Absorption and Excretion of Streptomycin Para-aminosalicylate.* (17465)

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(Introduced by C. C. Sturgis)

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Streptomycin has become firmly established as a valuable agent in the treatment of selected cases of human tuberculosis. Para-aminosalicylic acid (hereafter designated PAS) is effective in the treatment of experimental tuberculosis in animals[1] and appears to enhance the effectiveness of streptomycin when it is used in combined therapy in experimental infections.[2] Recent evidence from clinical trials[3,4] suggests that a combination of parenteral streptomycin and oral PAS acts to delay the accumulation of strains of tubercle bacilli resistant to either drug.

Basic streptomycin can be combined with 3 molecules of PAS to form streptomycin para-aminosalicylate‡ (hereafter designated streptomycin PAS); one gram of streptomycin combining with 0.8 g of PAS. The resulting chemical is very slightly, if at all, more irritating when injected intramuscularly than is an equivalent amount of streptomycin. It has been found to be superior to streptomycin in experimental tuberculosis in the mouse;[5] a daily dose of streptomycin PAS, equivalent to 0.4 mg of streptomycin affording the same degree of protection as that produced by 2 to 3 mg of streptomycin sulphate. The present report concerns the results of investigation into the fate of streptomycin PAS in human subjects.

Materials and methods. The subjects were young patients with minimal or moderately advanced pulmonary tuberculosis as the only significant deviation from normality. Streptomycin PAS was injected intramuscularly in quantities equivalent to 1.0 g of streptomycin (0.8 g of PAS) in 4 subjects, and 0.5 g of streptomycin (0.4 g of PAS) in 4 other subjects. Plasma specimens (heparinized) were obtained for control purposes, and at 15 minutes, 30 minutes, and 1, 2, 4, 8 and 12 hours following injection of the material. Urine was collected in 3 pooled specimens each covering a 4 hour period. For purposes of comparison 2 of the above subjects were given an amount of PAS (0.8 g) orally equivalent to that contained in the streptomycin PAS which they had received by intramuscular injection in a previous experiment. Two subjects were given streptomycin PAS orally and in this case plasma specimens were collected only at 30, 60 and 120 minutes. Concentrations of PAS in plasma were determined by the method described by Marshall[6] using sodium para-aminosalicylate as standard material for calibration of the Evelyn colorimeter. Streptomycin concentrations were measured by the cup-plate method of bioassay in use in this laboratory. According to custom, concentrations of PAS are expressed as mg per 100 ml while concentrations of streptomycin are expressed as γ per ml. The control plasma in most individuals contains interfering substances which give readings for PAS estimated as near, but slightly less than 0.05 mg per 100 ml. This figure has, there-

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1 Feldman, W. H., Karlson, A. G., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clin.*, 1947, **22**, 473.

2 Youmans, G. P., Youmans, A. S., and Osborne, R. R., *Journal-Lancet*, 1947, **67**, 403.

3 Karlson, A. G., Pfuetze, K. H., Carr, D. T., Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clin.*, 1949, **24**, 85.

4 Delaude, A., Karlson, A. G., Carr, D. T., Feldman, W. H., and Pfuetze, K. H., *Proc. Staff Meet., Mayo Clin.*, 1949, **24**, 341.

‡ Prepared and furnished by the Charles Pfizer Company, Brooklyn, N. Y.

5 Hobby, G. L., and Lenert, T. F., Presented at the 7th Streptomycin Conference, Veterans Administration, Denver, April 1949.

6 Marshall, E. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 471.

fore, been accepted as a baseline. The addition of streptomycin PAS to water or serum gives recoveries, by the above methods, very near the expected values for both PAS and streptomycin. This would appear to indicate that the chemical union does not interfere with the biologic activity of streptomycin nor with the chemical determination of PAS.

Results and discussion. The plasma streptomycin concentrations, measured in the subjects who received streptomycin PAS intramuscularly, varied but were within the range expected for an equivalent amount of streptomycin. The values obtained for plasma PAS likewise varied considerably, particularly in the early minutes of absorption. Peak values developed between 15 and 30 minutes after intramuscular injection and averaged 1.35 mg per 100 ml in the subjects receiving an amount of streptomycin PAS equivalent to 1.0 g of streptomycin. In those subjects receiving a dose one-half this size the peak plasma concentrations averaged 0.6 mg per 100 ml. Concentrations fell rapidly and were at the base line after 4 hours in the group receiving the smaller dose and only very slightly above the base line in those who received the larger amount. This data is illustrated in Fig. 1. In Fig. 2, the curve of plasma PAS following ingestion of 0.8 g of PAS is compared with that obtained, in the same individual, following intramuscular administration of an equivalent amount of PAS as streptomycin PAS. The curves are very similar save that the peak concentration appeared later when the material was taken orally. Virtually the same relationships were observed in a second individual.

One fairly consistent feature of these experiments was the disassociation between the curves for PAS and for streptomycin concentrations in plasma. Peak levels for PAS appeared between 15 and 30 minutes while those for streptomycin occurred from 1 to 2 hours after injection (Fig. 3). Presumably this is due to rapid conjugation of the PAS radical[7] although it may be due, at least in part, to

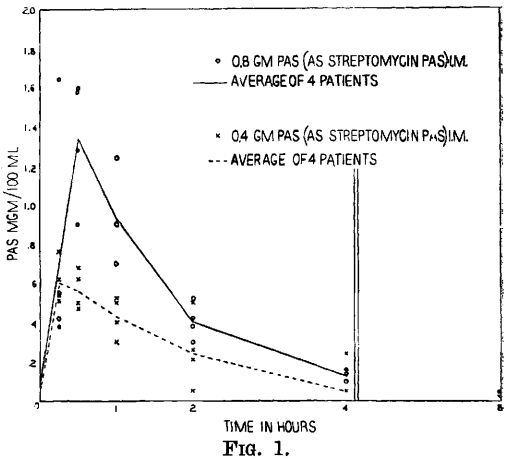

FIG. 1.

Plasma concentrations of PAS following intramuscular administration of streptomycin PAS in amounts equivalent to 1 g of streptomycin (0.8 g PAS) and 0.5 g streptomycin (0.4 g PAS). The lines indicate average values for each dosage level.

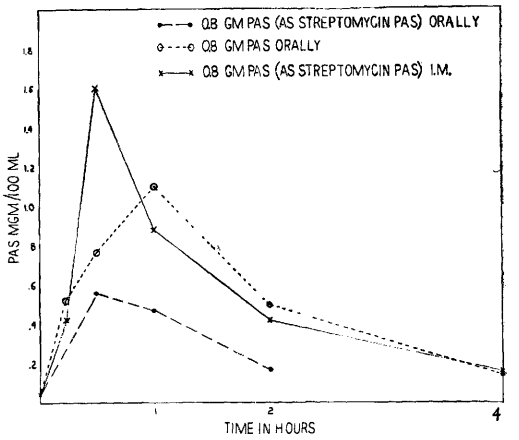

FIG. 2.

Comparison of plasma concentrations of PAS following equivalent amounts of: (1) Streptomycin PAS intramuscularly. (2) Streptomycin PAS orally. (3) PAS orally.

breakdown of the compound followed by independent behavior of streptomycin and PAS or its derivatives. Calculations of the expected levels of PAS, based on the concentrations of streptomycin in the plasma, were often fairly close to the observed levels of PAS for specimens taken 15 minutes after injection of the material. Later specimens usually exhibited PAS levels far below the calculated values, which might be expected if the PAS were undergoing rapid conjugation.

[7] Way, E. L., Smith, P. K., Howie, D. L., Weiss, R., and Swanson, R., *J. Pharm. and Exp. Therap.*, 1948, **93**, 369.

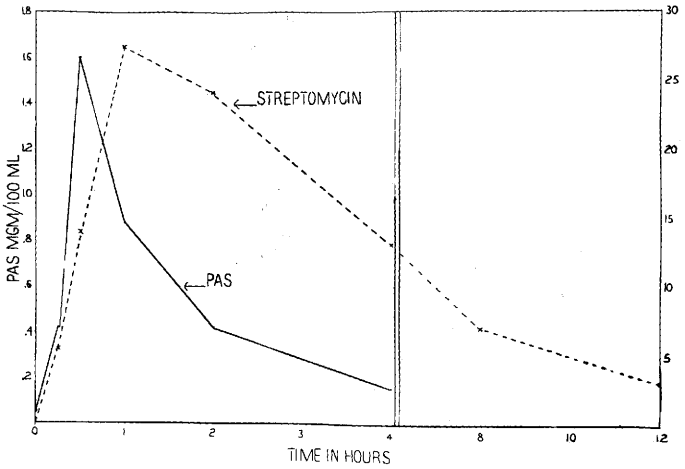

FIG. 3.
Plasma values for PAS and for streptomycin following intramuscular administration of an amount of streptomycin PAS equivalent to 1 g of streptomycin.

Streptomycin could not be detected in the plasma following oral administration of streptomycin PAS. The concentrations of PAS which developed were considerably lower than those expected from an equivalent amount of PAS, taken by mouth, or injected intramuscularly as streptomycin PAS, indicating that the material is certainly broken down to its components in the gastro-intestinal tract, though possibly not completely.

Urinary recovery of PAS following intramuscular administration of streptomycin PAS ranged from 9 to 24% (average 14%) with virtually the entire amount appearing within 4 hours of administration. Recovery of streptomycin averaged the expected 60%.

The concentrations of PAS in plasma following intramuscular administration of streptomycin PAS are not particularly impressive when compared with those following large amounts of PAS given orally (peak concentrations of 4 to 8 mg per 100 ml following doses of 3 g). They assume somewhat greater significance, however, when considered in relation to the sensitivity of newly isolated strains of tubercle bacilli which are reported[4] to be sensitive to PAS in concentrations in the neighborhood of 0.012 mg per 100 ml of medium. These results, coupled with the effectiveness of the material in experimental tuberculosis[5] and the simplicity of administration, would seem to indicate that streptomycin PAS is worthy of clinical trial in human tuberculosis on an experimental basis.

Summary. Streptomycin PAS may be given intramuscularly without undue irritation. Chemical assay of PAS, and bioassay of streptomycin, each give virtually quantitative recovery of streptomycin PAS *in vitro*. Following intramuscular administration of streptomycin PAS in human subjects, streptomycin was demonstrated in the plasma and urine within the expected range. PAS also appeared in significant concentrations, but the quantitative relation to streptomycin was altered.

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