The fluid inside the capsule (±5 cc) was inoculated with the fungus under investigation, while the outside fluid was inoculated with a recently isolated strain of virulent tubercle bacilli. While a rapid and easy exchange of metabolic product occurred through the alundun wall, the coarse fungus filaments did not as a rule penetrate it and invade the outer fluid. In the few cases in which this happened, the filtrate from the fungus growth was tested for the presence of growth inhibiting substances.

Of a large number of fungi, recently isolated from a variety of sources, only one, a Fusarium, sp., inhibited growth of the tubercle bacillus.

The inhibiting substance passes through fritted glass filters and Mandler filters but is partially inactivated by passage through Seitz filters. The crude filtrate is active up to a dilution of 1/20 and withstands heating to 100° for 15 minutes without loss of activity.

Experiments are now in progress to isolate the active principle and determine its activity *in vivo*.

C. D. Sherbakoff, Head Dept. of Plant Pathology, The University of Tennessee, Agricultural Experiment Station, has kindly identified the Fusarium as *Fusarium scirpi* Lamb. et Fautr. v. accuminatum (Ell. et Ev.) Wr. Wollenweber in his monograph on Die Fusarien, Z. f. Parasitenkunde, Berlin, 1931, places this Fusarium as No. 930 in a list of 1100.

## 15671 P

## Urinary Excretion of Orally Administered Pteroylglutamic Acid.*

RUTH STEINKAMP, CARROLL F. SHUKERS, JOHN R. TOTTER, AND PAUL L. DAY.

*From the Department of Physiological Chemistry, School of Medicine, University of Arkansas, Little Rock.*

*Introduction.* Few studies on the urinary excretion of pteroylglutamic acid have been reported.[1-4] No published information concerning the excretion of this vitamin after single large doses is available. Consequently, we are reporting results of such studies on 9 normal subjects and 9 hospitalized patients representing assays on 54 24-hour urine samples.

*Methods.* Since the primary purpose of the study was to determine the recovery of the free pteroylglutamic acid, no resort was made to the use of enzymes to liberate any conjugated vitamin.

One or more preliminary 24-hour urine samples were obtained in all but 2 instances. The test dose of synthetic pteroylglutamic acid was given orally (5.0, 5.1, 10 or 16 mg) and samples of urine were collected at varying intervals for the 24-hour period immediately following. The samples were preserved under benzene and aliquots were suitably diluted for determining the PGA microbiologically[5] with *Streptococcus faecalis*

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TABLE I.
Preliminary Twenty-four-hour Urinary Excretion of Pteroylglutamic Acid.

| Normal Subjects | | | | Hospital Patients | | | | |
|---|---|---|---|---|---|---|---|---|
| Subject No. | Sex | 24-hour excretion level pteroylglutamic acid, μg | Avg, μg | Subject No. | Sex | Diagnosis | 24-hour excretion level pteroylglutamic acid, μg | Avg, μg |
| 1 | M | 2.4; 2.9 | 2.65 | 10 | M | Pituitary deficiency | 2.3; 5.5 | 3.9 |
| 2 | M | 2.4; 3.4 | 2.9 | 11 | M | Lymphatic leukemia | 0.9; 1.1; 1.9; 1.4; 1.0; 2.3; 0.8 | 1.3 |
| 3 | F | 6.0; 2.8; 3.4 | 4.07 | 12 | F | Hemorrhagic anemia | 1.7 | 1.7 |
| 4 | F | 3.0; 1.5 | 2.25 | 13 | M | Myelogenous leukemia | 1.0; 2.3; 1.8 | 1.7 |
| 5 | M | 2.0 | 2.0 | 14 | M | Anemia | 1.8 | 1.8 |
| 6 | F | 2.7; 1.5 | 2.1 | 15 | F | Lymphosarcoma | 8.5; 4.7; 2.3; 2.6; 1.0; 1.3; 6.9; 11.9 | 4.9 |
| 7 | F | 2.9 | 2.9 | 16 | M | Anemia | 0.9; 0.7 | 0.8 |
| 8 | M | 0.07 | 0.07 | 17 | M | ,, | | |
| 9 | F | 2.0 | 2.0 | 18 | M | Cardiac insufficiency | | |
| | | Mean | 2.34 μg | | | Mean | | 2.3 μg |
| | | Standard deviation | ±1.08 μg | | | Standard deviation | | ±1.5 μg |

(American Type Culture Collection No. 8043). Synthetic pteroylglutamic acid was used as the standard. Turbidimetric readings were made with a Coleman spectrophotometer.

*Results.* The assay values for the preliminary 24-hour periods are given in Table I. The mean value for 15 determinations on normal subjects was 2.34 $\gamma \pm 1.08\ \gamma$; that for the 24 studies on the hospitalized patients was 2.3 $\gamma \pm 1.5\ \gamma$. These values are in satisfactory agreement with results obtained by Denko *et al.*[1] and by Wright and Welch[3] on normal individuals.

Data on the rate of excretion of the test doses are given in Fig. 1. The data are presented in terms of the percentage of test dose excreted, since this appears to be independent of the size of the test dose used.

The curve is drawn through the cumulative average recoveries from the normal individuals for 4, 8 and 24 hours. The greater part of the excretion of the vitamin took place between the second and eighth hours following oral administration. The total 24-hour percentage recovery for the normal subjects averaged 28.5 (range 24.5-37.5%). With the exception of one patient who had received 5 mg of "Folvite"* 12 days previously, the total 24-hour recoveries for the 8 samples from hospital patients fell below 11% (range for all patients 1.75-26.9%). The diagnoses and the preliminary urinary pteroylglutamic acid excretion levels are presented in Table I. The surprisingly small percentage recoveries of the vitamin in these patients may indicate either a low degree of saturation, increased destruction, increased requirement, or the conjugation of pteroylglutamic acid before excretion. The data at hand do not permit a choice among these possibilities.

*Summary.* Fifty-four urine samples for 24-hour periods have been assayed microbiologically for free pteroylglutamic acid. For normal subjects the average 24-hour excretion was 2.34 γ. Following an oral dose of pteroylglutamic acid the mean percentage recovery was 28.5%. Although the hospitalized patients studied had a similar excretion level (average 2.3 γ for 24 hours) prior to dosage with pteroylglutamic acid, the per-

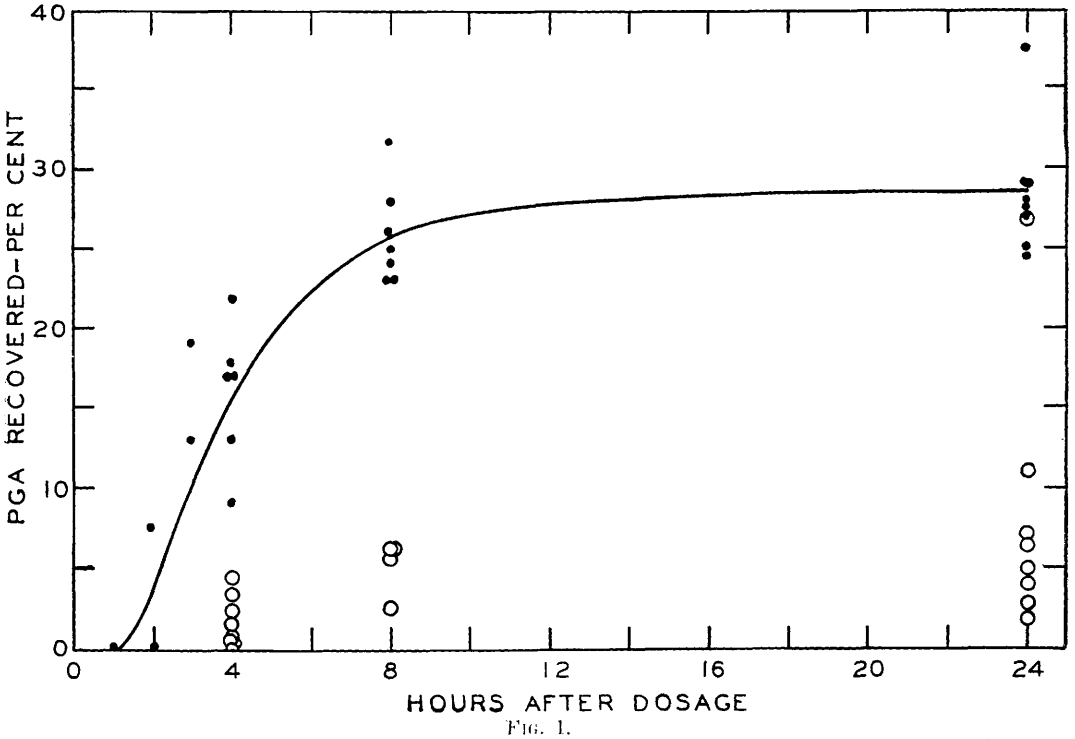


FIG. 1.

The percentage urinary recovery of pteroylglutamic acid after single oral doses of 5.0, 5.1, 10 or 16 mg. ●—Normal subjects; ○—hospital patients.

centage returns from single oral doses were much lower than in the normal subjects, a finding which is possibly indicative of a low degree of saturation.

15672 P

## Effect of Folic Acid upon Primitive Erythrocytes *In vitro*.*

EDWIN E. HAYS.†

*From the Department of Biochemistry, University of Vermont, College of Medicine, Burlington, Vermont.*

Since the antianemic properties of pteroylglutamic acid (synthetic folic acid) were reported[1] there have been many studies to determine how this substance acts upon the bone marrow to stimulate the production of cells and to ascertain the relationship of pteroylglutamic acid to the antipernicious anemia principle. Efforts to demonstrate significant amounts of pteroylglutamic acid in highly potent antipernicious anemia liver fractions have failed.[2,3] Attempts made to liberate free pteroylglutamic acid from ground muscle treated with normal gastric juice according to the technic of Castle and Town-

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† With the technical assistance of Miss Betty Paulsen, Mrs. Francena Galbraith, and Miss Constance Brownell.

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