3. The remedy stays the destructive and onward march of syphilitic ulcerations and causes their healing in a surprisingly short time.

4. It is more rapid in its effect on specific disease than any other known remedy.

5. It is likely to prove more valuable than any other remedy in the treatment of the specific diseases of most internal organs.

6. It cannot replace cicatricial tissue, the result of Nature's reparative processes; neither does it affect favorably chronic degenerative diseases of the nervous system, such as paresis, system-diseases of the cord, in which there is a break in the continuity of nerve structure, though in some cases it seems to influence favorably the continuous crises of locomotor ataxia.

7. In all cases it causes a leukocytosis and the formation of antibodies.

8. It materially modifies, and in most cases ultimately negatives, the Wassermann reaction.

9. It unquestionably floods the circulation with endotoxins resulting from the death of millions of spirochetes, and in all probability an antitoxin is developed in the blood-serum. These facts must be thoroughly considered in connection with the treatment.

10. In acute and threatening deposits in vital organs the effect of "606" will often prove life-saving because of its prompt action, and for that reason it is preferable to the iodids or mercury.

11. It ought not to be given to ambulatory patients; neither is it safe in the hands of the careless or those who have not seen it used and learned the difficult method of its preparation for injection.

12. The hospital, where all things required in the preparation of the mixture of "606" can be sterilized, and where the centrifuge can be used, is preferable to any other place for its injection.

13. Patients injected should be kept quiet and in bed during seven days under close observation, and for a longer period if indications demand.

14. Second injections, if indicated, should not be given in less than eight weeks after the first.

15. Contra-indications should be carefully considered before using the remedy. Patients with any other infection than that of syphilis should not be injected, however mild the former may be, until a safe period has lapsed after their recovery; neither should the feeble or old or those with other than syphilitic organic disease be injected.

16. Congenital syphilis demands the treatment, either directly or indirectly, as suggested in the paper.

17. The living contagion is destroyed by "606"; hence its early use can prevent the spread of syphilis. This subject demands the immediate attention of sanitarians and those directly interested in public health.

18. In occasional well-selected cases the use of the iodids after the method of Wechselmann will increase the efficacy of "606" when second injections are necessary. From two to three weeks should lapse after thorough mercurial treatment, when this has been used, before the injection of arsenobenzol.

19. In spite of the fact that nearly all agree that the effect of arsenobenzol is magical, sufficient time has not lapsed to justify the conclusion that a single injection of "606" will prevent what we now recognize as the secondary and tertiary stages of syphilis. Only after long years of careful observation shall we be able to reach a positive conclusion. It will be necessary therefore to keep injected patients under close observation.

400 East Genesee Street.

THE CULTIVATION OF TISSUES OF THE CHICK-EMBRYO OUTSIDE THE BODY *

MONTROSE T. BURROWS, M.D.
NEW HAVEN, CONN.

In 1907 Harrison described briefly before the Society of Experimental Biology and Medicine[1] a method which he had found successful for the growing of certain tissues of the frog embryo outside the body. Essentially the method consisted in dissecting the central nervous system, myotomes and skin of frog embryos free from the surrounding tissues and transplanting them to a drop of lymph taken from the lymph-sac of an adult frog and contained within a hollow slide. The lymph immediately clots about the tissue elements into a loose fibrin network. Harrison watched the growth of the axis-cylinder processes of nerves and the proliferation and wandering of epithelial and connective-tissue cells within this matrix. He further observed striated embryonic muscle cells to become differentiated from the preexisting cells of the myotomes. In other words, he was able to observe a certain amount of differentiation of the tissues over a short period of time.

Other fluids were employed, however, without success, viz., physiologic salt solution, Locke's solution, and gelatin; and the transplantation of the embryonic tissues to the ventricles of the brain of the frog was not followed by proliferation and development. It appears as though the fibrin network was essential in order to afford a supporting framework on which the cells can attach themselves and thus retain their tension. The fluid within the meshes of the network corresponds to the fluid bathing the cells in the living animal and provides the nutriment. The full details of Harrison's work are now in press and will appear in the *Journal of Experimental Zoology,* 1910. In the spring of 1910 Professor Harrison kindly permitted me to spend a few months in his laboratory in order that I might acquire under his own supervision the method of growing tissues outside the body. He had already concluded that lymph was not a wholly satisfactory medium for growth. Some of the reasons were the following: The clots produced from it were neither firm nor uniform. Small quantities of lymph could be obtained from a single animal that sufficed only for one or two preparations. Hence, large series of control preparations could not be secured. It became necessary, therefore, to obtain a more uniform and abundant medium in order that the conditions underlying the growth and differentiation of tissues might be subjected to close analysis. Such an abundant medium of constant composition would, theoretically, be supplied by the blood-plasma provided it could be obtained in suitable condition.

The particular object of my study with Professor Harrison was to adapt, if possible, his method to the investigation of the growth of the tissues of warm-blooded adult animals in order to continue and extend the study of the laws of the healing of wounds and regeneration of nerves, which subjects were at that time being actively studied by Dr. Carrel, with whom I was associated at the Rockefeller Institute.

In repeating the original experiments of Harrison I succeeded in substituting the blood-plasma of the adult frog for the lymph, and thus in overcoming some of the

* From the Sheffield Biological Laboratory, Yale University.

1. Harrison, R. G.: Observations on Living Developing Nerve Fibers, Proc. Soc. Exper. Biol. and Med., 1905-1907, iii, 140; the Outgrowth of the Nerve Fiber as a Mode of Protoplasmic Movement, Jour. Exper. Zool., 1910, ix.

chief drawbacks which Harrison had encountered in his earlier work. The attempt was then made to cultivate tissues of chick embryos. The embryo of the chick offered the especial advantage of being procurable at any time throughout the year, and provided the opportunity for making observations on a warm-blooded species. Moreover, the tissues of the chick embryos are nourished at an early period from an extra-cellular yolk through the means of a well-established vascular system. Hence the removal of pieces of tissue from the embryo interrupts the vascular connections and eliminates all the nutriment derived from the yolk, so that opportunity is afforded not only for the study of growth of tissue, but also of problems of self-nutrition.

TECHNIC

The technic employed consists in placing a carefully isolated fragment of tissue of the chick-embryo in a drop of uncoagulated plasma derived from a chicken on a cover-glass. The cover-glass is inverted and sealed to a hollow slide and the preparation incubated at 39° C. The plasma immediately coagulates about the tissue and holds the fragment firmly fixed in a fibrin network. Preparations made in this way can be readily observed at all time under the microscope.

The success of the method depends on maintaining absolute asepsis and preventing undue chilling of the embryos or the completed specimens either during preparation or the later observation. In excising the fragment of tissue from the embryos they were floated in Ringer's solution and the operation carried out under a binocular microscope covered with an oven heated to 39 C.

The blood for the preparation of the plasma was obtained from young healthy adult chickens under ether anesthesia. The carotid artery is exposed and a cannula previously sterilized in olive-oil is inserted. The blood is collected in sterilized, paraffin-coated tubes which are cooled immediately by being plunged into an ice salt-bath. The blood is next centrifugalized by placing the tubes in larger centrifuge tubes which contain a mixture of salt and ice. The supernatant plasma is removed by means of paraffin-coated pipettes and transferred to paraffin-coated receptacles which are kept in a refrigerator until used. The plasma so obtained is highly stabile and can be preserved in a fluid state for many days or even weeks. It should be stated, however, that in making control experiments plasma over four days old should never be used.

RESULTS

The method as described was employed especially during the past summer in the study of the growth of tissues of sixty-hour-old chick embryos. For this purpose isolated neural tubes, heart myotomes, and skin were employed. The results which we obtained can be stated briefly as follows:

The most actively growing elements in the preparations is the interstitial connective-tissue cells. These cells begin to spread into the plasma either as single cells or a layer of cells between the second and twelfth hours of incubation, as a rule, and the growth continues for from six to fourteen days. It often happens that a large part of the drop of fluid is filled with these cells. On being fixed and stained the preparations show mitotic figures to be very common in the proliferating cells. The muscular elements grow much less frequently and cellular outgrowths from them were observed in only about 3 per cent. of the experiments. The outgrowths take place from the myotomes and the heart and appear in the form of short chains of striated cells. The striated cells which are outgrowths from the heart contract rhythmically along with the portion of the heart from which they arise. The outgrowth from the nerve cells consists of long axis-cylinder processes which present the same morphologic appearances and react in the same way to specific nerve stains as those of the chick-embryo. The full account of these studies will appear in a forthcoming number of the *Journal of Experimental Zoology*.

The technic as here described for the frog and chick embryos has now been applied by Dr. Carrel and myself to the cultivation of tissues derived from embryonic and adult mammalian species, as has already been described in THE JOURNAL.[2]

I wish to express my great obligation to Professor Harrison, first for extending to me the privileges of his laboratory for the purpose of studying the method which he had developed for growing animal cells outside the body, and next, for the ready personal assistance which he gave me at all times. I wish also to thank Professor Mendel and Professor Rettger for permitting me to use the chemical and bacteriologic apparatus needed for this work.

THE RENAL ACTIVITY IN PREGNANT AND PUERPERAL WOMEN AS REVEALED BY THE PHENOLSULPHONEPHTHALEIN TEST *

F. C. GOLDSBOROUGH, M.D., AND F. C. AINLEY, M.D.
BALTIMORE

Rountree and Geraghty[1] have recently recommended the use of phenolsulphonephthalein as a means of determining the functional activity of the kidneys, and consider it greatly superior to methylene blue, rosanilin, indigocarmin and various other substances which have heretofore been used for that purpose.

The results which they obtained by its means were so satisfactory that we were led to hope that similar determinations might throw further light on the prognosis and treatment of certain cases of toxemia of pregnancy by showing some characteristic change in the functional activity of the kidneys. As it is known that marked alterations in the metabolism normally occur in the maternal organism under the influence of pregnancy and the puerperium, we subjected a number of normal pregnant and puerperal women to the test in order to determine to what extent their kidneys reacted to it, before applying it under diseased conditions. We employed the technic recommended by Rountree and Geraghty, which is as follows:

Twenty to thirty minutes before administering the test the patient is given 300 or 400 c.c. of water by mouth in order to insure free urinary flow, as otherwise a delay in the appearance of the drug in the urine might be simply due to lack of secretion. The bladder is then completely emptied by means of a catheter introduced under aseptic precautions, and 1 c.c. of a carefully prepared solution containing 6 mg. of phenolsulphonephthalein to the cubic centimeter is administered subcutaneously either in the arm or the buttocks by means of an accurately graduated syringe. The time is

2. Carrel, Alexis, and Burrows, Montrose T.: Cultivation of Adult Tissues and Organs Outside the Body, THE JOURNAL A. M. A., Oct. 15, 1910, p. 1379; Cultivation of Sarcoma Outside of the Body, THE JOURNAL A. M. A., Oct. 29, 1910, lv, 1554.

* From the Obstetrical Department of the Johns Hopkins Hospital and University.

1. Geraghty, J. T., and Rountree, L. G.: An Experimental and Clinical Study of the Functional Activity of the Kidneys by means of Phenolsulphonephthalein, Jour. Pharmacol. and Exper. Therap., July, 1910, p. 579.