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Multiple *N*-Acetyltransferases and Drug Metabolism

TISSUE DISTRIBUTION, CHARACTERIZATION AND SIGNIFICANCE OF MAMMALIAN *N*-ACETYLTRANSFERASE

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Investigations in the rabbit have indicated the existence of more than one *N*-acetyltransferase (EC 2.3.1.5). At least two enzymes, possibly isoenzymes, were partially characterized. The enzymes differed in their tissue distribution, substrate specificity, stability and pH characteristics. One of the enzymes was primarily associated with liver and gut and catalysed the acetylation of a wide range of drugs and foreign compounds, e.g. isoniazid, *p*-aminobenzoic acid, sulphamethazine and sulphadiazine. The activity of this enzyme corresponded to the well-characterized polymorphic trait of isoniazid acetylation, and determined whether individuals were classified as either 'rapid' or 'slow' acetylators. Another enzyme activity found in extrahepatic tissues readily catalysed the acetylation of *p*-aminobenzoic acid but was much less active towards isoniazid and sulphamethazine. The activity of this enzyme remained relatively constant from individual to individual. Studies *in vitro* and *in vivo* with both 'rapid' and 'slow' acetylator rabbits revealed that, for certain substrates, extrahepatic *N*-acetyltransferase contributes significantly to the total acetylating capacity of the individual. The possible significance and applicability of these findings to drug metabolism and acetylation polymorphism in man is discussed.

Liver *N*-acetyltransferase catalyses the acetylation of a number of commonly used drugs and foreign compounds such as isoniazid, sulphamethazine, sulphadiazine, *p*-aminobenzoic acid, diaminodiphenylsulphone and hydralazine in man and certain other mammalian species (Weber, 1971*b*). The activity of this enzyme varies widely between individuals. Genetic studies with man (Evans *et al.*, 1960) and rabbit (Frymoyer & Jacox, 1963*a*) have shown that individuals are either 'rapid' or 'slow' acetylators. Slow acetylators have low *N*-acetyltransferase activity and are homozygous for an autosomal recessive gene. The identification of the acetylator phenotype of an individual as either rapid or slow has been clearly demonstrated in population studies in which the ability of individuals to acetylate isoniazid (Evans *et al.*, 1960), sulphamethazine (Evans & White, 1964) and sulphadiazine (Frymoyer & Jacox, 1963*a*) has been measured and clear bimodal frequency-distribution histograms have been obtained. This phenomenon has become known as the isoniazid-acetylation polymorphism.

Previous studies (Jenne, 1965; Weber, 1971*b*) of the biochemical basis of the acetylation polymorphism have not shown any difference in the characteristics of *N*-acetyltransferases prepared from rapid- and slow-acetylator livers. The enzymes can be purified by the same procedure and their pH characteristics, heat stabilities, kinetic properties, substrate specificities and reaction mechanisms are indistinguishable. Thus Jenne (1965) has proposed that the same enzyme is present in the liver of both phenotypes and that the slow acetylator has less of it. The following observations are difficult to account for by this concept.

(1) In man and rabbit some drugs are acetylated polymorphically whereas others are acetylated monomorphically. In man, polymorphically acetylated drugs are isoniazid, sulphapyridine (Schroder & Evans, 1972), sulphamethazine, hydralazine, diaminodiphenylsulphone and probably also phenelzine (see Weber, 1971*b*). Monomorphically acetylated drugs include *p*-aminobenzoic acid, *p*-aminosalicylic acid and sulphanilamide (see Weber, 1971*b*). Certain other drugs such as sulphamethoxypyridazine (White & Evans, 1968) appear to follow the isoniazid-acetylation pattern to some extent.

(2) Slow acetylators are especially susceptible to dose-related toxicity from polymorphically acetylated drugs owing to their low acetylating capacity (Hughes *et al.*, 1954; Devadatta *et al.*, 1960; Evans *et al.*, 1965; Perry *et al.*, 1967). Susceptibility to adverse effects among slow acetylators, however, is subject to considerable variability. This is evident from the observation that the incidence of toxicity among this group is appreciably lower than their frequency in

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populations of patients exposed to these drugs (Harris, 1961).

(3) The difference in liver *N*-acetyltransferase activity in rapid and slow acetylators is much greater than the difference in their acetylating capacity *in vivo*. In the rabbit (Frymoyer & Jacox, 1963*a*,*b*), the ratio of the average drug-acetylation rates measured *in vivo* in rapid- versus slow-acetylator animals is small (2:1) compared with the ratio of drug-acetylating-enzyme activity measured *in vitro* (80:1) in liver samples from the two acetylator phenotypes. Much less information from humans is available for comparison, but from what is available a similar disparity appears to exist (Evans *et al.*, 1960; Jenne, 1965). It should be recognized in connexion with these differences that the values determined *in vitro* only take into account the metabolic contribution of the liver whereas the values *in vivo* reflect the contribution of extrahepatic as well as hepatic drug-metabolizing systems. In addition, the value *in vivo* is further affected by renal mechanisms of elimination of drug from the body.

These observations indicate that individual variability in the rate and pattern of drug acetylation depends on factors additional to the isoniazid-acetylator status. The drug-acetylating enzymes of liver clearly make an important contribution to the total acetylating capacity of some individuals. Investigating the importance of renal factors, Jenne *et al.* (1961) suggested that they are likely to be small compared with the acetylation of the drug. As such, renal factors would probably not account for the disparity between methods for assessing the acetylating capacity of an individual *in vivo* and *in vitro*. Several investigators (Evans & White, 1964; Motulsky, 1964; Jenne *et al.*, 1961) have obtained evidence for the existence of drug-acetylating enzymes in extrahepatic tissues and they have suggested that they may be important in explaining this disparity, but a comprehensive study of these enzymes and their possible significance in the acetylation of drugs was not carried out.

The studies reported here were designed to determine: (*a*) whether more than one *N*-acetylating enzyme contributes to the total drug-acetylating capacity of the rabbit; (*b*) the relative importance of hepatic and extrahepatic *N*-acetylating enzymes to the total drug-acetylating capacity of tissues obtained from rapid and slow isoniazid-acetylator rabbits; (*c*) the distribution and some of the characteristics of partially purified *N*-acetylating activity in the tissues of rapid and slow isoniazid-acetylator rabbits.

Experimental

Animals

New Zealand White rabbits (5 months of age or older) of both sexes, maintained on a standard diet, were used throughout.

Reagents

Isoniazid, sulphamethazine, sulphadiazine, *p*-aminobenzoic acid and acetyl-CoA were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. *N*-1-(Naphthyl)ethylenediamine dihydrochloride and routine laboratory chemicals were obtained from Fisher Scientific Co., Springfield, N.J., U.S.A. DEAE-cellulose was obtained from Nutritional Biochemicals Corp.

Determination of acetylator phenotype

The half-life of sulphadiazine in the blood of rabbits was determined by a modification of the method described by Frymoyer & Jacox (1963*a*). Sulphadiazine (20mg/kg body wt.) was administered intravenously via a marginal vein in one ear and blood samples (0.100ml) were obtained at approx. 20, 30, 40, 50, 60 and 70min after injection. Experimental animals in which the half-life for sulphadiazine was greater than 70–80min were classified as slow acetylators and those in which the half-life was shorter than 35min were classified as rapid acetylators. It has been shown (Frymoyer & Jacox, 1963*a*) that slow acetylators are characterized by a half-life for sulphadiazine of greater than 60–70min.

Enzyme purification

Animals were killed (by injection of 20ml of air into a marginal ear vein) and tissues were removed, homogenized (25%, w/v) in 0.1M-sodium pyrophosphate buffer, pH6.8 at 4°C, and centrifuged at 100000*g* for 60min; the supernatant fraction was retained for analysis. Additional purification of the enzyme was required for some studies and was carried out as described by Weber (1971*a*). For the preparation of enzyme from the gut, the duodenum was removed, washed, incised longitudinally and the mucosal tissue was scraped away with a sharp scalpel blade. The muscular gut-wall tissue was discarded and the mucosa preparation was homogenized as described above.

Determination of acetylating activity in vitro

In these studies two substrates, sulphamethazine and *p*-aminobenzoic acid, were used. In contrast with the determinations *in vivo* and to obtain maximal assay sensitivity sulphamethazine was used in preference to sulphadiazine, additional studies (D. J. Hearse & W. W. Weber, unpublished work) having demonstrated that there was a close correlation between the results obtained with sulphadiazine and sulphamethazine.

Purified samples (100000*g*-supernatant fraction) were suitably diluted and assayed for sulphamethazine- or *p*-aminobenzoic acid-acetylating activity by using a micro-modification of the Bratton & Marshall (1939) procedure for the determination of

aromatic amines. In each experiment, time–activity curves were constructed and enzyme activity was determined from the linear portion of the curve. The rate of acetylation was monitored by measuring the rate of disappearance of the amine from an incubation mixture. Radioisotope and t.l.c. studies confirmed that the disappearance of the amine was accompanied by the appearance of the corresponding amount of acetylated product. For the micro-assay, all incubations (37°C) were carried out in 0.40ml capped polyethylene micro-test tubes (Beckman Inc., Palo Alto, Calif., U.S.A.). The reaction mixture (0.090ml) consisted of 0.050ml of suitably diluted enzyme solution (diluted in 0.1M-sodium pyrophosphate buffer, pH6.8), aqueous acetyl-CoA (0.20μmol) and an aqueous substrate solution (*p*-aminobenzoic acid or sulphamethazine, 0.0040μmol). Control tubes contained no acetyl-CoA. The reaction was initiated by the addition of the enzyme preparation and the tubes (two for each time-period) were capped and incubated for 5, 10, 15, 20 or 30min. The reaction was then terminated by the addition of 0.050ml of aq. 10% (w/v) trichloroacetic acid. The precipitated protein was sedimented by centrifugation (10000*g*, 1min; Beckman Microfuge). All the non-acetylated amine in the supernatant was diazotized by the addition of 0.020ml of aq. 0.1% (w/v) $NaNO_2$, followed by mixing and leaving for 3min. Excess of nitrite was removed by the addition of 0.020 ml of aq. 0.5% (w/v) ammonium sulphamate, mixing and after a further 3min, coupling was effected by the addition of 0.100ml of aq. 0.05% (w/v) *N*-1-(naphthyl)ethylenediamine dihydrochloride. The mixture was left for 10min and the E_{540} was measured (Beckman 151 micro-spectrocolorimeter) against water as a blank. The extent of acetylation was obtained by subtracting the experimental reading from the control reading. Under the conditions of this assay and with the micro-spectrocolorimeter, a decrease in E_{540} of 87.5 corresponds to the acetylation of 1μmol of substrate. Enzyme activity was expressed as μmol of substrate acetylated/15min incubation period.

Specific activity of enzyme preparations

Activity was usually expressed as μmol of substrate acetylated/15min per mg of protein, μmol of substrate acetylated/15min per organ or μmol of substrate acetylated/15min per ml of enzyme. Protein concentration was determined by the method of Warburg & Christian (1942).

Results and Discussion

Tissue distribution of sulphamethazine N-acetyltransferase activity

Studies were undertaken with rabbit tissues from rapid and slow acetylators (phenotyped at least 7 days before the study). Sulphamethazine *N*-acetyltransferase activity was widely distributed in the tissues of a female rapid-acetylator rabbit, most of the activity being localized in the liver, duodenum (mucosal scraping) and lung, but activity was also detected (in order of quantitative significance) in thymus, ovary, spleen, uterus, adrenal gland, leucocytes, kidney, bone marrow, salivary gland, pancreas, pineal gland, erythrocytes and brain. No activity was detected in plasma, skeletal muscle or fat.

Tissue-distribution studies on a slow sulphamethazine acetylator showed that in contrast to liver and gut, where the sulphamethazine *N*-acetyltransferase activity was at an expected low or undetectable value, the specific activity in certain tissues, notably the spleen, kidney and pineal gland, were at a value comparable with that found with the rapid sulphamethazine acetylator, and thus did not reflect the sulphamethazine-acetylator phenotype of this individual.

Evidence for multiple forms of N-acetyltransferase

Hepatic and extrahepatic acetylation of sulphamethazine. The above observations were investigated further in a separate study on eight rabbits of known acetylator phenotype, selected to include a wide range of sulphamethazine-acetylating activities from very rapid to very slow acetylators. For each animal, several tissues, including liver, gut, spleen and kidney, were collected, prepared and assayed for sulphamethazine *N*-acetyltransferase activity. The results (Fig. 1) are arranged in decreasing order of liver sulphamethazine *N*-acetyltransferase specific activity (black shading). For convenience, the specific activity is expressed on a logarithmic scale, and there was a difference of several hundredfold between the specific activity of the liver sulphamethazine *N*-acetyltransferase of the slow acetylators (animals W and B) and the rapid acetylators (animals C and F). In contrast, the activity of spleen and kidney sulphamethazine *N*-acetyltransferase fluctuated very little between individuals. These observations may be interpreted as evidence for the existence of an additional sulphamethazine *N*-acetyltransferase activity in extrahepatic tissue, which differs from that of the liver. Further, it may be proposed that the enzyme associated with the isoniazid-acetylation polymorphism is localized predominantly in the liver.

The results for the gut activity present a more complex and interesting situation. In rapid-acetylator rabbits (C, F and G) an excellent correlation existed between the liver and gut sulphamethazine *N*-acetyltransferase activity. In slow-acetylator rabbits (D, W and B) with little or no liver sulphamethazine *N*-acetyltransferase activity, a significant baseline value for activity was always present in the gut. It

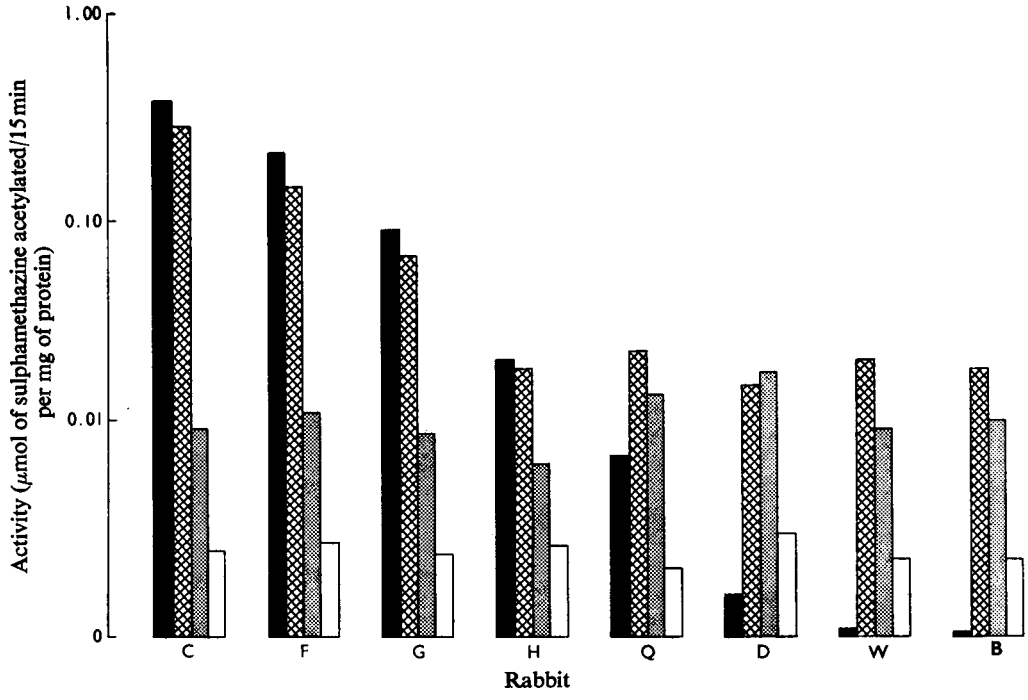

Fig. 1. *Relative sulphamethazine-acetylating activity in tissues obtained from eight rabbits of known acetylator phenotype selected to include a wide range of sulphamethazine-acetylating activities from very rapid (rabbit C) to very slow (rabbit B) acetylators*

Tissues used (100000*g*-supernatant fraction) were liver (■), gut (▩), spleen (▨) and kidney (□). Specific activity is expressed as μmol of sulphamethazine acetylated/15 min per mg of protein. For further details see the Experimental section.

would therefore appear that the gut contained a second activity, possibly similar to that in spleen and kidney. The value of this activity, as observed in other extrahepatic tissues, did not appear to vary greatly from individual to individual or to be associated with the isoniazid-acetylation polymorphism.

Studies of hepatic and extrahepatic acetylation in vitro with different substrates. As described in the introduction, the population-distribution curves for the acetylation of sulphamethazine and *p*-aminobenzoic acid differ markedly. It is possible that this difference could be explained by the presence of multiple forms of *N*-acetyltransferase. This possibility was investigated further in experiments in which the relative contributions of the extrahepatic and hepatic *N*-acetyltransferase systems to the metabolism of sulphamethazine and *p*-aminobenzoic acid were evaluated.

Tissue-distribution studies with rabbits of known acetylator phenotype were repeated with both *p*-aminobenzoic acid and sulphamethazine as substrates. Acetylating activity for both substrates was measured in liver and a convenient extrahepatic tissue (spleen). From the results illustrated in Fig. 2, two important observations can be made. First (Fig. 2*a*), the sulphamethazine *N*-acetyltransferase activity in the liver varies between individuals over a wide range, as shown in Fig. 1. In contrast (Fig. 2*b*), the *p*-aminobenzoic acid *N*-acetyltransferase activity from the same livers was high in all of these animals. Secondly, the *p*-aminobenzoic acid *N*-acetyltransferase activity was much greater than the sulphamethazine *N*-acetyltransferase activity in the spleen from the same animal. From these two observations and those in Fig. 1 it is apparent that not only did the relative enzyme activities for *p*-aminobenzoic acid and sulphamethazine vary from individual to individual but they also varied from tissue to tissue within the same individual. These findings are consistent with the concept that at least two enzymes are present in different tissues in different relative proportions.

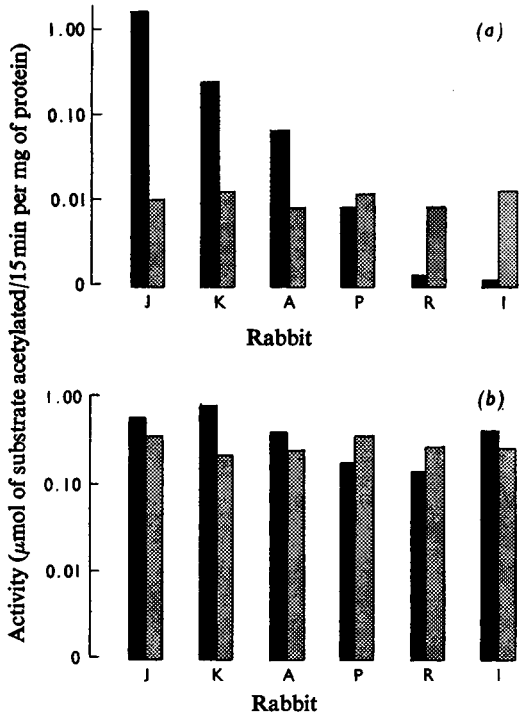

Fig. 2. *Relative sulphamethazine- and p-aminobenzoic acid-acetylating activity in tissues obtained from six rabbits of known acetylator phenotype selected to include a wide range of sulphamethazine-acetylating activities from very rapid (rabbit J) to very slow (rabbit I) acetylators*

Tissues studied (100000*g*-supernatant fraction) were liver (■) and spleen (▩). Specific activity is expressed as μmol of substrate acetylated/15 min per mg of protein. For further details see the Experimental section.

They also suggest that some degree of cross-specificity for sulphamethazine and for *p*-aminobenzoic acid exists for both enzymes. One enzyme, the sulphamethazine *N*-acetyltransferase, fluctuates widely in its activity between individuals and is responsible for the isoniazid-acetylation polymorphism, whereas the second enzyme, primarily responsible for the acetylation of *p*-aminobenzoic acid, appears subject to less variation between individuals.

Relative contribution of hepatic and extrahepatic tissues to the total acetylating capacity of the individual

The finding of significant amounts of *N*-acetyltransferase activity in extrahepatic tissues raises the possibility that the liver may not always be the primary site of drug acetylation, particularly for the acetylation of *p*-aminobenzoic acid. Experiments were therefore designed to evaluate, as far as possible, the quantitative contribution of the extrahepatic *N*-acetyltransferase to the total acetylating capacity of both slow and rapid acetylators for sulphamethazine and *p*-aminobenzoic acid.

Initially a slow-acetylator rabbit was subjected to phenotyping with sulphamethazine *in vivo*. The half-life of sulphamethazine in the blood was 185 min. After 7 days, sufficient time for clearance of the drug, the half-life of *p*-aminobenzoic acid was determined in the same rabbit and was then 15 min. After a further 7 days, the rabbit was killed and the tissues were collected. A similar experiment was also carried out on a rapid-acetylator rabbit.

The total acetylating capacity of the slow-acetylator rabbit (Table 1) expressed in terms of the contribution from liver and from extrahepatic tissues was determined with sulphamethazine and with *p*-aminobenzoic acid. In 15 min, approx. 19 μmol of sulphamethazine and 581 μmol of *p*-aminobenzoic acid were

Table 1. *Total acetylating capacity in vitro for p-aminobenzoic acid and sulphamethazine in tissues (100000g supernatant) obtained from a slow-acetylator rabbit*

Total activity is expressed as μmol of substrate acetylated/15 min per organ. For other details see the text.

Tissue	*p*-Aminobenzoic acid		Sulphamethazine	
	(μmol acetylated/15 min per organ)	(% of total)	(μmol acetylated/15 min per organ)	(% of total)
Gut	283.2	43.7	12.9	68.6
Blood	35.8	6.1	0.7	3.7
Brain+testes+thymus	23.4	4.0	0.7	3.7
Kidney	21.9	3.8	0.3	1.6
Lung	19.7	3.4	1.0	5.3
Spleen	10.2	1.8	0.3	1.6
Extrahepatic total	394.2	62.8	15.9	84.5
Liver	186.9	37.2	2.9	15.5
Total	581.1	100.0	18.8	100.0

acetylated *in vitro*. Thus the contribution of the liver is smaller than the contribution of the combined extrahepatic tissues for each substrate, and in both instances accounts for no more than one-third of the capacity of all tissues.

In tissues obtained from the rapid-acetylator rabbit (Fig. 3) the total amount of *p*-aminobenzoic acid acetylated and the relative contribution of the extrahepatic enzyme was comparable with that found in the slow acetylator. In contrast, however, with the acetylation of sulphamethazine not only does the total amount of sulphamethazine acetylated increase from approx. 19 μmol to approx. 600 μmol, but also the percentage contribution of the hepatic enzyme to the overall acetylation increases from 16% in the slow to 82% in the rapid acetylator.

These findings strongly suggest that the amount of *N*-acetylating activity that is present in extrahepatic tissues is adequate to account for a major proportion of the total acetylating capacity of a slow-acetylator rabbit for sulphamethazine and *p*-aminobenzoic acid. This evidence from studies *in vitro* alone does not necessarily indicate the actual extent to which the extrahepatic activity contributes to the acetylating capacity of the animal *in vivo*. It would be of interest to compare the amount of sulphamethazine and *p*-aminobenzoic acid acetylated *in vivo* over a 15-min period with the values *in vitro*, but this information is not readily obtainable. It is possible to estimate the maximum quantity of substrate that could undergo acetylation in this time-period from a knowledge of the dose of the drug given and its half-life in the body. On this basis, and for the slow-acetylator rabbit, it was estimated that up to 11 μmol of sulphamethazine and up to 219 μmol of *p*-aminobenzoic acid could have been acetylated. The relative magnitude of these values is very similar to that obtained from measurements *in vitro* with tissues from the slow-acetylator animal. This finding, although not conclusive, lends support to the possibility that *N*-acetylating activity in the extrahepatic tissues is functional *in vivo*. When taken together the findings both *in vitro* and *in vivo* suggest that extrahepatic *N*-acetyltransferase activity probably does contribute significantly to the total acetylating capacity of the individual, particularly for the acetylation of *p*-aminobenzoic acid in the slow-acetylator rabbit.

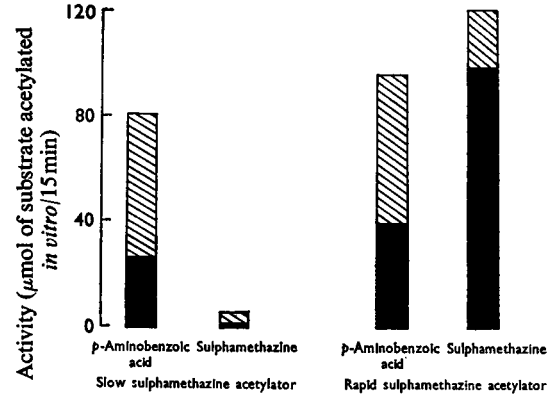

Fig. 3. *Relative contributions, determined in vitro (100000g supernatant), of liver and extrahepatic N-acetyltransferase systems to the total acetylating capacity of the individual for p-aminobenzoic acid and sulphamethazine in both rapid- and slow-acetylator rabbits*

Liver (■) and extrahepatic (■) activity is expressed as μmol acetylated/15 min. For details see the Experimental section.

Partial characterization and attempted separation of multiple forms of N-acetyltransferase

Investigations were undertaken with partially purified *N*-acetyltransferase preparations (100000*g*-supernatant fraction) from various tissues in an attempt to separate and characterize their respective *N*-acetyltransferases. Liver tissue was obtained from animals of either sex. For extrahepatic *N*-acetyltransferase, blood or spleen preparations were used, as they appeared to be typical of extrahepatic *N*-acetyltransferase and on the basis of the characteristics studied were indistinguishable. Preparations were studied at various stages (Weber, 1971*a*) of purification (all fractions were screened for *p*-aminobenzoic acid *N*-acetyltransferase activity), from the relatively crude 100000*g*-supernatant fraction of tissue homogenates to preparations purified over 700-fold from this stage.

At all stages, and particularly at higher stages of purification, the extrahepatic enzyme activity with *p*-aminobenzoic acid as substrate was much less stable to storage at 4°C for 6h (Fig. 4). Extensively purified (700-fold over 100000*g*-supernatant fraction) extrahepatic *N*-acetyltransferase lost approx. 80% of its activity, whereas the liver preparation lost a negligible amount. Mixing experiments demonstrated that the presence of extrahepatic *N*-acetyltransferase did not increase the rate of loss of activity of liver *N*-acetyltransferase. Further characterization was therefore severely impeded by the lack of stability of the purified extrahepatic enzyme because, at this time, stabilization procedures (H. Kelker, W. W. Weber & G. Drummond, unpublished work) had not yet been developed.

Fig. 5 shows the pH–activity profiles obtained for liver and extrahepatic *N*-acetyltransferase, purified 35-fold over the 100000*g* supernatant, with sulphamethazine as substrate. Several noteworthy features are apparent. Comparison of the profiles obtained at

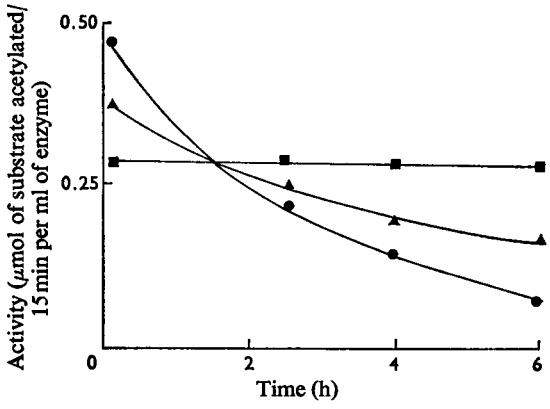

Fig. 4. *Relative stability to storage at 4°C of partially purified (700-fold over 100000g supernatant) N-acetyltransferase preparations*

For details see the Experimental section. ■, Liver *N*-acetyltransferase; ●, extrahepatic *N*-acetyltransferase; ▲, mixture of liver and extrahepatic *N*-acetyltransferase activity. Activity is expressed as μmol of *p*-aminobenzoic acid acetylated/15min per ml of enzyme preparation.

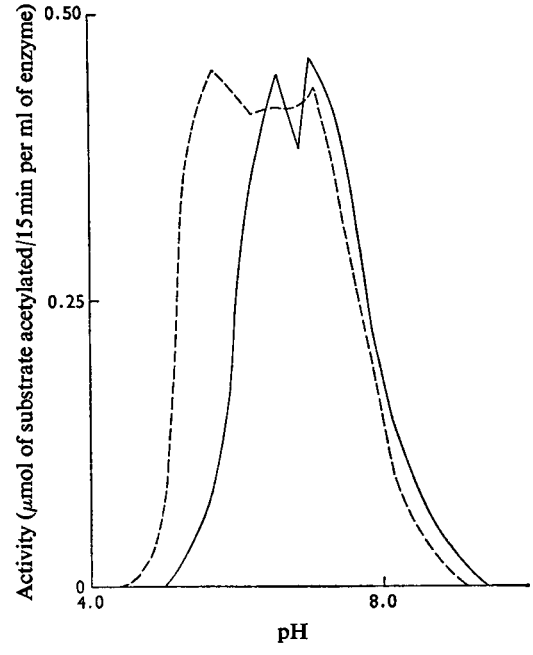

Fig. 5. *pH–activity curve with sulphamethazine as substrate for liver and extrahepatic N-acetyltransferase preparations (purified 35-fold over the 100000g supernatant)*

For details see the Experimental section. ----, Liver *N*-acetyltransferase; ——, extrahepatic *N*-acetyltransferase. Activity is expressed as μmol of substrate acetylated/15min per ml of enzyme preparation.

pH7.0 and above revealed that the profiles for extrahepatic and hepatic enzymes were completely superimposable and both exhibited pH optima at pH7.2. Below pH7.0, however, marked differences exist, with the liver enzyme exhibiting a significantly broader pH profile and having a second peak at pH5.6, a pH value at which the extrahepatic enzyme possesses very little activity. The similarities and differences observed provide additional evidence for the existence of multiple forms of *N*-acetyltransferase. Further, they may indicate either the existence of more than one *N*-acetyltransferase in the liver, or possibly the existence of a structural feature common to both the hepatic and extrahepatic enzymes. Electrophoretic evidence for the latter possibility has recently been obtained (G. Drummond & W. W. Weber, unpublished work).

Attempts were made to separate hepatic and extrahepatic *N*-acetyltransferases by gel filtration on Sephadex G-100. Although a slight separation was achieved with difficulty, complete separation was beyond the resolving power of the technique. These findings may be interpreted as evidence that the hepatic and extrahepatic enzymes are probably very similar in size and shape.

Implications resulting from the existence of multiple forms of N-acetyltransferase

The results described in the present paper provide evidence for the existence of two or more *N*-acetyltransferases that exist in various relative proportions in rabbit tissues. It is proposed that one enzyme is localized predominantly in the liver and gut and its variation between individuals is responsible for the isoniazid-acetylation polymorphism. This enzyme readily catalyses the acetylation of isoniazid, sulphamethazine, *p*-aminobenzoic acid and *p*-aminosalicylic acid. It is also proposed that another *N*-acetyltransferase is localized in the extrahepatic tissue. With the small number of animals used in this study this enzyme was not subject to such wide variation between individuals as was observed for the hepatic enzyme. In view, however, of the relatively small sample population in this study, it was not possible to preclude the possibility that a small proportion of individuals may have a very different enzyme activity. The extrahepatic *N*-acetyltransferase activity readily catalyses the acetylation of *p*-aminobenzoic acid and *p*-aminosalicylic acid, but is considerably less active towards sulphamethazine and isoniazid.

The unexplained observations associated with mammalian *N*-acetyltransferase discussed in the introduction may now be examined in the light of these findings. It should, however, be pointed out that these observations were derived from studies in both man and rabbit, whereas the findings in the present paper were obtained in studies with the rabbit alone. Although there is strong evidence that the rabbit *N*-acetyltransferase represents a good model for that of man, caution should be exercised in extrapolation between the two species.

The existence of more than one *N*-acetyltransferase readily accounts for the observation that a comparison of liver *N*-acetyltransferase activities *in vitro* between rapid and slow sulphamethazine acetylators reveals a very large difference whereas a comparison *in vivo* reveals a much smaller difference in the acetylating capacities of the two acetylator phenotypes. This is particularly evident when the large contribution that the extrahepatic tissues make to the total acetylating capacity in the slow sulphamethazine acetylator is considered. Further, the observation that *p*-aminobenzoic acid is a far better substrate than sulphamethazine for the extrahepatic enzyme and also that the activity of the extrahepatic *N*-acetyltransferase does not fluctuate widely between individuals may readily account for the absence of a bimodal frequency distribution for the acetylation of *p*-aminobenzoic acid. The results, although derived from studies with the rabbit, may stimulate an investigation of extrahepatic acetylation in man, for such a study may shed light on the observation of the occurrence of isoniazid toxicity in some but not all human slow acetylators. Owing to the difference in substrate specificity between the liver and the extrahepatic *N*-acetyltransferase it would seem reasonable to expect a toxic response in any slow-acetylator individual receiving isoniazid or a foreign compound that is metabolized primarily by the liver enzyme. In contrast, toxicity would not be so readily expected, even in slow acetylators, from compounds such as *p*-aminobenzoic acid or *p*-aminosalicylic acid, which are readily metabolized by the extrahepatic *N*-acetyltransferase irrespective of the isoniazid-acetylator phenotype of the individual. The observation that only a small proportion of slow acetylators actually exhibit a toxic response may be explained if the acetylating capacity for both the liver and the extrahepatic system is low. It is conceivable that individuals with low activities of extrahepatic *N*-acetyltransferase exist. Although our findings indicate that the extrahepatic *N*-acetyltransferase activity remains relatively constant between individuals, some variation exists, and the results do not preclude the possibility of the occurrence, at a low frequency, of individuals with typically low activities of extrahepatic *N*-acetyltransferase. In this connexion, Motulsky (1964) has reported a population study on the distribution of *p*-aminosalicylic acid *N*-acetyltransferase activity of human erythrocytes in 131 individuals, and although the overall distribution curve appears to be unimodal, it is noteworthy that two individuals have low enzyme activities. In addition, we have reported (Hearse *et al.*, 1970) details of a human population study in which one individual among 225 screened had an unusually low activity of erythrocyte *p*-aminobenzoic acid *N*-acetyltransferase. Clearly, additional studies on humans, particularly slow acetylators, are required to ascertain whether individuals known to exhibit a toxic response possess low extrahepatic *N*-acetyltransferase activity. If this is proved to be the case then it could be proposed that any individual simultaneously exhibiting low activities of liver and extrahepatic *N*-acetyltransferase would be particularly susceptible to toxicity from isoniazid and other drugs which are inactivated by acetylation.

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