sorptive state and therefore the serum amino nitrogen is probably higher than the average for the 24-hour period.)

It will be seen from Table 1. that there was no evidence of rapid extravascular proteolysis.

8567 C

Glycogen Content of Freshwater Mussels During Prolonged Starvation.

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In view of the high glycogen content of the hepatopancreas of freshwater mussels,[1] the glycogen contained in the hepatopancreas and in the pedal muscle ("foot"), of 206 mussels representing 20 species, was determined during periods of starvation ranging from 2 to 536 days, to ascertain whether the utilization of stored glycogen by these invertebrates during starvation is comparable to the utilization of stored glycogen by mammals during periods of inanition.[2]

The mussels in this series were collected in September and October before the water became cold, from beds where colonies of healthy mussels were thriving, so that each animal was started in these starvation tests after a summer of normal feeding, which had prepared the animal presumably for the winter period of reduced activity. The individual mussels, between 4 and 7 years of age, were isolated either in glass hatchery jars or metal hatchery tanks, through which well-aerated water from deep wells was flowing. This water, which had been found previously to constitute a satisfactory environment for mussels as regards inorganic salts, pH, and dissolved gasses, contained no plankton, organic detritus, or organic salts. The recording thermograph showed that the water temperature fell from 18°C. in October to 11°C. in February, rising slowly to 22°C. in August and dropping to 18°C. again by October, *i.e.*, the animals under observation were subjected to a slowly changing temperature cycle comparable to that of their natural habitat.

The analyses were made by a modification of the Sahyun-Alsberg technique, using tissue frozen in carbon dioxide snow immediately

1 Calvin, D. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 96.

2 Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

TABLE I.

% Glycogen, Dry Weight, in Hepatopancreas and in Foot of Freshwater Mussels During Starvation.

	1st month					2nd to 7th mo. incl.*					At end maximum starvation period, 5-18 mo.					
		Glycogen Hepatopan.		Foot			Glycogen Hepatopan.		Foot		Days of starvation		Glycogen Hepatopan.		Foot	
	Cases	Max.	Aver.	Max.	Aver.	Cases	Max.	Aver.	Max.	Aver.		Cases	Max.	Aver.	Max.	Aver.
Lampsilis anodontoides	4	18.5	7.5	7.9	5.8	10	25.3	15.2	7.8	5.6	536	8	2.1	1.1	3.0	0.5
" *carinata*	8	41.7	22.5	8.0	3.5	17	34.1	15.6	6.8	2.6	231	3	33.0	30.8	3.2	2.8
" *recta*	—	—	—	—	—	—	—	—	—	—	189	1	43.5	43.5	4.4	4.4
" *ventricosa*	—	—	—	—	—	—	—	—	—	—	197	1	31.1	31.1	4.9	4.9
Proptera capax	—	—	—	—	—	—	—	—	—	—	203	1	31.1	31.1	6.7	6.7
" *purpurata*	—	—	—	—	—	—	—	—	—	—	338	1	41.4	41.4	14.5	14.5
Tritogonia verrucosa	9	27.6	11.6	8.0	4.9	17	28.5	12.6	8.2	4.6	187	5	2.0	1.9	8.2	4.9
Cyprogenia aberti	1	32.0	32.0	—	—	1	30.5	30.5	TS	TS	182	1	30.5	30.5	TS	TS
Obliquaria reflexa	1	26.1	26.1	10.0	10.0	3	22.8	13.4	8.0	6.6	163	1	6.4	6.4	8.0	8.0
Quadrula metanevra	1	28.6	28.6	7.1	7.1	4	41.6	24.3	8.9	6.8	189	2	41.6	32.3	7.6	7.0
" *pustulosa*	4	52.2	37.8	10.6	7.2	4	18.2	7.9	10.0	3.9	161	2	1.0	0.5	TS	TS
Megalonaias gigantea	1	52.1	52.1	7.4	7.4	2	49.8	49.0	12.6	10.1	197	1	49.8	49.8	12.6	12.6
Amblema costata	5	50.8	29.1	10.2	5.9	8	23.9	11.9	10.5	6.4	238	1	TS	TS	3.7	3.7
" *dombeyana*	3	46.0	39.1	9.2	8.0	3	44.0	27.9	5.6	4.5	189	2	44.0	36.6	5.6	5.2
Fusconaia undata	8	35.6	27.8	8.1	4.4	2	20.5	15.2	6.1	4.9	133	2	20.5	15.2	6.1	4.9
Elliptio dilatatus	1	44.6	44.6	15.2	15.2	—	—	—	—	—	231	1	TS	TS	TS	TS

*Analyses were made at intervals of 15 days or less throughout this period.

TS = too small to be measured accurately by method used.

after removal from the animal.[3] Data from 134 specimens representing 16 species have been condensed in Table 1 as typical of the findings.

From all of the analyses made on these 206 specimens it was found that, for the thick-shelled species of mussels, the maximum value of stored glycogen in terms of dry weight of the hepatopancreas lay between 25 and 61%, values between 25 and 40% being common during the first month of starvation. Although the general average glycogen value fell slowly after the first month of starvation, many individuals were found to have maintained a glycogen level above 30% during 180 to 338 days of starvation, pointing either to a slow utilization of stored glycogen, or to its replacement from within by some form of autodigestion of body tissues. However, as these mussels having high glycogen after long starvation uniformly were found to be in good condition judging by the vigorous heart action and pedal muscle activities, slow utilization of the glycogen seemed the more probable explanation for the long-sustained high level of stored glycogen.

Individuals of *L. anodontoides* were subjected to the longest period of starvation. In some specimens of this species the glycogen content of the hepatopancreas remained above the 25% level for over 200 days of starvation. After 536 days of starvation however the glycogen in the hepatopancreas averaged only 1.1%, with a maximum of 2.1% for the 8 mussels surviving this period, although the hearts of these 8 mussels were still beating normally and the pedal musculatures showed good irritability responding with weak contractions when stimulated. The actual amount of glycogen present in the hepatopancreas of 3 of these 8 mussels was too small to measure accurately by the method used, as was the glycogen in the foot of 6 of these specimens.

These specimens of *L. anodontoides* were typical of the entire starvation series when the stored glycogen in the hepatopancreas fell to 4% or lower. Although all of the species of thick-shelled mussels studied maintained in general a glycogen level in the hepatopancreas above 15 or 20% for 5 to 7 months of starvation, eventually if the starvation were continued the animals became moribund. This condition was associated with a rather sudden drop in the stored glycogen level to below 4%. This low glycogen level was attended with an oedematous condition and a discoloration of the tissues, although the heart continued to beat normally. The loss of glycogen seemed to proceed more rapidly from the hepatopan-

[3] Ellis, M. M., and Calvin, D. B., *Am. J. Physiol.*, 1932, **101**, 32.

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