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.

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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-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.

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

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

Blood vessels were removed from the explants as completely as possible. Renewal of the medium was carried out once or twice a week depending on the state of the cultures. With each feeding the medium to be used as well as the supernates of the cultures were tested for mucin clot formation(8). 0.2 ml of 1 N acetic acid was added to 1 cc of the fluid to be tested. A tight mucin clot, the formation of which could be prevented by testicular hyaluronidase (Wyeth), indicated the presence of highly polymerized hyaluronic acid. By gradual dilution of samples of known hyaluronic acid content, the minimum concentration of hyaluronic acid which gives a positive mucin clot test has been found to be about 30 μg/ml. Fresh undiluted embryo extract prepared in this laboratory gave a mucin clot, the formation of which could be prevented by hyaluronidase. 20% of half diluted embryo extract used in this work contained about 20 μg/ml which is below the minimum concentration for a positive mucin clot test and, in fact, never gave a mucin clot. On the other hand, undiluted chick embryo extract no longer produced a mucin clot after 1 to 2 days incubation at 37°C. Consequently a positive mucin clot test after that time indicates the presence of hyaluronic acid newly produced *in vitro* by fibroblasts. Chick embryo extract may thus be used in any concentration in the culture medium, in which production of highly polymerized hyaluronic acid will be tested by the mucin clot method.

The lowest concentration of hyaluronic acid giving a positive mucin clot test is about 30 μg/ml. Multiplication by 30 of the minimal amount of fluid (equal parts of serum and saline) which prevents mucin clot formation, allowed a rough estimation of the concentration of hyaluronic acid in the original undiluted sample: $X = (1 + y)\ a$, where $y =$ the minimal amount of fluid added to the 1 ml sample for preventing mucin clot formation, and $a =$ the concentration of hyaluronic acid in the diluted sample, assumed to be about 30 μg/ml. The results roughly agree with those obtained by turbidimetric determination† (Table I).

Results. Growth usually started at the second or third day of incubation, when at first spindle-shaped cells and some round cells appeared. Subsequently more or less dense cell mats and sheets consisting of long and slender cells were observed. No neurons could be found. Positive tests for mucin clots in the supernates were not obtained before the third or fourth change of the medium, and at times only after 3-4 weeks. The mucin clot formation could be prevented by addition of 0.10 TRU of hyaluronidase (Wyeth) to 1 ml of the supernate.

Hyaluronic acid produced *in vitro* in different cultures and at varying periods of time was estimated to amount to about 45 to 210 μg/ml depending on the size of the cultures, the growth rate and other unidentified factors. The hyaluronic acid evidently was produced by the outgrowing cells (glia and/or "fibroblasts"), and not by cells inside the explants, since supernates from cultures without outgrowth or with very scarce outgrowth did not give a positive mucin clot test. (Table II).

Discussion. The spaces between the ramifications of axons, myelin, dendrites and neuroglia are not empty. Campbell referred to them as "that terra ignota which remains over when cells, fibers and neuroglia are subtracted"(5). But little has been known about

TABLE I. Order of Magnitude of Hyaluronate Concentration Estimated by Sample Dilution.

Source of hyaluronate		Turbidimetric determination, μg/ml	Sample dilution $x = (1 + y^*)\ 30$, μg/ml
Human synovial fluid	#1	3200	3030
Idem	#2	1520	1230
"	#2 2.5%	40	30
Beef EE_{66}		339	240
Chick EE_{100}		260	240
" EE_{50}		105–120	120
Cultures of rat subcut. tissue		90–140	90
Cultures of chick embryo bone		29–240	75–140

* y = minimal amt in cc of equal parts of saline and horse serum which prevents mucin clot formation, added to 1 cc sample.

† I am grateful to Miss Phyllis Sampson of Dr. Karl Meyer's laboratory for carrying out the turbidimetric determinations.

TABLE II. Positive Mucin Clot Test in Supernate of Cultures of Chick Embryo Brain Tissue.

	Tubes after 10 days		Tubes after 30 days		Tubes after 47 days	
Tubes	Good growth	Mc +	Good growth	Mc +	Good growth	Mc +
10	8	4	7	6	3	3
10	6	5	6	6	4	3
20	11	7	13	9	8	7
12	10	2	8	6	8	8
20	18	0	18	3	18	15

the existence of a ground substance in the central nervous system, and half a century later the presence of a ground substance in the central nervous system has been denied (2). On the other hand, increased spreading (6) in the cerebral cortex was found after treatment with hyaluronidase(3). Recently the presence of mucopolysaccharide between the elements constituting the central nervous system was assumed on the basis of histochemical reactions(4.7). The demonstration by Hess of a positive periodic acid-Schiff (PAS) reaction between the cells of the central nervous system suggested the existence of a ground substance between neuroglia fibrils, axon terminations and dendrites(9). Since hyaluronidase did not alter the red coloration of the PAS reaction, it was assumed that the ground substance consisted of a neutral mucopolysaccharide and not of hyaluronic acid(9). Again more recently, the existence of a substance in the cerebral cortex of the cat which is susceptible to the action of hyaluronidase has been reported(1). The present results show that hyaluronic acid is produced in tissue culture by cells growing from explants of embryonic brain. This constitutes further evidence for the existence in the central nervous system of a hyaluronic acid containing ground substance.

Summary. The supernatant fluid obtained from tissue cultures of avian brain, on addition of 0.2 ml of 1 N acetic acid to 1 ml sample, continued to give for over 2 months a mucin clot the formation of which could be prevented by testicular hyaluronidase. This indicates production of hyaluronic acid in tissue culture by cells, other than neurons, growing from explants of embryonic brain.

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Received September 5, 1957. P.S.E.B.M., 1957, v96.

Effect of 5-Nitro and 5-Chloro Benzimidazole Derivatives on Heme Synthesis *in vitro*.* (23628)

LYNN D. ABBOTT, JR. AND R. ARTHUR GINDIN†
Department of Biochemistry, Medical College of Virginia, Richmond

We have reported that certain 5-methylbenzimidazole derivatives, particularly 2-ethyl-5-methylbenzimidazole, 2,5 - dimethylbenzimidazole and 5,6 - dimethylbenzimidazole prevented incorporation of N^{15} from N^{15}-glycine into heme by chicken erythrocytes during *in vitro* incubation(1,2). Since the same benzimidazole derivatives also were found to inhibit virus duplication(3), we proposed that they might be inhibiting an important metabolic reaction fundamental to more than one biosynthetic process(1,2). They ap-