

Timonin. The antibacterial spectra of the two substances are similar and the activity of mold SD-17 may be due to citrinin. The active material produced by mold SD-17 has not been obtained in a crystalline form, and its identity has not been determined.

*Summary.* Filtrates of 13 mold cultures

were found to possess antibiotic activity against *Br. abortus in vitro*. The active material from one of these molds, tentatively identified as *Aspergillus terreus*, after partial purification, inhibited growth of *Br. abortus* in a dilution of 1:64,000.

## 15720

### Effect of Para-Aminohippurate on Mannitol Determinations by the Periodate-Iodide-Thiosulfate Method.\*

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In using the combination of mannitol<sup>1</sup> and para-aminohippurate<sup>2</sup> in renal function studies we noticed discrepancies which suggested to us that the presence of para-aminohippurate (PAH) might be affecting the chemical analyses for mannitol. Therefore, we prepared solutions containing no mannitol but in which known amounts of free para-aminohippuric acid (PAHA) were added in varying concentrations to water and to sugar- and protein-free filtrates of human plasma and of urine. These solutions were then analyzed by the technic used for estimation of mannitol.<sup>1</sup> This involves oxidation of mannitol with a known excess of  $\text{KIO}_4$  and determination, by titration with thiosulfate, of the remaining  $\text{KIO}_4$  together with the  $\text{KIO}_3$  produced in the reaction. Fig. 1 shows the results of these analyses. It can be seen that for every mg % of free PAH present, there is an apparent mannitol concentration of from 0.25 to 0.30 mg %.

In order to illustrate the extent to which this error can be expected to influence the various data of clinical interest, in a representative renal work-up with mannitol and PAH, we have prepared Table I. The data are calculated for an hypothetical patient based on a plasma mannitol level of 1 mg per cc throughout; a plasma PAH level of 0.02 mg per cc during the "plasma flow period" and 0.75 mg per cc during the "Tm period." The glomerular filtration rate is taken as 130 cc per min., the effective renal plasma flow as 670 cc per min., and the  $\text{Tm}_{\text{PAH}}$  as 75 mg per min. It is assumed that there is a false elevation of 0.27 mg % in mannitol levels for each mg % of PAH present.

One study in the literature<sup>3</sup> reports the glomerular filtration rate (mannitol) on each of 2 different days (4 in 2 cases) in 23 patients. In each instance, the mannitol clearance was done during a Tm determination with PAH on one day and during a diodrast Tm on the other day. In the 25 pairs, the mannitol clearance was higher on the PAH day in 21 cases and lower in 4 instances. The mean  $C_M$  was 9.2% higher when PAH was present at Tm levels than on days when diodrast was used. This agrees well with the 10% expected error listed in Table I.

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<sup>1</sup> Smith, W. W., Finkelstein, N., and Smith, H. W., *J. Biol. Chem.*, 1940, **135**, 231.

<sup>2</sup> Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. Clin. Invest.*, 1945, **24**, 388.

<sup>3</sup> Chasis, H., Redish, J., Goldring, W., Ranges, H. A., and Smith, H. W., *J. Clin. Invest.*, 1945, **24**, 583.

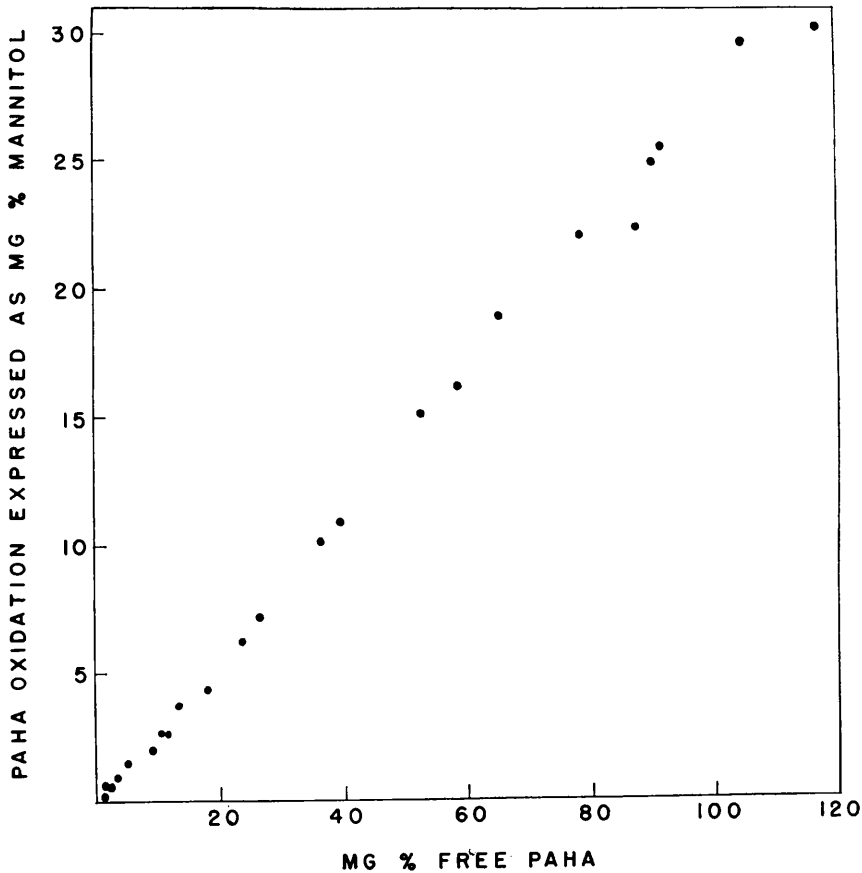


FIG. 1.

Para aminohippuric acid solutions analyzed by the unmodified method used for mannitol determination.

In the same paper an attempt was made to establish the  $Tm_{PAH}$  for normal subjects. One series of 10 cases gave a mean  $Tm_{PAH}$  of 67.6 using mannitol to determine glomerular filtration rate and another series of 21 cases

gave a mean of 82.2 using inulin for filtration rate estimation. It would seem that the normal  $Tm_{PAH}$  is probably higher than 77.5 (the average for the 2 series) and the standard deviation is less than 12.9, as there

TABLE I.

Errors in Clinical Data to Be Expected from the Unmodified Mannitol Method When Used in the Presence of PAH.

|                                                                         | Correct | Apparent         |                   | % error          |                   |
|-------------------------------------------------------------------------|---------|------------------|-------------------|------------------|-------------------|
|                                                                         |         | During $C_{PAH}$ | During $Tm_{PAH}$ | During $C_{PAH}$ | During $Tm_{PAH}$ |
| Mannitol excretion ( $U_M V$ ) mg/min                                   | 130     | 134              | 173               | 3                | 30                |
| Plasma mannitol level ( $P_M$ ) mg/cc                                   | 1       | 1.006            | 1.2               | 0.6              | 20                |
| Glomerular filtration rate ( $C_M$ ) cc/min                             | 130     | 133              | 143               | 2                | 10                |
| Max. tubular excretory capacity ( $Tm_{PAH}$ ) mg/min                   | 75      |                  | 67                |                  | 10                |
| Filtration fraction ( $C_M/C_{PAH}$ )                                   | 0.194   | 0.20             |                   | 3                |                   |
| Filtration rate per functioning unit ( $C_M/Tm_{PAH}$ )                 | 1.9     |                  | 2.6               |                  | 40                |
| Effective renal plasma flow per functioning unit ( $C_{PAH}/Tm_{PAH}$ ) | 8.9     |                  | 10                |                  | 10                |

See text for plasma levels, etc., assumed in preparation of this table.

TABLE II.  
Mannitol Recoveries by the Modified Method.

| Substance to which<br>mannitol and PAH<br>were added | PAH mg %<br>by weight | Mannitol mg % |             | Error<br>% |
|------------------------------------------------------|-----------------------|---------------|-------------|------------|
|                                                      |                       | by weight     | by analysis |            |
| Water                                                | 0                     | 10.0          | 9.92        | -1         |
| "                                                    | 8.72                  | 8.35          | 8.28        | -1         |
| "                                                    | 8.72                  | 8.35          | 8.10        | -3         |
| "                                                    | 10.46                 | 10.02         | 10.06       | +0.04      |
| "                                                    | 10.46                 | 10.02         | 10.17       | +2         |
| Urine filtrate                                       | 10.46                 | 10.02         | 9.92        | -1         |
| Plasma "                                             | 10.46                 | 10.02         | 9.87        | -1         |
| "                                                    | 77.0                  | 96.6          | 99.6        | +3         |
| "                                                    | 77.0                  | 96.6          | 97.7        | +1         |

Each recovery was run in duplicate and the analytic results are the averages. Samples with like concentrations represent separate dilutions in different volumetric flasks, but from the same stock solutions.

reported.

Unfortunately the oxidation of PAH is not sufficiently quantitative to enable accurate correction of mannitol figures on the basis of that reaction.

Acetylation of PAH was found to prevent its oxidation and it was possible to accomplish this without detectable acetylation of mannitol taking place.

Hydrolysis of acetylated PAH to free PAH occurs when it is boiled in the  $\text{KIO}_4\text{-H}_2\text{SO}_4$  solution usually used, but this can be avoided by replacing the sulfuric acid with acetic acid in the periodate solution. The sulfate which persists from the  $\text{CdSO}_4\text{-H}_2\text{SO}_4$  solution used for precipitation of proteins does not seem to affect results.

**Method.** A modification of the periodate-iodide-thiosulfate method<sup>1</sup> which we have found to be satisfactory is as follows: 1. Glucose is removed from plasma and urine samples by yeast fermentation and protein is precipitated with cadmium sulfate. 2. Duplicate 2 cc samples of filtrate are pipetted into pyrex ignition tubes. 3. Roughly 1 cc of acetic anhydride is added from a bacterial pipette and the tubes are shaken several times during the next 5 or 10 minutes. 4. Exactly 5 cc of  $\text{KIO}_4$ -acetic acid solution (0.06 Gm

$\text{KIO}_4$  dissolved in 100 cc of 20% acetic acid) is pipetted into the tubes. The tubes are covered with glass tears and boiled in a water bath for 20 minutes. 5. After cooling, about 0.5 cc of a 10% KI solution is added and the liberated iodine titrated immediately with sodium thiosulfate, using a starch indicator.

Recoveries of mannitol determined by this method in the presence of PAH are shown in Table II.

**Summary.** 1. The periodate-iodide-thiosulfate technic for mannitol determinations requires revision when para-aminohippurate is present. For each mg % of free PAH present, the mannitol recovery will be falsely elevated by from 0.25 to 0.30 mg %. 2. A modification of the technic is presented by which mannitol can be determined in the presence of PAH.<sup>§</sup>

<sup>§</sup> A method for mannitol estimations has been advanced<sup>4,5</sup> which measures quantitatively one of the products of the reaction between mannitol and periodate. The presence of PAH should make no difference in such a procedure if a sufficient excess of periodate is used.

<sup>4</sup> Corcoran, A. C., and Page, I. H., *Fed. Proc.*, 1946, **5**, 130.

<sup>5</sup> Page, I. H., personal communication.