to 80° C. for 20 minutes, using deep brain medium for the initial culture and deep agar for isolation of the anaerobes.

This culture of *B. histolyticus* shows all of the usual morphologic, cultural and pathogenic characteristics of the species.[2] It also grows definitely though delicately upon the surface of meat infusion blood agar slants exposed to the air. Such aerobic cultures failed to show hemolysis as do anaerobic cultures, and the stained organisms from them were atypical fusiform rods with few spores as in our former observations. This species, though not always strictly anaerobic in the sense of refusing to grow upon the surface of solid media, evidently prefers anaerobic conditions, as shown by these facts, as well as in the greater size and number of colonies in the depths of deep agar shake cultures.

This strain is exceedingly virulent; the leg of a guinea pig injected intramuscularly with 2 cc. of a 24 hour glucose broth culture was on the following day almost completely disarticulated and denuded of flesh from the knee down in addition to an area of about 15 sq. cm. on the abdomen from which the skin had sloughed.

The finding of this species in human feces, as well as in cultivated soil, establishes evidence, albeit a slender thread, to explain its occurrence in wounds where conditions are favorable for contamination with fecally polluted soil.

101 (2333)

Studies on inorganic salt metabolism.

By MARTHA R. JONES (by invitation).
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Studies on inorganic salt metabolism in dogs and rats which have been in progress in this laboratory during the past two

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2 Hall, I. C., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 501.

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years indicate that a bone condition comparable in all essential respects to that seen in rachitic children may be produced in young puppies on a diet, which, as far as we have been able to determine, is adequate in respect to protein, fat, carbohydrate, inorganic salts and vitamins but possesses a high potential alkalinity; while parallel experiments with the same diets on rats have invariably resulted in normal bone development.

Our basal diet consisted of bread, cooked lean beef, skimmed milk powder, butter fat and fresh orange juice made into a mush with distilled water. To this was added a salt mixture, the composition of which was based on ash analyses of cow's milk, except that the phosphorus was omitted and different proportions of calcium were used. The phosphorus content of the diets was varied by adding dibasic potassium phosphate to the above salt mixture, and the degree of alkalinity regulated by the addition of HCl. The first lot of salt mixture prepared proved to be alkaline in reaction and when added to the food mixture as described above and fed to six young puppies resulted in severe rickets in all cases, our diagnosis being based upon blood and bone analyses for calcium and phosphorus, radiographic, clinical and histological evidence. The parallel experiment on rats was negative. Another group of three puppies of the same litter received the identical diet with the addition of 0.8 gm. of phosphorus daily. They too developed rickets, the clinical signs appearing earlier than in the former group, while the rats fed similarly were normal. The experiment was repeated as nearly as possible a year later on two litters of puppies, 11 in number, except that the excess of alkali was neutralized with HCl. No signs of rickets were detected at any time in either puppies or rats during the experimental period of six months. We again produced well marked rickets in three puppies of the same litter on the basal diet with the addition of an alkaline salt mixture, the calcium content of which was higher than that of the first two lots, and cured two of these simply by adding sufficient HCl to the salt mixture to neutralize the excess of alkali, while the third animal was kept on the original diet and became steadily more rachitic. Here again the rats failed to develop any signs of the disease.

The fact that different litters of puppies and even individuals of the same litter vary tremendously in their susceptibility to rickets is generally conceded. A factor which has not been

stressed in the reports of recent researches on the etiology of rickets but which we have found to have a very important bearing on the ease and rapidity with which the disease may be induced in an animal, is the diet of the mother during pregnancy. In our experience, brood bitches which are fed on diets deficient in calcium give birth to litters which have marked rachitic tendency—some individuals developing clinical signs even before weaning. In these puppies there is a marked retardation in the appearance of the epiphyses and carpal bones of the feet and remarkable skeletal changes can be demonstrated by radiographs after two weeks on the experimental diet. Another brood bitch living under comparable conditions and fed in a manner similar to those mentioned above except that a well balanced inorganic salt mixture was added to the diet gave birth to normal young, our conclusions being based on the number and size of the epiphyses and carpal bones appearing in the fore feet at different ages.

It has been shown by a number of investigators that in active rickets the distribution of calcium and phosphorus between urine and feces is different from that which occurs in normal individuals. The amount of phosphorus excreted in the feces of a normal puppy is relatively small, while in rickets the quantity lost in this manner is tremendously increased. Experimentally, we have been able to so regulate the amount of alkaline salt mixture added to our basal diet as to divert the phosphorus almost entirely from urine to feces. If a large excess of phosphorus free salt mixture is given, only a trace of phosphorus appears in the urine, the retention of this element may be increased in the body and healing occur. On the other hand, a smaller amount of the salt appears to produce the optimal degree of alkalinity for the activation of the rachitic process and severe rickets may result.

If rickets can result from too high a degree of alkalinity in the intestines of an individual on a well constituted diet, as is frequently the case of artificially fed infants, it is possible that this condition may be the result of insufficient HCl in the gastric secretion or an excess of alkali in the intestinal juices. Those infants who have relatively little HCl in the gastric juice may develop normally when breast fed, but if the diet is changed to foods having a higher potential alkalinity the amount of acid present may be insufficient for normal mineral metabolism. Since cow's milk is potentially alkaline in reaction and contains a

large amount of buffer substance as compared with human milk, on the above hypothesis we can explain why infants fed on cow's milk are more prone to rickets than those who are breast fed. Individual differences in HCl secretion may also explain why one of a pair of breast fed twins may be rachitic and the other not. Furthermore, the fact that inanition greatly retards the rachitic process can also be explained on this hypothesis since the products of katabolism of body tissue are acid in reaction and may help to restore the normal acid-base balance.

While we do not consider our experiments sufficiently numerous, and the many factors involved in so complex a salt mixture as we have used well enough controlled to permit us to draw final conclusions, they have suggested lines for further study. Experiments cited above, supplemented by others now under way, will be published in detail at a later date.

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Anaphylactic reactions in isolated rabbit lungs.

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It was shown by Coca[1] that the dominant physological reaction in acute anapylaxis in rabbits is an occlusion of the pulmonary blood vessels with red blood corpuscle masses. Coca has evidence that this conclusion is not due to thrombosis or hemagglutination (embolism). He therefore concludes that it is due to an acute pulmonary vasoconstriction, comparable with the acute bronchoconstriction in anaphylactic guinea pigs. We have endeavored to test this conclusion by our routine perfusion technique.

On perfusing the isolated lungs of horse serum sensitized guinea-pigs with 2 per cent horse serum in Locke's solution, a distinct bronchoconstriction is demonstrable within thirty seconds, increasing to a complete bronchial stenosis by the end of two minutes.[2] At this stage of the reaction, the lungs can neither

1 Coca, A. F., The Mechanism of the Anaphylaxis Reactions in the Rabbit. *J. Immunol.*, 1919, iv, 219.

2 Manwaring, W. H., and Kusama, Y., Analysis of the Anaphylactic and Immune Reactions by Means of the Isolated Guinea-pig Lungs. *J. Immunol.*, 1917, ii, 157.