Anti-penicillin effect of casein hydrolysate: Casein hydrolysate[7] of a given concentration was added to Gladstone medium containing various amounts of penicillin; as well as various concentrations of the casein were tested against 1.5 O.U. of penicillin representing 1.5 times the amount necessary for complete inhibition of *E. coli,* strain No. 42, in Gladstone medium alone. The initial number of organisms was 0.75×10^6 cells per ml. In the presence of 2.5×10^{-3} and 1.25×10^{-3} ml of casein there were required 15 and 7.5 times greater amounts of penicillin, respectively, than in the absence of casein (*i.e.,* 15 and 7.5 O.U. as compared to 1 O.U.). Furthermore, 1.25×10^{-4} ml of the hydrolysate was capable of antagonizing 50 per cent. of the activity of 1.5 O.U. of penicillin per ml. It is obvious from the above that casein hydrolysate is an active antagonist of the effect of penicillin upon *E. coli.*

Effect of certain amino acids and asparagine upon inhibition of E. coli by penicillin: In this group of experiments there was studied the effect of various concentrations of some of the amino acids[8] entering into composition of casein hydrolysate and that of asparagine[8] upon the inhibition of *E. coli* by different concentrations of penicillin in synthetic medium. Glycine, 1.5×10^{-2}M and 0.75×10^{-2}M; histidine, 2×10^{-4}M; phenylalanine, 6×10^{-4}M; and serine, 1×10^{-4}M produced a somewhat variable antagonistic effect upon the minimal inhibitory dose of penicillin in synthetic medium (*i.e.,* 1 O.U. per ml), little effect having been observed with greater concentrations of penicillin. Glutamic acid, 6.2×10^{-3}M, possessed a greater but also variable anti-penicillin property. However, a distinct antagonism against penicillin was shown by asparagine. Thus 2 mg per ml of the substance completely antagonized the effect of 1 O.U., 50 per cent. of 1.5 O.U., 10 per cent. of 3 O.U. and 2 per cent. of 6 O.U. of penicillin. Valine, 1×10^{-2}M and 0.5×10^{-2}M, and methionine, 2.5×10^{-2}M, were totally devoid of anti-penicillin activity in Gladstone medium. It is evident that the anti-penicillin property of the substances described bears no relation to molar concentration and nitrogen contents.

Effect of dl-methionine upon anti-penicillin activity of casein hydrolysate and asparagine: In synthetic medium alone methionine failed to enhance the susceptibility of *E. coli* beyond one O.U. per ml. Methionine, 2.5×10^{-2}M, appeared capable of removing 50 to 60 per cent. of the anti-penicillin activity of casein hydrolysate, 1.25×10^{-4} ml and about 75 per cent. of the activity of asparagine, 1.5×10^{-2}M.

[7] Manufactured by SMACO as "Vitamin-free" Acid Hydrolysed Casein for use in culture media.

[8] l-Histidine, Hoffmann La Roche; dl-Serine, Eastman Kodak; Glycine, l(+)Glutamic acid, dl-Methionine, dl-Phenylalanine and dl-valine, SMACO; and Asparagine, Difco standardized.

Relation of rate of growth of E. coli to the penicillin effect of broth and casein digest: In these experiments there was studied at frequent intervals of time the optical density of cultures of *E. coli* in Gladstone medium alone, Gladstone medium containing various concentrations of casein hydrolysate and in meat infusion broth. The same inoculum was used in all media, all other conditions having been kept as uniform as possible (*i.e.,* size of tubes, amount of medium, H-ion concentration, etc.). In the absence of penicillin there was less than 10 per cent. difference in bacterial concentration of all the cultures studied at the expiration of 16 hours of incubation at 37° C. The initial lag period was 3½ to 4 hours. The generation time was 2 to 3 times longer during the initial 3 hours of the log phase in cultures made in the Gladstone medium than in the remaining media. Delay in growth is known to antagonize the effect of penicillin.[9] Inasmuch as the prolongation of generation time occurred in Gladstone cultures, obviously the fact could not be held responsible for the greater activity of penicillin in these cultures. In order to bring further support to this contention, a series of tubes with casein hydrolysate in Gladstone medium, and the same mixture containing 1.5 O.U. penicillin per ml were placed in the refrigerator for periods of time varying from zero to 5 hours prior to incubation at 37° C. for 16 hours. Variations in the amount of growths in the respective tubes did not exceed 5 per cent. It may be concluded that the differences in penicillin activity observed were not caused by changes in the rate of growth in the media used.

Summary: The susceptibility of certain strains of *E. coli* and Salmonella is significantly greater in synthetic medium than in meat infusion broth. Casein hydrolysate, asparagine, glutamic acid to a lesser degree, and possibly some other aminoacids partially antagonize the effect of penicillin upon *E. coli* in synthetic medium. The antagonism of casein hydrolysate and asparagine can be removed in greater part by methionine. The observations suggest that the refractoriness of Gram-negative bacilli to penicillin is at least to some extent extrinsic in nature.

GREGORY SHWARTZMAN

THE CHANGES IN RAT KIDNEY COCARBOXYLASE ASSOCIATED WITH THE INJURIOUS EFFECTS OF dl-SERINE[1, 2]

AN injurious action of *dl*-serine in rats has been observed following its administration by stomach tube[3]

[9] M. H. Dawson, *et al., Jour. Clin. Invest.,* 20: 434, 1941; G. L. Hobby and M. H. Dawson, *Proc. Soc. Exp. Biol. and Med.,* 56: 178 and 181, 1944; C. P. Miller and Foster A. Zimmerman, *Proc. Soc. Exp. Biol. and Med.,* 56: 205, 1944.

or by parenteral injection.[4] Clinically, the animals exhibited anorexia, a rapid loss in weight, albuminuria and marked weakness. Death occurred in over 50 per cent. of the male animals between the third and the eighth day of serine administration. Pathologically, the most characteristic findings were severe necrotizing lesions of the kidney tubules.[5]

The mechanism of the production of the toxic action of serine is still very obscure. We do know that a sudden elevation of the level of serine in the blood and tissues is necessary for serine to exert its injurious action.[4, 6] It is conceivable, therefore, that some essential metabolic process may be hampered. Thus, the serine molecule may exert a mass action effect on certain essential respiratory enzymes; *e.g.*, it may behave as a competitive inhibitor. The resultant breakdown of these important enzyme systems would lead to tissue damage and necrosis.

One of us had noted in unpublished work connected with other studies,[7] that several amino acids are apparently able to function as energy rich[8] phosphate carriers for the synthesis of cocarboxylase in anaerobic liver homogenate. On the basis of this observation and other considerations, we were led to investigate the cocarboxylase systems of rat kidney as affected by serine administration.

Experiments are described here which show: (*a*) the effect of orally administered serine on the concentration of cocarboxylase in rat kidney *in vivo*. (*b*) the effect of orally administered serine on the disappearance of cocarboxylase in anaerobic rat kidney homogenate *in vitro* (probably dephosphorylation of cocarboxylase and possibly a measure of cocarboxylase phosphatase activity).[7]

Experimental

Albino rats (Rockland) weighing between 120 and 190 grams, in groups of three or more animals, were maintained on an experimental diet consisting of "Labco" vitamin-free casein 10 parts, dextrin 37, sucrose 37, Crisco 5, cod liver oil 5, "Ruffex" 2 and salt mixture (Osborne and Mendel) 4. After seven days on this diet, certain groups received 100 milligrams of *dl*-serine (Merck), administered by stomach tube daily for periods of four or more days. Other groups on a stock diet (Rockland rat diet, complete), received the amino acid in a similar manner. Control groups of animals on both diets not receiving serine are included.

At the end of the experimental period the animals were decapitated, and the kidneys rapidly weighed and homogenized in a modified Potter and Elvehjem apparatus (Scientific Glass Apparatus Company). Cocarboxylase was determined by the Warburg technique, using the split enzyme as described by Green *et al.*[9] In each experiment, the level of the cocarboxylase present in the fresh homogenate and that remaining after 1 hour at 37° C under N_2 in the presence of thiamin (100γ/cc final concentration), and sodium pyrophosphate (0.01 M final concentration), was obtained. The samples were shaken in Warburg vessels during the period of incubation. In a number of experiments, the influence of *dl*-serine (0.05 M final concentration) added to the homogenate, on the cocarboxylase present in the tissue was studied. Cocarboxylase is expressed as γ/gram of dry tissue.

Results

The administration of serine to rats on the stock diet (Fig. 1a) resulted, after two doses, in a fall of cocarboxylase from 50 γ/gm to 34 γ/gm or to 68 per cent. of the amount normally present in the rat kidney. A return to the normal level takes place by the fourth day.

In the control groups on the experimental diet (Fig. 2a), there was a progressive decrease in kidney cocarboxylase. This may be explained by the reduced intake of thiamin, since the animals here are on a vitamin B deficient diet. However, serine administration resulted in a further decrease in the cocarboxylase. Unlike similar groups on the stock diet, there was no return to normal levels.

The effect of anaerobic incubation on the cocarboxylase level of the kidney homogenate is indicated in Figs. 1b and 2b. In the groups which received serine on the stock diet, the relative amount of cocarboxylase remaining unchanged in the digest after incubation increased to a maximum by the second day and thereafter returned to the resting level. As for the corresponding groups on the experimental diet, a similar phenomenon was evident. Thus again the breakdown of cocarboxylase was least on the second day.

Although it is not indicated in the figures, the addition of serine to the homogenate of kidney from animals on the stock diet or experimental diet in the absence of orally administered serine, resulted in no significant or characteristic change. However, in some

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[2] This investigation was aided by a grant from the John and Mary R. Markle Foundation.

[3] W. H. Fishman and C. Artom, *Jour. Biol. Chem.*, 145: 345, 1942.

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[5] R. P. Morehead, W. H. Fishman and C. Artom, *Am. Jour. Path.*, in press.

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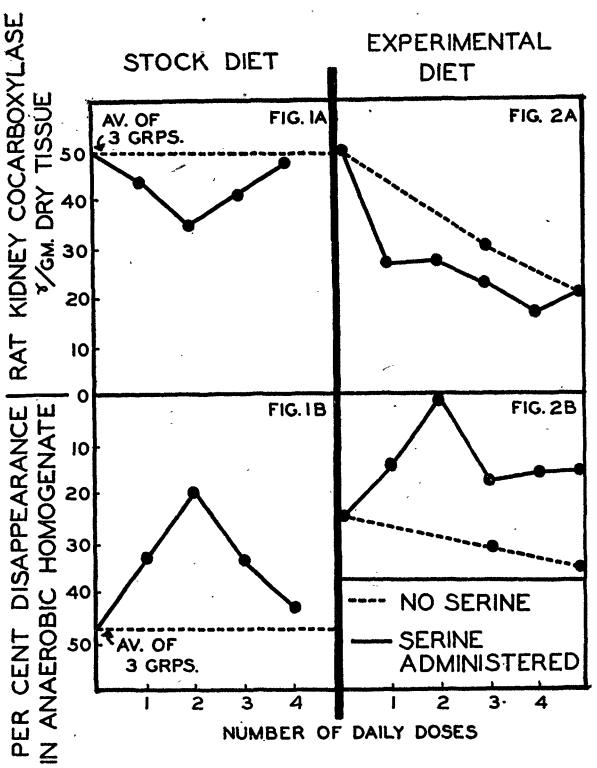

Changes in rat kidney cocarboxylase in animals receiving *dl*-serine. In figs. 1a, 1b, males (150 to 190 grams) were employed, as deaths due to serine have not been observed in rats on stock diet.[3] In figs. 2a, 2b, most of these groups consisted of smaller female rats (120 g) since on the experimental diet their mortality is less upon serine administration as compared to male rats.[3] Both these measures permitted the experiments to be completed satisfactorily. With respect to the changes in cocarboxylase, unpublished experiments have shown no sex difference.

kidney homogenates from animals on experimental diet which had received oral serine, it was possible to show more cocarboxylase in the digest than in the fresh tissue (in one case, an increase of 32 per cent.). The pyrophosphate and thiamin added to the homogenate represent the source of the extra cocarboxylase.

Discussion

In the animals receiving serine there are two phenomena evident, the reduction in kidney cocarboxylase *in vivo,* and a lessened tendency for this substance to disappear from the homogenate on anaerobic incubation *in vitro.* These biochemical events may now be correlated with the pathological findings.[5] The first dose of serine leads to profound necrotizing lesions in the kidneys of rats on either experimental or stock diets. At this time there occurs a drop in cocarboxylase and a reduction in the breakdown of cocarboxylase in anaerobic homogenate. Processes of repair are very extensive and almost completed by the fourth day in animals on the stock diet, and here we see a return to normal of the tissue cocarboxylase and of the rate of its disappearance on anaerobic incubation. In the animals on the vitamin B deficient diet, however, the kidneys never return to their former healthy state and the necrotic tissue is replaced by calcium deposits. Here we find no return to normal cocarboxylase levels or to a normal rate of its disappearance on incubation.

With reference to the reduction of kidney cocarboxylase *in vivo,* there may be several explanations, all equally worthy of consideration. Thus, the diminution in cocarboxylase may be the result of tissue damage, normal levels (Fig. 1a) returning when the tissue is repaired. Again, the observations may be ascribed to a specific involvement of cocarboxylase in serine metabolism, or perhaps to an indirect action of the amino acid on tissue respiratory systems. At present, there is no direct evidence which would permit us to select any one hypothetical mechanism in preference to the others.

In the case of the lessened tendency of cocarboxylase to disappear in homogenates from animals receiving oral serine, and where actual cocarboxylase synthesis occurs in the presence of added serine, the mechanisms involved seem even more obscure. One has the feeling, however, that these reactions involve the functioning of tissue cocarboxylase phosphatase.

Summary

When rats on either a stock or a vitamin B deficient diet are given the amino acid *dl*-serine by stomach tube, there is a fall in the cocarboxylase concentration of the kidney. This organ exhibits marked damage. There is, also, a lessened tendency for cocarboxylase to disappear from the tissue homogenate upon anaerobic incubation *in vitro.* In animals on the stock diet, only, there is a recovery of the normal cocarboxylase level. The probable relation of these findings to the mechanism of the production of the injurious action of serine is discussed.

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CASTRATION EFFECTS OF THE INHERITED HORMONAL INFLUENCE[1, 2]

WOOLLEY, Fekete and Little[3, 4] observed evidence of

[1] A preliminary report of material being compiled for a thesis in partial fulfilment of the requirements for the degree of doctor of philosophy at the University of Minnesota.

[2] Aided by a grant from the Graduate School Cancer Research Fund made to Dr. Bittner.

[3] E. Fekete, G. Woolley and C. C. Little, *Jour. of Exp. Med.,* 74: 1–8, 1941.