Author—average, 21 days.
Other authors—average, 21 days.

Complete healing: Author—6 weeks plus.
Other authors—5 weeks plus.

Return of calcium and phosphorus to normal: Author—14-21 days; other authors—14-28 days.

It is hoped that our dairies will be requested either to radiate the milk or preferably to add irradiated ergosterol so that we may use this newer knowledge to advantage in attempting prophylactically to prevent the extensive occurrence of rickets.

3962

Effect of Glucose on Urea Decomposition by B. Proteus.

A. A. DAY, W. M. GIBBS AND RUTH WESTLUND.

From the Department of Bacteriology, Northwestern University Medical School.

A typical strain of *B. proteus vulgaris* was grown in sugar free beef infusion broth, 1.0% glucose infusion broth, 0.5% urea infusion broth, and 1.0% glucose, 0.5% urea infusion broth, respectively. Incubation was practiced at 37° C. for 1, 3 and 5 days, at which times the following determinations were made:

a—Titratable acidity or alkalinity expressed as cc. of normal acid or alkali per 100 cc. of media.

b—Hydrogen-ion concentration.

c—Quantitative ammonia expressed as mg. of ammonia nitrogen per 100 cc.

d—Quantitative sugar expressed in percentage.

Results—For simplicity only the maximum readings without respect to days of incubation are here reported.

The reaction of the plain broth became 1.8 cc. alkaline, pH 7.3 to 8.3 and the glucose broth 3.0 cc. acid, pH 7.2 to 5.3. The amount of ammonia produced in the glucose broth was 8.9 mg. as against 36 mg. in the same broth without sugar. The alkalinity of the plain urea broth increased 6.7 cc., pH 7.3 to 9.2, and the ammonia 179.3 mg. The reaction of the glucose urea broth changed 1.5 cc. alkaline, pH 7.2 to 8.0, in contrast to the acidity of the glucose broth but not nearly to the degree of alkalinity manifested by the plain urea broth. Nevertheless 40.1 mg. more ammonia was produced in the glucose urea broth than in the plain urea broth,

which is still more significant in view of the fact that the sparing action of glucose for protein is here also undoubtedly operative. Clearly the presence of glucose accelerated the decomposition of the urea.

In the glucose broth the amount of glucose was reduced from 0.999% to 0.604%, while in the glucose urea broth the reduction was from 0.971% to 0.058%. Plainly then the urea in turn accelerated the destruction of the glucose.

A reasonable explanation of this acceleration of disintegration would appear to be that in the glucose urea broth the breakdown products of the 2 agents, glucose and urea, by maintaining the reaction nearer neutrality permit the microorganisms to act to a greater extent. To support this hypothesis the following 2 experiments were conducted:

1. Two flasks of plain urea broth of pH 8.9 were inoculated with *B. proteus*. At the end of 24 hours incubation, sufficient acid was added to one flask to reduce the pH to 6.7, reincubated for 24 hours, the reaction again adjusted to pH 6.7 and incubation continued 48 hours. It was then found that the ammonia in the corrected flask had increased 200.9 mg. as against 168.7 mg. for the uncorrected flask, which had incubated for a like period of time. This would indicate that an immoderate alkalinity restrains the breakdown of urea and supports the assumption that the greater amount of ammonia formed in glucose urea broth is due to the neutralizing action of the acid resulting from the glucose fermentation.

2. Glucose broth was distributed in 20, 50, 75, 100, 150 and 200 cc. quantities in 250 cc. Erlenmeyer flasks, one gram of finely divided $CaCO_3$ added to each, inoculated with *B. proteus* and incubated 24 hours. The insoluble $CaCO_3$ soon settles to the bottom of the flask; therefore, the larger the volume of broth in a flask of given size the further the acid must diffuse to reach the neutralizing agent. Thus if acidity was retarding glucose breakdown by *B. proteus,* there should be more glucose after 24 hours incubation in the flask containing 200 cc. of medium than in those containing lesser amounts. Such proved to be the case, the quantity of sugar being found in decreasing amounts from 0.565% in the flask with 200 cc. of medium to 0.072% in the flask with 20 cc. This favors the supposition that urea hastens the decomposition of glucose by preventing the development of acidity.

Repeated attempts to split urea in plain or glucose broth or in aqueous solution by the use of Berkefeld filtrates of cultures of *B. proteus* resulted in failure. To obtain the endo-enzyme the organ-

isms in 48-hour broth cultures of *B. proteus* were collected, washed repeatedly with saline, dehydrated with acetone and ether and then finely powdered. Testing for urease activity, by adding 0.5 gm. of the extracted organisms to 100 cc. of 0.5% urea dissolved in water, resulted in the production of 177.8 mg. of ammonia. Controls consisting of the urea solution alone and of urea solution plus 0.5 gm. of the extracted organisms, previously boiled for 10 minutes, showed no increase in ammonia. Therefore the splitting of urea, in the foregoing experiments, was brought about by an endo-urease.

Summary. Evidence is here presented that glucose and urea simultaneously present in broth media interact to accelerate the breakdown of each other by *B. proteus;* that this is in large part due to their end products maintaining the reaction at a more optimum range and that the splitting of urea is accomplished by an endocellular urease.

3963

Calcium and Phosphorus Balances of Epileptic Children Receiving a Ketogenic Diet.

MARTHA V. NELSON. (Introduced by P. C. Jeans.)

*From the Department of Pediatrics, State University of Iowa.**

Three epileptic children, 3, 7 and 9 years of age, receiving a ketogenic diet were made the subjects of a metabolic study with special reference to the calcium and phosphorus intake and output. The diets furnished approximately 1½ gm. of protein per kg. of body weight per day, and an energy value sufficient for average activity. The diet of the youngest child contained 2 gm. of potential fatty acid to each gm. of potential glucose. In the diets of the older children this ratio was 2.5:1. Each patient was excreting ketone bodies in considerable quantities and no epileptic symptoms were manifested during the period of observation. The diets were planned to include as much calcium and phosphorus as their limited carbohydrate content would allow, and were composed of cream, meat, eggs, butter and low carbohydrate vegetables and fruits. The usual metabolic procedures were employed.

In each instance the calcium and phosphorus output exceeded the intake, and the usual excess of excretion of calcium and phospho-

* Assisted by a fund from the Child Welfare Research Station, Iowa City, Ia.