fect on murine tuberculosis was greatly in excess of the equivalent dose customarily employed in management of human disease. On a weight basis, five-tenths mg/day of cortisone for a 20 g mouse for example, is 17 times the 100 mg/day maintenance dose frequently used for man.

The position of INHR organisms in human disease is still a matter of controversy although they have been isolated on primary culture from sputum and spinal fluid(3,4). The increase in virulence of INHR tubercle bacilli and of some chromogens when mice were treated with cortisone might have its parallel in human infections under conditions of prolonged stress or in anxiety states. In this connection, Steenken and co-workers have shown(5) that INHR organisms can cause progressive disease in silicotic guinea pigs.

Summary. 1) The virulence for mice of INHR laboratory strains of H37Rv was increased when cortisone was administered. 2) Cortisone also increased the pathogenicity for mice to some extent of 2 of 4 scotochromogens and 3 of 9 photochromogens tested. Seven Battey strains were unaffected.

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Stability of Isoniazid in Aqueous Solutions and Plasma. (25907)

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In our work on isoniazid (INH) blood level determinations in men and animals, the impression was gained that lower levels were found if the chemical test could not be completed immediately after blood sample was obtained. This raised the question of stability of the drug in solution. The following factors were considered: (a) Influence of temperature and pH in aqueous solution. (b) The possibility that the drug after becoming absorbed through the intestinal tract might be in a state in serum different from that in a simple solution; *e.g.,* INH could be bound by a protein fraction which might influence its stability.

Methods. The method used has been published(1). We have since modified this method as follows: Before medication was given patient's plasma was collected and used for blank and standard determinations to avoid the influence of the coloration by bile constituents which vary from one person to another.* Analytical error of the method is $\pm$ 0.3 μg. Two patients with polycythemia and healthy volunteers were available for blood donations. In each case 250 cc of blood was taken before breakfast and before medication for blank determinations. Ninety minutes after oral administration of 4 or 8 mg INH/kg another 250 cc of blood was obtained and first determination was made without delay. A pool of rabbit plasma was obtained from the carotid artery by exsanguinating male rabbits kept on a standard diet. Four rabbits received 20 mg INH/kg intravenously, their total blood was taken after 10 to 15 minutes and the plasma pooled. Another batch of pooled, untreated rabbit plasma was divided into 2 equal portions for the blank and for *in vitro* tests in which INH was added. Plasma specimens were kept at tem-

* This same modification has been introduced, independently, by Dr. John W. Jenne, Veterans Adm. Hosp., Minneapolis, Minn. Personal communication.

TABLE I. INH in Phosphate Buffer Solutions. Added 8 and 16 μg/cc.

Temp.	pH	μg found after days 0	1	3	7	14
25°	4.5	7.9	8.0	7.8	8.0	7.9
	6	7.9	7.9	7.9	7.5	7.8
	7	7.7	7.9	7.8	7.7	7.6
	8	7.9	7.8	7.0	5.2	4.0
	4.5	16.0	15.8	15.5	15.5	15.8
	6	15.7	15.4	15.5	15.6	15.6
	7	15.8	16.0	15.8	15.4	15.5
	8	15.6	15.5	15.0	13.0	9.9
37°	4.5	7.9	8.0	7.8	7.9	8.0
	6	7.9	7.9	7.6	7.1	7.3
	7	7.7	7.8	7.5	6.8	6.5
	8	7.9	7.3	5.0	3.5	2.7
	4.5	16.0	15.8	15.3	15.5	15.5
	6	15.7	15.7	15.3	15.0	15.2
	7	15.8	15 7	15.3	14.9	14.2
	8	15.6	15.3	13.1	11.8	10.7

At temperatures of 7° or in frozen state the solutions showed no changes at pH ranges 4.5-8.

TABLE II. INH in Rabbit Plasma (3 Run Separately), Taken 30 Min. after Intravenous Injection of 20 mg/kg.

Rabbit	Temp.	Found μg INH/cc after days of storage 0	4	7	11
I	37°	22.4	20.3	5.4	1.0
II		20.4	18.6	10.2	9.6
I	7°	22.4	15.6	13.3	9.2
II		20.4	15.0	14.2	13.4
III		24.3	17.6	15.2	14.3

peratures indicated in the tables, and INH determined at convenient intervals. Analog stability tests were performed in neutral aqueous solution and in buffered solutions at pH 4.5 to 8. While adequate quantities of blank and INH containing plasma were kept in bulk at temperatures as indicated, small samples of each sufficient for one test were kept frozen to avoid repeated melting and refreezing.

Results. Aqueous INH solutions of 8 and 16 μg/cc kept at 37°, 25°, 7° and frozen showed no changes within 14 days. INH is stable in aqueous and buffered solutions at pH below 8 (Table I) and if stored at low temperature, hence standard solutions may be kept refrigerated for several weeks.

INH is not stable in plasma at any temperature. Rate of disappearance is greater at 37° than at low temperature (Tables II, III), but even in frozen state is significant (Tables IV, V). Plasma from rabbits or humans contains a substance which changes INH into a form not detected by the reaction used in the analytical method. It has been claimed that metabolites of INH are isonicotinic acid, acetyl INH and conjugates of isonicotinic acid (2,3). Neither of these gives this reaction. However, none of these metabolites have been identified satisfactorily.

TABLE III. INH in Human Plasma, Taken 90 Min. after Oral Administration of 4 mg/kg (Patients) and 8 mg/kg to Volunteers.

Patient	Origin of blood	Temp.	Found μg/cc after days of storage 0	1	2	3	4	7	8	16	21	24	28	36
A	Polycythemia	25°		3.5					1.4	.8		0		
		7°		4.0					3.5	2.7		2.1	2.3	2.0
		frozen		3.7					4.0	3.4		3.1	2.9	2.6
B	"	25°	3.3	3.1	2.1		1.8			.02				
		37°	3.3	3.3	2.3		2.0			.4				
		7°	3.3	3.3	2.6		2.3			1.6				
		frozen	3.3	2.3	2.0		2.1			1.4				
Volunteer Normal		25°	6.1	4.7				3.9			3.0		2.1	
		37°	6.1	4.6				2.5			2.5		1.7	
		7°	6.1	4.7				3.2			2.7			
		frozen	6.1	4.7				3.4			3.0			
R		25°	5.4	4.9		2.8		2.6			1.0			
		37°	5.4	4.7		3.6		2.0			.0			
		7°	5.4	4.6		3.8		3.1			2.6			
		frozen	5.4	4.2		4.0		4.1			3.9			
J		25°	9.1	7.8		5.1		3.8			.0			
		37°	9.1	9.1		5.6		2.9			.1			
		7°	9.1	8.6		7.5		5.8			5.5			
		frozen	9.1	7.7		7.5		7.5			7.2			

This finding is important with reference to biological assay of INH in patients' plasma. For this assay the plasma usually is kept frozen or refrigerated for variable periods preceding testing. Adding the error caused by bacteriological technic of dilution it appears questionable whether the bioassay carries any value. Whether the factor contained in plasma is still active at the dilution resulting from distribution in the culture medium, cannot be answered because the chemical method is not sensitive enough for dilutions in that range. Should it be the case, INH would decrease in concentration during process of incubation, which usually lasts about 3 weeks. The presence of the postulated factor in plasma in larger or smaller quantities may explain why some patients show quicker inactivation of INH than others.

TABLE IV. 10 and 20 mg/cc INH Respectively Added to Pooled Rabbit Plasma.

	Found μg/cc after					
Temp.	4 hr	1 day	3 days	7 days	9 days	14 days
25°	7.2		4.4		2.2	2.0
37°	7.1		4.8		.3	.0
7°	7.9		5.9		3.2	3.0
	18.4	17.3	13.6	7.5		6.7
frozen	5.2		4.7		3.5	3.4

TABLE V. 10 mg/cc INH Added to Human Plasma.

		Found μg/cc after time				
	Temp.	0	1 day	3 days	7 days	21 days
R	25°	9.3	6.8	4.4	4.2	.9
R	37°	9.3	6.0	4.8	3.0	.0
J	7°	9.3	7.2	4.9	4.4	4.0
R	7°	8.8	7.2	6.1	5.8	5.1
R	frozen	9.3	8.0	6.9	6.0	5.7

Summary. INH is stable for several weeks in aqueous and buffered solution at pH values below 8 and at low temperature. It is unstable in human and rabbit plasma whether added to pure plasma or present after oral or parenteral administration. Instability is enhanced by increased temperature, but is quite marked at refrigerator temperature. The presence of a factor in blood causes changes in chemical state of INH so that it no longer reacts with reagent used in the assay method.

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Effect of β-3-Thienylalanine on Antibody Synthesis. I. Preliminary *in vitro* and *in vivo* Studies.* (25908)

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Experiments of Wissler *et al.* have shown that feeding β-3-thienylalanine (β-3-TA) in absence of dietary phenylalanine results in almost complete inhibition of antibody response to a single intravenous dose of particulate antigens in rats(1). In these experiments the amino acid analogue was incorporated in a liquid diet and fed by stomach tube for a period of 6 days beginning 2 days before antigen injection and continuing up to day before sacrifice. Antibody titer at this time was always considerably lower than that of control animals. The histologic transformations in rat spleen which accompany synthesis of antibody(2) were consistently absent in animals fed β-3-TA. Our experiments originated during study of antibody production in tissue culture(3). The purpose was to study the effect of β-3-TA on antibody synthesis *in vitro*

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