similar to those found in kidney tissue. In view of the high overall metabolic activity of kidney tissue, the low S.A. of G-6-P would indicate that, in the kidney, this compound may not occupy a central position in the metabolism of carbohydrate.

Conclusions. 1. A "single pass" technic is described for studying the intrinsic metabolism of the kidney. 2. It is shown that P uptake by ADP-ATP and G-1-P is very rapid, more so in the cortex than in the medulla. G-1-P probably is derived from glycogen via the phosphorylase reaction. 3. The very low relative S.A. of the G-6-P seems to indicate that most, if not all, of the G-6-P found in the kidney is formed elsewhere in the body and carried to this organ by the blood.

1. Sacks, J., *J. Biol. Chem.*, 1949, v181, 655.
2. Fiske, C. H., and SubbaRow, Y., *ibid.*, 1925, v66, 375.
3. Dratz, A. F., and Handler, P. P., *ibid.*, 1952, v197, 419.
4. György, P., Keller, W., and Brehme, T. L., *Biochem. Z.*, 1928, v200, 356.
5. Dickens, F., and Malherbe, H., *Biochem. J.*, 1936, v30, 659.
6. Glass, B., in McElroy, W. D., and Glass, B., *A Symposium on Phosphorus Metabolism*, v1, 673, Johns Hopkins Press, Baltimore, 1951.
7. Hanes, C. S., and Maskell, E. J., *Biochem. J.*, 1942, v36, 76.
8. Le Page, G. A., *Am. J. Physiol.*, 1946, v146, 267.
9. Sacks, J., *Arch. Biochem.*, 1951, v30, 423.
10. Guest, C. M., and Rapaport, S., *Physiol. Rev.*, 1941, v21, 410.
11. Gourley, D. R. H., *Arch. Biochem.*, 1952, v40, 1.

Received July 30, 1953. P.S.E.B.M., 1953, v84.

Antagonism of Chemotherapeutic Activity of p-Aminosalicylic Acid in Experimental Tuberculosis by p-Aminobenzoic Acid.* (20700)

R. H. HUBBLE AND L. W. HEDGECOCK.† (Introduced by Lloyd R. Jones.)

From the Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

In vitro antagonism of the tuberculostatic properties of para-aminosalicylic acid (PAS) by para-aminobenzoic acid (PABA) has been established(1-3). The *in vivo* relationship of PAS to PABA has not been reported. The purpose of this communication is to present the results of an investigation of the effect of dietary PABA on the chemotherapeutic activity of PAS in experimental tuberculosis.

Methods. The H37Rv strain of *M. tuberculosis* was utilized as the infectious agent in this study. Male CF_1 mice weighing 14 to 16 g each were employed as test animals. The basal ration represented a modification of a synthetic ration devised by Pearce *et al.*(4). Its composition was as follows: vitamin-free casein, 20%; Wesson's salt mixture(5), 4%; Ruffex, 10%; corn starch, 55.68%; sucrose, 5%; lard, 5%; and supplement, 0.313%. The following components of the supplement are expressed in terms of mg per 100 g of basal ration: pyridoxine hydrochloride, 1.0; nicotinic acid, 1.0; thiamine chloride, 0.6; riboflavin, 0.6; calcium pantothenate, 2.0; folic acid, 0.05; biotin, 0.05; PABA, 30; inositol, 50; choline chloride, 100; cystine, 100; alpha tocopherol, 3.5; and menadione, 2.5. Concentrations of 1,500 I.U. of vit. A and 200 I.U. of vit. D were added per 100 g basal ration. Adjustment of the concentrations of PABA and PAS in the rations was accomplished by the addition or omission of equal weights of starch. Five rations were studied in Exp. A and were as follows: 1) basal ration; 2) basal ration, PABA-free;‡ 3) basal ration containing 2% PABA; 4) basal ration containing

* This paper represents portion of dissertation submitted by Ralph H. Hubble to Graduate School of St. Louis University in partial fulfillment of requirements for degree of Doctor of Philosophy.

† Present address: Veterans Administration Hospital, Kansas City, Mo. and Department of Microbiology, Kansas University School of Medicine, Kansas City, Kan.

‡ The term, "PABA-free" refers only to omission of PABA from the supplement.

TABLE I. Comparison of Weights and Survival Times of Tuberculous Mice Receiving a Basal Ration and Rations Containing Varied Amounts of PAS with and without PABA.

	Rations	Avg wt mice (g) A*	B*	C*	T_{50} and confidence limits	% surviving on 55th day
A.	B.R.†	13.1	18.2	13.9	16.0 (14.0–18.0)	0
	"	13.7	19.8	13.5	18.7 (17.0–20.6)	0
	" + 2% PABA	13.9	15.0	12.4	15.6 (13.8–17.5)	0
	" + .5% PAS	13.4	19.2	15.5	0‡	69.6
	" + 2% PABA + .5% PAS	13.7	13.0	11.2	21.4 (18.8–24.6)	0
B.	B.R.	16.2	18.4	12.6	16.3 (14.3–18.6)	0
	" + .125% PAS	16.7	17.8	11.9	25.0 (22.5–30.0)	0
	" + .25% PAS	16.0	18.3	11.5	35.0 (30.6–40.0)	0
	" + 1% PABA + .125% PAS	15.6	17.2	11.9	19.4 (16.6–22.7)	0
	" + 1% PABA + .25% PAS	14.8	15.7	10.9	24.3 (22.4–26.4)	0

* A—Wt of mice on arrival. B—Wt of mice at inoculation. C—Wt of mice at death.
† B.R. = Basal ration.
‡ T_{50} was not attained.

0.5% PAS, PABA-free; 5) basal ration containing 2% PABA and 0.5% PAS. In Exp. B the following rations were included: 1) basal ration; 2) basal ration containing 0.125% PAS, PABA-free; 3) basal ration containing 0.25% PAS, PABA-free; 4) basal ration containing 1% PABA and 0.125% PAS; 5) basal ration containing 1% PABA and 0.25% PAS. Each ration was furnished *ad libitum* to a group of 20 mice. In Exp. A and B prefeeding periods of 8 and 5 days respectively, were instituted prior to injection of organisms. The animals were infected by intravenous injection of 0.15 mg (dry weight) of *M. tuberculosis*. The organisms had been cultured according to the method of McKee *et al.*(6). The survival time of each animal was recorded in terms of days following injection of organisms. The mortality rate for each group was plotted on an arithmetic-probability scale from which the 50% survival time (T_{50}) was determined (7). The death-rate response of each test group was examined statistically by the method of Litchfield(8).

Results. The data of Exp. A presented in Table I suggested antagonism of the tuberculostatic properties of PAS by orally-administered PABA. The results of this experiment did not permit definite conclusions since the concentration of PABA in rations furnished Groups III and V proved to be toxic as evidenced by the retarded gain in weight of the animals. However, a comparison of the T_{50} of Groups I and III indicated that the toxicity of the ration containing 2% PABA did not increase the susceptibility of the mice to experimental tuberculosis significantly. This finding tended to support the validity of the difference in percentages of mice surviving in Groups IV and V at the termination of the experiment.

In Exp. B the reduction of the concentration of PAS in the rations yielded a suitable T_{50}. Reduction in concentration of PABA resulted in minimal toxicity. The data of Exp. B illustrate antagonism of the tuberculostatic activity of PAS by PABA. The chemotherapeutic activity of PAS was evidenced by the increased T_{50} of the animals in Groups II and III as compared with those in Group I. Antagonism of the tuberculostatic properties of PAS by dietary PABA was likewise apparent in the reduced T_{50} of the animals in Groups IV and V as compared with the T_{50} of those in Groups II and III.

Under natural conditions it is doubtful if the dietary intake of PABA in man or animals ever approximates the amounts employed in this study. Among foodstuffs, yeast has been found to be one of the richest sources of PABA, containing up to 100 μg per g and of which 70 to 80% is present in the free form(9). The addition of such a foodstuff to the diet would result in a concentration of PABA much less than 1%. Denko *et al.*(10) have provided evidence that PABA is synthesized by microorganisms in the intestine of man and thereby made available to the host. These investigators have reported that the urinary and fecal excretion of PABA may exceed the dietary intake by approximately 100%. However, since the average daily urinary and fecal ex-

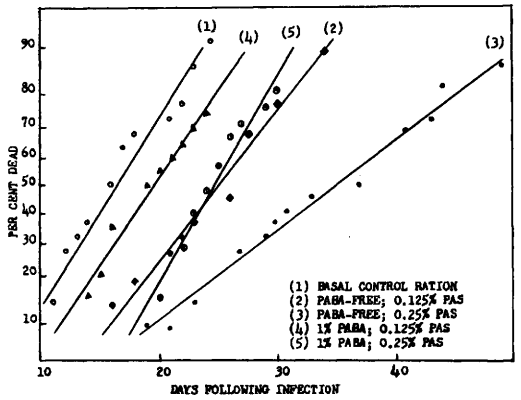

FIG. 1. Rate of death of tuberculous mice fed basal ration and rations containing varied amounts of PAS with and without PABA.

cretion was 737 μg the amounts involved were relatively small. The excretion of PABA in the urine and feces of the mice used in this study was not determined.

In studies of the relationship of PABA to the sulfonamides in the metabolism of *E. coli* and other organisms, it has been shown that the observed inhibition index SA/PABA(11, 12) may be progressively increased in the presence of end-products of the inhibited reaction(13,14). If such a relationship should exist with respect to PAS and PABA in the metabolism of *M. tuberculosis,* the presence of end-products of the inhibited reaction might render relatively small amounts of PABA effective in antagonizing the chemotherapeutic activity of PAS.

McKee *et al.*(6) reported that the H37Rv strain of *M. tuberculosis* was unsuitable for therapeutic studies in CF_1 mice since the animals did not die at a uniform rate under conditions which yielded extended survival. Youmans and Youmans(15) found, however, that infection of the "Strong A" strain of mice with H37Rv resulted in a uniform rate of death of the animals providing a sufficiently large infecting dose was used and that the uniform response was maintained under conditions of therapy in which the T_{50} was extended. The data presented in Fig. 1 indicate that under the conditions of the present study in which a limited number of animals were used, infection of CF_1 mice with H37Rv results in rates of death which yield approximately straight lines when plotted on probability paper indicating a normal frequency distribution curve.

Summary. 1. Infection of CF_1 mice with the H37Rv strain of *M. tuberculosis* produced a uniform response in rate of death. 2. Antagonism of the chemotherapeutic activity of PAS in experimental tuberculosis by dietary PABA was demonstrated.

1. Youmans, G. P., Raleigh, G. W., and Youmans, A. S., *J. Bact.,* 1947, v54, 409.

2. Goodacre, C. L., Mitchell, B. W., and Seymour, D. E., *Quart. J. Pharm. & Pharmacol.,* 1948, v21, 301.

3. Ivanovics, G., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 462.

4. Pearce, E. L., Sauberlich, H. E., and Baumann, C. A., *J. Biol. Chem.,* 1947, v168, 271.

5. Wesson, L. G., *Science,* 1932, v75, 339.

6. McKee, C. M., Rake, G., Donovick, R., and Jambor, W. P., *Am. Rev. Tuberc.,* 1949, v60, 90.

7. Donovick, R., McKee, C. M., Jambor, W. P., and Rake, G., *Am. Rev. Tuberc.,* 1949, v60, 109.

8. Litchfield, J. T., *J. Pharm. and Exp. Therap.,* 1949, v97, 399.

9. Landy, M., and Dickin, D. M., *J. Biol. Chem*, 1942, v146, 109.

10. Denko, C. W., Grundy, W. E., Porter, J. W., Berryman, G. H., Friedemann, T. E., and Youmans, J. B., *Arch. Biochem.,* 1946, v10, 33.

11. McIlwain, H., *Brit. J. Exp. Path.,* 1942, v23, 95.

12. Shive, W., and Mason, J., *J. Biol. Chem.,* 1946, v162, 451.

13. Shive, W., and Roberts, E. C., *ibid.,* 1946, v162, 463.

14. Winkler, K. C., and de Haan, P. G., *Arch. Biochem.,* 1948, v18, 97.

15. Youmans, G. P., and Youmans, A. S., *Am. Rev. Tuberc.,* 1951, v64, 541.

Received August 24, 1953. P.S.E.B.M., 1953, v84.