Our results do not necessarily conflict with those of authors who report that dehydrated animals show a prolonged action of insulin.[2] We measured only the effect of dehydration on the basal insulin requirement, the insulin given intravenously. All these authors cited gave the insulin subcutaneously; it is not improbable that dehydration slows up the absorption rate of insulin from the tissues. Others have shown that dehydration slows up the return of the blood sugar to normal after administration of glucose by stomach or intravenously.[3] Drabkin and Shilkret[4] report that spastic convulsions were not observed in desiccated animals after insulin although they appeared in those given fluids.

Summary. Depancreatized dogs maintained on a standard regime were dehydrated by producing glycosuria along with restricted water intake. It was found that the basal insulin requirement of the animals in this state was increased above that which they had normally without dehydration, but with other conditions the same.

If the dogs be given enough water so that no dehydration results from the glycosuria no increase in the basal insulin requirement results.

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Choline Esterase and Esters of Thiamine.

DAVID GLICK AND WILLIAM ANTOPOL.

From the Laboratories of the Newark Beth Israel Hospital, Newark, N. J.

The possible relationship between the physiological rôles of thiamine and choline esterase led the authors, in a previous study,[1] to investigate the inhibition of the enzyme by thiamine. Since thiamine occurs in the animal body largely as the pyrophosphate ester, cocarboxylase, a study of the effect of this compound upon choline esterase was undertaken, and it was found that, in contrast to the pronounced inhibitory action of thiamine, the pyrophosphate ester produced very little inhibition.

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2 Andrews, E., *Arch. Int. Med.*, 1926, **38**, 136; 1927, **40**, 637.

3 Tisdale, F. F., Drake, T. H., and Brown, A., *Am. J. Dis. Child.*, 1925, **30**, 837; *ibid.*, 1926, **32**, 854.

4 Drabkin and Shilkret, *Am. J. Physiol.*, 1927, **83**, 141.

1 Glick, D., and Antopol, W., *J. Pharm. and Exp. Therap.*, 1939, **65**, 389.

It has been reported[2] that although ethers are inactive, the esters of thiamine are physiologically active upon polyneuritic animals. There is the possibility that the ester is hydrolyzed *in vivo,* and the effects observed are due in fact to liberated thiamine. An attempt to elucidate this point was made by an investigation of both the enzymatic and hydroxyl-ion hydrolysis of the acetyl ester of thiamine chloride. A slow enzymatic splitting at a pH of 7.4, accompanied by a non-enzymatic scission of practically the same magnitude, was observed. Hence it cannot be stated with certainty that the physiological activity of the ester is an intrinsic property of the compound itself. However, the probability that such is the case gains credence by analogy to the physiological effects of somewhat related esters such as acetylcholine, atropine, and cocaine. As is well known, esterification enhances the physiological response to many substances. The inhibition of choline esterase by acetylthiamine chloride was also studied, the conditions employed being such as to obviate the disturbing influence of hydrolysis of the inhibitor.

Kuhn, Wieland, and Huebschmann[3] have raised the question of chemical mediation of nerve impulses by acetylthiamine. One would expect that the mediator would be capable of rapid removal from the site of its action. The relatively slow rate of hydrolysis, both enzymatically and non-enzymatically, reported below does not speak in favor of this rôle for acetylthiamine.

The pure thiamine chloride pyrophosphate and the acetyl ester of thiamine chloride were kindly supplied by Dr. R. T. Major of the Merck Research Laboratory, Rahway, N. J.

Measurements of ester hydrolysis were carried out in essentially the same manner as previously by the manometric method employing the Warburg apparatus.[1] All of the experiments were conducted at a pH of 7.4 and 30°. For the inhibition investigations 3 ml of acetylcholine chloride solution (5 mg of ester per ml in bicarbonate-Ringer solution) were employed with 0.5 ml of inhibitor solution and 0.5 ml of enzyme solution (0.4 ml of horse serum diluted to 10 ml with bicarbonate-Ringer solution). The inhibitor solutions were prepared by adding 0.1 n NaOH to the weighed substances until neutralized to pH 7.4, and the appropriate dilutions were then made with bicarbonate-Ringer solution. Readings were taken at 5-minute intervals for 30 minutes and the slopes of the linear activity-time curves were used to compare the effects of various concentrations of

2 Cline, J. K., unpublished data quoted by Williams, R. R., and Speis, T. D., in *Vitamin B_1 and Its Use in Medicine,* p. 173, The Macmillan Co., New York, 1938.

3 Kuhn, R., Wieland, T., and Huebschmann, H., *Z. Physiol. Chem.,* 1939, **259**, 48.

the inhibitors. The data obtained are shown in Fig. 1. The curve for the inhibition by thiamine has been taken from a previous publication[1] and was included to enable direct comparison with the inhibitory actions of the esters. Data in Fig. 1 were used to construct the curves in Fig. 2, which in turn were used to calculate the affinity of the inhibitors for the enzyme in the same manner as previously.[1] The calculation follows that given by Ziff, Jahn, and Renshaw.[4] The ratio of the slopes of the two lines to their respective intercepts on the 1/K axis is equal to their affinities for choline esterase relative to that of acetylcholine taken as 1. Hence, the affinity of the pyrophosphate ester is 1143/690 or 1.7, while that of the acetyl ester is 3500/665 or 5.3, as compared to 26 found previously[1] for the free thiamine.

For the study of the hydrolysis of acetylthiamine 1.5 ml of ester solution (1⅓%) and 0.5 ml of enzyme solution (0.5 ml horse serum diluted to 10 ml) were employed. The quantity of free thiamine liberated by hydrolysis had no demonstrable effect upon the course of the enzymatic reaction since the linearity of the activity-

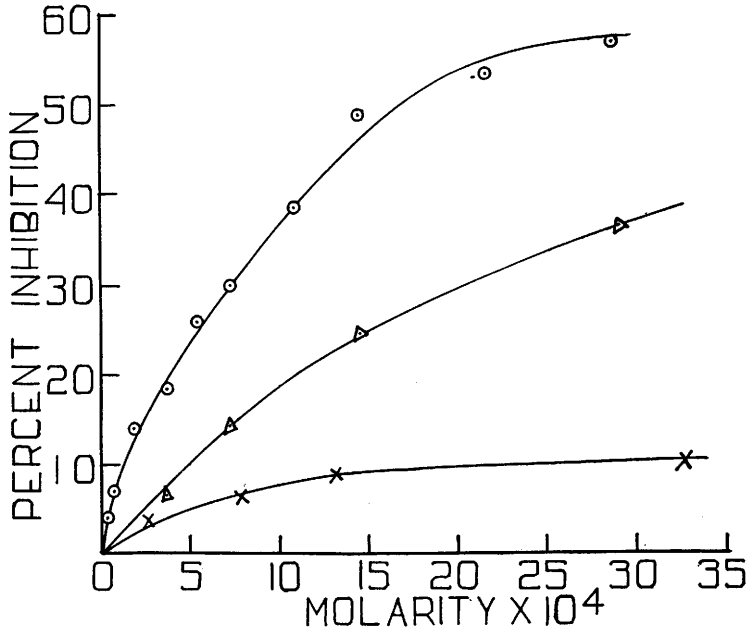

FIG. 1.

Inhibitions of choline esterase by thiamine (⊙), thiamine pyrophosphate (×), and acetylthiamine (△).

[4] Ziff, M., Jahn, F. P., and Renshaw, R. R., *J. Am. Chem. Soc.*, 1938, **60**, 178.

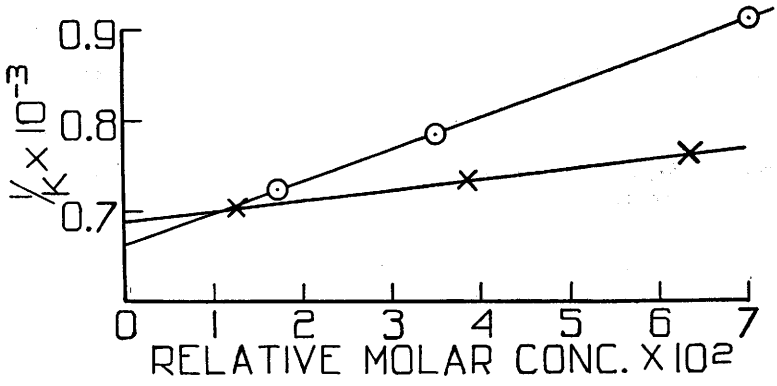

FIG. 2.

Inhibition of choline esterase by thiamine pyrophosphate (×) and acetylthiamine (⊙) in relation to the acetylcholine concentration. K, monomolecular velocity constant ($^{2.3}/_{t}$ log $^{a}/_{a-x}$). Abscissæ, ratio of molar concentration of thiamine ester to that of acetylcholine.

time curve was maintained for the duration of the experiment, 300 minutes. Furthermore, maximum velocity of enzymatic hydrolysis was obtained by employing a sufficiently high substrate concentration. The purely enzymatic hydrolysis was equivalent to the liberation of 26 cmm CO_2 in 300 minutes, and the scission due to hydroxyl-ion in the same period was 22 cmm.

After this paper had been submitted for publication, two others appeared concerning the enzymatic hydrolysis of acetylthiamine. Süllmann and Birkhäuser[5] reported that brain extract was able to split the ester, though horse serum had practically no effect. Their failure to observe hydrolysis in the latter case was due, for the most part, to the fact that measurements, under the conditions employed, were made for too short a reaction period (60 minutes). These authors also observed the inhibition of choline esterase by thiamine and acetylthiamine. Massart and Dufait[6] found that horse serum hydrolyzes acetylthiamine, and that eserine inhibits the action.

Summary. The acetyl ester of thiamine chloride was found to be a poorer inhibitor of choline esterase than free thiamine, while the pyrophosphoric ester had even less inhibitory action. The affinity for the enzyme of the former was calculated to be 5.3, and of the latter 1.7, times that of acetylcholine. The acetyl ester was hydrolyzed slowly by horse serum, the magnitude of the enzymatic splitting being approximately equal to that due to the hydroxyl-ion scission at the same pH (7.4).

[5] Süllmann, H., and Birkhäuser, H., *Schweiz. Med. Woch.*, 1939, **69**, 648.

[6] Massart, L., and Dufait, R., *Naturwissenschaften*, 1939, **27**, 567.