creas than from the foot of these moribund animals, so that during this stage the percent of glycogen in the foot frequently exceeded that in the hepatopancreas. *Amblema costata* and *Tritogonia verrucosa* after 238 and 187 days respectively of starvation (Table 1), are examples of this condition.

Only a limited number of thin-shelled mussels were included in these starvation tests, but in general the thin-shelled species were found less able to withstand prolonged starvation than the thick-shelled species. For example, the maximum amount of glycogen in the hepatopancreas for a group of 8 papershells (*Proptera lævissima* and *Leptodea fragilis*), after 49 days starvation was only 6.8%, and no glycogen was found in the hepatopancreas of 3 of these individuals, and none in the foot of any of the 8.

As expected the data showed some species variation among both the thin-shelled and thick-shelled forms, but considering only the thick-shelled species it seems that the utilization of the stored glycogen by these freshwater mussels during starvation proceeds quite slowly during the first 30 to 200 days of starvation.

As may be observed, all of the glycogen values presented are based on dry weight, in an effort to avoid errors due to variation in the water content of the tissues. It was found however that variations in tissue total solids were much less pronounced even at the end of the long periods of starvation, than were the observed changes in glycogen storage, making the relative changes in glycogen even more evident.

8568 P

Action of Choline Ester on Embryonic Iris and Development and Maintenance of Reactivity.

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In a search for the mechanism of the action of choline esters, it was desired to find the earliest stage at which a choline ester produces constriction in the iris, and to follow the rate of change, if any, in the amount of constriction as the iris develops. It was also desired to find what relation this action has to the development of the muscle and of the innervation.

For the study here reported, embryo pigs were obtained at the

packing house at the time of removal from the dead mother, still at approximately body temperature. Some of them were kept at body temperature, some allowed to cool at room temperature, and others were placed in Locke or other physiological solution at 0°C. Carbaminoyl choline chloride solution was placed on the cornea of the right eye and the left eye was used as a control. Sometimes the eyes were left in the head but usually they were excised and placed in glass cups. The amount of constriction produced was determined by measuring the diameter of the pupil with a pair of fine dividers and a millimeter scale and comparing it with the diameter of the control pupil.

TABLE I.
Iris (Embryo) Reactions to Carbaminoyl Choline.

——Embryo—— Length mm.	*Age da.	No. of eyes tested	Aver. Constr. (%) at 21°	at 37°	Lysis (Notches in iris)
76	52	3	0		
101	63	4	0		2-3 sml.†
103	64	10	0		?
104	64	10	0		2-4 sml.
122	71	12	3		2-7
136	76	11	6		1-6
151	81	16	13		5-6 lg.
164	87	4	20		3-4 v. lg.
177	90	5	20		
190	94	4	58		
196	95	2		0	
219	102	1	70		
”		2		5	
220	102	2	63		
”		1		0	
222	103	1	38		
”		4		0	
226	104	1	78		
261	112	8	53		

*Average of values given by Lebreton and Schaeffer[6] and by Warwick.[7]
† sml., small; lg., large; v., very.

In the accompanying table some of the characteristic results are shown. It will be seen from the table (1) that at room temperature no constriction by the drug in the iris has been noted before the 71st day of gestation; (2) that there was a gradual increase in constriction from that time at least up to the 94th day; (3) that there

[6] Lebreton, E., and Schaeffer, G., *Trav. de l'Institut. Physiol. du Fac. Méd.*, Univ. Strasb., 1923.

[7] Warwick, B. L., *J. Morph. and Physiol.*, 1928, **46**, 59.

was little or no constriction when the iris was kept at body temperature; and (4) that there was a disintegration or lysis of iris tissues at the ages from 63 to 87 days.

The fact that little or no constriction was produced, by the choline ester before the 71st day, suggests that at about that time the choline-labile receptive substance was beginning to be formed. What relation this beginning of drug action has to the contact of the nerve with the muscle, we hope to determine with histologic studies now in progress.

The gradual increase in constriction from the 71st to the 94th day might by due to a gradual development of one or more of the following structures: contractile mechanism, receptive substance, nerve fiber.

The absence of constriction at body temperature suggests that the receptive substance is very labile to heat in the absence of circulating blood.

Evidence that blood may inhibit the lytic or catalytic action is found in our observation that at times the only tissue remaining after lysis is blood vascular tissue.

The lysis was often grossly observed to begin as a minute hole at the pupillary margin of the iris; *i.e.*, near the location of the sphincter muscle, on the receptive substance of which the choline ester acts. The holes developed into notches that more or less rapidly enlarged, depending on the conditions. Up to this time, lysis has been seen only in embryos from 63 to 87 days old. Since the lysis was nil or very slight in ages before constriction was produced, and was not observed in irises that might be considered completely functional, it seems that it might be due to a substance produced by the action of a choline ester on a maturing receptive substance, or on the hypothetical enzyme whose function is to build up that receptive substance. Atropine prevents the lysis and also stops it after it has begun. We are studying further the action of atropine, of physostigmine and of other drugs on this lytic process. We are also studying histologically the effects on the various tissues of this lytic action.

The finding of this lytic action seems to lend support to the hypothesis of Shaklee, Christensen and Kaplan *(l.c.)*, that the normal action of the choline ester liberated at the nerve endings is a catalytic action on the receptor substance. The hypothesis of a catalytic action appears to be in harmony, not only with the hypothesis of receptive substances by Langley,[2] so well supported by his evidence,

2 Langley, J. N., *J. Physiol.*, 1905, **33**, 374.

but also with all the findings in this connection that we have examined; for example, to name only a few, the findings of Rosenblueth,[3] that there are two substances produced by the action of "sympathin" on receptive substances; the findings of Brown and Feldberg,[4] that potassium ions stimulate the curarized sympathetic ganglion which is not acted upon by acetylcholine; and the findings of Lánczos,[5] that calcium ions in addition to "sympathin" are liberated by stimulation of the sympathetic fibers to the frog's heart, and that these same calcium ions in the strength (0.01%) found in Ringer solution produced a marked action in the ergotaminized heart.

Further details, other drug actions and the histological studies will be presented in a following paper. We wish to thank Merck & Co. for the carbaminoyl choline.

8569 C

Bromsulphalein Dye Retention Test as a Measure of Functional Activity of Reticulo-Endothelial System.

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In the course of studies of the reticulo-endothelial system, it became apparent that some method of measurement of the function or degree of functional impairment obtained with the present so-called blockading methods, would be of great value in interpreting the experimental results. Of the various methods which offered themselves as tests, a dye excretion or clearance test, which depended upon the integrity of the reticulo-endothelial system seemed most suitable for adaptation to our needs.

Various dyes have been suggested as being selectively removed from the blood stream by the reticulo-endothelial system. Landsberger[1] reported using Congo Red for this purpose, but obtained inconstant results. Merklan and Wolf,[2] Saxl and Donath,[3] Shellong

[3] Rosenblueth, A., *Am. J. Physiol.*, 1932, **101**, 149; **102**, 12.

[4] Brown, G. L., and Feldberg, W., *J. Physiol.*, 1935, **86**, 10 P.

[5] Lánczos, Anna, *A. f. Path. u. Pharm.*, 1936, **180**, 312.

* Frederich Robert Zeit Fellow in Pathology.

[1] Landsberger, J., *Z. f. Immunitatsforsch.*, 1930, **65**, 385.

[2] Merklan and Wolf, *Bull. et Mem. d. l. Soc. Med. des Hosp.*, 1925, **49**, 1180.