A survey among 20 inbred mouse strains revealed large variation (up to ~20-fold) for the N-acetylation of p-aminobenzoic acid by blood N-acetyltransferase and for the aromatic amine carcinogen benzidine by both liver and blood N-acetyltransferase. Of 20 strains surveyed, three are classified as slow acetylators (A/J, AHe/J, and X/Gf) and 17 are classified as rapid acetylators (AuSwJ, Castaneous, ST/BJ, C57BL/6J, Molosinus, SF, SWR/J, 129/SV, RF/J, RIII/2J, IsCam, SOL/J, Balb/cJ, C3H/HeJ, CBA/J, AKR/J, and DBA/J). The rapid acetylator strains possessed ~10 times greater liver benzidine N-acetyltransferase specific activity than the slow acetylator strains. Intercross and backcross matings of A/J and C57BL/6J mice indicate that a single gene with two major alleles is responsible for differences in N-acetyltransferase activity in blood for p-aminobenzoic acid or the alternate aromatic amine carcinogen aminofluorene, and in liver for aminofluorene. Analysis of 11 recombinant inbred strains derived from matings of A/J with C57BL/6J mice support this conclusion and demonstrate the existence of minor modifying genes that segregate independently of the major N-acetyltransferase gene.

In man and rabbit, the rate of N-acetylation of arylamine drugs such as sulfamethazine,isoniazid, hydralazine, and procainamide is subject to a genetic polymorphism, attributable to a difference in the amount of N-acetyltransferase activity (EC 2.3.1.5) in liver (Weber, 1973; Weber et al., 1978; Du-Souich and Erill, 1976; Glowniski et al., 1980). In contrast, liver N-acetylation of the aromatic amine p-aminobenzoic acid is considered monomorphic in these species, displaying relatively little variation between individuals. Recent work has shown that blood PABA N-acetyltransferase activity is distinctly polymorphic in certain inbred mouse strains, despite the fact that no strain differences are apparent with regard to liver PABA N-acetyltransferase activity (Tannen and Weber, 1979). Of nine strains tested, only the A/J mouse lacked blood PABA N-acetyltransferase activity. Low SMZ N-acetyltransferase activity was apparent in either blood or liver of all strains, and thus, no strain differences were seen for this substrate in direct contrast to the case in man or rabbit. Using the A/J and C57BL/6J inbred mouse strains as models of slow and rapid acetylators, respectively, Tannen and Weber (1980a) showed that the expression of PABA acetylation in blood is consistent with simple Mendelian inheritance of two codominant alleles.

In human populations, the acetylation polymorphism plays an important role in determining the variability seen in the incidence of toxic responses to certain arylamine drugs such as INH, procainamide, and hydralazine (Drayer and Reidenberg, 1977). Recent studies have indicated that the acetylation polymorphism may play a role in differential drug toxicity in animal models as well as man. Using the A/J and C57BL/6J mouse model, it was shown that procainamide was acetylated to a lesser degree by the slow acetylator strain (A/J); that A/J mice had a higher incidence of spontaneous antinuclear antibodies than C57BL/6J mice; and that these antibodies could be induced by oral procainamide (Tannen and Weber, 1980b). In C57BL/6J mice, procainamide tended to suppress antinuclear antibody formation. Although it is clear that the ability to N-acetylate procainamide is not the sole factor controlling its ability to induce antinuclear antibodies in this genetic mouse model, it is equally clear that acetylator phenotype does play a role in this regard.

The conversion of compounds such as AF and BZ to metabolites capable of interaction with cellular constituents is important with respect to their carcinogenic properties (Miller, 1970; Miller, 1978). Metabolic activation of aromatic amine carcinogens is a complex process which is generally recognized as involving a number of enzymatically mediated steps, including N-hydroxylation and subsequent esterification. In addition, these compounds may also undergo N-acetylation in a number of different species, including hamster, guinea pig, rabbit, mouse, and rat (Lotlikar and Luha, 1971; Lower and Bryan, 1973; Morton et al., 1979). The dog, incapable of acetylation certain arylamine drugs (Williams, 1959), is also incapable of acetylating these carcinogens (Lower and Bryan, 1973; Poirier et al., 1963).

In human and rabbit populations, certain aromatic amine carcinogens such as AF, BZ and 2-naphthylamine are acetylated by the same polymorphic N-acetyltransferase that is responsible for the acetylation of arylamine drugs (Glowniski et al., 1978). This has led to the speculation that rapid and slow acetylator populations may differ in susceptibility to aromatic amine carcinogen-induced chemical carcinogenesis, parallelizing the situation for the drug-induced toxicities (Glowniski et al., 1980). One preliminary report suggests that slow acetylators may be at greater risk of urinary bladder tumors from
Genetics of Aromatic Amine N-Acetylation in Inbred Mice

Aromatic amine carcinogen exposure (Lower et al., 1979), although further studies in appropriate human populations are necessary to establish this. In an attempt to develop new animal models for the N-acetylation polymorphism and thereby possibly assess its effect on tumor susceptibility, these studies were undertaken to analyze the genetic basis of strain differences in aromatic amine carcinogen N-acetylation in inbred mice.

Experimental Procedures

Chemicals and Reagents

Reagent grade sulfamethazine (free acid) was obtained from Nutritional Biochemicals, Cleveland, OH; p-amino-benzonic acid from Sigma; acetyl coenzyme A, lithium salt from P & L Biochemicals, Milwaukee, WI; [acetyl-14C]coenzyme A (3 Ci/mmol) and Omnifluor from New England Nuclear; 2-aminofluorene from K & K Labs, Plainview, NY; and benzidine from Matheson, Coleman-Bell, Cincinnati, OH. All other chemicals and reagents were reagent grade.

Animals

Mice used in the strain survey (Table I) were obtained from The Jackson Laboratory, Bar Harbor, ME, through the help of Dr. Loren Skow. Mice of the X/Gf strain were donated by Dr. Anna Goldfelder, Columbia University, NY. F1, F2, and backcross animals used for genetic analysis were bred at The University of Michigan, Department of Pharmacology from parental A/J and C57BL/6J mice received from The Jackson Laboratory. The recombinant inbred strains of mice designated RXA and AXB, derived by inbreeding the F2 generation of crosses C57BL/6J X A/J (Taylor, 1976) were obtained through the generosity of Dr. Muriel Nesbitt, Department of Biology, University of California, San Diego, CA.

All animals were housed 2 to 8/standard shoe box cage with food (sulfur-free Purina Mouse Chow) and water ad libitum. Bedding consisted of cedar wood shavings which were changed once per week. Animals were maintained on a light-dark schedule with lights on from 6 AM to 8 PM. All mice were 8-24 weeks of age at the time of experimentation.

Tissue Preparation

Blood—Whole blood was collected by orbital sinus puncture in a 0.075-ml heparinized hematocrit tube. Fifty μl were added to 0.5 ml of distilled water and allowed to hemolyze for 3 min. One-half ml of chilled 50 mM potassium phosphate buffer, 1 mM dithiothreitol, pH 7.4, was added and the hemolysate kept on ice.

Liver—Animals were killed by decapitation, livers removed and homogenized by hand in 4 volumes of 50 mM potassium phosphate buffer, 1 mM dithiothreitol, pH 7.4, in a ground glass homogenizer. Homogenates were centrifuged at 10,000 × g for 20 min, and the resulting supernatants at 105,000 × g for 1 h.

Determination of Acetyling Activity in Vitro

Colorimetric Assay for Acetylation of p-Aminobenzonic Acid

The amount of PABA N-acetyltransferase activity was determined by the procedure of Hearse and Weber (1973), which is a modification of the diazotization procedure of Bratton and Marshall (1939). All incubations were carried out at 37 °C in capped polyethylene microtest tubes (0.4 ml) (Kew Scientific, Columbus, OH). The reaction mixtures (0.09 ml) contained 0.05 ml of suitably diluted enzyme, aqueous CoASAc (0.02 ml, specific activity, 0.02 ml of PABA, 0.2 mM). Control tubes contained no CoASAc. Following diazotization (Hearse and Weber, 1973), absorbance was measured at 540 nm in 1-ml cuvettes of 1-cm light path against a water blank in a Beckman 35 spectrophotometer.

The extent of acetylation was obtained by subtracting the experimental reading from the control reading. Under the conditions of this assay a decrease of 1 absorbance unit corresponds to the acetylation of 6.08 nmol of PABA. Specific activities are expressed as nanomoles of acetylated product formed/minute/milligram of protein, unless otherwise indicated.

Blood—Whole blood lysates (as described under "Tissue Preparation") were diluted 2.25-fold with 50 mM potassium phosphate buffer, 1 mM dithiothreitol, pH 7.4, and used as enzyme source. Incubations were carried out for up to 3 min.

Liver—Liver cytosol was diluted 20-fold with 50 mM potassium phosphate buffer, 1 mM dithiothreitol, pH 7.4. Incubations were carried out for up to 1.5 min.

Radioassay for Acetylation of Benzidine, Aminofluorene, and Sulfamethazine

The amount of N-acetyltransferase activity for these substrates was determined by a radioassay procedure (Glowinski et al., 1978). The reaction mixture (0.2 ml) consists of 0.1 ml of suitably diluted enzyme, 0.05 ml of aqueous [14C]CoASAc (2 mm, specific activity, 4.5 × 10^-3 μCi/mmol), and 0.05 ml of amine substrate solution in capped tubes.

| Strain | PABA | Benzidine | SMZ | Liver | PABA |
|--------|------|-----------|-----|-------|------|
| A/J    | 0.094 ± 0.026 (7) | ND  | 0.011, 0.011 | 0.058 ± 0.024 (3) | 17.9 ± 4.0 (3) |
| AHe/J  | 0.112 ± 0.012 (3) | ND  | 0.017 ± 0.007 (3) | 0.002 ± 0.004 (3) | 17.3 ± 1.8 (6) |
| X/Gf   | 0.045 ± 0.026 (7) | ND  | 0.020, 0.018 | 0.027 ± 0.035 (6) | 19.4 ± 2.2 (6) |
| AuSa/J | 1.01 ± 0.21 (3) | 0.020, 0.018 | 0.015, 0.033 | 0.041 ± 0.008 (3) | 21.0 ± 2.3 (4) |
| Castaneous | 0.93, 1.11 | 0.027, 0.198 | 0.013 ± 0.004 (3) | 0.123, 0.187 | 24.7 ± 2.4 (4) |
| ST/Bj  | 0.85, 1.92 | 0.036 ± 0.008 (3) | 0.017 ± 0.005 (3) | 0.364 ± 0.075 (4) | 24.7 ± 2.4 (4) |
| C57BL/6J | 1.47 ± 0.23 | 0.040 ± 0.003 (3) | 0.010 ± 0.002 (3) | 0.215 ± 0.033 (4) | 24.7 ± 2.4 (4) |
| Molossinus | 1.49, 1.56 | 0.033 ± 0.009 (3) | 0.012 | 0.287 ± 0.179 | 26.7 ± 4.7 (3) |
| SP     | 1.88 ± 0.45 (4) | 0.039 ± 0.008 (3) | 0.016 ± 0.003 (3) | 0.338 ± 0.051 (4) | 20.8 ± 1.8 (5) |
| SWR/J  | 1.72 ± 0.21 (4) | 0.039 ± 0.008 (3) | 0.022, 0.016 | 0.114, 0.187 | 22.3 ± 4.2 (3) |
| 129/SV | 1.29, 2.23 | 0.039 ± 0.008 (3) | 0.18 | 0.411 | 31.2, 24.3 |
| RF/J   | 1.86 ± 0.27 (4) | 0.039 ± 0.008 (3) | 0.011 | 0.254 ± 0.240 | 24.3 ± 2.9 (3) |
| RII/2J | 1.82, 1.95 | 0.060 ± 0.010 (8) | 0.020 ± 0.006 (5) | 0.314 ± 0.114 (7) | 25.3 ± 4.9 (7) |
| IsCam  | 2.03 | 0.064 ± 0.008 (5) | 0.015 ± 0.001 (3) | 0.362 ± 0.026 (5) | 23.1 ± 3.6 (5) |
| SJL/J  | 2.31, 1.95 | 0.048, 0.067 | 0.019 ± 0.005 (4) | 0.314 ± 0.090 (4) | 24.4 ± 1.7 (5) |
| BALB/c/J | 2.30 ± 0.24 (11) | 0.052 ± 0.005 (6) | 0.015 ± 0.003 (5) | 0.345 ± 0.085 (7) | 21.0 ± 3.4 (8) |
| C57/HeJ | 2.36 ± 0.14 (5) | 0.057, 0.071 | 0.018 | 0.220 ± 0.070 (4) | 24.4 ± 2.8 (6) |

* The potassium phosphate buffer (50 mM) used did not contain dithiothreitol.
* Number of animals is in parenthesis.
* ND, nondetectable.
polypolypropylene micro sample tubes (1.5 ml) (Kew Scientific, Columbus, OH). The final concentrations of BZ, AF and SMZ in the assay were 0.25 mM and that for CoASAc was 0.5 mM. BZ and AF were dissolved in Me3SO and SMZ in 50 mM potassium phosphate buffer, pH 7.4. All incubations were carried out at 37 °C. Incubations were terminated by addition of 1 ml of N-ethylmaleimide (2 mM) in ethylene dichloride. 

After extraction of acetylated product, an aliquot of the organic layer was counted. Under the conditions of this assay, 5500 dpm corresponded to the acetylation of 1 nmol of substrate and 0.042 nmol were detectable. Values were corrected for both the counting efficiency of the counter (42.5%) and the extraction efficiency of each substrate (Glowinski et al., 1978). Specific activities are expressed as nanomoles of acetylated product formed/minute/milligram of protein, unless otherwise indicated.

Blood—Whole blood lysates (as described under “Tissue Preparation”) were used as source of enzyme. Incubations were carried out for up to 12 min.

Liver—Cytosol was diluted 2-fold with 50 mM potassium phosphate buffer, 1 mM dithiothreitol, pH 7.4. Incubations were carried out for up to 6 min for BZ and AF. For SMZ, incubations were carried out for up to 15 min.

**Treatment of Inheritance Data**

For Figs. 1, 2, and 4, additive inheritance of N-acetyltransferase genes in both parental strains was used as a first approximation. The calculated value for N-acetyltransferase activity in the F1 generation was thus half-way between the two parental mean values. For classification of animals according to genotype and for comparison of observed and expected segregation ratios, a line was drawn at the midpoint of the means between the A/J and the calculated F1 values, and also between the F1 and C57BL/6J values.

**Protein Determinations**

Blood protein concentrations were determined by the method of Warburg and Christian (1941). Liver protein concentrations were determined by the modified method of Lowry et al., 1951.

**RESULTS**

**Strain Survey of N-Acetyltransferase Activity**—Twenty selected inbred strains of mice were surveyed for initial N-acetylation rates of a number of aromatic amine substrates in liver and blood (Table I). This data reveals wide interstrain variation in N-acetylation in these tissues. A/J, AHe/J, and X/Gf mice have no detectable blood BZ N-acetyltransferase activity, while the other strains tested have very low activities for this substrate (0.020-0.060 nmol/min/mg).

Liver BZ N-acetyltransferase, also highly variable among these strains, correlates significantly with blood BZ activity (p<0.001). A/J and AHe/J mice have very low activity (0.058 and 0.032 nmol/min/mg) while the other strains tested have considerably higher activities for this substrate. The blood N-acetyltransferase activity pattern of PABA follows that of BZ. A/J, AHe/J, and X/Gf strains exhibit very low blood N-acetyltransferase activity (0.045-0.112 nmol/min/mg) and the other strains exhibit activity which is ~10-fold higher.

The acetylation of PABA by mouse liver cytosol reveals a different pattern. This activity is higher in all strains tested than that seen in the livers of either man or rabbit. In contrast to the situation in mouse blood, however, the interstrain variation in liver PABA activity is relatively small (17.3-35.4 nmol/min/mg). The A/J and AHe/J mouse strains appear to be on the low side of this range and are only 1-2-fold lower than the other strains tested. Liver SMZ N-acetyltransferase activity is at the limit of sensitivity for this assay and thus, no differences between strains were detected. It is clear that mice can be classified as rapid or slow acetylators from blood BZ or blood PABA N-acetyltransferase activities since either is correlated with liver BZ activity.

Table I reveals other acetylator variants among the seventeen “rapid” inbred mouse strains. The ST/bJ and RI/NJ/J mice have intermediate liver BZ N-acetyltransferase activity, despite the fact they have high blood PABA activity; the SF strain has high blood PABA and blood BZ activities, yet has a low liver PABA activity; and the X/Gf strain, which has a low liver-like activity, like the “slow” acetylator A/J and AHe/J strains, for both blood PABA and BZ N-acetyltransferase, exhibits intermediate liver BZ activity. Thus, there are both tissue-specific and substrate-specific differences between strains in the expression of N-acetyltransferase activity.

**Inheritance of N-Acetyltransferase Activity in A/J and C57BL/6J Mice**—Mice of the A/J (slow acetylator) and C57BL/6J (rapid acetylator) strains were chosen for the analysis of inheritance of N-acetyltransferase. AF, an alternate substrate for BZ, was used for the inheritance studies. Assays of N-acetyltransferase with AF in tissue preparations from the two strains revealed no differences from BZ with respect to N-acetylation. All animals were phenotyped for PABA and AF activity in blood; several days later they were killed and liver N-acetyltransferase determinations were performed using the same two substrates. Thus, the same mice are represented in each of Figs. 1-4.

In Fig. 1 shows the segregation of the N-acetylation capacity for PABA in blood of A/J, C57BL/6J, AC57F1, AC57F2, and backcross animals. There is ~16-fold difference between the A/J (mean 100 pmol/min/mg) and C57BL/6J (mean 1660 pmol/min/mg) parental strains, and no overlap between the two. The mean blood PABA N-acetyltransferase activity in AC57F1 hybrids is 1160 pmol/min/mg which is clearly intermediate (the calculated value is 880 pmol/min/mg) (see “Experimental Procedures”), but nearer the activity of the
was determined by the radioassay at "Experimental Procedures." Aminofluorene N-acetyltransferase activity is again difficult. In addition to overlap between the "rapid" and "slow" acetylators among AC57F2 and backcross animals, there is considerable overlap between the F1 hybrids and the C57BL/6J parental strain. Identification of the phenotypically "slow" acetylators among AC57F2 and backcross animals presented no problem as this phenotype is clearly distinct from the others. However, overlap between the intermediate and "rapid" phenotypes makes definitive assignment of some animals uncertain. The 46 animals in the AC57F2 generation segregate into three classes with a ratio of 1 low: intermediate: high of 10:22:1, which obviously deviates from the expected 1:2:1 segregation of phenotypes in the C57BL/6J parental strain. Identification of the phenotypically "rapid" phenotype for some animals clearly deviates from the expected 1:1 ratio (C57 × AC57F1: χ² = 8.1, p < 0.5; A × AC57F1: χ² = 0, p < 0.5), and are thus not significantly different from that which is expected.

Fig. 2 shows the segregation of N-acetylation capacity for AF in blood of A/J, C57BL/6J, AC57F1, AC57F2, and backcross animals. A pattern similar to that seen for blood PABA is observed. The AF N-acetyltransferase activity in A/J mice is, without exception, nondetectable by the conditions of the assay. C57BL/6J mice have a mean activity of 110 pmol/min/mg which is consistent with additive inheritance (the calculated value is 697 pmol/min/mg) (see "Experimental Procedures"). However, there is some overlap...
between the intermediate type and both the low and high parental types which makes classification of individuals in the backcross generations of respectively. Initial velocities were calculated by extrapolating the time-activity curves back to time zero. Dotted lines were drawn as described under "Experimental Procedures."  

Liver and blood p-aminobenzonic acid N-acetyltransferase activity in A/J, C57BL/6J, AC57F1, AC57F2, and backcross animals. Liver cytosol, prepared as described under "Experimental Procedures," was diluted 2-fold. Aminofluorene N-acetyltransferase activity was determined by the radioassay at 37°C for up to 6 min. Final concentrations of CoASAc and AF were 0.5 and 0.25 mM, respectively. Initial velocities were calculated by extrapolating the time-activity curves back to time zero. Dotted lines were drawn as described under "Experimental Procedures."  

Thus, the distributions of phenotypes in the F1, F2, and backcross generations of A/J and C57BL/6J mice are consistent with the hypothesis that AF N-acetyltransferase activity in liver and blood and PABA N-acetyltransferase activity in blood are all controlled by a single major gene. The fact that in some instances, the observed ratios deviate from that which is expected provides evidence for the existence of modifying influences.  

Inheritance of N-Acetyltransferase Activity in A/J and C57BL/6J Recombinant Inbred Strains—Table II summarizes N-acetyltransferase activities for PABA in liver and blood of A/J, C57BL/6J, and AC57F1 mice, as well as 11 RI strains derived by inbreeding the F2 generation of A/J and C57BL/6J crosses. The RI strain distribution patterns can be divided into two principal classes with respect to blood PABA N-acetyltransferase. Five strains (BXA-1, AXB-2, -5, -6, and -17) have high activity resembling the C57BL/6J progenitor, while the other six strains have low activity resembling the A/J progenitor. These results provide additional evidence for the single gene inheritance hypothesis of blood PABA N-acetyltransferase. The pattern of liver PABA N-acetyltransferase is inconsistent with additive inheritance, although A/J animals have a slightly lower value (18.8 nmol/min/mg) than do C57BL/6J animals (22.6 nmol/min/mg). However, the RI strains that resemble the A/J parent for blood PABA N-acetyltransferase activity (BXA-3, -6, -15, AXB-3, -4 and -9) appear to have ~2-fold lower liver activity for this substrate than the RI strains that resemble the C57BL/6J parent, with the exception of RI strain BXA-3. Thus, there may be modifying genes operating in A/J mice that enhance liver PABA N-acetyltransferase activity.  

Table III summarizes AF N-acetyltransferase activities in liver and blood of A/J, C57BL/6J, and AC57F1 mice as well as 11 RI strains. For both tissues, the RI strains can be divided into two distinct classes. Five strains (BXA-1, AXB-2, -5, -6, and -17) have high AF activity resembling the C57BL/6J  

![Diagram](https://via.placeholder.com/150)  

**Fig. 4. Inheritance of liver aminofluorene N-acetyltransferase activity in A/J, C57BL/6J, AC57F1, AC57F2, and backcross animals.** Liver cytosol, prepared as described under "Experimental Procedures," was diluted 2-fold. Aminofluorene N-acetyltransferase activity was determined by the radioassay at 37°C for up to 6 min. Final concentrations of CoASAc and AF were 0.5 and 0.25 mM, respectively. Initial velocities were calculated by extrapolating the time-activity curves back to time zero. Dotted lines were drawn as described under "Experimental Procedures."
parent, while the other six strains have low activities for this substrate resembling the A/J parent. The strain distribution pattern is identical to that seen for blood PABA N-acetyltransferase activity (Table II). Thus, the probability is high that there is a significant relationship between the expression of N-acetylation capacity in these two substrates in both liver and blood. These results also provide evidence for the existence of modifying genes. The liver AF N-acetyltransferase activity in the AXB-3 strain (0.059 and 0.061 nmol/min/mg), is approximately one-half that of the A/J parent (0.134 nmol/min/mg), although it is still of the low type (Table III). As in the case for liver PABA activity (Table II), A/J mice may possess a modifying gene which acts to enhance this activity. The C57BL/6J-like RI strains (BXA-1, AXB-5, -6, and -17) possess higher blood AF N-acetyltransferase activities (0.162 to 0.173 nmol/min/mg) than the C57BL/6J progenitors, although none are as high as that seen in the AXB-3 strain. This may provide evidence for the existence of modifying genes operating in the C57BL/6J mouse which lowers its AF activity in blood.

**DISCUSSION**

The concept that N-acetylation may be capable of modulating the susceptibility to carcinogenesis is supported by a number of observations. Dogs develop tumors of both liver and bladder upon administration of acetylated aromatic amines, but only urinary bladder tumors when the unacetylated parent compound is administered (Poirier et al., 1963). Oral administration of 4-aminobiphenyl leads to primarily urinary bladder tumors (Walpole et al., 1954; Deichmann et al., 1958) while administration of 4-acetylaminobiphenyl leads to tumors of both liver and bladder (Jabara, 1963). In contrast, oral administration of 2-acetyl-naphthylamine fails to induce urinary bladder tumors (Conzemian and Flanders, 1972), even though the unacylated parent compound 2-naphthylamine is a well established canine urinary bladder carcinogen (Deichmann and Radomski, 1963). Thus, it is possible that the capacity for N-acetylation may play a role in the susceptibility to aromatic amine-induced carcinogenesis.

Previous studies of N-acetylation of carcinogenic aromatic amines have dealt with the capacity of a species to perform this reaction, but comparatively little attention has been paid to host factors affecting individual variability of this metabolic pathway in any species. One major goal in surveying inbred mouse strains was to detect intraspecies variation in N-acetylation capacity which could be developed into new genetic models of the human acetylator polymorphism. These models may facilitate investigations of genetic differences in susceptibility to aromatic amine-induced carcinogenesis in animals and perhaps in man. They may also be useful in studies of genetic differences in aryamine drug metabolism and related toxicities.

A survey of 20 inbred strains of mice (Table I) indicates that high N-acetyltransferase activity is more prevalent than low N-acetyltransferase activity by a ratio of 6:1. Two of the low strains (A/J and AHe/J) have a common origin. The X/Gf mice are known for their low susceptibility to a number of potent carcinogenic agents, including the aromatic amine acetylaminofluorene (Goldfeder, 1974), and it would be interesting to learn more about other possible biochemical similarities of X/Gf to the A/J and AHe/J strains.

It is clear from Table I that there is a significant correlation between blood PABA and blood BZ activities (p < 0.001). Distributions of these activities suggest the presence of two acetylator phenotypes. This is especially noteworthy as the A/J, AHe/J, and X/Gf strains stand alone in having no blood BZ activity, and in having blood PABA activity which is at least 10-fold less than that seen in the other strains. The occurrence of genetically controlled differences in blood PABA N-acetyltransferase activity has been demonstrated in rapid and slow acetylator rabbits (Szabadi et al., 1978).

Liver N-acetyltransferase activity for SMZ, the prototype polymorphic substrate in both rabbit and man, was not detectable in mice and thus, no interstrain variation was observed. The level of liver N-acetyltransferase activity as measured by either BZ or PABA is quite different from that of SMZ. Liver BZ N-acetyltransferase activity is bimodally distributed with A/J and AHe/J animals exhibiting an ~8-fold lower activity than the lowest of the rapid acetylator strains. It is interesting to note that the differences in SMZ N-acetylation capacity between rapid and slow human liver samples are approximately the same (Glowinski et al., 1978). The pattern of liver PABA N-acetyltransferase activity is similar, although the differences are 2-fold or less depending on the strains compared.

Liver BZ N-acetylation appears to correlate well with blood N-acetyltransferase activity for either PABA or BZ, with some exceptions. Such strains as ST/bJ and RI/J/23, which have approximately one-half the liver BZ activity of the other rapid acetylator strains, or as the X/Gf strain which has ~4 times the liver BZ activity of the other slow acetylator strains, may prove useful in studies of variant forms of the N-acetyltransferase molecule.

The results shown in Table I are in complete agreement with those of Tannen and Weber (1979), although only four strains of inbred mice were surveyed using SMZ and PABA in that study. Their values for liver PABA N-acetyltransferase are slightly lower which may be due to the fact that the enzyme preparation used for assay was a 30,000 x g homogenate in contrast to the 105,000 x g cytosol used for the studies reported here.

The data on the inheritance of N-acetyltransferase in A/J and C57BL/6J mice presented in Figs. 1-4 shows that F1 are intermediate, F2 are divisible into three classes corresponding to the two parental types and an intermediate type, and backcross animals fall into the parental and intermediate types. This is consistent with the conclusion that a single major gene with two alleles is responsible for this activity in blood (AF and PABA) and liver (AF). The gene symbol Nat has been proposed for this variation with Nat" as the common rapid acetylator allele and Nat* as the slow acetylator allele.3 The data in Fig. 1 on the inheritance of blood PABA activity is in agreement with that reported by Tannen and Weber (1980a). The data in the RI strains (Tables II and III) provide further support for the hypothesis of single gene inheritance.

The 2-fold variation in N-acetyltransferase activity in either blood or liver of the C57BL/6J parental types, irrespective of substrate, must be due to environmental influences or the presence of modifying genes. The RI strain data (Tables II and III) indicate that an appreciable part of this variation may be attributable to the presence of modifier genes and that these genes segregate independently of the major Nat gene. While liver PABA N-acetyltransferase appears unimodal in all offspring of the appropriate crosses (Fig. 9), data in the RI strains (Table II) reveals the existence of two groups of animals. RI strains BXA-3, -6, -15, and AXB-3, -4, and -9, which can be classified as A/J types from their blood PABA N-acetyltransferase data, all exhibit liver PABA N-acetyltransferase activity ~2-fold lower than that seen in the C57BL/6J-like strains. This is consistent with the presence of a modifier in A/J mice, increasing the apparent liver PABA N-acetyltransferase activity, which is not present in these RI strains.

3 Mouse News Letter (1981) Number 64 (February), p. 71.
strains. The data in Tables II and III also support the presence of modifier genes in the C57BL/6J parental types, but their effect is in the opposite direction of that seen in A/J mice.

The concept of repeatedly passing a selected gene from one inbred strain into the genome of another strain has recently been reviewed (Nebert, 1980). The ultimate congenic "inbred line" (developed after 120 generations) will have the selected gene "fused" and theoretically in more than 99.99% genome of the host background strain. Placement of the N-acetyltransferase-C57BL/6J allele into the low acetylator strain and placement of the N-acetyltransferase-A/J allele into the high acetylator strain would clearly be of interest with respect to the question of modifier genes. The development of these lines within this laboratory is now in progress. They may provide an improved means to evaluate the effect of differences in acetylator phenotype on aromatic amine-induced tumorigenesis, and also to study the possible effect of genetic modifiers on this process.

Acknowledgments—We would like to express our sincere appreciation to Oksana Lockridge for valuable comments on the manuscript and to Jeannie Brewer for technical assistance.

REFERENCES

Braiton, A. C., and Marshall, E. K., Jr. (1939) J. Biol. Chem. 128, 547-550

Conzelman, G. M., and Flanders, L. E. (1972) Proc. West Pharmacol. Soc. 15, 96-99

Deichmann, W. B., Radomski, J., Anderson, W. A. D., Coplan, M., and Woods, P. (1958) Ind. Med. Surg. 27, 25-26

Deichmann, W. B., and Radomski, J. L. (1963) Ind. Med. Surg. 32, 161-166

Drayer, D. E., and Reidenberg, M. M. (1977) Clin. Pharmacol. Ther. 22, 251-258

DuSouich, P., and Erill, S. (1976) Eur. J. Clin. Pharmacol. 10, 283-287

Glowinski, I. B., Radtke, H. E., and Weber, W. W. (1978) Mol. Pharmacol. 14, 940-949

Glowinski, I. B., Weber, W. W., FYsh, J. M., Vaught, J. B., and King, C. M. (1980) J. Biol. Chem. 255, 7883-7890

Goldfeder, A. (1974) Trans. N. Y. Acad. Sci. 36, 59-77

Hearse, D. J., and Weber, W. W. (1973) Biochem. J. 132, 519-526

Jabara, A. G. (1963) Cancer Res. 23, 921-927

Lottikar, P. D., and Laha, L. (1971) Biochem. J. 123, 287-299

Lower, G. M., and Bryan, G. T. (1973) Biochem. Pharmacol. 22, 1581-1588

Lower, G. M., Nilsson, T., Nelson, C. E., Wolf, H., Gamsky, T. E., and Bryan, G. T. (1979) Environ. Health Perspect. 29, 71-79

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275

Miller, E. C. (1978) Cancer Res. 38, 1479-1496

Miller, J. A. (1970) Cancer Res. 30, 559-576

Morton, K. C., King, C. M., and Baetcke, K. P. (1979) Cancer Res. 39, 3107-3113

Nebert, D. W. (1980) J. Natl. Cancer Inst. 64, 1797-1798

Szabadi, R. R., McQueen, C. A., Drummond, G. S., and Weber, W. W. (1978) Drug Metab. Dispos. 6, 16-20

Tannen, R. H., and Weber, W. W. (1979) Drug Metab. Dispos. 7, 274-279

Tannen, R. H., and Weber, W. W. (1980a) J. Pharmacol. Exp. Ther. 213, 480-484

Tannen, R. H., and Weber, W. W. (1980b) J. Pharmacol. Exp. Ther. 213, 485-490

Taylor, B. A. (1976) Genetics 83, 373-377

Walpole, A. L., Williams, M. H. C., and Roberts, D. C. (1954) Br. J. Ind. Med. 11, 105-109

Warburg, D., and Christian, W. (1941) Biochem. Z. 310, 384-421

Weber, W. W. (1973) in Metabolic Conjugation and Metabolic Hydrolysis (Fishman, W. H., ed) Vol. 3, pp. 250-294, Academic Press, New York

Weber, W. W., Michel, J. N., Hearse, D. J., and Drummond, G. S. (1976) Drug Metab. Dispos. 4, 94-101

Williams, R. T. (1969) in Detoxication Mechanisms, vol. 2, pp. 428-471, John Wiley & Sons, New York
Genetic regulation of aromatic amine N-acetylation in inbred mice.
I B Glowinski and W W Weber

J. Biol. Chem. 1982, 257:1424-1430.

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