gen, although at the same time there was a marked decrease in the glycogen of skeletal muscle.

These two findings seem to indicate that heart glycogen is under different control than that of skeletal muscle; the alternate conclusion would be that if cardiac and skeletal muscle glycogen are under essentially the same control, then some additional factor not readily recognized when dealing with skeletal muscle is entering more largely in the case of the heart.

Cardiac glycogen is therefore set aside as an interesting subject for study, not only because of the possible importance to the heart itself, but because such an investigation might also bring new light to the views regarding carbohydrate and muscle metabolism generally.

Summary. Cardiac glycogen is found to be raised in depancreatized cats, and to be well maintained in fasting phloridzinized rats, both with and without an accompanying injection of epinephrine. Attention is drawn to the importance of these findings to current views of carbohydrate change in the body.

8862 C

Effects of Chilling on Structure and Behavior of Embryos of *Amblystoma punctatum* Cope.

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In my study of the development of the behavior of Amblystoma it became imperative to know whether or not eggs or embryos that had been subjected to a low temperature, in order to prolong the experimental season by retarding growth, followed the normal order of development of movements. To meet this requirement I conducted experiments in 1930. Regarding behavior the results were conclusively in the negative, but it seemed desirable to vary the conditions in further experiments before publishing the results, especially in consideration of the numerous and extreme structural defects that appeared in the experimental animals. But inasmuch as I have not been able, and probably shall not be, to repeat the experiments the most obvious results are here presented on account of their bearing on experimental morphology. The work was done in the biological laboratory of the Effingham B. Morris Biological

Farm of the Wistar Institute of Anatomy and Biology. The special advantages of this laboratory are gratefully acknowledged.

Embryos from 2 clutches of eggs, designated 30M and 30E, were used for the experiments. They were collected from Gould's mill-pond in the Pocono Mts., Pa., April 13 and removed from the egg-masses, but not from the individual envelopes, on April 14. On April 20 they were placed in a mechanical refrigerator, the clutches in separate finger-bowls, where they remained till May 27. On this date the refrigerator was found not to be operating and defrosted, although it was operating on the previous day. The period of chilling was, therefore, 36 days. The temperature of the interior of the refrigerator fluctuated considerably with that of the room, which was also subject to rather marked changes according to the weather. However, the temperature of the embryos could not have been far above freezing for on one occasion (May 12) ice formed in the dishes.

30M. In this clutch there were 93 embryos between Harrison's stages 28 and 29 when placed in the refrigerator. April 29 2 had died. The others lived through the period of chilling. May 12 they had not reached stage 29. When finally removed from the refrigerator they were in approximately stage 34 and in apparently normal condition. They were then removed from the membranes. The ciliary action of the skin was strong but there was no muscular motility demonstrably in any of the specimens. Several specimens having been preserved for anatomical study, 71 were kept under observation. By June 3 structural abnormalities had appeared in 54; and by June 13, in all. The more obvious defects, and the number of embryos in which each occurred, were as follows: (A) gills more or less fused together, 41; (B) gills bent ventrad, 40; (C) tail bent dorsad near the middle, 35; (D) caudal fin reduced or wholly suppressed, 23; (E) trunk arched dorsad, 22; (F) abdomen transversely constricted, 20; (G) gills bent abruptly laterad at base, 15; (H) tail bent dorsad at tip, 13; (I) balancers bent caudad, 13; (J) tail bent dorsad at base, 10; (K) pericardium edematous, 7; (L) IV ventricle edematous, 6; (M) tail bent laterad, 4; (N) tail bent ventrad, 3; (O) arm fused with the body, 2; (P) gills bent abruptly dorsad, 1; (Q) balancer fused with the first gill, 1; (R) first gill completely suppressed, 1.

By June 13, 13 specimens had died. In these the distribution of defects was as follows: E in 10; D in 8; C in 6; I in 5; F in 3; A in 2; G in 2; B, M, N, P each in 1; H, K, L, O, Q, R, in none. Two to 5 of these defects occurred combined in single individuals.

30E. There were 45 embryos in this clutch. When put in the refrigerator they did not differ perceptibly from 30M in age; when removed they were in stage 31 or a little in advance of this. All survived the period of chilling in apparently normal condition. Forty-two were continued under observation for the effects of the treatment. The structural defects indicated above by letters occurred as follows: A in 31; B in 27; D in 16; C in 12; F in 10; I in 8; N in 7; H in 5; E in 4; R in 4; G in 3; O in 3; M in 1; Q in 1; J, K, L, and P in none. All had from 2 to 6 of these defects. In addition to these abnormalities, which appeared also in 30M, the balancers were grossly affected in 19, varying from short club-shaped structures to mere nodules. Nine died while under observation. In these the defects were distributed as follows: J in 8; D in 5; C in 4; E in 3; N in 2; A, B, G, and K each in 1; F, H, I, L, M, O, Q, and R in none. Beyond this period of special observation the surviving specimens were not given particular attention but it was noted that 14 of them were apparently normal individuals on the following Sept. 17. One of these had survived a combination of 6 of the above defects: A, C, D, I, M, N.

Obviously exact staging of these individuals according to Harrison was impossible. Nevertheless it was clearly established that in practically all the specimens the tail grew in length out of proportion to the dimensions of the head. This indicates that the ectodermal tissues were more retarded than the mesodermal. This difference in rate of growth of the 2 tissues offers an explanation of the structural defects of the gills, balancers, caudal fin, and fusion of the arm with the body wall. Also the dorsal curvature of the tail may have been due to longitudinal tension on the mesoderm due to a more slowly growing spinal cord. It is noteworthy that the embryos of 30E were more retarded during the period of chilling (probably due to a different position in the refrigerator) than those of 30M, and that the balancers were more affected in the former group. The edematous conditions were probably due to the failure of circulatory channels to open up in the normal manner because of some such retardation of growth as appeared in the transverse constriction of the abdomen. The death of approximately 20% of the specimens seems to indicate that in general the defects have more or less lethal action although the data are too limited for a statistical treatment of this question. However, a high degree of regulation is demonstrated.

The chilling in this case is recognized as relatively extreme in time and degree. Nevertheless the results demonstrate the need of exact studies of this nature with critical control of temperature if

chilled embryos of Amblystoma are to be used in experimental morphology, for retarding of the normal rate of growth by temperatures much below the optimum for the species must introduce variables of unknown value. At the same time this method may be used as a tool in experimental morphology in problems related to those attacked by Gilchrist,[1] and, correlated with cytological studies, it should yield significant results in regard to structure-function relations in development. It is known that localized acceleration of development within a relatively even gradient of growth in the central nervous system of Amblystoma accounts for the origin of its chief functional centers.[2] The development of the medullary plate also appears to take place according to the same principle within a wide field of potentially nervous ectoderm. The "organizer" in this case, and possibly in others, may be only an accelerator. The use of the method here proposed should throw new light on problems of this nature.

8863 C

Virucidal (Rabies and Poliomyelitis) Activity of Aqueous Urea Solutions.

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In an attempt to bring the suspended particles of an aqueous spinal cord suspension containing rabies or poliomyelitis virus into more intimate contact with inactivating agents for vaccine production trials a "solution" of the cord was made with the aid of urea. The effect of urea in strong concentration on these viruses proved interesting. As first recorded by Spiro[1] and in more detail by Ramsden[2] urea in aqueous solution has a remarkable ability to "dissolve" proteins. When spinal cord of the rabbit or monkey, moist with saline, is placed in a mortar and urea crystals added the cord on trituration quickly passes into an opaque syrupy "solution" containing innumerable lipid droplets in suspension. In this manner a 50%

1 Gilchrist, Francis J., *J. Exp. Zool.*, 1933, **66**, 15.

2 Coghill, G. E., *J. Comp. Neurol.*, 1926, **40**, 47; 1926, **42**, 1; 1928, **45**, 227; 1933, **57**, 327; 1936, **64**, 135.

1 Spiro, *Z. f. Physiol. Chem.*, 1900, **30**, 182.

2 Ramsden, W., *J. Physiol.*, 1902, **28**, xxiii.