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Research Article

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Rate of inactivation by obligatory heterozygotes was determined. Such heterozygotes could not be adequately differentiated from homozygous rapid inactivators.

The gene frequency for slow inactivation was 0.46 ±0.04 in Alaskan Eskimos and 0.62±0.06 in Athabaskan Indians.

The Discrimination of Phenotypes for Rate of Disappearance of Isonicotinoyl Hydrazide from Serum

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ABSTRACT Expression of the rate of inactivation of isonicotinoyl hydrazide (INH) as a first-order constant gave better discrimination of slow and rapid inactivators than did the level of INH at the end of 6 hr. The most probable division point between the two classes was at $k = 0.130$.

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INTRODUCTION

The rate of disappearance of isonicotinoyl hydrazide (INH, isoniazid) from the serum of human subjects is a polymorphic hereditary trait of clinical interest in treatment of tuberculosis, as well as of anthropologic significance. People can be classed as either fast or slow inactivators of INH, and slow inactivation has been shown to be an autosomal recessive trait (1). It has also been claimed that subjects can be classified into three groups—fast, intermediate, and slow inactivators—the intermediate group being heterozygotes (2, 3). Human ethnic groups vary in the proportion of fast inactivators. Some of the proportions reported are: U. S. whites, 0.42 (3), Japanese, 0.88 (2), and Canadian Eskimos 0.95 (4).

Using a sensitive fluorophotometric assay for INH, we have examined the following problems: (*a*) the methodology of determining rate of inactivation, (*b*) the degree of reliability of classification of individuals into phenotypes, and (*c*) the proportion of slow inactivators in Alaskan ethnic groups.

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METHODS

The persons studied were patients under treatment for tuberculosis at the Alaska Native Medical Center, Anchorage, plus a few Eskimo and Indian families. In most cases, INH was given orally in the amount of 4 mg/kg body weight to the nearest 25 mg. Samples of serum were obtained 2 and 6 hr later, and in some cases 4 hr later. INH was determined fluorophotometrically (5). A second method was evaluated, where INH was given intramuscularly in the amount of 2 mg/kg body weight to the nearest 10 mg, and INH measured after 0.5 1.5, and 2.5 hr.

Rate of inactivation was calculated as though the process was first order:

$$k = \frac{1}{t} \log \frac{I_1}{I_2}$$

where k = rate constant of inactivation in reciprocal hours, t is time between samples in hours, and I_1 and I_2 are INH concentrations of the first and second samples.[1]

RESULTS

Intramuscular injection of INH. This method took less time and avoided possible differences in rate of absorption of the drug. The results in 87 persons are shown in Fig. 1 in terms of k calculated from levels 0.5 hr and 2.5 hr after injection. The distribution showed an antimode near 0.2. In 30 people, k was determined both after intramuscular injection (k_{1m}) and after oral ingestion (k_o) (Table I). The latter rate was calculated from levels at 2 and 6 hr after ingestion. The correlation coefficient of k_{1m} and k_o was 0.88.

[1] Strictly, $k = \frac{1}{t} \ln \frac{I_1}{I_2}$ in a first-order process. Since the application of this equation to disappearance of INH is empirical, the use of natural logarithms is of no significance.

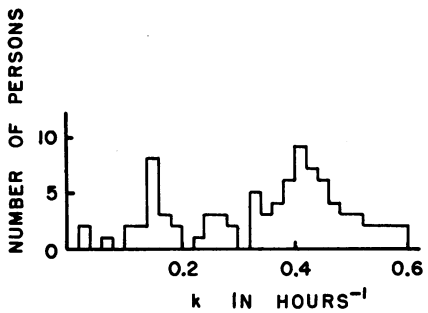

FIGURE 1 First-order rate constant for disappearance of of INH from serum of 87 persons. INH was given intramuscularly (2 mg/kg body wt) and serum level determined after ½ and 2½ hr.

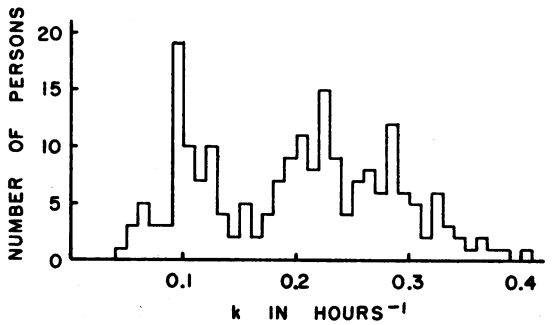

FIGURE 2 First-order rate constant for disappearance of INH from serum of 204 persons. INH was given orally (4 mg/kg body wt) and serum level determined after 2 and 6 hr.

A disadvantage of the intramuscular method was that the value of k fell rapidly during the 2 hr test period. In Table I, rates calculated from 0.5 to 1.5 hr ($k_{0.5-1.5}$) are compared with those from 1.5 to 2.5 hr ($k_{1.5-2.5}$). In 86 persons, the correlation coefficient of the two rates was 0.65. The inconstancy of k, plus the inconvenience of giving injections, appeared to outweigh possible advantages of the intramuscular method.

Oral ingestion of INH. In previous studies of inactivation, the results have nearly always been expressed in terms of concentration of INH remaining in serum 6 hr after ingestion. The log of this value is proportional to the rate constant, provided the inactivation is a first-order process and the initial concentration is the same. One study expressed results as half-life (6), based on the relationship: $t_{\frac{1}{2}} = 0.301/k$. Since the genetic difference in rate of inactivation is undoubtedly due to a difference in amount of enzyme activity, and the disappearance approximates a first-order reaction, the first-order reaction constant should be nearly proportional to amount of enzyme activity. We have therefore chosen k as a measure of rate.

The rates calculated between 2 and 6 hr after ingestion in 204 persons are shown in Fig. 2. The curve shows an antimode near 0.13. The corresponding levels at 6 hr are shown in Fig. 3. Levels previously suggested as distinguishing homozygous from heterozygous rapid inactivators (2, 3) did not correspond to an antimode. The correlation coefficient between k and the log of the 6 hr level was − 0.88 and the least squares equation was:

$$\log I_2 = -6.525k + 0.538$$

with a standard error of the slope of 0.25. The corresponding half-lives are shown in Fig. 4. The antimode is near 2 hr. The half-lives for slow inactivators were

TABLE I

Comparison of k for Two Methods of Administration at Different Times (mean and SD)

	Slow	Rapid
I.m. administration		
Number	22	64
$k_{0.5-1.5}$	0.163 ±0.088	0.453 ±0.115
$k_{1.5-2.5}$	0.122 ±0.039	0.379 ±0.099
Difference	0.041 ±0.083	0.074 ±0.134
Oral administration		
Number	17	52
k_{2-4}	0.088 ±0.018	0.266 ±0.078
k_{4-6}	0.113 ±0.032	0.258 ±0.056
Difference	−0.025 ±0.039	0.008 ±0.076
Oral and i.m.		
Number	6	24
k_{im}	0.113 ±0.058	0.406 ±0.087
k_o	0.103 ±0.010	0.261 ±0.068
Difference	0.009 ±0.059	0.135 ±0.068

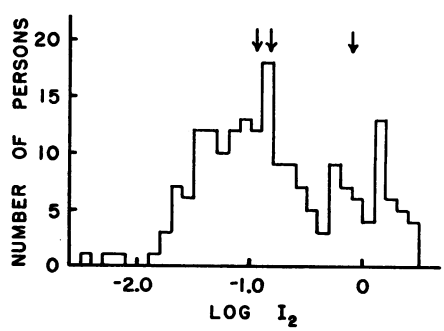

FIGURE 3 Level of INH in serum (I_2) in μg/ml 6 hr after oral INH (4 mg/kg body wt). Arrows indicate levels suggested (2, 3) for distinguishing phenotypes. The arrows on the left are levels suggested for distinguishing homozygous and heterozygous rapid inactivators; arrow on right for distinguishing slow and rapid inactivators.

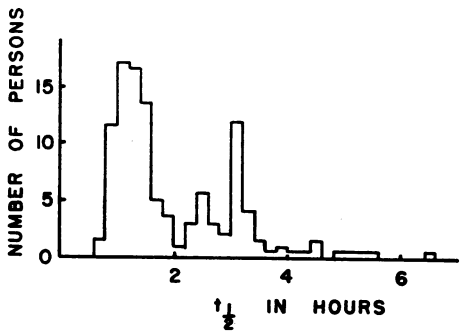

FIGURE 4 Half-life of disappearance of INH from serum of 204 persons after oral INH (4 mg/kg body wt).

spread widely into a skewed distribution while those of rapid inactivators were closely bunched.

To test the constancy of k, a comparison was made of the values calculated from the 2- and 4-hr levels (k_{2-4}) and those from the 4- and 6-hr levels (k_{4-6}) in 69 persons (Table I). The correlation coefficient was 0.73.

Inactivation of obligatory heterozygotes. Rate of inactivation was determined in several families and 13 persons selected from them who were both rapid inactivators and either children or parents of slow inactivators. The mean value of k for these obligatory heterozygotes was 0.217 with a standard deviation of 0.043. The mean level of INH at 6 hr was 0.11 μg/ml.

Ethnic groups. The results obtained in unrelated individuals from two ethnic groups are summarized in Table II. The gene frequency for slow inactivation is higher in Indians than in Eskimos.

DISCUSSION

Considering the complexity of events in the body after INH ingestion, which includes absorption, diffusion to and from tissues, excretion, enzymatic inactivation, and the nonenzymatic reactions of INH, all of which may be concentration dependent, one could hardly expect the disappearance of the drug to be a kinetic process with an integral order. The use of logarithmic disappearance rates is therefore empirical.

TABLE II

Fast and Slow Inactivators in Alaskan Ethnic Groups

	No. of fast	No. of slow	Gene frequency*
Eskimos, Southern	91	21	0.433 ±0.043
Northern	33	12	0.516 ±0.064
All	124	33	0.459 ±0.037
Indians, Athabaskan	29	18	0.618 ±0.057

* For slow inactivation.

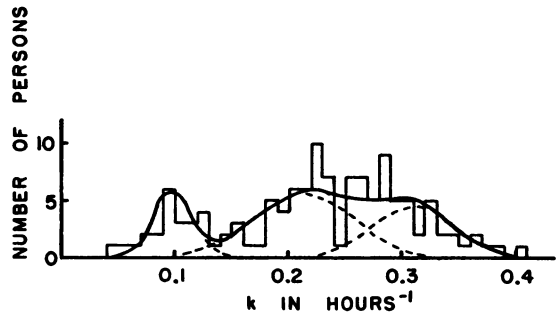

FIGURE 5 First-order rate constants for disappearance of INH in 125 Eskimos. The curves were drawn from the following values of n, $\bar{k}$, and σk: slow inactivators, 24, 0.098, 0.016; heterozygotes, 61.5, 0.217, 0.043; rapid homozygotes, 39.5, 0.310, 0.036.

The value of k appears to decline with time in the intramuscular test, and when the oral and intramuscular tests are compared. In the oral test, k is constant in rapid inactivators but increases with time in slow inactivators. The increase of k in slow inactivators was not due to a linear decrease in INH levels with time, however. Average levels found in 17 such persons were 3.91, 2.62, and 1.60 μg/ml at 2, 4, and 6 hr respectively.

In 63 slow inactivators, the mean value of k was 0.098 with a standard deviation of 0.016; the mean of I_2 at 6 hr was 0.96 μg/ml. Using the measured means and variances for slow inactivators and obligatory heterozygotes instead of the true values, the dividing point between heterozygotes and slow inactivators which appears to minimize the probability of misclassification is at $k =$ 0.130. The corresponding probable division point using 6-hr levels is 0.41 μg/ml.

Rate of inactivation was determined by the oral method on 125 Eskimos with the result shown in Fig. 5. 24 of these were slow inactivators, giving a gene frequency of 0.438. If the sample is genetically homogeneous, the Hardy-Weinberg law predicts 61.5 heterozygous inac-

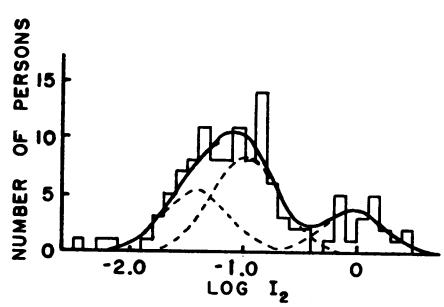

FIGURE 6 Levels of INH in serum after 6 hr in 120 Eskimos. The curves were drawn from the following values of n, $\overline{\log I_2}$, and σlog I_2; slow inactivators, 23, −0.02, 0.25; heterozygotes, 59, − 0.97, 0.28; rapid homozygotes, 38, − 1.41, 0.28.

TABLE III

Predicted Levels of INH in Serum after 6 hr

	Log I_2	Mean concentrations of INH (I_2)
		μg/ml
13 obligatory heterozygotes		
Found	−0.96 ±0.39	0.110
Calculated	−0.97 ±0.28	0.114
Homozygotes		
Calculated	−1.41 ±0.28	0.039

tivators and 39.5 homozygous rapid inactivators in the sample. Assuming that $n = 61.5$, $\bar{k} = 0.217$, and $\sigma k = 0.043$, a normal curve was drawn and the numbers of heterozygotes calculated for each value of k. These numbers were subtracted from the total number of rapid inactivators. The excess rapid inactivators, presumed to be homozygous, gave a mean value of k of 0.310 with a standard deviation of 0.036. From this, the most probable discrimination point between homozygous and heterozygous rapid inactivators was 0.268.

The corresponding 6-hr levels of these Eskimos are shown in Fig. 6. The mean level of all rapid inactivators in this group was 0.071 μg/ml. This level was not sufficiently different from the mean level of obligatory heterozygotes to allow a calculation of the mean of the presumed homozygotes as was done with k. However, from the least squares equation for 135 rapid inactivators:

$$\log I_2 = -4.78k + 0.07$$

the predicted values shown in Table III were calculated. From this, the probable level for discrimination was 0.065 μg/ml.

The practical usefulness of phenotypic classification can be expressed as the per cent of the total individuals who can be correctly classified with reasonable certainty. For most applications, at least 90% of the individuals should be so classified with 95% certainty. The summary in Table IV is based on a relatively small population, and the calculated values shown are only first approximations. The results indicate, however, that the first-order rate constant is more efficient in discrimination of phenotypes than is the 6 hr level, and that the discrimination between the two rapid phenotypes is inadequate by either method.

TABLE IV

Expected Per Cent of Persons Who Cannot Be Classified into Phenotypes with 95% Certainty

Method of calculation	Slow and rapid inactivators		Rapid inactivators	
	Slow	Rapid	Homozygous	Heterozygous
k	2.3	2.1	40.1	30.4
6 hr level	23.6	12.4	65.3	65.3

ACKNOWLEDGMENTS

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