understanding of them. Kurzrok,¹³ for example, has shown that whereas in cases of artificial menopause by radio-destruction or removal of the ovaries the ovarian hormones are necessarily absent, in cases of spontaneous menopause these hormones may or may not be absent. Manifestly, it is only where they are absent that their therapeutic injection can be expected to do good. Many papers have been published in the last two or three years on the treatment of menopausal symptoms, by oestrin-containing substances in which there is no evidence of uniform success, just as in earlier years there was the same discrepancy of results in the use of the solid and largely inert ovarian extracts. The psychical factor in the menopause is probably as important as in dysmenorrhoea, and must always be taken into account in estimating the success of any treatment, especially treatment by hypodermic injections. But, when all is said and done, it is certain that if only patients whose urine showed an absence of oestrin were treated by oestrin therapy, the results would be more uniform, the treatment would be rationalized, and our understanding of the real physiology of the menopause would be increased.

REFERENCES

- ¹ Crew: Pregnancy Diagnosis Station, Report on First Year's Working, *British Medical Journal*, 1930, i, 662.
- ² Phillips: *Zentralbl. f. Gynäk.*, 1930, liv, 1858.
- ³ Ehrhardt and Mayes: *Ibid.*, 1930, liv, 2949.
- ⁴ Zondek: *Amer. Journ. Obstet. and Gynecol.*, xxiv, 6, 836.
- ⁵ Cook, Dodds, and Kennaway: Quoted in *Lancet*, 1933, i, 373.
- ⁶ Smith, P. E., and Engle: *Proc. Soc. Exp. Biol. and Med.*, 1931-2, xxix, 1225.
- ⁷ Horder: *British Medical Journal*, 1933, i, 859.
- ⁸ Johnstone, Wiesner, and Marshall: *Lancet*, 1932, ii, 569.
- ⁹ Brown, John: *Horae Subsecivae*.
- ¹⁰ Knaus: *Arch. f. Exper. Path. u. Pharmacol.*, 1930, cli, 371.
- ¹¹ Robson and Illingworth: *Quart. Journ. Exper. Physiol.*, xxi, i, 93.
- ¹² Reynolds and Allen: *Amer. Journ. Physiol.*, 1932, cii, 39.
- ¹³ Kurzrok: *Endocrinology*, xvi, 366.

ISOLATION OF ENTERIC BACILLI FROM SEWAGE AND WATER AND ITS BEARING ON EPIDEMIOLOGY*

BY

W. JAMES WILSON, M.D., D.Sc.

PROFESSOR OF HYGIENE AND PUBLIC HEALTH, QUEEN'S UNIVERSITY,
BELFAST

In these islands the control of major outbreaks of typhoid fever had been secured before the specific bacillus had been isolated. Budd (1856) recognized that the living poison of the disease was present in the stools of the patient and of the convalescent, and that the disease was mainly acquired through the consumption of water, milk, and vegetables contaminated with such excreta. The measures which would reduce the occurrence of the disease were those which would secure pure water and clean soil; hence steps were taken to obtain water supplies from clean catchment areas or from pure wells, and to provide drains and sewers to remove filth rapidly from the premises. Abolition of privy middens and cesspools followed. The purification of sewage was studied, and it was found possible to purify it to the extent that secondary decomposition was prevented in the streams into which it was discharged. The control of typhoid was brought about by the water engineer and town surveyor, following the advice of the clinician. The bacteriologist had no part, since many decades elapsed before the typhoid bacillus was cultivated by Gaffky in 1884.

* Read in the Section of Public Health at the Annual Meeting of the British Medical Association, Dublin, 1933.

The measures taken—good housing, good drainage, pure water supplies—were expensive but lasting, and were useful for the eradication of many other diseases. It was probably an advantage that bacteriology developed later than sanitary engineering, as it is possible that active immunization might have been advocated as being cheaper than the great public health schemes which were undertaken. Bacteriology has contributed much to our knowledge of the aetiology and diagnosis of enteric fevers, assisting in their diagnosis and calling attention to the part played by the "carrier" in their dissemination. The findings of many bacteriologists, however, if they had been taken seriously, would have indicated that much of the expense incurred to secure pure water was unnecessary; in fact, from laboratory investigations it was shown that the typhoid bacillus rapidly died out in the water, that 99 per cent. of the bacilli were dead after twenty-four hours, and that probably after three or five days all of them had lost their vitality. According to these results, a water-borne epidemic of typhoid fever was a practical impossibility, but epidemiological inquiries, on the other hand, showed that such outbreaks were by no means infrequent.

During the last five years the introduction of improved methods of isolation of the bacillus has thrown fresh light on the question, and has shown that to keep the disease in check the efforts of the sanitary and water engineer must not be relaxed, but must become more strenuous. Murchison, like Budd, believed that typhoid fever might be transmitted by water or milk infected with the excreta of a patient, but he also believed that the disease might arise *de novo*. He believed that the faeces from healthy persons, and other forms of filth, undergo fermentation outside the body, and that the virus of the disease is produced in this process of putrefaction. This "pythogenic" theory, if translated into terms of modern medicine to mean that the *B. coli* may undergo a ripening process and be converted into the *B. typhosus*, has no experimental evidence in its favour; but if it is taken to mean that the stools of normal individuals frequently contain the typhoid bacillus, and that in consequence the sewage of a moderately large institution or small town is liable to contain them, and that this sewage, if it gains access to a water supply, is liable to cause the disease, then it has, as recent work has shown, much to support it.

DISCOVERY OF TYPHOID BACILLI IN SEWAGE

In this paper I shall refer to the discovery of typhoid and paratyphoid bacilli in sewage and in water, and this when no clinical cases of the disease were recognized in the district. Whether the typhoid bacillus in sewage becomes more virulent it is impossible to say in the present state of our knowledge. Up to 1928 typhoid bacilli had never been found in sewage, but in that year, by means of a new bismuth sulphite medium, I was able to isolate typhoid bacilli from Belfast sewage and cockles, and Adams (1928) isolated them from the droppings of seagulls. In 1929 Gray, and in 1930 Begbie and Gibson, isolated *B. paratyphosus* B from the sewage of Edinburgh. Houston, by means of the bismuth sulphite medium, on two occasions in 1928 and on two occasions in 1929, succeeded in isolating the *B. typhosus* from the River Thames. In 1931 Wilson and Blair reported the almost constant presence of *B. typhosus* during two years in Belfast sewage, and also the isolation of *B. typhosus* and *B. paratyphosus* B from the River Lagan, the water being taken below the point of entrance of the effluent of Lisburn sewage works.

In 1930 Houston, on four occasions out of thirty-four experiments, isolated the typhoid bacillus, but no para-

typhoid bacilli, from 0.1 c.cm. of the crude sewage of London at Barking outfall, but he added:

"Since writing these notes an opportunity occurred of examining the sewage effluent (land treatment) from a town during a paratyphoid B outbreak. The picture here presented was totally and almost unbelievably different, and enabled one to visualize how easily an epidemic might arise from the contamination of a drinking-water supply with even traces of such a liquid. The effluent was proved to contain initially seventy-three paratyphoid bacilli per cubic centimetre, and the infection persisted for months."

In his report for 1931 Houston gives the results of the weekly examination of the sewage of Epping and of the effluent for the presence of paratyphoid bacilli, on one occasion finding as many as 2,880 bacilli in 1 c.cm. of the sewage, and 355 in the effluent. He calculated that on February 18th, 1931, over thirty-three thousand million paratyphoid bacteria were being discharged daily into Cobbins Brook, a tributary of the Lea. In the same report Houston refers to the negative results as to the presence of paratyphoid bacilli in the sewage of five towns, but to the discovery of paratyphoid bacilli in the sewage of Luton, and of *B. aertrycke* (Mutton) in a mixed sample of sewage from Luton, Hertford, and Harlow, and of Welwyn and Bishop's Stortford.

In his report for 1932 Houston records the persistence of paratyphoid bacilli in Epping sewage and effluent, and the discovery of paratyphoid bacilli in Hendon sewage and of typhoid bacilli in Havant sewage. The discovery of typhoid bacilli in Havant effluent was made on January 8th and 29th, 1932, when sixty and forty *B. typhosi* were found in each cubic centimetre. In June, 1930, a party of 118 Portsmouth school children and ten adults had visited the district, and of these, ninety-nine persons drank of the untreated water of a stream into which the effluent of the Havant rural district sewage works discharged. Seventy per cent. of the children who drank of the water were subsequently ill with gastro-intestinal symptoms, and eighteen of them developed enteric fever.

In the annual reports for 1930 and 1931 of the Chief Medical Officer of the Ministry of Health, reference is made to the persistence of paratyphoid bacilli in Epping sewage after the milk-borne epidemic of paratyphoid which occurred in February and March, 1931, and which included 233 cases, all of which were treated in hospital. The paratyphoid bacilli were found not only in the sewage, but also in the sediment deposited on the irrigation area. The 1930 report notes:

"We know from the work of Wilson and Blair that it is possible for the *B. typhosus* and *paratyphosus* which is in sewage under natural conditions to survive for over five weeks (thirty-eight days) when kept bottled at room temperature and exposed to light. These observers found that the organisms seemed in time to disappear from the supernatant liquor and to survive in the sediment. It is suggested that envelopment of the organisms in particles of faeces favours this survival. The experience at Epping affords striking confirmation under field conditions of these experiments."

In the 1931 report is announced the discovery by Dr. Scott of *B. aertrycke* and *B. newport* in Ipswich sewage, and of *B. enteritidis* Gaertner in that of Daventry, of *B. paratyphosus* B in that of Stoke, and of *B. paratyphosus* B not only in the sewage of Wroxall, but in the River Var twenty yards below the outfall in 2 c.cm., and in the river three and a half miles below the outfall in 100 c.cm.

Using the method of Wilson and Blair, Stewart and Ghosal (1932-3) at Calcutta isolated the *B. typhosus* from cesspools, and Fleming (1933) at Hereford isolated *B. typhosus* and *B. paratyphosus* from the sewage of a mental hospital. The recent outbreaks of enteric fever at Ecclefechan and Malton emphasize the danger of access

of even small quantities of sewage matter to a water supply.

The greatest danger is where the water is consumed soon after the contamination—that is, where no storage is available. Where the contamination is continuous, even although most of the bacilli die in the water, a number may survive, and may give rise to sporadic cases of the disease.

ISOLATION OF *B. TYPHOSUS* FROM A WATER SUPPLY

On May 26th, 1932, a sample of water reached my laboratory from a place almost 100 miles distant. It was not examined until twenty-four hours after its collection, and it was not packed in ice. The water had been taken from a dam in the course of a stream which was used for domestic purposes, and probably not infrequently for drinking purposes; it was not a public water supply. This water was found to contain on an average in every 3 c.cm. one typhoid bacillus, two *B. welchii*, and thirty *B. coli*. The *B. typhosus* had the usual cultural characters—motile, formed acid in glucose and mannite media, without action on lactose and saccharose, failed to liquefy gelatin or to form indole. It was agglutinated to full titre by two different anti-typhoid serums. It is sometimes assumed that the chance of typhoid bacilli being present in water would necessitate the presence of large numbers of *B. coli*, but in this instance there were only thirty *B. coli* to every one typhoid.

I may briefly refer to the method by which the typhoid bacilli were isolated. To 100 c.cm. of 3 per cent. nutrient agar were added 10 c.cm. of a stock glucose-phosphate-sulphite-bismuth mixture, and 1 c.cm. of a 1 per cent. solution of brilliant green. Equal volumes (25 c.cm.) of the medium, cooled to 55° C., and water were mixed and poured into Petri dishes. After the medium had set and been incubated, black colonies consisting of typhoid bacilli developed in its substance. These were subcultured on to MacConkey plates, and cultural and serological tests applied.

The bismuth media are very easily prepared when a stock solution is available, and such stock solutions keep for months. The method of preparation which I have found most convenient is as follows. Dissolve 6 grams bismuth-ammonio-citrate scales in 50 c.cm. boiling distilled water, and neutralize by the addition of about 2 c.cm. of 10 per cent. caustic soda. Mix with a solution obtained by boiling 20 grams sodium sulphite anhydrous in 100 c.cm. of water, and then, while the mixture is boiling, add 10 grams of sodium phosphate anhydrous. To the sulphite-bismuth-phosphate mixture, when cool, add a solution of glucose obtained by dissolving 10 grams of commercial glucose in 50 c.cm. of boiling distilled water. For preparation of old standard medium, add to 100 c.cm. of a hot melted 3 per cent. nutrient agar 20 c.cm. of stock mixture, then 1 c.cm. of an 8 per cent. solution of ferrous sulphate crystals in water, and 0.5 c.cm. of a 1 per cent. solution of brilliant green in distilled water; pour into Petri dishes, and when the medium has set inoculate the surface. Isolated colonies of *B. typhosus* and *B. paratyphosus* are black, the typhoid colonies usually appearing within twenty-four hours, and the paratyphoid within forty-eight hours.

SUMMARY

The bacilli of the typhoid-paratyphoid and food-poisoning groups are present in considerable numbers in sewage of institutions and towns.

When cases of enteric fever have occurred in such institutions and towns the numbers of enteric bacilli in the sewage are enormous.

Recent bacteriological work, especially the use of the bismuth-sulphite media, has given an explanation of the risk involved in the consumption of water, shellfish, and vegetables exposed to contamination with sewage.

The presence of large numbers of typhoid and paratyphoid bacilli in sewage in the absence of clinical cases of the disease may be due either (a) to the presence of large numbers of "carriers," or (b) to the sewage works acting as media and incubators for the organisms.

Up to the present time no evidence has been advanced to show that the organisms multiply in sewage, so that in the present state of our knowledge alternative (a) is more probably correct.

BIBLIOGRAPHY

- Adams, B.: *The Medical Officer*, 1928, xxxix, 185.
 Begbie, R. S., and Gibson, H. J.: *British Medical Journal*, 1930, ii, 55.
 Fleming, G. W. T. H.: *Ibid.*, 1933, i, 412.
 Gray, J. D. A.: *Ibid.*, 1929, i, 142.
 Houston, A. C.: Metropolitan Water Board, Twenty-third Annual Report, 1928; Twenty-fourth Annual Report, 1929; Twenty-fifth Annual Report, 1930; Twenty-sixth Annual Report, 1931.
 Stewart, A. D., and Ghosal, S. C.: *Indian Journ. Med. Research*, 1932-3, xx, 341.
 Wilson, W. J., and Blair, E. M. McV.: *Journ. of Hyg.*, 1927, xxvi, 374; *ibid.*, 1931, xxxi, 139.
 Wilson, W. J.: *British Medical Journal*, 1928, i, 1061.
 Annual Report of the Chief Medical Officer, Ministry of Health, 1930 and 1931.

RECENT EXPERIMENTAL WORK ON THE AETIOLOGY OF HODGKIN'S DISEASE*

BY

C. Æ. VAN ROOYEN, M.B.

ASSISTANT, DEPARTMENT OF BACTERIOLOGY, EDINBURGH
UNIVERSITY

Recent work on the aetiology of Hodgkin's disease has served to bring fresh interest to bear on the nature of this condition.

In 1930 L'Esperance reported having isolated the avian tubercle bacillus from cases of Hodgkin's disease and claimed to have reproduced the condition in chickens. These claims have been investigated by Davidson (1933), the workers of the Rose Research on Lymphadenoma (1932), van Rooyen (1933), and others, all of whom failed to reproduce the disease in birds or to isolate the avian tubercle bacillus from human lesions in the disease. In 1930 Twort found that the subcutaneous inoculation of a guinea-pig with a suspension of Hodgkin lymphatic tissue produced after three to four days a swollen inflammatory nodule, in which no bacteria could be demonstrated by the application of ordinary methods. The workers of the Rose Research on Lymphadenoma (1932) next published an extensive series of observations on the pathology of the condition, showing that neither spirochaetes, yeasts, diphtheroids, nor acid-fast organisms were responsible for the causation of the disease.

THE ENCEPHALITIC SYNDROME IN RABBITS

Among the many findings of the last-named workers those of M. H. Gordon were perhaps of the greatest significance. He found that the intracerebral inoculation of rabbits with suitable suspensions of lymphadenomatous tissue was followed in three to four days by highly characteristic changes affecting the central nervous system of the animal. The sequence of events was: initially, slight impairment of locomotion, this being followed by progressive ataxia, muscular incoordination with spasm, spastic paralysis of the hind limbs, and frequently accompanied by incontinence of urine and faeces. The severity of these lesions produced in the rabbit were to some extent dependent on the acuteness of the case from which material was derived. Thus tissue from an acute case of Hodgkin's disease produced, in addition to the foregoing changes, violent convulsive movements, head retraction, opisthotonos, and grinding of the teeth. Animals affected to such an extent inevitably died, but in the less severe type of lesion several recovered completely, and a few passed into a chronic state in which severe muscular atrophy and wasting were much in evidence.

This "encephalitic" syndrome, as described by Gordon (1932), was found to be highly specific in character, and

could not be elicited by the intracerebral inoculation of rabbits with material derived from cases other than those of Hodgkin's disease. This fact has been utilized by the writer (1933), who attempted to use this finding to form the basis of a biological test for the more accurate diagnosis of Hodgkin's disease.

The test has proved of value as an aid to the clinical and post-mortem diagnosis of this disease, particularly when the appearances presented are not entirely typical of the condition. In view of the satisfactory results obtained from the Gordon biological test, it has been proposed that material removed at biopsy and necropsy from suspected cases of Hodgkin's disease should be subjected not only to histological examination, but also to this animal inoculation test. It must be emphasized, however, that a rabbit which exhibits the encephalitic syndrome reveals no cultivable bacterium in either its brain or meninges. Hence, prior to the acceptance of a positive result in any animal, the possibility of bacterial infection being responsible for the effects manifested should first be excluded by appropriate methods of bacteriological examination.

ITS AETIOLOGY

From the theoretical standpoint one or other of the following causes might be responsible for the production of the condition.

- (a) Trauma following damage by mechanical irritation.
- (b) A specific neurotoxin present *in vivo* in lesions of Hodgkin's disease, or produced *in vitro* by changes in the tissue of the lesion after removal from the body.
- (c) The action of a virus specifically associated with the disease and producing an effect on the nervous system of the rabbit.

The complexity of this problem, combined with the incomplete state of our knowledge regarding diseases produced by ultra-microscopic viruses in general, render it extremely difficult to express a dogmatic view regarding the nature of the pathogenic agent under discussion. I propose, therefore, merely to discuss the arguments relative to the above-mentioned hypotheses, guided by the experimental evidence so far advanced.

HISTOLOGICAL CHANGES IN THE RABBIT'S BRAIN

Below is a discussion on trauma and the histological changes produced experimentally following intracerebral inoculation with Hodgkin tissue and with a variety of other substances.

The chosen site of inoculation lies in the occipital lobe of the rabbit, and is situated well behind the motor area of its brain. Histo-pathological changes following the inoculation of Hodgkin tissue into this area were relatively slight in character. They revealed the occurrence of some localized haemorrhage occupying the path of the needle, and surrounded by a zone of lymphocytic infiltration. Occasionally similar infiltration of the meninges overlying the point of inoculation was also noticed, but this finding was too inconstant to be regarded as a regular feature. It may be stated that the slight histo-pathological changes produced in the experimental animal were not sufficient to account for the progressive degree of disability produced in the living animal. On the contrary, it could be shown that very severe damage could be inflicted to the brain tissue of the occipital lobe without in any way reproducing Gordon's encephalitic syndrome.

Such experimental damage may be deliberately produced by the intracerebral introduction into this area of chemical and mechanical irritants, such as turpentine, aleuronat, sodium nucleinate, quinine-urethane, or powdered glass. Likewise, the use of milk, dead bacteria—for example, *B. pyocyaneus*, *B. typhosus*, and *B.*

* Read in the Section of Pathology and Bacteriology at the Annual Meeting of the British Medical Association, Dublin, 1933.