Manometric Studies on Oxidation of Choline by Avian Liver Homogenates.* (20083)

V. M. Doctor, Patricia G. Sparks, H. O. Kunkel, and J. R. Couch.

From the Departments of Biochemistry and Nutrition and Poultry Husbandry, Texas Agric. Exper. Station, Texas Agric. and Mechanical College System, College Station, Texas.

The presence in chick liver of an enzyme system oxidizing choline has been reported (1). The importance of this enzyme system has become evident in recent years in that reports have appeared indicating that choline may have to be oxidized to betaine before its methyl groups can become available to the organism for transmethylation reactions(2, 3). Williams *et al.*(4) have developed an assay method for the determination of choline oxidase in rat liver homogenates. This method, however, has limited usefulness in studies with chick tissues. The endogenous oxygen uptake for the chick liver homogenate is relatively high and the chick liver homogenate is quite sensitive to the influence of tonicity. The present report concerns the study of factors which influence the oxidation of choline by homogenates and washed suspensions of chick liver.

Experimental. Unless otherwise indicated, New Hampshire chicks of 8 to 10 weeks of age, fed a commercial type broiler ration,† were used. In the preliminary experiments, the method of Williams *et al.*(4) was used to measure the choline oxidase activity of chick liver homogenate. All enzyme activity was assayed in a Warburg bath at 37°. This method utilizes a system containing 1.0 ml of the 16.7% homogenate, 1.0 ml of distilled water, and 10 mg of choline chloride. Endogenous respiration is measured in a similar system containing no added choline. The center well contained 0.2 ml of 10% KOH in all flasks. The water and choline chloride were added from the side arm at the end of a 10-minute equilibration period. Since the oxygen uptake was maximum during the first 20 minutes and then dropped rapidly, the oxygen uptake during this period was employed as a measure of choline oxidase activity. All enzyme activity is reported on the basis of fresh liver weight.

Results. Factors influencing oxidation of choline by homogenates of chick liver. 10 mg choline chloride per flask was the optimum concentration Contrary to the results of Williams *et al.*(4) the addition of 17.5 mg niacinamide per flask did not affect the endogenous oxygen uptake or choline oxidase activity of chick liver homogenate. The addition of betaine increased the endogenous oxygen uptake but this was probably a tonicity effect since 16 mg KCl could also produce a similar increase. The addition of 22 mg betaine to choline chloride depressed by 28% the choline oxidase activity. Therefore, the procedure for the determination of choline oxidase in chick liver homogenate was modified to eliminate the high endogenous respiration and the influence of tonicity.

Factors affecting oxidation of choline by washed suspension of chick liver. A 16.7% homogenate of chick liver was prepared in isotonic KCl solution (1.13%) containing 0.04% $NaHCO_3$ with the Potter-Elvehjem glass homogenizer, and this was centrifuged at 1700 x *g* for 10 minutes at 5°C. The supernatant was found to have less than 5% of total choline oxidase activity and was thus discarded. This finding is in accord with earlier reports(5,6). The residue was thoroughly mixed with 4 ml of ice cold isotonic KCl solution and recentrifuged as above. This operation was repeated 3 times, finally the residue being resuspended in enough isotonic KCl to give a suspension equivalent to a 33.3% homogenate. Chick liver suspension prepared according to this procedure was found to give only 10 to 15% of the endogenous O_2 uptake of that noted with an equivalent amount of the whole homogenate. Addition of niacinamide and betaine and further addition of KCl had no

* Supported in part by grant-in-aid, U. S. Public Health Service, National Institute of Health, Bethesda, Md.

† Texas A & M Poultry Farm Stock Ration.

effect on endogenous O_2 uptake or choline oxidase activity.

The influence of choline chloride concentration on the O_2 uptake during the 20-minute period was studied with a suspension equivalent to 333 mg fresh tissue. The values obtained were as follows: 2 mg, 47 μl of O_2; 10 mg, 60 μl; 20 mg, 77 μl; and 50 mg, 40 μl. Thus 20 mg of choline chloride in 2 ml was optimal. A straight line was obtained by using a substrate concentration ranging from 0.2 mg-2 mg of choline chloride and plotting the reciprocal of choline oxidase activity against the reciprocal of the substrate concentration. Michaelis constants for 4 separate experiments were 2.0, 2.0, 2.1 and 1.7 x 10^{-3} M, which are within the range of those reported by Eadie and Bernheim(7) using rat liver suspensions.

With the washed preparation no significant differences were obtained in the oxygen uptake with pH values ranging from 6.8-7.6. At pH 7.3 liver suspensions gave a maximum O_2 uptake in the first 20-minute period and then gradually decreased with time. Therefore, pH 7.3 was employed for routine determinations of choline oxidase in chick liver suspensions. Cytochrome *C* did not increase the oxygen uptake under these conditions.

Washed suspensions of liver were prepared in isotonic KCl solution and in isotonic sucrose solution (0.25 M). In both cases centrifugations were done at 9500 x *g*. In 4 different experiments, 5 to 10% more O_2 uptake was obtained with suspensions of liver made in isotonic KCl in comparison to the one in isotonic sucrose. To determine the influence of centrifugal force on the choline oxidase activity, 2 liver suspensions were prepared with 2 different centrifugal forces, one at 1700 x *g* and another at 9500 x *g*. The choline oxidase values for the 2 liver suspensions using 20 mg choline chloride were 79 and 80 μl of O_2 per 20 minutes respectively. 1700 x *g* was therefore used for routine determinations.

TABLE I. Choline Oxidase Activity of Avian Tissue Suspensions.

Preparation	Choline oxidase, μl O_2/20 min.
Chick liver	85
" kidney	74
Turkey liver	110
" kidney	81

Flask components: 1 ml tissue suspension equivalent to a 33.3% homogenate in isotonic KCl solution along with .5 ml of .08 M sodium-potassium phosphate buffer (pH 7.3) and .2 ml of 10% choline chloride solution. Water was added to a total vol of 2 ml. Two-tenths ml of 10% KOH was placed in center wells. Prior to closing stopcocks, flasks were equilibrated for 10 min. at 37°C.

Determinations of choline oxidase were made on liver and kidney suspensions of chick (New Hampshire), turkey (Beltsville small white) and turtle (Chelydra serpentina) using the same procedure. The results are shown in Table I. Turtle liver and kidney were devoid of any detectable choline oxidase activity.

Summary. Factors influencing the manometric determination of choline oxidase activity in chick liver homogenate and washed liver suspension have been studied and a procedure is outlined for the measurement of this enzyme system in washed suspensions.

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Received December 29, 1952. P.S.E.B.M., 1953, v82.