there was some variation among litters in values obtained from animals at the first 3 age groups. This variation was associated with the number of animals in the litter, since animals from the larger litter showed a higher initial acetal phosphatide content in the adipose tissue. The changes associated with increased age for each litter were parallel and of the same order of magnitude. In the experiment described above and where tissues were pooled, the variation among litters was minimized by keeping the number of animals from any one litter the same for each age group.

Discussion. The changes in the lipid fractions of the liver with increased age in newborn rats were within normal adult limits and little significance can be attributed to them. These data, however, do not exclude the possibility that acetal phosphatides may be involved in the lipid metabolism of the liver by means of a turnover mechanism. In adipose tissue, on the other hand, the maximum changes occurred during the first 5 days of life. The initially high levels of acetal phosphatide and total phospholipid decreased rapidly to only trace amounts as the adipose tissue filled with fat. The results confirm and extend the observation of Möckel(7). The inverse changes in acetal phosphatide and phospholipid, as compared to total lipid, indicate that they are in some way involved in the laying down of fat in adipose tissue. Acetal phosphatides could be involved directly in this mechanism or they could be involved in the synthesis of other phospholipids which could then play some role in the fat metabolism of adipose tissue.

Summary. The changes in the acetal phosphatide, phospholipid and total lipids have been followed in the adipose tissues and livers of newborn rats over the first 3 weeks of life. As the adipose tissue fills with fat, the acetal phosphatide and total phospholipid, initially quite elevated, decrease rapidly to only trace amounts by the fifth to eighth day of life. These results indicate that acetal phosphatides and phospholipids are involved in some manner in the laying down of fat in adipose tissue.

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Effect of Para-Aminosalicylic Acid on Serum Isoniazid Levels in Man. (22278)

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Previous investigations(1,2) with chemical methods have shown wide variations in the rate of metabolic alteration of isoniazid (INH) from person to person, but a relatively constant rate in any one individual. This observation is of clinical importance because only the free, unaltered isoniazid has significant antimicrobial activity against tubercle bacilli(3). One of the major pathways of metabolic disposition of INH is by acetylation(1). *In vitro* experiments by Johnson (4) have shown that para-aminosalicylic acid (PAS) inhibits the acetylation of INH by a competitive mechanism.

The purpose of this report is to present observations on the application of a specific microbiologic assay technic to determine the effect of PAS on the level of antimicrobially active INH in human subjects.

Materials and methods. The technic of microbiologic assay for INH was as follows: 1 ml of serum from aseptically collected specimen of clotted venous blood was added to 9 ml of oleic acid-albumin medium ST-T* containing 10 μg PABA/ml. From this tube of 1:10 serum dilution, 2.5 ml aliquots of serum dilutions of 1:10, 1:20 and 1:40 were prepared in duplicate in ST-T medium in screw-capped culture tubes. Each of 6 tubes for any one determination was then inoculated with 0.1 ml of fully-grown, dispersed, stock culture of an INH-susceptible, streptomycin-resistant strain of tubercle bacilli (H37RvS-RSM) grown in ST-T medium containing 0.05% Tween 80 and 10 μg streptomycin/ml. The inoculated tubes, along with standard control test of susceptibility of the strain to known concentrations of INH (0, .02, .04, and .08 μg INH/ml), were incubated at 36°. After 5 days, a Ziehl-Neelsen stained smear from each tube was prepared and the tubercle bacilli examined for loss of acid-fastness (AF). This is a specific test for antimicrobially active INH as neither human sera nor any known antimicrobial agent other than INH has this effect on tubercle bacilli(6). The results were read as follows: (1) No loss of AF at 1:10 dilution (<0.3 μg INH/ml); (2) Moderate loss of AF at 1:10 dilution, no loss at 1:20 (0.3 μg INH/ml); (3) Marked loss of AF at 1:10, no loss at 1:20 (0.4-0.6 μg INH/ml); (4) Moderate loss of AF at 1:20, no loss at 1:40 (0.7 μg INH/ml); (5) Marked loss of AF at 1:20, no loss at 1:40 (0.8-1.4 μg INH/ml); (6) Moderate loss of AF at 1:40 (1.5 μg INH/ml); (7) Marked loss of AF at 1:40 (1.6 μg or more INH/ml). Preliminary studies with known amounts of INH added to human sera permit assignment of these values for INH equivalents in μg/ml. It was also shown that addition of as much as 100 μg streptomycin/ml and 1000 μg PAS/ml to undiluted human sera did not interfere with this microbiologic assay. A total of 76 assays were performed on 25 patients under treatment for tuberculosis. Patients were selected from a group who did not maintain serum INH levels in excess of 0.3 μg/ml 6 hours after oral loading dose of 4 mg INH/kg in tablet form (Nydrazid, Squibb). This standard loading dose regimen required that

TABLE I. Effect of PAS on Microbiologic Assay of Serum INH, in 25 Patients.

	Loading dose, mg/kg	Without PAS, μg INH/ml	With PAS, μg INH/ml	Result*
H.W.	4	<.3	.8–1.4	+
T.H.	"	"	.4– .6	"
C.H.	"	"	.8–1.4	"
W.C.	"	"	.4– .6	"
W.D.	"	"	"	"
W.S.	"	"	.3	"
P.M.	"	"	.4– .6	"
G.R.	"	"	<.3	0
M.P.	"	"	"	"
C.M.	8	"	.8–1.4	+
E.R.	"	.4– .6	"	"
M.B.	"	"	"	"
A.B.	4	<.3	.4– .6	"
	"	.3	"	"
S.P.	"	<.3	.8–1.4	"
	"	"	.3	"
M.H.	8	"	.4– .6	"
	"	"	.8–1.4	"
F.H.	"	.4– .6	"	"
	"	"	"	"
B.S.	4	<.3	.7	"
	8	.3	.8–1.4	"
J.E.	4	<.3	<.3	0
	8	"	.4– .6	+
S.C.	4	"	"	"
	8	.4– .6	.8–1.4	"
A.J.	4	<.3	.4– .6	"
	8	.4– .6	.8–1.4	"
B.Y.	4	<.3	.4– .6	"
	8	.3	"	"
R.C.	4	<.3	<.3	0
	8	.4– .6	.8–1.4	+
M.B.	4	<.3	.4– .6	"
	8	.8–1.4	.8–1.4	0
M.S.	4	<.3	<.3	"
	8	.3	.4– .6	+
M.G.	4	<.3	<.3	0
	8	"	"	"

* + = Increase; 0 = No detectable change.

* Same as "basic medium" previously described(5) except for addition of 0.4 g sodium citrate, 0.01 g ferric ammonium citrate and 2 g glycerol/l; 0.5% oleic acid-albumin complex plus 0.2% glucose (final concentrations) were added aseptically after autoclaving basic medium.

the patient have received total daily dose of INH from 8 to 16 mg/kg for at least one day prior to test and that, except for streptomycin, he receive no antimicrobial agent other than INH. The INH was given at 9 a. m. and blood was collected at 3 p. m. When PAS (Hellwig, PAS Sodium tablets) was administered, it was given in a total daily dose of 10 g of free PAS, 2.5 g at 9 a. m., 12 p. m., 4 p. m. and 8 p. m. Each study was performed under the same conditions with or without PAS which was given or withheld for 5 days prior to each determination.

Results. The results are listed in Table I. Twenty-two of the 25 patients showed a detectable increase in their microbiologically active serum levels of INH when PAS was also given. Repeat determinations with the same loading dose of INH were performed on 4 patients. They showed increases each time that PAS also was administered. Repeat determinations with different loading doses of INH were performed on 9 patients. Five were done because there was no detectable change in the first study. In 4 of these 5 subjects there was a detectable increase in INH level when PAS was added. The absence of a detectable increase in 3 of the 25 patients studied did not necessarily mean that PAS had no effect in these individuals for the minimum serum dilution employed (1:10) did not permit detection of possible changes below 0.3 μg INH/ml.

Discussion. The biologic phenomenon of one drug raising the blood level of another drug is not uncommon. The results of the present study demonstrate that concurrent administration of PAS with INH raises the serum concentration of INH when measured 6 hours after administration of INH. In view of Johnson's observation(4), it appears probable that PAS achieves this effect by decreasing the rate of acetylation of INH to the antimicrobially inactive acetyl derivative.

It seems fair to assume that delivery of effective concentrations of INH to multiplying populations of tubercle bacilli in tuberculous patients is determined in part by the concentration of INH in the circulating blood. Therefore, the problem of adequate dosage of INH presents itself in those tuberculous patients who dispose of INH so rapidly that effective antimicrobial levels cannot be achieved or maintained. The present studies show that these levels can be elevated by increasing the dosage of INH or by adding PAS.

The therapeutic superiority of treatment with INH plus PAS over INH alone in human beings(7) may be attributable not only to the antimicrobial activity of PAS itself, but also to the delivery of higher concentrations of INH to multiplying tubercle bacilli.

Summary. A method for the microbiologic assay of INH in the presence of high concentrations of streptomycin or PAS is described. The concurrent oral administration of PAS with INH resulted in detectable elevation of the antimicrobially active INH level in the blood serum of 22 of 25 tuberculous patients. The significance of this effect is discussed.

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