jections. However, no antisera were obtained from these pigs before the shock dose. Hence, the effect of the challenging injection on the formation of this type of antibody could not be assessed.

The skin-sensitizing activity of the sera bore no relation to their potency in passive sensitization of guinea pigs. This observation permits the speculation that skin-sensitization and anaphylaxis are mediated by different antibodies, the skin-sensitizing antibody being formed by only a fraction of the guinea pigs in response to a type of stimulation which consistently produced anaphylactic antibody.

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## Certain Relationships Between Pteroylglutamic Acid, Citrovorum Factor, and Cortisone. (18739)

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Recent investigations have shown that relatively large amounts of dehydroisoandrosterone were interchangeable with pteroylglutamic acid (PGA) for growth of *Streptococcus faecalis* and *Lactobacillus casei*(1). In view of these observations cortisone was tested with *S. faecalis* for its ability to replace PGA and with *Leuconostoc citrovorum* for its ability to replace citrovorum factor (CF)(2), a substance related chemically to PGA(3). Cortisone was found to replace both PGA and CF for the two organisms mentioned; moreover, it reversed the inhibitory effects of Aminopterin, a potent antagonist for both PGA and CF.*

*Experimental.* The cultures used were *Le. citrovorum* ATCC 8081 and *S. faecalis* ATCC 8043. The medium used for *Le. citrovorum* was that of Sauberlich and Baumann (2) substituting acid-hydrolyzed casein for part of the amino acid mixture. The basal medium of Rabinowitz and Snell(5) was used for *S. faecalis*. The organisms were grown at 37°C, growth being observed turbidimetrically at 20 hours using a Coleman Model 11A spectrophotometer. Both the clinical aqueous suspensions and crystalline preparations of cortisone were used.† Leucovorin, a synthetic substance with CF activity(3), was used as a source of CF.

*Results and discussion.* Preliminary experiments indicated that the method of dissolving and diluting cortisone had a marked influence on the amounts required to give growth of the organisms. Table I shows the results obtained with a variety of sample treatments. The mild acid treatment was used to destroy any citrovorum factor possibly occurring in the samples of cortisone. No evidence for the presence of CF was found. The acid treatment consistently enhanced the potency of the clinical cortisone preparation while in 2 experiments the acid treatment decreased the potency of the crystalline cortisone sample.

The most reproducible results were obtained by evaporating alcoholic solutions of crystalline cortisone in the assay tubes. In several experiments by this technic 1.5 γ per ml gave

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* Preliminary experiments on replacement of PGA and CF by cortisone were discussed at Federation Meetings, 1951(4).

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† The clinical suspension was "Cortone Merck". A sample of crystalline cortisone acetate was obtained through the courtesy of Merck and Co.

TABLE I. Amounts of Various Cortisone Preparations Required to Give Growth of *Leuconostoc citrovorum* and *Streptococcus faecalis*.

| Treatment | Amt needed in γ/ml of culture medium for ½ max growth* *Le. citrovorum* | *S. faecalis* |
|---|---|---|
| Clinical cortisone | | |
| Suspended in water | 25, 40 | |
| " " † | 45 | 30 |
| Dissolved in Tween 40 and steamed 30 min. at pH 2 | 10, 25, 50 | |
| Crystalline cortisone | | |
| Dissolved in Tween 40 | 1.5, 7.5, 15 | 50 |
| Dissolved in Tween 40 and heated | 1.5, 2.7, 7.5 | 300 |
| Dissolved in Tween 40 and autoclaved 5 min. at pH 2 | 50, 75, 100 | 100 |
| Dissolved in alcohol and evaporated in assay tube | 1.5, 2.5, 4.0 | 5 |

* Optical density of 0.55 on Coleman Spectrophotometer.

† Microbiological test medium contained Tween 40.

TABLE II. Comparison of Amounts of Cortisone, Leucovorin, and Pteroylglutamic Acid Needed for Growth of *Le. citrovorum* and *S. faecalis*.

| Compound | Amt needed in γ/ml of culture medium for one-half max growth *Le. citrovorum* | *S. faecalis* |
|---|---|---|
| Cortisone* | 1.5 | 5.0 |
| Leucovorin | 0.00015 | .0004 |
| Pteroylglutamic acid | 30.0 | .0002 |

* Crystalline product dissolved in alcohol and evaporated to dryness.

half-maximum growth of *Le. citrovorum*. Larger amounts of all the preparations of crystalline cortisone were required by *S. faecalis* to replace PGA.

In an occasional experiment with *Le. citrovorum*, particularly in the earlier phases of this study, cortisone in any amount tested failed to replace CF. The reason for this has not been determined.

Table II compares the amounts of cortisone, PGA and leucovorin needed for growth of the organisms. Although 10,000 times more cortisone than leucovorin is required by *Le. citrovorum*, cortisone is 20 times more potent for this organism than PGA, which is closely related chemically to leucovorin. Dehydroisoandrosterone which replaces PGA for *S. faecalis*(1) was found to replace CF for *Le. citrovorum* at 50 γ per ml.

The mechanism whereby cortisone replaces PGA and CF was studied by use of Aminopterin (4-aminopteroylglutamic acid). Cortisone eliminated the growth inhibition of *Le. citrovorum* produced by toxic amounts of Aminopterin. The ratio of metabolite:antagonist for half-maximum growth was changed from 0.0037 to 0.0025 by a four-fold increase in cortisone concentration (Fig. 1). This comparatively small change indicated that the relationship between cortisone and Aminopterin was competitive and that cortisone is probably a catalyst rather than an end product of the enzyme reaction inhibited by Aminopterin.

Rodney *et al.*(6) have shown that livers from rats rendered PGA-deficient by inclusion of 2% succinylsulfathiazole in their diet had a lowered capacity to oxidize tyrosine. These workers later showed that the oxidation of tyrosine can be partially restored *in vitro* by PGA(7). Rienits(8) has found that PGA will stimulate the disappearance of tyrosine from liver preparations from scorbutic guinea pigs. To test the effect of cortisone on tyrosine oxidation, albino rats (Carworth Farms) were fed for 5 weeks a purified diet(9) containing 1% succinylsulfathiazole but no PGA.

TABLE III. Effect of Cortisone on Oxidation of Tyrosine by Rat Liver Homogenates.*

| Time, min. | Rat fed diet: Without PGA — Tyrosine | Without PGA — Tyrosine + cortisone | With PGA — Tyrosine | With PGA — Tyrosine + cortisone |
|---|---|---|---|---|
| 30 | 3† | 35 | 7 | 30 |
| 60 | 12 | 58 | 15 | 55 |
| 80 | 17 | 72 | 15 | 65 |

* Flasks contain 2 ml of 14% liver homogenate in .067 M phosphate buffer. .1 mg cortisone was added to flask in alcohol and alcohol evaporated off. .5 mg tyrosine was added to side arm and tipped in after 10 min equilibration.

† Difference in oxygen uptake (μl $O_2$) between that of liver homogenates and liver homogenates plus test substances.

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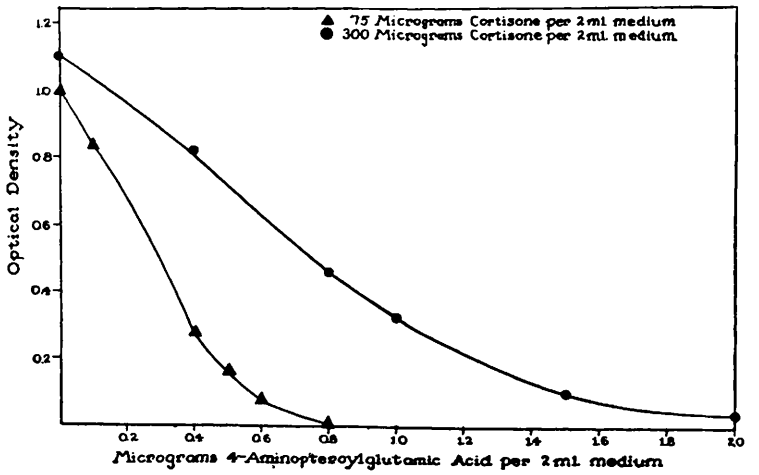


FIG. 1. Effect of Cortisone on inhibition of growth of *Leuconostoc citrovorum* by Aminopterin.

Control rats were fed the same diet with added PGA. As shown in Table III crystalline cortisone markedly stimulated the oxidation of tyrosine by liver homogenates from rats on both diets. Cortisone had no effect on endogenous respiration *i.e.* without added tyrosine. PGA and leucovorin had no discernible effect on tyrosine oxidation by liver homogenates from either group of rats. Presumably the rats fed the succinylsulfathiazole were not deficient enough to demonstrate the reported effect of PGA *in vitro*. The magnitude of the cortisone stimulation of tyrosine oxidation appears to be considerably greater than that reported for PGA(7).

*Summary.* 1. Cortisone was found to replace pteroylglutamic acid and citrovorum factor for growth of *Streptococcus faecalis* and *Leuconostoc citrovorum*, respectively. Cortisone reversed the growth inhibition of *Le. citrovorum* produced by toxic amounts of Aminopterin. 2. Cortisone *in vitro* stimulated the oxidation of tyrosine by liver homogenates from rats fed a purified diet containing 1% succinylsulfathiazole with or without PGA.

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## Effect of Parenterally Administered Glycine upon Action of Insulin in Rabbits.* (18740)

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Varying reports have been made of the effect of amino acids given orally and parenterally upon the blood glucose level. Izumi(1) found that glycine administered intravenously caused a slight rise in the level of glucose in the blood and that glycine administered perorally caused a slight lowering over a four-hour period. Schenck(2) found that 1 g of glycine administered to an animal perorally produced a hypoglycemia, whereas 1.5 g by the same route produced a hypoglycemia. This effect was more intense in an animal weighing 2810 g than in one weighing 1260 g. Hypoglycemia was produced in man by administration of glycine by the same route according

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