Fig. 3. In the bioassay of the aqueous humor from 2 of these eyes, a slight augmentative effect was noted. It thus appears that in contrast to the aqueous of non-glaucomatous eyes, no acetylcholine-like substance is demonstrable in the aqueous humor of eyes with chronic simple glaucoma when tested by this method.

Conclusions. The results are presented of the bioassay of aqueous humor from human eyes prepared according to the method applied by Engelhart to animals. In a series of 10 human eyes without glaucoma, every sample of aqueous humor contained a parasympathomimetic substance which, in the 2 cases so tested, was found to be similar to acetylcholine. In the aqueous humor of 7 human eyes with chronic simple glaucoma, no such parasympathomimetic substance could be demonstrated.

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Renal Clearance of β-Hydroxybutyric Acid in a Dog.

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Urinary excretion of β-hydroxybutyrate (βB) commonly occurs in ketosis of starvation and diabetes and may be induced experimentally by injections of anterior pituitary extracts or of certain of the ketone bodies and fatty acids. Evidence has been obtained from studies on rats[1] and humans[2] which indicates that excretion of total ketone bodies increases markedly when their concentration in the blood rises above 15 to 20 mg % in humans and above 25 to 30 mg % in rats. However, variable amounts of acetone and acetoacetate are found associated with βB and analyses of total ketone bodies cannot yield conclusive information regarding excretion of βB. Intravenous injection of 0.5 g of βB per kilo per hour into a dog was found necessary to cause an appreciable excretion of βB. At a rate of 0.4 g per kilo per hour only slight amounts of βB were found to have been excreted. None was found in the urine when the rate of infusion was 0.3 g per kilo per hour.[3]

Procedure. The renal clearance of *dl*-βB was investigated in 2 experiments on a trained female dog weighing 13.6 kg. In the first experiment an intravenous infusion of 1.9 cc per minute of a 7.5% solution of sodium *dl*-βB and 1% creatinine was maintained for 45 minutes, following an initial priming injection of 3 g of sodium *dl*-βB and 1.5 g creatinine. Creatinine was infused to permit the measurement of the glomerular filtration rate. About 500 ml of water were given by stomach tube 45 minutes before the infusion was begun. In a second experiment 13 g of sodium *dl*-βB in 25% solution were given in a single initial intravenous dose. Creatinine, 100 mg per kg, was given subcutaneously 30 minutes before collection of urine samples. Diuresis was maintained with water given at 45-minute intervals. Because of the difficulty of obtaining the compound, the time allowed in each experiment for mixing was shortened to 12 minutes. The levels of creatinine and βB were followed in each experiment for about 1.5 hours with urine collections approximately every 10 minutes and blood samples every 15 minutes.

Analyses. The plasma proteins were precipitated by $CdSO_4$.[4] The carbohydrates in

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60 ml of filtrate were precipitated by the addition of 6 ml of 25% $CuSO_4$ and an excess of dry $Ca(OH)_2$.[5] The carbohydrates in 30 ml of urine were precipitated by addition of 15 ml of 25% $CuSO_4$ and 15 ml of 10% lime suspension. The Cu-lime filtrate acidified with 0.5 cc of 50% H_2SO_4 was freed of acetone and acetoacetic acid by boiling for 5 minutes without reflux. The method of Barnes and Wick was modified by addition of approximately 0.25 g of ferrous alum to the Hg precipitate before distillation to prevent formation of free chlorine. Creatinine was determined[6] in the Cd plasma filtrates and in diluted urines.

Results. In the first experiment the plasma level of βB was maintained at nearly 40 mg % with only a slight fall during the infusion, after which the level fell to 6 mg % one hour after the infusion was shut off. The rate of tubular reabsorption of βB ($T_{\beta B}$) during the first 3 ten-minute periods was between 30 and 35 mg per minute when the plasma level was between 40 and 45 mg % and gradually fell to 5 mg per minute when the plasma level was 8 mg %. The load/$T_{\beta B}$ (plasma concentration × filtration rate/rate of tubular reabsorption) ratios were between 1.1 and 1.2 during the first 3 periods. Reabsorption of βB approached but did not reach completeness as the plasma level fell to 8 mg %. At this level reabsorption was incomplete by only 2%, while at a plasma level of 21 mg % reabsorption failed of completeness by 9%.

In the second experiment the plasma concentration of βB was 95 mg % 15 minutes after injection and the level fell to 32 mg % during the following 69 minutes. The rate of tubular reabsorption ($T_{\beta B}$) ranged between 28 and 38 during the 5 periods when the plasma level of βB was above 40 mg %. The load/$T_{\beta B}$ ratios ranged from 1.3 to 2.0 during these periods. At a plasma level of 35 mg % reabsorption was only 80% complete while at a plasma level of 96 mg % reabsorption was 50% complete. The maximum value ($Tm_{\beta B}$) for tubular reabsorption would appear to lie between 35 and 40 mg per minute for a dog weighing 12 to 15 kg.

Discussion. The finding of a gradual rather than a precipitate increase in the rate of excretion by the kidney of *dl*-βB with increasing plasma levels would not be anticipated from previous work on ketonuria. However, it is in accord with recent work on the excretion of amino acids[7] and of lactic acid (F. N. Craig, unpublished). The failure of reabsorption of 2% of the βB in the tubular filtrate, as occurs at a plasma level of 8 mg %, involves the loss of about 10 mg of βB per hour by a 15 to 20 kilogram dog. This is a not inconsiderable amount since at ordinary urine pH values it involves the loss of approximately 10 m.eq. of sodium. At higher plasma levels the rate of loss would lead increasingly rapidly to acidosis, unless sodium was supplied in a suitable form. For example, at a plasma level of 35 mg % 20% fails of reabsorption, leading to excretion of βB at the rate of 400 mg per hour, equivalent to approximately 400 m.eq. per hour, by a 15 to 20 kg dog.

Conclusion. Two renal clearance experiments in a dog indicate that *dl*-β-hydroxybutyrate is excreted by the kidney in a fashion resembling many amino acids. The maximum rate of tubular reabsorption ($Tm_{\beta B}$) appears to lie between 2 and 3 mg per minute per kg of animal. An appreciable excretion of β-hydroxybutyrate occurs at a plasma level of 8 mg %.

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