

ity of the eccrine sweat glands. The nature of the mechanism is largely unknown. The effect seems to be of a permissive or facilitatory nature such that the action of efferent impulses upon the sweat glands is enhanced. There may be two explanations: (i) The local excitatory state of sweat glands is increased by heat and decreased by cold directly or through a type of axon reflex. (ii) The facilitation occurs by the interaction of impulses from heat receptors and efferent sympathetic fibers by some unknown mechanism.

Lloyd (12), studying the influence of temperature upon the response of sweat glands in the footpad of the cat, found a shorter latency of sweat emergence with higher local temperature; he concluded that for each nerve impulse the glands yield an amount of sweat proportional to the local temperature.

In many studies the influence of skin temperature on sweating was investigated by changing the temperature of large areas of skin or that of the entire surface; such a change, however, influences the mean body temperature and presumably the hypothalamic temperature. Thus, separation of surface effects and central regulation has been difficult. We differ with Benzinger *et al.* (9) in that our results indicate that skin temperatures over 33°C have a marked effect on sweating. Their suggestion that the inhibitory effect of cooling is produced only by impulses from cold receptors inhibiting the efferent outflow of the hypothalamus should therefore be reconsidered.

The local thermal effect on sweat gland activity appears to be an important factor in thermoregulatory control. The magnitude and response time of the immediate changes in sweating with variations in mean skin temperature (6, 7) must have been influenced by the local excitatory state of the sweat glands.

W. VAN BEAUMONT

ROBERT W. BULLARD

Department of Anatomy and
Physiology, Indiana University,
Bloomington

References and Notes

1. J. D. Hardy, *Physiol. Rev.* **41**, 521 (1961); H. T. Hammel, D. C. Jackson, J. A. J. Stolwijk, J. D. Hardy, S. G. Stromme, *J. Appl. Physiol.* **18**, 1146 (1963); T. H. Benzinger, *Proc. Natl. Acad. Sci. U.S.A.* **45**, 4 (1959).
2. Y. Kuno, *Human Perspiration* (Churchill, London, 1934).
3. H. C. Bazett, in *The Physiology of Heat*

Regulation and the Science of Clothing, L. H. Newburgh, Ed. (Saunders, Philadelphia, 1949), pp. 109-192.

4. W. C. Randall, *Am. J. Phys. Med.* **32**, 292 (1953).

5. S. Robinson, in *The Physiology of Heat Regulation and the Science of Clothing*, L. H. Newburgh, Ed. (Saunders, Philadelphia, 1949), pp. 193-232.

6. H. S. Belding and B. A. Hertig, *J. Appl. Physiol.* **17**, 103 (1962).

7. R. D. Wurster, R. D. McCook, W. C. Randall, *Physiologist* **7**, 291 (1964).

8. R. W. Bullard, *J. Appl. Physiol.* **19**, 137 (1964).

9. T. H. Benzinger, A. W. Pratt, C. K. Kitzinger, *Proc. Natl. Acad. Sci. U.S.A.* **47**, 730 (1961).

10. R. W. Bullard, *J. Appl. Physiol.* **17**, 735 (1962).

11. W. van Beaumont and R. W. Bullard, *Science* **141**, 643 (1963).

12. D. P. C. Lloyd, in *Advances in Biology of Skin* (Pergamon, New York, 1962), vol. 3, pp. 127-151.

13. Supported by the Office of the Surgeon General, Department of the Army, Contract DA-49-0070, MD 947.

25 January 1965

Hepatic Glycogen Depletion in *Amphiuma* during Induced Anoxia

Abstract. *Giant salamanders, Amphiuma* means, measuring 240 to 280 millimeters from snout to vent, tolerate induced anoxia for 6 hours. After 3 hours of anoxia, hepatic glycogen units are reduced in size and concentration; after 6 hours the glycogen units are almost completely depleted. Greater development and changes in the density of the endoplasmic reticulum indicate that this structure participates in the mobilization of glycogen from the cell.

Recent studies of the respiratory metabolism of the giant salamander, *Amphiuma* means *tridactylum*, indicated that these animals could tolerate anoxia for periods up to 13 hours at 22°C. It therefore appeared that *Amphiuma* would be a suitable animal in which to study depletion of hepatic glycogen with the aid of the electron microscope.

Nine animals were placed individually in a nitrogen chamber filled with oxygen-free water. A continuous flow of nitrogen forced the water out of the test chamber and flushed out exhaled oxygen. The chamber was placed in a water bath at 22°C.

Since there was a definite correlation between size and ability to tolerate anoxia in this species, only animals measuring between 240 and 280 mm from snout to vent were used. *Amphiuma* within this size limit tolerated anoxia for about 6 hours. Animals were removed after 3 and 6 hours of anoxia, their body cavities opened,

and comparable portions of liver removed. The tissue was fixed in 2-percent osmium tetroxide buffered in Millonig's phosphate buffer (pH 7.6), embedded in Epon (1), sectioned, and stained for 10 minutes in saturated aqueous uranyl acetate and then for 5 minutes in lead citrate (2). Animals maintained in the laboratory for 5 days were used as controls.

As aptly reviewed by Revel, two basic types of glycogen can be recognized with the electron microscope (3). Alpha units are complex conglomerates of glycogen particles that assume a characteristic rosette-shape. Beta units are smaller and have smoother edges than alpha units, and they may be analogous to the glycogen particles that comprise the alpha units. Hepatic glycogen in amphibians is usually distributed as individual particles (beta units) (3), but this was not the case in *Amphiuma*.

Liver cells of the control animals were packed with glycogen rosettes (Fig. 1A). The glycogen was stored in the cytoplasm and the smooth endoplasmic reticulum appeared as short, transparent areas (cisternae) within the cells.

In the animals subjected to anoxia for 3 hours there was an obvious decrease in the size and concentration of glycogen rosettes (Fig. 1B). Also, the cisternae were considerably lengthened by the union of shorter sections to form anastomosing channels. The edges of the rosettes were smoother than in the controls, suggesting that the most externally exposed portions had dissolved, leaving a smaller, more rounded unit. At this stage the endoplasmic reticulum appeared more dense than the cytoplasm.

Liver cells of the animals subjected to anoxia for 6 hours were almost completely devoid of glycogen (Fig. 1C) and further anastomosing of the endoplasmic reticulum had occurred.

At higher magnifications the glycogen particles of the rosettes appeared to be composed of still smaller, punctate particles (inserts, Fig. 1). These smaller subunits may be either normal facets of glycogen particles, or the effects of nonspecific areas of lead deposition; alternatively, they may be caused by electron-beam bombardment of the specimen (3).

There was definite decrease in the number of visible subunits per rosette with increased time of anoxia. Thus, ten of the largest glycogen units had

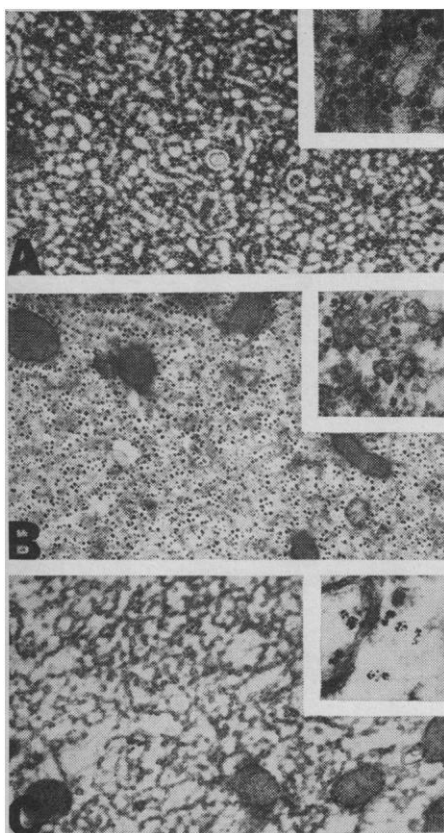


Fig. 1. Sections of liver from *Amphiuma means tridactylum* showing depletion of glycogen during induced anoxia. (A) From control; (B) from animal subjected to anoxia for 3 hours; (C) from animal subjected to anoxia for 6 hours ($\times 5400$; inserts, $\times 40,000$).

a mean of 29 (18 to 35) subunits per rosette in the control animals; 15 (10 to 30) subunits per rosette in the animals exposed to anoxia for 3 hours; and 9.3 (3 to 16) subunits per rosette in the animals exposed to anoxia for 6 hours. If these subunits are subsequently found to be normal facets of each glycogen unit, then they are probably the smallest visible particles converted to glucose.

The smooth endoplasmic reticulum is generally considered to be a channeling network, since it is best developed when the cell is actively secreting some substance, for example, during salt secretion from the gills of fish (4). During storage periods one would expect the endoplasmic reticulum to be less developed than during periods of utilization of the storage product. Greater development of the endoplasmic reticulum during anoxia is probably a response by the cell to more effectively mobilize glycogen out of the liver.

Glycogen is generally considered a quick energy reserve in mammals; however, when there is complete ab-

sence of oxygen, death probably ensues before hepatic glycogen is mobilized. That some reptiles and amphibians can utilize stored glycogen during anoxia more effectively than others suggests that glycolysis may play a more predominant respiratory role in these animals than in mammals.

Amphiuma means tridactylum in southern Louisiana are collected from sewage and swampy ditches—areas of low oxygen tension. Many of these ditches dry out during the summer and the *Amphiuma* go underground to avoid desiccation. Under these conditions the animals lower their metabolic rate and rarely use pulmonary respiration (5).

Stored lipid is at a minimum during the summer months; even during periods of maximum storage the amount stored is extremely low (5), yet *Amphiuma* can withstand starvation for extended periods (6). Thus glycogen may be a long-term storage product in *Amphiuma*, as in some molluscs, and may serve as a lipid substitute, since the oxidation of fats requires more oxygen per gram than the oxidation of glycogen. In a habitat of low oxygen tension, glycogen would have a definite advantage over lipid as a storage product. Possibly a balance exists between the energy derived from glycolysis and the small amount of aerobic energy that allows these animals to sustain themselves during extended droughts. The low metabolic rate during unfavorable conditions would also be a sustaining factor.

FRANCIS L. ROSE

Department of Zoology,
Tulane University

JOSEPH ZAMBERNARD

Department of Pathology,
University of Colorado Medical
School, Denver

GILBERT S. POGANY

Department of Zoology, Tulane
University, New Orleans, Louisiana

References and Notes

1. J. H. Luft, *J. Biophys. Biochem. Cytol.* **9**, 409 (1961).
2. E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
3. J. P. Revel, *J. Histochem. Cytochem.* **12**, 104 (1964).
4. C. W. Philpott and D. E. Copeland, *J. Cell Biol.* **18**, 389 (1963).
5. F. L. Rose, in preparation.
6. W. E. Brode and G. Gunter, *Science* **130**, 1758 (1959).
7. Supported in part by NIH fellowship (1 F1 GM-20, 096-01) and a grant from the Society of the Sigma XI to F.L.R. We thank Eugene Copeland, Harold Dundee, Milton Fingerman, Gerald Gunning, Austin Fitzjarrell, Jean P. Revel, and Franklin Sogandares-Bernal for assistance during the preparation of this report.

29 January 1965

Kinin-Induced Parthenocarpy in the Fig, *Ficus carica* L.

Abstract. *Parthenocarpic Calimyrna fig fruits induced with a kinin were identical morphologically to those previously produced parthenocarpically with auxin or gibberellin. Thus, the three types of endogenous hormones thought to originate in the seeds and to stimulate directly fruit growth can be supplied by plant parts other than seeds.*

Kinins occur naturally in a number of fruits (1, 2). Although they have been implicated in the cell division phase of fruit growth (2), it is not known whether they actually govern this type of growth. Weaver and van Overbeek (4) reported that Black Corinth grape clusters dipped in a kinin solution 4 days after anthesis produced berries about three times as large as untreated berries. Since no histological studies were made, it is not known whether the increase in berry size was due to stimulated cell division or cell enlargement, or both. Kinins have not heretofore been reported to induce parthenocarpy. However, the synthetic kinin SD 8339 (4), having the structural formula shown in Fig. 1, has proved very effective for promoting parthenocarpy in the *Calimyrna* fig.

At concentrations of 100 and 500 parts per million, SD 8339 was applied in aqueous solution by spraying the young syconia and foliage or by injecting the fruit with a hypodermic syringe, the needle being passed through the ostiole and into the central cavity which was then filled with 1 to 2 ml of the solution. Three to four fruits on each of five trees were used for each treatment. At the time of treatment, the female flowers within the syconia were receptive to pollination. Cross-pollination was prevented by covering the branches bearing the fruits to be treated with muslin bags. The bags enclosed the branches for about 2 weeks, the period during which pollination of unbagged fruits by the insect *Blastophaga psenes* took place. Bagged but untreated fruits served as controls. Unless cross-pollination occurs or the syconia are treated with certain growth regulators (5), they cease growth and wither, and abscission occurs in about 2 weeks.

As shown in Table 1, SD 8339 was very effective for inducing parthenocarpy, particularly when injected into the syconia. After spraying with SD