Klyne, W. & Wright, A. A. (1956*b*). *J. Endocrin.* **14**, xxxiii.
Marker, R. E., Kamm, O., Oakwood, T. S., Wittle, E. L. & Lawson, E. J. (1938*a*). *J. Amer. chem. Soc.* **60**, 1061.
Marker, R. E., Lawson, E. J., Wittle, E. L. & Crooks, H. M. (1938*b*). *J. Amer. chem. Soc.* **60**, 1559.
Marrian, G. F. & Bauld, W. S. (1955). *Biochem. J.* **59**, 136.
Oppenauer, R. (1941). *Hoppe-Seyl. Z.* **270**, 97.
Pearlman, W. H. (1948). In *The Hormones*, vol. 1, p. 407. Ed. by Pincus, G. & Thimann, K. V. New York: Academic Press.
Ramirez, F. & Stafiej, S. (1955*a*). *J. Amer. chem. Soc.* **77**, 134.
Ramirez, F. & Stafiej, S. (1955*b*). *Chem. & Ind.* p. 1180.
Ramirez, F. & Stafiej, S. (1956). *J. Amer. chem. Soc.* **78**, 644.
Wright, A. A. & Klyne, W. (1955). *Abstr. 3rd Int. Congr. Biochem., Brussels*, p. 139.
Zimmermann, W. (1935). *Hoppe-Seyl. Z.* **233**, 257.
Zimmermann, W. (1936). *Hoppe-Seyl. Z.* **245**, 47.

Studies on Sulphatases

15. THE ARYLSULPHATASES OF HUMAN SERUM AND URINE*

By K. S. DODGSON and B. SPENCER
Department of Biochemistry, University of Wales, Newport Road, Cardiff

(*Received* 3 *October* 1956)

Conflicting reports concerning the arylsulphatase activity of serum and urine have appeared in the literature. Thus Pantlischko & Kaiser (1952), using phenolphthalein disulphate as substrate, could detect no arylsulphatase activity in urine, whereas Russo (1947), using phenyl sulphate, stated that the arylsulphatase activity of urine towards this substrate was quantitatively comparable with that of animal tissues excluding liver. Huggins & Smith (1947) found activity equivalent to the liberation of 0·3–15·0 and 0·9–19·7 μg. of *p*-nitrophenol/hr./ml. from *p*-nitrophenyl sulphate (NPS) by serum and urine respectively, and Abbott & East (1949), using the same substrate, observed increased serum arylsulphatase activity after induced liver damage in the rat. On the other hand, Abbott (1947) could detect no hydrolysis of phenyl sulphate by human serum over a period of 18 hr. Dodgson & Spencer (1954) were unable to confirm the observations of Huggins & Smith (1947), the activities recorded corresponding to the release of only 0–0·2 μg. of *p*-nitrophenol/hr./ml. by either urine or serum. More recently Boyland, Wallace & Williams (1955) used potassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) as the assay substrate, and reported that the arylsulphatase activities of normal urine and serum were respectively 0–0·82 and 1·2–3·3 μg. of nitrocatechol liberated/hr./ml. These authors observed increased activity in several infected urines and in urines from patients with carcinoma of the bladder and other sites and from patients with tuberculosis. There was no parallel rise in serum arylsulphatase activity.

Recently, the arylsulphatase activity of mammalian tissues has been shown to be due to three separable enzymes which can be distinguished by their relative substrate affinities, behaviour towards inhibitors and ease of solubilization (Dodgson, Spencer & Thomas, 1955; Dodgson, Spencer & Wynn, 1956). Two of the enzymes (arylsulphatases *A* and *B*) are easily obtained in solution from mammalian livers after rupture of the mitochondria of the liver cells (cf. Viala & Gianetto, 1955) and have been called 'soluble' or mitochondrial arylsulphatases (Dodgson *et al.* 1955, 1956). They have a high affinity and activity towards NCS but have little affinity or activity towards NPS or potassium *p*-acetylphenyl sulphate (APS). The substrate specificity of the other arylsulphatase (arylsulphatase *C*) is the converse of that shown by the 'soluble' enzymes. This enzyme is found exclusively in the microsomes of the liver cell (Dodgson, Spencer & Thomas, 1954, 1955) and has been obtained in a soluble form only after treatment with crude pancreatic extracts in the presence of a non-ionic surface-active agent (Dodgson, Rose & Spencer, unpublished results).

A re-assessment of the urinary and serum arylsulphatase patterns has now been made in the light of these findings.

* Part 14: Dodgson, Lloyd & Spencer (1957).

EXPERIMENTAL

Serum and urine samples. Blood samples from humans, dogs and rabbits were collected intravenously and from rats by cardiac puncture. Ox and sheep bloods were fresh slaughter-house specimens. Whenever possible sera were separated and assayed within 1 hr. of collection of the blood. Urines were assayed immediately after voiding.

Assay of arylsulphatase activity. The substrates APS, NPS and NCS were prepared as previously described (Dodgson *et al.* 1955). No attempt was made to purify the NCS

further, as recently suggested by Dodgson & Spencer (1956*a*). The method of determination of the enzyme activity of human-liver preparations by spectrophotometric measurement of the phenols liberated from these substrates (Dodgson *et al.* 1955) was adapted to the low activities found in urine and serum. To 0·4 ml. of the substrate, dissolved in 0·5 M sodium acetate–acetic acid buffer at the required pH and contained in a 15 ml. tapered centrifuge tube, was added 0·4 ml. of serum or urine which had been adjusted to the same pH with acetic acid or NaOH. After incubation at 38° for the required period, 2 ml. of 0·2 N-NaOH was added for APS and NPS and 2 ml. of alkaline quinol mixture for NCS. When NCS was used as the substrate the alkaline quinol mixture was made up immediately before use according to the directions of Roy (1953), except that the $Na_2SO_3,7H_2O$ was dissolved in 0·5 N-NaOH. The tubes were centrifuged at 2500 *g* for 10 min. and spectrophotometric readings at 323 mμ. (APS), 400 mμ. (NPS) and 515 mμ. (NCS) were made within 30 min. with serum or at leisure with urine. All assays were carried out in duplicate with duplicate controls in which serum or urine and the buffered substrate solution were incubated separately and mixed immediately before the addition of alkali. The amounts of the phenols liberated were calculated as follows, the appropriate molecular extinction coefficient being used (see Dodgson & Spencer, 1953):

μg. of phenol liberated/hr./ml. of urine or serum

$$= \frac{(E_t - E_c) \times \text{mol.wt.} \times 2{\cdot}8 \times 10^6}{T \times \epsilon_{\text{max.}} \times 0{\cdot}4 \times 10^3},$$

or

$$= \frac{(E_t - E_c) \times F}{T},$$

where E_t and E_c are the $\log_{10} I_0/I$ readings of the test and control determinations respectively, and F is a factor which is 47·6 for *p*-hydroxyacetophenone, 53·5 for *p*-nitrophenol and 96·06 for 4-nitrocatechol; T is the time of incubation in hr. and was usually 18–24 hr. for APS and NPS and 2 hr. for NCS.

An APS, NPS or NCS unit of arylsulphatase activity is defined as that which liberates 1 μg. of the respective phenol in 1 hr. under the stated conditions.

Non-enzymic hydrolysis of NPS by serum at alkaline pH. In the assay of serum with NPS, N-NaOH was originally used to stop enzyme action and to convert the liberated *p*-nitrophenol into the anionic form ($\lambda_{\text{max.}}$ 400 mμ.). However, under these conditions an increase in optical density at 400 mμ. occurred on allowing the final alkaline mixture to stand. It can be seen from Fig. 1 that this increase was due to three factors: the development of turbidity in the mixture, a slow liberation of *p*-nitrophenol owing to the alkaline hydrolysis of NPS (cf. Burkhardt & Wood, 1929) and hydrolysis of NPS by a reaction catalysed by serum at alkaline pH. The onset of the serum-catalysed reaction and the increase in turbidity were both preceded by an initial lag period (see Fig. 1) the duration of which was greatly extended by decreasing the concentration of NaOH or by lowering the temperature. By using 0·2 N-NaOH in the assay method the duration of the lag period was so lengthened that the increase in optical density at 400 mμ. was negligible over 1 hr. Increasing the temperature or the concentration of NaOH not only decreased the duration of the lag phase but also increased the rates of turbidity formation and of *p*-nitrophenol liberation. The lag phase could be eliminated by pre-incubating the serum with alkali before addition of the NPS, the time required being dependent on temperature and the concentration of alkali used.

The sera and plasma of man, ox, dog, sheep, rat and rabbit possessed the NPS-hydrolysis factor. The factor was heat-stable and non-dialysable and did not catalyse the hydrolysis of APS, NCS, potassium *m*-nitrophenyl sulphate or phenolphthalein disulphate. Both the albumin and globulin fractions of serum were capable of catalysing the hydrolysis although albumin appeared to be the more active of the two. Later the catalysis was seen to be a property of many proteins, although high concentrations were usually necessary before the effect was shown. It seemed possible that the ability of proteins to catalyse the alkaline hydrolysis of NPS might depend on the presence of certain amino acids. Of a series of amino acids tested, cystine, cysteine, methionine, proline and hydroxyproline were capable of catalysing the liberation of *p*-nitrophenol from NPS in the presence of N-NaOH.

A similar phenomenon, the alkaline hydrolysis of *p*-nitrophenyl acetate, phosphate and benzoate catalysed by albumin and sulphur-containing amino acids, has been observed by Perenyi (1954*a–c*). Perenyi examined the reactions in detail and proposed a general reaction mechanism which involved the SH groups present in the protein or the amino acids. The lag phase observed with serum was thought to be due to the gradual release of SH groups by alkali. It is possible that a similar mechanism might explain

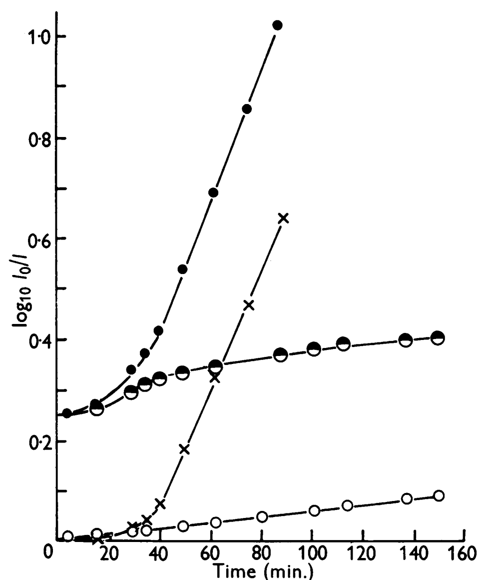

Fig. 1. The effect of NaOH on the turbidity of serum and the serum-catalysed liberation of *p*-nitrophenol from NPS. ●, Increase in the $\log_{10} I_0/I$ value at 400 mμ. for a mixture of 0·4 ml. of human serum, 0·4 ml. of 0·016 M NPS in 0·5 M acetate buffer, pH 7·5, and 2 ml. of N-NaOH. This curve is the sum of three factors: ○, the alkaline hydrolysis of NPS (water substituted for serum); ◓, the increase in the turbidity of the serum (water substituted for the NPS solution); ×, the serum-catalysed alkaline hydrolysis of NPS (curve ●, minus curves ○ and ◓).

the catalysed hydrolysis of *p*-nitrophenyl sulphate observed in the present work although the effect of proline and hydroxyproline, which were not examined by Perenyi, cannot be explained in this way.

Potassium *p*-nitrophenyl sulphate has been used freely in the past as a substrate for the assay of mammalian and other arylsulphatases (Dodgson *et al.* 1955; Dodgson, Melville, Spencer & Williams, 1954). It was not inconceivable that erroneous results might have been obtained in such assays owing to the failure to recognize any protein-catalysed alkaline hydrolysis of NPS which might have occurred during the period between addition of alkali at the end of the incubation period and the spectrophotometric measurement. However, when 10% (w/v) suspensions of rat liver, kidney, brain and intestine were substituted for serum in the assay procedure no release of *p*-nitrophenol from NPS after the addition of alkali could be detected. The concentration of protein used in this experiment was much greater than that used in previous assay work (Dodgson *et al.* 1955) and it can be assumed that the arylsulphatase activities measured in the previous studies are therefore correct. It is recommended, however, that NPS as an assay substrate should not be used under new conditions or for new sources of the enzyme until preliminary experiments have shown that non-enzymic liberation of *p*-nitrophenol under the alkaline conditions necessary for the formation of the *p*-nitrophenol anion does not occur.

RESULTS

Activity of serum and urine towards APS and NPS

The arylsulphatase *C* of human tissues is very active towards APS and NPS compared with aryl sulphatases *A* and *B*, which have little activity towards these substrates. The use of APS and NPS under the optimum conditions for human arylsulphatase *C* (0·005M APS, pH 6·9; 0·008M NPS, pH 7·5) provides a reasonably accurate measure of the activity of arylsulphatase *C*, since under these conditions the activity of, and competition by, arylsulphatases *A* and *B* are negligible (Dodgson *et al.* 1955, 1956). Assays of human sera and urines under these conditions showed that, apart from infected urines and those from adult females, none of the samples assayed had an activity greater than 0·24 NPS unit/ml. or 0·21 APS unit/ml. (Table 1). Three samples of sera from each of ox, sheep, dog, rabbit and rat were assayed with both substrates, but the activities found were no greater than those of human sera. The low level of arylsulphatase activity of urine and sera can be assessed by comparison with the APS activity of fresh human liver, which has varied between 4000 and 20000 units/g. wet wt. in the samples so far tested.

Apart from the higher activities found in the urine of adult females, the activities of normal urine and sera found with both substrates after a 20 hr. incubation period correspond to $E_t - E_c$ readings in the range 0–0·09. It is difficult to decide whether these small differences between test and control readings can be completely attributed to the activity of an arylsulphatase since the assay controls

Table 1. *Activity of urine and serum towards APS, NPS and NCS*

Urine and serum were assayed under the conditions described in the text. In some cases urines were centrifuged for 15 min. at 3000 *g* at the pH of assay and the cell-free supernatants assayed. The urines were not 24 hr. specimens. Ranges are quoted in parentheses. M, male; F, female.

Sample	Sex	No. of samples	Activity (μg. of phenol/hr./ml.) APS	NPS	NCS
Serum					
Normal	M	10	0·11 (0–0·21)	0·14 (0–0·21)	—
Normal	F	6	0·09 (0–0·19)	0·13 (0–0·23)	—
Normal	M	7	—	—	4·4 (2·8–7·8)
Normal	F	5	—	—	4·7 (2·2–6·5)
Two months before parturition	F	12	0·09 (0–0·18)	—	—
Urine					
Normal	M	26	0·06 (0–0·13)	—	—
Normal	M	12	—	0·12 (0·03–0·24)	—
	After centrifuging		—	0·10 (0·03–0·20)	—
Normal*	F	10	—	0·41 (0·09–0·82)	—
	After centrifuging		—	0·18 (0·07–0·26)	—
Normal	M	10	—	—	8·1 (6·3–11·9)
	After centrifuging		—	—	8·1 (6·3–11·9)
Normal	F	8	—	—	6·4 (3·4–10·3)
	After centrifuging		—	—	6·4 (3·4–10·3)
Infected	M	4	—	0·53 (0·27–1·06)	11·3 (7·8–17·6)
	After centrifuging		—	0·09 (0·01–0·16)	11·3 (7·8–17·6)

* The urine from one female had an NPS activity of 444, which was reduced to 32·4 on removing cellular debris by centrifuging. This urine was found to be contaminated with menses and has not been included in the table.

do not cover the possibility of a weak non-enzymic hydrolysis of the arylsulphate substrate by factors present in serum and urine. Separate experiments with boiled serum were designed to test this possibility but no satisfactory conclusion could be reached, since the coagulum formed by boiling the serum, even when diluted five times, would not dissolve in the assay incubation mixture. When assayed with either APS or NPS, boiled urines from males gave $E_t - E_c$ readings which were about one-third of those given by the corresponding untreated urines.

Arylsulphatase activity of urines from females. The urines from females consistently showed higher APS and NPS activities than those from males although there was no corresponding sex distinction between the enzyme activities of the sera (Table 1). Female urine generally contains large numbers of squamous epithelial cells derived mainly from the vagina and urethra. On the other hand, male urine normally contains very few epithelial cells, most of which are derived from the urinary tract. After removal of the cellular debris by centrifuging at 3000 *g* for 15 min. at the pH of assay, the NPS activity of the female urines fell from an average of 0·41 unit/ml. to an average of 0·18 unit/ml. (Table 1). The latter value is close to that found for untreated male urines (0·12 unit/ml.). There was no appreciable difference between the activities of centrifuged and untreated male urines. Four infected male urines containing large numbers of leucocytes showed high NPS activity, which could be removed by centrifuging (Table 1).

Effect of serum and urine on the activity of arylsulphatase C. It was possible that the arylsulphatase activities of urine and serum towards APS and NPS were being affected by endogenous inhibitors or activators. The low arylsulphatase activity of these fluids precluded the search for endogenous inhibitors by the method of Maengwyn-Davies & Friedenwald (1954), but it was possible to assess the effects of serum and urine on the arylsulphatase *C* of human liver in the following system: 0·2 ml. of APS (0·025 M) in 0·5 M acetate, pH 6·9; 0·4 ml. of acetone-dried human liver suspension in M acetate, pH 6·9 and 0·4 ml. of water, serum or urine adjusted to the assay pH. Serum had no effect on the activity of arylsulphatase *C* but urine inhibited strongly (30–60 %), the degree of inhibition varying with the sample. Assuming that the activity of urine towards NPS and APS is due to an enzyme similar to arylsulphatase *C* of liver, the true urinary activity, although possibly 50 % greater than the apparent value (Table 1), is still extremely low.

Activity of serum and urine towards NCS

Arylsulphatases *A* and *B* of human tissues are highly active towards NCS (Dodgson *et al.* 1956) and, since arylsulphatase *C* has only weak activity towards this substrate (Dodgson *et al.* 1956), NCS can be used to obtain an approximate measure of the combined activity of the two mitochondrial enzymes. Boyland *et al.* (1955) have already shown that serum and urine possess considerable NCS-hydrolysing activity and it has been possible to confirm many of their findings.

The optimum conditions for NCS-hydrolysing activity of urine were found to be 0·03 M NCS and pH 5·6 when measured over an incubation period of either 2 or 20 hr. Under these conditions a slight decrease in the rate of enzyme action occurred during the first 30 min. of incubation, but thereafter, up to 20 hr., a straight-line relationship existed between the nitrocatechol liberated and the time of incubation. The activities of male and female urines measured over a 2 hr. incubation period under the optimum conditions are shown in Table 1.

The substrate-concentration curve for serum acting on NCS was similar to that found for ox sulphatase *B* (Roy, 1954), the curve still ascending at 0·06 M NCS, above which concentration it is not practical to proceed owing to the insolubility of the substrate. In the presence of 0·04 M NCS the optimum pH for the arylsulphatase activity of serum towards NCS was in the region of 5·6. The activities of several different samples of serum under these conditions are recorded in Table 1.

Effect of urine on arylsulphatases A *and* B. Boyland *et al.* (1955) have shown that the arylsulphatase activity of urine towards NCS is markedly affected by endogenous inhibitors present in the urine. This was confirmed in the present work, there being a marked decline in the curve obtained by plotting enzyme activity (V) against enzyme concentration ($[E]$). Moreover, the plot of $[I]/V$ against $[I]$, where $[I]$, the inhibitor concentration, is proportional to $[E]$ (see Maengwyn-Davies & Friedenwald, 1954), was not a straight line, thus showing that the inhibition was not due to the effect of one inhibitor on a single enzyme. It has recently been shown that normal centrifuged urine contains two arylsulphatases which are active towards NCS and which possess electrophoretic mobilities identical with those of purified human-liver arylsulphatases *A* and *B* respectively (Dodgson & Spencer, 1956*b*). One of these enzymes, the electrophoretic mobility of which is identical with that of liver arylsulphatase *A*, when separated from urine, exhibits anomalous enzyme kinetics similar to those shown by the latter enzyme (Dodgson & Spencer, 1956*c*). It seems certain, therefore, that the NCS-hydrolysing activity of urine is due to the presence of both arylsulphatases *A* and *B*, and for this reason the effect of urine on the activity of purified liver arylsulphatases *A* and *B* was investigated (Table 2). It can be seen from this table

Table 2. *Effect of urine on arylsulphatases* A *and* B

Electrophoretically purified arylsulphatases *A* and *B* (Dodgson *et al.* 1956) were diluted with water, urine, boiled urine or urine which had been dialysed against running tap water for 24 hr. The mixtures were adjusted to the assay pH (5·0 for *A* and 6·0 for *B*) and 0·4 ml. was incubated with 0·4 ml. of NCS solution (0·01 M NCS in 0·5 M acetate, pH 5·0, for arylsulphatase *A* and 0·04 M NCS in 0·2 M acetate, pH 6·0, for arylsulphatase *B*) for 1 hr. at 38°. Alkaline quinol mixture (Roy, 1953), 2 ml., was added and the liberated 4-nitrocatechol measured at 515 mμ. Suitable controls were made to allow for the NCS-hydrolysing activity of the various urine preparations under these conditions.

	Arylsulphatase *A*		Arylsulphatase *B*	
	$\text{Log}_{10}(I_0/I)$	Inhibition of enzyme (%)	$\text{Log}_{10}(I_0/I)$	Inhibition of enzyme (%)
Purified enzyme	0·622	—	0·268	—
Urine	0·057	—	0·099	—
Boiled urine	0·016	—	0·003	—
Dialysed urine	0·041	—	0·055	—
Enzyme + urine	0·243	70·2	0·120	92·1
Enzyme + boiled urine	0·193	70·6	0·034	88·4
Enzyme + dialysed urine	0·638	3·6	0·318	1·9

that both enzymes retain activity in the presence of urine. Both enzymes therefore contribute towards the arylsulphatase activity of urine, although the greatest contribution would appear to be made by enzyme *A*. Although the NCS-hydrolysing activity of urine does not show the anomalous time–activity curves which are characteristic of purified liver arylsulphatase *A* (Dodgson & Spencer, 1956*c*) and does not show the same optimum conditions of pH and substrate concentration (Dodgson *et al.* 1956), these discrepancies can almost certainly be attributed to the presence of enzyme *B* in the urine and, more particularly, to the high concentrations in urine of inorganic phosphate and sulphate, both of which are known to modify considerably the kinetics of human-liver arylsulphatase *A* (Dodgson & Spencer, 1956*c* and unpublished results).

The urinary inhibitors of both liver arylsulphatases *A* and *B* were thermostable and dialysable (Table 2) and may be presumed to consist mainly of phosphate and sulphate ions, both of which, when present in concentrations similar to those obtaining in urine, are known to inhibit these enzymes (Dodgson *et al.* 1956 and unpublished results).

DISCUSSION

The presence in urine of arylsulphatases possessing similar properties and identical electrophoretic mobilities (see Dodgson & Spencer, 1956*b*) to those of arylsulphatases *A* and *B* of human liver accounts for the hydrolytic activity of urine towards nitrocatechol sulphate. Both these enzymes are known to occur in many human tissues (Dodgson *et al.* 1956) and although, for practical reasons, no close examination of serum has been attempted, it is probable that the same enzymes are responsible for the NCS-hydrolysing activity of serum. It is doubtful whether serum or urine contains an enzyme similar to the 'insoluble' or 'microsomal' arylsulphatase *C* of liver and other tissues (Dodgson *et al.* 1956), since both serum and urine show almost negligible activity towards NPS and APS under conditions of assay which are optimum for this enzyme. However, appreciable NPS-hydrolysing activity is occasionally observed in urine, but in these cases it can be attributed to the presence of cellular debris or bacteria and can therefore be removed from the urine by centrifuging.

Since the arylsulphatase activities obtained for urine and serum with NPS during the present work (0–0·8 μg. of *p*-nitrophenol liberated/hr./ml.) differed considerably from those obtained by Huggins & Smith (1947) (0·3–19·7 μg./hr./ml.) careful repetition of their work was undertaken. The procedure employed by Huggins & Smith differed from that of the present authors in four respects: the conditions used by the former, 0·001 M NPS at pH 5·8, were those established for the arylsulphatase of *Aspergillus oryzae*; an antibacterial agent, thymol, was incorporated into the incubation mixture; the volumes of substrate, buffer, serum or urine sample and NaOH were different; liberated *p*-nitrophenol was measured with a 420 mμ. filter. It was possible that these differences in experimental procedure might account for the conflicting results, but exact repetition of the method of Huggins & Smith (1947) by ourselves and several independent workers in this Laboratory have failed to disclose an activity in serum or urine greater than 0·14 μg./ml./hr. Four separate preparations of NPS gave similar results. In this connexion it is worth noting that Dr H. Cohen (personal communication) failed to detect arylsulphatase activity in horse serum by the method of Huggins & Smith (1947). The discrepancies in the observations of the two groups of workers are therefore still unexplained.

The results of any quantitative estimation of the arylsulphatase activities of serum and urine must be

interpreted with caution. When enzyme activity is measured with NCS, two arylsulphatases (*A* and *B*) compete for the same substrate. Moreover, in urine, natural substrates (the so-called ethereal sulphates) are also present. The activity of both arylsulphatases *A* and *B* is affected by certain inorganic ions, including sulphate, phosphate and chloride, and these are present in very variable amounts in urine. It is also possible that the pH optima of the two enzymes may vary slightly with the relative concentration of inorganic ions, as shown for arylsulphatases *A* and *B* of ox liver (Roy, 1955). The anomalous kinetics of arylsulphatase *A*, which are markedly affected by varying concentrations of inorganic ions (Dodgson & Spencer, 1956*c* and unpublished results), must also be borne in mind.

Although ureteric urine is known to possess arylsulphatase activity (Boyland *et al.* 1955) it is possible that some of the NCS-hydrolysing activity of urine is derived from the cells of the urinary and genital tracts. It is known that the NPS-hydrolysing activity of female urine which has been collected in a normal manner is largely due to the presence of vaginal epithelium cells, and the NPS-hydrolysing activity of infected urines is also due to sedimentable material, whether bacteria, leucocytes or erythrocytes. Such factors apparently contribute little to the NCS-hydrolysing activity of urine, since there is little difference in this activity between whole and centrifuged urines. However, it seems pertinent to point out that both arylsulphatases *A* and *B* are readily obtained in soluble form from liver cells by simple treatment such as incubation with various saline solutions, whereas the NPS-active arylsulphatase *C* is insoluble and remains attached to the particulate material in the cells (Dodgson *et al.* 1956). An approximate idea of the amount of NCS activity which could arise from the cellular debris of urine can be obtained by multiplying the difference between the NPS activity of urine and centrifuged urine by a factor (1·5), which represents the approximate average NPS:NCS ratio for human tissues [range, 1:0·39 (liver) to 1:4·1 (brain); Dodgson *et al.* 1956]. Thus the average NPS activity of female urine is 0·41 μg. of *p*-nitrophenol/hr./ml., and that of the centrifuged urines 0·18. The difference will correspond to an NCS activity of 0·34 μg. of 4-nitrocatechol/hr./ml., which is small when compared with the average NCS activity of female urines of 4·86. It is clear then, that in normal male and female urine only a small contribution is made to the NCS activity by the urinary sediment. Indeed, Boyland *et al.* (1955) found no correlation between NCS activity and red and white corpuscles and epithelial-cell counts in normal urine. However, in disease the urinary cellular debris may be substantial and it is suggested that in the interpretation of elevated arylsulphatase levels account should be taken, in the manner suggested above, of the difference in NPS activity between whole and centrifuged urine.

SUMMARY

1. Human serum and urine exhibit little arylsulphatase activity towards potassium *p*-acetylphenyl and *p*-nitrophenyl sulphates. In certain circumstances urine does show activity towards these substrates but the activity resides in sedimentable material such as cellular debris and bacteria.

2. Both serum and urine exhibit appreciable arylsulphatase activity towards nitrocatechol sulphate. The activity in urine is a feature of the urine *per se* and probably arises only in part from cellular debris.

3. Two enzymes, corresponding to arylsulphatases *A* and *B* of human tissues, are responsible for the bulk of the arylsulphatase activity of urine.

4. Some observations on the non-enzymic catalysis by serum of the alkaline hydrolysis of *p*-nitrophenyl sulphate are reported.

This work was supported by The Medical Research Council, The Royal Society and Imperial Chemical Industries Ltd.

REFERENCES

Abbott, L. D. (1947). *Arch. Biochem.* **15**, 205.
Abbott, L. D. & East, M. K. (1949). *Fed. Proc.* **8**, 178.
Boyland, E., Wallace, D. M. & Williams, D. C. (1955). *Brit. J. Cancer*, **9**, 62.
Burkhardt, G. N. & Wood, H. (1929). *J. chem. Soc.* p. 141.
Dodgson, K. S., Lloyd, A. G. & Spencer, B. (1957). *Biochem. J.* **65**, 131.
Dodgson, K. S., Melville, T. H., Spencer, B. & Williams, K. (1954). *Biochem. J.* **58**, 182.
Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **53**, 444.
Dodgson, K. S. & Spencer, B. (1954). *Biochem. J.* **56**, xiii.
Dodgson, K. S. & Spencer, B. (1956*a*). *Biochim. biophys. Acta*, **21**, 175.
Dodgson, K. S. & Spencer, B. (1956*b*). *Clin. Chim. Acta*, **1**, 478.
Dodgson, K. S. & Spencer, B. (1956*c*). *Biochem. J.* **62**, 30P.
Dodgson, K. S., Spencer, B. & Thomas, J. (1954). *Biochem. J.* **56**, 177.
Dodgson, K. S., Spencer, B. & Thomas, J. (1955). *Biochem. J.* **59**, 29.
Dodgson, K. S., Spencer, B. & Wynn, C. H. (1956). *Biochem. J.* **62**, 500.
Huggins, C. & Smith, D. R. (1947). *J. biol. Chem.* **170**, 391.
Maengwyn-Davies, G. D. & Friedenwald, J. S. (1954). *Arch. Biochem. Biophys.* **53**, 29.
Pantlischko, M. & Kaiser, F. (1952). *Mh. Chem.* **83**, 1140.
Perenyi, L. (1954*a*). *Acta physiol. hung.* **5**, 87.
Perenyi, L. (1954*b*). *Acta physiol. hung.* **5**, 97.
Perenyi, L. (1954*c*). *Acta physiol. hung.* **5**, 103.
Roy, A. B. (1953). *Biochem. J.* **53**, 12.
Roy, A. B. (1954). *Biochem. J.* **57**, 465.
Roy, A. B. (1955). *Biochem. J.* **59**, 8.
Russo, F. (1947). *Fisiol. e Med.* **15**, 289.
Viala, R. & Gianetto, R. (1955). *Canad. J. Biochem. Physiol.* **33**, 839.