

lus of hyperglycemia.

Conclusions. Rats fasted for 1-6 days failed to show a progressive decrease of oral glucose tolerance. Return of blood sugar levels to normal after intravenous glucose was somewhat delayed after 1-6 days of fasting as compared with the fed state, but not proportionately with the duration of fast. Prolonged acute fasting therefore did not lead to typical "starvation diabetes" in rats. Chronically underfed rats fasted for 1 day and given glucose orally or intravenously exhibited a more fleeting elevation of blood sugar than *ad libitum* fed-fasted controls. This was not associated with any marked deficiency in hepatic glycogen formation from fed glucose.

nor with glycosuria.

1. Staub, H., *Z. Klin. Med.*, 1922, v93, 89.
2. du Vigneaud, V., and Karr, W. G., *J. Biol. Chem.*, 1925, v66, 281.
3. Hill, R., Baker, N., and Chaikoff, I. L., *ibid.*, 1954, v209, 705.
4. Wertheimer, E., and Ben-Tor, V., *Brit. J. Nutrition*, 1950, v4, 1.
5. Halmi, N. S., and Spirtos, B. N., *Am. J. Physiol.*, 1956, v187, 432.
6. Spirtos, B. N., and Halmi, N. S., *ibid.*, 1957, v190, 239.
7. Montgomery, R., *Arch. Biochem.*, 1957, v67, 378.
8. Scow, R. O., and Foglia, V. G., *Am. J. Physiol.*, 1951, v166, 541.

Received August 26, 1957. P.S.E.B.M., 1957, v96.

Blood Chemistry and Parietal Eye of *Anolis carolinensis*.* (23626)

KENNETH E. HUTTON AND ROBERT ORTMAN[†] (Introduced by H. S. Mayerson)

Department of Zoology, Tulane University, New Orleans

Although the parietal eye of reptiles has received considerable histological study (Tilney and Warren(8); Von Haffner(9)) its possible function remains unclear. In the present study, quantitative determinations of various organic components of the blood of *Anolis carolinensis* were made to ascertain if removal of the parietal eye affected these components. Previous studies concerning possible functions of this structure have indicated that surgical removal of the parietal eye of adult *Anolis carolinensis* caused testicular stimulation (Clausen and Poris(3)) and modification of the rate of oxygen consumption (Clausen and Mofshin(2)).

Materials and methods. The lizards (*Anolis carolinensis*) were collected in New Orleans several days prior to use. Four experiments, each involving 3 groups, were performed between February and April. Two of these experiments were of 26 hours duration and 2 were of 18 days duration. The

parietal eye was removed by thermal cauterization of an etherized lizard. A drop of colloidion was used to seal the wound in operated and sham-operated lizards. The sham-operation was performed by cauterization of a small midline area on the surface of the head at the level of the anterior border of the lateral eyes. Only lizards in 18-day experiments were supplied with meal worms (larvae of *Tenebrio molitor*) daily except on the last day. Droplets of water were sprinkled on the cage and on dead vegetation within the cage. At the end of each experiment, the blood of each group was analyzed. Blood was obtained by decapitation; potassium oxalate was used as the anticoagulant. Determination of uric acid was according to the method of Folin(7). Other determinations followed the technics put forth in the *Manual of Standardized Procedures for Spectrophotometric Chemistry*, edited by Fister(6). Accordingly, the lipid-phosphorus method was basically that of Fiske and Subbarow, the urea method that of Gentzkow and Masen. The determinations of glucose, total nitrogen and non-protein nitrogen were based upon the methods and

* This study was supported by grant from Louisiana Heart Assn.

[†] The work was equally divided and neither person is to be considered as the senior author.

TABLE I. Blood Chemistry of *Anolis carolinensis* after 26 Hour Parietal Eye-Ectomization.

	Sham-operated				Sham-operated		
	Intact 6 ♂, 12 ♀ 1.8 g*	4 ♂, 16 ♀ 1.9 g	Operated 4 ♂, 16 ♀ 1.9 g		Intact 6 ♂, 4 ♀ 3.2 g	6 ♂, 4 ♀ 3.1 g	Operated 6 ♂, 4 ♀ 3.2 g
Ending 3/22/57				Ending 3/27/57			
Non-protein nitrogen, mg %	73.9 (2)†	63.0 (2)	76.9 (3)	Glucose, mg %	226 ± 11† (4)	192 ± 11 (4)	179 ± 10 (4)
Glucose, mg %	170 (1)	197 (2)	124 (2)	Uric acid, mg %	5.80 ± .21 (3)	6.10 ± .25 (4)	6.58 ± .21 (3)
Uric acid, mg %	6.95 (1)	5.15 (2)	3.40 (1)				

* Mean wt of group.

† No. of determinations.

‡ Mean followed by stand. error of mean.

modifications of Folin and Wu. The determinations were made on the pooled blood of each group. This was necessary due to the small amount of blood obtainable from each animal (0.05 to 0.2 ml). All lizard heads were fixed in Bouin's fluid. Selected heads were embedded in tissuemat and sections studied histologically. Bodian's(1) protargol method was used for parietal eye "nerve fibers." All testes of lizards in the second 18-day experiment were weighed after fixation. A testis from each experimental group was studied histologically.

Results. Tables I and II set forth the results of the 4 experiments. In most instances the values obtained are similar to the normal values obtained of *Anolis carolinensis* by Des-sauer(4,5). The data of the first 26-hour experiment, while insufficient to be analyzed statistically, suggested that removal of the parietal eye 26 hours earlier reduced the blood level of glucose and uric acid. These changes were not confirmed when the experiment was repeated. There are no statistically signifi-

cant differences between the sham-operated and the operated groups in the second 26-hour experiment. In the experiments in which the parietal eye had been removed 18 days earlier no statistically significant difference between sham-operated and operated lizards in either total nitrogen, non-protein nitrogen or glucose were found. However, significant differences in uric acid, urea and lipoid-phosphorus were detected between sham-operated and operated lizards. These alterations in blood-chemistry can be interpreted as a reflection of the weight and age differences of the experimental groups.

Histological examination of serial sections of the 9 lizard heads in the first 18-day experiment in which the parietal eye was removed, indicated that the parietal eye was completely destroyed in all cases. No obvious differences were noted in the color or locomotor ability of parietal eyeless lizards and their controls. Sham-operated and parietal eye-ectomized lizard groups ate less during the first part of the 18-day experiments, but these groups were

 TABLE II. Blood Chemistry of *Anolis carolinensis* after 18 Day Parietal Eye-Ectomization.

	Intact		Sham-operated	Operated
	Ending 3/9/57	5 ♂, 2 ♀ 5.9 g*	5 ♂, 3 ♀ 4.5 g	5 ♂, 4 ♀ 4.8 g
Total nitrogen, %	2.45 ± .16†(4)‡		2.70 ± .14 (3)	2.44 ± .04 (3)
Non-protein, nitrogen, mg %	—		71.6 ± 1.1 (3)	84.7 ± 5.4 (4)
	Intact		Sham-operated	Operated
	Ending 4/6/57	20 ♂, 1 ♀ 5.6 g	15 ♂ 3.9 g	16 ♂ 5.0 g
Non-protein nitrogen, mg %	63.8 ± 1.1 (3)		—	—
Glucose, mg %	203 ± 4 (10)		188 ± 10 (7)	200.4 ± 4.0 (9)
Uric acid, mg %	3.78 ± .07 (9)		5.19 ± .25 (8)	3.57 ± .17 (9)
Urea, mg %	5.80 ± .52 (9)		3.36 ± .31 (8)	4.32 ± .37 (10)
Lipoid-P, mg %	21.4 ± .8 (9)		15.5 ± .2 (7)	19.3 ± .4 (8)

* Mean wt of group.

† Mean followed by stand. error of mean.

‡ No. of determinations.

eating as much as the intact animals toward the end of the experiments.

Mean testicular weights for lizards in the 18-day experiment were 47 mg (intact), 33 mg (sham-operated), and 43 mg (parietal eyeless). The lesser testicular weight of the sham-operated lizards may likely be explained by the lesser mean body weight of the group. Histological examination of a testis in each group showed very similar pictures of seasonal stimulation; the seminiferous tubules were well developed and spermatozoa were abundant in each testis.

Several parietal eyes were studied histologically to ascertain the condition of lens, retina, and the presence or absence of parietal eye nerve. In the central region of the lens, deposition of pigment in the cytoplasm of the lens cells was found. In the retina, circumscribed areas of lens-like cells were found where normal differentiation of retinal elements did not take place. Examination of 4 μ sections clearly revealed that the sensory cells (rods) do not contain melanin granules. The melanin pigment of the retina is contained within separate pigment cells. These pigment cells appear to be completely filled with melanin granules. This agrees with the work by von Haffner(9) on *Lacerta vivipara*. An abundantly nucleated, "nerve-like" structure attaches to the connective tissue capsule of the parietal eye and runs caudally into the region of the epiphysis. Study of serial sections failed to reveal a convincing connection

of this "nerve-like" structure to the retinal elements, on the one hand, or to the brain in the region of the habenular ganglia, on the other hand. After staining with protargol, no nerve fibers were seen in this "nerve-like" structure, whereas nerve fibers in the brain were clearly demonstrated.

Summary and conclusions. In the present experiments on *Anolis carolinensis*, no significant quantitative alteration, which could be attributed solely to the complete ablation of the parietal eye, was found in the organic components of the blood which were studied. If the parietal eye influences physiological processes at certain times, it probably must do so by means of a secretion produced by the sensory cells themselves.

1. Bodian, D., *Anat. Rec.*, 1936, v65, 89.
2. Clausen, H. J., and Mofshin, B., *ibid.*, 1936, v67, Suppl. 1, No. 175.
3. Clausen, H. J., and Poris, E. G., *ibid.*, 1937, v69, 39.
4. Dessauer, H. C., *Proc. Soc. Exp. Biol. and Med.*, 1952, v80, 742.
5. ———, *ibid.*, 1953, v82, 351.
6. Fister, H. J., (ed.), *Standard Scientific Supply Corp.*, N. Y., 1950.
7. Folin, J., *J. Biol. Chem.*, 1934, v106, 311.
8. Tilney, F., and Warren, L. F., *American Anatomical Memoirs*, Wistar Inst. of Anatomy, Philadelphia, 1919, No. 9, p257.
9. Von Haffner, K., *Mitt. Hamburg Zool. Mus. Inst.*, 1955, v53, 25.

Received August 27, 1957. P.S.E.B.M., 1957, v96.

Positive Mucin Clot Test in Supernates of Cultures of Avian Embryonic Brain.* (23627)

HENRY GROSSFELD (Introduced by Charles Ragan)

Departments of Medicine and Orthopedic Surgery, Columbia University College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hospital, N. Y. City

Methods. Tissue cultures in Carrel flasks and in roller tubes were prepared from the lateral walls (corpus striatum) of the hemispheres of the telencephalon of 12- and of 14-

* Aided in part by U.S.P.H.S. grant.

Reported in part at Annual Tissue Culture Meeting, Baltimore, April 16, 1957.

day-old chick embryos. The explantation was carried out in a thin plasma layer, and after several hours, when the plasma coagulated, the liquid phase of the culture medium consisting of 20% chick embryo extract, 20% chick amniotic fluid, 30% horse serum, and 30% Earle's balanced salt solution was added.