betes was reported by Carrasco-Formiguera.[22]

The metabolism of diabetic rabbits requires comment. When fed they excrete only a moderate amount of carbohydrate. On fasting the glycosuria ceases, there is no ketonuria, and the nitrogen excretion is somewhat greater than that of normal fasted rabbits. These facts are compatible with partial pancreatic diabetes, and the persistence of some islet tissue makes this a probable explanation. However, pancreatic diabetes as seen in the goat[23] prompts one to ask whether alloxan may not produce a severe diabetes similar to that following pancreatectomy. In depancreatized goats there is no glycosuria or ketonuria on fasting and little or none when they are fed an ample amount of greens. After pancreatectomy or phlorhizin the nitrogen excretion is approximately equal in the goat and it is the same after alloxan or phlorhizin in the rabbit. It is thus possible that the seemingly mild diabetes of the rabbit may at times be severe diabetes for this species.

Summary. Diabetes has been produced in rabbits by the intravenous injection of alloxan.[1,2] The effectiveness of the repeated daily injections of doses as low as 50 mg per kg indicates that alloxan has a cumulative action or that a gradual extension of pancreatic injury takes place.

When alloxan was given to rabbits with alloxan diabetes no effect on the blood sugar was observed. This supports the favored hypothesis that the hypoglycemic phase of alloxan poisoning is due to the liberation of insulin from damaged cells.

Metabolic studies indicate that alloxan diabetes in rabbits corresponds to partial pancreatic diabetes. In this connection the factor of species is discussed.

Hydropic degeneration was observed in the islet tissue of 2 animals. This change has been attributed to the effect of hyperglycemia on beta cells which escaped destruction by alloxan.

22 Carrasco-Formiguera, R., *J. Lab. and Clin. Med.*, 1944, **29**, 510.

23 Lukens, F. D. W., *Am. J. Physiol.*, 1938, **122**, 729.

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Bacteriostatic Action of Sulfonamide-Penicillin and Urea-Penicillin Mixtures *in vitro*.

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The effectiveness of penicillin* in the treatment of certain types of infections has become well established. In some instances, however, particularly general sepsis and local wound infections, it is possible that combinations of penicillin with other chemo-therapeutic agents will prevent unsuccessful results that are occasionally encountered with penicillin alone. The situation is analogous to that of the sulfonamides, in which urea-sulfonamide mixtures are highly effective for topical application to infected wounds.

The following experiments were designed to study the bacteriostatic action of sulfonamide-penicillin and urea-penicillin mixtures *in vitro*.

Method. Growth curves were measured turbidimetrically under rigidly controlled conditions for a period of 24 hours, according to a method developed in this laboratory and described elsewhere in detail.[1] In the present studies the organisms were a coagulase-positive strain of *Staphylococcus aureus*, and a

* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

1 Kirby, W. M. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.

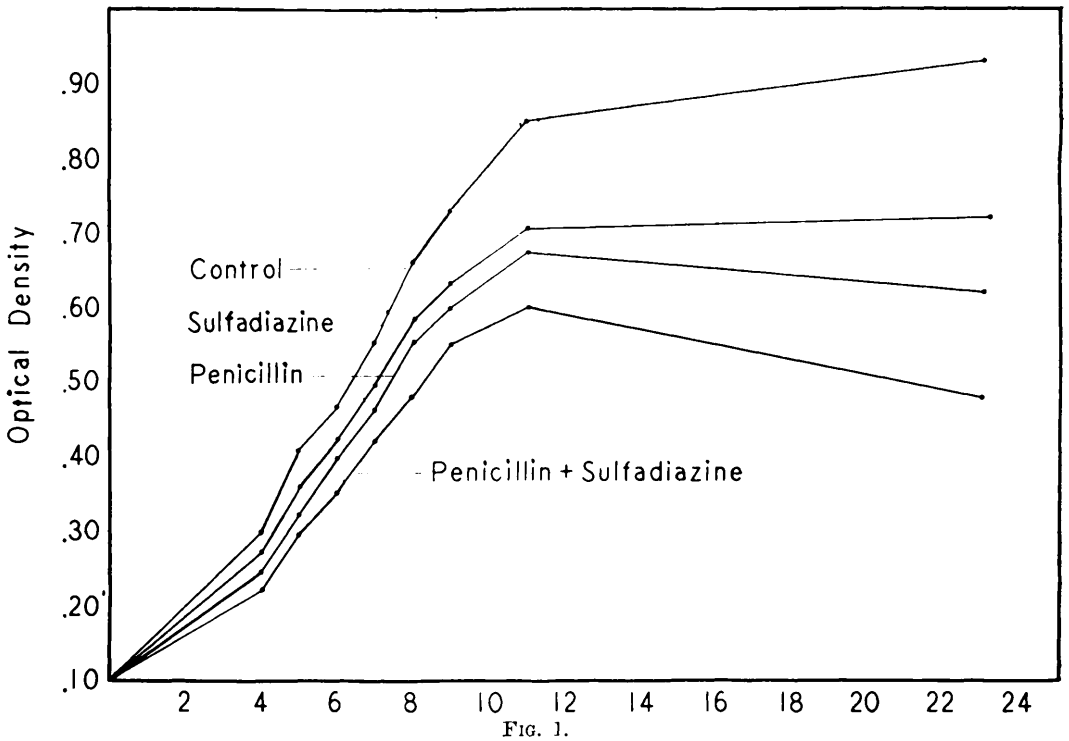

FIG. 1.

The effect of penicillin and sulfadiazine separately and in mixture upon the growth of *Staphylococcus aureus*.

Lancefield Group A hemolytic streptococcus. For the staphylococcus, 0.5% peptone was added to the synthetic medium previously described, and the streptococcus was grown in beef infusion broth. The pH of the media was not altered by the addition of urea and sulfonamide solutions.

Concentrations of penicillin, sulfadiazine, and urea that produced moderate bacteriostasis during the stage of logarithmic multiplication of the organisms were determined, and added singly and in mixtures to standardized cultures for the various experiments. For one staphylococcus urea 2%, sulfadiazine 50 mg % and penicillin 0.01 unit per cc were used, and for the streptococcus penicillin 0.05 u./cc, sulfadiazine 100 mg %, and urea 2% were found to produce adequate bacteriostasis. The number of viable bacteria at the beginning of each experiment was, for both organisms, approximately one million per cc.

Results. Sulfadiazine, urea, and penicillin in concentrations which were not bacteriostatic separately, did not cause inhibition of growth when mixed together. If concentrations were used that were bacteriostatic separately, sulfonamide-penicillin and urea-penicillin mixtures produced greater bacteriostasis than did any of these substances alone. However, in no instance was the bacteriostatic action of the mixture greater than the sum of the inhibitory effects of the individual constituents. In other words, there was no actual potentiation of penicillin action. Fig. 1 illustrates this for the staphylococcus. It is also evident from the figure that lysis of staphylococci was no greater with the mixtures than with penicillin alone.

Discussion. The results of these experiments are similar to those found previously with urea-sulfonamide mixtures; namely, that the bacteriostatic action of the mixtures represents merely the additive effects of the separate constituents.

Divergent views have been expressed concerning urea-sulfonamide mixtures,[2,3] largely as a result of differences in technical methods and in interpretation. In the opinion of the present author true potentiation can be best demonstrated when the following conditions are fulfilled: (1) The medium should support luxuriant growth of the organisms, (2) measurements of bacteriostasis should be made frequently during the phase of active growth, and (3) concentrations of the bacteriostatic agents should not be so great in relation to the size of the inoculum that, either separately or in combination, active growth of the bacteria is prevented. The last point is especially important and requires further elucidation. When the bacteriostatic action of the mixture is too strong good growth of the organism never occurs; the effect is that of rendering the medium unsatisfactory. Under these circumstances valid comparisons cannot be made with experiments in which there is good initial bacterial growth. Superficially there may appear to be true potentiation, but actually there is not. This important fact, namely, that in experiments such as these bacteria must have certain minimal conditions favoring good growth, has been previously emphasized.[4] It is believed that such pitfalls and artefacts can best be avoided by adhering to the conditions listed above. If true potentiation exists, bacteriostasis of mixtures should be greater than merely the additive effects of the separate constituents when there is active growth of all the organisms. It is clear from Fig. 1 that, under these conditions, there is no actual synergism or potentiation; the effects are merely additive.

The potential clinical significance of these observations is difficult to predict. However, in seriously ill patients who are not responding well to penicillin it would seem not unreasonable to add the effect of another bacteriostatic agent such as sulfadiazine.

Summary. *In vitro,* sulfonamide-penicillin and urea-penicillin mixtures have been shown to produce greater bacteriostasis than penicillin alone with the *Staphylococcus aureus* and hemolytic streptococcus. This increased inhibition represents the additive effects of the separate bacteriostatic agents; there is no actual potentiation of penicillin action.

[2] Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 262.

[3] Lee, S. W., Epstein, J. A., and Foley, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 105.

[4] Bloomfield, A. L., and Felty, A. R., *J. Exp. Med.*, 1924, **39**, 367.

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Folic Acid and Biotin Synthesis by Sulfonamide-sensitive and Sulfonamide-resistant Strains of *Escherichia coli.*

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When certain poorly absorbed sulfonamides, such as succinylsulfathiazole or sulfaguanidine, are incorporated in highly purified diets and fed to rats, bacteriological studies show the total number of organisms in the feces of such rats to be unaffected by the presence of the sulfonamide, although certain types of bacteria, such as the coliform organisms, may diminish or even disappear.[1] The feeding of such sulfonamide rations to weanling rats also results in the production of a nutritional deficiency[2,3] which may be corrected by feed-

[1] Gant, O. K., Ransone, B., McCoy, E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 276.

[2] Welch, A. D., *Federation Proc.*, 1942, **1**, 171.

[3] Black, S., Overman, R. S., Elvehjem, C. A., and Link, K., *J. Biol. Chem.*, 1942, **145**, 137.