port adequate egg production. No dermatitis was observed during the experiment.

Summary. Evidence is presented to show that biotin is essential for normal embryonic development in the hen's egg. A satisfactory synthetic ration for use in studies of the vitamin requirements of laying and breeding hens is also presented.

13713

Studies on Inhibition and Activation of Atropinesterase.*

DAVID GLICK.

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

In an earlier communication dealing with the enzymatic hydrolysis of atropine by rabbit serum a study was made of the kinetics of the reaction, its enzyme-substrate affinity relations, and effects of pH and temperature.[1] Subsequently the occurrence of atropinesterase in rabbits, its distribution in blood and organs, and certain of its specificity characteristics were reported.[2] The present study extends these observations to include the influence on activity of certain salts, dyes, sulfhydryl compounds, substances affecting sulfhydryl groups, and physostigmine.

Rabbit serum, used as a source of enzyme, was dialyzed in 10 ml portions against distilled water for 3 days at 5-10° in cellophane tubes. After dialysis the volume was about 15 ml and the serum was centrifuged to remove precipitated globulins. Each batch, kept in a refrigerator, was used within a week. A loss in activity could be observed when the material was used after the first week.

The enzyme activity was determined at 30° and pH 7.4 by the manometric method employing the Warburg apparatus[1, 2] with the substitution of 0.20% $NaHCO_3$ for bicarbonate-Ringer solution in order to eliminate possible effects of KCl, NaCl, and $CaCl_2$. Three ml of substrate solution, containing 10 mg atropine sulfate dissolved in the $NaHCO_3$ solution, were placed in the main chamber; 0.5 ml of the dialyzed serum preparation and 0.5 ml of a solution of the compound to be tested, dissolved in $NaHCO_3$ solution, were placed in the side

* Aided by a grant from the Sidney C. Keller Research Fund.

1 Glick, D., *J. Biol. Chem.*, 1940, **134**, 617.

2 Glick, D., and Glaubach, S., *J. Gen. Physiol.*, 1941, **25**, 197.

arm. Saturation with 95% N_2-5% CO_2 was carried out as usual. In parallel control experiments 0.5 ml of distilled water was used instead of 0.5 ml of enzyme solution, and corrections for non-enzymatic hydrolysis were applied.

The compounds, whose effects on atropinesterase were studied, were obtainable in pure form with the exception of Congo red and methylene blue.† The Congo red was purified by 3 recrystallizations from 70% alcohol, and the methylene blue by 3 recrystallizations from 95% alcohol and washing with petroleum ether.

In the experiments dealing with the effect of calcium ion, atropine could not be used in the form of its sulfate due to precipitation of calcium sulfate; hence the free base of the alkaloid was employed, and HCl was added, after the $CaCl_2$, to bring the pH to 7.4.

From Fig. 1, it is apparent that equimolar concentrations of KCl, NaCl, NaBr, or NaI produce equivalent activations of the enzyme. The concentration-activation curve is very much like that obtained for the effects of KCl and NaCl on cholinesterase in dialyzed rabbit serum.[3] In both cases, with increasing concentration, the curves approach 50% activation in an asymptotic manner. It might be sup-

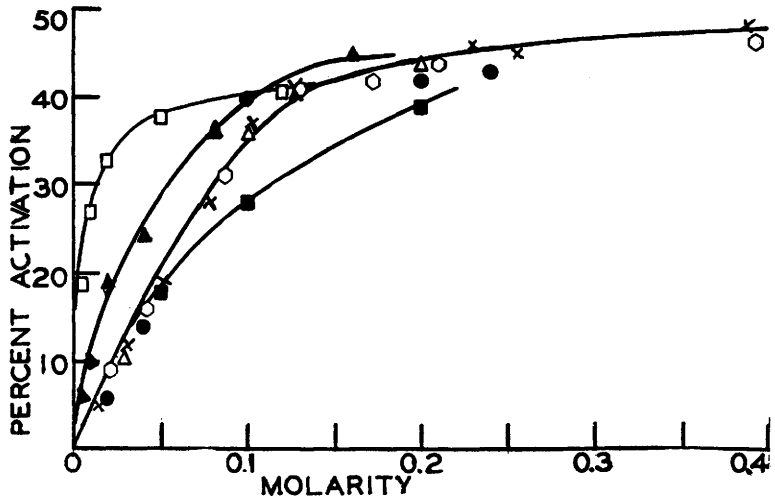

FIG. 1.
Activation of Atropinesterase by Salts.
(○) NaCl, (×) KCl, (●) NaBr, (△) NaI, (▲) Na_2SO_4, (□) $MgSO_4$, (■) KCNS.

† p-Chloromercuribenzoic acid, o-iodosobenzoic acid, and phenylmercurihydroxide were graciously supplied by Dr. Leslie Hellerman of the Department of Physiological Chemistry, Johns Hopkins School of Medicine.

3 Glick, D., *Nature*, 1941, **148**, 662.

posed that these activations result from effects of the ions on the colloidal enzyme protein. The greater activations produced by equimolar concentrations of the salts with divalent radicals, Na_2SO_4 and $MgSO_4$, are compatible with this view, although Mg^{++} is known to activate certain esterases more specifically.

From Table I it may be seen that the sulfhydryl compounds, cysteine and glutathione, inhibit the enzyme, whereas the effect is reversed with cystine. Since agents capable of eliminating sulfhydryl groups, such as cyanamide,[4] p-chloromercuribenzoic acid,[5] o-iodosobenzoic acid,[6] phenylmercurihydroxide,[5] had no effect, it follows that the dialyzed serum contained no sulfhydryl groups capable of affecting the enzyme activity, nor were sulfhydryl radicals an essential part of the enzyme itself. How much of the cyanide inhibition may be due to reduction of -S-S-groups possibly present in the enzyme preparation cannot be stated.

The inhibition produced by KCN was noted earlier by Van der Heyde[7] although no really quantitative study was made. Bernheim and Bernheim[8] reported an 80% inhibition of the hydrolysis of homatropine by guinea pig liver in the presence of 1.5% (0.36M) NaF, as well as strong inhibitions by physostigmine in concentrations of 0.7×10^{-5} and 2.1×10^{-5} M. The time course of the reactions inhibited by physostigmine in the present investigation is given in Fig. 2. It may be seen that the inhibitions produced by the lower

TABLE I.
The Effect of Certain Compounds on the Activity of Atropinesterase.

Compound	Range of concentration (molarity)	% change
Calcium chloride	.010	15 activation
Trisodium citrate	.030-0.170	0
Sodium cyanide	.005-0.015	10-58 inhibition
Sodium fluoride	.005-0.100	6-59 "
Cysteine	.050-0.200	13-75 "
Glutathione	.008-0.040	17-100 "
Cystine	.005-0.010	10-24 activation
Cyanamide	.006-0.300	0
p-chloromercuribenzoic acid	.25% suspension	0
o-iodosobenzoic acid	"	0
Phenylmercurihydroxide	"	0
Congo red	.00018	0
Methylene blue	.00033	0
Physostigmine salicylate	.0000024-0.00242	36-100 inhibition

[4] Glaubach, S., *Arch. Exp. Path. Pharm.*, 1926, **117**, 247, 257.

[5] Gemmill, C. L., and Hellerman, L., *Am. J. Physiol.*, 1937, **120**, 522.

[6] Hellerman, L., Chinard, F. P., and Ramsdell, P. A., *J. Am. Chem. Soc.*, 1941, **63**, 2551.

[7] Van der Heyde, H. C., *Arch. neerl. Physiol.*, 1921, **5**, 380.

[8] Bernheim, F., and Bernheim, M. L. C., *J. Pharm. Exp. Therap.*, 1938, **64**, 209.

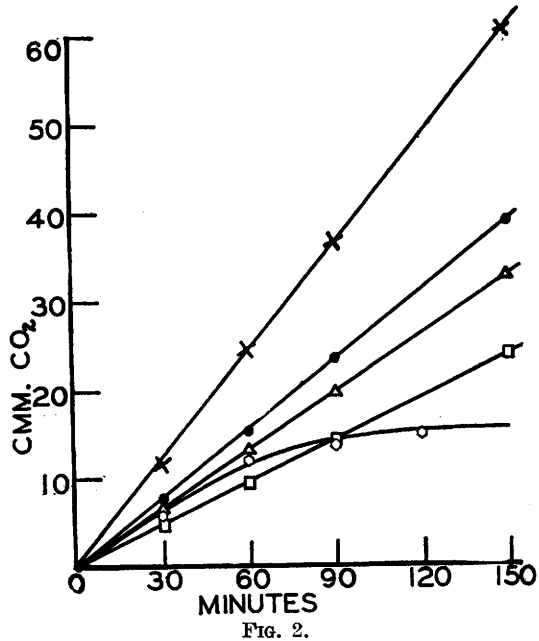

FIG. 2.

Inhibition of Atropinesterase by Physostigmine.

(×) control without drug, (●) 0.24 × 10^{-5} M, (△) 2.42 × 10^{-5} M, (□) 24.2 × 10^{-5} M, (○) 242. × 10^{-5} M physostigmine salicylate.

concentrations of physostigmine reach their maximum during the period of setting up the experiment (about 30 min.) since the hydrolysis-time curves are linear. But, with the highest concentration of inhibitor (242 x 10^{-5} M), the maximum inhibition is not attained for about 120 min. The lag in obtaining inhibitions of cholinesterase with this drug is well known.

Massart and Dufait[9] found Congo red to be representative of certain acid dyes having no effect on cholinesterase, whereas methylene blue, containing a quaternary ammonium base, strongly inhibited the enzyme in very high dilutions. Rentz[10] had previously shown that methylene blue inhibited cholinesterase as powerfully as physostigmine. Although the concentration of methylene blue used in this case completely inhibited the cholinesterase in the serum, it had no influence on the atropinesterase. This finding affords another mark of differentiation between the two azolesterases.

Summary. Atropinesterase of rabbit serum was found to be activated to the same degree by equimolar concentrations of KCl, NaCl, NaBr, and NaI. Activations were also produced by KCNS,

[9] Massart, L., and Dufait, R. P., *Enzymologia*, 1941, **9**, 364.

[10] Rentz, E., *Arch. exp. Path. Pharm.*, 1940, **196**, 148.

$MgSO_4$, and $CaCl_2$. Trisodium citrate had no effect. Cyanide, fluoride, physostigmine and sulfhydryl compounds inhibited the enzyme. Compounds capable of eliminating sulfhydryl groups present in the enzyme preparation had no influence, nor did Congo red or methylene blue.

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Inactivation of Pressor Amines by Quinones and Related Diketones.

SAUL SOLOWAY* AND KURT A. OSTER.† (Introduced by Harry Sobotka.)

From the Department of Chemistry, Laboratories of The Mount Sinai Hospital, New York City.

In the metabolism of certain aromatic l-amino acids in the kidney pressor amines are formed by decarboxylases as intermediary products. In the normal kidney the enzyme amine oxidase deaminizes these amines to inert substances. This oxidase is not effective in the ischemic kidney, and the process is arrested at the stage of the pressor amine. This mechanism is thought to be one of the causes of experimental hypertension (Holtz,[1] Bing[2]).

It was believed that an approach to the problem of hypertension could be effected by causing the oxidative destruction of pressor amines circulating in the blood stream. Kisch[3] has shown that certain quinones act as catalysts in the oxidative deamination of amino acids and polypeptides.

Since catechol derivatives and aliphatic enediols form quinones and diketones respectively on aeration in the presence of certain metallic ions as catalysts, it was of interest to examine their effect on various pressor amines *in vitro*. The quinones (or diketones) formed could act either by deaminating the amines or by forming compounds of the azophenine type.

Experimental. Solutions containing tyramine, indolethylamine,

*Hernsheim Fellow in Chemistry.

† George Blumenthal, Jr., Fellow.

1 Holtz, P., Heise, C., and Luedtke, C., *Arch. exp. Path. u. Pharm.*, 1938, **191**, 87.

2 Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

3 Kisch, B., *Oppenheimer's Handb. der Biochem.*, Jena, 1933, Ergw. **1**, 563.

4 Beyer, K. H., *J. Pharm. Exp. Ther.*, 1941, **71**, 394.