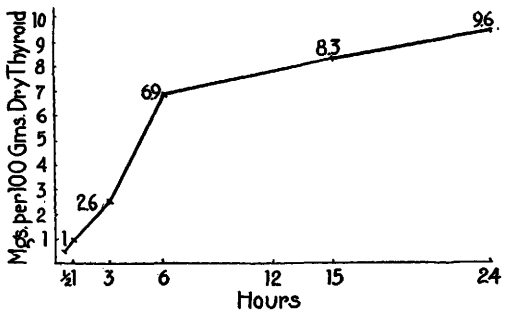

FIG. 1.

Manganese deposition in the thyroid gland of the guinea pig following a single subcutaneous injection of manganese chloride in a dosage of 10.0 mg per kg of body weight.

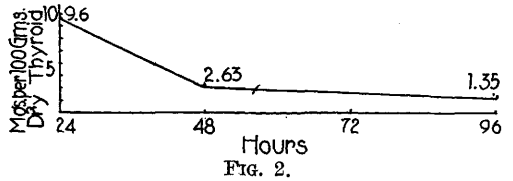

FIG. 2.

Rate of elimination of manganese from the thyroid gland of the guinea pig.

content of the thyroid gland was determined by the procedure of Ray.[2] The results are recorded graphically in Fig. 1 and 2. Each point on the graphs represents the average amount of manganese found in the thyroids of 3 animals. A detectable increase over the level found in earlier tests[1] was noted in 30 minutes, and a measurable increment was found in one hour. Fig. 1 shows further that the accumulation of manganese was rapid during the early stages of absorption. Of the total amount deposited in a 24 hour period, about 70% of it accumulated within the first 6 hours. Fig. 2 shows that the elimination of manganese from the thyroid, once it began, was very rapid. More than 70% of the 24 hour accumulation disappeared within 48 hours after the injection, and 85% of it disappeared within 96 hours.

Summary. Subcutaneous injection of manganese chloride into the guinea pig caused manganese to accumulate in all organs of the body tested, but the deposition was especially great in the thyroid gland where it began to appear within 30 minutes after administration. Absorption was particularly rapid during the first few hours, but the element continued to accumulate for at least 24 hours. Of the total amount of manganese deposited in the thyroid gland about 70% accumulated during the first 6 hours. Elimination of manganese from the thyroid, once it began, was rapid. Seventy per cent of the 24 hour accumulation disappeared within 48 hours after the injection; 85% of it vanished in 96 hours.

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[2] Ray, T. W., *J. Biol. Chem.*, 1940, **134**, 677.

17129. Effect of the D- and L-isomers of Isoamidone* on the Permeability of Dog Erythrocytes.†

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We have previously reported that changes in the permeability or erythrocytes *in vitro* appeared to be correlated with changes in the activity of the acetylcholine-cholinesterase system in the red cell.[1,2] This conclusion was based on the observation that physostigmine as well as methadon,§ both of which are chol-

* 1-dimethylamino-2-methyl-3,3-diphenyl hexanone-4.

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[1] Greig, M. E., and Holland, W. C., submitted for publication to *Arch. Biochem.*

[2] Greig, M. E., and Holland, W. C., *Fed. Proc.*, 1949, **8**, 297.

§ Methadon, amidone, 2-dimethylamino-4,4-diphenyl-heptanone 5. The methadon and isoamidone were kindly supplied by the Mallinckrodt Chemical Works.

TABLE I.

Effect of the D- and of the L-isomers of Isoamidone on Cholinesterase Activity of Dog Erythrocytes.

The media used were Ringer Krebs bicarbonate saline (R.K.),[7] sodium bicarbonate saline (Na) consisting of 100 parts 0.9% NaCl and 21 parts 1.3% $NaHCO_3$, and potassium bicarbonate saline (K) consisting of KCl and $KHCO_3$ in the same proportions as for the sodium buffer. 0.2 cc packed dog erythrocytes, and acetylcholine in a final concentration of 0.01 M in a volume of 2 cc were used. Carbon dioxide evolution was measured in Warburg manometers.

Medium	Duration of exp., min.	Conc. of drug, M		Mm³ CO_2 evolved				
		D	L	Control	D	% effect	L	% effect
Na	60	.006	.0002	115	84	27	84	26
K	60	.006	.0002	124	87	30	67	46
RK	60	.00087	.00087	202	124	39	98	51
RK	60	.00087	.00087	228	168	26	102	55

TABLE II.

Effect of the D- and the L-isomers of Isoamidone on Glycolysis.

The experiments were carried out in a Ringer Krebs bicarbonate medium[7] in Warburg manometers. The concentration of glucose in the experiment with erythrocytes was 0.014 M, that in the experiment with brain 0.01 M. The amount of tissue was 1.0 cc packed dog erythrocytes or 150 mg homogenized rat brain in a final volume of 2 cc. The gas phase was 95% N_2, 5% CO_2.

Tissue	Duration of exp., min.	Conc. of isoamidone, M	CO_2 evolved in mm³				
			Control	D	% effect	L	% effect
Erythrocytes	40	.00174	89	70	21	77	14
Rat Brain	60	.00174	130	107	18	101	22

inesterase inhibitors[3,4] produced changes in permeability of dog erythrocytes under certain conditions. Physostigmine is considered to be a specific inhibitor of cholinesterase. This drug produced most marked changes in the permeability of erythrocytes under the conditions where it had its maximum effect on cholinesterase activity. Methadon is not a specific inhibitor of cholinesterase as it also inhibits glycolysis of glucose[5] as well as certain oxidative processes.[6] However, under our experimental conditions, the inhibition of cholinesterase rather than the inhibition of glycolysis by methadon seemed to account for its effect on permeability of erythrocytes.

As the D- and L-isomers of isoamidone have been found to be equally effective in inhibiting glycolysis, but the L-isomer is a much more effective inhibitor of cholinesterase than is the D, it was assumed that if the effects on permeability were different with the different isomers, this action could be more readily attributed to their effect on cholinesterase than to that on glycolysis.

The present communication describes experiments dealing with the effects of the D- and of the L-isoamidone on the permeability of dog erythrocytes.

Results. In Table I are presented results of experiments on the effects of the D- and L-isomers of isoamidone on the cholinesterase of dog erythrocytes. As in the case of brain cholinesterase the L-isomer has a much greater inhibitory action than has the D- in the same concentrations.[3]

From Table II it may be seen that there is little or no difference between the effects of the D- and L-isomers on glycolysis of glucose by rat brain or by dog erythrocytes.

[3] Greig, M. E., and Howell, R. S., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 352.

[4] Eadie, G. S., Bernheim, F., and Fitzgerald, D. P., *J. Pharm. Exp. Therap.*, 1948, **94**, 19.

[5] Greig, M. E., *Arch. Biochem.*, 1948, **17**, 129.

[6] Greig, M. E., and Howell, R. S., *Arch. Biochem.*, 1948, **19**, 441.

[7] Krebs, H. A., and Henseleit, K., *Z. physiol. Chem.*, 1932, **210**, 33.

In our experiments the hemoglobin liberated has been taken as a criterion for changes in permeability of erythrocytes. If percent hemolysis be plotted against time, six possible arrangements of curves representing the effects of the D- and L-isomers of isoamidone on erythrocytes could be obtained. If C represents the hemolysis in the control suspension, D- that with the D-isomer and L- that with the L-isomer the 6 possible types obtainable would be (1) L>D>C, (2) D>L>C, (3) C>D>L, (4) C>L>D, (5) D>C>L, (6) L>C>D.

Experimentally we found that all 6 types occurred to some extent under different conditions. The factors controlling the type of curve obtained appeared to be mainly the proportions of Na and K in the medium and the pH. There seemed to be some variation in results obtained with red cells from different dogs under the same experimental conditions, although the results obtained with different samples of blood from the same dog under the same experimental conditions were quite consistent.

In a medium containing a high proportion of KCl compared with NaCl, curves of type 3 were most frequently obtained, while those of type 5 and type 1 occurred less often. Of 23 experiments carried out in a medium consisting of isotonic KCl or a mixture of isotonic KCl and isotonic NaCl in the proportions of 9:1, fifteen fell in type 3 and 8 in type 5. It was frequently observed that early in an experiment the results would follow curves of type 3, later the curves would cross and follow those of type 5 and still later those of type 1 and occasionally type 2.

In a medium containing a high proportion of Na compared with K there was little difference in the effects produced by D- and L-isomers. Usually curves of types 1 and 2 were obtained.

Results of 3 typical experiments are presented graphically in Fig. 1-3.

Discussion. It may be seen from the above results that:

1. the D- and L-isomers of isoamidone in the same concentrations inhibit the glycolysis of glucose by rat brain and by dog erythrocytes to about the same extent.

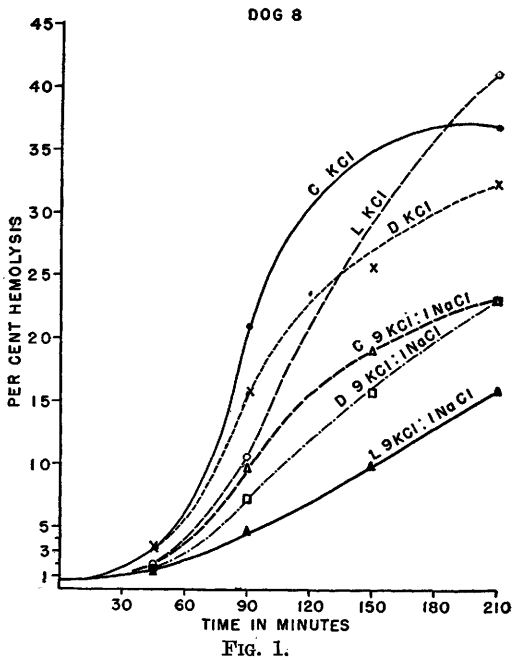

FIG. 1.

One cc packed dog erythrocytes were suspended in 10 cc isotonic saline in Erlenmeyer flasks. The final concentration of the isoamidone was 0.00022 M, that of acetylcholine 0.01 M. Samples of suspension of 1 cc were removed at intervals and after dilution and centrifugation the hemoglobin content of the supernatant fluid was determined using a photoelectric colorimeter.

C—Control.
D—D-isoamidone.
L—L-isoamidone.
Ac ch—Acetyl choline.

2. the D- and L-isomers of isoamidone inhibit the cholinesterase activity of erythrocytes to different extents, the L- being a more effective inhibitor than the D-isomer.

3. the D- and L-isomers of isoamidone have different effects on the permeability of dog erythrocytes.

As the stereoisomers have the same chemical properties, *e.g.*, molecular weight, solubility, molecular radius, rate of diffusion, the different effects on the permeability of erythrocytes could not be attributed to these factors.

If one assumes that the permeability of living cells is controlled by metabolic activity, then of the two metabolic reactions investigated which are affected by isoamidone the inhibition of cholinesterase activity would seem best able to account for the changes in permeability in view of the different effects pro-

duced by the two isomers on this enzyme.

In a medium containing a high proportion of KCl compared with NaCl the L-isomer usually produced greater resistance to hemolysis than the D at the start of the experiment, (type 3 and Fig. 1).

In a bicarbonate medium, pH 8, the L usually increased hemolysis, relative to the control and the D-isomer (Fig. 2). These results are similar to those found with physostigmine.[1,2] Occasionally curves of type 5 were found early in the experimental period, in which the L-isomer caused increased resistance and the D-increased fragility relative to the control.

The addition of acetylcholine increases the resistance of the control suspension in a K medium and tended to increase the differences between the effects produced by the D- and L-isomers relative to the control (Fig. 2).

In a medium consisting of either NaCl or high Na compared with K the differences between the effects of the D- and L-isomers were usually less marked (Fig. 3) and the D occasionally produced slightly greater hemolysis than the L (curves of type 2).

We also found that physostigmine had little

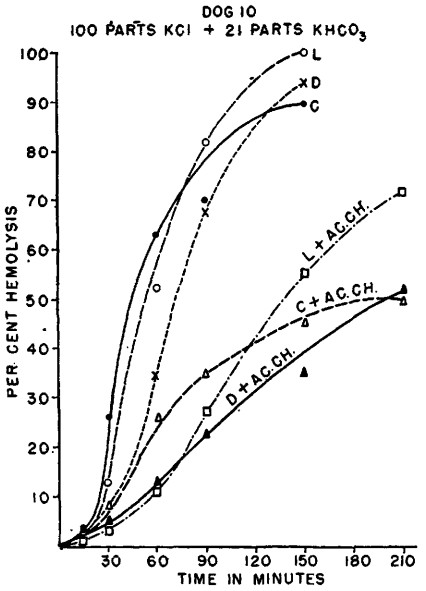

FIG. 2.

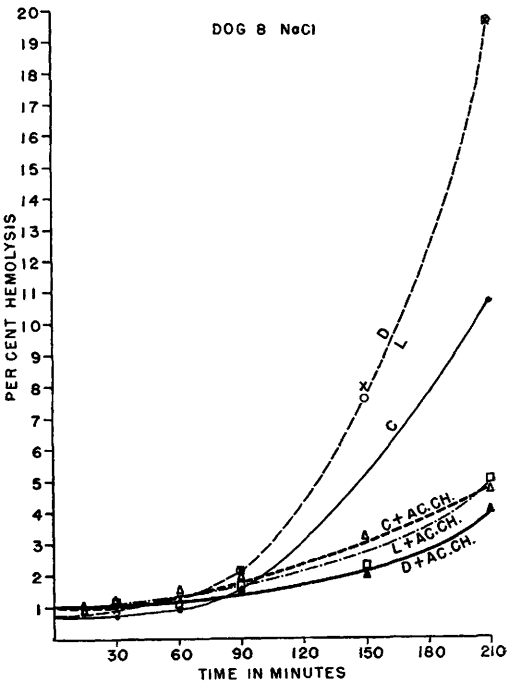

FIG. 3.

effect on permeability in a medium of NaCl as evidenced by hemolysis but that it produced very marked changes in permeability in a high K medium, in the presence of acetylcholine and a pH of 8, under which conditions it produced maximum inhibition of cholinesterase.[1]

Investigations of ionic transfer between erythrocytes and the medium in the presence of cholinesterase inhibitors are under way.

Summary. The theory that the acetylcholine-cholinesterase system is concerned with membrane permeability is further substantiated by the finding that the L-isomer of isoamidone which has a greater inhibitory action on cholinesterase than has the D-isomer also changes permeability of dog erythrocytes to a different degree than does the D-isomer.

The conditions under which the L has a greater effect than the D-isomer on permeability are a medium containing a high proportion of K ions relative to Na ions.

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