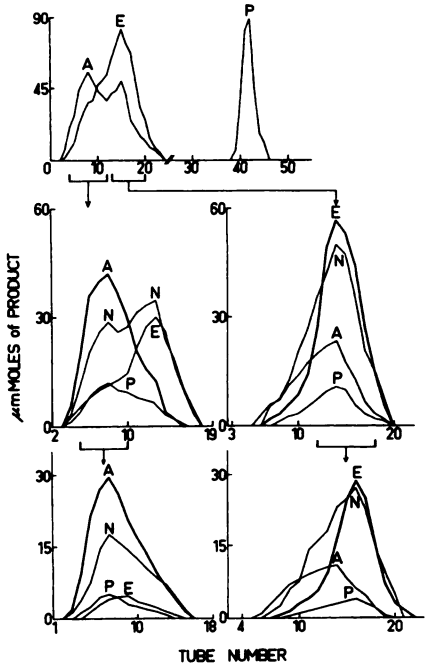

FIG. 4. *The separation of the various sulfotransferase activities in a partially purified extract of guinea pig liver by chromatography on DEAE-Sephadex*

The sulfotransferase activities (determined as in Table 1) were measured with androstenolone (A), estrone (E), *p*-nitrophenol (P), and 2-naphthylamine (N). The break in the abscissa in the top section indicates the change in the buffer used for the elution (see text). Chromatographic procedure as described in text.

androstenolone and estrone sulfotransferases, were separately collected and rechromatographed on DEAE-Sephadex exactly as before, giving the elution pattern shown in the center section of Fig. 4. Again the androstenolone and estrone sulfotransferase peaks were separated and rechromatographed, giving the pattern shown in the bottom section of the figure. It is quite clear from those results that androstenolone sulfate and estrone sulfate were formed by two independent enzymes although their complete separation could not be achieved under these conditions.

It is likewise clear from Fig. 4 that the synthesis of 2-naphthyl sulfamate and of *p*-nitrophenyl sulfate occurred with both the androstenolone and estrone sulfotransferases. This is made more obvious in Fig. 5 which shows the ratios in the various fractions obtained during the second passage through DEAE-Sephadex (shown in Fig. 4) of the amounts of 2-naphthyl sulfamate produced to the corresponding amounts of, first, androstenolone sulfate, secondly, estrone sulfate, and thirdly, androstenolone plus estrone sulfates. Fig. 6 shows similar ratios for *p*-nitrophenyl sulfate and the steroid sulfates. Only when the naphthyl sulfamate or the *p*-nitrophenyl sulfate was compared with the total steroid sulfate—that is, androstenolone plus estrone sulfates—were constant ratios obtained. Confirmation of this constancy was provided by considering all the appropriate ratios obtained from the chromatograms

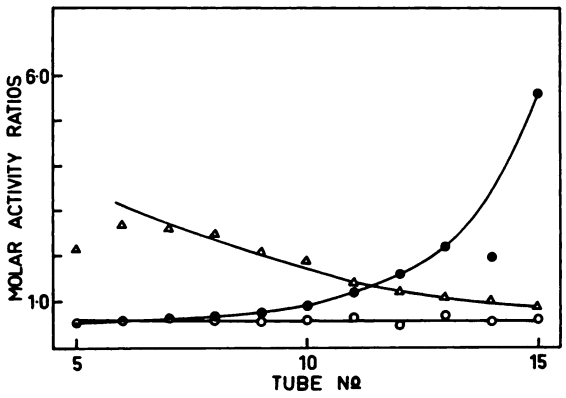

FIG. 5. *The amount of 2-naphthyl sulfamate synthesis in each of the appropriate fractions of the center section of Fig. 4 expressed as a ratio with the amounts of androstenolone sulfate (●), estrone sulfate (△), and androstenolone plus estrone sulfates (○) similarly synthesized*

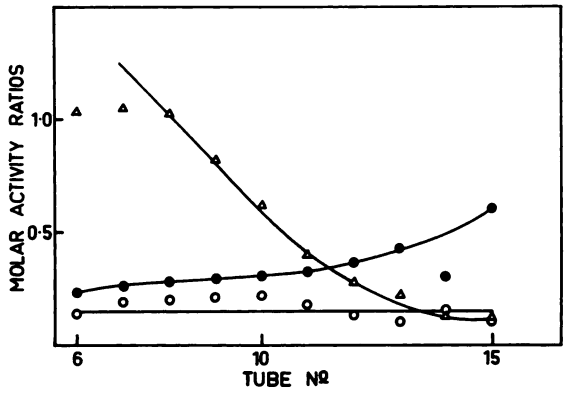

FIG. 6. *The amount of p-nitrophenyl sulfate synthesis in Fig. 4 expressed as in Fig. 5*

shown in Fig. 4 and randomizing them into two groups: application of Student's t test showed that the two group means were not significantly different, even at the 50% level. For 17 degrees of freedom the value of t was 0.42 in the case of the ratios of naphthyl sulfamate to total steroid sulfate and 0.55 for the corresponding ratios with p-nitrophenyl sulfate. The mean ratios, with the 95% confidence limits, were 0.65 ± 0.05 and 0.15 ± 0.02 for 2-naphthyl sulfamate and p-nitrophenyl sulfate, respectively. These results strongly suggest that the synthesis of 2-naphthyl sulfamate and of p-nitrophenyl sulfate by the steroid sulfotransferase fraction were in fact catalyzed by both androstenolone and estrone sulfotransferases.

All the activities shown by the latter two sulfotransferases required the presence of 2 mM Mg^{2+} ions (in excess over the EDTA added with the enzyme) for their full activity. Increasing the Mg^{2+} ion concentration to 10 mM gave no further rise in activity. The pH curves for the several activities are shown in Figs. 7 and 8 which clearly support the hypothesis that p-nitrophenyl sulfate and 2-naphthyl sulfamate are formed by both the steroid sulfotransferases. It should be noted that estrone sulfotransferase has an optimum pH of 6.2, both with estrone and with 2-naphthyl-

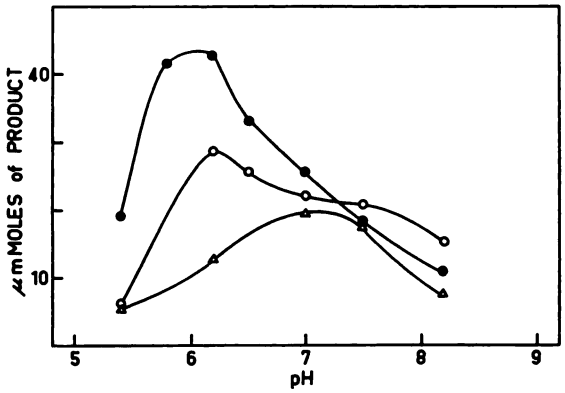

FIG. 7. *The effect of changes in pH on the synthesis of estrone sulfate* (●), *2-naphthyl sulfamate* (○), *and p-nitrophenyl sulfate* (△) *by estrone sulfotransferase*

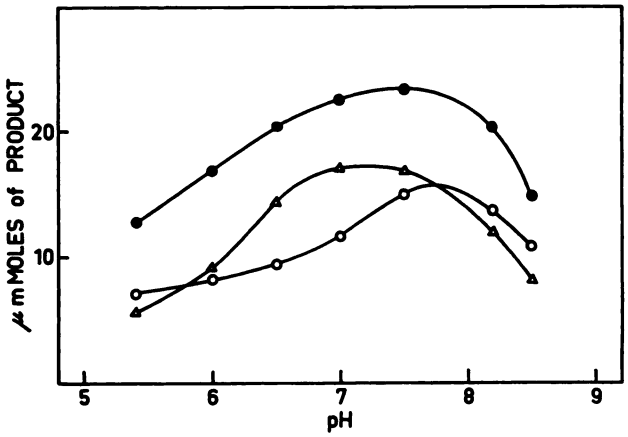

FIG. 8. *The effect of changes in pH on the synthesis of androstenolone sulfate* (●), *2-naphthyl sulfamate* (○), *and p-nitrophenyl sulfate* (△) *by androstenolone sulfotransferase*

amine as substrates, so that the conditions used for the assay of estrone sulfotransferase (Table 1) are not in fact optimal. This cannot alter the significance of the results presented here. Both steroid sulfotransferases had failed to reach an optimum activity at 0.2 mM PAPS: they were likewise unsaturated at 2 mM 2-naphthylamine but were saturated by 0.1 mM concentrations of the appropriate steroids.

It has previously been shown (9, 16) that the synthesis of 2-naphthyl sulfamate by crude preparations from guinea pig liver was powerfully inhibited by 3β-methoxyandrost-5-en-17-one (androstenolone methyl ether). The effect of this compound on the more purified enzyme preparations now available was therefore investigated. The results are summarized in Table 3 which shows that the formation of 2-naphthyl sulfamate by both androstenolone and estrone sulfotransferase was inhibited by 2 μM concentrations of androstenolone methyl ether. In the case of the estrone sulfotransferase, the synthesis of *p*-nitrophenyl sulfate was also inhibited, but to a lesser extent.

A detailed study of the specificity of the androstenolone and estrone sulfotransferases would clearly be premature at this stage because of their incomplete separation. The only other sulfate acceptors which have been tested are androsterone, cholesterol, testosterone, and deoxycorticosterone. Androsterone and cholesterol seem to be substrates for androstenolone sulfotransferase, as shown by the constancy of the ratios of the various activities through the stages of the purification (Table 4). With testosterone the situation was not so clear: the rate of formation of testosterone sulfate was much lower than that of the other steroid sulfates, but the activity was clearly associated with the androstenolone sulfo-

TABLE 3

The effect of 2 μM 3β-methoxyandrost-5-en-17-one on sulfotransferase activity

The activities were determined as in Table 1, and the values are expressed as rates relative to that (1.00) found in the absence of the added steroid, the absolute values of which were 27.1 μmmoles androstenolone sulfate and 64.9 μmmoles estrone sulfate, respectively, synthesized per milligram protein per 30 min.

	Activity	
Substrate	Androstenolone sulfotransferase	Estrone sulfotransferase
Androstenolone	1.00	0.98
Estrone	0.99	0.99
p-Nitrophenol	1.00	0.75
2-Naphthylamine	0.45	0.49

TABLE 4

Relative steroid sulfortransferase activities with various substrates

The activities were determined as in Table 1, and the rates of sulfate ester formation with the various steroids are expressed relative to the rates with androstenolone and with estrone.

	Androsterone	Cholesterol	Testosterone	Deoxycorticosterone
1. Ratios with androstenolone				
pH 5 ppt.	0.69	0.19	0.22	0.26
G-200 eluate	0.70	0.15	0.27	0.53
DEAE eluate [a]	0.71	0.13	0.23	0.27
[b]	0.79	0.14	0.24	0.50
2. Ratios with estrone				
pH 5 ppt.	2.3	0.97	0.75	0.82
G-200 eluate	0.88	0.19	0.33	0.66
DEAE eluate [a]	1.2	0.22	0.39	0.46
[b]	0.55	0.10	0.17	0.34

[a] Androstenolone sulfotransferase fraction.

[b] Estrone sulfotransferase fraction.

transferase rather than with estrone sulfotransferase. With deoxycorticosterone as substrate, it seemed clear that a separate sulfotransferase was involved because the ratios shown in Table 4 were far from constant.

DISCUSSION

The methods described above provide a useful means of partially separating the various sulfotransferases of guinea pig liver: phenol sulfotransferase can be obtained free of any detectable steroid sulfotransferase activity whereas androstenolone sulfotransferase and estrone sulfotransferase can be partially separated. These various fractions will prove useful starting points for the more intensive purification upon which further progress depends. During the preliminary work considerable difficulty was encountered because of the apparently great instability of the sulfotransferases, but when the enzymes were kept in 0.03 M EDTA at pH values close to 7 their stability was greatly increased. Although all the sulfotransferases studied here seem to be SH enzymes (unpublished observations) it seems unlikely that the EDTA can be acting by removing heavy metals in view of the high concentration of the chelating agent which is required to stabilize the enzyme.

All the sulfotransferases are eluted together from Sephadex G-200, either under the conditions described above or on a long column with a length:diameter ratio of 100. They are eluted considerably behind sulfatase A of ox liver, which has a molecular weight at pH 7.5 of 107,000 (17) and somewhat in front of sulfatase B of ox liver which has a molecular weight of about 40,000 (Roy, unpublished observations). The sulfotransferases of guinea pig liver therefore probably have molecular weights of about 70,000. This value should be contrasted with that of greater than 200,000 claimed by Carroll and Spencer (7) for the sulfotransferases of rat liver.

With regard to the synthesis of *p*-nitrophenyl sulfate, it is clear that this can be catalyzed by at least three enzymes, the main one apparently being a specific phenol sulfotransferase and the other two steroid sulfotransferases. The activity of the phenol sulfotransferase is maximal at pH 5.8 and is independent of the presence of the Mg^{2+} ions which are required by the other two enzymes having optima between pH 6.5 and 7.5. If this complex situation is typical of mammalian livers in general then the reasons for many of the inconsistencies in previous work on the enzymatic synthesis of aryl sulfates become clear. Such inconsistencies are, for instance, the requirement for Mg^{2+} ions claimed by Segal (18) but disputed by Gregory and Lipmann (19) or the position of the pH optimum, which has variously been reported between 6 and 8.5. The only previous mention of a dual pH optimum for the synthesis of *p*-nitrophenyl sulfate is that of SubbaRao *et al.* (20), who reported optima at pH 6 and 7.5 with preparations from rat liver. Such conflicting reports are immediately explicable if more than one enzyme is involved in the synthesis of aryl sulfates. It is interesting to compare this situation, where several enzymes of overlapping specificity occur, to that of the synthesis of glucuronides by liver preparations, where again several enzymes of different, but overlapping, specificity are involved (21).

The data of Fig. 2 clearly show that, like the choline sulfotransferase of *Aspergillus nidulans* (22), the phenol sulfotransferase of guinea pig liver must have two independent substrate-combining sites, one for PAPS and one for the phenol. The fact that the reciprocal plots shown in Fig. 2 give families of intersecting straight lines shows that the reaction catalyzed by the enzyme cannot be a nonsequential one nor a simple random one but must be one of the other sequential types (23). It is tempting to suggest that it may in fact be a rapid equilibrium random reaction such as occurs with some phosphotransferases (24, 25).

The present work confirms the separate identity of the androstenolone and estrone sulfotransferases which was first noted by Nose and Lipmann (6). More detailed studies of these enzymes must await their complete separation, a procedure which will probably be complicated by the fact that

androstenolone sulfotransferase apparently forms an interacting system of some type. This is suggested by the data of Fig. 4 which show that the androstenolone sulfotransferase zone gives a consistent tailing, whether in the main androstenolone sulfotransferase fraction where it trails backward, sometimes to the extent of giving a second peak, or in the estrone sulfotransferase zone, where it trails forward. The nature of this interaction will become known only when the pure enzyme is obtained.

Little need be said of the specificity of the steroid sulfotransferases at this stage except to point out that this work gives the first demonstration that cholesterol can be sulfurylated by enzyme preparations from mammalian tissues. As pointed out by Baulieu *et al.* (3), the previous failure to detect this synthesis made it difficult to visualize cholesteryl sulfate as the starting point for the biosynthesis of androstenolone sulfate and so perhaps of estrogens. This difficulty now disappears. The synthesis of androsterone sulfate by the enzyme that forms androstenolone sulfate is perhaps surprising in view of the different configurations of the hydroxyl groups but appears to be established by the data in Table 4.

As already pointed out it seems to have been shown beyond doubt that androstenolone sulfotransferase and estrone sulfotransferase can both act as phenol sulfotransferases and arylamine sulfotransferases, forming *p*-nitrophenyl sulfate and 2-naphthyl sulfamate, respectively. Definite proof of this hypothesis will be hard to obtain, but it is difficult to see what other interpretation can be put on the data: certainly if the two steroid sulfotransferases do not both show these other types of sulfotransferase activity then the several enzymes involved must be closely associated indeed. It therefore seems that the previously postulated arylamine sulfotransferase does not have a separate existence, its apparent activity simply being a reflection of the activity of the steroid sulfotransferases, and it is perhaps of interest that Holcenberg and Rosen (26) have recently suggested that estrone and *p*-nitrophenol might be sulfurylated by the one enzyme in ox tissues. Certainly the data previously obtained by Roy (9, 16) for the action of 17-oxosteroids on the synthesis of 2-naphthyl sulfamate can have little absolute significance because of the participation of more than one enzyme in the process. Nevertheless, the powerful inhibition of the synthesis of 2-naphthyl sulfamate by androstenolone methyl ether has been confirmed in the present work and if, as seems certain, this synthesis is due to the steroid sulfotransferases, then added weight is given to the suggestion (8) that this effect is an allosteric one.

The present study therefore suggests that the sulfotransferases of liver may not be quite as numerous as had been thought. In guinea pig liver there certainly exist a phenol sulfotransferase, an androstenolone sulfotransferase, and an estrone sulfotransferase. The phenol sulfotransferase is apparently specific for the synthesis of aryl sulfates whereas the two steroid sulfotransferases can each utilize not only the appropriate steroid, but also *p*-nitrophenol and 2-naphthylamine. The question of the nature of the enzymes involved in the syntheses of testosterone sulfate and of deoxycorticosterone sulfate requires further investigation, but it seems clear that in guinea pig liver a separate sulfotransferase is involved in the sulfurylation of the corticosteroid. In the case of testosterone the evidence is inconclusive one way or the other. No study has yet been made of the synthesis of alkyl sulfates because the technique used here is not suitable for this, but it should be recalled that Carroll and Spencer (7) showed an association between aryl sulfamate synthesis and alkyl sulfate synthesis in rat liver.

REFERENCES

1. R. T. Williams, "Detoxication Mechanisms." Chapman & Hall, London, 1959.
2. K. D. Roberts, L. Bandi, H. I. Calvin, W. D. Drucker and S. Lieberman, *Biochemistry* **3**, 1953 (1964).
3. E. E. Baulieu, C. Corpechot, F. Dray, R. Emiliozzi, M. C. Lebeau, P. Mauvais-Jarvis and P. Robel, *Recent Progr. Hormone Res.* **21**, 411 (1965).

4. J. D. Gregory, *Proc. 1st Intern. Pharmacol. Meeting, Stockholm 1961* Vol. 6, p. 53. Pergamon Press, Oxford, 1962.
5. J. R. Gillette, *Progr. Drug Res.* **6**, 11 (1963).
6. Y. Nose and F. Lipmann, *J. Biol. Chem.* **233**, 1348 (1958).
7. J. Carroll and B. Spencer, *Proc. 6th Intern. Congr. Biochem. New York, 1964*, abstract VI-18 (1964).
8. A. B. Roy, *J. Mol. Biol.* **10**, 176 (1964).
9. A. B. Roy, *Biochem. J.* **74**, 49 (1960).
10. E. G. Brunngraber, *J. Biol. Chem.* **233**, 472 (1958).
11. P. W. Robbins and F. Lipmann, *J. Biol. Chem.* **229**, 837 (1957).
12. A. B. Roy, *Biochem. J.* **63**, 294 (1956).
13. A. B. Roy, *Biochem. J.* **62**, 41 (1956).
14. H. A. Sober, F. J. Gutter, M. M. Wycoff and E. A. Peterson, *J. Am. Chem. Soc.* **78**, 756 (1956).
15. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
16. A. B. Roy, *Biochem. J.* **79**, 253 (1961).
17. L. W. Nichol and A. B. Roy, *Biochemistry* **4**, 386 (1965).
18. H. Segal, *J. Biol. Chem.* **213**, 161 (1955).
19. J. D. Gregory and F. Lipmann, *J. Biol. Chem.* **229**, 1081 (1957).
20. K. SubbaRao, P. S. Sastry and J. Ganguly, *Biochem. J.* **87**, 312 (1963).
21. I. D. E. Storey, *Biochem. J.* **95**, 209 (1965).
22. B. A. Orsi and B. Spencer, *J. Biochem. (Tokyo)*, **55**, 49 (1964).
23. W. W. Cleland, *Biochem. Biophys. Acta* **67**, 104 (1963).
24. A. M. Reynard, L. F. Hass, D. D. Jacobsen and P. D. Boyer, *J. Biol. Chem.* **236**, 2277 (1961).
25. H. J. Fromm and V. Zewe, *J. Biol. Chem.* **237**, 3027 (1962).
26. J. S. Holcenberg and S. W. Rosen, *Arch. Biochem. Biophys.* **110**, 551 (1965).