appeared to be approximately as effective as were larger amounts in producing reticulocytosis and an increase in erythrocyte count. The administration of 30 mg daily did not cause significant improvement over that seen with 15 mg a day (cases 4-5-6, Table II). However, the only patient (Case 3) whose red cell count reached 5 million was given very large amounts of pteroylglutamic acid initially. The 7 patients who have been followed for prolonged periods have received only 5 mg daily for the past 3 to 6 months. During this time the erythrocyte counts have remained essentially constant. This dosage seemed to be sufficient and may be more than the minimal amount needed for maintenance.

The initial leukocyte count was low (less than 3500 per mm^3) in 6 patients included in this study. In each instance there was a return to normal when pteroylglutamic acid was administered and the percentage of granulocytes increased. The rise in white cells began during the first week of therapy and reached a maximum in 1 to 3 weeks.

The bone marrow was studied in most of the patients treated with pteroylglutamic acid and showed a rapid return to normal. In pernicious anemia the marrow is hyperplastic, megaloblasts being the predominant cell. In one patient in whom serial studies were undertaken, megaloblasts had decreased in number at the end of 5 days and were rarely observed at the end of 2 weeks. In patients with nutritional macrocytic anemia the marrow was at times hyperplastic and at others hypoplastic. The predominant cell was the megaloblast in some instances, the normoblast in others. Regardless of the findings prior to therapy, the bone marrow became normal within 4 to 6 weeks.

Conclusion. The administration of pteroylglutamic acid was followed by definite improvement in 17 of 23 cases of human macrocytic anemia. The hematologic effects of pteroylglutamic acid closely resembled those of liver extract but differed in that oral administration was equally or more effective than parenteral and that restoration of the blood picture to normal was often incomplete.

15719 P

Antibiotic Activity of Certain Molds Against *Brucella.**

GRACE A. BEAL.† (Introduced by S. A. Koser.)

From the Department of Medicine and the Department of Bacteriology and Parasitology, The University of Chicago.

Although a number of mold antibiotics have been described,[1] few reports indicate activity against *Brucella spp.*[2,3] The ineffectiveness of penicillin against bacteria of this group was early recognized,[4] and this observation has received repeated confirmation. Despite the demonstration of inhibition of Brucella by high concentrations of penicillin,[5,6] resistance of these organisms is so great that the drug has been successfully used in concentrations of 2-5 Oxford units per ml for selective isolation of Brucella from material containing other more sensitive bacteria.[6]

Experimental. In a preliminary study 202 mold cultures,‡ including strains of Aspergillus, Penicillium, Absidia, Rhizopus, Mucor, Chaetomium, Trichoderma, Sporotrichum,

* Aided by a grant from Swift and Company.

† The early part of this study was made at The Brucellosis Research Laboratory, Clayton Foundation, The University of Texas.

1 Waksman, Selman A., *Microbial Antagonism and Antibiotic Substances,* The Commonwealth Fund, 1945.

2 Kocholaty, Walter, *J. Bact.,* 1942, **44**, 469.

3 T'ung, Tsun, PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 8.

4 Fleming A., *Second Int. Congress for Microbiology,* Report of Proceedings, London, 1936, 33.

5 Hobby, Gladys L., *Science,* 1944, **100**, 500.

6 Beal, G., unpublished data.

‡ Cultures were kindly made available by Dr. M. B. Morrow, Department of Botany and Bacteriology, The University of Texas.

TABLE I.
Antibiotic Activity of Certain Molds Against Brucella and Staphylococcus.

Mold culture	Source of mold	Activity of Filtrates*	
		Brucella abortus 295	*Staphylococcus aureus* 209
Aspergillus terreus No. 1	Stock collection	+	0
" " No. 2	" "	+	0
" " No. 3	" "	+	0
" " No. 4	" "	+	+
" *sydowi* No. 1	" "	0	+
" *nidulans* No. 1	" "	0	+
Penicillium carminoviolaceum No. 1	" "	+	+
" *sartoryi* No. 1	" "	+	+
" *chrysogenum*	" "	0	+
" sp., SD-1	Soil†	+	+
" " SD-21	"	+	+
Aspergillus glaucus, SD-3	"	+	+
" *versicolor*, SD-5	"	0	+
" *terreus*, SD-16	"	+	+
" " SD-17	"	+	+
" *fumigatus*, SD-19	"	0	+
" *flavipes*, SD-16	"	+	+
" *humicola*, SD-23	"	+	0
" *versicolor*, SD-7	"	0	+

* Filtrates of mold cultures in Czapek-Dox medium were tested against 0.1 ml of 1:10 dilution of bacterial culture in 100 ml tryptose—0.1% dextrose agar. + = inhibition; 0 = no inhibition.
† Freshly isolated from lot inclosing Brucella-infected cattle.

Pullularia, Hormodendrum, Curvularia, Helminthosporium, Stemphylium, Alternaria, Spondylocladium and Fusarium, were tested for inhibition of *Br. abortus* 295 (Huddleson) and *Staph. aureus*. Molds were grown at room temperature in a tryptose-0.1% dextrose broth, pH 7.3. Metabolite samples, diluted in Locke's solution, were tested for activity after 6, 9, 12, 19, and 26 days against *Staph. aureus* and *Br. abortus* by a modified cup method,[7] using 20 ml of tryptose agar seeded with 0.1 ml of a 24-hr culture of the test bacteria. One culture of *Penicillium spinulosum* inhibited growth of *Staph. aureus* and one *Aspergillus flavus* inhibited both *Staph. aureus* and *Br. abortus* by this method.

Thirty-two strains of Penicillium and Aspergillus found to be inactive in the above tests were grown in modified Czapek-Dox medium[8] and the undiluted filtrates were assayed against *Br. abortus* 295 and *Staph. aureus* 209, using 0.1 ml of 1:10 dilution of bacterial culture in 100 ml tryptose agar. Filtrates of cultures of nine of the molds were active against one or both of the bacteria. Of 24 molds isolated from soil of an inclosure in which Brucella-infected cattle were kept, 10 were found to be active by the same method (Table I). In some instances activity was slight or inconsistent.

Mold SD-17, tentatively classified as *Aspergillus terreus*, was particularly active against Brucella. On Czapek-Dox medium this culture produces a tan-colored colony which is brown on the reverse side; microscopically the heads are columnar, and the conidia smooth and globose. Further studies were made on cultures of this mold grown in Czapek-Dox broth. Active filtrates of a 14-day culture were wine-colored. The active material is found in the ether soluble fraction, is heat stable (boiling 10 minutes), and after partial purification inhibits *Br. abortus in vitro* in a dilution of 1:64,000.

Since citrinin is produced by a related species,[9] comparative studies have been made with crystalline citrinin supplied by Dr.

[7] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

[8] Hobby, Gladys L., Meyer, Karl, and Chaffee, Eleanor, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

[9] Timonin, M. I., *Science*, 1942, **96**, 494.

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.*

HAROLD G. BARKER† AND JOHN K. CLARK.‡ (Introduced by C. F. Schmidt.)

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia.

In using the combination of mannitol[1] and para-aminohippurate[2] 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.[1] This involves oxidation of mannitol with a known excess of KIO_4 and determination, by titration with thiosulfate, of the remaining KIO_4 together with the 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 Tm_{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[3] 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.

* The expenses of this investigation were defrayed in part by a grant from the Life Insurance Medical Research Fund to the Department of Pharmacology, University of Pennsylvania.

† Research Fellow in Pharmacology and Fellow in the Harrison Department of Surgical Research.

‡ Research Fellow in Pharmacology.

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

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

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