TABLE II.—Showing Results of Investigation by Graham's Method of Four Cases who were not Operated on.

Case.	X-ray Findings.								Deduction.	Remarks.
	Gall-bladder Shadow after Injection.									
	Intensity.				Size.					
D. J.	4 hrs. 1	8 hrs. 3	24 hrs. 3	36 hrs. 1	4 hrs. 4	8 hrs. 4	24 hrs. 3	36 hrs. —	Normal gall bladder	Normal individual with no gall-bladder symptoms.
A. H.	—	—	—	—	—	—	—	—	Pathological gall bladder	Clinically and biochemically—obstructive jaundice.
R. F.	—	—	—	—	—	—	—	—	Pathological gall bladder	Clinically and biochemically—carcinoma head of pancreas.
I. W.	—	—	—	—	—	—	—	—	Pathological gall bladder	Two gall stones shown by ordinary x-ray examination.

deformity of contour of the shadow; or mottling, or central defects (indicating stones in the gall bladder itself, but not causing obstruction, or papilloma).

RESULTS OF INVESTIGATION.

Of the series of cases in which the gall bladders were investigated by Graham's method ten were subsequently operated on and four were not operated on. Our radiological findings and deductions together with the operative or other findings are shown in Tables I and II.

In only one of the ten patients operated on were the surgeon's findings at variance with our radiological deductions. In this case (J. O.) the evidence obtained by an examination of the gall bladder by Graham's method (on two occasions) indicated a pathological condition of the gall bladder. Yet at operation no gross evidence of pathology was obtained. The only abnormality found was a slender adhesion over the common duct; when this had been divided the gall bladder, previously full, was seen to empty. It is doubtful whether this could be sufficient to prevent the dye from passing into and being concentrated in the gall bladder. We trust that a further examination of the gall bladder by Graham's method after the patient has recovered sufficiently from the effects of the operation may throw further light on the question.

CONCLUSIONS AS TO THE VALUE OF GRAHAM'S METHOD.

In estimating the value of this method of radiologically examining the gall bladder three facts must be borne in mind:

1. That only a maximum of 35 to 40 per cent. of gall stones show evidence of their presence by the ordinary method of direct radiography.
2. That a negative finding by the ordinary method does not exclude the presence of gall stones.
3. That "gall stones are incidental and not essential to a cholecystitis which may necessitate surgery."

It is therefore in those cases which by the ordinary direct method of radiography fail to show the presence of gall stones that this new method is of particular value. By no other means short of operation can we ascertain whether this negative finding signifies a normal condition of the gall bladder or not. In other words, it should be used as an adjunct rather than as an alternative to the ordinary method of examination.

A striking feature of the cases shown in Table I is the fact that of all seven cases where a pathological condition of the gall bladder was found at operation, only one case gave the slightest evidence of this before injection, whilst all gave evidence of a pathological condition when examined by Graham's method. The two cases where a definitely normal gall bladder was found at operation both gave definite indication of a normal gall bladder by Graham's method.

The single remaining case where a pathological gall bladder was deduced from the findings by Graham's method, and where at operation no gross evidence of a pathological condition was found, as already stated, needs further investigation.

However, the successes encountered in all the other cases are, in our opinion, a striking testimony to the value of this method as a diagnostic aid in the examination of the gall bladder.

We wish to express our thanks to Dr. Leggate for his valuable assistance, and to those physicians and surgeons of the Liverpool Royal Infirmary who have allowed us to investigate their cases.

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LEPTOSPIRA IN LONDON WATERS.

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THE occurrence in the British Isles of sporadic cases of spirochaetal jaundice, although apparently few in number, shows that under certain conditions the disease may be acquired in this country, and therefore any observations throwing light on the origin of these outbreaks may help to eradicate the infection. The following notes on the presence of a leptospira, morphologically identical with *Leptospira icterohaemorrhagiae*, in London tap water, Thames river water, and water from several other sources in the neighbourhood, are published in the hope that they will draw attention to these interesting organisms, which under certain conditions may assume grave importance from the point of view of public health.

Attention was first directed to this subject by the discovery of leptospira in one of Professor Leiper's cultures at the London School of Hygiene and Tropical Medicine, which had been prepared to show the ciliated embryos of the broad tapeworm, *Dibothriocephalus latus*. The faeces of a patient infected with this worm, and containing large numbers of ova, had been mixed with ordinary London tap water in a Petri dish, then incubated at 30°C. for sixteen days, and subsequently kept at room temperature for about one week. When examined by dark-ground illumination, the water was found to be swarming with leptospira, there being as many as thirty to forty organisms in each field of the microscope. Under the impression that the leptospira might have had a human origin, the faeces of a number of patients in the Seamen's Hospital, and also from other sources, were mixed with tap water in Petri dishes and the mixtures incubated at 25°C. In every case without exception large numbers of leptospira appeared in the water after an incubation period of ten to fourteen days. Subsequently cultures were prepared by mixing faeces with boiled tap water and incubating, when it was found that these organisms never appeared, thus showing that they were present in the water and not in the faeces. Finally, cultures were made by mixing tap water with faeces, both human and animal, which had been sterilized by boiling, and in every case leptospira appeared after incubating at 25°C. for not less than ten days.

From these experiments it was evident that leptospira is present in London tap water, and an attempt was made to determine in what numbers. Although, subsequently, leptospira was found in the slime on the inside of a dripping tap in the laboratory, the direct examination of water under the microscope gave negative results, even

after centrifuging. Therefore a mixture of sterilized water and sterilized faeces was poured into a number of Petri dishes, and subsequently a measured quantity of unboiled tap water added to each; the resulting mixture was incubated at 25° C. It was found that the addition of less than 0.5 c.cm. of tap water produced inconstant results, whilst the addition of this amount, or more, invariably resulted in the development of leptospira. Consequently, at the time of the experiment (March) London water must have contained approximately two leptospira in every cubic centimetre.

Subsequently water from other sources was examined, and by employing the method above described leptospira were found in the following: Tring tap water; a rain-water tank in Major S. S. Flower's conservatory at Tring; and water storage tanks at a school in Buckinghamshire. In addition, these organisms were found by direct examination, in London sewage water, in Thames river water off the landing stages at Gravesend and Tilbury respectively; and in the slime at the outlet of one of the marine tanks in the aquarium of the London Zoological Gardens, which was examined through the kindness of Mr. E. G. Boulenger. Finally, my friend Dr. A. Robertson drew my attention to the presence of leptospira, in considerable numbers, in the slime on the inside of a bowl which received the water draining from the melted ice of an ice-chest in the laboratory.

Culture Experiments.

Up to the present I have not succeeded in isolating a pure culture of this water leptospira, and therefore it has been grown only in the presence of other bacteria. All attempts to grow it on any of the ordinary culture media used for *L. icterohaemorrhagiae* failed owing to the more rapid growth of the contaminating bacilli and cocci, which decomposed the media before there was any sign of leptospira.

By far the simplest and most successful way of growing these organisms is the coprozoic method mentioned in the earlier part of this article. A portion of human faeces about the size of a pea is mixed in a Petri dish with about 20 c.cm. of the water to be examined and the mixture kept in the dark at a temperature of 25° to 30° C. Leptospira can generally be found after ten days, and reach their maximum growth about the twentieth day. They usually persist for a period of four to five weeks, gradually diminishing in number, until they seem to disappear, about two months after the preparation of the culture. This disappearance, however, is only apparent, due possibly to the organisms becoming so scanty that they are not easily detected. In order to produce their reappearance it is only necessary to add a small quantity of fresh faeces and continue the incubation at 25° to 30° C.

With this method the organisms can readily be obtained in considerable numbers, and it affords a convenient way of growing them for demonstration. The cultures should be kept either in the dark or in a shaded corner of the laboratory, for exposure to a bright light is rapidly fatal. It is essential to employ Petri dishes, as the same mixtures of tap water and faeces, when placed in test tubes, or flasks, and incubated at 25° C. never showed any development of leptospira. Moreover, when a culture containing large numbers of these organisms was poured into a test tube the leptospira gradually lost their motility and died off in four or five days. In parenthesis, it may be noted that this property of requiring a large surface and not growing in tubes is in marked contrast with that of *L. icterohaemorrhagiae* cultured on Wenyon-Noguchi medium, in which Dr. Okell has found that although growth takes place readily in tubes, the organism does not grow in Petri dishes or Roux bottles exposing a large surface. Obviously, it is difficult to compare the conditions when one organism is growing in association with many kinds of bacteria and protozoa, whilst the other is growing in pure culture; but the ordinary pathogenic strain of *L. icterohaemorrhagiae* may be grown in a mixture of faeces and water, and in these circumstances requires the same large surface as the water leptospira.

The luxuriant growth of leptospira in the presence of

faeces may be due to the production of sulphuretted hydrogen in the water, for Dobell¹ observed that this substance favoured the growth of other water spirochaetes, and the same effect has been noticed by Zuelzer.² These organisms will also grow in a 0.1 per cent. solution of potassium nitrate in sterile tap water, but only comparatively small numbers have been obtained in this medium. The food supply of organisms growing in such a medium is a little obscure; the leptospira may obtain its nourishment either from traces of organic matter present in the water, or by synthesis of food substances from the inorganic salts. Further experiments are necessary to decide this question, which is of some interest from a theoretical point of view.

Filtration Experiments.

The water leptospira will pass through an L3 Pasteur-Chamberland filter, and may be observed by direct examination of the filtrate. With an L5 filter of the same make leptospira, as such, could not be detected in the filtrate, even after employing the centrifuge. Nevertheless, when the filtrate was poured into a Petri dish and incubated for about ten days at 25° C., leptospira appeared in the liquid. This experiment has been repeated successfully on three separate occasions, and although it is conceivable that leptospira, as such, may have been present in the filtrate in extremely small numbers, so that they were almost impossible to detect by microscopical examination, the more probable hypothesis is that the organisms were present in some other form. The L5 candle is of such fine texture that it is very difficult to believe that an ordinary leptospira could pass through, and the negative results of direct examination of the filtrate support the view that some other stage must have been present.

Animal Experiments.

The results of these few experiments are inconclusive, as up to the present I have not succeeded in isolating a pure culture of the water leptospira, and therefore the animals have been inoculated with a mixture of organisms. In no case were leptospira recovered from the inoculated animals, except in three guinea-pigs which died the day after an intraperitoneal injection of 4 c.cm. of a water culture. The peritoneal fluid of these animals, examined after death, contained actively motile and apparently healthy leptospira. In addition, the following results may be mentioned:

(a) Two guinea-pigs, each inoculated intraperitoneally with 0.5 c.cm. of a suspension of leptospira in water. Both showed febrile symptoms and one died on the tenth day with general darkening of the viscera, but subinoculations into two other guinea-pigs gave negative results. The other individual showed rectal haemorrhages on the twelfth, thirteenth, and fourteenth days, but no other symptoms.

(b) A young guinea-pig was given by mouth 4 c.cm. of a suspension of leptospira in water. Its temperature gradually rose to 104° F. and was accompanied by epistaxis on the ninth day. Two days later it was killed, and showed slight jaundice and two haemorrhagic spots on the lungs. Blood from the heart and liver was inoculated into two other guinea-pigs, both of which showed febrile symptoms, and one of them had rectal haemorrhages on the seventh to the tenth days inclusive.

Owing to the necessity of bringing the investigation to a premature termination, due to the author's sudden departure for China, it has not been possible to continue these experiments, and it is doubtful whether any conclusions can be drawn from the results. Case (b) is the most suggestive, as the symptoms resembled those of a mild case of spirochaetal jaundice, and subinoculations produced fever, in one case accompanied by rectal haemorrhages. The difficulty of recovering leptospira from inoculated guinea-pigs is not sufficiently recognized by authors who have worked with exceptionally virulent strains. Buchanan⁴ only recovered the organism from one guinea-pig out of 44 inoculated with leptospira from proved cases of spirochaetal jaundice, which shows the necessity of long-continued observations before their presence can be definitely excluded. Up to the present very little attention has been paid to the minor clinical symptoms of guinea-pigs

inoculated with leptospira, but in view of the existence of strains with varying degrees of virulence, they would probably repay closer attention.

DISCUSSION OF RESULTS.

Although water leptospira have been recorded from various parts of the world, the only record, so far as I am aware, of their occurrence in this country is by Coles,¹² who found them at Bournemouth. J. G. Thomson and D. Thomson² described "a new variety of *Treponema* found in an old specimen of human faeces," which is almost certainly the organism under discussion, but assumed that it came from the faeces and not from the tap water in which the faeces had been kept. In addition, the interesting observations of Buchanan³ demonstrated the existence of a free living leptospira in the slime on the roofs of coal mines in East Lothian. In the latter case, although the organisms were not discovered in surface water, there is presumptive evidence, in view of their habitat, that they are identical with the water leptospira.

Considering the variety of sources from which this leptospira has been obtained it is evidently a very widely distributed organism, and there is little doubt that further observations will result in its being found in the majority of water supplies; therefore it is of some interest to determine whether it possesses any pathogenic significance. Morphologically it is impossible to distinguish the common water leptospira from *L. icterohaemorrhagiae*, although, perhaps, too much importance should not be attached to such a resemblance in organisms presenting so few morphological characters. On the other hand, there is a biological relationship between them, for agglutination of a leptospira from London tap water was produced by a 1 in 40 dilution of an antileptospira serum, prepared at the Wellcome Physiological Laboratories for a virulent strain of these organisms, isolated by Buchanan. For the supply of this serum I am indebted to my friend Dr. Okell, who had used it in his researches on jaundice in dogs.

If we turn our attention to the history of recent outbreaks of spirochaetal jaundice it is obvious that there must be some very widespread source of infection in nature, for it is impossible to explain the origin of the cases in any other way. Certain outbreaks, notably that in Edinburgh described by Lyon and Buchanan,⁴ have been traced to human carriers of the infection, and once the disease becomes established in man it seems to be contagious, especially among children. The exact mechanism of transmission in such cases is not obvious, but presumably the infection is conveyed by the urine, which often contains living leptospira. No such explanation, however, can be offered for the origin of most outbreaks, which generally seem to be associated with conditions that would favour the saprophytic growth of leptospira.

The best example of this nature is the outbreak in East Lothian, described by Buchanan,³ who was able to show that certain coal mines were the source of infection. In the infected mines typical *L. icterohaemorrhagiae* were obtained from the slime on the roof, in situations that appeared inaccessible to rats. Moreover, the inoculation of this slime into guinea-pigs produced the characteristic symptoms of spirochaetal jaundice, to which the animals succumbed, and abundant leptospiral organisms were present in all their organs.

On the Continent various epidemics have been traced to insanitary bathing establishments, amongst which may be mentioned the outbreak near Magdeburg described by Körner,⁵ which was arrested by closing certain bathing sheds. The Vesle outbreaks in Northern France were traced to the river, and leptospiral organisms were found by Etchegoin⁶ in the river water and mud. During the great war epidemics of the disease occurred in wet ill drained trenches, where conditions, especially in dug-outs, would undoubtedly favour the growth of leptospira.

The disease has also been recorded from among workers in sewers, or, in one or two cases, in persons who had fallen into filthy water.

The best example of such a case is that described by Manson-Bahr⁷ of a seaman who fell into the Thames off Gravesend, and was nearly drowned. Five days later he developed a typical attack of spirochaetal jaundice, and

Wenyon and Brown, who made a laboratory examination of this case, were able to recover the leptospira from guinea-pigs inoculated with the patient's blood. A subsequent examination, recorded in the earlier part of this paper, supports Dr. Manson-Bahr's view that the infection had originated from immersion in contaminated water, for I found the Thames water at Gravesend to contain as many as one leptospira in every 40 fields of the microscope.

The work of Zuelzer⁸ and others on water leptospira shows that non-pathogenic forms may acquire pathogenic properties after prolonged culture, although Buchanan³ is the only observer who has succeeded in finding virulent leptospira living saprophytically in nature.

The occurrence of a similar infection in wild rats, in which the organism can exist as a harmless commensal, has led to the view that they may serve as a reservoir, and in view of the usual habitat of these animals it seems reasonable to assume that they acquire the infection by drinking filthy water containing leptospira. The observations of Foulerton,⁹ Stevenson,¹⁰ and Balfour¹¹ show that a considerable proportion of London rats are infected with the disease, Balfour¹¹ finding leptospira in 22.6 per cent. of 154 brown rats. Since the organisms are passed out in the urine, these animals may serve to spread the infection, but their agency is not essential, as outbreaks have occurred in which their presence was definitely excluded.

Considering all the known facts concerning outbreaks of spirochaetal jaundice, there is reasonable evidence for the assumption that certain strains of water leptospira may acquire pathogenic properties, and therefore constitute a potential source of human infection. The conditions under which they acquire such properties are not clear, but one important factor is the dose, as the results of inoculation experiments with the water leptospira, as well as with blood spirochaetes, have shown that it is necessary to introduce a minimal number of organisms in order to produce infection. For example, Buchanan³ failed to infect guinea-pigs by the inoculation of 1 c.cm. of slime containing leptospira, but with four times this amount produced fatal infections. On the other hand, the occurrence of laboratory infections among experienced bacteriologists, who have worked with this disease in guinea-pigs, suggests that after passage through these animals the virulence for man is considerably increased, and it is possible that similar variations may occur in nature.

The susceptibility of the host is another important factor, for the inoculation of identically the same dose into a number of guinea-pigs produces very different results, ranging from a complete absence of any obvious effects up to the development of a fatal attack of intense haemorrhagic jaundice. Probably the human host is equally variable in susceptibility to infection, and it is a noteworthy feature that most of the recent outbreaks of the disease have been confined to children or young adults.

The presence of leptospira in water in small numbers is obviously harmless, otherwise a considerable proportion of the population would be affected, but under insanitary conditions, and especially when faecal contamination of the water takes place, the organism may increase in numbers and constitute a real source of danger. Under modern sanitary conditions there is little likelihood of the disease assuming any great importance; but under abnormal conditions, such as war, or in countries where sanitation has not yet developed, the infection is likely to continue, as the causative agent is evidently capable of living indefinitely as a saprophyte, either in water or in damp localities containing suitable organic matter. Spirochaetal jaundice may be added, therefore, to the long list of diseases which owe their origin to insanitary conditions and lack of attention to public hygiene.

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