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STUDIES ON ISONIAZID ACETYLATION AND POLYMORPHISIM IN HUMANS

by

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For their patience, love and support
this thesis is dedicated to members of
my family

This thesis by Mohammad Saleem is accepted in its present form by the Department of Biological Sciences as satisfying the thesis requirements for the degree of Master of Philosophy in Biochemistry/Molecular Biology.

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CONTENTS

ABSTRACT

Phyenotype frequencies of fast and slow acetylators of the drug isoniazid (INH) were determined in 157 subjects including normal (80) and tuberculosis (77) patients selected from twin cities of Rawalpindi and Islamabad. The drug, INH, was administered orally to the subjects. The plasma INH concentration was determined spectrophotometrically. The results show that INH is metabolized in humans at extremely variable rates. On this basis, two types of phenotypes have been identified: the slow acetylators with less than or equal to (2.5 µg INH/ml), and fast acetylators with more than (2.5 µg INH/ml). Of the 157 subjects investigated, 50 (31.8 percent) were found to be fast acetylators and 107 (68.2 percent) slow acetylators of INH.

An attempt was made to determine co-relation, if any, between various blood groups and acetylators of INH. The data obtained show non association between blood groups and acetylators ($P<0.05$). The importance of the findings is discussed with reference to the rational use of INH in tuberculosis treatment.

INTRODUCTION

INTRODUCTION

Tuberculosis remains a major health problem in all the developing countries. In some areas of Africa, Asia and Oceania the reported annual incidence of pulmonary tuberculosis is 200 to 350 cases per 100,000 inhabitants. Since innumerable number of cases remain undetected, the actual prevalence of the disease is suspected to be atleast twice as high. The number of infectious cases of tuberculosis in the world is estimated to be in the range of 15 to 20 millions (World Health Organization 1974). Approximately 80 percent of the world's population is located in the third world (Garrett, 1981). A large proportion of this population lives under conditions of poverty, inadequate medical care, poor sanitation and malnutrition. These factors are the primary cause for the spread of a number of communicable diseases, including tuberculosis (Kunin, 1985).

Tuberculosis is also a serious problem in many of the technologically advanced countries. In countries where it is considered rare, it usually causes more deaths than all other notifiable infectious diseases combined together. (World Health Organization 1974).

Wherever the problem of tuberculosis has been studied it has been shown that the incidence of this disease is low in persons under 20 years of age but rises with age and is highest in people over 50 years of age. (Benenson, 1975). However, numerous countries have shown a downward trend of mortality rates ranging from 5 to 100 deaths per 100,000 people per year. Both mortality as well as the morbidity rates are higher in males as compared to females and much higher in non-whites than in whites (Benenson, 1975).

Pakistan being a developing country is also confronted with the menace of tuberculosis. To assess the tuberculosis problem in the community two tuberculosis prevalence surveys have been conducted in the country. The first survey was carried out in 1960-1962. The second survey was conducted during 1974-1978 (Report of Tuberculosis Survey, 1978). According to the latter survey the prevalence of pulmonary tuberculosis in Pakistan was reported to be a lot higher compared to the third world average. However it follows the same pattern as that in the other parts of the world that is, age and sex seems to be co-related to the occurance of tuberculosis in the Pakistani population. The males are more vulnerable to tuberculosis which may be attributed to a wide contact amongst males for socio-economic commitments. The prevalence of pulmonary

in people according to the sputum positivity rate was found highest in the age group of 40 to 44 years both in males as well as in females. Sexwise the sputum positivity rate was higher in males. The incidence of the disease in the same age group of both urban and rural areas was found to be 14.75 and 6.35 percent of the population respectively. The National Tuberculosis Programme started in Pakistan in the early 70's has shown a great impact on the control of this disease. It seems that as a result of this programme the prevalence of pulmonary tuberculosis has been reduced from 4.6 percent (Survey 1961-62) to 2.9 percent (1974-1978) of the population. However this present 2.9 percent prevalence of pulmonary tuberculosis is still unacceptably high. Therefore further concerted efforts are required so that the incidence of the disease could be brought down to a negligible level (Report of Tuberculosis Survey, 1978).

Chemotherapeutic Aspect of Isoniazid

Since the introduction of effective chemotherapy, mortality rate has declined tremendously all over the world. Because of the wide application of chemotherapy and with the addition of new drugs the standards of

chemotherapy has improved, with the result that the level of primary drug resistance has stablized to a single drug. Furthermore, the response of the patients to standard triple drug chemotherapy is usually good (World Health Organization, 1974).

The main drugs used for the treatment of tuberculosis are isoniazid (INH), streptomycin (SM), Para amino salicylic acid (PAS) and thiacetazone. These drugs have been used to formulate highly effective and inexpensive regimens. More recently, rifampcin (RIF) and ethambutol have been introduced in Pakistan,as in other parts of the world.

The early stages of chemotherapy are crucial for the final outcome of the treatment, especially in patients who suffer from tuberculosis. The standard practice to treat tuberculosis is an initial course of triple drug chemotherapy (including isoniazid) for a period from 1 to 3 months followed by the two drugs including INH regimen (World Health Organization, 1974).

Isoniazid

Isoniazid (1-isonicotinyl hydrazide) was first

synthesized by Meyer and Mally in 1912. However, its chemotherapeutic value was not discovered until 40 years later. In 1952 Grunberg and Schnitzer showed that the compound when tested *in vitro*, was bacteriostatic against Mycobacterium tuberculosis H37RV. It protected mice from developing tuberculosis when the mice were injected with tubercle bacilli intraveneously. In the same year it was shown that the compound was effective in the treatment of human tuberculosis. These findings brought revolution inthe management of tuberculosis (Evans, *et al*. 1960 and Hinshaw, 1969). Since then the treatment of tuberculosis and chemoprophylaxis have been based on isoniazid either in monotherapy, or, in combination with other drugs and antibiotics. (Braun, *et al*. 1984). The drug isoniazid is effective against the intracellular and extra-cellular organism. (Neill and Musser 1969). The mode of action of isoniazid against Mycobacterium tuberculosis, essentially remains unclear. Nor is it clear why the drug is selectively active for tubercle bacilli (Freeman, 1985).

Metabolism of Isoniazid

The metabolism of isoniazid has been studied in animals as well as in humans. It has been demonstrated that isoniazid is rapidly and completely absorbed from

the gastro-intestinal tract soon after its ingestion leading to its initially high level in blood. However these high blood levels of INH do not persits for long. It is uniformly distributed in the blood plasma, spinal fluid, and such tissue as the brain, lungs, liver and spleen. INH is rapidly cleared from the blood by the liver and kidneys (Evans, et al. 1960 and Neill, et al. 1969). In the liver, like most other drugs, INH is enzymatically inactivated, while the kidney filter the drug without any change (Raghupati Sarma, et al. 1980). INH as well as its metabolite are thereafter excreted in the urine.

Studies conducted in monkeys show that as much as 90 percent of the ingested INH may appear in urine within 24 hours (Hughes, 1953). In the liver,INH is converted into its acetyl derivative by the enzyme acetyltransferase. (Eidus, et al. 1971). The major acetylated metabolite beeing 1-isonicotinyl-2-acetyl hydrazine which in monkeys and in humans may make up as much as 94 percent of the total metabolite (Hughes, 1953).

Toxicitity of Isoniazid

Persistant high levels of INH as well as its acetylated metabolite have shown to be toxic. The slow acetylators will have higher plasma concentration of the

parent drug and lower concentration of the acetylated metabolite.For this reason, the slow acetylator will be at a greater risk from the overdose toxic reaction of INH, and is likely to suffer from peripheral neuropathy (Devadatta, et al. 1960). Fast acetylators on the other hand will be at a greater risk from the adverse reaction of the acetylated metabolite and is likely to suffer from hepatitis (Mitchell, et al. 1975).

It may be emphasized that acetylation of INH is probably a significant reaction, as acetylation results in a striking loss in chemotherapeutic activity of the drug; the acetyl derivative of INH shows less than one five hundreth the activity of unacetylated INH in vitro against tubercle bacilli. However, the difference is reduced to one hundreth in vivo (Hughes, 1953).

Since triple drugs therapy is commonly used in the treatment of tuberculosis the toxic effects of INH are further exagerated. In rodents either pretreated with rifampcin and subsequently treated with INH or treated with RIF and INH together was reported to cause an earlier and more pronounced hepatitis, compared with the control animals treated with INH alone(Skakun, et al. 1985 and Noda, et al. 1983).

Slow and Fast Acetylators

The rates of acetylation of INH are genetically controled and exhibit a bimodal pattern. Based on the elimination of free INH from the host organism, individuals have been divided into groups of fast and slow acetylators (Eidus, et al. 1971). Studies have been conducted in humans to determine slow and fast acetylators (polymorphisim). The incidence of rapid acetylators among various ethnic groups has been reported as in 85 percent Japanese, 63 percent in Burmese (Ishizaki, et al. 1981 and Tunkyi, et al. 1970) and 89 percent Koreans and 39 percent in South Indians respectively (Wade, 1977). The fast acetylators have been further divided into intermediate acetylators and fast acetylators. For instance, a greater proportion of the Eskimos are fast acetylators and, the Canadian students are mainly slow acetylators of INH. However the intermediate only comprise about 1/3 of the both races (Eidus, et al. 1974). Among Chinese population three types of polymorphic individuals are found.The percentage of fast, slow and intermediate acetylators are 21.5, 25.7 and 52.4 respectively. (Yan et al. 1983). In French children the distribution of fast and slow acetylators are 44.5 and 55.5 percent respectively. This classification of acetylation polymorphisim is similar to that of adults. (Advenier, 1981). It is

evident from the above that there is a wide variations of acetylators polymorphisimexists between different ethnic groups all over the world.

Present Work

The introduction of INH as a component of anti tuberculosis drug regimen has become the corner stone of treatment of active tuberculosis. Time and experience has proved the efficacy of this drug as a potent, cheap and less toxic. Although persistently high level of INH in the blood may prove to be toxic, the concentration of INH in the dose range used for the treatment of tuberculosis is comparatively safe, side effects like nausea, skin rashes vomiting and other gastro-intestinal effects have not usually been reported. In contrast to other anti tuberculosis drugs commonly used inconjuction with INH for treatment of tuberculosis have been reported to produce certain side effect like nausea, vomiting, rarely hepatitis and anorexia.

As reviewed in the preceding sections that INH has been studied extensively for determining the acetylators polymorphisim among humans in different ethnic groups around the world. No specific work in this regard has so far been undertaken in Pakistan.

An attempt has been made in the present investigation to obtain base line data on the distribution of fast and slow acetylators (polymorphisim) in the local population. Also, effort has been made to explore the relationship if any, between genetic markers, (blood group polymorphisim) and slow and fast acetylators.

It is hoped that this study would generate interest among medical practioners for treating tuberculosis, and it will be possible for the practioners to assess the patient with respect to INH acetylation (polymorphisim) rate before commencing the treatment with INH.

MATERIALS AND METHODS

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Subjects

The sample of human subjects consisted of: (1) 80 males volunteers from the National Institute of Health, Islamabad, weighing 50-70 kg and ranging in age from 20-60 years, (2) 77 males from the Tuberculosis Centre, Government of Pakistan who were to be treated for pulmonary tuberculosis. They were registered as out door patients, they were in the age range of 17-60 years and weighed 40-55 kg. All the subjects were motivated to participate in the study and gave informed consent.

Dose of Isoniazid (INH)

Isoniazid was given orally in a dose of 10 mg/kg body weight using the commercial tablets containing 100 mg of INH.

Blood Sampling

Blood (10 ml) was collected asceptically from the antecubtial vein two hours after ingestion of the drug. Clotting was prevented by adding EDTA (Oser, 1965).

Preparation of Plasma

The blood was centrifuged at 3000 rpm for 15 minutes. The plasma was seperated immediately. The analysis of isoniazid was perferably conducted the same day or otherwise the plasma was stored over night at $-20^{o}C$ and analysed the next day.

Erythrocytes

Erythrocytes were used for blood group determination.

Drug and Chemicals

Isoniazid (4-pyridine-carboxylic acid hydrazide pharmaceutical grade) was obtained from VEB BERLIN-CHEMIE (Berlin). Isonex (Isoniazid) tables, a commercial preparation containing 100 mg. INH per tablet, was the product of Pfizer Laboratories Limited, Pakistan. Kit for the determination of blood groups, were obtained from American Dade, Division of American Hospital supply corporation, Miami, USA. All chemicals used in the stuty were of analytical grade.

Estimation of Isoniazid in Plasma

The spectrophotometric determination of the concentration of isoniazid in blood plasma was essentially based on the method of Braun et al.(1984). Reagents used were:

Zinc sulphate (5.5% solution w/v in distilled water); Barium hydroxide (4.5% solution w/v in distilled water); ammonium vandate (0.1% solution w/v in sulphuric acid); stock isoniazid standard (137 µg/ml in distilled water), and working isoniazid (13.7 µg/ml in distilled water).

Standardization of Isoniazid Estimation

0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard was placed in a screw cap tubes. The volume was brought to 3 ml with distilled water. After slight shaking, 1 ml of 5.5% Zinc sulphate was added, followed by 1 ml of 4.5% barium hydroxide. the tubes were stoppered and the precipitate was allowed to settle for 5 minutes, the contents of the tubes were filtered, & 2 ml of filterate was taken in a spectrophotometric cuvettes. To this was added 0.1% of ammonium vandate, followed by thorough mixing. Absorbance was measured at 430 mµ against a reagent blank.

For Determining INH in Plasma

1 ml of plasma was placed in a screw capped tube and the volume was made to 3 ml with distilled water. To this was added 1 ml of 5.5% zinc sulphate, followed by 1 ml of 4.5% barium hydroxide solution. The tubes were stoppered, and shaken vigorously. The precipitate was allowed to stand for 5 minutes and then filtered. The clear protein free filterate (2 ml) was placed in spectrophotometric cuvettes and 1.0 ml of 0.1% ammonium vandate was added. The contents were thoroughly mixed. Absorbance was measured at 430 mμ against the reagent blank. All the determinations were done in duplicate. The INH concentration of plasma were read directly from the standard curve.

Chemical Assessment of Quality of Drugh Isoniazid (Isonex) Tablets

The identification of the drug, determination of weight variation and disintegration time were carried out in accordance with the procedures described in the British Pharmacopoeia (1973). The chemical quality test of the drug was performed according to the following method.

Twenty tablets were weighed and powdered. The powdered was (0.4 g of INH) was dissolved as completely as possible in distilled water and filtered. The residue was washed thoroughly with water (final volume 250 ml) Twenty five ml of the solution was transfered to a glass-stoppered flask, 25 ml of 0.1N bromine and 5 ml of hydrochloric acid were added, the contents were shaken for one minute, and allowed to stand for 15 minutes. 10 ml of 10% potassium iodide solution was added and then subsquently titrated the librated iodine with 0.1N sodium thiosulphate, using starch as indicator. This operation was repeated without isoniazid, the difference between the titrations represent the amount of 0.1N bromine required by the Isoniazid. Each ml of 0.1N bromine is equivalent to 0.003429 of $C_6 H_7 N_3O$ (Isoniazid).

RESULTS

RESULTS

Standardization of Method

Pure INH was used at various concentrations to prepare the standard curve. The representative results are shown in Figure 1. The data presented clearly show that the concentration of INH is directly proportional to absorbance. A linear curve is obtained with in the concentration range of 1.37 to 13.70 µg/ml. The data of several such experiments are shown in Table 1. The data indicates that the results obtained by this method are reproducible within a standard deviation of 0.0010-0.0023.

The same experiment was repeated by dissolving various concentration of INH in normal plasma. The results obtained are shown in Figure 2. It may be seen that the use of plasma does not effect the linearity of curve. Several experiments were performed under this condition. The results are presented in Table 2. The two linear curves, are obtained by dissolving INH in distilled water and an other obtained by dissolving INH in normal plasma are compared in Figure 3. Slight deviation in slope is possibly results from the adsorption of INH (5 percent) on denatured plasma proteins. No correction was made in the final calculations.

Recovery of Isoniazid from Plasma

Reliability of the method was further tested by recovery experiment in which a known concentration of INH was dissolved in plasma from different normal subjects. The recovery of INH under this condition was of the order of 95.43 percent. Data are presented in Table 3.

Absorbance of Drug Free Plasma

A series of experiment were conducted to determine the absorbance of drug free plasma. The results of investigatons are presented in Table 4. The mean absorbance was 0.0046 ± 0.0014 which indicates that absorbance of drug free plasma is almost negligible at the wave length used (430 mu).

Quality Assurance of Drug Isonex

The drug isonex (isoniazid) was evaluated for its quality in order to ensure its correct dosage for the subjects. The drug was tested physico-chemically according to the methods, specified in the British pharmacopoeia Table 5 shows the results of assay of the drug. The drug was found to be 97 percent pure isoniazid in compliance

with B.P. (1973) standard.

Fast and Slow Acetylators

Table-6 shows the result of estimated levels of plasma isoniazid found among (80) normal subjects and (77) tuberculosis patients after administration of INH 10 mg/kg body weight after 2 hours, mean plasma INH concentration of 4.566 ± 1.885 µg/ml was found among normal subjects, whereas the tuberculosis patients, the mean plasma INH concentration was 4.230 ± 1.805 µg/ml. The statistical analysis of data do not reveal any significant difference among normal subjects and that of tuberculosis patients ($t = 0.4397$ $P < 0.05$). The data of these subjects was further analysed to find out fast and slow acetylators. Those subjects in whom the INH concentration after 2 hours was equal to or less than 2.5 µg/ml were considered as fast acetylators. In those subjects in which the concentration of INH was more than 2.5 µg/ml were considered as slow acetylators. Of the 157 subjects, 31.8 percent were fast acetylators and 68.2 percent were slow acetylators. Among fast acetylators, the mean plasma INH was 2.32 ± 0.18 µg/ml where as that of slow acetylators the mean plasma INH was 5.32 ± 1.373 µg/ml. The data are recorded in Table-7.

In order to identify the effect of prolonged treatment of tuberculosis patients with INH, the data of chronic fast and slow acetylators and recent fast and slow acetylators were compared. As a result it has been observed (Table 8) that chronic subjects are not different from the recent subjects, this inference was based on tests of significance between the recent fast acetylators and chronic fast acetylators and chronic slow acetylators and recent slow acetylators (Table 8).

In Table 9 are shown the number and percentages of various blood group types among normal subjects and tuberculosis patients. As recorded the relative percentage of O, A, B and AB blood groups in normal subjects is 33.802%, 5.633%, 40.84% and 19.718% respectively. In the tuberculosis patients, the relative percentages of O, A, B and AB is 36.231%, 11.594%, 42.028% and 10.144% respectively. It may be observed that the relative proportion of various blood groups in normal and tuberculosis patients does not differ significantly from each other ($\chi^2_5 = 3.64$).

The distribution of fast and slow acetylators in relation to various blood group types (B,O,(A,AB)) is shown in Table 10. The statistical analysis, performed

as 2x2 Contingency Chi-square tests shows that the polymorphisim of fast and slow acetylators is not associated with the blood groups.

The percentage of fast and slow acetylators found among 157 subjects studied is shown in Table 11. It has been found that 31.8 percent of the subjects were fast acetylators, while 68.2 percent were slow acetylators. The table also shows the relative percentages (in parenthesis) of fast and slow acetylator found among normal subjects and tuberculosis patients. The number of fast and slow acetylators segrated into various blood groups type are shown in Table 12. It is interesting to note that both fast and slow acetylators have the same dominating blood group types B and O that was found in various blood groups of normal and tuberculosis patients.

The results of various studies dealing with INH acetylators in different ethnic population including the present study are compared in Table 13. Caucasian and Negroes show the higher percentage of slow acetylators while Japanese and Koreans the lowest percentage of slow acetylators. The data obtained in this study show that the Pakistani sample approaches the Caucasian and South Indians sample in their percentage of slow acetylators.

FIGURES

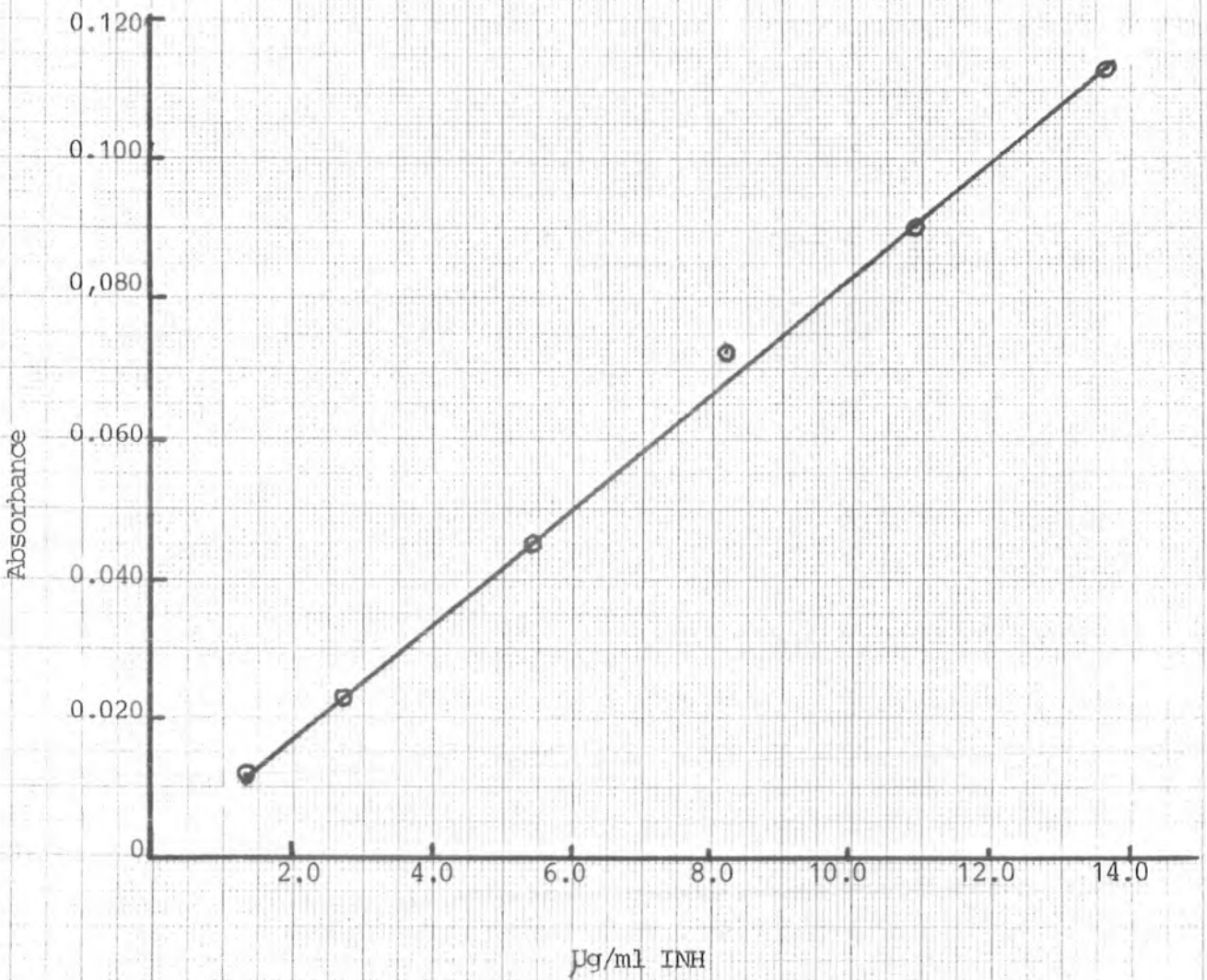

Figure 1 Standard curve of isoniazid in aqueous medium.

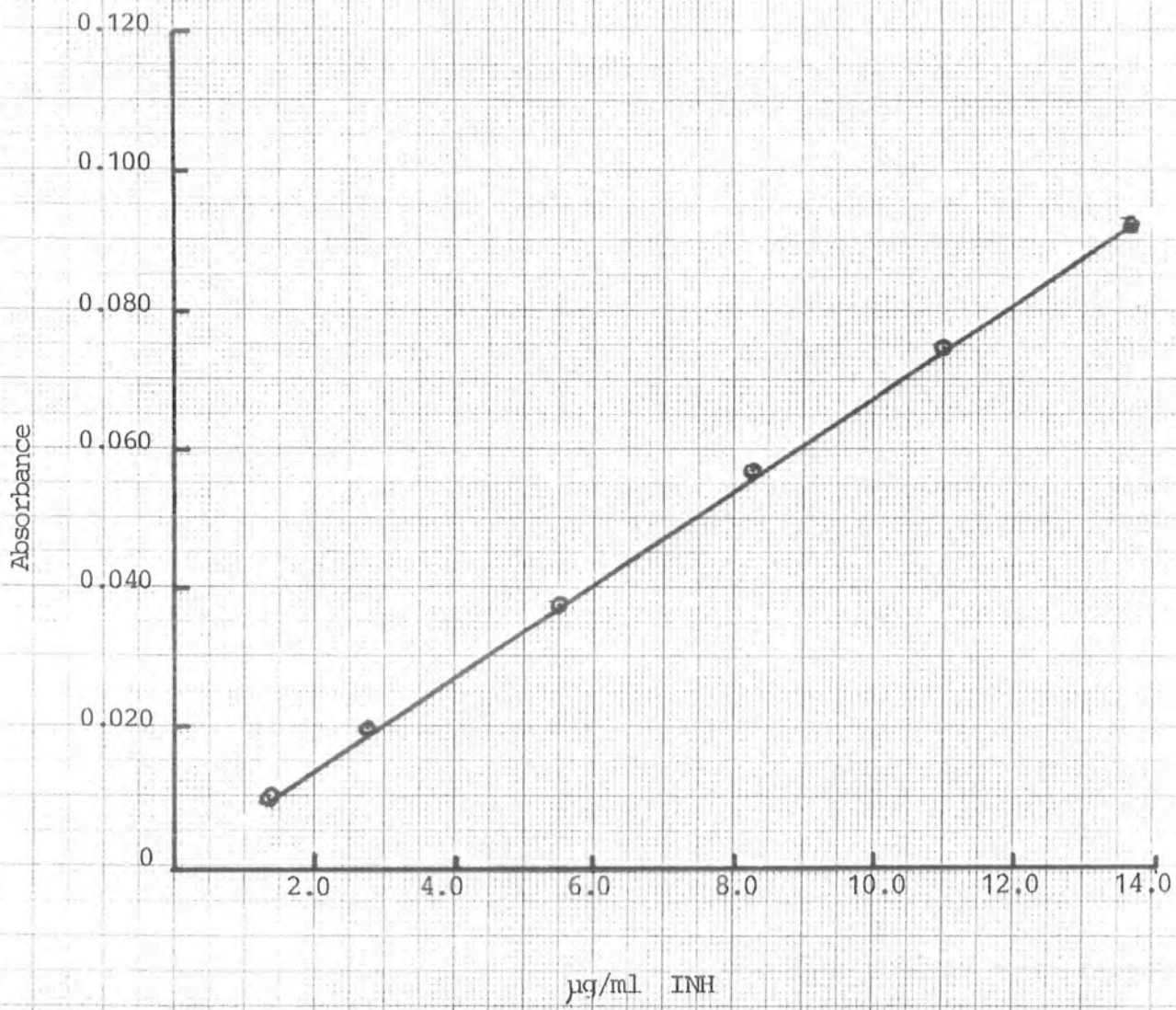

Figure 2. Standard curve of isoniazid in human plasma.

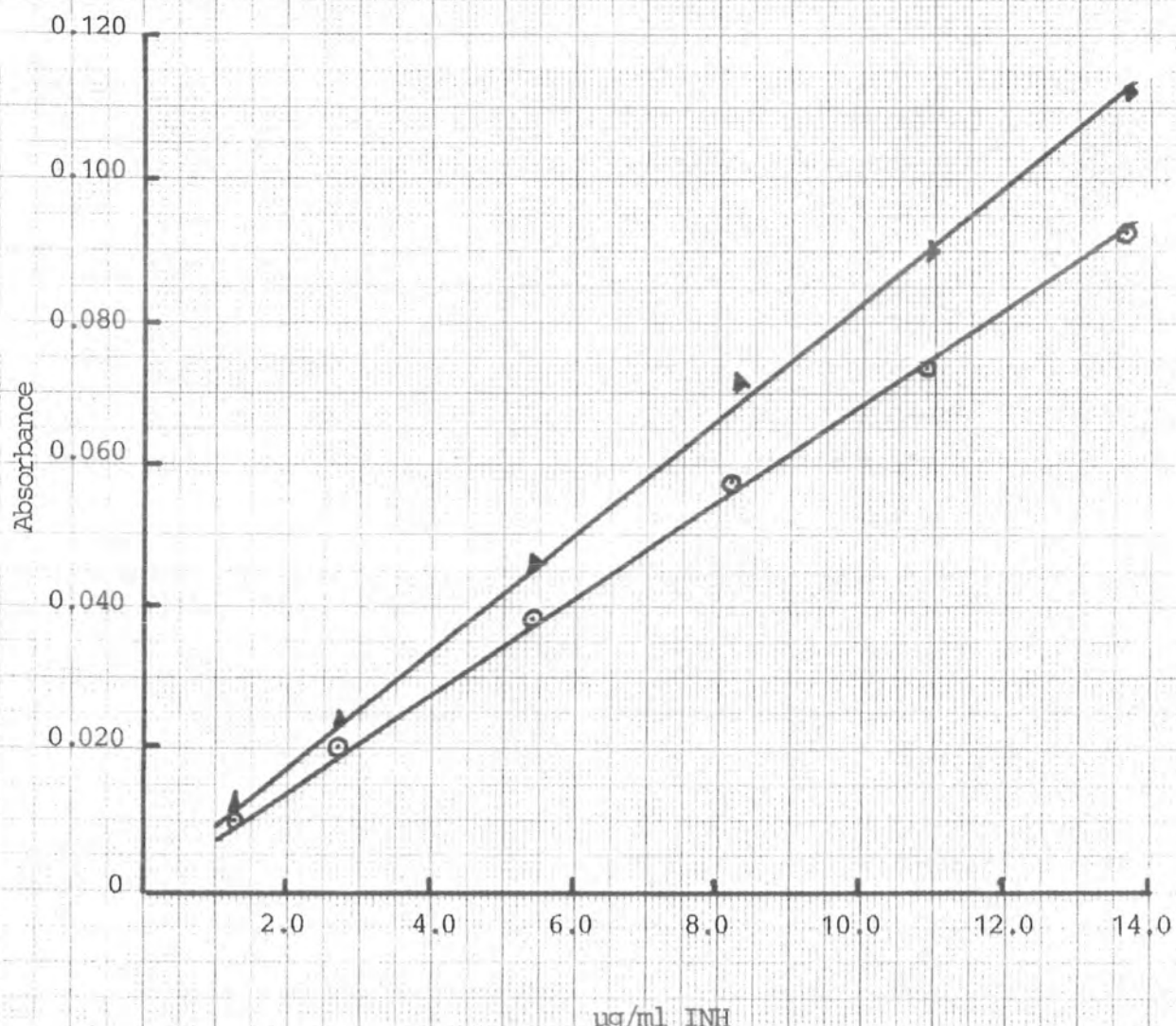

Figure 3. Comparative absorbance of standard isoniazid in aaqueous medium and in human plasma.

TABLES

TABLE: 1 Absorbances of Standard Isoniazid Solution in Aqueous Medium

Concentration of Isoniazid µg/ml	Absorbance (430 mµ)
1.37	0.012 ± 0.0012 (6)
2.74	0.023 ± 0.0014 (5)
5.48	0.045 ± 0.0020 (7)
8.72	0.072 ± 0.0012 (7)
10.96	0.090 ± 0.0022 (5)
13.70	0.113 ± 0.0024 (6)

The data are shown as mean ± SD.
The number in parenthesis is the number of samples.

TABLE: 2 Absorbances of Standard Isoniazid in Normal Human Plasma

Concentration of Isoniazid µg/ml	Absorbance (430 mµ)
1.37	0.010 ± 0.0019 (5)
2.74	0.020 ± 0.0010 (5)
5.48	0.038 ± 0.0020 (5)
8.72	0.057 ± 0.0012 (5)
10.96	0.075 ± 0.0021 (5)
13.70	0.092 ± 0.0023 (5)

The data are shown as mean ± SD

The number in parenthesis is the number of samples.

TABLE: 3 Recovery Data of Isoniazid

Sample Number	INH (µg/ml) *Actual	Recovered	% Recovery
1	5.48	4.66	85.03
	5.48	5.21	95.07
2	5.48	4.93	89.96
	5.48	4.93	89.96
3	5.48	5.48	100.00
	5.48	5.75	104.92
4	5.48	6.03	110.03
	5.48	4.93	89.96
5	5.48	5.48	100.00
6	5.48	4.93	89.96
	Mean	5.23 (n = 10)	95.43 ± 8.2

* Added to the plasma of normal, non-tuberculosis donors.

TABLE: 4 Absorbance of Drug Free Plasma

*Subject Number	Absorbance (430 mμ)
1	0.003
2	0.007
3	0.005
4	0.005
5	0.003
6	0.006
7	0.003
8	0.003
9	0.004
10	0.007
	**0.0046 ± 0.0014

* Normal healthy donors.

** Represents mean ± standard deviation.

TABLE: 5 Chemical Quality** of Isonex (Isoniazid) Tablets*

Test+	Values
Identification	Identified as Isoniazid
Weight variation	Maximum = 0.191 Minimum = 0.165
Desintegration time	7 minutes
Assay	stated 100 mg; found 97 mg

* 100 mg INH per tablet.

** The chemical quality parameters analysed comply with the British Pharmacopocia standards (B.P., 1973).

\+ For the procedures used see materials and methods.

TABLE: 6 The estimated levels of (μg/ml) plasma INH found in normal and tuberculosis patients*

Normal subjects (μg/ml)				Tuberculosis Patients (μg/ml)			
S.No.	Level of INH	S.No.	Level of INH	S.No.	Level of INH	S. No.	Level of INH
1	5.610	41	7.026	1	5.121	41	5.692
2	4.108	42	7.741	2	5.240	42	5.692
3	3.984	43	7.384	3	5.811	43	3.720
4	3.490	44	2.490	4	6.312	44	4.100
5	5.359	45	4.525	5	3.930	45	2.450
6	6.669	46	2.385	6	5.735	46	7.278
7	5.597	47	4.285	7	2.500	47	2.257
8	4.359	48	6.074	8	3.620	48	7.631
9	7.419	49	4.108	9	5.121	49	1.992
10	4.764	50	7.146	10	2.237	50	6.809
11	5.359	51	3.984	11	1.871	51	7.045
12	2.364	52	5.716	12	4.406	52	6.431
13	2.237	53	5.478	13	4.406	53	5.478
14	4.357	54	1.743	14	5.610	54	4.525
15	6.431	55	8.098	15	4.760	55	5.955
16	4.406	56	8.572	16	2.486	56	2.336
17	4.357	57	2.440	17	6.550	57	7.326
18	2.237	58	5.984	18	2.336	58	5.359
19	4.407	59	4.287	19	4.644	59	4.168
20	2.410	60	2.230	20	6.341	60	2.237
21	2.361	61	4.525	21	2.363	61	2.440
22	6.550	62	6.550	22	6.074	62	2.100
23	4.644	63	1.992	23	5.478	63	4.000
24	7.146	64	4.406	24	5.359	64	2.300
25	6.512	65	2.450	25	6.193	65	2.100
26	2.490	66	2.490	26	5.716	66	2.450
27	4.644	67	7.146	27	6.193	67	4.600
28	6.809	68	2.460	28	4.644	68	2.336
29	4.764	69	6.809	29	5.716	69	6.431
30	2.375	70	7.400	30	2.410	70	10.012
31	6.312	71	4.644	31	5.230	71	6.512
32	5.002	72	8.572	32	1.250	72	3.811
33	4.525	73	5.121	33	2.450	73	2.442
34	2.490	74	7.503	34	4.200	74	4.126
35	3.984	75	3.984	35	3.620	75	2.500
36	2.257	76	4.357	36	2.320	76	2.316
37	2.490	77	2.250	37	2.320	77	2.421
38	3.620	78	2.500	38	4.287		
39	2.450	79	4.764	39	4.883		
40	2.361	80	4.108	40	5.835		

n=80; $\bar{x}$ = 4.560; SD = 1.885

*Students t. test comparision gives t=0.4397; non significant (P<0.05) level

n=77 $\bar{x}$ =4.2305; SD=1.805

TABLE: 7 Estimated level of (µg/ml) plasma INH found among fast and slow acetylators

Rapid Acetylators (µg/ml)				Slow Acetylators (µg/ml)					
.No.	Level of INH	S.No.	Level of INH	S.No.	Level of INH	S.No.	Level of INH	S. No.	Level of INH
1	2.490	26	2.237	1	5.611	41	5.984	81	4.200
2	2.364	27	1.250	2	4.108	42	4.287	82	3.620
3	2.237	28	2.486	3	3.984	43	4.525	83	4.287
4	2.237	29	2.336	4	5.359	44	6.550	84	4.883
5	2.410	30	2.363	5	6.669	45	4.406	85	5.835
6	2.361	31	2.410	6	5.597	46	7.146	86	3.692
7	2.490	32	1.871	7	4.359	47	6.809	87	3.692
8	2.375	33	2.450	8	7.419	48	7.400	88	3.720
9	2.490	34	2.320	9	4.764	49	4.644	89	4.100
10	2.237	35	2.320	10	5.359	50	8.572	90	7.278
11	2.490	36	2.450	11	4.357	51	5.121	91	7.631
12	2.450	37	2.337	12	6.431	52	7.503	92	6.309
13	2.361	38	1.992	13	4.406	53	3.984	93	7.045
14	2.490	39	2.336	14	4.357	54	4.357	94	6.431
15	2.385	40	2.336	15	4.407	55	4.764	95	5.478
16	1.743	41	2.440	16	6.550	56	4.108	96	5.525
17	2.440	42	2.100	17	4.644	57	5.121	97	5.955
18	2.250	43	2.300	18	7.146	58	5.240	98	7.326
19	1.992	44	2.100	19	6.312	59	3.811	99	5.359
20	2.450	45	2.450	20	4.644	60	6.312	100	4.168
21	2.490	46	2.336	21	6.809	61	3.930	101	4.000
22	2.460	47	2.442	22	4.764	62	3.735	102	4.600
23	2.250	48	2.500	23	6.312	63	3.620	103	6.430
24	2.500	49	2.316	24	5.002	64	5.121	104	10.012
25	2.500	50	2.421	25	4.525	65	4.406	105	6.312
				26	3.984	66	4.406	106	3.811
				27	7.026	67	3.610	107	4.126
				28	4.764	68	4.760		
				29	7.741	69	6.550		
				30	7.384	70	4.644		
				31	4.525	71	6.341		
				32	4.285	72	4.074		
				33	6.074	73	5.478		
				34	4.108	74	5.359		
				35	7.146	75	6.193		
				36	3.984	76	5.716		
				37	5.716	77	6.193		
				38	5.478	78	4.644		
				39	8.098	79	5.716		
				40	8.572	80	5.250		

n= 50; $\bar{x}$ = 2.32; SD= 0.181

n=107; $\bar{x}$ =5.32; SD=1.373

TABLE: 8 The estimated levels (µg/ml) of plasma INH found among tuberculosis patients recent (fast and slow acetylators) and chronic tuberculosis patients fast and slow acetylators*

Recent				Chronic			
Fast		Slow		Fast		Slow	
S.No.	Level of INH	S.No.	Level of INH	S.No.	Level of INH	S.No.	Level of INH
1	2.363	1	5.121	1	2.500	1	5.811
2	2.410	2	5.240	2	2.257	2	6.312
3	2.450	3	5.930	3	1.871	3	5.620
4	2.320	4	3.735	4	2.486	4	4.406
5	2.450	5	5.121	5	2.556	5	4.406
6	2.257	6	5.610	6	1.250	6	4.760
7	2.257	7	6.550	7	2.520	7	4.644
8	2.100	8	6.541	8	1.992	8	6.074
9	2.500	9	5.478	9	2.556	9	5.559
10	2.516	10	5.716	10	2.440	10	6.193
11	2.421	11	4.644	11	2.100	11	5.716
		12	4.200	12	2.450	12	5.230
		13	5.620	13	2.556	13	4.883
		14	4.287	14	2.442	14	5.855
		15	5.692	15	2.500	15	5.720
		16	5.692			16	4.100
		17	7.631			17	7.278
		18	4.525			18	6.809
		19	5.955			19	7.045
		20	4.168			20	6.431
		21	4.600			21	5.478
		22	6.431			22	7.326
		23	5.811			23	5.359
		24	4.126			24	4.000
						25	10.012
						26	6.312
						27	4.201
Mean=2.327;SD=0.1077		Mean=4.842; SD=1.1199		Mean=2.246;SD=0.5259		Mean=5.583;SD= 1.1427	

*Comparison between recent fast and chronic fast acetylators gives students t test value t=0.083 (n.s.)
Comparison between recent slow and chronic slow acetylators gives students t test value: t=0.367 (n.s.)

TABLE: 9 Number and Percentage of Various Blood Groups(ABO) Found in Normal Subjects and Pulmonary Tuberculosis Patients

Blood Group	Normal subjects	Patients
O	(24) 33.802%	(25) 36.231%
A	(4) 5.633%	(8) 11.594%
B	(29) 40.845%	(29) 42.028%
AB	(14) 19.718%	(7) 10.144%

The data were analysed by 2 x 3 Contingency Chi-square test ($X^2_3 = 3.64$) not significant at 0.05 level of significance.

TABLE: 10 Showing Non-association Between Blood Groups and Acetylators

Acetylators	Blood Groups		
	B	O	A,AB
Rapid	17	18	9
Slow	41	31	24

The data were analysed by 2x2 Contingency Chi-square test ($\chi^2_2 = 1.03$) not significant at 0.05 level of significance.

TABLE: 11 Number and Distribution of Fast and Slow Acetylators (Polymorphs) Among Normal Subjects and Tuberculosis Patients

Types of subjects	Total numbers	Acetylators	
		Fast	Slow
Normal	80	24 30.0%	56 70.0%
Tuberculosis patients	77	26 33.8%	51 66.2%
	157	50 31.8%	107 68.2%

TABLE: 11(a) Number and Distribution of fast and slow Acetylators (Polymorphs) among recent and chronic tuberculosis patients

Types of subjects (Tuberculosis patients)	Total number	Acetylators	
		fast	slow
Recent	35	11 31.42%	24 68.58%
Chronic	42	15 35.71%	27 64.28%
	77	26 33.8%	51 66.2%

TABLE 12 Segration of Fast and Slow Acetylators Found Among Blood Group

Type of subject	No. of Samples	Fast Acetylators ABO				Slow Acetylators ABO			
		A	B	AB	O	A	B	AB	O
Normal	71	1	7	7	7	3	22	7	17
Tuberculosis patients	69	1	10	-	11	7	19	7	14
Total	140	2	17	7	18	10	41	14	31

TABLE: 13 Frequencies for Isoniazid Acetylation in Different Populations

Race	No	Percent Slow Acetylation	References
Caucasian and Negro	484	52	Evans,Manley & McKusick (1960)
Caucasian	105	57	Dufour, Knight & Harris (1964)
Negro	116	53	Dufour, Knight & Harris (1964)
Japanese	209	8	Dufour, Kinght & Harris (1964)
Japanese	1808	11	Sunahara, Urano & Ogawa (1961)
Ainu	86	9	Sunabara, Urano & Ogawa (1961)
Korean	65	11	Sunahara, Urano & Ogawa (1961)
Ryukyan	124	16	Sunahara, Urano & Ogawa (1961)
Thai	108	29	Sunahara, Urano & Ogawa (1961)
Burmese	121	37	Tun Kyi & Smith (1970
S. Indians	321	61	Gangadharam, Bhatia Radhakrishna & Selkon (1961)
Present Study	157	68	Saleem M (1986)

DISCUSSION

DISCUSSION

Several earlier studies have shown the polymorphic nature of human populations with respect to the acetylation of commonly used anti-tuberculosis drug, INH (Wade, 1977). Accordingly, it has been found that in the human body, either the rate of acetylation is slow (slow acetylators) or fast (fast acetylators). The relative ratio of slow and fast acetylators has been shown to vary in different ethnic groups (Ake Lanngran et al., 1970). The highest ratio of slow acetylators has been found amongst Caucausian (57 percent Gangadharam et al., 1961). The highest percentage of fast acetylators has been found amongst Koreans (89 percent Sunahara et al., 1961) and Ainuans (91 percent Sunahara et al., 1961). The clinical significance of both slow and fast acetylators is widely recognized (Evans et al., 1960), since, the prolonged retention of the drug in slow acetylators, causes neuropathy (TunKyi., and Smith, 1970), and its rapid acetylation in fast acetylators causes hepatitis (Mitchell et al., 1975). For these reasons, it has been considered appropriate to adjust the dose of INH in tuberculosis patients according to the polymorphic capacity of the liver (Evans et al.,1960).

In the present investigation 157 subjects (80 normal, and 77 tuberculosis patients) were studied. Our

data demonstrate that in the limited population studied, both slow and fast acetylators are present. In this regard, there was no significant difference in the normal population (fast acetylators; 30.0 percent; slow acetylator 70.0 percent) and subject, suffering from tuberculosis (chronic fast acetylators 35.71% percent; chronic slow acetylators 64.28 percent; recent fast acetylators 31.42% percent; recent slow acetylators 64.28 percent).These data are in agreement with those reported by others (Sharma et al., 1976; Tunkyi and Smith, 1970; and Wade, 1977). Since no difference was found in normal subjects and subjects suffering from tuberculosis, the pooled data of 157 subjects was analysed and compared with similar data obtained in different ethnic groups, we have found that the Pakistani population studied (slow acetylators 68.2 percent; fast acetylators 31.8 percent) compares favourably with the South Indian population (slow acetylators 61 percent; fast acetylators 39 percent, Gangadharam et al., 1961) and Caucasian population (Slow acetylators 57 percent; fast acetylators 43 percent; Dufour et al.,1964). In other populations, for example Burmease, Japanese, Koreans and Thais, the fast acetylators predominate (Wade, 1977; TunKyi and Smith, 1977).

Since the polymorphisim is an autosomal, inherited trait, the dichotomy in polymorphisim seems to be related

Since the polymorphisim is an autosomal, inherited trait, the dichotomy in polymorphisim seems to be related to the gene flow in various human races, the Caucasian from which the Indo-Pakistan group has descended, receiving a gene pooled with dominant slow acetylators. Our data on Pakistani population is therefore not surprising and receives further support from the studies of Sharma et al. (Sharma et al., 1976) who demonstrated a distribution 39.0 percent fast acetylators and 61 percent slow acetylators in an Indian population, though the INH was estimated from urine, in these studies.

Since earlier studies have been confined essentially to normal subjects, the information provided by us on tuberculosis patients, using normal subjects as controls, assumes high clinical importance. When the chronic tuberculosis patients were compared with those who had contacted the disease recently, no significant difference was found in the ratio of slow and fast acetylators in the either group. The relative ratios were identical to those observed in pooled data (chronic patients: 35 percent fast acetylator; recent patients 31 percent fast acetylators). It can be concluded, that prolonged exposure of the patient to the drug does not effect the extent of acetylation. Although we have not studied the relative distribution of slow and fast acetylators amongs male and female populations,

but the previous studies have indicated that such a difference does not exist (Evans et al., 1960) for this reason we have, for ease of recruiting subjects confined our studies only to the males. However, for clinical use, data in the male may well be interpreted for the female populations as well. Since acetylation of INH is an autosomally inherited trait, we have made an attempt to find out whether blood group (ABO), could be use as genetic marker's for this polymorphic trait. Our statistical analysis (Table 10) shows that there is no association between blood groups, ABO, and acetylation in the population studied. Since no other comparable data are available, it is difficult to speculate about the association or disassociation of blood groups, or, of any other genetic marker with the polymorphic acetylation trait. In the absence of any definite information on this count, it may be worth while to study the polymorphic nature of the enzyme N-acetyltransferase.

The present study clearly brings out the clinical use of studying the distribution of slow acetylators and fast acetylators in a population. Although our study was made on a limited number of geographically restricted population yet, the data of this investigation suggest that a geographically more wide spread population should be studied in Pakistan, in order to find difference, if

any, so that the use of the drug INH can be rationalized. The study also emphasizes that, since, both in slow and fast acetylators, the indiscriminate use of INH is likely to cause either hepatitis or neuropathy, the clinician will be well advised to find out the rate of acetylation of INH in a patient for prescribing a dose regimen of INH.

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