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Icterus of Sheep caused by a
Bacterium (Bacterial Icterus).**

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THE investigations to be described were commenced as a result of an inquiry made during February, 1926, into deaths occurring in a flock of sheep subsequent to inoculation for blue-tongue. The sheep were on a farm in the Klerksdorp district of the western Transvaal, and the owner reported that some of the cases showed a jaundiced condition. Dr. G. de Kock, of this Laboratory, visited the farm, and reported on a case on which he was able to make a post-mortem. This animal showed a swelling the size of an orange at the site of inoculation in the left thigh. The swelling was completely encapsuled and contained a large amount of greenish creamy pus. Smears of this pus showed the presence of small oval bacteria, which is of interest in view of the bacteriological findings in one of the other cases to be described. No other lesions were found in this sheep, and it did not show icterus. A further case which had undergone decomposition to a considerable degree did not show icterus, but the site of inoculation in the left thigh showed a diffuse firm swelling with a sero-sanguinous infiltration of the surrounding tissues.

A further case (sheep 14421), which was sent to this institute, on arrival showed a swelling on the inside of the left thigh at the site of inoculation. Three days later the swelling was punctured with a sterile needle, and a few cubic centimetres of a reddish fluid were obtained. From this fluid a culture was obtained, which consisted almost entirely of colonies of a small oval bacillus with a few colonies of a white staphylococcus. The sheep itself died the night after the cultures were made, and at post-mortem a very well-marked icteric condition of the carcass was observed.

The organism mentioned as having been isolated in almost pure culture was then obtained in a pure state, and in order to see whether it had any pathogenicity, emulsions of it were inoculated into sheep. The results, as will now be described, were striking, and formed the commencement of a series of experiments which are still in progress.

EXPERIMENTAL WORK CARRIED OUT WITH THE ORGANISM ISOLATED FROM THE CASE OF ICTERUS (14421).

The primary experiment carried out with a pure culture was the inoculation of sheep subcutaneously and intravenously with emulsions of the bacterium. In all the experiments to be described the bacterial emulsions were made by washing the surface growths from blood agar slopes after three to four days' incubation at 37° C. In this experiment quantities of 5 c.c. and 2 c.c. were inoculated intravenously into each of

two sheep and the same quantities subcutaneously into two sheep. All four sheep showed a rise of temperature to 106°–108° F. within twenty-four hours after inoculation. After periods varying from forty-eight to seventy-two hours after inoculation, symptoms of dullness appeared with marked dyspnoea. The mucous membranes were of a brownish-yellow colour, best marked in the sheep done subcutaneously. In all four sheep haemoglobinuria was observed, and in the sheep inoculated subcutaneously there was marked redness and swelling at the inoculation site in the thigh. At post-mortem the carcasses of the sheep showed a well-marked icterus, and a reddish-brown urine was observed in the bladder. A detailed description of the symptoms appears in the appendices.

The results of this experiment were so striking and unexpected that further experiments were immediately undertaken. It should be mentioned at this point that a condition characterized by a very well-developed icterus has been observed to occur amongst the sheep imported to this station from the Karroo for the making of blue-tongue vaccine. This condition, which has provisionally been called enzootic icterus, has an obscure etiology, but the symptoms during life and the post-mortem lesions are very similar in many respects to those seen in cases of icterus produced by an organism. This disease is fully discussed in the paper of Dr. De Kock in the present report. Experiments carried out with the bacterial icterus were therefore designed with the object of seeing whether any relationship between the two diseases could be established and if a study of bacterial icterus would throw any light on the etiology of enzootic icterus.

An experiment (Experiment 2) was then undertaken to see the effect of dosing the sheep per os with virulent cultures of the organism, subcutaneous inoculation being undertaken as a control. Two sheep were each given the growth from a whole blood agar slope per os, while four sheep were inoculated subcutaneously with culture emulsion. Of the four sheep, one received 2 c.c., one 1 c.c., one 0.1 c.c., and the fourth 0.01 c.c. The two sheep dosed by the mouth never showed any symptoms subsequently. The sheep given 2 c.c. and 1 c.c. respectively both developed very typical icterus and died (sheep 13847 and 13989). The sheep which received 0.1 c.c. and 0.01 c.c. respectively both survived and showed only slight lameness, with swelling and reddening of the inoculation site. When subsequently inoculated subcutaneously with 2 c.c. of the culture both again survived, the first inoculation having apparently immunized the animals (sheep 13957 and 13821).

Experiment 3.

In connexion with the previous experiment another was carried out to see whether the blood of a case of bacterial icterus was infective for normal sheep. Sheep 13989 in the previous experiment was bled, and four sheep were inoculated each with 10 c.c. intravenously (sheep 9605, 13992, 12376, and 13636). No symptoms were observed in the sheep subsequently.

Experiment 4.

In this experiment an attempt was made to produce cases of bacterial icterus by various methods with a view to possibly throwing some light on the source of natural infection in "enzootic icterus."

Two sheep (8603 and 13951) were inoculated each with 1 c.c. of culture emulsion into the interdigital sac of the left hind foot. Two sheep were scarified over an area the size of the palm of the hand on the inner side of the right thigh, and culture emulsion was rubbed into the areas (sheep 8725 and 9498).

The two sheep (11445 and 13853) dosed per os with culture in Experiment 2 were again dosed per os each with one whole culture. Two sheep, both susceptible to blue-tongue, were used as controls, one receiving 2 c.c. of culture emulsion subcutaneously and one 1 c.c. (sheep 14312 and 9505). Blue-tongue susceptible control sheep were used, as it was intended to inoculate blood from one sheep showing icteric symptoms into blue-tongue susceptible sheep, which were subsequently to receive blue-tongue vaccine.

Both the control sheep developed typical icterus, resulting in death. From one sheep (14312) blood was taken when icterus was well developed, and 10 c.c. were inoculated intravenously into each of six blue-tongue susceptible sheep (see Experiment 5).

The two sheep dosed by the mouth did not subsequently show any symptoms.

Both the sheep inoculated into the interdigital sac developed marked lameness, with swelling of the interdigital region, but no other symptoms. The sheep which were scarified did not develop any symptoms, except slight lameness.

Experiment 5.

As mentioned in the previous experiment, blood was taken from a case of bacterial icterus (14312) and inoculated into five sheep, each getting 10 c.c. intravenously. The object was to repeat the blood inoculation done in Experiment 3, but at the same time to see whether there might be a latent infection produced by the blood and which would perhaps be brought into activity by a reaction to blue-tongue. After the blood inoculation, at an interval of five days, two of the sheep were inoculated with blue-tongue vaccine; five days later two more, and again five days later the last sheep. All the sheep showed blue-tongue reactions, but did not develop any symptoms of icterus.

Experiment 6.

The object of this experiment was to see whether a disease, such as heartwater in a mild form, would bring out a latent infection with bacterial icterus if it existed. The two sheep dosed with cultures per os and six sheep previously inoculated with blood of a case (four of them from Experiment 3) were used. The strain of heartwater was one which usually only produced a temperature reaction, but occasionally was fatal. Five of the sheep developed typical heartwater reactions and two died of the disease. No symptoms of icterus during life or post-mortem were noticed in any of the animals.

Experiment 7 (9.4.26).

In order to see whether other animals besides sheep were susceptible to infection with the organism of bacterial icterus in sheep, a horse, ox, pig, dog, and goat were inoculated with emulsions of it subcutaneously. In addition, rabbits, guinea-pigs, and white rats were inoculated.

Of the latter animals, the rabbits and white rats showed no symptoms, but one guinea-pig died a month after infection, showing abscesses in the liver and spleen from which the organism was recovered.

Horse 15002.—The animal was inoculated subcutaneously with the growth from a whole blood agar slope. The temperature rose to 106° F. on the second day after inoculation, but dropped day by day until by the 4th day it was normal. A hot, painful swelling occurred at the inoculation site, which developed into a large abscess. This burst during the night of the 10th day after inoculation, so cultures were not made from the pus.

Ox 1135.—Received the same dose as the horse. In this case the temperature rose to 106° F. about thirty-six hours after inoculation, and slowly fell until it was normal about the 9th day. A hot, painful swelling developed at the inoculation site, and the whole of the right side of the neck, where the inoculation was done, became swollen and painful. An abscess formed, which was opened on the 12th day, and from the pus the organism was recovered.

Pig 535.—In this case, and again in the dog and goat, only 2 c.c. of an emulsion of the organism were inoculated subcutaneously. The pig showed a marked swelling at the inoculation site, which was hot and painful, and the animal was very stiff in its gait when made to move.

The temperature went up to 105° F., and did not drop until the 5th day after inoculation. By the end of a week the animal was normal again, and no abscess formation took place.

Dog 396.—The only symptoms noticed were a painful swelling of the inoculation site, with a rise of temperature to 104° F. No abscess formation took place, and by the 5th day after inoculation the animal was again normal.

Goat 13995.—Inoculated subcutaneously on the inner side of the right thigh. There was no rise of temperature, but a slightly painful swelling developed at the inoculation site and there was marked lameness. By the 6th day after inoculation the animal was normal again, and no abscess formed.

In none of the animals was any sign of icterus noticed, and of all the species inoculated only one died, a guinea-pig, in which abscesses were found, but it was not certain whether they were the only cause of death.

The organism of bacterial icterus in sheep, therefore, has not produced jaundice in any animal species but the sheep.

Experiment 8.

This was a repetition of Experiment 4, and again no success was experienced in infecting sheep by the mouth, scarification, or intradermal inoculation.

Two sheep (8734 and 10227) were each given two whole blood agar cultures by the mouth. Two sheep (12201 and 13712) had the skin of the thigh scarified and culture emulsion rubbed in. One sheep (9063) was given 1 c.c. of the emulsion into each of the interdigital sacs of the hind feet.

Experiment 9.

In order to see whether one could produce bacterial icterus in splenectomized sheep, one (10511) was inoculated subcutaneously with 2 c.c. of culture emulsion. There was a subsequent rise of temperature to 107° F., the site of inoculation became red, hot, and painful, causing slight lameness, which by the 4th day had disappeared, as had the swelling. The control sheep (13825) inoculated at the same time and with the same amount of culture emulsion developed a very high temperature, and the inoculation site became hot and painful. On the 2nd day there was slight icterus, which became very marked by the 3rd day, and, in addition, there was haemoglobinuria. Death occurred early on the 4th day.

The result of this experiment suggested that splenectomized sheep might perhaps be more resistant to the infection than normal sheep, so the experiment was at once repeated.

Experiment 10.

This was a repetition of the previous one. In this case a normal sheep (13833) and a splenectomized one (9119) were each inoculated subcutaneously with 2 c.c. of culture emulsion in the right thigh. Both developed marked redness and a painful swelling at the inoculation site with marked lameness. Sheep 13833 showed a temperature of 105° F. on the day following inoculation, but was normal again by the 8th day, when a fresh rise took place with a subsequent drop.

Complete recovery took place in about ten days.

The splenectomized sheep developed marked icterus on the morning of the 3rd day and died during the afternoon.

In this experiment the results of the previous one were reversed, so it was decided to undertake a further similar experiment.

Experiment 11.

Two sheep (13987 and 10743) (splenectomized) were given 1 c.c. each of culture emulsion subcutaneously into the right thigh. Apart from an initial rise of temperature to 105° F., with a subsequent rapid fall and slight redness and swelling at the inoculation site, neither sheep showed any symptoms.

It is possible the dose in these cases was too small, but the explanation of this result and those of the previous two experiments is very likely that many individual sheep have marked resistance to the organism.

Quantities of less than 1 c.c. of culture emulsion have not produced any symptoms, so that in the last experiment a dose somewhere close to the minimum lethal was used.

Experiment 12.

In this experiment an attempt was made to determine whether any toxin or haemolysin was produced by the organism in culture media. At the same time a sheep was given a sublethal dose of culture emulsion subcutaneously with the addition of lactic acid to see if the organism would develop if the tissues were damaged.

Sheep 7377 received 0.1 c.c. of culture emulsion subcutaneously and at the same time 1 c.c. of lactic acid. A rise of temperature to 106° F. on the 4th day and again on the 8th were the only symptoms noticed.

Sheep 11338, given 5 c.c. filtrate from serum broth cultures of the organism intravenously, showed no symptoms subsequently, except a rise of temperature to 107° F. on the 6th day and again to 106° F. on the 12th.

Conclusions.

These experiments show that the organism of bacterial icterus in sheep will produce the typical symptoms when inoculated subcutaneously or intravenously in a dose of emulsion not less than 1 c.c. per os; by scarification and intradermal inoculation icterus has not been produced. Some of the experiments tend to show that there is considerable individual variation in susceptibility to inoculation with the organism.

In animals, other than sheep, no icterus has been produced, but in some animals abscesses have developed.

DESCRIPTION OF THE ORGANISM.

Morphology.—The organism is a cocco-bacillus, oval in shape, and usually occurring in pairs or small clumps. The average length is 1.5 μ and varies from 1 μ to 2 μ ; width, 0.5 μ to 0.8 μ . In old cultures on glucose liver agar, longer forms have been seen, up to 4 μ in length and club-shaped. These forms have not been seen in cultures in other media and probably are involution forms.

There is no motility, and spores are not formed.

Staining Reactions.—Gram positive and not easily decolourized. The usual weak basic aniline dyes, such as methylene blue, carbol thionin, etc., stain it easily and intensely.

CULTURAL CHARACTERISTICS.

The organism grows well aerobically as well as anaerobically. A good growth is obtained on most of the media in common use, but particularly luxuriant on media containing blood or serum. In shake cultures the colonies grow in the depths of the medium, but are thickest about $\frac{1}{4}$ in. below the surface. There is no evidence of liquefaction in gelatine media, and on blood containing media there is no evidence of haemolysis. The optimum temperature for growth is about 37° C., but it occurs slowly at room temperature.

Agar and Glucose Agar.—Growth fairly good, and takes the form of a glistening white layer with a tendency to the formation of discrete colonies. The colonies are dew-drop like in form, with flattened edges in old cultures.

Glucose Liver Agar.—Growth as on agar. On this medium the bacteria frequently show involution forms as described in morphology.

In deep shake cultures in glucose agar or glucose liver agar (Vignal tubes) lenticular colonies develop in the depths of the medium, but, as previously stated, they are more frequent just below the surface of the medium.

Blood Agar and Serum Agar.—The growth on these media is thick and better than on any other medium so far used. It is white and glistening,

and after a few days' incubation it is rather difficult to detach from the medium, and an emulsion made from it in normal saline appears full of clumps.

One-tenth per cent. Agar.—The growth was not very good. It took the form of an irregular turbidity best marked close to the surface.

Gelatin.—No growth was observed in shake, stab, or surface cultures.

Ordinary Broth and Glucose Broth.—Good growth. The medium becomes turbid, and on close inspection very numerous fine granules can be seen in the fluid. These settle out after about two days' incubation, leaving the fluid clear, and a dense white deposit forms at the bottom of the tube. This clumping is seen in emulsions from agar slopes in normal saline.

In *Chopped Meat Medium* or *Hibler* a slight turbidity occurs, which settles out. No gas is formed in these media.

In litmus milk acid is formed and coagulation occurs. No indol formed in peptone water.

Sugar Reactions.—Very few of the sugars, etc., used were affected. Acid was produced in glucose, galactose, and dextrin only.

The classification of the organism has been found difficult, and it does not appear to correspond to any well-known type. It apparently belongs to the group classified as the corynebacteria, and a name suggested for it is *Corynebacterium haemolyticum ovis*.

So far it has not been found possible to produce haemolysis of sheep's red corpuscles by cultures of the organism. Washed sheep's corpuscles were used, and both fluid cultures and emulsions of growths from blood or serum agar in normal saline were added to them. Even undiluted, the fluid cultures produced no haemolysis even after twelve hours' incubation at 37° C. The addition of fresh sheep serum to act as complement was likewise without effect on the culture corpuscles mixtures.

Filtrate from cultures has not proved successful in setting up icterus, but more experiments are necessary to clear up this question.

On account of the marked haemolysis occurring in the sera of sheep with "enzootic icterus," it has not been possible so far to carry out serological tests with them on the organism of "bacterial icterus."

NOTE.—For a description of the post-mortem findings in cases of bacterial icterus and the pathology of the condition, reference should be made to the article by G. de Kook on "A Study of the Reticulo-endothelial System of the Sheep," which appears in this report.

NOTE.—Since this article was written, the writer has been able to identify the organism as a type of the Preisz Nocard bacillus (*Corynebacterium ovis*). With strains of the latter organism similar lesions to those seen in bacterial icterus have been set up in sheep.

APPENDIX.

Sheep 10791.—2 c.c. subcutaneously in right thigh emulsion of culture of icterus organism on 10.2.26.

11.2.26. Inner aspect of thigh red, hot, and painful. Lame.

12.2.26. Inner aspect of thigh red, hot, and painful. Lame.

13.2.26. Dull, not eating. Marked dyspnoea. Marked haemoglobinuria. Visible mucous membranes brownish-yellow. Died at 11 a.m.

Sheep 10255.—2 c.c. culture of icterus organism intravenously on 10.2.26.

11.2.26. Dull, respiration rapid, not eating.

12.2.26. Dull, respiration rapid, not eating. Mucous membranes pale.

13.2.26. Lying on side, marked haemoglobinuria. Visible mucous membranes deep yellow. Died at 11 a.m.

- Sheep* 10731.—10.3.26. 5 c.c. culture intravenously.
 11.2.26. Respiration rapid, grunting, not eating.
 12.2.26. Respiration rapid, grunting, not eating. Haemoglobinuria.
 13.2.26. Died previous night.
- Sheep* 13980.—10.2.26. 5 c.c. culture subcutaneously.
 11.2.26. Thigh swollen, painful and reddened on inner aspect. Lamé.
 12.2.26. Thigh swollen. Haemoglobinuria. Mucous membranes deep yellow.
 13.2.26. Dead at 7 a.m.
- The culture emulsion used for these and the following sheep was obtained by washing surface growths from blood agar slants with normal saline after three to four days' incubation at 37° C.
- Sheep* 13853 and 11445.—15.2.26 and 1.3.26. Each received the growth from one whole blood agar per os. No result.
- Sheep* 13847.—15.2.26. 1 c.c. subcutaneously of culture emulsion in right thigh.
 16.2.26. Inoculation site red and swollen. Very lame in right hind leg. Temperature 106° F.
 17.2.26 and 18.2.26. No change.
 19.2.26. Dull, visible mucous membranes yellow.
 20.2.26. Dull, visible mucous membranes yellow. Died in the night.
- Sheep* 13989.—2 c.c. culture emulsion subcutaneously in right thigh.
 16.2.26. Very lame. Pale, slight icterus. Inoculation site red, hot, and swollen. Temperature 106° F.
 17.2.26. Very lame, dull, marked icterus. Bled for inoculation purposes. Died in the night.
- Sheep* 13957.—15.2.26. 0.1 c.c. culture emulsion subcutaneously. No symptoms.
Sheep 13821.—15.2.26. 0.01 c.c. No symptoms.
- Sheep* 9605, 139 2, 12376, and 13636.—1.3.26. Each inoculated intravenously with 10 c.c. blood of sheep 13989. No symptoms subsequently.
 8.3.26. Inoculated with mild strain of heartwater. No symptoms shown.
- Sheep* 8603 and 13951.—Each inoculated with 1 c.c. culture emulsion into the interdigital sac of the left hind foot. Apart from marked lameness and swelling and suppuration at the inoculation site, no definite symptoms were seen. Slight icterus was seen from the 8th to 10th day after inoculation.
- Sheep* 8725 and 9498.—Scarified on inner side of right thigh and culture emulsion rubbed in. No symptoms noticed.
- Sheep* 13957.—1.3.26. 2 c.c. culture subcutaneously. No symptoms.
Sheep 13821.—1.3.26. 1 c.c. culture subcutaneously. No symptoms.
Sheep 14312.—1.3.26. 2 c.c. culture emulsion subcutaneously.
 2.3.26 to 5.3.26. Marked lameness, thigh red and swollen.
 6.3.26. Slight icterus. Blood taken and inoculated into normal sheep.
 9.3.26. Well-marked icterus. Temperature 106° F.
 10.3.26. Dead.
- Sheep* 9505.—1.3.26. 1 c.c. subcutaneously of culture emulsion.
 2.3.26. Thigh red and swollen, very lame. Temperature 107° F.
 4.3.26. Thigh red and swollen, very lame. Temperature 106° F.
- Sheep* 14275, 14335, 14322, 14309, and 14340.—6.3.26. Each 10 c.c. blood intravenously from sheep 14312 (bacterial icterus).
 12.3.26. Sheep 14275 and 14335 inoculated with blue-tongue vaccine.
 19.3.26. Sheep 14309 and 14340 inoculated with blue-tongue vaccine.
 26.3.26. Sheep 14322 inoculated with blue-tongue vaccine.
- All five sheep reacted to blue-tongue, but did not show any symptoms of icterus.
- Sheep* 11445.—Twice dosed with culture emulsion per os.
 8.3.26. Mild strain of heartwater subcutaneously. Died of heartwater on 23.3.26.
- Horse* 15002.—9.4.26. Growth from one blood agar slope subcutaneously.
 10.4.26. Large painful swelling at inoculation site on the neck. Temperature 105° F.
 12.4.26. Swelling less painful, becoming diffuse. Temperature 100° F.
 15.4.26. Abscess formed. Lanced. No symptoms apart from abscess formation.
- Ox* 1137.—9.4.26. Growth from one blood agar slope subcutaneously.
 10.4.26. Large hot, painful swelling at inoculation site. Temperature 106° F., but dropped to normal in five days. An abscess formed, which was opened on 19.4.26. The icterus organism was in pure culture in the pus.
- Pig* 535.—9.4.26. 2 c.c. culture emulsion subcutaneously.
 10.4.26. Temperature 105° F. Diffuse painful swelling at inoculation site. Swelling disappeared by 14.4.26.
- Dog* 396.—9.4.26. 2 c.c. culture emulsion subcutaneously.
 10.4.26. Temperature 104° F. Symptoms as in pig 535. Swelling disappeared by 15.4.26.

Goat 13995.—9.4.26. 2 c.c. culture emulsion subcutaneously. The only symptom subsequently noticed was slight lameness in the inoculated leg.

Sheep 8734 and 10227.—9.4.26. Each given emulsion of growth from two whole cultures on blood agar per os. No symptoms subsequently.

Sheep 9063.—9.4.26. 1 c.c. culture emulsion intradermally in the intradigital sacs of both hind feet. Slight lameness was shown for about a week. No other symptoms, except a rise of temperature to 105° F. in both cases about the 5th day after inoculation, with a subsequent drop to normal in three to four days.

Sheep 12201 and 13712.—9.4.26. Scarified on the inside of right thigh and culture emulsion rubbed in. Beyond an initial rise of temperature to 105° F. no other symptoms were noticed.

Sheep 10511 (splenectomized).—10.5.26. 2 c.c. emulsion of icterus organism subcutaneously in right thigh.

11.5.26. Red and painful at inoculation site. Lame. Temperature 107° F.

12.5.26. Temperature 105° F. Still red and painful at inoculation site.

13.5.26. Temperature normal. Still red and painful at inoculation site.

14.5.26. Temperature normal. Slight icterus. Recovery subsequently took place without further symptoms.

Sheep 13825.—10.5.26. 2 c.c. emulsion of icterus organism in right thigh.

11.5.26. Slight redness and pain at inoculation site. Temperature 106° F. Slight icterus.

12.5.26. Dull. Haemoglobinuria. Mucous membranes brownish-yellow. Died in the night.

Sheep 9119 (splenectomized).—17.5.26. 2 c.c. culture emulsion subcutaneously in right thigh.

18.5.26. Very lame. Inoculation site red and swollen.

19.5.26. Temperature 106° F. Very lame, slight icterus.

20.5.26. Temperature 106° F. Very lame, well-marked icterus. Died at 4 p.m.

Sheep 13833.—17.5.26. 2 c.c. icterus culture emulsion subcutaneously in right hind thigh.

18.5.26. Very lame. Inside of thigh red, hot, and swollen. Mucous membranes injected. Dull, not eating.

19.5.26. Temperature 106° F. Symptoms unchanged.

20.5.26. Temperature 103° F. Marked improvement. Recovery without further symptoms.

Sheep 10743 (splenectomized) and 13987.—Each 1 c.c. subcutaneously culture emulsion on 24.5.26. *Sheep 10743* showed an initial rise of temperature to 106° F., with slight lameness. *Sheep 13897* did not show any symptoms, except slight lameness. Both recovered.

Sheep 7377.—24.5.26. 0.1 c.c. icterus culture emulsion and 1 c.c. lactic acid subcutaneously. No symptoms subsequently.

Sheep 11338.—24.5.26. 5 c.c. filtrate from cultures of the icterus organism in ser m broth inoculated intravenously. No symptoms shown subsequently.