puseles present. In every examination numerous active trichomonades were noted until the last week of the disease, during which they gradually decreased in number and lost their motility. There were still a few present in the sputum on the day of the patient's discharge (August 5, 1910) from the hospital. At no time were there more than a few bacteria present in the sputum.

Course of the Disease.—At no time during the patient's confinement in the hospital did the temperature rise above normal after the first twenty-four hours, but ranged between 97.6 and 98.5 F. The pulse and respiration were normal throughout. The sputum and pain in the side and over the root of the left lung were the only noteworthy symptoms, and on discharge (August 5, 1910) only a slight soreness over the root of the lung remained, the foul and bloody sputum and the pain having quite disappeared. The patient has reported twice since discharged, at intervals of a week, for observation, and at the last visit seemed to be in perfect health.

THE NON-OVIPAROUS FEMALE HOOKWORM*

WILLIAM LITTERER, A.M., M.D.
NASHVILLE, TENN.

In every higher animal we recognize certain more or less definite periods of physiologic activity, and we roughly divide the span of life into three stages, which are in no way sharply outlined. These we call the stages of youth, adolescence, and old age. Youth, characterized by a high degree of vitality, is the period of rapid cell multiplication and growth; organs are formed and perfected, functions are unimpaired and active, and the body is a perfect living thing. The second period is characterized by functional and sexual maturity; the multiplication of tissue cells is less rapid; the organs strengthen and their functions are more perfectly correlated; growth comes to an end. In the perfected animal it is a period for perpetuation of the race, and in conformity with this great function sexual differentiation is fully established. The third period, old age, brings a marked change, the potential of vitality wanes; degenerations of all kinds appear, and cumulative weakness ends natural death. These three periods are all characteristic of all of the higher-celled animals, the last period being rarely seen in nature, because in the wild animals a violent death follows the early functional weakening and inability to fight off enemies (Calkins).

Do we find the same sequence of physiologic changes in the lower many-celled animals, and can we distinguish periods of youth, maturity and old age? Since the fundamental biologic laws are much the same, on a *priori* grounds alone we should expect to find the same series of changes in the lower metazoa and likewise in the protozoa.

Of late I have been greatly interested in the study in the development of the *Uncinaria americana* with special reference to the non-oviparous female, endeavoring to ascertain whether their inability to produce eggs is due to the "old age period" or to other influences not attributable to senile degeneracy. It is affirmed by Bass that more than 7 per cent. of females out of the 247 dissected by him failed to produce ova. He maintains that during the latter third of their existence they cease to lay eggs. This observation, if substantiated, is of paramount importance for the reason that individuals

may be harboring the worm though no ova can be found in their stools.

The limited amount of work that I have done along this line does not correlate with the result of Bass's investigations, as shown from the examinations of specimens obtained from cases coming from the Tennessee Industrial School. At this juncture I wish to express my deep indebtedness to Dr. Thomas Weaver, the physician in charge, for the privilege of studying these cases. Children are brought here from practically every county in the state and are kept under surveillance until they have attained their majority. So far as we could ascertain, there is no evidence that the disease has ever been contracted in the institution. Ground-itch, dew-poison, etc., are unknown among them and the water-supply is perfectly pure. This offers a fruitful field of research for ascertaining just how long the infection may have persisted in a given case and likewise in determining the oviparous and non-oviparous females, with especial reference to their ages. The report in detail follows:

CASE 1.—L. D., a girl, aged 17, admitted March 8, 1899, from Putnam County; eleven years in the institution without interruption; two treatments by thymol (on Feb. 15, 1910, and March 18, 1910) recovered 27 hookworms—22 females and 5 males; all of the females contained many eggs.

CASE 2.—M. G., a girl, aged 18, admitted from Rutherford County; thymol treatment recovered 19 hookworms—13 females, 6 males. The females were all oviparous.

CASE 3.—M. C., a girl, aged 9, admitted May 27, 1905, from Overton County. Only 1 hookworm recovered by thymol. This worm was an egg-bearing female.

CASE 4.—R. S., male, aged 18, admitted Feb. 12, 1904, from Pickett County; six years in school. Three courses of thymol given (March 4, 1910; March 11, 1910, and March 17, 1910). Forty-five hookworms—36 females and 9 males—were recovered. All the females contained many eggs, except one. This non-oviparous female was of average size and, so far as could be ascertained macroscopically, was perfectly normal. Microscopically, the vulva was abnormally situated just in front of the lower two-thirds of the body. The two uterine and ovarian tubes were observed to be quite atrophic, and very much shorter than normal.

CASE 5.—D. C., a boy, aged 13, admitted May 13, 1909, from Blount County. Thymol treatment recovered 18 hookworms—13 females and 5 males. All the females were oviparous.

CASE 6.—F. M., a boy, aged 16, admitted July 13, 1909, from Davidson County. Thymol treatment recovered only 1 oviparous female.

CASE 7.—W. D., a boy, aged 12, admitted Jan. 1, 1910, from Cannon County. Thymol treatment revealed only 1 oviparous female.

CASE 8.—F. C., a boy, aged 16, admitted Feb. 10, 1910, from Scott County. Thymol treatment recovered 3 oviparous females.

CASE 9.—K. B., a girl, aged 9, admitted July 1, 1908, from Macon County. Thymol treatment recovered 2 oviparous females and 1 male.

CASE 10.—B. J., a boy, aged 14, admitted Jan. 25, 1909, from Greene County. Thymol treatment recovered 2 oviparous females and 1 male.

CASE 11.—L. M., a girl, aged 13, admitted Dec. 28, 1907, from Lawrence County. Thymol treatment recovered 7 oviparous females and 4 males.

SUMMARY

In summarizing the above cases it will be seen that 132 worms were recovered and examined—101 females and 31 males. Only one non-oviparous female was ob-

* From the Pathological Laboratory of Vanderbilt University, Medical Department.

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.