were made frequently from this cage and placed in a refrigerator until a quantity sufficient for extraction was secured. Since all specimens were used promptly after collection, only refrigeration was used for preservation.

Table I shows the results of extractions of chimpanzee urine and clinical specimens. All letters refer to the clinical cases. The practice of occasional alternation of tests with chimpanzee and clinical samples was considered necessary in order to eliminate the possibility that negative findings might be due to chance variations or inadequacies of technic. This control was indicated especially by the fact that the method was being used for the first time in these laboratories.

Pregnandiol was recovered from all of the human specimens except that from Subject B, a menstrual cycle specimen. It was never found in chimpanzee urine. The amount of final residue from the chimpanzee urines was negligible in every instance.

11888

Neutralization (*in vitro*) of Bacteriostatic Activity of Sulfonamides by P-Aminobenzoic Acid.

MAURICE LANDY AND JURO WYENO. (Introduced by Paul György.)
From the Research Laboratories of the S.M.A. Corporation, Chagrin Falls, Ohio.

Many theories have been advanced to explain the bacteriostatic activity of sulfanilamide and related compounds, but few have convincing evidence in their support. Among the more recent theories advanced is that of Fildes,[1] who proposes that inhibitors of bacterial growth are effective as a result of interference with an "essential metabolite", a substance which has an essential part in the chain of synthesis necessary for bacterial growth. The interference in the case of sulfanilamide as has been demonstrated by Woods,[2] consists in the competition for an enzyme associated with the essential metabolite and the inhibition occurs in this way because of a chemical similarity to the essential metabolite.

Woods and Fildes[2, 3] prepared a number of compounds in which

[1] Fildes, P., *Lancet*, 1940, **1**, 955.
[2] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.
[3] Woods, D. D., and Fildes, P., *Chem. Ind.*, 1940, **59**, 133.

the sulfonic residue of sulfanilamide was replaced by the carboxylic residue and tested for sulfanilamide neutralization. Of all the compounds of this type tested, p-aminobenzoic acid was found to be by far the most active.

The results of our study on the effect of p-aminobenzoic acid on the bacteriostatic activity of sulfanilamide, sulfapyridine and sulfathiazole on streptococci, pneumococci, and staphylococci are reported here. In addition, the isomers and 2 derivatives of p-aminobenzoic acid have been examined for anti-sulfanilamide activity.

Anti-Sulfanilamide Activity of p-Aminobenzoic Acid. In Table I there is shown the results of a typical experiment in which p-aminobenzoic acid was examined for anti-sulfanilamide activity using *Streptococcus hemolyticus*. From this experiment it will be noted that the amount of sulfanilamide used completely inhibited the growth of streptococci in heart infusion broth. Complete neutralization of the sulfanilamide growth inhibition was brought about by the maximum concentration of p-aminobenzoic acid since the amount of growth obtained when this quantity was present approached that found in the inoculated infusion broth. Decreasing concentrations of p-aminobenzoic acid resulted in progressively diminished growth.

Anti-Sulfapyridine Activity of p-Aminobenzoic Acid. Table II gives the results of an experiment in which the ability of p-aminobenzoic acid to neutralize the bacteriostatic action of sulfapyridine on the pneumococcus was determined. As in the previous experiment, the amount of growth was in general, proportional to the concentration of the p-aminobenzoic acid.

TABLE I.

Effect of P-aminobenzoic Acid on Sulfanilamide Inhibition of *Streptococcus hemolyticus* Growth. Inoculum Streptococcus S-20, ±32.*

Para-aminobenzoic acid μg	Sulfanilamide μg	Growth—Colonies/1 cc in 48 hr
500	1000	690,000,000
100	"	491,000,000
50	"	440,000,000
40	"	401,000,000
30	"	310,000,000
20	"	270,000,000
10	"	145,000,000
5	"	100,000,000
1	"	373,000
.5	"	130,000
.1	"	4,000
.05	"	0
—	"	0
—	—	840,000,000

**Streptococcus hemolyticus* S-20. Western Reserve University. Inoculum—1 cc 1 × 10-7 of a 24-hour broth culture. Medium—Difco heart infusion broth.

TABLE II.
Effect of P-aminobenzoic acid on Sulfapyridine Inhibition of *Diplococcus pneumoniæ* Growth. Inoculum Pneumococcus NY-5 ± 96.*

Para-aminobenzoic acid μg	Sulfanilamide μg	Growth—Colonies/1 cc in 48 hr
100	2000	221,000,000
50	"	94,000,000
25	"	28,000,000
10	"	2,080,000
1	"	14,200
.5	"	1,680
—	"	1,000
—	—	260,000,000

**Diplococcus pneumoniæ* NY-5. Ohio State University. Inoculum 1 cc 1×10^{-7} of a 24-hour broth culture. Medium—Difco dextrose heart infusion broth.

Anti-Sulfathiazole Activity of p-Aminobenzoic Acid. The effect of the concentration of p-aminobenzoic acid on growth inhibition of staphylococcus by sulfathiazole is demonstrated in Table III. As in the 2 previous experiments the growth was proportional to the concentration of p-aminobenzoic acid, the larger the concentration of this compound, the greater the amount of growth in the presence of a constant amount of sulfathiazole.

TABLE III.
Effect of P-aminobenzoic Acid on Sulfathiazole Inhibition of *Staphylococcus aureus* Growth. Inoculum—Staphylococcus 6-14, ± 38.*

Para-aminobenzoic acid μg	Sulfathiazole μg	Growth—Colonies/1 cc in 24 hr
250	2000	440,000,000
100	"	196,000,000
50	"	85,000,000
25	"	42,000,000
10	"	36,200,000
1	"	17,200,000
0.1	"	6,080,000
—	"	4,200,000
—	—	1,502,000,000

**Staphylococcus aureus* 6-14. Ohio State University. Inoculum—1 cc 1×10^{-7} of a 24-hour broth culture. Medium—Difco heart infusion broth.

Anti-sulfanilamide activity of compounds related to p-aminobenzoic acid. It was of considerable interest to determine whether the 2 isomers of p-aminobenzoic acid exhibited similar activity; accordingly o-aminobenzoic acid and m-aminobenzoic acid were tested in concentrations as high as 1 mg. No anti-sulfanilamide effect was observed. In addition, p-aminophenyl acetic acid and p-aminophenyl glycine dihydrochloride were tested in the same manner and concentration with no discernible activity.

Para-aminobenzoic acid controls were included in all of the experiments. In all cases p-aminobenzoic acid in the absence of

sulfonamide inhibitor had no discernible effect on the growth of the test organisms.

Our findings on the reversal of sulfonamide inhibition of streptococcal growth by p-aminobenzoic acid are in good agreement with those of Woods. We have in addition demonstrated the *in vitro* neutralization of the bacteriostatic effect of sulfapyridine and sulfathiazole by p-aminobenzoic acid.

The data indicate that the same neutralization mechanism is apparently effective regardless of the organism or inhibitor used in these experiments. There were quantitative differences in the ability of p-aminobenzoic acid to neutralize the 3 inhibitors tested but probably not much greater than could be accounted for by the difference in the molar concentrations of the inhibitors. This suggests that the various inhibitors (sulfonamides) effect micro-organisms in a similar way even though their efficiency varies from one organism to another.

Summary. The bacteriostatic effect, as measured *in vitro,* of sulfanilamide, sulfapyridine and sulfathiazole on streptococci, pneumococci, and staphylococci respectively, has been found to be completely neutralized by p-aminobenzoic acid. This observation suggests that this neutralization mechanism is similar for any of the organisms and inhibitors investigated. The specificity of the sulfonamide neutralization by p-aminobenzoic acid is demonstrated by the inactivity of its isomers and 2 related compounds.

11889 P

Correlation Between Density and Ash, Organic, and Water Contents of Calcified Tissues.*

MARTIN DEAKINS.† (Introduced by H. C. Hodge.)

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York.

There are two reliable methods for determining the density of minute particles of calcified tissues, namely (a) comparison of the refractive index with that of a liquid of known density,[1] and (b)

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† Now at University of Pennsylvania, School of Dentistry, Philadelphia, Pa.

1 Manly, R. S., and Hodge, H. C., *J. Dent. Res.*, 1937, **16**, 311.