suspensions of *A. indologenes* on *l*-malate, succinate, pyruvate, acetate, and aconitate. Aconitate was attacked aërobically only after an induction-period and anaërobically not at all. Citraconic, itaconic, tricarballylic, and α-OH isobutyric were not dissimilated either aërobically or anaërobically.

Other microrespirometric studies have shown that the respiratory quotients of oxidations of citric, aconitic, oxaloacetic, *l*-malic, fumaric, succinic, and pyruvic acids by *A. indologenes* decrease with time, indicating that the first steps in the oxidation of these acids are anaërobic.

Schemes for oxidation of citric acid by animal tissue[4] in which the citrate is oxidized stepwise through α-ketoglutaric, succinic and oxaloacetic acids cannot apply to the bacterial oxidation because the latter produces more acetate and less CO_2 in the early stages (Table I) than required by the former.

The oxidation of citric acid by *coli-aërogenes* bacteria proceeds through the normal anaërobic fermentation, to products that are dehydrogenated to CO_2, H_2O and assimilated to complex carbohydrate-like materials. The synthesis of "carbohydrate" is inhibited by NaN_3 as evidenced by increased O_2 uptake and CO_2 production.

10614 P

Some Effects of Low Choline Diets.

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The importance of choline in the prevention of "fatty livers" has been previously demonstrated by the investigations of Best[1] and of Channon.[1] The following experiments indicate that the production of a fatty liver on a low choline diet is only one manifestation of a more fundamental deficiency condition.

Male rats, 40 g in weight and 24 days of age, were used in groups of ten. The basal diet consisted of fibrin-4, casein-8, dried egg white-3, salt mixture[2]-4, calcium carbonate-1, codliver oil-5, lard-35, agar-2, and sucrose-38. The water soluble vitamins were supplied

4 Martius, C., *Z. physiol. Chem.*, 1937, **247**, 104.

1 Best, C. H., and Channon, H. J., *Biochem. J.*, 1935, **29**, 2651.

2 Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

by a daily supplement of 0.02 mg of thiamin chloride, 0.02 mg of riboflavin, 0.04 mg of nicotinic acid and 0.1 cc each of concentrated extracts of rice polish and hog liver. The rats consumed 4 to 5 g of food per day. The term fatty liver refers in every case to enlarged livers containing from 8 to 12 times the normal weight of chloroform-soluble substances.

This newly recognized effect of choline deficiency was brought to light when rats were killed and examined at the end of a 10-day experimental period. At this time 90% of the rats showed markedly hemorrhagic kidneys as well as fatty livers unless choline was added to the diet. Similar results were obtained when the vitamin supplement was omitted, when it was fed separately, when it was mixed with the basal ration and when it was doubled in amount. Although the minimum effective level has not yet been determined, the degeneration of the kidneys was prevented if the rats received 2 mg of added choline daily. This amount had no effect on the liver fat. Ten mg of choline per day were required to prevent the fatty liver. A high fat diet was not required for the production of the deficiency due to low dietary choline. The typical fatty livers and hemorrhagic kidneys occurred on a diet in which the lard was decreased from 35 to 15%. The deficiency condition was severe if all of the dietary protein was supplied by fibrin. The livers were fatty but the kidneys were normal if all of the protein consisted of casein alone or of dried egg white alone.

Other indications of a severe pathological deficiency condition were observed in addition to the gross hemorrhagic appearance and enlargement of the kidneys. The rats on the low choline diet were noticeably sick, the spleen was enlarged and the thymus was uniformly decreased to approximately one-half its normal weight. The microscopic examination of the kidney tissue showed extensive glomerular and tubular degeneration with hemorrhagic areas in the cortical region particularly.

The importance of the absolute and relative amounts of certain amino-acids in determining the choline requirement was suggested by the difference in results obtained with casein or egg white, and with fibrin. The fact that fatty livers occurred in these young choline deficient rats regardless of the protein used and the fact that much larger amounts of choline were required to prevent the fatty liver than to prevent the renal lesion indicated that certain amino-acids in casein and in the protein of egg white might have "spared" the small amount of choline in the basal ration so that none of the deficiency symptoms appeared except the fatty liver when these proteins were used.

Beeston and Channon[3] have observed an enhancing effect of cystine on fatty liver production and Tucker and Eckstein[4] have shown that methionine has the opposite effect. Furthermore, Newburgh and Curtis[5] and Cox, Smythe and Fishback[6] have reported the occurrence of hemorrhagic kidneys on a diet containing casein and added cystine and Hartwell[7] noted a similar effect on diets containing edestin. Our data suggest that these renal lesions were in reality due to choline deficiency and experiments to answer this question are now in progress.

The remarkable effect of small amounts of choline in preventing a severe pathological state associated with hemorrhagic degeneration of the kidneys demonstrated its important rôle in the maintenance of normal kidney structure in young rats. The choline requirement is dependent upon certain other factors, among which the methionine-cystine ratio of the protein may be particularly important. It is provisionally suggested that the choline requirement is increased by dietary protein relatively richer in cystine than in methionine.

10615 P

Observations Indicating Absence of Glomerular Intermittence in Normal Dogs.*

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Khanolkar[1] and Hayman and Starr[2] have reported experiments on rabbits which they interpreted as showing that usually only a fraction of the renal glomeruli are open to circulating blood. The principle in both sets of experiments was essentially the same, the injection of hemoglobin or dye into the blood stream with subsequent examination of the kidneys to determine what fraction of the capsules or glomeruli

3 Beeston, A. W., and Channon, H. J., *Biochem. J.*, 1936, **30**, 280.

4 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

5 Newburgh, L. H., and Curtis, A. C., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 963.

6 Cox, G. J., Smythe, C. V., and Fishback, C. F., *J. Biol. Chem.*, 1929, **82**, 95.

7 Hartwell, G. A., *Biochem. J.*, 1928, **22**, 1212.

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1 Khanolkar, V. R., *J. Path. and Bact.*, 1922, **25**, 414.

2 Hayman, J. M., Jr., and Starr, Isaac, Jr., *J. Exp. Med.*, 1925, **42**, 641.