The effect of the extract upon *Alcaligenes fecalis* is entirely negative. This organism is known to have no carbohydrate-fermenting ability. As would be expected, the pH increases rather than decreases.

From the facts that the extract causes a decrease in the final pH, the production of gas, and has no stimulating effect on the growth of *Alcaligenes fecalis,* the assumption can be made that the stimulating agent, probably pantothenic acid, is related to the carbohydrates. The stimulation observed with the extract, however, can in no way be attributed to glucose or other hexoses which stimulate *E. coli* in a manner similar to glucose.

Conclusion. 1. Rice bran contains a growth-stimulating agent for *Escherichia coli,* but not for *Alcaligenes fecalis.* 2. This stimulating agent is probably related to the sugars. 3. It is not a hexose. 4. The substance is probably identical with the pantothenic acid of Williams.

7602

Isolation of Glycocyamine from Urine.

C. J. WEBER. (Introduced by E. A. Doisy.)

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The isolation of glycocyamine (guanido acetic acid) from urine is of interest because of its possible relationship to the origin of creatine. Hunter has critically reviewed the literature on this subject in his monograph on "Creatine and Creatinine".

My interest in this subject began with the finding that an extract of urine prepared with Lloyd's reagent gave a positive Sakaguchi reaction. I also found that the Lloyd's extract of urine from a case of pseudohypertrophic muscular dystrophy gave a stronger Sakaguchi reaction than did urine from normal individuals. Urine from this patient (8-year-old boy) was therefore used in attempting to isolate the substance responsible for the positive reaction. The patient received 15 gm. of glycine daily during the period of urine collection. The urine was collected in 2- or 3-day periods using toluene as a preservative.

The procedure used is briefly as follows: The urine after filtering was made acid to congo red with hydrochloric acid and extracted

with 100 gm. Lloyd's reagent. The extractives were removed from the Lloyd's with barium hydroxide. The barium was removed from the filtrate with sulphuric acid. The filtrate containing creatine, creatinine and substances giving a positive Sakaguchi reaction was concentrated *in vacuo* to a volume of approximately 300 ml. This concentrated extract was kept in the refrigerator until there had accumulated a series of extracts from a total of 14.8 liters of urine representing a collection period of 18 days. These concentrates were then combined and evaporated to a volume of 1400 ml. and treated in the usual manner with phosphotungstic acid. Sixty per cent of the substances responsible for the positive Sakaguchi reaction remain in the phosphotungstic acid filtrate. The filtrate is freed of phosphotungstic acid with barium hydroxide and the excess barium removed with sulfuric acid. The filtrate is evaporated almost to dryness and extracted with absolute alcohol. The residue insoluble in alcohol was dissolved in water and sufficient sulphuric acid added to give a 2% concentration. This solution was autoclaved at 15 pounds pressure for 30 minutes and was then again treated with phosphotungstic acid. The filtrate was evaporated to 25 cc. The addition of picric acid to this concentrated filtrate resulted in the formation of a picrate which was recrystallized once from water. Six hundred and sixty mg. of the picrate was obtained which retained its crystalline form after drying at 100°C. for several hours. The picrate melted with decomposition at 200.6° and showed no depression of melting point when mixed with glycocyamine picrate. This picrate gave a positive Sakaguchi reaction and gave the same amount of color as glycocyamine picrate when equal quantities were compared.

Three hundred and fifty mg. of the picrate was suspended in 10 ml. of 20% sulfuric acid and extracted with benzene. The sulphuric acid was removed with barium hydroxide and the solution evaporated to a volume of 5 ml., from which, on cooling, crystals were obtained in the form of rhomboid plates. These were recrystallized once from water and dried at 90°C. Dr. S. A. Thayer of St. Louis University analyzed these crystals and obtained the following results:

3.813 mg. gave 2.02 mg. H_2O and 4.312 mg. CO_2
Glycocyamine, $C_3H_7O_2N_3$ (117) calculated C 30.77 H 5.98
found C 30.83 H 5.93
1.890 mg. gave 0.632 cc. N at 30° C., B.P. 734 at 0°
calculated N 35.87
found N 35.68

In another case of muscular dystrophy (12-year-old boy) receiv-

ing 15 gm. of glycine daily, 274 mg. of glycocyamine picrate was isolated from the urine over an 8-day period. In this case the urine was collected daily and extracted with Lloyd's reagent on the same day. The quantity of glycocyamine picrate isolated represents 23% of the original Sakaguchi reacting substances in the Lloyd's extract using glycocyamine as the standard for comparison.

In a control experiment 600 mg. of glycocyamine was added to a solution containing creatin, creatinine, glycine, urea and salts in concentrations approximating that found in the above 8-day urine collection. This mixture was treated by exactly the same procedure used above. Three hundred and ninety-six mg. of glycocyamine picrate was isolated which represents a recovery of approximately 33% of the glycocyamine extracted by Lloyd's reagent as determined by the Sakaguchi reaction. This suggests that probably the major part if not all of the Sakaguchi reacting substance in the Lloyd's extract from urine is glycocyamine.

Glycocyamine is not produced during the process of isolation, at least, from creatine, creatinine or glycine and urea. The addition of creatine, creatinine, glycine or urea together, or separately, to urine causes no increase in the Sakaguchi reaction of the Lloyd's extract.

The feeding of glycine to a patient with pseudohypertrophic muscular dystrophy results in an increase of approximately 60% in the Sakaguchi reacting substances in the urine.

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Relation of Cytoplasmic Structure to Growth and Respiration in Plasmodium.

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Recently I have shown that if a small piece of the plasmodium of *Physarum polycephalum* be forced through a gauze with sufficiently small pores (less than 0.25 mm. in diameter) subsequent growth of this material does not occur, whereas if the pores are larger, growth takes place when the material is put on the culture medium. The experiments were repeated and confirmed for *P. rigidum*. During the present summer I have confirmed in the