moderate number of round cells. There was extensive degeneration of the nerve cells throughout the several layers of the cortex, most marked in the large antler cells, of which groups were completely degenerated, and a similar state of affairs in the cerebellum and cord.

The cervical root ganglia showed a striking picture. While most cells appeared normal, a number scattered throughout the ganglion stood out deeply stained, the nucleus being with difficulty recognizable from the cell body. In others there was marked eccentricity or vacuolation of the nucleus and cytoplasm. There was no accumulation of round cells such as seen in poliomyelitis. No lesions were observed in other organs with the exception of the kidney, which showed glomerular and tubular nephritis, together with hyaline thickening of the arteries.

GENERAL CONCLUSION.

The several diseases were reproduced in an attenuated form in the first place. While not all, certainly many of the lesions described can be referred to the consequences of ischaemia, others appear to be toxic in origin.

In the case of polyneuritis a curious homogeneous or hyaline change was noted in the vessels of the cord, particularly the arteries in a monkey which lived for thirty weeks after inoculation. A similar or closely related change has been noted in polioencephalitis. In influenza and nephritis vascular lesions are the predominating features, and by reinoculation after complete recovery acute and chronic vascular lesions can be produced side by side—for example, in the kidney and lungs. The diseases of vessels are most imperfectly understood, and little is known of the stages which may precede or determine the changes observed clinically or after death by the microscope. In the case of experimental nephritis it appears that the vascular changes are concomitant with the onset of the disease and are not a late development.

It may be that new insight is about to be obtained into the pathology of the blood vessels, the production of oedema, and other phenomena associated with alterations in vessel walls, such as the rashes of the exanthemata, but, until further work has been done in some other diseases and the material already accumulated more fully worked out, it seems inadvisable to enter into a detailed discussion of phenomena which are at present not a little confused and whose relations to one another are not at all clear.

THE BACTERIOLOGY OF CERTAIN FILTER-PASSING ORGANISMS.

BY

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THE filter-passing organisms here discussed have been under investigation during the past ten months. The cultivation of such an organism is no new thing, yet it is possessed of a certain novelty. In 1913 Flexner and Noguchi¹ cultivated a filter-passing organism from the nervous tissues of fatal cases of acute anterior poliomyelitis. Their method was that introduced by Noguchi² in 1911 for the cultivation of the spirochaete of syphilis. The original technique has been modified, but the underlying principle remains the same, and constitutes one of the greatest additions to bacteriological technique within recent years.

It is the "Noguchi technique" which has been employed in the present investigation, but before discussing its details it is necessary to refer to one or two points in the preliminary treatment of the infected material.

Preparation of Materials for Culture.

Blood.—The withdrawal of blood is accomplished in the usual way, 10 to 15 c.cm. being withdrawn from a vein in the arm and added to an equal volume of sterile 2 per cent. sodium citrate in normal saline. For cultural purposes the whole blood, the plasma, or the washed red corpuscles may be used. When it is not convenient to put up the cultures on the day on which the blood is withdrawn there are two possible procedures—namely, the separated plasma and red corpuscles may be mixed with equal parts of sterile 50 per cent. glycerin in citrated saline, or the whole blood on withdrawal may be added to an equal volume of the glycerin solution.

Pleural Fluid and Cerebro-spinal Fluid.—In the case of these two fluids a portion is centrifugalized and the sediment microscopically examined to determine the presence or otherwise of

an added infection. If there is no evidence of other infection the fluids may be used in their natural state, or they may be centrifugalized and the sediment taken for the culture, but it has to be remembered that the latter method increases the risk of contamination. Where other organisms are present in the specimen it may be filtered, or the sediment (obtained by centrifugalization) may be emulsified in three volumes of the 50 per cent. glycerin solution and allowed to stand at room temperature, and in the dark, for a period of three or four days, when the culture may be put up.

Sputum.—The sputum is collected in sterile vessels. The most satisfactory method of excluding the many other organisms present is to filter the specimen, either in its natural state or, if it be too glutinous, after emulsification in normal saline, through Berkefeld N or V filters, these forms having been found most suitable. Where a large number of sputa are under observation the strain on the filters is a heavy one, and it becomes necessary to resort to the glycerin method, one part of sputum being emulsified in three parts of the 50 per cent. glycerin solution. The sputum, however, sometimes contains glycerin-resistant organisms, more especially in cases of bronchopneumonia, and this process entirely fails.

Urine.—The sediment obtained by centrifugalization of a catheter specimen is invariably used. It may be filtered, but emulsification in glycerin is usually an adequate method.

Post-mortem Specimens.—Such specimens being, as a rule, grossly contaminated, require prolonged treatment in the glycerin solution. The method is eminently suitable for nervous tissues from fatal cases of polyneuritis, encephalitis, and rabies, in which diseases the specific organism is resistant over long periods. In nephritis and influenza the glycerin method is not, as a rule, available; it is necessary to emulsify the tissues in normal saline and to filter, as in the case of sputa.

Louse Excreta.—The only other material to be considered is the louse excreta, for which the glycerin method gives excellent results, about one milligram of excreta being emulsified in each cubic centimetre of the glycerin solution. The period required to kill off the other organisms present varies from four to eighteen days.

Technique of Culture.

Into each of a series of sterile test tubes (6 by $\frac{1}{2}$) a fragment, about the size of a pea, of sterile rabbit kidney is placed, and alongside it a piece of the nervous tissue, or a drop or two of the filtrate, whichever is under examination. Serum agar, in the proportion of one part of inactivated serum to ten parts of melted agar, at a temperature between 53° C. and 56° C., is added to each tube in an amount just sufficient to cover the tissue. After solidification of the serum agar has taken place there are added to each tube about 15 c.cm. of serum bouillon, also one part in ten, and at a similar temperature. Complete anaërobiosis is obtained by running on to the surface of the medium sterile liquid vaseline at a temperature of about 60° C. and to a depth of half an inch. The completed medium, therefore, consists of a thin layer of serum agar containing a fragment of sterile kidney tissue and the infective material, with a superimposed column of serum bouillon, the whole being sealed with a layer of vaseline.

In the case of blood, pleural fluid, and cerebro-spinal fluid, the cultures are put up in conical Erlenmeyer flasks of a capacity of 50 c.cm. It is sufficient to add 10 c.cm. of the blood or other fluid, the amount of agar being about 15 c.cm. In the case of blood and pleural fluid ordinary agar and bouillon are used; for cerebro-spinal fluid the serum constituent must be added.

It is essential to put up a series of controls involving all the constituents of the medium.

It may be matter of surprise that such an apparently simple method has not been universally employed in the investigation of diseases of unknown etiology, yet in practice it is one of the most difficult and disheartening things in bacteriology.

One of the chief sources of trouble is the *sterile kidney tissue*, which is not always sterile. Quite apart from the risk of contamination in removal of these organs, it sometimes happens that they are infected with *B. pseudotuberculosis*, or with a bacillus of the Gaertner group, even though they appear perfectly healthy. The most careful examination of the abdominal viscera must be made before using the kidneys. Any hyperaemia of the intestinal canal or enlargement of the mesenteric glands should be sufficient to condemn the organs for cultural purposes.

Another danger is to be found in the fact that in the making of the complete culture the tube has to be opened five times; the risk of contamination is obviously not a light one.

The *serum factor* in the medium is important; in all cases it should be inactivated and sterilized, by heating for two hours at 56° C. on three successive days. Fresh serum has an inhibitory effect on the growth of filter-passing organisms, hence the necessity of bleeding the animal whose kidneys are to be employed as thoroughly as possible.

In the case of the organisms of *polyneuritis*, *encephalitis*, and *nephritis*, any serum will do, but it is not so in the case of the organisms of influenza, trench fever, and rabies, the best results being obtained with human and horse serum in the first, with human and rabbit in the second, and, in the case of rabies, with rabbit and dog serum.

The bouillon in the medium is made from beef or mutton. It and the agar have a reaction of + 10 on the Eyre scale.

Characters of the Growth.

It is not necessary to describe the features of the cultures of each organism, inasmuch as the six organisms fall into two groups, the first containing the organisms of *polyneuritis* and *encephalitis*, the latter those of influenza, trench fever, *nephritis*, and rabies.

Taking a culture containing a fragment of tissue from a case of *polyneuritis* first: No obvious change takes place in the medium until the fourth or fifth day, when a faint granular opacity is to be seen in the vicinity of the tissue. The haze deepens, and, extending throughout the serum agar, reaches the surface on the sixth or seventh day, where it appears as minute yellowish semi-translucent colonies. These minute colonies rapidly increase in size, become confluent, and form a yellow-brown continuous layer showing slight elevations on its surface. By this time, that is, about the twelfth day, the serum agar has become definitely opaque and dirty-brown in colour, from the autolysis of the kidney tissue. The lower levels of the serum broth now show slight opacity, but unless the surface growth has been disturbed it is not a marked feature. The subsequent changes occur in the serum bouillon and consist of an increasing turbidity.

The same features are developed in subcultures, but the growth becomes scantier with each succeeding generation. No turbidity of the bouillon is produced in third generation cultures.

The second type of growth may be represented by influenza, and it is conveniently observed in a plasma culture. The growth makes its appearance as early as the third day in the form of a fine granular haze in the vicinity of the rabbit tissue. The haze becomes intensified, spreads throughout the agar, and reaches the surface on the fourth or fifth day. The appearance on the surface is dramatic, and it is confluent almost from the outset. The surface growth has a fine granular appearance, is yellow-white in colour, and is moderately adherent. The growth does not extend into the broth.

In subcultures the growth appears as minute greyish-white colonies in the substance of the serum agar after forty-eight hours' incubation. The colonies unite and convert the medium into a greyish-white opaque mass. By the fifth day the surface growth is established. The organism usually dies out in the fourth generation.

Conditions of Growth.

It will be observed that the conditions of growth are of a specialized character, and they remain so. The ordinary methods of culture familiar to laboratory workers have failed to give any growth. In subculture, however, growth may be obtained in a medium in which the serum element is replaced by glycerin in the proportion of 5 per cent. This modification is specially useful in *polyneuritis*, *encephalitis*, trench fever, and in rabies.

As regards temperature, growth takes place only in the vicinity of 37° C.

The organisms are strict anaerobes both in primary culture and in subcultures.

Microscopic Characters and Distribution in the Body.

Polyneuritis.—The organism presents the appearance of a minute rounded or oval body having a darkly stained rounded spot, surrounded by a narrow faintly stained area. In size it varies between 0.2 μ and 0.5 μ . The organisms are grouped in irregular colonies, and it is sometimes difficult to determine the limits of the individual elements. More usually the individual elements can be made out, when it is found that they are arranged in pairs and in small groups of three to five. By the overlapping of elements bacillary forms may be produced. This organism has been recovered from the blood during the febrile stage, but not from the cerebro-spinal fluid. *Post mortem* it has been recovered from the brain, the spinal cord—in both of which its distribution is very wide—from the sciatic nerve, from the lymphatic glands, but not from the liver or spleen.

Encephalitis.—The organism is about the same size as that of *polyneuritis*, but it is less definitely "globoid" in character. It is more frequently found in pairs, and there is not the same tendency to overlapping. In its growth it is more luxuriant, and makes its appearance at an earlier date, usually on the third day. It is present in the blood during the febrile stage, and in some cases in the cerebro-spinal fluid; in the latter it has been observed microscopically. *Post mortem* it has been obtained from the brain, spinal cord, nerves, and lymphatic glands.

Influenza.—The organism is a minute rounded or oval coccus-like body of very definite outline and of uniform appearance. In size it varies from 0.15 μ to 0.5 μ , the former predominating in young cultures, the latter in old ones. They show a tendency

to occur in pairs, and sometimes in short chains of four elements. Involution takes place very rapidly after the seventh day. This organism is present in the blood at the onset of the disease and in the sputum. In the sputum during the early stages of the disease it is present in large numbers, and there is no difficulty in picking it up microscopically. In pleurisy it is to be seen in the mononuclear leucocytes and in the endothelial cells, as well as in the free state. In arrangement it is essentially diplococcal. In fatal cases its distribution is very wide. It has been seen microscopically, and cultivated from the lungs and related glands, from the heart, liver, spleen, kidney, and in one case from the brain and spinal cord.

Trench Fever.—The virus is a minute oval coccus-like body, occurring in pairs with the opposing surfaces flattened. In size it varies between 0.3 μ and 0.5 μ . In cultures it is arranged in colonies, each pair being surrounded by a halo suggestive of a capsule. The organisms, even in very young cultures, show a considerable variation in the degree with which they take up the stain. The organism has been recovered from the blood as early as two hours after the onset of the illness, and is present throughout the febrile stage. It can be recovered also from the blood during the afebrile stage—that is, in the interval between the relapses—in about 30 per cent. of cases. It can be seen in suitably stained films free in the plasma, in the red corpuscles, and in the mononuclear leucocytes. In louse excreta it is present about twenty-four hours after an infective feed. The earliest period at which an infective feed may take place is about forty hours after onset, but the optimum time is somewhat later, between the third and fourth day of the illness.

Nephritis.—This organism has the appearance of an oval coccus-like body arranged in twos and fours, the long axes being approximated. In size it varies between 0.3 μ and 0.6 μ in diameter. In cultures there is a suggestion of capsulation, but it is not confirmed by staining methods, nor by dark-ground illumination. There is not the same rapid involutionary changes seen in the organism of trench fever. The organism has not, so far, been seen in the blood, though it can be cultivated therefrom during the febrile period, and in some cases during afebrile relapses. In the urine it is present in considerable numbers, especially during the acute exacerbations, arranged in small colonies, lying free, and also in the epithelial cells and mononuclear leucocytes. No fatal cases have been examined.

Rabies.—This is the smallest that has so far been seen. It appears as a minute rounded and undifferentiated body of 0.1 μ to 0.3 μ in diameter. It, too, shows a colonial arrangement, but there is not the suggestion of pairing that is seen in the others. The individual elements do not stain with the same intensity, a fact most probably due to the early involution change which is a feature of the organism. This organism has been obtained only from animals inoculated with infected tissues, and those inoculated with cultures, no other material being available. It has been recovered from the nervous tissues, from the salivary and lymphatic glands, and in some cases from the stomach.

Certain Common Features.

The whole group of filter-passing organisms has certain features in common. It will be convenient to discuss the question of staining first.

Staining Reactions.—The ordinary dilute solutions have but little effect on them, even when applied for long periods. The most suitable and the easiest method of producing satisfactory films is to stain with 1 per cent. methylene blue, or, better, Kühne's methylene blue, after washing the fixed film in ether for two minutes. Giemsa's stain, when applied for some hours, stains the organisms a purplish-blue, but it has the disadvantage of staining the background. In young cultures and in the tissues the organism retains the stain in Gram's method; old cultures show large numbers of Gram-negative elements. They are not acid-fast.

Filtrability.—The salient feature of the organisms is that they pass through bacteriological filters. The filters generally used in this investigation were the Berkefeld N and V and the Massen porcelain filters, and they served their purpose excellently. The preparation of the filters before use is a matter of supreme importance. They are first of all scrubbed with soft soap and water, then immersed in distilled water made slightly alkaline with caustic soda. The water is changed every day for a week, when hot distilled water is run through under pressure. Finally they are boiled in pure distilled water for a period of three hours. In the process of filtration advantage is taken of the negative pressure produced in an autoclave which has been heated up to a temperature of 120° C. and then allowed to cool. By a series of Y tubes connected to the exhaust nozzle by stout rubber tubing several filtration experiments may be carried out at the same time. The degree of permeability of the filter is next determined; this is accomplished by centrifugizing the filtrate and determining in the usual way the size of the organisms which have passed through. The six organisms under review pass through the Berkefeld N and V filters and through most Massen filters. The time required in the case of the Berkefelds is from half an hour to an hour; in the case of the Massens from three to four hours. They can, of course, be cultivated from such filtrates.

Resistance to Heat.—A third feature which is common to the group is the fact that they present a certain degree of resistance to heat. In the tissues and in culture they resist the application of a temperature of 56° C. for thirty minutes. In filtrates, on the other hand, the resistance is not of so marked a character; the longest period the organisms will resist is about twenty

minutes at this temperature. The thermal death-point is between 65° and 70° C.

Resistance to Glycerine.—An important feature, and one which can be put to practical use, as already noted, is the resistance of the organisms to the action of 50 per cent. glycerin. It is not of the same degree in all instances. In the case of polyneuritis, encephalitis, and rabies the organisms remain viable in glycerin for some months. The virus of trench fever resists its action for about three months. On the other hand, the organisms of influenza and nephritis show a feeble resistance, at the most three or four weeks being the viable period.

CONCLUSION.

Such, then, is a brief account of certain filter-passing organisms. In three of the diseases—*influenza*, *trench fever*, *trench nephritis*, and possibly in *encephalitis*—microscopical examination is sufficient for the purposes of clinical diagnosis. In *polyneuritis*, and in most cases of *encephalitis*, blood during the febrile stage is the only means of diagnosis.

As far as can be determined these organisms are definite entities—that is, they are not stages in the life-history of a more complex organism. No evidence could be obtained that they were mere products of the breaking down of larger cocci, as suggested by Rosenow.

In rabies no naturally acquired cases have come under review, nor has there been time to go into the microscopic distribution of the virus in the tissues.

REFERENCES.

- ¹ Flexner and Noguchi, *Journ. Exp. Med.*, 1913. ² Noguchi, *ibid.*, 1911.

ULCERO-MEMBRANOUS LARYNGITIS OF STREPTOCOCCIC ORIGIN.

BY

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During October and November, 1918, a series of cases of laryngitis was admitted to No. 3 Canadian General Hospital, Boulogne, both from the front and base areas, presenting unique features both in their laryngeal pictures and in the almost complete absence of general symptoms. No attempt will be made in this brief report to trace their connexion with any of the influenzal or other streptococcic infections prevalent at that time.

Similar conditions of the larynx were not seen in patients suffering from influenza in hospital at that time, nor has the condition been noted in any reports of necropsies following death from influenzal infection that I have seen.

Colonel Haven Emerson, M.R.C.U.S.A.¹, has classified eleven diseases as respiratory infections among members of the American Expeditionary Force, but, while mentioning acute pharyngitis and tonsillitis, he does not include laryngitis, yet of the cases observed 30 per cent. were seen in American troops. Fifty cases were observed in this hospital.

Symptoms and Course.

Onset.—There is usually a feeling of malaise or vague chilly sensations, headache, and pains in limbs. The temperature reached its maximum in from forty-eight to sixty-two hours, and in ten cases rose to 102°. Twenty-five cases had no elevation of temperature, and in fifteen the temperature varied from 99° to 101°, subsiding within forty-eight hours. Nearly all cases were admitted as walking cases, and complained only of the laryngeal discomfort.

Laryngeal Symptoms.—Cough, hoarseness, or aphonia appeared in from one to five days. Aphonia at some stage of the disease was present in forty cases, ten being hoarse only. Cough was uniformly present and was spasmodic and unproductive. Recovery was complete in from eight to twenty-five days, the average being seventeen days. Functional aphonia followed in eight cases.

Objective Signs.—The fauces might present no changes, but were usually somewhat red and engorged; the epiglottis was not involved. The vocal cords showed ulcero-membranous changes, usually the middle third of the cords was involved, and in this respect the picture resembled that of traumatic laryngitis from gas. The greyish translucent membrane lay symmetrically on both cords, and might involve as much as two-thirds of the upper surface and free edges of the cords. This membrane may be removed with difficulty by grasping with forceps or briskly rubbing with cotton tightly rolled on an appli-

cator. Its removal leaves a raw bleeding surface, which is quickly covered by the re-forming membrane. After the third or fourth day the edges of the membrane are seen to be curling up, or may have been removed, and a shallow ulcerated surface is exposed. The free edges of the cord were invariably involved, and very early showed thickening, and later a "mouse nibbled" appearance.

Oedema of the glottis, supra-glottic or infra-glottic tissues was not observed, and the arytenoids and ventricular bands were not involved in any way. Aphonia, at first due to mechanical disturbance, thickening or ulceration of the cords, may continue as a functional condition.

Treatment.

Treatment consisted of three factors, which we have arranged in the order of their importance: (1) Voice rest; (2) no smoking; (3) inhalations of medicated steam.

Inhalations may relieve the cough, but probably have no influence on the course of the ulcero-membranous process. Abstinence from smoking, especially army cigarettes, probably relieves irritant symptoms. Voice rest is most important and should be complete. It is evident that the greatest possible quietude of the vocal cords is essential, and even whispering was prohibited. Not only is recovery hastened by voice rest, but the patient is not likely to adopt a whispering voice after the cords have healed, which frequently leads to a condition of functional aphonia, as has often been seen after enforced or permitted whispering during the course of laryngeal disease.

The cases of functional aphonia generally yielded quickly, at the first treatment, to *persuasive* suggestion as used in pure functional aphonia cases seen among soldiers, and as opposed to the "*peremptory*" suggestion treatment.

After the disappearance of the membrane the cords were red, injected, and somewhat thickened or roughened on the edges. In a few cases recovery was hastened by the daily application of some astringent to the cords. Partial rest of the voice was enjoined for a convalescent period of a week or ten days. Those patients who might have to use the voice in giving or transmitting commands to troops were detailed to duties not entailing the use of a loud voice for such time as would permit the cords to resume their normal appearance. Too early use of the voice appeared likely to lead to a subacute or chronic laryngitis with permanent damage to the cords.

Bacteriology.

Cultures were taken from the cords by three methods. An attempt was made to adapt an applicator moving in a curved glass tube, as in taking smears from the nasopharynx; this was not successful. Some of the smears were made by indirect laryngoscopy, care being taken to reach the larynx without contaminating the sterile cotton on the applicator. Other smears were made by direct laryngoscopy through a short laryngoscopic tube.

Out of 50 cultures, 35 showed streptococci in almost pure growth; 10 showed the same as the prevailing organism, and the remainder were indefinite. Of the 35 streptococcic growths, 25 were classified as *Streptococcus haemolyticus* and 10 were *Streptococcus viridans*. Further subdivision of these groups was not attempted owing to difficulty in securing carbohydrate media.

REFERENCE.

- ¹ U.S. Army Medical Bulletin, December, 1918.

SHELL WOUND OF LEFT SIDE OF NECK: INJURY OF GREAT VESSELS AND DIVISION OF VAGUS NERVE:

RECOVERY AND RETURN TO FIRING LINE.

BY

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THE following case appears to be worthy of record on account of the nature and severity of the injury, and as an example of recovery after complete division of the vagus. Owing to the additional injuries to the great vessels, necessitating ligature of the left common, external and internal carotid arteries as well as of the internal jugular vein, the fact that the man recovered sufficiently