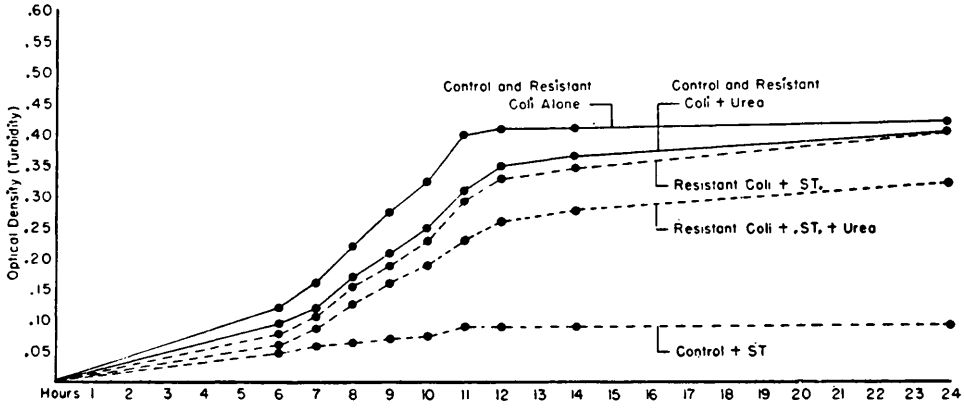

FIG. 3.

Effect upon a non-resistant and a sulfonamide-resistant strain of *E. coli* of adding urea 1% in the presence of sulfathiazole (ST) 10 mg%. See text for explanation.

the effect of urea, a bacteriostatic agent itself in high concentrations, is purely additive.

It is not to be inferred that urea-sulfonamide mixtures are of no value. On the contrary, the fact that urea is in itself a bacteriostatic agent, in addition to its solvent, peptizing, lytic, and deodorant actions, would seem to make the combination highly desirable for topical application.

Summary. Contrary to previous reports the present experiments indicate that concentrations of urea which are not bacteriostatic do not counteract sulfonamide inhibitors, nor do they potentiate the bacteriostatic action of the sulfonamides against resistant organisms. High concentrations of urea have a bacteriostatic action of their own which may be of value when they are used in combination with the sulfonamides.

14208

Presence of Hippuric Acid in Milk.

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The non-protein nitrogen, or residual nitrogen of cow's milk is known to consist in part of urea, uric acid, creatinine, amino acids, and purine bases. The remainder is composed to a large extent of compounds of unknown structure. We wish to report that one of these heretofore unidentified substances of the residual nitrogen fraction of cow's milk is hippuric acid.

In connection with the isolation of crystalline biotin from milk in this laboratory,[1] we had occasion to fractionate a biotin concentrate from milk, generously supplied by the S.M.A. Corporation of Chagrin Falls, Ohio. This material had been prepared by charcoal adsorption from a commercial milk residue which remained after the removal of protein and sugar from milk. One gram of the biotin concentrate represented approximately 15 kg of milk.

The first steps in the fractionation of this concentrate for the isolation of biotin included esterification of the concentrate with alcoholic HCl and then extraction with ethyl acetate of a slightly alkaline solution of the esterified material. The ethyl acetate extracts were concentrated to remove solvent and the residue

[1] Melville, D. B., Hofmann, K., Hague, E., and du Vigneaud, V., *J. Biol. Chem.*, 1942, **142**, 615.

TABLE I.

	M.P.	Neutral equivalent	% nitrogen	M.P. methyl ester	M.P. *p*-bromophenacyl ester
Acid isolated from milk	188°	185	7.7	84°	151-2°
Hippuric acid	188°	179	7.8	85°	152°

was dissolved in chloroform and chromatographed on a column of Decalso.[1]

It was found that the chloroform which passed through the columns contained appreciable amounts of a substance which separated in crystalline form when the solutions were concentrated. Recrystallization of this material from an alcohol-ether mixture yielded colorless needles of a nitrogen-containing compound, m.p. 84°, which by alkaline hydrolysis yielded an acid, m.p. 188°. This acid contained 7.7% N and possessed a neutral equivalent of 185. Additional amounts of the free acid were obtained by acidification of the ethyl acetate-extracted esterification residues. These data indicated that the substance isolated from the esterified milk concentrate was the methyl ester of hippuric acid. A mixture of the isolated free acid with hippuric acid showed no depression of the melting point. Further confirmation of the identity of the isolated compound was obtained by preparation of the *p*-bromophenacyl ester from the sodium salt of the acid. The melting point of this derivative agreed with that of *p*-bromophenacyl hippurate. The data are summarized in Table I.

Quantitatively, only a minimum value for the amount of hippuric acid in milk can be derived from this work, inasmuch as there was no way of knowing how much hippuric acid had been lost during the various procedures used for the preparation of the biotin concentrate from milk, and no attempt was made to obtain a quantitative separation of the hippuric acid present in the biotin concentrate. From 75 g of concentrate, representing approximately 1160 kg of milk, there were obtained 11 g of hippuric acid; this would indicate an original minimum concentration of 10 γ hippuric acid per gram of milk.

14209

Distribution of Poliomyelitis Virus in Central Nervous System of Mice Paralyzed After Intracerebral Inoculation.*

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Tests using mice inoculated with the Lansing strain of poliomyelitis virus have been handicapped by irregularities both in morbidity and in the length of incubation periods. Some of the factors related to these difficulties such as the strain of mice[1] and the pH of the inoculum[2] have been reported. Also it has been noted that serial passage of cord and brain stem material from the first mice of an inoculated group to become paralyzed resulted in shorter incubation periods while passage from the last mice to become paralyzed resulted in longer incubation periods. The age of mice was not found to be an important

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† Fellow of the Pan-American Sanitary Bureau.

1 Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 86.

2 Hammon, W. M., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 579.