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Immunochemical Studies of Rabbit *N*-Acetyltransferases[1]

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SUMMARY

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Immunochemical studies were undertaken to investigate qualitative and quantitative differences in *N*-acetyltransferases from rapid and slow isoniazid acetylator rabbits. Goat anti-*N*-acetyltransferase antiserum was prepared against partially purified liver *N*-acetyltransferase from a rapid acetylator rabbit. *N*-Acetyltransferases found in rabbit erythrocytes, lymphocytes, intestinal mucosa, and livers were tested against this antiserum by Ouchterlony double diffusion. Liver and intestinal mucosal enzyme preparations yielded reactions of complete identity between enzymes in the two tissues and also between enzymes in the two phenotypes, whereas erythrocyte and lymphocyte *N*-acetyltransferases were nonimmunoreactive. Precipitin reactions between goat anti-*N*-acetyltransferase and 100,000*g* liver supernatant fractions from each acetylator genotype yielded equivalent amounts of precipitated protein at nearly identical equivalence points. The antibody titer required to inactivate the sulfamethazine and *p*-aminobenzoic acid *N*-acetyltransferase activity in 100,000*g* liver supernatant fractions was not significantly different for rapid and slow phenotypes, although sulfamethazine *N*-acetyltransferase activity in rapid acetylator preparations was 10 to 407 times greater than in slow acetylator preparations. Sulfamethazine *N*-acetyltransferase and *p*-aminobenzoic acid *N*-acetyltransferase activities were inactivated at the same rate in both phenotypes. Radial immunodiffusion studies with hepatic supernatant fractions containing equal amounts of *p*-aminobenzoic acid *N*-acetyltransferase activity produced precipitin rings with equivalent areas for both rapid (137.5 ± 16.3 mm^2) and slow (163.0 ± 46.2 mm^2) acetylators. Equal amounts of sulfamethazine *N*-acetyltransferase activity produced much larger precipitin ring areas for slow acetylator preparations (2443 ± 1389 mm^2) than for rapid preparations (27.6 ± 4.1 mm^2). These observations are consistent with the following hypotheses: (i) The livers of rapid and slow isoniazid acetylator rabbits contain approximately the same number of *N*-acetyltransferase molecules, but contain different forms of *N*-acetyltransferase with markedly different catalytic activities; and (ii) monomorphic (*p*-aminobenzoic acid) and polymorphic (sulfamethazine) substrates are acetylated by the same *N*-acetyltransferase molecule.

INTRODUCTION

Genetic variation in man and rabbits determines the ability of individuals to acetylate arylamine drugs such as isoniazid (INH) and sulfamethazine (SMZ) *in vivo*. The variation is attributed to large differences in the level of liver *N*-acetyltransferase (EC 2.3.1.5) activity. Similar differences have also been observed for the *N*-acetylation of arylamine carcinogens, such as aminofluorene and benzidine (1, 2). The low levels of drug acetylation observed in slow acetylators could not be attributed to substrate affinity differences in *N*-acetyltransferases partially purified from autopsy specimens of human rapid and slow INH acetylator livers, nor could it be attributed to the presence of enzyme inhibitors. It was suggested that the acetylator polymorphism might be due to differences in the amount of an identical enzyme molecule produced and these differences could be regulated by a genetic locus that controls the rate of enzyme synthesis (3). Further comparisons of the enzymes from human and rabbit livers with respect to purification, pH optimum, heat stability, and substrate specificity revealed no distinguishing features (3–5). Extensive electrophoretic studies using polyacrylamide gel and column isoelectric focusing techniques yielded phenotypically

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different zymograms for rapid and slow acetylator rabbit liver *N*-acetyltransferase (6). The interpretation of these results was complicated by a low recovery of the enzymes and by a marked variability within the patterns obtained, as well as by the inability to reproduce these results by use of slightly modified procedures.

Differences in the rates of acetylation of *p*-aminobenzoic acid (PABA) are smaller between rapid and slow isoniazid acetylators than observed with polymorphic substrates such as INH and SMZ. Because the rate of acetylation of PABA is so similar in rapid and slow acetylator phenotypes, it has been referred to as a "monomorphic" substrate. To account for the occurrence of the relative lack of individual variation in PABA acetylation by the liver in the presence of wide fluctuations in the capacity of liver to acetylate sulfamethazine, it has been proposed that the rabbit livers contained multiple forms of drug acetylating enzymes (7). Similar observations obtained from human liver biopsies have suggested that the same is true for human livers (2, 8).

The fact that *N*-acetyltransferase preparations catalyze the acetylation of compounds with such widely different structures as PABA, SMZ, and various carcinogenic arylamines indicates that mammalian liver contains either a single enzyme with a very broad substrate specificity or a group of isozymes. Both the number of isozymes in the liver that contribute to the acetylation of these compounds and how they are related to the genetic variation in their rates of acetylation have yet to be determined. In this paper, the immunochemical characteristics of *N*-acetyltransferase isolated from genetically rapid and slow isoniazid acetylator rabbits are compared and described. The results indicate that rapid and slow liver *N*-acetyltransferases are intrinsically different enzymes. These results are also consistent with the hypothesis that the catalytic specificities for monomorphic (PABA) and polymorphic (SMZ) *N*-acetyltransferase activities coexist on the same molecule.

MATERIALS AND METHODS

Reagents. (Acetyl-^{3}H)coenzyme A (3 Ci/mmol) and Omnifluor were obtained from New England Nuclear Company, Boston, Massachusetts. Sulfamethazine (free base) was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Acetyl CoA ($LiAcCoA \cdot 3H_2O$) was obtained from P-L Biochemicals, Milwaukee, Wisconsin. *p*-Aminobenzoic acid was purchased from Sigma Chemicals, St. Louis, Missouri. Noble agar was obtained from Difco Laboratories, Detroit, Michigan.

Animals. Selected lines of mature New Zealand white rabbits, bred and maintained in our laboratory, were used. The animals were fed a sulfonamide-free diet and water was available *ad lib.* The rabbits were caged individually at temperatures of 18–22°C on a light/dark schedule with lights on from 6 AM to 9 PM.

Antiserum. Liver *N*-acetyltransferase was purified 300-fold from a homozygous rapid acetylator rabbit as previously described (5). Antiserum against this enzyme was prepared in a goat by Cappel Laboratories, Cochranville, Pennsylvania, and refrigerated as the lyophilized powder until use. Aliquots of antiserum were reconstituted to the original volume with 0.1 M potassium phosphate buffer, pH 7.4, with 0.15 M sodium chloride added.

Partially purified N-acetyltransferase preparations. Rabbits of known genotypes were sacrificed by the injection of 50 ml air into the marginal ear vein. The livers were removed, washed in cold water, and homogenized in 4 vol of ice-cold 0.1 M potassium phosphate buffer, pH 7.4, for 1 min in a Waring Blendor. The liver homogenates were centrifuged at 10,000*g* for 20 min, followed by centrifugation at 100,000*g* for 1 h. Further purification of the 100,000*g* liver supernatant fraction involved ammonium sulfate precipitation (45–60%), Sephadex G-100 gel filtration, DEAE-cellulose anion-exchange chromatography, and isoelectric focusing as previously described (5, 6). The proximal 10-cm segment of the duodenum was washed in cold water, everted, and washed in cold water again. The mucosal surface was scraped away using a scalpel blade and homogenized in 10 ml 0.1 M potassium phosphate buffer, pH 7.4, with a Kontes hand homogenizer and a Teflon pestle. This homogenate was centrifuged at 100,000*g* for 1 h.

Lymphocyte and erythrocyte preparations were isolated according to the procedure of Boyum (9) as modified in our laboratory (10). The erythrocytes were washed with 0.1 M potassium phosphate buffer, pH 7.4, and recentrifuged at 10,000*g* for 10 min at 4°C. The supernatant was discarded and the erythrocytes were lysed with an equal volume of water at 4°C. This mixture was centrifuged at 100,000*g* for 1 h. The supernatant was diluted with 0.1 M potassium phosphate buffer, pH 7.4, to obtain the desired *p*-aminobenzoic acid *N*-acetyltransferase activity. The lymphocyte enzyme preparation was prepared from a packed lymphocyte pellet resuspended in 0.1 M potassium phosphate buffer, pH 7.4, and was lysed by freeze–thawing (8–10 times) in dry ice–methanol and water at 37°C. This mixture was then centrifuged at 10,000*g* for 5 min.

Enzyme assays. PABA *N*-acetyltransferase activity was determined colorimetrically as previously described (2, 5). SMZ *N*-acetyltransferase activity was determined by the radiolabeling method of Glowinski *et al.* (2).

Ouchterlony double diffusion. A 1% Noble agar containing 1% sodium chloride and 0.001% merthiolate was used for Ouchterlony double diffusion analysis. Wells, 3 mm in diameter, were punched 5 mm apart in a hexagonal pattern around a center well. Experiments were initiated by filling the center well with 5 μl of antiserum and filling the outer wells with 5 μl of an *N*-acetyltransferase enzyme preparation. The gels were kept in a moisture chamber at 23°C for 24 h. Excess protein was removed over a 2-day period by serial washing in three baths containing 1% sodium chloride in water. The gels were then air-dried under lintless paper strips. The gels were stained in a solution of 0.5% Coomassie blue, decolorized in an aqueous solution containing 10% isopropranol and 7% acetic acid for 2 h, and air-dried at room temperature.

Precipitin assays. Varying amounts of 100,000*g* liver supernatant were added to sufficient volumes of 0.1 M potassium phosphate buffer, pH 7.4, to bring the total volume to 500 μl in a 1.5-ml polyethylene tube. A 100-μl aliquot of antiserum was added and the mixture was vortexed for 10 s. The mixture was incubated at 4°C for

24 h and centrifuged at 10,000*g* for 5 min. The supernatant fraction was discarded and the precipitated protein was resuspended in 55 μl of 0.1 M potassium phosphate buffer, pH 7.4, vortexed for 10 s, and centrifuged at 10,000*g* for 10 min. The precipitated protein was measured using a modified biuret procedure (11). Nonimmune goat serum was used as a control. The amount of protein precipitated using nonimmune goat serum was subtracted from the protein precipitated using immune serum. Equivalence points were expressed as micrograms precipitated protein per milligram antiserum protein.

Immunotitration. Varying amounts of antiserum were added to a sufficient quantity of 0.1 M potassium phosphate buffer, pH 7.4, to bring the total volume to 300 μl in a 1.5-ml polyethylene tube. Liver 100,000*g* supernatant, 300 μl, was added and the mixture was incubated for 24 h at 4°C. The mixture was centrifuged at 10,000*g* for 5 min and the PABA and SMZ *N*-acetyltransferase activities remaining in the supernatant fraction were measured. Goat serum from nonimmunized animals was used as a control, and enzymatic activity was expressed as a percentage of the activity present in the nonimmune serum-treated samples. Goat nonimmune serum did not reduce *N*-acetyltransferase activity as compared with samples in which 0.1 M phosphate buffer, pH 7.4, was substituted for goat nonimmune serum. A least-squares regression analysis was performed on the linear section of the immunotitration curve and was used to calculate an equivalence point of enzyme inactivation.

Radial immunodiffusion. Glass plates, 8.3 × 10.2 cm, were coated with 12.5 ml of 1% Noble agar containing 5.34% sodium chloride, 0.001% merthiolate, and 400 μl antiserum. The agar was prepared and allowed to cool to 62°C before the antiserum was added, mixed for 10 s by vortexing, and poured over the glass plates. The gels were allowed to harden at 4°C for 12–24 h before use. Thirty 3-mm-diameter wells were punched into each plate and 0.2–10 μl of a rabbit 100,000*g* supernatant fraction was added to each plate. After overnight incubation at room temperature, the diameter of the precipitin ring was measured under magnification using a Model 2743 calibrating viewer (Transidyne Corp.).

RESULTS

N-Acetyltransferases from different tissues. Rabbits of the three isoniazid acetylator genotypes (homozygous rapid, *RR*; heterozygous rapid, *Rr*; and homozygous slow, *rr*) were sacrificed and the *N*-acetyltransferase activities of the liver 100,000*g* supernatant fractions were determined. Rapid acetylator preparations possessed from 10 to 407 times greater SMZ *N*-acetyltransferase activity than did slow acetylator preparations. The *N*-acetyltransferase activity toward the monomorphic substrate, PABA, however, was equivalent in 100,000*g* liver supernatant fractions of rabbits from all three genotypes (Table 1). Whole blood PABA *N*-acetyltransferase activity was greater in slow acetylators than in rapid acetylators (0.113 ± 0.116 vs 0.048 ± 0.024 nmol acetylated produce/min/ml red blood cells). Slow acetylators also possessed greater levels of lymphocyte PABA *N*-acetyltransferase activity than did rapid acetylators (87 ± 20 vs 39 ± 14 nmol acetylated product/h/mg protein). These results are consistent with previous findings (10, 12).

TABLE 1

Specific activities of rabbit liver N-acetyltransferases

Specific *N*-acetyltransferase activity was determined in 100,000*g* liver supernatant fractions for SMZ and for PABA as previously described (2). For SMZ specific activity determinations, the reaction mixture (0.20 ml) contained 0.10 ml of suitably diluted enzyme and the initial concentrations were 5×10^{-4} M SMZ and 5×10^{-4} acetyl CoA. For PABA specific activity determinations, the reaction mixture (0.090 ml) contained 0.050 ml of suitably diluted enzyme and the initial concentrations were 4.4×10^{-5} M PABA and 2.2×10^{-3} M acetyl CoA. Incubations were conducted at 37°C.

Rabbit No.	Genotype	Sulfamethazine	*p*-Aminobenzoic acid	Protein
		nmol acetylated product/min/mg protein		*mg/ml*
86	*RR*	4.9	1.4	27.0
1	*RR*	8.2	1.1	28.0
3	*RR*	12.2	1.9	19.5
81	*RR*	7.6	2.0	31.3
101	*Rr*	6.4	2.5	26.7
102	*Rr*	5.8	—	21.0
60	*rr*	0.03	1.7	18.6
106	*rr*	0.19	1.8	40.7
107	*rr*	0.23	1.2	14.6
108	*rr*	0.33	1.4	17.6
109	*rr*	0.47	1.5	20.3

Ouchterlony double diffusion. Various purification fractions of liver *N*-acetyltransferase were tested by Ouchterlony double diffusion. Fractions tested included 100,000*g* liver supernatant, Sephadex G-100 gel filtrate, ammonium sulfate-precipitated protein, DEAE-cellulose purified protein, and an isoelectric focusing peak, *p*I 5.3. This represents stepwise 1200-fold purification of *N*-acetyltransferase activity from the 100,000*g* supernatant fraction. Reactions of identity were obtained from all purification fractions from individual rapid ($N = 2$) and slow ($N = 3$) acetylator liver enzyme preparations. No immunologically precipitated material was detected in the cruder enzyme preparations that was not present in the more highly purified enzyme preparations.

The 100,000*g* liver supernatant fractions from various rapid (*RR* and *Rr*) and slow (*rr*) acetylators were tested against goat anti-*N*-acetyltransferase antiserum (Fig. 1). Reactions of identity were seen between all 100,000*g* liver supernatant fractions from rapid and slow acetylator rabbits. No immunological differences were noted between liver *N*-acetyltransferases from rapid and slow acetylator rabbits.

Erythrocyte, lymphocyte, and intestinal mucosal *N*-acetyltransferases were also tested by Ouchterlony double diffusion. Similar amounts of PABA *N*-acetyltransferase activity were added to each of the outer wells. Erythrocyte and lymphocyte *N*-acetyltransferases from both phenotypes were nonimmunoreactive with goat anti-*N*-acetyltransferase antiserum. Intestinal mucosal *N*-acetyltransferase yielded a reaction of complete identity when compared with liver *N*-acetyltransferases from rapid or slow acetylator rabbits. These results are summarized in Table 2.

Precipitin reactions. Increasing amounts of 100,000*g* liver supernatant fraction from rapid and slow acetylator rabbits were added to a constant amount of anti-*N*-acetyltransferase antiserum. Precipitated protein was

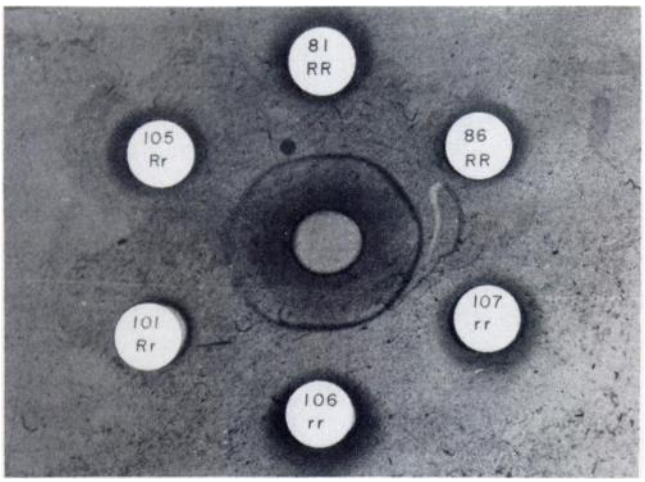

FIG. 1. *Ouchterlony double diffusion*

Antiserum (5 μl) was placed in the center well. Starting with the top well and proceeding in a clockwise direction, the outer wells were filled with 5 μl of 100,000*g* liver supernatant fractions from the following rabbits: 81 (*RR*), 86 (*RR*), 107 (*rr*), 106 (*rr*), 101 (*Rr*), and 105 (*Rr*). Reactions of identity were seen when the three genotypes were compared.

measured and plotted against the amount of 100,000*g* supernatant protein added. The equivalence points and the amount of protein precipitated at equivalence were not significantly different for 100,000*g* supernatant fractions from livers of rapid and slow acetylator rabbits (Table 3 and Fig. 2).

Immunotitration. Increasing amounts of anti-*N*-acetyltransferase antiserum were added to a constant amount of 100,000*g* liver supernatant fraction and the residual SMZ and PABA *N*-acetyltransferase activities were measured. The linear segment of the resultant sigmoidal curve was extrapolated to the point where *N*-acetyltransferase activity was completely eliminated (Fig. 3). Equivalence points for enzyme preparations from rapid and slow acetylators were identical for both SMZ and PABA *N*-acetyltransferase activity (Fig. 3 and Table 4). Also, SMZ and PABA *N*-acetyltransferase activities were proportionally inactivated by antiserum in both rapid and slow acetylator 100,000*g* liver supernatant fractions (Fig. 4).

TABLE 2

Summary of double diffusion (Ouchterlony) analysis

The presence or absence of a reaction of identity is marked by a (+) or (−). No reactions of partial identity or nonidentity were seen when any two enzyme preparations were tested.

N-Acetyltransferase preparation tested	Acetylator genotype	Reaction
Liver fractions		
100,000*g*	*rr*	+
Ammonium sulfate precipitate	*rr*	+
Sephadex G-100	*rr*	+
DEAE-cellulose	*rr*	+
Isoelectric focusing peak (*pI* 5.3)	*rr*	+
100,000*g*	*Rr*	+
100,000*g*	*Rr*	+
100,000*g*	*RR*	+
100,000*g*	*RR*	+
Small intestine		
100,000*g*	*rr*	+
100,000*g*	*RR*	+
Erythrocyte lysate	*rr*	−
Erythrocyte lysate	*RR*	−
Lymphocyte lysate	*rr*	−
Lymphocyte lysate	*RR*	−

TABLE 3

Precipitin curve results

A 100-μl aliquot of antiserum containing 5.5 to 6.1 mg of protein was added to varying amounts of 100,000*g* liver supernatant fraction and the quantity of precipitated protein plotted against the amount of 100,000*g* supernatant protein added. Equivalence points and precipitated protein at equivalence are similar for the three enzyme preparations shown from rapid (*RR* and *Rr*) and slow (*rr*) acetylators.

	Equivalence point ratio[a]	Precipitated protein
		μg
Rapid acetylators ($N = 6$)	3.28 ± 0.32	152 ± 12
Slow acetylators ($N = 5$)	3.09 ± 0.23	142 ± 16

[a] 100,000*g* supernatant protein/antiserum protein.

Radial immunodiffusion. The applicability of this method was first tested with various purification fractions from the livers of individual rapid and slow acetylator rabbits. For the various purification fractions, the precipitin ring area was shown to be linearly related to the amount of either SMZ or PABA *N*-acetyltransferase activity added to the wells ($R = 0.965$, $P < 0.05$; $R = 0.955$, $P < 0.05$). Dilutions of a single enzyme preparation also yielded a linear relationship between the precipitin ring area and the amount of SMZ or PABA *N*-acetyltransferase activity added to the well ($R = 0.985$, $P < 0.01$; $R = 0.986$, $P < 0.01$).

Equal amounts of PABA *N*-acetyltransferase activity (0.15 nmol acetylated product/min) yielded nearly identical precipitin ring areas for rapid (137.5 ± 16.3 mm²) and slow (163 ± 46.2 mm²) acetylators. Equal amounts of SMZ *N*-acetyltransferase activity (0.008 nmol acetylated product/min) from 100,000*g* liver supernatant fractions, however, yielded significantly different ($P < 0.02$) precipitin ring areas for rapid (27.6 ± 4.1 mm²) and slow (2443 ± 1389 mm²) acetylators.

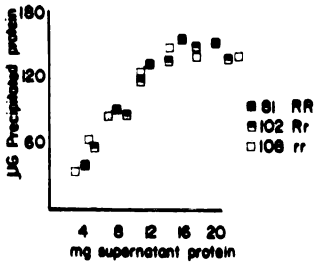

FIG. 2. *Protein precipitin reaction*

The quantity of precipitated protein is plotted against the amount of 100,000*g* supernatant protein added to a constant amount of antiserum. Equivalence points and precipitated protein at equivalence are equivalent for the three enzyme preparations shown from rapid (*RR* and *Rr*) and slow (*rr*) acetylators.

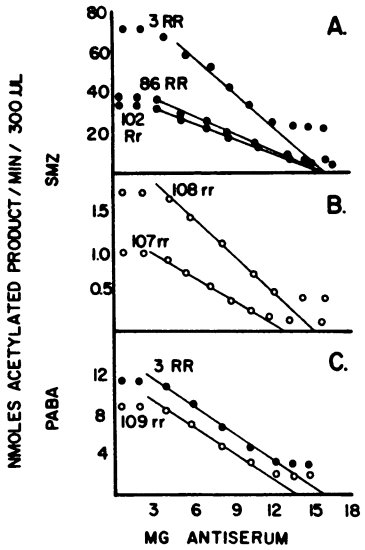

FIG. 3. *Immunotitration*

Increasing amounts of antiserum were added to a constant amount of 100,000*g* supernatant from rapid and slow acetylator livers. Sulfamethazine and *p*-aminobenzoic acid *N*-acetyltransferase activities were measured in the supernatant fraction.

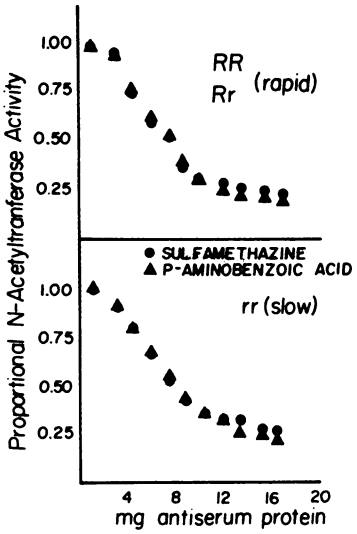

FIG. 4. *Immunotitration*

The proportion of the original *N*-acetyltransferase activity is plotted against the amount of antiserum protein added.

DISCUSSION

The capacity for acetylation of arylamine drugs and foreign compounds is determined primarily by the level of tissue *N*-acetyltransferase activity in a variety of mammalian species. Furthermore, a genetically determined difference in the capacity of individuals to acetylate INH *in vivo* is manifested in man and the rabbit, enabling them to be classified as rapid and slow INH acetylators (1, 13–16). This difference is due to a difference in the level of hepatic *N*-acetyltransferase activity. One unanswered question concerning this polymorphism is: What is the nature of the biochemical difference between the *N*-acetyltransferases that are responsible for this trait? One possible explanation could be the existence of a quantitative difference in the amount of a single hepatic *N*-acetyltransferase present in individuals of both acetylator phenotypes (3). Another possibility could be the existence of structurally different forms of the enzyme in the two acetylator phenotypes. The immunochemical studies reported here were undertaken as a means of obtaining further information for characterizing and distinguishing liver *N*-acetyltransferase in rapid and slow acetylator rabbits with the purpose of answering this question.

TABLE 4

Immunotitration of rabbit liver N-acetyltransferase activity

Equivalence points were determined by least-squares regression of the linear segment of the immunotitration curves.

Rabbit No.	Genotype	Equivalence points	
		SMZ	PABA
		mg antiserum protein	
86	*RR*	15.0	14.3
1	*RR*	14.4	14.7
3	*RR*	15.6	15.7
81	*RR*	13.8	13.7
101	*Rr*	13.3	14.6
102	*Rr*	16.0	16.1
60	*rr*	15.7	15.3
106	*rr*	13.8	13.5
107	*rr*	12.5	12.8
108	*rr*	15.0	15.0
109	*rr*	13.6	13.5

In order to evaluate the relative content of *N*-acetyltransferase in the two acetylator phenotypes, immunotitration, protein precipitin studies, and radial immunodiffusion studies of liver preparations of each phenotype were carried out with goat anti-*N*-acetyltransferase antiserum. The immunotitration equivalence points were found to be essentially the same for rapid and slow acetylator 100,000*g* liver supernatant fractions (Table 4) in which the SMZ *N*-acetyltransferase activity differed by more than 400-fold (e.g., *RR* rabbit number 3 vs *rr* rabbit number 60; Table 1). Similar results are shown graphically in Figs. 3A and B. In the precipitin experiments considered in Table 3 and Fig. 3, the amount of protein precipitated at nearly identical equivalence points was essentially the same. The immunotitration and precipitin results show that liver *N*-acetyltransferase preparations from both acetylator phenotypes contain approximately the same amount of antigen that reacts with the anti-*N*-acetyltransferase antiserum. Radial immunodiffusion results support these findings. The precipitin ring areas for slow acetylator liver preparations were from 68 to 88 times larger than the precipitin ring areas for rapid acetylators when equal SMZ *N*-acetyltransferase activities were added to the wells. The area of the precipitin ring was shown to be linearly related to the amount of *N*-acetyltransferase added to the wells; therefore, these data indicate that the livers of rapid and slow acetylator rabbits contain similar numbers of antigenically similar molecules of SMZ *N*-acetyltransferase molecules and that slow acetylator rabbit livers contain a form of *N*-acetyltransferase with specificity for SMZ that is catalytically less active than that of rapid acetylator livers.

The immunochemical studies of PABA *N*-acetyltrans-

ferase activity in rapid and slow liver preparations provide further insight into the nature of the acetylating enzymes. Immunotitration equivalence points (Table 4 and Figs. 3C and 4) were found to be identical for PABA when *N*-acetyltransferases from rapid and slow acetylator liver preparations were compared. This indicates that these liver preparations contain equal numbers of PABA *N*-acetyltransferase molecules. On the basis of these immunochemical observations and the similarity of the specific catalytic *N*-acetyltransferase activity for PABA in the two acetylator phenotypes (Table 1), it appears that the livers of rapid and slow acetylator rabbits also contain similar catalytic forms of PABA *N*-acetyltransferase.

In addition, equal amounts of anti-*N*-acetyltransferase antiserum were observed to inactivate equal proportions of SMZ *N*-acetyltransferase and PABA *N*-acetyltransferase activity in rapid and slow acetylator liver preparations (Fig. 4). This is consistent with the hypothesis that SMZ and PABA *N*-acetyltransferase activities coexist on the same enzyme molecule.

We have provisionally considered the three models summarized in an attempt to account for these findings in rapid and slow acetylators (Fig. 5).

Model I: Rapid and slow acetylator liver preparations both possess an *N*-acetyltransferase with identical catalytic specificity for polymorphic substrates such as SMZ, but rapid liver preparations contain more of this enzyme than do slow preparations. Both preparations contain equal amounts of another *N*-acetyltransferase with specificity for PABA acetylation.

Model II: Rapid and slow acetylator liver preparations contain *N*-acetyltransferases with significantly different catalytic activities for polymorphic substrates, and both phenotypes also contain equal amounts of an *N*-acetyltransferase with specificity for PABA acetylation.

Model III: Rapid liver preparations contain an *N*-acetyltransferase that readily catalyzes on the same molecule the acetylation of both SMZ and PABA, while slow preparations contain another *N*-acetyltransferase that is capable of acetylating PABA to a remarkably greater extent than SMZ.

Since the *N*-acetyltransferase antigen content in the livers of rapid and slow acetylators is approximately equal despite the large phenotypic differences in SMZ *N*-acetyltransferase activity, Model I appears to be a very unlikely interpretation of the data and has been eliminated from further consideration. Models II and III are both consistent with the following findings: (i) Liver preparations from rapid and slow acetylators contain equal numbers of SMZ *N*-acetyltransferase molecules; (ii) slow acetylators contain SMZ *N*-acetyltransferase molecules with significantly lower SMZ catalytic activity than *N*-acetyltransferase molecules in rapid acetylators; and (iii) rapid and slow acetylator liver preparations contain equal numbers of PABA *N*-acetyltransferase molecules with similar catalytic activities. However, the proportion of SMZ activity precipitated by antiserum was the same as that of PABA activity, suggesting that SMZ and PABA *N*-acetyltransferase activities may be contained on the same enzyme molecule, as is proposed in Model III.

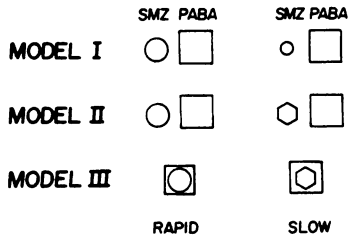

FIG. 5. *Molecular models of proposed mechanisms for the isoniazid acetylator polymorphism*

Sulfamethazine *N*-acetyltransferase (SMZ) and *p*-aminobenzoic acid *N*-acetyltransferase (PABA) are schematically drawn for rapid and slow acetylators.

Immunotitration failed to precipitate all of the *N*-acetyltransferase activity in the 100,000*g* liver supernatant fractions of both rapid and slow acetylators (Figs. 3 and 4). Further addition of antiserum in excess of twice the amount of antiserum at equivalence failed to further reduce the residual *N*-acetyltransferase activity in the supernatant. A possible explanation of this finding is that other soluble arylamine *N*-acetyltransferases which are antigenically distinct from the rapid and slow *N*-acetyltransferases may be present in the 100,000*g* supernatant fraction of liver. It may be pertinent to note in this connection that immunochemically distinct forms of intestinal arylhydroxamic acid *N*-acyltransferase, an enzyme with properties which are very similar to those of rabbit liver *N*-acetyltransferase (17), have been demonstrated in the rat (18).

Immunochemical comparisons of *N*-acetyltransferase activities of selected extrahepatic tissues with *N*-acetyltransferases of liver were carried out because of correlations known to occur between them. INH *N*-acetyltransferase activity in human jejeunal mucosa varies more than 20-fold from one individual to another and correlates highly with liver INH *N*-acetyltransferase activity (3), whereas PABA *N*-acetyltransferase activity varies over a 3-fold range and does not correlate with polymorphic liver activity (2, 8). This has also been shown to occur in the rabbit (8, 12). Since the jejeunal and liver activities are both polymorphic for INH and monomorphic for PABA, comparisons of intestinal and liver preparations were especially interesting. A reaction of complete identity was seen with jejeunal and liver preparations from rapid and slow acetylators when compared to Ouchterlony double diffusion. Observations in rabbits have also indicated that *N*-acetyltransferase activity for PABA in blood and INH acetylation in the liver are interrelated (12). Previous studies of isoenzyme patterns and certain other biochemical properties of the partially purified enzymes from both tissues have indicated that these enzymes may possess certain features in common (5). However, *N*-acetyltransferases from red blood cells and lymphocytes do not react with the antibody prepared to rabbit liver *N*-acetyltransferase. These observations indicate that rabbit liver and jejeunal mucosal *N*-acetyltransferases may be the same enzyme but are different from peripheral blood *N*-acetyltransferase.

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