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Enzoötic Jaundice (yellows) is a troublesome disease often brought to the notice of veterinary surgeons. It occurs in all parts of Britain, particularly in hunting districts, while some areas, e.g. London, are comparatively free. More cases are met with in country districts than in towns, especially amongst sporting dogs, though all kinds of dogs are liable to attack. Sporadic cases are met with amongst adult dogs, but most of the reported cases come from foxhound and breeding kennels where many dogs are housed together. Here it particularly affects young dogs as an enzoötic. Wallis Hoare (1915) says: "Except in mild cases the mortality is very high, and we frequently find that an entire litter of puppies may succumb to the infection." This statement agrees with our own experience, for in the outbreaks we have investigated the mortality has reached at least 95 per cent.

We think that our experiments and observations have reached a convenient stage for publication, though many points remain for further investigation.

Symptoms in Naturally Occurring Cases.

The following description of the symptoms seen in naturally infected dogs is based on personal observations made during the outbreaks we have studied. We believe that careful clinical observation shows that the disease can be divided into two main types with every gradation between. We have named these types the hyperacute hæmorrhagic type and the icteric type.

(*a*) The Hyperacute Hæmorrhagic Type.

The chief characteristics are:—

(1) *Sudden Onset.*—A puppy may be seen playing with its fellows, and an hour later may be found huddled up in the darkest corner of its shelter.

(2) *Temperature.*—When taken at the onset, the temperature is high, varying from 104° F. to 106° F. Death is always preceded by a sudden fall of the temperature to 97° F. or 98° F.

(3) *Nervous Symptoms.*—There is intense depression. Shivering is occasionally exhibited. Manipulation of the muscles of the neck or abdomen often causes pain. In a few cases symptoms of meningitis have been observed. Occasionally hiccough is met with.

(4) *Respiratory Symptoms.*—Epistaxis is common. The respirations are increased and may be laboured in severe cases. Bronchial râles are very evident, and may sometimes be heard several yards away from the patient, and there may be a soft repressed cough.

(5) *Alimentary Symptoms.*—Hæmorrhagic herpes of the lips is common, and frequently there is bleeding from the gums. Vomiting occurs in all cases, and is specially marked when water is allowed. Blood may be found in the vomitus. Thirst is always a prominent symptom. In most cases there is frequent passage of soft fæces in small amounts, often blood-stained and accompanied by tenesmus. Hæmorrhage into the alimentary tract has apparently been the cause of death in some cases.

(6) *Urine.*—The urine is passed in small amounts and occasionally contains bile pigment. All samples examined after death have shown an abundance of albumen.

(7) *Other Symptoms.*—The face may be swollen below the eyes. The cervical lymphatic glands are not infrequently enlarged. Petechiæ have been observed on the skin of the flank and under the arms. An intense conjunctivitis may be present; some cases have shown a mild inflammatory condition of the eye with a slight purulent discharge. As a rule icterus is not present. The disease runs a rapid course, death occurring in a few hours after the onset, but occasionally the dog lives for two or three days.

(*b*) The Icteric Type.

(1) *Onset.*—In this type the onset varies. In the rapidly fatal form with jaundice the onset is acute, while in other cases the disease may be so insidious that the dog is not thought to be sick until the development of obvious jaundice.

(2) *Temperature.*—In the majority of cases that show well-marked jaundice the initial temperature is generally 102° F. or 103° F. As icterus becomes apparent on the gums and conjunctivæ, the temperature falls to normal or subnormal. A subnormal temperature is practically constant before death.

(3) *Nervous Symptoms.*—There is a tendency for affected dogs to

hide away and rest and to avoid muscular exercise. There is not the marked depression seen in the hyperacute type, and affected puppies often appear at times quite lively. As the disease develops depression becomes more marked. In uncomplicated cases there is no very obvious muscular tenderness, but when intussusception has occurred the abdominal muscles are held rigid.

(4) *Respiratory Symptoms.*—Generally cough is absent, but bronchial râles can be detected easily in the more acute cases. Epistaxis has been observed, but is uncommon.

(5) *Alimentary Symptoms.*—The mucous membrane of the mouth usually appears healthy and free from mucus. The gums are yellow, the degree of pigmentation depending on the stage of the disease at the time of examination. Vomiting is fairly constant, and blood may be found in the vomitus. Constipation is usually marked, and the fæces when removed by lavage are pale and clay-like. As the disease progresses the motions become loose and blood-streaked. Intussusception of the small intestine can often be diagnosed by palpation of the abdomen, and is frequently the immediate cause of death. Prolapse of the rectum has been observed.

(6) *Urine.*—The urine is usually darkly coloured with bile pigment, and contains much albumen.

(7) *Other Symptoms.*—The superficial lymphatic glands are not often swollen in this type. Pruritus appears to be fairly common. Conjunctivitis is often present in the more acute cases.

The day of the disease upon which jaundice appeared in each of the dogs we have had under observation is shown in Table I.

Associated with outbreaks of enzoötic jaundice, cases occur where dogs show a slight fever and transient gastro-intestinal disturbance. The fact that these cases occur so frequently in association with enzoötic jaundice, in our view suggests strongly that they are mild cases of the same infection, but experimental proof is as yet lacking.

Acute cases may die within two days from the onset; milder cases may survive a week or more. Dogs which recover often develop conjunctivitis or keratitis as sequelæ.

Morbid Anatomy in Naturally Occurring Cases.

As a rule, there is marked jaundice of the walls of the abdomen, the plantar surface of the paws, inside the ears, etc. All degrees, from a faint yellow tinge to a deep chrome colour, have been observed. In the hyperacute case jaundice may be absent. Shreds of blood stained mucus often protrude from the anus. There may be a thick catarrhal discharge from the eyes or nose or both, and a frothy blood-

stained saliva may be discharged from the mouth. Punctate hæmorrhages may be seen under the arms. The subcutaneous fat is icteric. The *peritoneum* may appear normal or may show punctate hæmorrhages, either discrete or confluent, forming red patches on an icteric background. The *peritoneal fluid* may appear normal, or may be bloodstained and increased in amount. All the abdominal blood-vessels are markedly congested. The *stomach* in many cases appears normal, in others it shows petechiæ, which may involve any part, including the pylorus. Occasionally the whole of the stomach is affected. These hæmorrhages are seen from the exterior of the stomach, and can be shown by dissection to be both subserous and submucous. In cases of sudden death, the stomach may contain coagulated blood. The *intestines* usually show varying degrees of inflammation, varying from bright red to a dark blue or almost black colour. Petechiæ may be seen in any part of the small or large intestine, and are submucous and subserous. Large portions or even the whole of the intestine may be thus affected. The small intestine suffers more than the large, and the jejunum and ileum more than the duodenum.

Intussusceptions are very frequent, and occur chiefly in the small intestine ; a prolapse of the rectum was seen in one case. The *liver* is usually enlarged and congested, or on the other hand it may be pale and icteric. The *gall bladder* usually contains much bile. The *kidneys* may be congested or may be pale and icteric. Petechial hæmorrhages may be present in the cortex. The *urinary bladder* usually contains some bile-stained urine. The bladder wall may show punctate hæmorrhages. The *lungs* in all cases show a very characteristic appearance. Bright red, punctate hæmorrhages are found scattered all over the surface, and are present in the substance of the lung. They may be small and discrete, or consist of irregular areas up to an inch in diameter. When such lesions are few in number, they are found mostly near the thin margins of the lung. In some cases a few lesions only can be found, in others the whole lung is involved.

The *pleura, pericardium, and endocardium* may appear normal or show hæmorrhages. Exudates of a serous or sero-fibrinous character may be found in the pleural and pericardial cavities. There may be small hæmorrhages in the *heart muscle*. The *spleen, pancreas, and thyroid* are usually normal in appearance. The *suprarenals*, though often apparently normal to the naked eye, generally contain hæmorrhages in their substance. The *thymus* may appear normal or may contain petechiæ. The *lymphatic glands* of the thorax and abdomen are almost invariably enlarged, dark, and hæmorrhagic ; all glands

may be affected. The *eye* may be normal or inflamed; the sclerotic is usually icteric. Hæmorrhages and jaundice may be seen on the *gums*. Hæmorrhages, mainly interstitial, may occur in the *skeletal muscles*. The *brain and spinal cord* show no abnormalities.

Examination of Dogs with Special Reference to Etiology.*

Dog 1.—The liver ground up in a mortar while in a fresh condition was examined by dark-ground illumination, and several typical motile leptospiræ about 10 μ in length were seen. The ground-up spleen and mesenteric glands were also examined, but no leptospiræ were found. In Giemsa smears no leptospiræ were seen. Examination of sections of liver, lung, and spleen by Dobell's modification of Levaditi's method showed no leptospiræ.

Five guinea-pigs were inoculated intraperitoneally with 1 c.c. of ground-up liver; all died about one month later, but not with the typical picture of leptospiral jaundice. In the ground-up tissues of these guinea-pigs no leptospiræ were found except in the liver of one animal, where a few doubtful forms were seen.

Dog 2.—Leptospiræ were not found in ground-up liver, in Levaditi sections of liver, lung, spleen, mesenteric gland, suprarenal, or in cultures. Three guinea-pigs were each inoculated intraperitoneally with 1 c.c. of ground-up liver. All three died with the typical post-mortem picture of leptospiral jaundice, i.e. jaundice with hæmorrhages in practically all the tissues, including the lungs.

The further history of this strain and its passages is shown in Chart I. What appeared to be a pure culture of the organism was obtained, and this culture produced the typical disease in both dogs and guinea-pigs. A serum obtained by injecting a horse with a known strain of rat leptospira (Rat 2 leptospira) was found to give complete protection against the organism, and neither normal horse serum nor normal dog serum had this power. It will be seen that one dog (Exp. Dog 2), which had been injected with this strain and had developed definite jaundice, recovered after a short illness. A month after inoculation, and about three weeks after the appearance of jaundice, the dog showed no jaundice, and was apparently perfectly well in every particular. It was then killed. In the lungs were present the remains of old hæmorrhages. No abnormalities were present elsewhere. The ground-up liver showed no leptospiræ, but many freely motile and typical leptospiræ were found in the ground-up kidney. The urine was also examined and contained what appeared to be dead leptospiræ. Guinea-pigs inoculated with the ground-up

* For the method of numbering the dogs we have examined, *vide* p. 21.

kidney of this dog failed to contract the disease. The serum of this dog was used in subsequent cross-protection experiments, and is referred to as Dog 2 leptospira serum. This dog serum showed high protective properties against Dog 2 leptospira, Dog 3 leptospira, and Rat 2 leptospira, our standard rat strain. The cross-protection of the Dog 2 leptospira serum and the rat leptospira serum was indeed complete, and both sera were protective against Dog 3 leptospira.

A further experiment is indicated in the chart, where the strain was inoculated into two guinea-pigs without serum and into one guinea-pig which had previously been given the serum of a dog which had recovered from the natural disease. This sample of serum was, unfortunately, very small, and had become contaminated. The control animals died on the fourth day, while the guinea-pig which received the serum of the dog which had recovered remained quite well until the end of the fifth day. It then died apparently from a peritonitis due to a Gaertner-like bacillus. No signs of jaundice or hæmorrhage were found post-mortem, and no leptospiræ could be found by dark-ground examination. The strain from this dog is referred to as Dog 2 leptospira.

Dog 3.—After a prolonged search, several definite leptospiræ were seen by dark-ground examination of the various ground-up organs of this animal. Four guinea-pigs were injected intraperitoneally with 1 c.c. of ground-up liver substance, but none died of the disease. A culture taken directly from the liver of this dog showed, however, a pure growth of leptospiræ. The further history of this strain (Dog 3 leptospira) is shown in Chart II. When given intraperitoneally 1·5 c.c. of culture, two guinea-pigs developed the typical disease. Five days later they died with typical symptoms and post-mortem appearances, and numerous leptospiræ were found in the ground-up livers. A dog was also infected with this culture (Exp. Dog 5), and died nine days later with typical symptoms and post-mortem appearances. The ground-up liver of this dog showed a number of forms somewhat resembling leptospira, but a definite opinion as to their nature could not be expressed. Four guinea-pigs inoculated from the dog's liver did not develop the disease. Thus, even where an apparently pure culture of leptospira was injected into a dog and the typical disease produced, the same difficulty in retrieving the leptospira from the dog was met with as in a number of cases of the natural disease. The chart further shows that guinea-pigs are protected from the leptospira by anti-Rat-2-leptospira horse serum, and by the serum of the dog which had recovered after infection with the leptospira from Dog 2. Normal horse and dog sera gave no such protection.

Levaditi sections of the liver, lung, spleen, kidney, and suprarenal of Dog 3 showed no undoubted leptospiræ, but sections of one of the hæmorrhagic lumbar glands of this dog showed numerous leptospiræ. These appearances are shown in a photograph of a section shown in Fig. 2. The strain from this dog is referred to as Dog 3 leptospira.

Dog 4.—Dark-ground examination of ground-up liver showed a number of doubtful elements resembling leptospira, but a definite decision could not be made. The ground-up spleen, glands, kidneys, suprarenals, and also the heart blood showed no forms resembling leptospira. Leptospira could not be cultivated from the liver, and none were seen in Levaditi preparations of the important organs, or in blood-smears stained with Giemsa stain.

Four guinea-pigs were inoculated with ground-up liver and with a mixture of the organs; none developed the typical disease, though two died thirteen days later without any typical appearances. A dog inoculated with 4 c.c. of a mixture of ground-up liver and spleen remained unaffected.

Further histological work on the materials from this dog is in progress, but up to the present we have been unable to demonstrate the presence of leptospira, though the clinical history and post-mortem appearances were typical of leptospiral jaundice in dogs.

Dog 6.—Dark-ground examination of the ground-up liver of this dog showed a number of forms very closely resembling leptospira, but they did not satisfy our criteria of characteristic motility, and they could not be properly resolved under a high power into primary spirals. We thus registered them as probably, but not certainly, leptospiræ.

Four guinea-pigs were injected intraperitoneally with the ground-up liver of this dog, and all died about three weeks later, two of them showing definite hæmorrhages in the lungs, but no jaundice. No leptospiræ were seen in the organs of these guinea-pigs. A puppy was also injected with 3 c.c. of the ground-up liver of this dog, but it remained unaffected.

No leptospiræ were seen in Levaditi preparations of liver, lung, kidney, spleen, suprarenal, or lymphatic glands. In this dog, as in Dog 4, it has been impossible to isolate leptospira, though the clinical and post-mortem pictures were typical.

Dog 7.—The post-mortem of this puppy was not done in the laboratory. Small pieces of liver, a kidney, and a lung only were received. The lungs contained typical punctate hæmorrhages. The case was regarded by the veterinary practitioner who attended it as one of "distemper with jaundice." A few suspicious forms were seen in the liver and lungs. The specimens were, however, contami-

nated, and the guinea-pigs inoculated with the material died from peritonitis within forty-eight hours. No leptospiræ were seen in Levaditi preparations of the liver, lung, kidney, spleen, and lymphatic gland of this dog.

Dog 8.—An adequate examination of fresh tissues for leptospira was not possible in this case. No leptospiræ were seen in Levaditi preparations of liver, lung, kidney, and other organs. Six guinea-pigs and two dogs were inoculated with ground-up liver and other organs, but no infection took place.

Dog 9.—A very exhaustive examination of the fresh material from this puppy was undertaken, but only in the liver were some suspicious elements seen by the dark-ground method. Four guinea-pigs were inoculated intraperitoneally, each with 1 c.c. of ground-up liver. All four subsequently died with slight but definite jaundice, and otherwise with the typical post-mortem appearances of leptospiral jaundice. Numerous definite leptospiræ were seen in the liver by dark-ground illumination. The guinea-pig's liver was ground up and injected into a further series of guinea-pigs, and produced the typical disease. Four guinea-pigs were protected with anti-Rat 2 leptospira horse serum and inoculated with a test dose of ground-up liver rich in leptospiræ. All remained well. Four control guinea-pigs injected at the same time with the same inoculum died typically in three days.

The anti-horse serum made from a rat s[redacted] therefore gives complete protection against this strain in the same way as it gives protection against Dog 2 and Dog 3 strains. Further experiments with this strain are in progress. The histological examination is not yet completed. The strain seems to be definitely more virulent to guinea-pigs than our standard rat strain, the test dose killing guinea-pigs within three days, whereas our rat strain kills almost constantly in four days.

Dog 10.—This puppy belonged to the same litter as Dog 9, and showed an almost exactly similar clinical and post-mortem picture. No leptospiræ were found, and eight inoculated guinea-pigs remained healthy. Histological examination has not yet been completed.

The Experimental Infection of Dogs with a Leptospira Strain of Rat Origin.

Three puppies were inoculated with a rat strain (Rat 2 leptospira), kindly sent to us by Dr. George Buchanan. The rat had come from a Scottish mine in which cases of leptospira infection had occurred in human beings. This gave rise to the typical clinical

course and post-mortem appearances of the natural disease (*vide* account of Exp. Dogs 1, 15, and 16). The infection was fatal to Exp. Dogs 15 and 16. Exp. Dog 1 was killed *in extremis*. In the case of the latter dog, exhaustive search of the organs was undertaken by dark-ground method, and only after four hours' search could a few undoubted leptospiræ be found. This illustrates the frequent difficulty of finding the leptospiræ even in cases where the disease is produced by the inoculation of definite leptospiræ of rat origin. Four guinea-pigs inoculated from the organs of this dog died typically, and leptospiræ were seen in the liver of all of them. In the case of Exp. Dog 15, fairly numerous leptospiræ were seen in the ground-up liver. A prolonged search through the organs of Exp. Dog 16 failed to reveal any leptospiræ. Death followed in all of the cases within six to seven days after the inoculation, and the third dog was dying six days after inoculation. Jaundice developed in each dog on the fifth day after inoculation.

Symptoms in Experimentally Infected Dogs.

(*a*) *Dog Strain Leptospira Infections.*—(Exp. Dogs 2, 3, 4, 5, 6, 7, 12, 13, 17, and 18, *vide* pp. 24–26.) Puppies inoculated with a strain of leptospira isolated from a dog suffering from jaundice present symptoms indistinguishable from those of the naturally occurring disease. The hyperacute hæmorrhagic type has not been observed in these experimentally infected animals. Symptoms usually develop in the following sequence: pyrexia, constipation, serous or purulent discharges from the eyes and nose, depression, loose clay-coloured stools, often blood-stained, vomiting, jaundice, a rapid fall in temperature to normal or sub-normal, dark-coloured urine, increase in the intensity of jaundice, great depression, intussusception, a weak rapid pulse, death. A fall in temperature at the time of the appearance of jaundice is constant. It is not uncommon to find the inoculated dog apparently perfectly healthy for several days after inoculation, when the above train of symptoms develops suddenly, and death occurs within twenty-four hours of their onset. Intussusception can generally be found by palpation of the abdomen. The superficial lymphatic glands may be enlarged. Pruritus has been observed. One dog (Exp. Dog 2) developed mild symptoms and subsequently recovered.

(*b*) *Rat Strain Leptospira Infections.*—(Exp. Dogs 1, 15, and 16, *vide* pp. 26, 27.) Puppies inoculated with strains of leptospira obtained from rats show symptoms indistinguishable from those presented by puppies inoculated with dog strains or suffering from the natural disease.

*

Incubation Period in Experimentally Infected Dogs.—The period between infection and the development of jaundice in the experimental series of dogs is shown in Table II. The day of death is also indicated in the table.

Morbid Anatomy in Experimentally Infected Dogs.

(*a*) *Dog Strain Leptospira Infections.*—(Exp. Dogs 2, 3, 4, 5, 6, 12, 13, 17, and 18, *vide* pp. 22–26.) A perusal of the individual case histories will show that the general post-mortem lesions agree with those found in the naturally occurring disease. One dog developed little or no jaundice, but presented all the other lesions associated with leptospiral infection (*vide* Exp. Dog 13). The *lung* lesions have been most typical, and have varied from a condition where the whole lung was involved (Exp. Dogs 3 and 4) to that where hæmorrhages could only be found after a careful search (Exp. Dog 13). Punctate hæmorrhages on the serous membranes were constantly present. Enlarged *lymphatic glands* have been seen in all cases, many showing discrete hæmorrhages, others being highly congested and dark blue or almost black. The *mesenteric, sublumbar, hepatic, and bronchial glands* have been almost always affected. The *liver* may be either congested or pale and in an obviously degenerated condition. The *intestines* have always shown hæmorrhages and inflammatory lesions. Intussusception was present in most of the cases.

(*b*) *Rat Strain Leptospira Infections.*—The post-mortem appearances in dogs infected with a strain of leptospira of rat origin have been identical with those dying from the natural disease or as a result of inoculation with a strain of canine origin.

General Conditions of Experimental Work.

The ground-up organs used throughout all our experiments on both the natural and the experimental disease were as fresh as possible, and usually only an hour or two old. Only young puppies which we believed had never had distemper or other grave disease were used for our experiments. The ease with which artefacts may be mistaken for leptospiræ in blood, freshly ground-up organs, and also in stained sections was always in our minds and where we state that definite leptospiræ were present, we mean such forms were present as could not possibly be confused with non-living artefacts. Our criteria were that the leptospiræ should have the usual morphology, should have the characteristic appearance of rotary motility, and should be capable of resolution into primary spirals under a dark-ground system, consisting of a Beck compensated (XII) eyepiece, a 2 m.m. apochro-

matic objective (1·2 NA Beck) and a Beck focusing dark-ground condenser (1·2 NA). Although we have noted less typical elements in some of the dogs, we have refrained from making any deductions as to their nature.

Horse Serum used in Experiment Work.—The anti-leptospira horse serum made from the rat strain (Rat 2 leptospira) has been titrated on many occasions against its homologous strain. The test dose consisted of 1 c.c. of liver rich in leptospiræ ground up in saline and filtered through a little cotton wool. The dose was given intraperitoneally. This somewhat severe test is the method employed by Griffith (1919). No preservative was present in the serum. Chart III shows a typical titration of this serum which has been used throughout our experiments as our anti-leptospira serum of rat origin. Chart IV gives a protocol of some typical experiments performed with the Rat 2 strain.

Dog Experiments.—The following precautions were taken in order to avoid fallacies due to uncontrolled infection of our animals. Dogs infected with rat leptospiræ were never placed in the same room as those infected with the natural disease or passages therefrom. Each dog was isolated from the others, and laboratory attendants always were completely dressed in rubber gowns, "gum boots," and rubber gloves. These were washed down with lysol before and after attending each animal, and mats impregnated with lysol were provided for the cleansing of boots. In some of the more critical experiments the dogs were isolated in separate buildings, and each animal entrusted to a special attendant. The general precautions were such as we thought might reasonably be expected to control the infection of any highly contagious disease.

Guinea-pig Experiments.—We have observed many hundred guinea-pigs infected with leptospira, and have never so far met with a contact infection of any kind. Many normal guinea-pigs have been allowed to intermingle freely with infected stock without contracting the disease. We have, however, taken the greatest care to guard against this possibility so far as this could be done in one guinea-pig house. Our senior laboratory assistant, Mr. Tottem, who has long experience in infectious animal work, has had almost complete care of the animals. They have never been handled with the naked hands. Rubber gloves have always been used, and the hands have been disinfected with lysol between the handling of every group of animals. As is well known, the leptospira is extremely sensitive even to diluted disinfectants. Needless to say, elaborate precautions were taken to ensure the exclusion of rats from the laboratories. From our previous experience of leptospiral infection we had reason to think that these

precautions were sufficient, and throughout our experiments we have had no reason to suspect that any cross-infection had taken place.

Examination of Normal Puppies.

We have examined by the dark-ground method and by guinea-pig inoculation a number of normal puppies, and have up to now found no evidence of the presence of leptospiræ. The sera of a number of dogs which had no history of jaundice gave no protection to guinea-pigs against our rat strain (cf. results with the serum of dogs which had recovered from jaundice).

General Discussion of the Experimental Results.

In three cases out of ten of enzoötic jaundice in dogs we were able to isolate leptospiræ which agreed in every respect with a known rat strain of *Leptospira icterohæmorrhagiæ*. In these cases we believe we have satisfied almost completely the postulates of etiology usually required in ascribing a disease to a given micro-organism. The very characteristic appearance of motile leptospiræ and the peculiar post-mortem picture have made it particularly easy to satisfy ourselves at every passage that we were dealing with a leptospiral infection.

We have been less successful in satisfying Koch's important "postulate" that "the parasitic micro-organisms are to be found in all cases of the disease in question." We have brought forward evidence, which is indeed familiar to most workers in this field of pathology, that the leptospira is often exceedingly difficult to recover from animals (other than guinea-pigs), even when the leptospira inoculated has caused a perfectly typical clinical and post-mortem picture. The same difficulty has been met with in demonstrating the leptospira in cases of Weil's disease in human beings (cf. Stokes, Ryle and Tytler (1917), Dawson, Hume, and Bedson (1917) and others). We might also refer to the difficulty that is so often met with in demonstrating *Treponema pallida* in typical syphilitic material. Leishman (1920), in his Horace Dobell lecture on *Spirochæta duttoni*, refers to the difficulty he met with in demonstrating the spirochætes in infected ticks, although the juices of these produced relapsing fever when inoculated into monkeys. In connection with *Leptospira icterohæmorrhagiæ*, we cannot resist mentioning the hypothesis formulated by Leishman in connection with *S. duttoni* that the micro-organism may pass through a stage in its life-history which is either granular or invisible. We find it difficult to explain our own experimental results and those of other workers on leptospira infection, except upon some such hypothesis.

The consistency of the characteristic clinical and post-mortem appearances, both in naturally and artificially infected dogs, makes it a strong presumption that all the cases were indeed due to leptospiral infection. It may be added that piroplasma could not be found in any of the dogs examined.

Prophylaxis.

It has been frequently shown that anything up to 30 per cent. of the adult rats in many areas both in England and abroad harbour virulent leptospiræ in their kidneys and excrete them in their urine (Stevenson, 1922; Balfour, 1922). The rats themselves may show scarcely any reaction to the parasite, and may be looked upon in the great majority of cases as "healthy carriers" of the disease. In human outbreaks of Weil's disease a close association between man and the rat can nearly always be established, and the large epidemics on the various fronts during the Great War, described by Martin and Pettit, Stokes, Ryle and Tytler, Dawson, Hume and Bedson, and others, were universally referred to the close association between man and infected rats which took place under the conditions of trench warfare.

In the infected kennels we have investigated rats were evidently present in large numbers, and the opportunities of rat contamination gross. In the only two outbreaks where we have had the opportunity of examining rats infesting the kennels, we have demonstrated the presence of virulent leptospira infection in the rats.

We believe that rats are probably the main vectors of the disease, and therefore anti-rat measures appear to be an essential in the eradication of the disease. We are endeavouring to discover by experiment how the natural infection takes place. We think that it is by means of food and bedding. Presumably the food and bedding of the dogs become contaminated with the urine of rat carriers of the disease, and from these materials the puppies become infected. The Japanese workers showed that guinea-pigs may be infected by feeding with infected material, and Courmont and Durand (1917*a*) showed that dogs could similarly be given the disease. It has been stated that leptospira infection may take place through the unbroken skin of guinea-pigs (Inada, etc., 1916*a*). Courmont and Durand (1917*b*) and the present writers have frequently infected guinea-pigs by rubbing a minute amount of material on a scarified area. Infection through bedding may therefore take place. Adult dogs apparently rarely become infected. Two explanations suggest themselves: either the greater acidity of the adult dog's stomach kills any leptospiræ

taken with the food, or adult dogs are immune because they have already passed through an attack ending in recovery.

Human beings are known to carry leptospiræ in their urine for some time during their convalescence from the disease, and rats are known to carry the infection in their kidneys and urine almost indefinitely. It seems very probable that dogs also may carry the infection in their urine for at least a short time after their recovery; that this actually takes place is strongly suggested by the discovery of leptospiræ in the kidneys, and probably in the urine, of Exp. Dog 2 three weeks after recovery from the disease.

In a recently published paper, Buchanan (1924) has shown that pathogenic leptospiræ may multiply in damp places independently of any mammalian host, and indeed he concludes that rats may owe their infection to this source. These very important observations of Buchanan explain the known effect of telluric conditions (Tohyama, 1924) on the spread of the disease in rats and human beings, and emphasise the importance of kennels being kept as dry and hygienic as possible, for not only may leptospiræ excreted by rats be kept alive for prolonged periods in warm damp kennels, but may even multiply. Possibly, too, under certain favourable conditions, pathogenic leptospiræ may occur in damp kennels and their surroundings without any immediate connection with a rodent host.

Passive Immunity.—We have clearly shown that guinea-pigs can be protected against certainly lethal doses of leptospiræ of canine origin by an immune serum, and our field tests go to prove that a similar protection can be obtained in dogs exposed to natural infection. Of twelve pups which were "contacts" in an enzoötic, seven were inoculated with 5 c.c. anti-leptospira serum, and five were left uninoculated. Two of the five uninoculated dogs developed the disease, while the seven inoculated remained healthy. We believe that anti-leptospira serum will play an important part in prophylaxis. When a dog with jaundice is discovered in a kennel, it should be removed at once from the remaining healthy dogs, and the latter should be isolated either in their present kennel or removed to new quarters. Each should be inoculated subcutaneously with 5–10 c.c. anti-leptospira serum, the dose depending on the size and age of the dog. Any suspicious case should be removed at once. We believe that a dose of serum will confer a passive immunity for about three weeks. Further doses may be desirable if there is any likelihood of the dogs having been exposed to infection since the first inoculation. To guard against anaphylactic symptoms developing if too long a period elapses between a first and a second dose of serum, and to confer an immunity of longer duration, we would provisionally

suggest an initial dose of 5-10 c.c. at once, followed by a dose of 1 c.c. a week later, and a further dose of 1 c.c. at the end of a fortnight.

Active Immunity.—From the work of Noguchi (1924) and others on guinea-pigs, it is highly probable that a lasting active immunity to the disease may be obtained by the use of vaccines consisting of dead or attenuated cultures of leptospira. We are now investigating the value of such vaccines in the prophylaxis of the disease in dogs, but as yet we are not in a position to make any definite statement as to their utility.

Treatment.

It is yet too early to state that the administration of anti-leptospira serum will affect cures in all cases of enzoötic jaundice. We have received records of ten instances in which serum was administered to dogs suffering from the disease. In every case jaundice was obvious at the time of the administration of the serum. Nine of these, in the opinion of the veterinary surgeons, were saved by the administration of the serum. We at present have an open mind on the question. We have tried to save dogs which we had artificially infected, but we have hitherto been unsuccessful in saving them after jaundice had developed. It may be that the 1 c.c. of ground-liver tissue with which we artificially infected produced a more severe infection than occurs in the natural disease, and that the serum therefore failed to save the animals.

We have found the following general treatment of advantage: The administration of liquid paraffin by mouth and the withholding of all drastic purgatives. Enemata of normal saline solution till a free action of the bowels is obtained. Liquid diet of milk, water, and raw eggs.

The Relationship of Enzoötic Jaundice to Distemper.

Gray (1915) says: "Indeed, it is permissible to doubt the connection between this enzoötic form of jaundice and distemper until some definite knowledge is forthcoming on the subject. No doubt some practitioners meet with cases of distemper that during the course of the disease develop evidence of jaundice, while hepatic lesions are found on post-mortem. In such instances the jaundice is usually regarded as a complication of the disease, but it must be remembered that a dog may be suffering from an unsuspected affection of the liver prior to the attack of distemper."

It is clear that Gray and other observers have realised that dis-

temper and enzoötic jaundice are two distinct diseases, a conclusion borne out by our findings.

With regard to diagnosis, further observation will probably put the veterinarian in a position to say that at one end of the scale are the typical cases of leptospiral disease, either the form with intense jaundice or the acute form with enteritis, and at the other end are the cases resembling distemper with marked catarrhal symptoms. Instances may, however, occur in which laboratory investigation alone can establish the diagnosis of leptospiral disease as distinct from distemper. Time only can determine the limits in this borderland.

The earlier in the course of the disease one sees the patient, the more difficult it is to make a differentiation between distemper and leptospiral disease. We have provisionally adopted the following points as of help :—

Temperature.—In distemper this is generally persistently high, while in leptospiral disease the early high temperature may drop even to normal or subnormal for several days, followed by a sudden short rise and sudden fall to subnormal.

Depression.—Is greater than in distemper, the patient lying possibly for days and taking no interest in its surroundings.

Hæmorrhagic Herpes and Blood-stained Vomit.—Are very characteristic of leptospiral disease.

Fæces.—In leptospiral disease there can be early diarrhœa, then constipation, followed by the passage of clay-coloured mucoid stools containing much blood, or perhaps only tinted or streaked. How many of the outbreaks of what has been considered intestinal distemper are due to leptospiral disease we cannot tell—probably many. If the serum in use proves in general practice to be as effective in both treatment and prophylaxis as it is at present proving, it will be possible to say that an epidemic that responds to leptospiral serum is not distemper. We shall, of course, take every opportunity available for investigating cases in what we may for the moment regard as the undefined territory between distemper and leptospiral disease.

Note on Weil's Disease in Human Beings.

Some brief mention should be made of the human disease (Spirochætal Jaundice, Weil's Disease) which is due to *Leptospira icterohæmorrhagiæ*. The disease is not common in this country, though occasional outbreaks occur. In Egypt and Japan and many other hot countries it is much more common. During the War many outbreaks took place on both the enemy and the Allied fronts,

The mortality in most European outbreaks has been low, never probably amounting to more than 5 per cent. (Martin and Pettit, 1919). In Japan, on the other hand, the mortality from the disease has in some outbreaks reached 40 per cent. (Inada, Ido, etc., 1916*b*). The possibility of the disease being conveyed from dogs to human beings must not be overlooked, particularly in the light of Krumbein and Frieling's case (1916), *vide Historical Note*. We hope that any further cases of jaundice in human beings associated with cases in dogs will be fully reported by veterinary and medical practitioners meeting with them.

It is advisable that every care should be taken in handling affected dogs, particularly in doing post-mortem examinations. The virus of the disease, though sensitive to disinfectants, is able to strike rapidly through small abrasions of the skin, and possibly even through the intact skin. Powerful disinfection of the skin a few minutes after the application of the virus is said to be ineffective (Inada, 1916*a*). Rubber gloves should therefore be worn when post-mortems are being performed. The handling of infectious guinea-pig material needs particular care. Cases of infection in laboratory workers have occurred in many laboratories where much work on experimental leptospiral infection has been done; and although apparently no fatality due to laboratory infection is on record, the disease in several of the reported cases has been severe.

For further particulars of Weil's disease in human beings, reference may be made to the monograph of Martin and Pettit, and to the papers by Dawson, Hume and Bedson, Stokes, Ryle and Tytler, and Uhlenhuth and Fromme, and to the classical papers by Inada and his co-workers.

Veterinarians who study the classical accounts of Weil's disease in human beings will, we think, be impressed by the close parallel between the disease in human beings and dogs.

Historical Note.—Courmont and Durand (1917*a*) showed that puppies were susceptible to leptospiral infection. They found that after subcutaneous or intraperitoneal inoculation, or ingestion of infectious material, the puppies contracted typical and fatal jaundice. They state that the evolution of the disease in old dogs is much less typical. Monti (1917) was able to confirm Courmont and Durand's observation. Krukenberg (quoted by Krumbein and Frieling, 1916) was unable to infect dogs from the blood of human patients suffering from Weil's disease. Nicolle and Lebailly (1918), also Uhlenhuth and Fromme (1919), were unable to infect dogs intraperitoneally with infectious material. One of the dogs inoculated by Uhlenhuth and Fromme subsequently developed conjunctivitis and keratitis.

Martin and Pettit (1919) state that dogs can be inoculated with the virus of Weil's disease, but that the virus can only pass from dog to dog with difficulty. They note that the liver and kidney emulsions only contain very few leptospiræ. Van de Velde (1923) attempted to infect a dog with 2 c.c. of a virulent guinea-pig emulsion, but succeeded only in producing a transient illness followed by keratitis.

Krumbein and Frieling (1916) report an exceedingly interesting association of human with canine jaundice. Two officers living in the same room as a dog suffering from jaundice developed what appeared clinically to be typical Weil's disease. The authors do not appear to have been able to confirm the nature of the infection by pathological methods.

Uhlenhuth and Fromme (1919), while they were investigating an area where cases of human Weil's disease had occurred, met with a case of jaundice in a dog. They considered the condition in the dog to be typical Weil's disease. Examination of the liver of this animal showed a few spirochætes resembling *Leptospira icterohæmorrhagiæ*. A guinea-pig was injected with ground-up liver and remained apparently normal. Seven and a half weeks later the guinea-pig was killed and showed no abnormal post-mortem appearances. Leptospiræ were recognised with certainty in smears from the kidney, and in the liver there were also forms which were probably leptospiræ. In reviewing their own results and the case of Krumbein and Frieling, they state that icterus is uncommon in dogs, and when it occurs it is usually due to duodenal catarrh. They, however, conclude that their own observations and Krumbein and Frieling's case "speak, if not with certainty, at least with great probability for the occurrence of Weil's disease in dogs under natural conditions."

It should be added that Lukês (1924) and Křiváček (1924) have recently stated that they found spirochætes, to which they give the name *Spirochæta melanogenes canis*, in the tissues of animals which had died of canine typhus (Stuttgart disease).

Summary and Conclusions.

On the basis of clinical and pathological observations and experimental work, the commonly occurring Enzoötic Jaundice of Dogs (Yellows) is attributed to infection with *Leptospira icterohæmorrhagiæ*, which is normally carried by a considerable percentage of rats in this country.

The following are the lines upon which we suggest combating the disease :—

Prophylaxis.—(*a*) Protection of kennels and food from rats. Disinfection of infected kennels. Dry and hygienic condition of floors and bedding.

(*b*) The elimination of "recovered cases" from kennels until it has been proved that they are not excreting leptospiræ in their urine.

(*c*) Complete but temporary protection against the disease is apparently given by passive immunity with a potent anti-leptospira serum.

(*d*) Active immunity will probably be given by suitable doses of a vaccine made from dead or attenuated leptospiræ.

Treatment.—The results obtained in the treatment of the disease with potent anti-leptospira serum have been encouraging.

We owe our thanks to Drs. C. M. Wenyon and R. A. O'Brien, who have given us much help and advice; to Dr. Stevenson, whose opinion we have sought on our histological material; and to Mr. Barnard, of the National Institute for Medical Research, who has kindly made the microphotographs of the leptospira which illustrate this paper. Our thanks are also due to several veterinary surgeons who have supplied us with material for investigation, in particular to Professor Hobday.

Case Index.

The naturally occurring cases of the disease are numbered throughout this paper as follows: Dog 1, *Dog* 2, *Dog* 3, *etc. The experimentally infected dogs inoculated with material of either canine or rat origin are numbered: Exp. Dog* 1, *Exp. Dog* 2, *etc.*

Dogs suffering from the natural disease.

Dog 1.—This animal was depressed and "off its food" for two days. It was at this time markedly icteric. The temperature, 101·4° F., fell gradually until death, which took place on the fifth day.

Post-mortem.—The pup died on its way to the laboratory. Post-mortem examination half an hour after death. Marked general jaundice. The peritoneal cavity contained a small quantity of blood-stained fluid. The *intestines* showed large areas of inflammation, especially in the jejunum and ileum, the contents being mixed with blood. Intussusception of the jejunum present. *Liver* congested and gall bladder full of bile; the duodenum in close proximity to the gall bladder was bile-stained. *Kidneys, spleen, suprarenals, and pancreas*

appeared normal. Ali *mesenteric glands* enlarged, congested, and showed small punctate hæmorrhages. Most of the lymphatic glands were in a similar condition, specially the sublumbar and mediastinal group. *Lungs* showed a typical "butterfly wing" appearance.

Ground-up liver showed sparse but unmistakable leptospiræ. Three guinea-pigs inoculated with ground-up liver did not develop jaundice; they died over a month after inoculation, and no leptospiræ were seen in any of their tissues.

Levaditi sections of the various tissues showed no leptospiræ.

Dog 2.—When first examined this pup showed great depression, vomiting, conjunctivitis, and passed liquid stools containing specks of blood and mucus. Temperature 102·5° F. Jaundice on the fourth day. On the seventh day an intussusception was palpable. Death on the eighth day.

Post-mortem.—Post-mortem two hours after death. Lesions practically identical with those of Dog 1, except that lungs were not so typical. A few fresh petechiæ were seen along the margins of the lungs. Smears made from ground-up liver, spleen, kidneys, and glands showed no leptospiræ. Inoculated guinea-pigs developed typical leptospiral infection. Levaditi-stained sections of mesenteric glands showed typical leptospiræ.

Dog 3.—This puppy became suddenly ill with slight rigors, great depression, anorexia, thirst, constipation, vomiting, with some blood in the mucous vomitus, and severe photophobia. Conjunctivitis on the third day. Temperature gradually fell from 103·5° F. on the days of onset to 101·2° F. on the fourth day. Jaundice well marked on this day, and increased in intensity to the seventh day. Clay-coloured and blood-stained fæces passed. On the seventh day intussusception was palpated. The puppy collapsed on the seventh day, the temperature then being 97° F.

Post-mortem.—Autopsy carried out immediately after death; there was marked general icterus, hæmorrhage into both *lungs* giving a "butterfly wing" appearance. Hæmorrhages into the *thymus gland, pleura, pericardium.* Slight effusion of serous fluid into *mediastinum. Thoracic lymphatic glands* enlarged and very congested. *Liver* enlarged and pale. *Stomach* appeared normal externally, but when opened distinct submucous hæmorrhages seen and contents blood-stained. *Pylorus,* submucous hæmorrhages. *Jejunum and ileum* showed many submucous hæmorrhages, and an intussusception was found at the first part of the ileum. *Large intestine* showed several discrete hæmorrhages near the cæcum. *Abdominal lymphatic glands* markedly congested and hæmorrhagic. There were a few discrete hæmorrhages in the *peritoneum. Spleen*

and pancreas appeared normal. *Kidneys* enlarged and slightly congested, and there were several small hæmorrhages into the cortex. Bladder contained highly albuminous urine. The ground-up liver showed several undoubted leptospiræ. Cultures of liver positive for leptospira. Inoculated guinea-pigs died with typical lesions.

A dog (Exp. Dog 5) was also inoculated.

Dog 4.—This was an adult dog about four years old and had been ill several days. It had vomited freely, and had become jaundiced two days before we examined it. Jaundice was general, pulse 84 and weak, temperature 98·6° F. The dog lay absolutely still, paying no attention to its surroundings. The bowels did not move, and it died suddenly.

Post-mortem.—Post-mortem immediately after death. Jaundice intense and general. Lesions in *lungs* and *lymphatic glands* were typical. *Stomach* and *intestines* appeared normal. Leptospiræ could not be isolated from this case.

Dog 6.—This pup showed depression, vomiting, thirst, and intense conjunctivitis when first seen. Temperature 102·4° F. Slight jaundice was noted on the fourth day, the day of its death.

Post-mortem.—Post-mortem immediately after death. Slight jaundice was present and catarrhal discharge from nose and eyes. The picture was similar to that seen in Dog 1, except that fewer hæmorrhages were present. *Lymphatic glands* congested but not hæmorrhagic, and lungs typical.

Ground-up liver showed definite leptospiræ. Inoculated guinea-pigs died three weeks later with hæmorrhagic symptoms but no jaundice, and no leptospiræ were seen in their tissues. One dog (Exp. Dog 12) was inoculated with ground-up liver.

Dog 7.—Pieces of liver, kidneys, and lungs only were received. The material was stated to have come from a beagle puppy aged three months which died of "Distemper with Jaundice."

Lungs typical. Leptospiræ could not be isolated.

Dog 8.—This pup showed great depression. Manipulation of the neck and abdomen caused pain. Temperature 104·2° F. Pulse rapid and weak. Constipation. Slight conjunctivitis. Bronchial râles were well marked and there was a soft cough. Complete anorexia. Second day, the temperature was 102·2° F., and hæmorrhagic herpes of the lips appeared. Cervical glands swollen. Jaundice marked. Temperature sank rapidly during the next six hours, gums bled, and blood was passed per rectum.

Post-mortem.—Autopsy within two hours of death. Jaundice intense and general. *Liver* enlarged and congested. *Spleen* was enlarged. *Lymphatic glands* much enlarged, highly congested, and

hæmorrhagic. *Kidneys* pale. Hæmorrhages in *lungs, pleura,* and *peritoneum. Mediastinum* contained a sero-fibrinous exudate. There were tracts of acute congestion in both *small* and *large intestine.* No leptospiræ were found in this case.

Two dogs (Exp. Dogs 13 and 14) were also inoculated.

Dog 9.—This pup died after a few hours' illness. No jaundice observed.

Post-mortem.—Hæmorrhages in the *lungs, pleura, and peritoneum. Lymphatic glands* congested and enlarged. *Spleen* appeared normal. *Kidneys* apparently enlarged. Petechiæ present in *stomach* and *small intestine.* No leptospiræ were found on direct examination of tissues. Inoculated guinea-pigs developed jaundice and died. Typical lesions were found and leptospiræ were isolated. Exp. Dog 17 was inoculated from this strain.

Dog 10.—History similar to that of Dog 9.

Post-mortem.—No jaundice. *Liver* irregularly mottled. A few hæmorrhages present in *lungs* and *pleura.* Abdominal cavity contained about 50 c.c. of a blood-stained fluid. *Large intestine* congested throughout. All *lymphatic glands* were enlarged and congested. No leptospiræ demonstrable on direct examination. Inoculated guinea-pigs remained healthy.

Dogs experimentally infected with Leptospira of Canine Origin.

Exp. Dog 2.—(Passage Dog 2 through guinea-pig to Exp. Dog 2). Inoculated September 25, 1924, intraperitoneally with 2 c.c. ground-up guinea-pig liver. Typical jaundice, October 10, 1924. Stools blood-stained. Temperature dropped to 98·6° F. from 103° F., and great depression was noted. Jaundice disappeared and pup gradually recovered. It was destroyed for examination on October 27, 1924.

Post-mortem.—No jaundice remained. In the *lungs* were several old punctate hæmorrhages. Other tissues normal. No leptospiræ in *liver,* but in *kidney* there were many definite leptospiræ, and the urine apparently contained dead forms. Guinea-pigs inoculated from kidney remained normal.

Exp. Dog 3.—(Passage Dog 2 through guinea-pig to Exp. Dog 3). This pup was inoculated as Exp. Dog 2. It developed jaundice, discharge from eyes and nose, constipation, and later passed clay-coloured stools showing streaks of blood and mucus. Temperature dropped from 102° F. to 100·8° F. before death. Great depression and wasting were noted. It was destroyed when *in extremis* on October 4, 1924.

Post-mortem.—Icterus intense and general. *Lungs* typical.

Lymphatic glands enlarged and hæmorrhagic. *Liver* enlarged and pale. Hæmorrhages in *intestines*. An intussusception in *ileum*. No leptospiræ could be isolated from this case.

Exp. Dog 4.—(Passage Dog 2 through guinea-pig to culture to Exp. Dog 4). Inoculated September 30, 1924, intraperitoneally with 1 c.c. culture grown from liver of infected guinea-pig. Pup appeared well until October 3, 1924, when the temperature rose to 101·8° F. Anorexia, soft cough, catarrhal discharge from nose and eyes, and constipation. On October 4, 1924, the temperature began to fall and slight jaundice was evident. Jaundice became intense: clay-coloured stools were passed. Depression marked and pain on manipulation. Death occurred on October 8, 1924.

Post-mortem.—Jaundice intense and general. *Lungs* typical. *Intestines* congested in parts. *Lymphatic glands* enlarged and hæmorrhagic. Leptospiræ found in the ground-up lungs and kidneys, but not in liver. Guinea-pigs inoculated with ground-up liver died typically, and leptospiræ found in their livers.

Exp. Dog 5.—(Passage Dog 3 through culture to Exp. Dog 5). Inoculated September 30, 1924, intraperitoneally with 1 c.c. culture grown directly from liver of Dog 3. Pup developed the typical disease, temperature falling to 98·4° F. before death on October 9, 1924.

Post-mortem.—Jaundice intense and general. *Lungs* typical. *Lymphatic glands* hæmorrhagic. *Liver* enlarged and congested. *Intestines* congested. Questionable forms resembling leptospiræ only seen.

Exp. Dog 6.—(Passage Dog 4 direct to Exp. Dog 6). Inoculated October 1, 1924, intraperitoneally with 4 c.c. ground-up liver and spleen of Dog 4. This dog, aged about nine months, has never shown any signs of disease and is still healthy. (December 12, 1924).

Exp. Dog 7.—(Passage Dog 2 through guinea-pig, through Exp. Dog 3 to Exp. Dog 7). Inoculated October 4, 1924, 4 c.c. ground-up liver Exp. Dog 3 intraperitoneally. Pup perfectly healthy till October 16, 1924, when blood was seen in the stools. Jaundice developed the same evening and temperature began to fall. Death on October 17, 1924, the temperature being 99·6° F.

Post-mortem.—Jaundice intense and general. *Liver* pale. *Spleen* congested and enlarged. *Large and small intestines* showed numerous punctate subserous hæmorrhages. Two intussusceptions were present. *Lungs* typical. Leptospiræ could not be demonstrated in this dog after death by direct methods, but a guinea-pig inoculated with ground-up liver died typically with numerous leptospiræ in the liver.

Exp. Dog 12.—(Passage Dog 6 direct to Exp. Dog 12). Inocu-

lated intraperitoneally with 3 c.c. ground-up liver Dog 6, October 9, 1924. This pup did not develop clinical symptoms of disease, and when destroyed on October 31, 1924, was apparently healthy. The post-mortem examination showed no evidence of disease.

Exp. Dog 13.—(Passage Dog 8 direct to Exp. Dog 13). Inoculated 2 c.c. mixture of ground-up organs of Dog 8 intraperitoneally October 29, 1924. Pup remained healthy till November 4, 1924, when it refused food, developed a thin watery discharge from nose and eyes, and suffered from diarrhœa with blood in the motions. Its temperature fell from 100·8° F. to 95·2° F. in twenty-four hours. No jaundice was evident. Death occurred on October 5, 1924.

Post-mortem.—No jaundice. *Liver* congested. Gall-bladder full of dark bile. The whole *intestine* markedly inflamed. Two intussusceptions were present. Mesenteric, sublumbar, and bronchial *lymphatic glands* enlarged and congested. *Lungs* showed six small fresh hæmorrhagic spots and four larger discrete similar areas near their borders. No leptospiræ were found. Guinea-pigs were not inoculated.

Exp. Dog 17.—(Passage Dog 9 through guinea-pig to Exp. Dog 17). Inoculated 0·25 c.c. ground-up guinea-pig liver intraperitoneally October 31, 1924. This pup appeared perfectly healthy till the evening of November 14, 1924, when its temperature was 102·6° F., and a thin discharge was observed from its nose and eyes. Food was refused. On November 15, 1924, marked jaundice developed, and the temperature was 100·2° F. The pup was killed.

Post-mortem.—Jaundice intense and general. *Lungs* typical. A sero-fibrinous exudate was present in the *mediastinum*. *Liver* congested and gall-bladder full. There were areas of congestion on *stomach* and *intestines*. No intussusceptions were present. Mesenteric *lymphatic glands* much enlarged and hæmorrhagic. Leptospiræ seen in the blood and ground-up liver. Guinea-pigs inoculated intraperitoneally with blood and with ground-up liver died from a typical leptospiral infection, and leptospiræ were found in their various organs.

Exp. Dog 18.—(Passage Dog 9 through guinea-pig to Exp. Dog 18). Inoculation, history, post-mortem, and results identical with those of Exp. Dog 17.

Dogs experimentally infected with Leptospira of Rat Origin.

Exp. Dog 1.—Inoculated intraperitoneally with 1 c.c. of ground-up liver from a guinea-pig dead of leptospira infection (Rat 2 leptospira) September 18, 1924.

The first signs of illness were noted on September 23, 1924, when icterus appeared in the usual situations. Constipation was marked, and this later gave place to diarrhœa, the stools being clay-coloured and streaked with blood. Great depression was noted and pain evinced on handling. There was a discharge from the eyes and nose. The pup was killed by chloroform on September 24, 1924. Temperature taken daily showed a rise on September 22, 1924 to 102·2° F. and a rapid fall before death to 99·6 F.

Post-mortem.—Jaundice intense and general. *Lungs* typical. *Intestines* showed punctate hæmorrhages, especially in the *duodenum* and *rectum*. Leptospiræ found with difficulty in the ground-up liver.

Exp. Dog 15.—Inoculated with 2 c.c. as Exp. Dog 1 on November 4, 1924. No signs of illness were observed until November 9, 1924, when slight jaundice developed. The course was then similar to that of Exp. Dog 1.

Post-mortem.—Jaundice intense and general. *Lungs* typical. The *peritoneal cavity* contained about 5 c.c. of hæmorrhagic fluid. Two intussusceptions were noted. *Small intestine* showed punctate hæmorrhages. *Lymphatic glands* enlarged and congested, many showing hæmorrhages in their substance. The *mediastinum* contained a sero-fibrinous exudate. Leptospiræ present in liver.

Exp. Dog 16.—Inoculation as in Exp. Dog 15 on November 4, 1924. On November 10, 1924, food was refused and blood passed in a clay-coloured semi-solid stool. At the same time jaundice developed. Depression extreme. The evening temperature was 95° F. Dog found dead on November 10, 1924.

Post-mortem.—Jaundice intense and general. *Lungs* typical. Extensive hæmorrhages into the *abdominal wall*. *Intestines* highly inflamed. Intussusception of jejunum. *Lymphatic glands* enlarged and hæmorrhagic. *Mediastinum* contained a sero-fibrinous exudate. Leptospiræ were seen in the *liver*.

FIG. 1.—Ground-up Liver of Guinea-pig showing Living Leptospira from a Case of Canine Jaundice (Dog 9). Dark-ground illumination. Magn. × 1300.

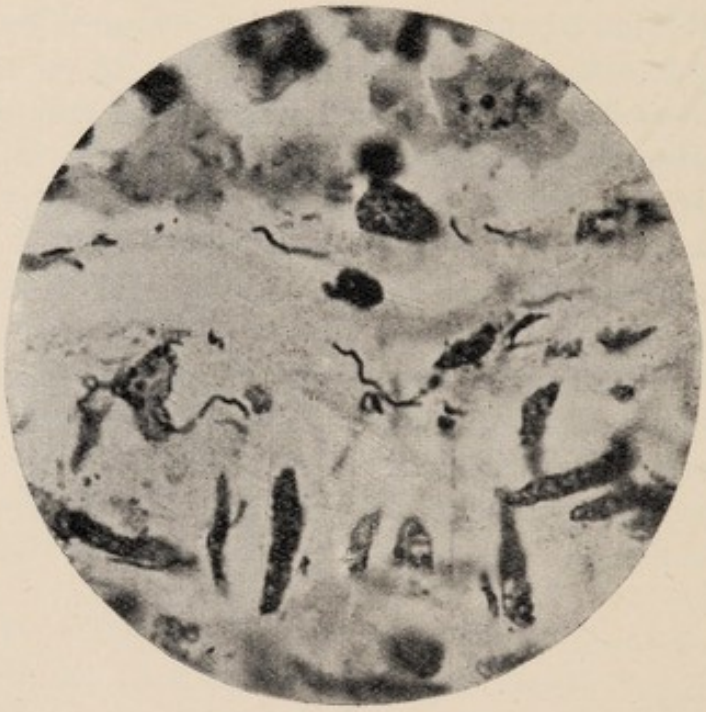
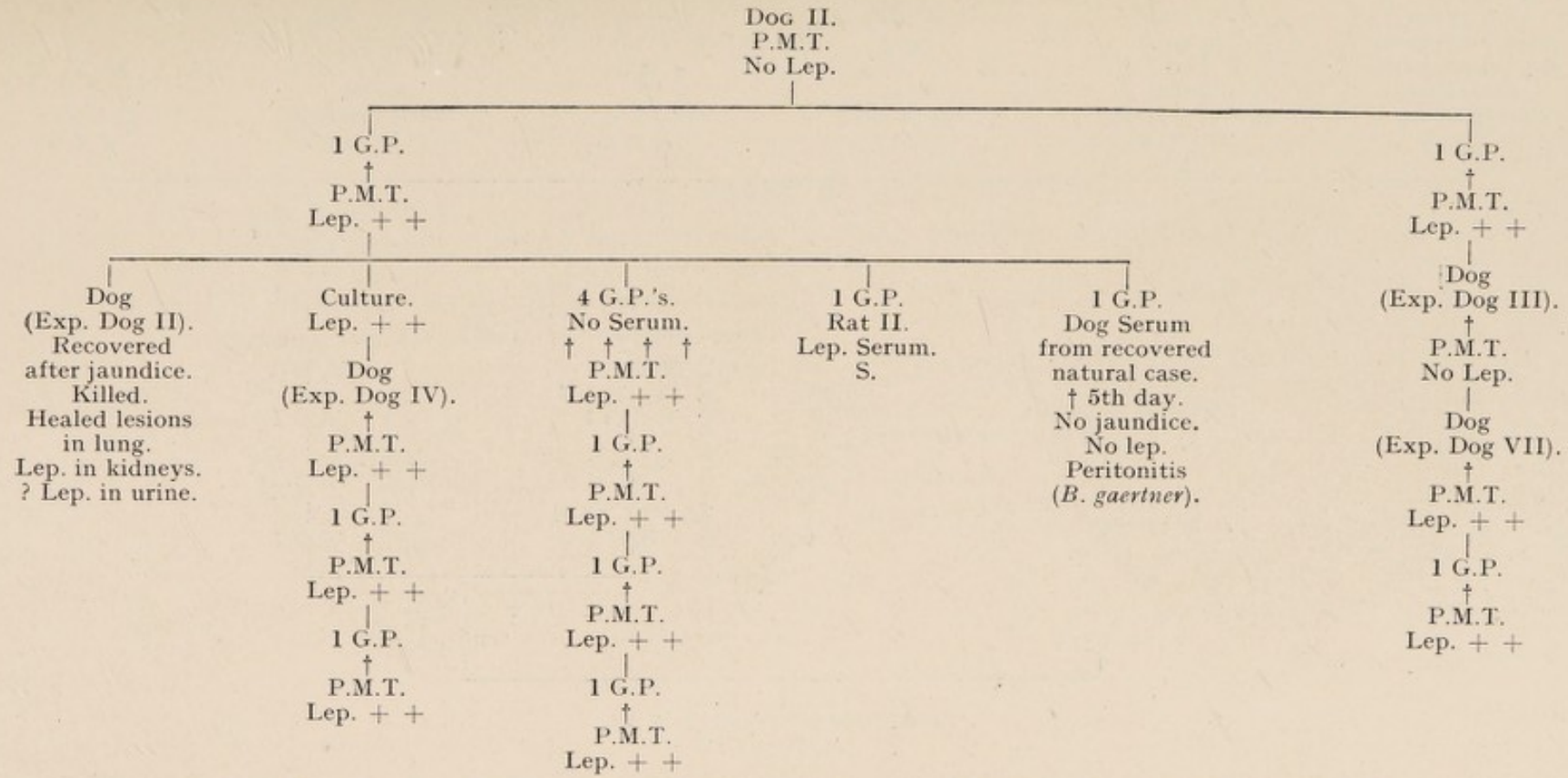


FIG. 2.—Section of Sublumbar Lymphatic Gland of a Dog affected with Canine Jaundice showing Leptospiræ (Dog 3). Dobell's modification of Levaditi's method. Magn. × 1300.

CHART I.

PASSAGE TREE OF DOG II LEPTOSPIRA.

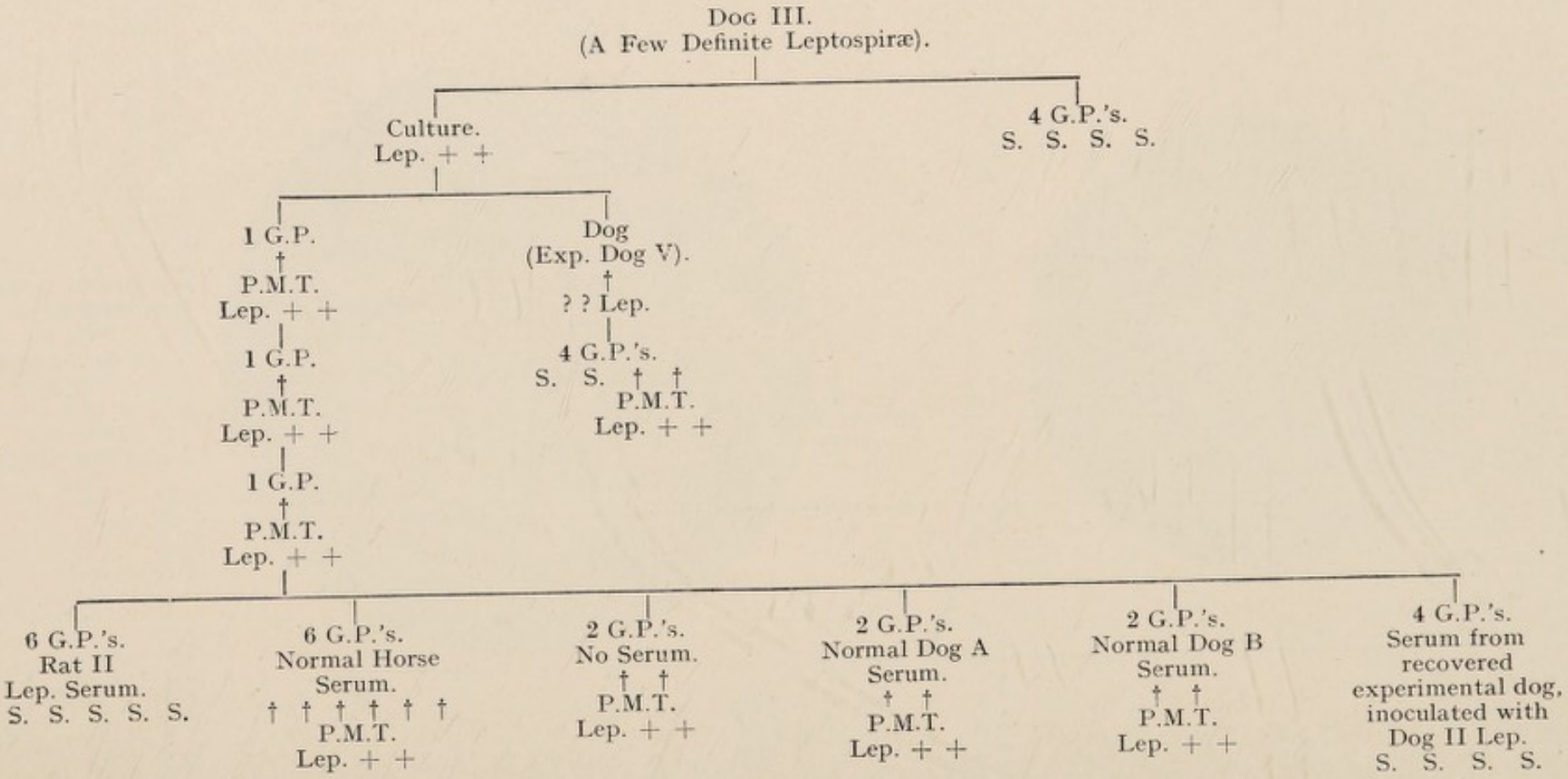


P.M.T. = Typical post-mortem appearances.
Lep. + + = Many leptospiræ present.

† = Died. G.P. = Guinea-pig.
S. = Animal survived with no symptoms.

CHART II.

PASSAGE TREE OF DOG III LEPTOSPIRA.



P.M.T. = Typical post-mortem appearances.
Lep. + + = Many leptospiræ present.

† = Died. G.P. = Guinea-pig.
S. = Animal survived with no symptoms.

CHART III

TITRATION OF SERUM OBTAINED FROM A HORSE IMMUNISED WITH RAT II LEPTOSPIRA STRAIN.

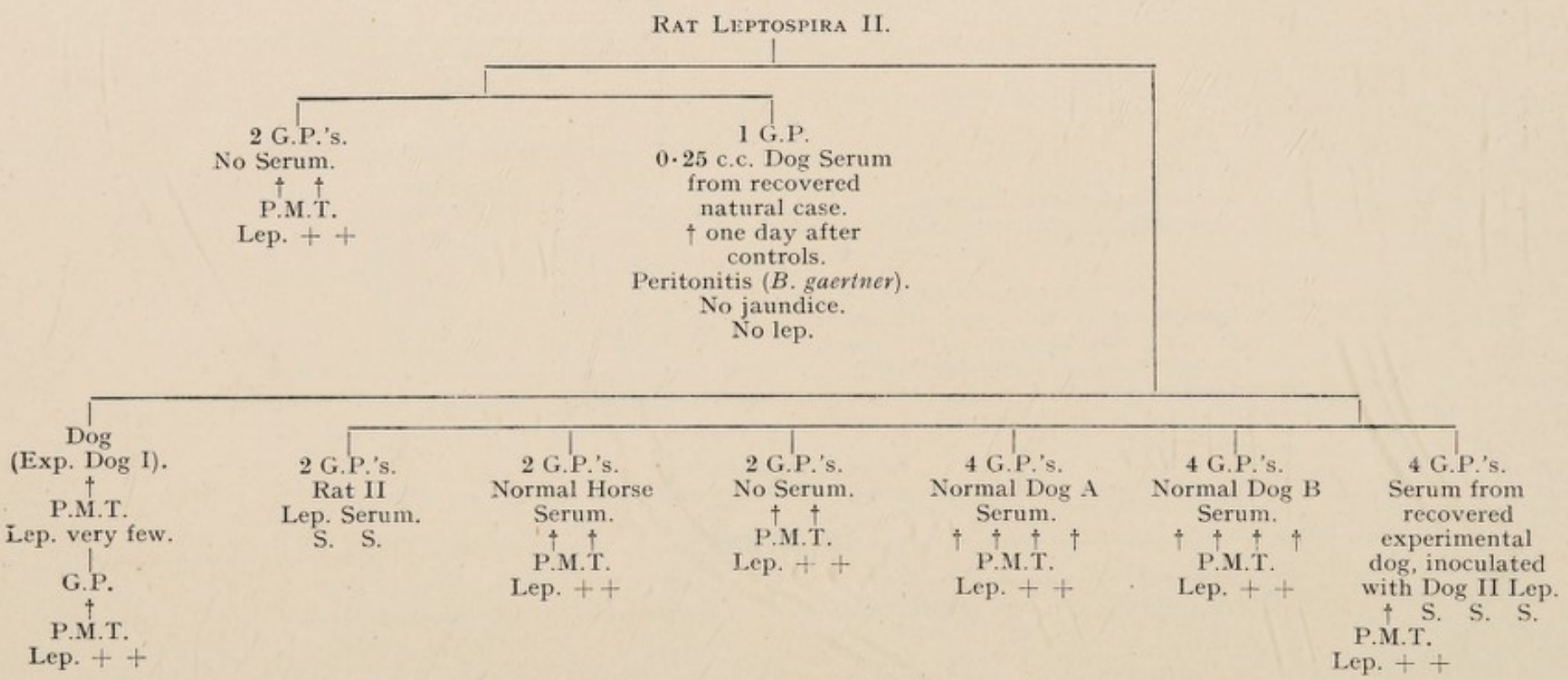
Amount of Serum	Guinea-pigs Injected	
1 c.c.	S	S
0·5 c.c.	S	S
0·4 c.c.	S	S
0·2 c.c.	S	†
0·1 c.c.	†	†
0·05 c.c.	†	†
0·025 c.c.	†	†

Two guinea-pigs on each dose of serum. Test dose of virus 1 c.c. of ground-up liver rich in leptospirae, injected intraperitoneally.

S = Survived.
† = Died with typical signs of infection with leptospira.

CHART IV.

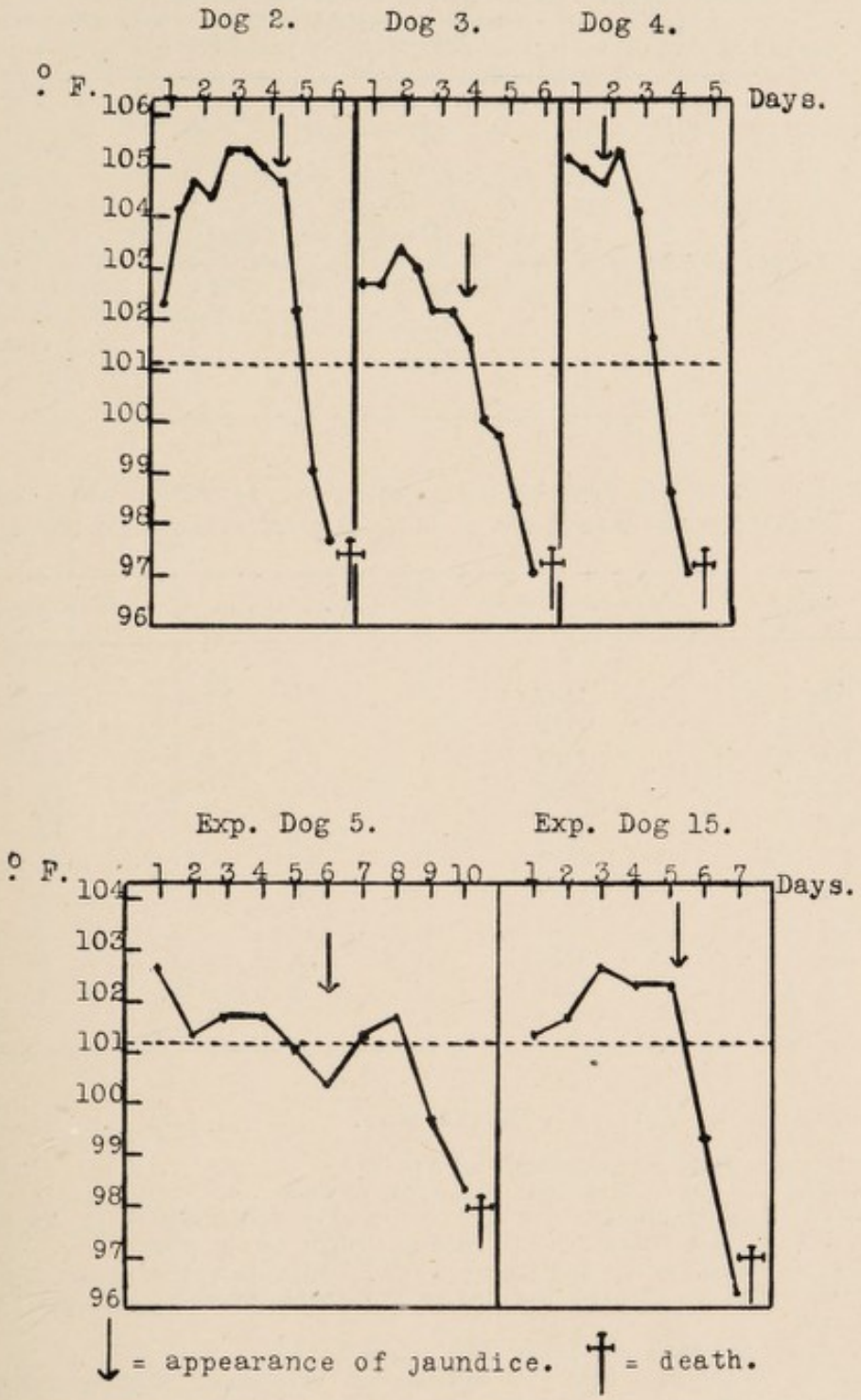
PASSAGE TREE OF [RAT] LEPTOSPIRA STRAIN.



P.M.T. = Typical post-mortem appearances.
Lep. + + = Many leptospiræ present.

† = Died. G.P. = Guinea-pig.
S. = Animal survived with no symptoms.

CHART V.



TEMPERATURE CHARTS (See Case Index).

TABLE I.—SHOWING TIME OF OCCURRENCE OF JAUNDICE AND TIME OF DEATH AFTER ONSET IN NATURALLY OCCURRING CASES.

Dog.	Occurrence of Jaundice (Days).	Time of Death (Days).
1	2	5
2	4	6
3	4	7
4	2	5
6	4	4
8	2	3
9	None	1
10	None	1

TABLE II.—SHOWING TIME OF OCCURRENCE OF JAUNDICE AND TIME OF DEATH AFTER INJECTION IN DOGS ARTIFICIALLY INFECTED.

Dog.	Leptospira Strain.	Jaundice (Days).	Death (Days)
Ex. 2	Dog	16	Lived
Ex. 3	Dog	7	10
Ex. 4	Dog	5	10
Ex. 5	Dog	6	11
Ex. 7	Dog	6	13
Ex. 17	Dog	16	17
Ex. 18	Dog	16	18
Ex. 1	Rat	5	6
Ex. 15	Rat	5	6
Ex. 16	Rat	5	7

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