served out of the entire number. The patient from whom the non-oviparous female was obtained has been in the institution for six years (Case 4). We can assume that the worms recovered from this patient are over six years of age, since there has been no evidence of infection while in the institution. Of absorbing interest is Case 1, possibly the longest infected case on record, Dr. Stiles (personal communication) related two cases in which the infection lasted about eleven years. Our patient has been constantly present at the institution for over eleven years and no outside source of infection was possible.

It is generally conceded that the average infection will last three or four years; the worms die or become dislodged from their firm attachment and are then passed out by the bowel. Two thymol treatments in Case 1 dislodged 27 hookworms—22 females and 5 males. Every female contained enormous numbers of ova. It is evident from the above case that the worms are more than eleven years old. Notwithstanding their extreme age they appear to be producing as many eggs as in the prime of life. This case, however, cannot be taken as a criterion, since it may be an exception rather than the rule.

About two years ago I recovered 89 worms from a patient giving a history of having had the disease a little more than one year. Recently I examined these worms and found 66 to be females and 23 males. Three of the 66 females proved to be non-oviparous. The history of the case follows:

CASE 12.—A boy, 12 years old, had always lived in Massachusetts except for the past year and a half. He had always been healthy and energetic. The family moved from Massachusetts to South Carolina in the early spring of 1907. During the summer the boy went barefooted and contracted ground-itch on two different occasions. Several months later he appeared not to be as well as usual, and throughout the winter months he exhibited a marked lack of energy, lassitude, and stood very poorly in his classes at school. Anemia was present, but was not very marked. In June, 1908, the family moved to Atlanta, and came to Nashville in November, 1908. The case came under my observation several weeks later. Examination of the blood showed red cells 4,115,000, hemoglobin 85; whites, 9,200. The differential leukocyte count revealed 17 per cent. of eosinophils. The increase in eosinophils led me to suspect uncinariasis, so the stools were examined, with the result that a great many hookworm ova were found. Thymol was given, which expelled 67 worms, 53 of which were females and 14 males.

One week later, another course of thymol was given, with the result that 19 worms were obtained—10 females and 9 males. Two weeks later, another course of thymol was given, which expelled 3 females. After this, repeated examinations of the stools failed to show the ova. The patient rapidly improved, and three months after the last treatment, was as healthy and robust as he had always been before the infection.

The unique feature in this case was the finding of three non-oviparous females out of 66 worms, which gave a history of being less than one and one-half years old. From this case it is evident that comparatively young females may be incapable of egg production.

I am of the opinion that the non-oviparous nature of the female is not entirely due to the "old-age period," but is, in all probability, caused by some defects in development, or occasioned by peculiarity in a given strain. Definite conclusions, however, are not warranted, as evidenced by the paucity of the material at hand. Further observations by the writer along this line will be forthcoming in the near future.

CULTIVATION OF ADULT TISSUES AND ORGANS OUTSIDE OF THE BODY*

ALEXIS CARREL, M.D.
AND
MONTROSE T. BURROWS, M.D.
NEW YORK

The solution of many problems of human pathology depends, in a large measure, on the finding of the still unknown physiologic laws of generation, growth and evolution of cells. We must, therefore, develop new methods which permit the discovery of these laws. A few weeks ago, we began to investigate systematically one of these future methods, namely, the cultivation of adult tissues outside of the body. The starting point of our researches was the beautiful work of Harrison on the embryonic tissues of the frog. Some years ago, Harrison observed the development of nerves from the central nervous system of frog embryos cultivated in a drop of lymph. In 1910, Burrows studying with Harrison improved very much this method and adapted it to embryonal tissues of warm-blooded animals. He succeeded in cultivating nerves and mesenchymatous cells of sixty-hour chick embryos.

Then, at the Rockefeller Institute, we tried to develop on this basis a general method which would be applicable to the adult tissues of the mammalia and thus to determine some of the laws of cellular physiology.

The experiments on which we wish to report now were performed on dogs and cats and an adult frog and consisted of extirpating small fragments of tissues of an animal, inoculating it aseptically into a plasmatic medium taken from the same animal, and sealing the materials in hollow glass slides. The slides were placed in an incubator, maintained at a constant temperature of 37 C. The microscope was also placed in a special thermostat which was kept at this temperature. The growth of the cells could, therefore, be observed, over a period of time, with the microscope, kept itself at the body temperature, and the multiplication of cells directly seen.

GENERAL CHARACTERS OF THE GROWTH

The plasmatic media were inoculated with many tissues or organs, of which all were found to multiply or grow. The cultures of the different tissues—as we shall call them—contain common characteristics. The time of the beginning of cellular proliferation depends on the nature of the tissue, the age of the animal and other more or less important factors. In the cultivation of glandular organs of adult dogs, the vegetation starts after thirty-six or forty-eight hours. But, if the young animal is only a few days old, new cells appear in the culture after ten or twelve hours. Four or five days after the inoculation of the medium, the cultures of thyroid, kidney, suprarenal, etc., are in full activity, and remain in this condition as long as the medium allows it. Tissues like cartilage or peritoneum grow, at first, very slowly. After three days, there are in the cultures very few new cells. But about one week after the inoculation, the cultures become very much more active, and are in full vegetation after about nine or ten days. There is also some analogies between the morphologic characters of the cultures of various tissues

*From the Laboratories of the Rockefeller Institute for Medical Research.

and organs. For all tissues, the first indication of growth is the appearance on the edges or the surface of the specimen of a few small and regular granulations. These granulations consist of the cytoplasm of cells, the nucleus and nucleoli of which soon become visible. The cells belong to two general types, spindle and polygonal. The spindle cells appear ordinarily at first and their morphology is about the same in all tissues, bone marrow or kidney, thyroid or cartilage. They are long and slender and radiate from the fragment of tissue or organ through the plasmatic medium. They are derived probably from the connective tissue framework of the organ. At the same time, or a little later, the cells of the second type appear. They are polygonal or multipolar cells in form, but their morphology varies widely according to each tissue and organ. They seem in part to be differentiated cells of epithelial nature. Cartilage produces cartilaginous cells, and thyroid generates cells which look like thyroid cells. Even in the renal cultures, this second type of cells congregates in tubular formations. By using a suitable technic, we can control the growth of one or another of these types. A small fragment of thyroid cleanly cut produces mainly spindle cells, while in tissues more finely divided (scrapings), epithelial-like cells appear.

In the first part of the work we found and studied the growth of adult tissues outside of the body. In the second part we attempted to cultivate thyroid cells in series, and also to activate the growth of a tissue by passage from one plasmatic medium to another. Connective tissue, cartilage, peritoneum, bone marrow and bone, skin, thyroid gland, spleen, suprarenal gland, kidney, ovary and lymph gland, were all cultivated successfully.

CULTIVATION OF TISSUES

Arterial Sheath.—Three days after inoculation of a fragment of arterial sheath, very delicate palm-like cells appeared on the edge of the tissue and ramified through the plasmatic medium in long filaments ending in spindle cells. Vegetation was very weak and stopped entirely after a few days.

Connective Tissue.—Most of the cultures of connective tissue remained inactive.

Conjugal Cartilage.—This started also to grow on the third day. For about one week, very few spindle and spider like cells were found slowly wandering along the edges of the cartilage. From the upper pole of the fragment of tissue, a mass of new cartilage protruded and invaded the plasmatic medium. After a few days, it became so large that it could be seen by the naked eye. Progressively, the rate of growth became faster. Many irregular cells with long arms now appeared in the plasma about the old cartilage.

After nine days, the culture was in full activity, and the old cartilage had thus generated outside of the body a piece of new cartilage two millimeters long.

Peritoneal Endothelium.—This underwent also a slow evolution. For several days, there were only a few beautiful and irregularly-shaped cells along the edges of the tissue. After a week they began to multiply more actively and many very large cells resembling endothelial cells slowly moving through the clear plasmatic medium were directly observed under the microscope. On the twelfth day, the culture was still in full vegetation.

Bone.—During the first hours of the cultivation of fragments of bone marrow and bone, the anatomic elements began to wander away from the tissue. After three or four days, the little pieces of bone hidden in the bone marrow became visible, because almost all the cells had invaded the plasmatic medium. Around the tissue, there were radiating spindle cells and many red blood corpuscles. Leukocytes with active ameboid motion and large cells with granular cytoplasm and long pseudopodia had reached the remotest part of the medium. A few large spindle cells were seen crawling along the edges of the fragments of bone.

Epidermis.—We studied the growth of epidermis by cultivating fragments of the skin of an adult frog. Masses of epithelial cells appeared on the edges of the cutaneous fragments after twelve or twenty-four hours. They grew very rapidly. After forty-eight hours, the area of new epidermis obtained in some cultures was twice larger than the old fragment of skin. A few cultures were fixed and stained, and it could be seen that many cells were dividing by karyokinesis.

CULTIVATION OF ORGANS

Thyroid, Spleen, Etc.—Many cultures of glandular organs were made and grew rapidly. The cultivation of the thyroid of adult dogs was very easy. After thirty-six or forty-eight hours, long fusiform cells protruded at one or several points from the edges of the tissue. Often new polygonal cells also could be seen on the upper surface or on the edges of the thyroid. After the fifth and the sixth days, the cultures were generally in full and sometimes wild vegetation, which lasted as long as the plasmatic medium was in good condition. A great many long fusiform cells or chains of fusiform cells radiated from the tissue through the plasma. Polygonal cells were generally closer to the tissue. In a few cultures there was an abundant proliferation of cells resembling epithelial cells, while the fusiform cells were in small number.

The cultivation of suprarenal and of spleen gave also excellent results.

Kidney.—But very much more important were the results of the cultivation of the kidney. Two plasmatic media were inoculated with small fragments of a kidney of a young cat. Twelve hours later, fusiform cells were protruding from the tissue. After twenty-four hours, a great many cells had invaded the plasma all about the renal substance. One day later, the cultures vegetated wildly. On the fifth day, one of the cultures was fixed and stained with hematoxylin. We saw many karyokinetic figures in the cells which had proliferated through the plasma. A tube had begun to grow from the tissue into the medium. The cells showed a condition of great activity.

The other culture was allowed to live to the sixth day and an exceedingly active growth of the cells took place. In the morning, we observed a few tubes growing from the renal substance into the plasma. In the evening, they were very much longer and curved at their blind ends. At the beginning of the seventh day, the culture was fixed and stained. Around the renal tissue a very large number of fusiform and polygonal cells had formed. A few tubes, composed of a lumen limited by epithelial-like cells, had passed from the fragment of kidney for a distance into the plasmatic medium. They had the appearance of renal tubules.

These experiments demonstrate that adult tissues grow very easily outside of the body. Tissues like cartilage, and even like renal substance, can be caused to develop in something like normal manner under entirely new conditions.

REACTIVATION AND CULTIVATION IN SERIES

The second part of our study consisted of modifying the rate of growth of tissues by passing them into a second medium. A few six and seven day old cultures of thyroid were used for the first series of experiments. The thyroid fragments were removed from the old cultures, cut into small pieces and placed into new plasma. Eleven and twelve hours after, new cells protruded from the previously inactive parts of the thyroid substance, as well as from the newly proliferated cells. We found, indeed, that the thyroid of an adult animal had now become as active as the thyroid of an animal a few days old. Afterward the cells invaded very quickly the new plasmatic medium. One of the cultures was fixed a little less than thirty-six hours after the passage into the new plasma and stained with hematoxylin. From one side of the old tissue there was a large mass of fusiform cells radiating through the plasma. From another point, several tubular formations had wandered far into the medium. The wall of these tubules was composed of epithelial-like cells. It seems, therefore, that the passage from one medium into another of the same kind increases the vegetative power of the thyroid cells.

In a second series of experiments, a plasmatic medium was inoculated with cells produced by the cultivation of a thyroid fragment, in order to obtain a second generation of cells. In several instances, this result was achieved. After twenty-four hours, we noted that a few cells had wandered from the old plasma into the new. In one experiment, less than four hours after the inoculation, the new plasmatic medium already contained new cells. One of these cells was fusiform and its activity was so great that we could follow under the microscope the motion of its cytoplasmic gravitations and the changes of its shape. In a few minutes, one end of the cell became very large, while a long tail grew at the opposite end. Finally the cell became multipolar. Other cells appeared at the same time in the new medium. Thirty-six hours later, the culture was fixed and stained and many active cells resembling epithelial and connective tissue cells were found to be present in the new plasma. We had, therefore, obtained a second generation of the first culture of thyroid cells.

CONCLUSION

The main results of these observations can be summarized in a few words: Adult tissues and organs of mammals can be cultivated outside of the animal body.

The cultivation of normal cells would appear to be no more difficult than the cultivation of many microbes. It remains, however, to be determined whether continuous series of cultures can be secured. This method can, therefore, be used for the study of many important problems. For instance, it may render possible the cultivation of certain micro-organisms in conjunction with living tissue cells or alone in plasmatic media. Then it will be of great value in the study of the problem of cancer. We have already succeeded in inoculating a plasmatic medium with sarcoma of the fowl; cells appeared in the surrounding plasma after nine hours and the culture is growing actively at present. We can assume, therefore, that the perfection of the method of cultivating adult tissues of mammals outside of the body will be helpful in the exploration of unknown fields of human pathology.

Work is in progress along the lines indicated, the results of which will be published from time to time.

Therapeutics

DIET IN TYPHOID FEVER

As typhoid fever has its most active pathology in the intestinal canal, and as all ordinary food must traverse this canal, and as in this almost invariably protracted fever nutrition is a matter of serious consideration, the character of the food given in this fever is always a live subject for discussion.

While this subject has been several times touched on in this department, the last word has not yet been said, as evidenced by the repeated discussion of the subject, both in medical journals and in medical societies.

Whether we have under-fed our typhoid patients or over-fed them, it seems that the evidence is very strong that milk alone is not the proper food for these patients. In fact, when we consider the frequent difficulty in its digestion, the large amount of it that must be given to satisfy the system either in calories or in protein, it would seem that we should rule against it as a typhoid diet. These facts immediately cause the decision that our old feeding of typhoid fever was wrong, and that we must select a new or modified food in this disease.

It can not be questioned that the high temperature, rapid pulse, delirium, and that association of nervous symptoms called typhoid are not caused by the typhoid germ alone, but by a double infection, and the double or secondary infection is due to toxins or the products of secondary germs absorbed from the intestines.

Tympanites is an indication not of typhoid fever, but of intestinal putrefaction and fermentation, and a mistake in the management of the bowels and of the food administered. Tympanites need not be a symptom of typhoid fever, and when it is present it is almost invariably a medical mistake. It is too easily a demonstrable fact to require anything more than the assertion that, if the abdomen is flat and not distended, if the bowels have properly moved, and if there is no troublesome diarrhea or obstinate constipation, then the tongue is less coated and is not dry, the temperature is less, the skin is moist, the pulse is better, the delirium is less or generally absent, and such symptoms as carphology and subsultus are absent. The temperature being lower than with such added bowel infection, there is less necessity for disturbing the patient with cold water antipyretic measures, and the less such disturbance the quieter the nervous system and the less loss of nutrition, to say nothing of the less irritation of the heart from rapidity caused by the exercise and nervous excitement due to cold applications.

It stands to reason, then, that primarily such food and arrangement of the movements of the bowels as cause the least tympanites and the least indigestion are of first importance in the management of typhoid fever. Secondly, the food which, so far as possible, satisfies the requirements of the body for nutrition and at the same time satisfies the above requirements of easy and thorough digestion, should be the food of choice.

It must always be considered, of course, that during the fever term there will be a progressive loss of weight, in other words, a denutrition. It is probably impossible to prevent this by any amount or any character of the food, as before sufficient nutriment could be absorbed to prevent loss of weight such indigestion would be caused as to preclude its administration. As it is impossible to determine exactly how much of all of the different elements of food the patient can absorb just inside of his limit of perfect digestion, we must have