SOCIETIES AND ACADEMIES

THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE. TWENTY-THIRD MEETING

New York University and Bellevue Hospital Medical College, May 22, 1907. President Flexner in the chair. Members present—Atkinson, Beebe, Brooks, Calkins, Carrel, Emerson, Ewing, Field, Flexner, Gibson, Gies, Lillie, Lusk, Meyer, Murlin, Salant, Shaffer, Teague, Wadsworth, Weil, Wolf, Yatsu.

Abstracts of the Communications[1]

The Osmotic Pressure of Colloidal Solutions and the Influence of Electrolytes and Non-electrolytes on such Pressure: RALPH S. LILLIE.

Determinations were made of the osmotic pressure of gelatin and egg albumin. The colloids were used (1) in approximately pure solution, and (2) after the addition of various electrolytes and non-electrolytes to the colloidal solution; in this case the employed substance was added in the same concentration to the outer fluid of the osmometer so as to pervade the entire system on both sides of the membrane in uniform concentration. The osmotic effects observed under these conditions can be due only to the colloid and not to the added substance. The colloidal solution was found, however, after the addition of an acid, alkali or neutral salt, to exhibit an altered osmotic pressure, the degree of alteration varying with the nature and concentration of the added electrolyte. Non-electrolytes were found to have no appreciable influence on the osmotic pressure of these colloids.

Hemolysis in Eclampsia: JAMES EWING.

The author's observations indicate that the eclamptic toxin is not a hemolytic agent derived from the placenta, and that hemolysis is not necessarily associated with the lesions of the viscera. Semb's experiments in which he demonstrates visceral lesions strongly resembling those of a hemolytic serum, can not be accepted as furnishing evidence of a specific eclamptic toxin. Histological study of the liver in eclampsia indicates that the characteristic lesions consist of fibrin thrombi and not in agglutination and hemolysis of red cells, and that when hemolysis occurs it results from the products of degeneration and necrosis of endothelial and hepatic cells. It is therefore probably an entirely secondary factor in the disease.

Glycocoll Nitrogen in the Metabolism of the Dog: JOHN R. MURLIN.

While attempting to explain the behavior of gelatin in metabolism it occurred to the author that much significance might be attributed to its high content of glycocoll. It is well known that the nitrogen of gelatin is not ordinarily retained in the body, but appears quantitatively in the urine, chiefly as urea. But when fed with meat and an abundance of carbohydrate, it is possible to establish nitrogen equilibrium near the fasting level, if two thirds of the total quantity of nitrogen fed is given in the form of gelatin and only one third is present in the meat. Would glycocoll, if given in the same way, behave as does gelatin? The author's experiments answered this question in the affirmative.

An Hydrodynamic Explanation of Mitotic Figures: ARTHUR B. LAMB.

The distinctly polar arrangement of the chromatin substance about the astral centers in dividing cells, combined with the pronounced curvature of the astral rays and of the spindle fibers, has demanded the assumption of some polar force as universally operative. On such an assumption it is of course necessary to assume further that astral centers represent either opposite or like poles. On the alternative of opposite poles, we should expect, with any force so far proposed, a configuration of astral rays simulating that of iron filings between *opposite* magnetic poles, coupled with a mutual *attraction* of the astral centers. On the other alternative, we should similarly expect a configuration of astral rays and spindle fibers simulating that of iron filings between *like* magnetic poles, coupled with a mutual

[1] The abstracts presented in this account of the proceedings have been greatly condensed from abstracts prepared by the authors themselves. The latter abstracts of the communications appear in Number 6 of Volume IV. of the Society's proceedings, which may be obtained from the Secretary.

repulsion of the astral centers. Actually, we have neither of these conditions, but instead, a configuration like that of iron filings between *opposite* magnetic poles *and at the same time an apparent repulsion between the astral centers or the centrosomes.*

This is not the case with the forces of attraction or repulsion existing between bodies oscillating or pulsating in a fluid medium. More specifically, if two spheres are pulsating synchronously, and in opposite phase, or oscillating synchronously and in the same phase, they will repel one another, *but at the same time the field between them will simulate the configuration of iron filings between opposite magnetic poles.*[2]

If then we assume that the centrosomes are pulsating in opposite phase, or better, oscillating in the same phase, we shall obtain the desired repulsion and at the same time have a configuration like that actually observed.

The configuration taken by the chromosomes is explicable on the same grounds. Indeed, it is not necessary to assume any independent motion on their part, but simply to consider it an induction phenomenon. The tri- and multi-polar spindles are also better explained on these hydrodynamic grounds than on previous assumptions.

The foregoing explanation is, of course, pure hypothesis, with no support other than the facts it seeks to explain. There is, however, nothing inherently impossible in it, and it may provoke fresh observation and new ideas.

Transfusion Experiments on Dogs, showing Artificially Implanted Tumors: GEORGE W. CRILE and S. P. BEEBE.

Direct transfusion of the whole blood from immune dogs to dogs with actively growing, artificially implanted tumors has been conducted in six animals. In the first set of three, sufficient time has elapsed to determine the outcome, which was as follows:

I. Dog 116. Planted January 7, 1907. Tumors were first seen on February 20; continued to grow slowly. March 20, transfusion experiment: dog was bled 400 c.c. and immediately transfused with 550 c.c. of blood from dog 244, in which implantation had occurred on January 18; tumors were first noticed on February 6, and had continued to grow until February 20, when they began to regress. Regression complete March 7. Three days after transfusion, dog 244 was again planted with tumor. Four plants were made with positive results in three and tumors are growing at the present time. The immunity which dog 244 possessed as a result of the previous growth and regression of the tumors could not have been very marked. The effect of this transfusion upon tumors of dog 116 was negative, since they continued to grow until the death of the animal in a cachetic condition four weeks later.

II. Dog 125. Weight 13 kilos. Tumors were planted December 6. All plants grew and continued to increase in size until the day of transfusion, March 20. On this day the dog was bled 500 c.c. and immediately transfused with the same quantity of blood from dog 163. Following the bleeding and transfusion the tumors of dog 125 became softer and began to regress. One tumor has entirely disappeared and the others are subsiding.

III. Dog 133. Weight 17 kilos. Tumors planted January 31; first growth noticed February 13, and continued active until day of transfusion. March 20, bled 600 c.c. and transfused 1,500 c.c. from dog 289. The latter animal was 19½ kilos in weight, in very good physical condition and naturally immune to the tumor. Following this transfusion, which was the largest and of the best quality that any animal in this series received, the tumors of dog 133 began to regress immediately, and at the present time the regression is complete.

The authors postponed discussion of these results.

Transplantation of the Thigh from One Dog to Another: ALEXIS CARREL.

On April 23, 1907, at 9:50 A.M., a medium-sized dog was killed with chloroform. At 10:20 A.M. the left thigh of the cadaver was amputated just below its middle part, perfused with Locke solution and placed on a

[2] See Bjerknes's text-book, "Hydrodynamische Fernkräfte," J. A. Barth, Leipzig, 1902.

table of the laboratory, the temperature being 88–90° F.

At 11 A.M. a medium-sized bitch was etherized; her left thigh was amputated and immediately replaced by the thigh of the dead dog. The reconstruction of the thigh began by the suture of the bone, the adductors and quadriceps. Then the femoral vessels were united and the circulation reestablished at 1 P.M. The operation was completed by the suture of the nerves, muscles, aponeuroses and skin, and the limb placed in a plaster of Paris apparatus.

On April 23, 24 and 25 the animal remained in good condition and walked on her three normal feet. The transplanted limb was warmer than the normal one and its circulation very active. On April 26 she appeared to be sick. There was a phlegmon of the thigh. Incisions were made in Scarpa's triangle and on the transplanted limb, which was warm. Hemorrhage of red blood occurred from the incisions in the transplanted limb.

During the succeeding days, the circulation of the limb remained active, the foot became swollen and the general condition of the animal declined. On May 1 a large abscess was detected near the pelvis and opened. A small incision having been made on the foot of the transplanted limb, hemorrhage of red blood occurred. The general condition of the animal was very low. On May 2 the animal died of septicemia.

Then it was found that the lumen of the femoral vessels was free from thrombus, and the intima smooth and glistening. There was no deposit of fibrin on the lines of suture. In spite of the infection, the union of the vessels was excellent. The skin and the muscles were cicatrized and the ends of the femur firmly united by the ligature.

The Bacteriotherapy of Leprosy: Paul G. Woolley (by invitation).

It seemed to the author that, lacking pure cultures for the purpose, he might make the leprosy patient serve as his own culture medium. It is well known how abundant are the bacilli in the lepra nodule. The author excised a nodule from the arm of an advanced and wretched case of the tubercular form of leprosy. The nodule was very rich in bacilli. It was ground with sand and salt solution; centrifugalized; heated to 65–70° C. for fifteen minutes, and treated with enough 5-per-cent. carbolic acid to make a suspension containing 0.5 per cent. of the acid. This suspension was rich in bacilli. Of it subcutaneous inoculations of 0.01 c.c. were made at intervals, the intervals depending on the general condition of the patient. Experience with the more exact methods possible with the analogous disease, tuberculosis, indicates that minimal inoculations of the dead bacilli must be continued over a long period before a genuine arrest is attained; even, therefore, with the most favorable outcome, the author does not expect to report the results of this treatment for months to come. The author communicated the method in order that others with fuller opportunities may test it.

Direct Silver Staining of Spirochetes and Flagellated Bacteria: Simon Flexner.

The discussion of the nature of the structure now called *Spirocheta (Treponema) pallida*—whether a microorganism or some histological elements—led Flexner to try to effect the silver staining directly upon smear preparations prepared from serum exudates obtained from syphilitic lesions. While engaged unsuccessfully in this endeavor, Stern,[3] of Prag, published a simple method for staining the spirochetes directly with silver nitrate. When the deposit of silver presents a metallic sheen the impregnation is regarded as sufficient. Flexner has found the method very simple and sufficient; but he has obtained better results from long (3–4 days) than from short (1–2 days) exposures. The length of exposure required will depend somewhat upon the weather (strength of light) and the thickness of the spread. Moderately heavy spreads have given him better results than thinner ones, and impression preparations better than smear preparations.

Other spirochetal organisms, from the buccal cavity, etc., may be silvered by this method, and bacteria may also be silvered. In a

[3] Stern, *Berl. klin. Woch.*, 1907, XLIV., 400.

few comparative tests which were made, the degree of impregnation was greatest with the pallida. Whether this is to be accounted for by elective affinity or difference of medium in which the organisms were embedded can not be said. In the course of these examinations the author came across examples of flagellated bacteria from the buccal cavity in which the flagella were distinctly silvered. He attempted to stain the flagella of certain bacteria—*B. typhosus, paratyphosus, pyocyaneus, hog cholera*—from pure cultures, but unsuccessfully. The terminal cilia of the pallida appeared not to be stained by the silver.

Flexner observed instances in which the silvered films showed many more *spirochetæ pallida* than the corresponding preparations stained by Giemsa's or Proca's methods.

On the Bacterial Production of Skatol and its Occurrence in the Human Intestinal Tract: C. A. HERTER.

A large number of facultative and strict anerobic organisms have been studied with respect to their ability to form skatol. The anerobes *B. putrificus* (strain isolated by Bienstock) and one strain of the bacillus of malignant edema (obtained from Professor Theobald Smith) were found to produce skatol in peptone bouillon, although it was not possible to determine the conditions under which skatol could be regularly obtained through the action of these bacteria. It was found that skatol is rarely present in the intestinal tract except in conditions of disease associated with intestinal putrefaction. Usually skatol is associated with indol in such conditions, although there are instances in which the intestinal contents exhibit little or no indol but, relatively speaking, considerable skatol. This has been observed heretofore only in putrefactive processes associated with pronounced clinical manifestations.

A Spirochete found in the Blood of a Wild Rat: W. J. MACNEAL.

Of thirty-nine wild rats (*Mus decumanus*) caught at Morgantown, W. Va., by MacNeal, one has shown a minute, actively motile, spiral organism in the blood. It is present in very small numbers and careful search with high magnification is necessary to detect its presence.

The parasite stains readily by the various modifications of the Romanowsky stain, and very intensely by the rapid method recommended for clinical staining of *Spirocheta pallida.*[4] It takes a uniform, deep, violet-red color. The measurement of a number of individuals shows a marked variation in length, the shortest forms, consisting of one and three quarters turns or nodes, having a length of 1.75 μ; the longest, consisting of three and one half turns, being 3.55 μ long.

The infection is readily transferred to other wild rats by intraperitoneal injection of a very small drop of infected blood in normal salt solution. In many cases, not more than ten or twenty parasites could have been present in the injection, yet, so far, the wild rats have always developed the infection. The parasites never become very numerous and disappear in from one to nine days. This apparent recovery is then followed by repeated relapses. The parasite may become more numerous in the blood during the relapse than in the primary invasion. Neither a certain recovery nor a fatal result has, as yet, been observed.

White rats are susceptible, with an incubation period of four to eight days according to the dose employed. The house mouse (*Mus musculus*) is apparently more resistant.

Similar spirochetes have been described by Carter (in the rat), by Lingard (in the bandicoot, *Mus giganteus*), by Nicolle and Comte (in the bat), by Wenyon and by Breinl and Kinghorn (in the house mouse); all these in the circulating blood. Borrel and Gaylord have described spiral organisms in mouse carcinomata, and one of the forms found by Borrel has been shown by Wenyon to be identical with his *Spirocheta muris,* found in the blood of mice. Morphologically the parasite found here in the rat is apparently identical with this one of Borrel and Wenyon. Its behavior in animals is somewhat different. MacNeal tentatively proposes for it the name *Spiro-*

[4] MacNeal, *Journal Amer. Med. Assn.,* February 16, 1907.

cheta muris, var. *Virginiana,* following the principle suggested by Calkins.[5] Its specific relation to that organism must be left for further work to determine.

Experimental Ligation of Splenic and Portal Veins, with the Aim of Producing a Form of Splenic Anemia: Aldred S. Warthin.

The author's results indicate that obstruction of the splenic veins of dogs by ligation is not followed by a fibroid hyperplasia of the spleen but by a partial atrophy. A more or less complete venous collateral circulation is always produced. The picture of splenic anemia as seen in man can not, therefore, be reproduced in the dog, by an obstruction to the venous outflow from the spleen.

An Experimental Control of Fischer's Attraxin Theory: C. Snow. (Communicated by Aldred S. Warthin.)

Fischer recently reported from Ribbert's laboratory[6] that by injecting a solution of Scharlach R, Sudan III. or Indo-phenol in olive-oil under the skin of the ears of rabbits he was able to get an epithelial proliferation which was not to be distinguished histologically from a squamous-celled carcinoma in man. He was not able to get this result with other substances acting as irritants, and therefore assumed the existence of specific bodies—attraxins—in the injected solution, which exerted a chemotactic influence on the epithelial cells.

His work has been repeated by Snow as nearly as was possible from the meager description given of his technic. Three old and three young rabbits received, under the skin of the ear, injections of the Scharlach R-olive-oil solution, and the injected tissue was excised and examined at times varying from 7 to 61 days. The results show that the solution has absolutely no influence on the epithelial elements, but acts as a mild irritant, inducing a chronic inflammation with slight reaction on the part of the connective tissue in the case of the old rabbits, and a greater reaction, with the formation of foreign-body giant cells, in the case of the young rabbits, the conclusion being that the attraxin theory is without sound foundation, so far as "Scarlet-oil" is concerned.

The Effects of Struggle on the Content of White Cells in the Lymph: F. Peyton Rous. (Communicated by Aldred S. Warthin.)

Preliminary determinations, with the animal (dog) quiet, showed that for any one individual the number of leukocytes per cubic millimeter of lymph, from the thoracic duct, was practically constant during the 1 to 4 hours during which observations were made. With struggle, as others have shown, the lymph flow increases sharply in amount for a few minutes. With this the author found a corresponding increase in cell content, an increase marked in "cell concentration" per cubic millimeter of lymph and in the total number of elements passed.

An additional conclusion was that, for a given individual, the lymph glands seem "set" to produce cells at definite rates. These rates have a wide range for reasons unknown. The cell increase with struggle comes from the peripheral lymph system rather than from sedimented cells in the receptaculum chyli, and is probably dependent on another factor besides increased lymph flow (a supposition upheld by later experiments with lymphagogues).

The facts elicited have a bearing on the "physiological mononucleosis" of the blood observed in man following active exercise, on the disappearance of this after prolonged exertion (25-mile run), and the absolute decrease in mononuclears sometimes seen.

A Lipolytic Form of Hemolysis: Hideyo Noguchi.

Lipase is, under some conditions, an efficient hemolytic agent which acts, however, not directly upon the red corpuscles, but indirectly through the liberation from available fats of the active fatty acids. Neutral fats are not hemolytic, but they become so under the influence of lipase.

[5] Calkins, *Journal of Infectious Diseases,* April 10, 1907.

[6] Fischer, *Münch. med. Wochenschrift,* October 16, 1906.

Potassium cyanide and sodium fluoride in 1:10,000 solution inhibit the action of lipase on the fats, and calcium chloride removes the lytic agent from an active mixture. Since the bile salts are known to increase lipolysis, the effects of the sodium salts of cholic, glycocholic and taurocholic acids in *n*/500 solutions were tested on lipolytic hemolysis. The rate of hemolysis was accelerated.

On the Mechanism by which Water is eliminated from the Blood Capillaries in the Active Salivary Glands: A. J. CARLSON, J. R. GREER and F. C. BECHT.

There is a spontaneous flow of lymph from the quiescent parotid gland of the horse. It is probable that part of the lymph that flows from the neck lymphatics in an anesthetized dog with all the salivary glands at rest comes from the salivary glands. When the parotid of the horse is thrown into activity by stimulation of the cranial secretory nerves, or by injection of pilocarpin into the blood, there is no appreciable increase in the output of lymph from the gland as compared with that from the gland at rest. This is true both of the spontaneous flow and of the flow aided by direct massage of the gland. The activity of the submaxillary does not appreciably influence the flow of lymph from the neck lymphatic in the dog. This conclusion is based on experiments on thirteen dogs. If the activity of the submaxillary gland increases the output of lymph from the neck ducts, the increase is too slight to be detected by present methods, and is not one tenth of the saliva eliminated by the gland, as Barcroft's observations would seem to demand.

In dogs under *light* ether anesthesia, perfectly quiescent and with all the salivary glands at rest, there is always a spontaneous flow of lymph from the neck lymphatics.

The osmotic pressure of the lymph from the active parotid of the horse is not the same in all animals. The lymph obtained from the active gland had in three cases considerably lower osmotic pressures than the serum, a fact which apparently eliminates osmosis as the factor effecting the transfer of water from the blood capillaries in the active gland. The osmotic pressure of the lymph from the neck lymphatics of the horse, collected with the animal under chloroform anesthesia, may be of slightly higher, of the same or of considerably lower osmotic pressure than the serum. The osmotic pressure of the lymph from the neck lymphatics of the dog is usually lower than that of the serum. It is rarely greater. The thoracic lymph was in one case of the same, in the other case, of a higher, osmotic pressure than the serum. It is therefore probable that the osmotic pressure of the thoracic lymph is usually greater than that of the neck lymph.

Under the conditions of the experiments—ether or chloroform anesthesia for from two to four hours—the osmotic pressure of the serum at the end of the experiments was in many cases greater than at the beginning. The same difference is sometimes exhibited by the lymph collected from the same lymphatic, but at different periods of the experiment.

On the Dissociation in Solutions of the Neutral Caseinates of Sodium and Ammonium: T. BRAILSFORD ROBERTSON.

In the case of the neutral caseinate of *sodium* the sum of the ionic velocities was found to be slightly greater than the velocity of the Na ion, indicating a specific velocity of 2.6×10^{-5} cm. per second for the casein anion at 25° C. In the case of *ammonium* caseinate, however, the sum of the ionic velocities was found to be considerably *less* than the specific velocity of the ammonium ion. This was interpreted as indicating the presence in this solution of complex cations containing ammonium. Other considerations show that the effect is not due to viscosity. If casein be regarded as an ampholyte of the type HXOH, the sodium salt would be of the type $Na^+ + XOH^-$; it is possible that the ammonium salt in solution forms ions of the type $NH_4X^+ + OH^-$ or $NH_4X^+ + XOH^-$.

The Altmann Granules in Kidney and Liver and their Relation to Granular and Fatty Degenerations: WILLIAM OPHÜLS.

In the kidneys of dogs, rabbits and guinea-pigs the author found the following arrange-

ment of the Altmann granules: In the connecting and convoluted tubules, and in the descending parts of the loops of Henle, the granules are rather coarse, very definitely rod-shaped and arranged in radial rows in the basilar two thirds of the cells, often so closely set end to end that it is difficult to make out the dividing lines between them. In the part of the cells directly adjoining the lumen there are few scattered short rod-shaped granules and none in the "Bürstenbesatz." These details are naturally more plainly shown in the large cells of the convoluted tubules, but in a general way the smaller cells in the connecting tubules and in the descending loops of Henle resemble them very closely. Some groups of convoluted tubules have much coarser granules than others. Ophüls has not been able to make out whether this is a constant anatomic difference or due to different functional stages. If the granules have any relation to the function of the cells, which seems probable, one would surmise that the connecting tubules can not purely serve the function of conducting urine from one place to another, all the more so since in the large ducts of the pyramids which serve this purpose alone, the granules are very scanty and irregularly arranged. In the large light cells of the ascending parts of the Henle loops, the granules are exceedingly small, also slightly rod-shaped, extremely numerous and scattered all through the cells in an irregular fashion. This condition might be used as an argument in favor of a difference in function of this portion of the tubules. In the cells of the liver of these animals the granules vary greatly in size from just visible to quite coarse granules. All of them are rods, some short, others quite long and more or less wavy. The granules are scattered irregularly all over the cells.

In granular degeneration, the characteristic macroscopic and microscopic pictures of which can be best produced by intravenous injection of potassium bichromate, the granules enlarge in size, become more or less spherical, lose their normal arrangement and stain very deeply with the Altmann stain, contrary to what has been generally assumed after the work of Schilling,[7] who seems to be the only investigator of this question. In the liver the change is similar, all cells being equally involved. The albuminous granules in granular degeneration, then, are not newly formed granules, but to a great degree the enlarged and disarranged normal Altmann granules. The author's observations on the kidneys and liver confirm the view that in fatty degeneration, fat in all cases appears first in and around the Altmann granules. It seemed more as if the granules were changed to fat *in toto*.

These observations indicate why granular degeneration and fatty degeneration so frequently occur simultaneously, for both appear to be the result of abnormal conditions in the Altmann granules.

The Relation of Anatomic Structure to Function: William Ophüls.

The kidney appeared to be the organ best suited for the study of this problem, for by collection of the urine directly after its discharge from the ureters, the exact moment of the occurrence of the disturbance could be ascertained. It is possible to produce albuminuria in dogs within a few hours by intravenous injection of potassium bichromate (about 2–3 c.c. of a 2-per-cent. solution). If Altmann specimens are made from the kidneys at this time no lesions are found. That the poison, nevertheless, acts upon the epithelial cells and the granules in them is shown by the subsequent development of severe lesions.

In phlorhizin glycosuria, likewise, no lesions are demonstrable by this method, although we are fairly certain that the excretion of sugar in this case is due to a lesion in the kidney.

The author believes that quite a number of the anatomic changes which we now look upon as primary, are the result rather than the cause of the functional disturbances, although the disarrangement brought about by them naturally often aggravates the original condition. It is questionable whether the real primary lesion in these cases is of such character as ever to be demonstrable by physical methods.

[7] Schilling, *Virch. Arch.*, 1897, CXXV., 410.

Protein Poisons: VICTOR C. VAUGHAN.

The author has been able by diverse methods to split proteins—bacterial, vegetable and animal—into poisonous and non-poisonous products.

The poisons obtained from the different proteins are similar, but are not identical. All are soluble in both water and absolute alcohol, more freely in the latter than in the former. The aqueous solutions are acid and slowly decompose sodium bicarbonate, forming salts, apparently, and these are less poisonous than the free acids. The aqueous solutions give the general color reactions for proteins with the exception of that of Molisch, and some of them give this reaction. However, most of the protein poisons obtained by cleavage of the protein molecule contain no carbohydrate and are free from phosphorus.

These poisons when injected into animals intra-abdominally, subcutaneously or intravenously induce characteristic symptoms and when administered in sufficient quantity kill promptly.

Death is due to failure of respiration and the heart often continues to beat for some minutes after respiration has ceased. It seems most probable that death is due to the direct action of the poisons on the respiratory center. It is inferred from the readiness with which recovery may follow non-fatal doses that the poison cripples, but does not destroy, the cells of the respiratory center.

All attempts to produce antitoxins with these protein poisons have, so far, failed. It is true that repeated treatments of animals with non-fatal doses of the poisons from the colon and typhoid bacilli enable animals to bear from two to four times the ordinarily fatal doses of living cultures of these bacteria, but this seems to be due to an increased resistance rather than to a true immunity. This condition is not specific and may be induced by the poisons obtained from peptone or egg white, as well as with that obtained by cleavage of the homologous bacterium.

Attempts have been made to ascertain the chemical constitution of the protein poisons by splitting them up with mineral acids, but at present these experiments have not yielded satisfactory knowledge, and work along this line is being continued. The physiologic action of the protein poisons leads to the suspicion that they contain a neurin group, but so far the author has not been able to demonstrate the presence of such a radical.

Observations on the Living, Developing Nerve Fiber: ROSS G. HARRISON.

The immediate object of the author's experiments was to devise a method by which the end of a growing nerve could be brought under direct observation while alive, in order that a correct conception might be had regarding what takes place as the fiber extends during embryonic development from the nerve center to the periphery.

The method employed was to locate pieces of embryonic tissue known to give rise to nerve fibers, as, for example, the whole or fragments of the medullary tube, or ectoderm from the branchial region, and to observe their further development. The pieces were taken from frog embryos about 3 mm. long, at which stage, *i. e.*, shortly after the closure of the medullary folds, there is no visible differentiation of the nerve elements. After carefully dissecting it out, the piece of tissue is removed by a fine pipette to a cover slip, upon which is a drop of lymph freshly drawn from one of the lymph sacs of an adult frog. The lymph clots very quickly, holding the tissue in a fixed position. The cover slip is then inverted over a hollow slide and the rim sealed with paraffin. When reasonable aseptic precautions are taken, tissues will live under these conditions for a week and in some cases specimens have been kept alive for nearly four weeks. Such specimens may be readily observed from day to day under highly magnifying powers.

While the cell aggregates, which make up the different organs and organ-complexes of the embryo, do not undergo normal transformation in form, owing no doubt in part to the abnormal conditions of mechanical tension to which they are subjected, nevertheless, the individual tissue elements do differentiate characteristically. Groups of epidermal cells

round themselves off into little spheres or stretch out into long bands, their cilia remain active for a week or more and a typical cuticular border develops. Masses of cells taken from the myotomes differentiate into muscle fibers showing fibrillæ with typical striations. When portions of myotomes are left attached to a piece of the medullary cord, the muscle fibers which develop will, after two or three days, exhibit frequent contractions. In pieces of nervous tissue numerous fibers are formed, though owing to the fact that they are developed largely within the mass of transplanted tissue itself, their mode of development can not always be followed. However, in a large number of cases fibers were observed which left the mass of nerve tissue and extended out into the surrounding lymph clot.

It has not yet been found possible to make permanent specimens which show the isolated nerve fibers completely intact. The structures are so delicate that mere immersion in the preserving fluid is sufficient to cause violent tearing and this very frequently results in the tearing away of the tissue in its entirety from the clot. Nevertheless, sections have been cut of some of the specimens and nerves have been traced from the walls of the medullary tube, but they were in all cases broken off short.

In view of this difficulty an effort, which resulted successfully, was made to obtain permanent specimens in a somewhat different way. A piece of medullary cord about four or five segments long was excised from an embryo and this was replaced by a cylindrical clot of proper length and caliber, which was obtained by allowing blood or lymph of an adult frog to clot in a capillary tube. No difficulty was experienced in healing the clot into the embryo in proper position. After two, three or four days the specimens were preserved and examined in serial sections. It was found that the funicular fibers from the brain and anterior part of the cord, consisting of naked axones without sheath cells, had grown for a considerable distance into the clot.

These and many other interesting observations described by the author show beyond question that the nerve fiber develops by the outflowing of protoplasm from the central cells. This protoplasm retains its amœboid activity at its distal end, the result being that it is drawn out into a long thread which becomes the axis cylinder. No other cells or living structures take part in this process. The development of the nerve fiber is thus brought about by means of one of the very primitive properties of living protoplasm, amœboid movement, which, though probably common to some extent to all the cells of the embryo, is especially accentuated in the nerve cells at this period of development.

The Presence of Allantoin in the Urine of the Dog During Starvation: Frank P. Underhill.

During the progress of an investigation upon intermediary metabolism, it became necessary to subject the experimental animals to periods of starvation lasting from ten days to two weeks. From the urine of these dogs allantoin separated spontaneously in pure white crystals and the presence of this substance in the urine was constant. The presence of allantoin in the urine during starvation has not been recorded hitherto. This observation makes it probable that allantoin is a constant constituent of the urine of the dog.

Alkaloidal Compounds of Mucoids, Nucleoproteins, and other Proteins: Walter H. Eddy and William J. Gies.

In continuation of their studies of protein compounds the authors have observed that nucleoprotein, mucoid, caseinogen and alkali albuminate form water-soluble products with alkaloids. By intimately mixing samples of the purified moist protein and the pure alkaloid, especially with the latter in considerable excess, soluble products are formed, which may be precipitated by alcohol or alcohol-ether, and which, after purification and drying, readily dissolve in water. Such aqueous solutions are neutral to litmus, and the proteins may be readily precipitated from the solutions by slightly acidifying them.

The purification of such products as well as their chemical and pharmacological study, is under way.

William J. Gies,
Secretary