THE SOUTH CAROLINA ACADEMY OF SCIENCE

THE South Carolina Academy of Science held its annual meeting at Winthrop College, Rock Hill, on April 25. At the business meeting, the following officers were elected for the ensuing year:

President, Professor A. C. Carson, University of South Carolina.

Vice-president, Dr. J. E. Mills, Sunoco Products Company, Hartsville, S. C.

Secretary-treasurer, Dr. F. W. Kinard, Medical College of South Carolina.

Executive Committee: Dr. Roe E. Remington, Medical College of South Carolina; Professor Franklin Sherman, Clemson Agricultural College; Professor Mary New, Greenville Womans College; Dr. Bruce Mayne, U. S. Public Health Service, State Hospital, Columbia, S. C.; Dean S. B. Earle, Clemson Agricultural College.

Librarian, J. E. Copenhaver, University of South Carolina.

A program of twenty-six papers was given. About 250 members and visitors attended. One of the outstanding features of the meeting was the awarding of the Phipps and Bird gold medal, for the best paper presented, to Drs. F. W. Kinard and F. N. Martin, Jr., Medical College of South Carolina. The subject of their paper was: "A Study of Blood Histamine in Normal and Burned Dogs." This paper will be sent in competition with similar winning papers from the academies of Georgia, North Carolina and Virginia for a $100 prize. The next two best papers will be given $25 each. These medals and prizes are given by Phipps and Bird, Inc., Richmond, Virginia, distributors of chemicals and laboratory apparatus.

The academy will meet at the University of South Carolina, Columbia, next year.

J. E. COPENHAVER,
Retiring Secretary and Treasurer

THE VIRGINIA ACADEMY OF SCIENCE

THE Virginia Academy of Science held its fourteenth annual meeting on May 1 and 2 at the Virginia Military Institute at Lexington, Va., with a registration of 408.

The address of the president, Professor Ida Sitler, of Hollins College, was on the subject of a Science Museum, which is a very live subject with the academy at this time. Dr. C. C. Little, of Bar Harbor, Me., gave the public address at the Friday night meeting on the subject of "Heredity in Experimental Cancer." One hundred and forty-two papers were presented before the sectional meetings.

The regular academy prize of fifty dollars and the recently established Jefferson Gold Medal were both awarded to Dr. Alfred Chanutin, of the University of Virginia, for a paper entitled "The Effect of Whole Dried Meat Diets on Renal Insufficiency Produced by Partial Nephrectomy."

The president for the coming year is Dr. H. E. Jordan, of the University of Virginia, the president-elect is Professor D. Maurice Allan, of Hampden-Sydney College, and the newly elected member of the council is Dr. Edward Steidtmann, of the Virginia Military Institute.

The next meeting will be held at the University of Virginia on the first Friday and Saturday of May, 1937.

E. C. L. MILLER,
Secretary

SPECIAL ARTICLES

THE CULTIVATION OF LARGE QUANTITIES OF ADULT TISSUE IN FLUID MEDIA

IF a technique could be devised for keeping large quantities of adult tissue in a state of functional survival for considerable periods in fluid media, it would provide a means of studying a great variety of physiological problems that could not be approached by using either cell strains or embryonic material. It would also rule out the necessity of having to contend with a plasma coagulum as an integral part of the culture medium. This has been accomplished. It is the purpose of the present communication to describe the procedures that have been developed.

The tissues are cut into fragments of such a size that it requires about 75 to weigh 100 mg. The cutting is done with cataract knives on a glass plate. For most purposes, it is unnecessary to weigh the tissue for each experiment. With a little practice, it is possible to cut uniform fragments and to judge their total weight by the number prepared. Thus, each culture comprising a given series receives the same number of fragments. The total number of fragments (50 to 75) intended for each individual culture are placed in separate depression slides containing glucosol[1] and allowed to stand until all the fragments have been prepared for a given experiment. After several changes of glucosol, they are ready to be transferred to their respective flasks. The flasks[2] are of the H-8 type

[1] "Glucosol" is a modified Tyrode solution that has been used in this laboratory for many years. It has the same composition as Tyrode solution, except that the $NaHCO_3$ is omitted.

[2] These flasks are 8 cm in height, have a flat bottom 5 cm in diameter and a capacity of 65 to 70 cc. The neck is eccentric, oblique (45°) and has an opening 1 cm in diameter. The oblique neck and the small opening offer protection from contamination when the flasks are unstoppered. The eccentric position of the neck renders it possible to examine the contents with the aid of the

designed three years ago by Carrel for the cultivation of viruses. These flasks are particularly sturdy, simple in design and of large capacity (65 to 70 cc). As a rule, 2 cc of culture medium is used. This amount is just sufficient to cover the tissue fragments, which are transferred to the flasks after the medium (see below) has been introduced and either remain suspended in the fluid or else loosely adherent to the glass. Finally, and in accordance with a routine procedure that has been followed in this laboratory for six years, the flasks are filled with a gas mixture[3] comprised of O_2, CO_2 and N. The gas mixture serves a double purpose: first, a certain amount of oxygen is required by the tissues as an aid to respiration; and second, a definite quantity of CO_2 is used to establish an appropriate hydrogen-ion concentration and to maintain it at a constant level. The composition of the gas mixture depends on the nature of the tissue, the composition of the medium and the purpose of the experiment. The moment the gas mixture has been introduced, the flasks are closed with rubber stoppers and sealed with waterproof cement[4] in the manner adopted several years ago for all cultures. The gaseous atmosphere is replenished daily.

At the termination of an experiment, and as a routine procedure, the tissue fragments are fixed, sectioned and stained for histological study. Just prior to fixation, the tissue from each individual culture flask is collected together in a flat, compact mass and embedded in a plasma coagulum on a piece of mica. This not only protects them from injury but also facilitates subsequent handling.

From time to time, comparative experiments have been made in which the same number of tissue fragments from the spleen of an adult rabbit were cultivated in equal amounts of plasma and serum prepared from the same sample of blood. Fragments placed in the solid plasma mixture showed less contraction than those cultivated in the fluid medium containing an equivalent amount of serum. Yet the state of preservation of the plasma cultures was only slightly better than those cultivated in serum. In fact, the differences were so slight that, for practical purposes, they seemed almost negligible.

A comparative study was also made of the survival of sister cultures of rabbit spleen carried in an atmosphere containing 21 per cent., 40 per cent. and 80 per cent. O_2. In these experiments, the CO_2 content of the gas mixture was held uniform, whereas the N was varied with the O_2. The cultures were gassed for 3 minutes on the first day and for 1 minute on each day thereafter. The results obtained were most striking. Of the three concentrations of O_2, 80 per cent. gave by far the best cell preservation. After 4 days, cultures carried in 21 per cent. O_2 showed almost complete degeneration and necrosis in the central portions of the tissue fragments, whereas sister fragments cultivated in 80 per cent. O_2 showed almost complete survival. In 40 per cent. O_2, the survival was better than in 21 per cent. O_2, but still quite inferior to that obtained in the 80 per cent. mixture. It would seem, therefore, that fluid cultures containing large quantities of organized tissue in suspension have very high oxygen requirements.

When 100 mg of rabbit spleen are cultivated in 2 cc of culture medium, special precautions have to be taken in order to prevent the medium from becoming too acid. This may be done by adding a slight excess of sodium bicarbonate. If, for example, the medium is made up to include 50 per cent. rabbit serum, 16 2/3 per cent. of an isotonic solution of sodium bicarbonate (1.4 per cent.), 33 1/3 per cent. Tyrode solution containing 4 times the usual amount of glucose and 0.005 per cent. phenol red (to serve as an indicator), it will be very alkaline in the beginning but may be adjusted immediately to pH 7.2 by the introduction of a gas mixture containing 8 per cent. CO_2. On the first day, it is usually necessary to gas the cultures for a period of 3 minutes in order to attain this. Thereafter, the time may be greatly reduced. When, as occasionally happens, the medium becomes too acid and it is necessary both to remedy this and, at the same time, to treat the cultures with a gas mixture containing a higher concentration of O_2 than is contained in the atmosphere, the flasks are unstoppered and left for a time in the incubator with their openings protected with sterile gauze. As a result of this treatment, the medium will lose a certain amount of CO_2 and become more alkaline. It may then be brought back to the desired hydrogen-ion concentration by introducing a mixture containing 3 per cent. CO_2 and the desired amount of O_2.

This system of cultivation has already been used to great advantage in a study of the formation of antibodies *in vitro*.[5] It offers a simple means of keeping

projectoscope. Also, the dorsal surface of the flask is made with a certain convex curvature that facilitates projection.

[3] The gas mixtures are made up in high pressure storage tanks by means of Hoke valves and a 100 lb. pressure gauge, each gas being led in separately until the desired pressure (percentage) for that particular gas has been obtained. Separate tanks are made up to contain varying concentrations of O_2 and CO_2, the balance in each case consisting of N. Eventually, the gas mixture is fed into the cultures through a saturation flask (containing 1 per cent. copper sulphate), a 1-inch "N" Berkefeld filter (to render the mixture sterile) and a sterile glass pipette. The sterile filter is replaced daily. The glass pipettes are changed much more frequently, and just as often as there is doubt of their sterility.

[4] Du Pont's clear airplane dope No. 5332.

[5] K. Landsteiner and R. C. Parker, unpublished experiments.

adult tissues in a state of functional survival rather than one of unlimited proliferation. It also renders it possible to study simultaneously both the effect of the medium on the cells and the effect of the cells on the medium. Thus, for example, the entire medium may be changed without removing the suspended fragments, or any part of it may be withdrawn at any time in order to test it for the presence of particular substances elaborated by the tissues.

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PULSATING BLOOD VESSELS IN THE OYSTER[1]

DESCRIPTION was recently published[2] of a pair of "accessory hearts" in the oyster. These structures are large, well-defined, thin-walled blood vessels in the mantle wall of the cloacal chamber and apparently pump blood from the excretory organs into the pallial arteries, which run around the borders of the mantle lobes. They pulsate independently of one another and at a rate considerably slower than that of the heart.

Further studies on *Ostrea lurida* have demonstrated that these organs are only the two most prominent of a great many pulsating peripheral blood vessels in the walls of the mantle. These vessels may be seen on the inner surface of each mantle lobe as radially arranged structures extending from the region of the adductor muscle and visceral mass to the tentacle-bearing periphery. They are of greater diameter toward their distal ends than centrally and are sometimes branched. Examination of sections shows that these vessels are directly associated with and partially surround the bundles of muscle fibers which function as retractors of the mantle.

A well-defined band of tissue, the cilia on which beat posteriorly, runs in each mantle lobe from a point adjacent to the labial palps to the postero-ventral border of the lobe. Underneath the ciliated epithelium of each of these bands, throughout at least a large part of its length, is a blood vessel which also pulsates rhythmically. At its posterior extremity each band with its underlying blood vessel becomes continuous with one of the radial blood vessels near the edge of the mantle, while along its course it crosses the radial vessels and appears to be directly continuous with many or all of them.

All these vessels (the accessory hearts, radial vessels and the horizontal bands) apparently open directly into the circumpallial vessel at the border of the mantle. These vessels are hardly more than indefinite blood spaces with such poorly defined walls that it is difficult to trace their origin, save in the case of the accessory hearts which originate in the blood spaces of the excretory organs. The pulsations of the radial vessels progress toward their distal ends as very distinct, relatively slow constrictions. The wave of pulsation of the vessel underlying the ciliated band begins anteriorly and as it crosses the radial vessels appears to be synchronous with pulsations of the latter.

Observation of the activity of these vessels is most difficult. When the oyster is removed from its shell the mantle becomes curled back and distorted because of contraction of the bands of muscle fibers. The structures were best observed by removing only one valve, leaving one mantle lobe still in contact with its shell. Also, small oysters, or spat 10 to 15 mm long, caught on glass plates were observed by transmitted light, making it possible to see both the waves of contraction and, in some cases, the direction of movement of blood corpuscles.

All pulsations proceed toward the periphery of the mantle, and during the contraction the blood cells go in the same direction. There appears, however, to be no effective valve action to maintain flow of blood in one direction, for as the vessels expand again the blood corpuscles reverse their direction of movement, though going more slowly.

The function of these pulsating vessels is as yet not entirely clear, though it may be to move the blood back and forth through the mantle to facilitate aeration. It is possible that the radial vessels, like the accessory hearts, receive their supply of blood from the excretory organs, though this has not been demonstrated. In the case of *O. lurida* it is doubtful that the marginal vessels of the mantle have a direct connection with the arterial system, as in *O. gigas*.

Blood in the marginal vessels (the pallial arteries or sinuses) may be observed to flow alternately back and forth, depending upon pulsations of the radial vessel as well as upon a pulsating activity of its own. Blood is collected in veins near the outer surface of the mantle and returned to the auricles.

A study of the anatomy of these contractile vessels is being made in order to establish what structures produce the pulsations. It appears probable that cells of the type of "Rouget cells," as investigated by Federighi[3] in *Nereis*, may be the agents responsible for the observed activity.

A. E. HOPKINS

U. S. BUREAU OF FISHERIES

[1] Published by permission of the Commissioner of Fisheries.

[2] A. E. Hopkins, *The Biological Bulletin*, Vol. 67: 3, 345–355, December, 1934; SCIENCE, 80: 2079, 411–412, November 2, 1934.

[3] Henry Federighi, *Jour. Exp. Zool.*, 50: 2, 257–294, February 5, 1928.