TABLE II.

	Av. Initial Blood lactic acid mg./%	Av. Final Blood lactic acid mg./%	Av. Increase in Blood lactic acid mg./%/hour
Normal	50.5	106.7	15.2
Depancreatized	118.1	183.2	21.0
Hypophysectomized	27.3	62.8	9.5

It is evident that the muscle glycogen of the hypophysectomized animal is relatively stable and is not as rapidly catabolized as in the normal and depancreatized animal.

Taking into account the influence of the blood sugar level upon the rate of utilization of sugar, our results clearly show the diminished utilization of carbohydrate by the hypophysectomized dog, as compared to both the normal and the depancreatized animal. These results are important in explaining the hypoglycemic effects of fasting in both the hypophysectomized and the hypophysectomized-depancreatized animal, and the amelioration of the diabetic syndrome in the latter animal. It is obvious that these effects of hypophysectomy cannot be due to an increased rate of sugar utilization. Sugar utilization is actually diminished and, therefore, the hypoglycemia and modification of the diabetes must be attributed to a proportionately greater decrease in the rate of new formation of sugar by the liver. This work confirms and extends our previous report upon the influence of hypophysectomy on gluconeogenesis in the normal and depancreatized dog.[3]

9719 P

A Method for Isolation of Ascorbic Acid Fermenting Bacteria.

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The objectives of this preliminary communication are two: to describe a procedure for the isolation of ascorbic acid fermenting bacteria, from suitable material, and to describe briefly the results obtained with this procedure. This preliminary communication does not presume completeness either with respect to the types or strains of bacteria which may actually utilize ascorbic acid for energy, or with regard to the patterns of fermentation of this substance by specific strains of bacteria.

Plain, nutrient broth is reinforced with ascorbic acid.* Fifteen to 20 mg. per 10 cc. of broth is a preferable amount, both because this is sufficient to indicate unequivocally that decomposition has taken place, and also because within these limits the initial reaction of the broth is not brought below pH 6.0, a point at which the bacteria thus far encountered grow readily. Also, a reaction slightly on the acid side is conducive to the stability of ascorbic acid. The broth, thus prepared is inoculated with stomach contents, feces, or other suitable material and incubated at 37° for 18-30 hours. Then, if ascorbic fermenting bacteria are present, a considerable growth will be noticed. From this first, or preliminary culture, a second broth culture is made, using 2 to 5 loops for the inoculum. After incubation and growth, plates are made either upon plain, or, preferably, glucose agar. (Ascorbic acid agar is not so well suited for this purpose.) Colonies are picked off, tested for purity and eventually for ascorbic acid utilization. The use of 2 successive cultivations in ascorbic acid broth is desirable both because it tends to eliminate non-fermenting organisms, and because it acclimatizes the bacteria to the medium. Inasmuch as bacteria isolated by this procedure are somewhat prone to dissociation some stability is obtained. Using this method, some 30 cultures of bacteria have been isolated, without difficulty, from gastric contents of achlorhydrics (a fruitful source of ascorbic acid fermenting bacteria) from a normal artificially fed baby, from the feces of a normal adult, and from the feces of guinea pigs. The cultures obtained are of 2 types, with some individual variation in minor details: members of the *Lactis aerogenes,* or pneumobacillus group, and members of the enterococcus group. These have been tested and retested for their ability to decompose ascorbic acid as follows:

Ninety cc. of plain broth, in an Erlenmeyer flask, is inoculated with the desired culture and incubated 18 hours, at which time a good growth is obtained. Ten cubic centimeters of ascorbic acid solution, filtered through a Berkefeld filter to insure sterility, and of such strength that the final concentration in the broth will be from 15 to 20 mg. per 10 cc. broth is added, and the medium is distributed in a series of sterile test tubes (preferably ⅝ inches in diameter or less, to reduce chances of oxidation), using exactly 10 cc. to each tube. These several tubes, together with control tubes containing exactly the same proportion of ascorbic acid, but no bacteria, are incubated, and at appropriate intervals examined for their respective amounts of this substance. The method of quantitation

* The ascorbic acid was very generously provided by Merck and Co., Inc.

of ascorbic acid is as follows: At the proper time, exactly 1 cc. of culture is removed, and mixed with approximately 1 cc. of glacial acetic acid to arrest growth. An equal amount of control uninoculated broth is treated in exactly the same manner. Each solution is then made up to precisely 50 cc. with distilled water. The most direct way is to place the culture (1 cc.) in a 50 cc. volumetric flask, add the acetic acid, then make up to the 50 cc. mark with water. Two aliquots, of 1 cc. each, are taken for analysis by titration against a freshly prepared, standardized solution of 2:6 dichlorophenolindophenol dye of which each cubic centimeter is equivalent to 0.02 mg. ascorbic acid. The end point for the titration is the first appearance of a faint, but distinct bluish color. From the amount of ascorbic acid medium required to elicit this color, it is a very simple matter to calculate the amount of ascorbic broth in the solution. It is good practice to repeat the determination of ascorbic acid upon a sample which has been saturated with H_2S prior to titration, thereby changing any reversibly oxidized ascorbic acid to the fully reduced, and titratable condition, and thus acting as an additional check on the decomposition of the substance by the bacteria.

9720 P

Pathogenesis of Arterial Hypertension in Coarctation of the Aorta.

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Experiments on albino rats show that the hypertension in coarctation of the aorta has the same pathogenesis as the hypertension in a Goldblatt dog (partial constriction of a renal artery). The criterion of hypertension was the presence of cardiac hypertrophy estimated in relation to body weight (left ventricle more than right) in non-anemic rats sacrificed 20 days after operation. Blood urea concentrations were normal and proteinuria was not increased over the control levels.

The experimental procedure consisted in a modification of Collins'[1] technique. The left renal artery was tied together with a wire 0.4 mm. diameter, or the aorta with a wire 0.9 mm. diameter; after tying, the wire was removed but the ligature left in place.

[1] Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.