dosage of Diamox, though still evident with the low dosage. The response to Diamox suggests, although it does not prove, that the light-induced buphthalmos in domestic fowl is associated with excessive intraocular pressure.

*Summary.* Neither miotics nor vision occluders corrected the buphthalmos seen in chicks reared under continuous light, but Diamox caused a marked decrease in eye weight, proportional to dosage.

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## Studies on the Metabolism of Isoniazid in Subhuman Primates.* (30594)

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Several studies have provided convincing evidence that the genetic polymorphism for isoniazid (INH) inactivation existing in human populations(1,2) is due to inherent capacities of human subjects to acetylate this drug to the metabolite, acetylisoniazid (AcINH)(3,4,5). Furthermore, a direct correlation between an individual's capacity to acetylate this drug, sulfamethazine (SMZ) (3,6) and hydralazine(3) was demonstrated. Unexpectedly, human subjects, whether exhibiting high or low abilities to form acetyl metabolites of the above drugs, were found to acetylate sulfanilamide(6) or p-aminosalicylic acid(7) to very nearly the same extent. Studies with liver tissue obtained by biopsy demonstrated a direct correlation between *in vitro* and *in vivo* capacities to acetylate the hydrazine derivatives and SMZ(3). However, the liver preparations were not active for converting sulfanilamide or p-aminobenzoic acid to acetyl metabolites(3). These studies have suggested that in human subjects different enzymes or tissues must be responsible for acetylating the group of compounds represented by the hydrazine derivatives and SMZ and the group comprising sulfanilamide, p-aminosalicylic, and p-aminobenzoic acids. Mechanistic studies to elucidate the bases for these observations would be greatly facilitated if an animal species could be found that exhibits a genetic polymorphism for acetylating substituted hydrazine derivatives such as INH or hydralazine, or aryl amines such as SMZ. Frymoyer and Jacox(8) reported a genetic polymorphism in rabbits for sulfadiazine acetylation. However, no clear polymorphism for acetylation was observed when INH was administered. Before their reports appeared, we had initiated studies with subhuman primates to find a species that exhibited varying capacities to acetylate INH like man.

Earlier studies, wherein plasma levels of INH and AcINH were measured in 6 rhesus monkeys shortly after intravenous or intramuscular administration of 5 or 20 mg INH/kg, suggested that these animals did not exhibit greatly divergent capacities to acetylate this drug(9). Later, 24-hour excretions of INH were determined following intramuscular injection of 10 mg INH/kg twice a day to 3 rhesus monkeys(10). These animals differed consistently in the amounts of unchanged drug eliminated in the urine. Thus, on 7 separate days of treatment, one monkey excreted between 10.4 and 12.9%, another between 8.0 and 9.5%, and the third between 5.4 and 7.0% of the daily dose. These results suggested that urinary excretion studies are more likely to demonstrate differences in inactivation capacities than determination of plasma levels of the drug.

This report describes investigations on the capabilities of rhesus and cynomolgus mon-

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keys, and mangabeys to metabolize INH. To relate these results to previous studies with human subjects, acetylation of SMZ also was investigated in some of the same rhesus monkeys. Urinary excretions of parent drug and metabolites were determined.

*Materials and methods.* INH and SMZ were commercial preparations that were purified as described previously(6,9). We used 17 rhesus monkeys (*Macaca mulatta*), weighing 3.0 to 6.1 kg; 12 cynomolgus monkeys (*Macaca cynomolgus*),‡ weighing 2.1 to 3.1 kg; and 12 mangabeys (*Cercocebus fulliginosus*), weighing 2.8 to 5.1 kg. They were housed in gang-cages and were given adequate water and food. All animals were free of tuberculous and malarial infections. Their ages cannot be stated with certainty since all had been imported from outside sources. For the metabolic studies, the animals were maintained singly in metabolism cages.§ The 50 ml of water provided 4 times daily was almost always consumed within 5 minutes. Access to food‖ was allowed for 30 minutes twice daily. This schedule was begun 1 day before treatment and control urine was collected from 0 to 12 hours and 12 to 24 hours in iced flasks containing 1 ml of toluene as a preservative. At zero hour on the next 3 days, the monkeys received intramuscular injections of an aqueous INH solution (40 mg/ml) to yield doses of 20 mg/kg. Food and water were provided and urine was collected as described. In the first group of 6 animals tested, urine was collected 24 to 36 hours after the third treatment with INH. Later, 6 of the rhesus monkeys were injected intramuscularly with appropriate volumes of a solution of SMZ (81.2 mg/ml of 0.3 N NaOH) to provide doses of 40.6 mg/kg (equimolar to 20 mg INH/kg). Control and treatment urines after SMZ were obtained as before. All urine collections were measured and appropriate volumes were stored frozen when analyses could not be performed immediately. We selected the 20 mg INH/kg dose because it is approximately equivalent to a dose of 10 mg/kg to human subjects when either dosage is expressed as mg/kg of "metabolically active mass," *i.e.*, mg/kg$^{0.7}$, in the respective species(11). The 10 mg/kg dose had provided an easily discernible separation of human subjects into acetylation classes(6).

Since this was a survey-type study, urinary analyses for INH, acid-labile INH and AcINH were performed by the rapid techniques of Hughes(12) rather than the newer, but slower, ion-exclusion method(13). The latter provides greater accuracy for determining INH and measures the contributions of individual INH hydrazones to the acid-labile INH fraction of metabolites. However, these advantages were counterbalanced by the large number of samples to be analyzed in this study. Total metabolites of INH, *i.e.*, all derivatives hydrolyzable to isonicotinic acid by strong acid, were determined as described previously(14). The difference between the total metabolites found and the total of all hydrazine-containing derivatives (INH, acid-labile INH, and AcINH) defines the amount of isonicotinic acid and its conjugates present in urine. For this study, such calculations were not thought to be important. Total metabolites were determined primarily to ascertain the completeness of excretion of administered INH. Urinary concentrations of SMZ and its acetyl metabolite were determined by published techniques(15). The results were expressed as percentage of the amount of drug given.

*Results.* Table I shows the excretion of INH and its metabolites by 17 rhesus monkeys. Since analyses of the two 12-hour urine collections showed only that most of the dose was excreted during the first period, the 24-hour values were derived by totaling the analyses of the two 12-hour urine collections. In the first group of 6 animals studied, the series of 3 successive treatments was repeated 1 month later to test the reproducibility and validity of the observations. Individual variations were no greater after 6 treatments than after 3. Therefore, data obtained from 3 treatments were used thereafter to determine the metabolic characteristics of individual animals. Since no more than

‡ Other designations used for this species are *Macaca irus philippinensis* or *Macaca philippinesis*.

§ Hoeltge, Inc., Cincinnati, Ohio. A rack holding 6 metabolism cages was employed.

‖ Purina Monkey Chow.

TABLE I. Urinary Excretion During 24 Hours of INH and Its Metabolites by Rhesus Monkeys Receiving INH (20 mg/kg) Intramuscularly.

| Monkey | No. of treatments | Mean % (± S.D.) of dose excreted as INH | Acid-labile INH | AcINH | Total metabolites |
|---|---|---|---|---|---|
| 1696 ♀ | 6 | 15.2 ± 2.8 | 36.0 ± 7.3 | 25.6 ±12.4 | 89.1 ±10.3 |
| 1499 ♂ | " | 8.1 ± .8 | 14.5 ± 3.2 | 61.8 ± 2.8 | 95.3 ± 4.4 |
| 1848 ♂ | 3 | 6.7 ± 3.0 | 18.7 ± 2.0 | 56.0 ± 1.5 | 88.4 ± 3.7 |
| 1815 ♀ | " | 5.9 ± .5 | 14.8 ± .7 | 55.3 ± 4.0 | 83.8 ± 2.0 |
| 1205 ♂ | " | 7.1 ± .4 | 12.2 ± 1.8 | 68.4 ± 3.5 | 91.2 ± 2.0 |
| 1699 ♀ | " | 7.0 ± .8 | 13.0 ± 2.8 | 67.5 ± 2.9 | 88.9 ± .5 |
| 1809 ♂ | " | 6.1 ± 1.2 | 8.0 ± 6.2 | 65.1 ± 6.3 | 84.8 ± 2.9 |
| 1497 ♂ | " | 5.8 ± .1 | 14.4 ± 4.2 | 66.3 ± 3.8 | 88.4 ± 2.4 |
| 1700 ♂ | " | 4.7 ± 3.2 | 14.9 ± 2.2 | 55.0 ± 2.3 | 84.7 ± 2.9 |
| 1817 ♀ | " | 4.5 ± .3 | 16.0 ± 3.5 | 52.7 ±10.3 | 86.9 ± 7.9 |
| 1703 ♂ | 6 | 4.6 ± 1.5 | 9.9 ± 2.7 | 52.2 ±10.2 | 82.6 ±10.4 |
| 1875 ♀ | " | 5.2 ± 1.3 | 13.1 ± 2.2 | 62.8 ± 3.6 | 90.7 ± 4.2 |
| 1842 ♂ | 3 | 5.0 ± 1.5 | 14.0 ± 3.0 | 61.3 ± .5 | 89.6 ± 4.4 |
| 1632 ♀ | 6 | 5.2 ± 1.0 | 12.7 ± 1.6 | 68.8 ± 8.0 | 94.4 ± 9.8 |
| 1851 ♂ | " | 4.8 ± .9 | 10.5 ± 3.5 | 71.0 ± 7.5 | 95.6 ± 4.1 |
| 1868 ♀ | 3 | 4.0 ± 1.5 | 10.3 ± .3 | 64.1 ± 6.7 | 88.7 ± 6.4 |
| 1273 ♂ | " | 3.8 ± 1.6 | 11.1 ± 4.7 | 63.8 ± 4.3 | 88.0 ± 2.2 |

2% of the daily dose was detected as total metabolites in urine collected 24 to 36 hours after the third treatment with drug, 24-hour values were used. No effect of earlier administration on the metabolic patterns found on later days was noted in any animal. Excretion of total metabolites of INH was almost the same for all rhesus monkeys. Nevertheless, they varied as much as 4-fold in INH excreted with somewhat lesser variations in acid-labile INH derivatives and AcINH. The animals are arranged in the Table by increasing ratio of AcINH to INH. This ratio, when used simply as a whole number, defines acetylation capacity; it has been used to advantage in studies with human subjects(6). With the rhesus monkeys, the acetylation capacity ranged from 1.7 (monkey 1696) to 16.8 (monkey 1273); it was not related to sex or weight. A plot of the frequency distribution of these values (lower third of Fig. 1), did not indicate a modality, which would be expected if the animals were polymorphic for INH acetylation.

Six of these monkeys were also given SMZ to determine their capacities to acetylate this drug. The 24-hour excretion of SMZ and its acetyl conjugate are shown in Table II. All animals except 1696 showed relatively high abilities to acetylate this drug. Monkey 1696 did not acetylate either SMZ or INH readily. However, comparison of the acetylation capacities for SMZ and INH in all 6 monkeys reveals no correlation. Thus, in contrast to human subjects, these rhesus monkeys failed to exhibit either a polymorphism for INH acetylation (Fig. 1), or a parallelism between acetylation of this drug and SMZ.

Similar studies on the metabolism of INH in cynomolgus monkeys provided the data of Table III. Again, large differences (nearly 4-fold) were observed in excretion of the parent drug, with somewhat smaller differences in excretion of acid-labile INH and AcINH. Total metabolites of INH excreted did not vary extensively. The animals ranged in acetylation capacity from 2.5 for monkey 36 to 19.5 for monkey 41. These capacities were not related to sex or weight. The fre-

TABLE II. Urinary Excretion During 24 Hours of SMZ and AcSMZ by Rhesus Monkeys Receiving SMZ (40.6 mg/kg) Intramuscularly. Comparison of SMZ and INH acetylation capacities.

| Monkey | Mean % (± S.D.)* of dose excreted as SMZ | AcSMZ | Acetylation capacity† after SMZ | INH‡ |
|---|---|---|---|---|
| 1696 ♀ | 43.2 ± 6.3 | 47.8 ± 9.1 | 1.1 | 1.7 |
| 1499 ♂ | 26.8 ± 3.2 | 61.0 ± 4.8 | 2.3 | 7.6 |
| 1703 ♂ | 21.1 ± 4.9 | 41.7 ±14.9 | 2.0 | 11.9 |
| 1875 ♀ | 29.7 ± 2.7 | 51.3 ± 5.6 | 1.7 | 12.1 |
| 1632 ♀ | 22.4 ± 5.9 | 64.4 ± 9.8 | 2.9 | 13.2 |
| 1851 ♂ | 29.1 ± 5.1 | 57.6 ± 4.3 | 2.0 | 14.8 |

* Each value is mean of 6 treatments.
† Acetylation capacity = % acetylated drug/% unchanged drug.
‡ Calculated from data of Table I.

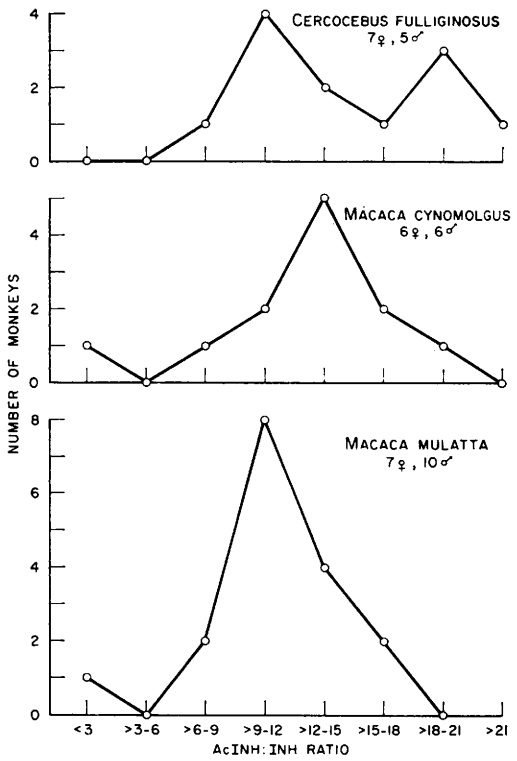


FIG. 1. Frequency distribution of INH acetylation capacities in subhuman primates.

quency distribution of these values (middle third of Fig. 1) was essentially unimodal. Thus, these cynomolgus monkeys did not exhibit a polymorphism for INH acetylation.

Table IV presents data for mangabeys, a species belonging to the same taxonomic family (*Cercopithecoidea*) as the macaques(16).

TABLE III. Urinary Excretion During 24 Hours of INH and Its Metabolites by Cynomolgus Monkeys Receiving INH (20 mg/kg) Intramuscularly.

| Monkey | Mean % (± S.D.)* of dose excreted as INH | Acid-labile INH | AcINH | Total metabolites |
|---|---|---|---|---|
| 36♂ | 13.3 ±2.0 | 21.7 ±4.1 | 33.8 ± 3.8 | 80.3 ± 8.1 |
| 29♂ | 8.3 ±1.1 | 12.7 ±1.0 | 62.6 ± 6.3 | 91.9 ± 5.0 |
| 21♀ | 5.3 ±1.0 | 12.4 ±1.6 | 62.9 ± 4.3 | 88.9 ± 5.1 |
| 19♂ | 5.0 ±1.1 | 8.7 ±1.3 | 59.9 ± 2.1 | 81.5 ± 1.4 |
| 33♂ | 4.5 ±1.0 | 9.2 ±3.0 | 56.4 ± 9.9 | 80.6 ±10.2 |
| 43♀ | 4.1 ±1.6 | 12.0 ±2.1 | 54.5 ±10.3 | 76.4 ±14.1 |
| 31♀ | 4.4 ± .6 | 10.8 ±1.0 | 59.6 ± 1.6 | 84.0 ± 8.3 |
| 2♀ | 4.6 ± .9 | 13.5 ±2.0 | 65.0 ± 4.5 | 86.9 ± 6.6 |
| 51♀ | 4.8 ± .7 | 12.7 ±3.4 | 70.3 ± 3.9 | 89.3 ± 1.7 |
| 46♀ | 4.6 ± .4 | 8.3 ±2.5 | 76.4 ± 8.6 | 90.3 ± 7.8 |
| 5♂ | 4.5 ± .4 | 7.6 ±1.1 | 74.5 ± 3.0 | 92.6 ± 1.6 |
| 41♂ | 3.6 ± .5 | 8.9 ± .3 | 70.3 ± 5.3 | 87.4 ± 4.4 |

* Each value is mean of 3 treatments.

The results were very similar to those obtained with the other monkeys, except that somewhat less parent, and more acetylated, drug was excreted. These changes were reflected in increased acetylation capacities, of from 8.5 to 33.3. Sex and weight were not determining factors. Plotting the frequency distribution of the acetylation capacities of these animals gave a bimodal curve (upper third of Fig. 1). Such a result suggests a polymorphism for INH acetylation comparable to that observed in man.

*Discussion.* Although these studies did not employ more accurate methods now available (13) to assess the acid-labile INH derivatives

TABLE IV. Urinary Excretion During 24 Hours of INH and Its Metabolites by Mangabeys Receiving INH (20 mg/kg) Intramuscularly.

| Monkey | Mean % (± S.D.)* of dose excreted as INH | Acid-labile INH | AcINH | Total metabolites |
|---|---|---|---|---|
| 33♀ | 6.7 ± .6 | 10.3 ±2.7 | 58.5 ± 1.2 | 96.8 ± 3.7 |
| 12♂ | 6.1 ±1.2 | 12.9 ±1.1 | 65.5 ± 3.8 | 92.3 ± 2.7 |
| 34♂ | 5.8 ± .6 | 11.3 ±1.3 | 60.4 ± 4.0 | 92.0 ± 3.3 |
| 32♀ | 5.5 ± .6 | 12.4 ± .4 | 62.4 ± 2.8 | 95.0 ± 1.3 |
| 13♀ | 4.8 ±1.3 | 11.1 ±2.6 | 55.5 ±10.9 | 78.3 ±19.3 |
| 24♀ | 5.1 ±1.1 | 9.9 ±1.4 | 68.6 ± 3.7 | 89.4 ± 3.2 |
| 38♂ | 4.9 ± .3 | 9.4 ± .7 | 70.8 ± 4.8 | 95.1 ± .9 |
| 20♀ | 4.2 ± .9 | 12.2 ±3.4 | 72.1 ± 3.8 | 95.0 ± 1.9 |
| 39♀ | 3.8 ± .1 | 10.0 ± .9 | 72.6 ± 2.6 | 90.4 ± 1.1 |
| 16♀ | 3.7 ± .7 | 8.8 ±1.2 | 72.7 ± 3.7 | 90.2 ± 3.8 |
| 29♂ | 3.7 ± .2 | 10.4 ±1.3 | 72.6 ± 1.3 | 91.3 ± 1.6 |
| 5♂ | 2.2 ± .6 | 6.4 ±1.1 | 73.3 ± 5.1 | 87.4 ± 5.4 |

* Each value is mean of 3 treatments.

or isonicotinic acid fraction of INH metabolites, we can tentatively conclude that the major differences between the metabolism of INH in monkeys and in man(5) are quantitative, not qualitative. The data suggest that monkeys, in general, are more capable of acetylating INH than man, but that human subjects exhibit a wider range of capacities than monkeys. The acetylation capabilities differed by as much as 16-fold between human subjects(6) and only 10-, 8-, and 4-fold in rhesus monkeys, cynomolgus monkeys, and mangabeys, respectively. The latter species, as a group, showed the greatest capacity to acetylate INH whether evaluated from the amounts of AcINH excreted (Table IV) or from the ratios of AcINH to INH (Fig. 1).

These results emphasize that widely different capacities to acetylate INH are ex-

hibited by individual members of the same simian species. It seems clear from the experimental design that these differences are based on stable individual characteristics. We can conclude that neither rhesus nor cynomolgus monkeys exhibit any modality of acetylation capacities; the finding that INH and SMZ acetylations are unrelated in rhesus monkeys supports this difference from the characteristic polymorphism of human populations. However, when the results with mangabeys were evaluated similarly, a clear bimodal distribution of acetylation capacities was obtained (Fig. 1). Unfortunately, these studies had to be concluded without testing the capabilities of this species to acetylate SMZ.

After our studies were terminated, a report appeared on INH metabolism in African green monkeys (*Cercopithecus aethiops*). Goedde *et al*(17) presented evidence for a possible polymorphism of INH inactivation based on plasma levels of the drug 3 hours after its administration. The work was not repeated to test the validity and reproducibility of the observations. However, the authors cited as supporting evidence *in vitro* experiments using 2 donor monkeys (one "rapid" and one "slow" inactivator) that demonstrated a direct correlation between plasma levels of INH and acetylation of INH by extracts of acetone powders derived from liver tissue. These *in vitro* observations are invaluable for clarifying the mechanism of INH acetylation, but fail to support a polymorphism of INH acetylation. Our studies have shown that widely divergent capacities to acetylate can exist in rhesus and cynomolgus monkeys without an apparent polymorphism. Obviously, more detailed research is needed before it can be concluded that a polymorphism for INH acetylation, corresponding to that in man, occurs in any other mammalian species.

*Summary.* Tests for a polymorphism of isoniazid acetylation were performed in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca cynomolgus*) monkeys and in mangabeys (*Cercocebus fulliginosus*) by studying the urinary excretion of isoniazid and its metabolites after intramuscular injection. In general, the patterns of metabolites excreted in urine of these species were qualitatively similar to those found previously in man. However, neither rhesus nor cynomolgus monkeys exhibited a polymorphism for isoniazid acetylation that is characteristic of human populations. Furthermore, rhesus monkeys showed no parallelism in capacities to acetylate isoniazid and sulfamethazine. Other results suggested that mangabeys exhibited a polymorphism for isoniazid acetylation.

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