Observations

ON

LABORATORY AND HOSPITAL INFECTIONS
WITH YELLOW FEVER IN ENGLAND

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Since the introduction of the rhesus monkey in the study of yellow fever by the American Commission in West Africa, various laboratory workers have acquired the disease, not through the agency of infected mosquitos, but by direct contamination with infective blood or tissues. Hindle, in a recent communication (1930), briefly referred to certain patients contracting the disease in this manner, but since the present outbreak, which started in July, 1930, involved, in addition, two of our hospital laboratory workers who had had no access to infected monkeys, we propose to give a more detailed account of the mode of infection. So important an experience justifies publication, as it forcibly illustrates the grave risk attending the routine collection and examination of human blood during the first four days in yellow fever cases.

Our first patient was a laboratory assistant from the Wellcome Bureau of Scientific Research, where experimental investigations on yellow fever were being undertaken. Infection occurred with a Brazilian strain which had been maintained by Dr. Hindle in monkeys and mosquitos for at least a year. At the time of contracting the disease, however, the only source of virus was *Macacus rhesus*.

CASE I

This patient, who was a delicate youth, aged 17, had never been abroad; he gave a history of measles, pertussis, and varicella in childhood, of suppurative appendicitis in 1923, and of an operation for right strangulated inguinal hernia in 1929. A mild attack of pneumonia occurred in the same year.

July 8th, 1930.—At 6.30 p.m. the patient, who was then quite well, went for a ride on his bicycle; about 10 p.m. he developed a headache, and later felt chilly, then hot.

July 9th.—The headache persisted, but he came to the laboratory in the morning. Half an hour after arrival he vomited bile, and repeated this forty-five minutes later. He returned home from work at 6 p.m.; was very restless that night, and vomited food once.

July 10th.—At 2.30 p.m. he was admitted to the Hospital for Tropical Diseases complaining of headache and fever. He sweated profusely, and at 6 p.m. the temperature was 104.8° F., the pulse was 100, and the respirations 24 per minute. The tongue was coated with fur, the face slightly flushed, and the conjunctival vessels were injected. No icteric tinge was present. The pulse was regular and bounding, and there was a blowing systolic bruit at the apex. The liver and spleen, though not enlarged, were definitely tender on deep palpation under the costal margin. The abdomen showed the scarred incisions of previous operations, but did not present any other features of clinical interest. That night the patient slept at intervals, the headache having abated somewhat by 10 p.m.

July 11th.—In the morning he was rather drowsy, and complained of photophobia. The tongue was dry, with yellowish fur laterally and a reddened centre. Herpes was present on the lower lip, and the eyes were definitely blood-shot. At 10 a.m. the temperature was 102.2° and the pulse 88 per minute. At 2 p.m. blood was taken for biochemical estimations of urea and bilirubin, and some was also sent to the Wellcome Bureau of Scientific Research for inoculation

into a monkey (*Macacus rhesus*). The specimen was collected sixty-four hours after the onset of the illness as judged by the initial headache. At 8.30 p.m. 12 c.c.m. of immune serum were injected subcutaneously into the abdominal wall. This was obtained from a person who was known to have contracted yellow fever some months previously, and whose blood had been proved to be protective to animals inoculated with the virus. The urine (specific gravity 1012) was acid in reaction, and contained only a slight trace of albumin; bile salts and pigments were absent. The total quantity passed that day was 42 oz. The van den Bergh test yielded a negative direct reaction, and an indirect reaction of only 0.8 unit. The blood urea figure was 46 mg. per 100 c.c.m. Haematological estimation showed that the red blood corpuscles numbered 4,400,000 per c.mm., the haemoglobin percentage was 80, and the colour index 0.9. The leucocyte count was 9,000 per c.mm.

July 12th.—The patient had slept during the night, but now again complained of photophobia. The pulse was 80 per minute, regular, and slow compared with the temperature (102°). No vomiting, rash, or jaundice was present, but the conjunctival vessels were congested. Slight twitchings of the arms and face were noted. The urine still contained only a trace of albumin, and 40 oz. were passed in the twenty-four hours. During the evening the patient was drowsy and slept at intervals; at 10 p.m. a loose stool containing mucus was passed.

July 13th.—The patient appeared somewhat weaker, the temperature was 102°, and the pulse still slowing—74 per minute. A distinct trace of jaundice was evident on the conjunctivae. The urine was acid, with a specific gravity of 1022, and the amount of albumin had increased. The total quantity passed in the twenty-four hours was 38 oz. Urobilin was present, but there were no bile salts or pigments. Vomiting occurred during the day, and indigestion was complained of at 10 p.m., but the patient slept satisfactorily despite it.

July 14th.—During the morning the breathing was somewhat irregular, and at times slight cyanosis developed. There was a good deal of muscular twitching, and the patient complained of abdominal pain. Vomiting occurred only once. The blood pressure was then: systolic 110, diastolic 66. The urine contained a considerable amount of albumin (0.3 per cent.), and also urobilin; the total amount passed in the twenty-four hours was 46 oz.

July 15th.—At 2 a.m. the temperature fell to 98.8°; subsequently during the day it rose and fell thrice. The patient was restless, troubled with nausea and abdominal pain, and vomited once. A saline enema was given at 6 p.m., with good result. The urine was acid, and contained a considerable quantity of albumin (0.5 per cent.); bile pigment in small quantity was present, and there was a marked excess of urobilin. The total quantity passed in the twenty-four hours was 36 oz.

July 16th.—The patient now remained afebrile, the general condition improving, and the tongue being moist for the first time. The urinary findings were almost identical with those recorded on the preceding day (albumin 0.45 per cent.), but microscopical examination showed granular casts and a few leucocytes. The van den Bergh test showed a negative direct reaction, the indirect registering 1.2 units.

July 17th.—No further rises of temperature were recorded, but the tendency to sweating was marked. The urine still contained a trace of bile pigments, much urobilin, and considerable quantities of albumin (0.4 per cent.). The blood urea figure was 29 mg. per 100 c.c.m.

July 19th.—The patient's condition was quite satisfactory, and the urine now contained only a trace of urobilin and albumin (less than 0.01 per cent.).

July 20th.—The patient was discharged from hospital. Convalescence was uneventful, and two months later normal health was attained.

Commentary

In addition to convalescent serum the patient was given 10 grains of calcium lactate three times daily, and glucose in his drinks. For the first few days plain water, albumin water, and diluted milk were alone allowed, and later Horlick's malted milk and peptonized milk. As much fluid as the patient could swallow was administered, the nursing staff being instructed to see that at least 80 oz. of fluid were taken

daily. Symptomatic treatment was instituted when necessary; an ice-bag proved useful for headache, glycerin suppositories and saline enemata for constipation, and sponging for reducing the temperature. On July 21st sago, eggs, and junket were permitted, and later toast, butter, pounded fish, and weak tea were added to the menu. A full diet was allowed ten days later.

This case ran a typical course of a moderate infection of yellow fever, the pyrexial bout lasting seven days, with bradycardia as a marked feature of the illness (see chart). Albumin appeared in the urine on the third day, and bile pigment on the seventh day of fever, though an icteroid tinge of the skin and conjunctivae was noted on the fifth day.

The low blood urea and the low van den Bergh findings are of special interest when compared with those found in black-water fever, where much higher values are recorded.

CASE II

The second patient, aged 28, first examined the blood of the preceding case on July 11th, 1930. The specimen was sent from the ward to the laboratory with the request for an estimation of bilirubin and urea. Blood collected at the same time was inoculated subcutaneously into a monkey of the species *Macacus rhesus* at the Wellcome Bureau, the animal succumbing subsequently to yellow fever. The only other occasion on which the second patient could have come into contact with yellow fever was on July 16th—five days later—when the biochemical tests were repeated. By this time, however, the blood was non-infective, so that the date of exposure in this case can be definitely fixed.

July 21st, 1930.—The patient was quite well during the day, then suddenly, about 10 p.m., he was taken ill with generalized headache. Shivering started about 11 p.m., and severe abdominal pain and discomfort followed. Flatus was passed with difficulty. The condition was as if "lumps of ice were located in the abdomen." Castor oil taken in the early morning brought some relief.

July 22nd.—Next morning he was seen by Dr. W. Williams, the temperature at 8 a.m. being 100.2°. The general condition remained much the same, the fever, headache, nausea, and other features persisting. In the afternoon the temperature was 100.8°, while at 8 p.m. it was 102°.

July 23rd.—Next morning at 10 a.m. the temperature was 102°, and at 5 p.m. 102.6°. Dr. Williams then got in touch with one of us (G. C. L.), who arranged for admission to the Tropical Diseases Hospital. The patient was by this time complaining of very severe supra-orbital headache, fleeting pains in the muscles, and pain in the lumbar region. The face was flushed, and there was some visible perspiration. No obvious anaemia or jaundice was present; the tongue was thickly covered with white fur. The temperature was 102.6°, and the respirations 18 per minute. The pulse, which was 72 per minute, was regular in time and force, and of good volume; the blood pressure was sustained, the systolic pressure being 126 and the diastolic 62. No abnormal physical signs were detected on examination of the heart or lungs. The abdomen was slightly distended and the parietal muscles were somewhat rigid, but no visceral enlargement or localized tenderness was elicited on palpation. The urine contained both albumin and phosphates.

July 24th.—In the morning the temperature was 103°, and the general condition unchanged. The pulse was now only 60 per minute; this bradycardia subsequently persisted throughout the pyrexial period. Blood was collected from the ear by a laboratory technician, who later developed the disease (Case III). The red blood corpuscles numbered 5,000,000 per c.mm., the haemoglobin percentage was 90, and the total leucocyte count was 5,000 per c.mm. The differential count revealed 81 per cent. of neutrophil polymorphs, and 19 per cent. of lymphocytes. The urine had a specific gravity of 1025, was acid in reaction, and contained a quantity of albumin and urobilin, but no sugar, bile salts, or acetone bodies. During the day the patient slept on and off, but still complained of headache, and was at times restless. About 11.30 p.m. 30 c.cm. of convalescent serum from the first case was injected subcutaneously, and calcium lactate and glucose were commenced, the patient being treated along the lines already indicated in Case I.

July 25th.—At 6.15 a.m. the patient vomited, but later the headache improved somewhat, and the temperature tended

to fall. The pulse kept at about 60 per minute throughout the day. At 3.15 p.m., 89½ hours after the onset of the illness, blood was taken for inoculation into a monkey, which subsequently died of yellow fever, necropsy revealing the typical pathological changes. The van den Bergh test showed a delayed direct reaction and an indirect reaction of 1.5 units of bilirubin (0.75 mg. per 100 c.cm.). At 9.30 p.m. the patient complained of pain in the lower abdomen, associated with a desire, but an inability, to micturate.

July 26th.—Hot fomentations failed to give relief, and at 1 a.m. 48 oz. of urine were withdrawn by catheter. Next morning the general condition had markedly improved; the temperature fell to 97°, but rose subsequently to 100°; the pulse remained slow, and the tongue somewhat dry, furred, and dirty. The urine still contained albumin in quantity, but no bile salts. Urobilin was present, and the Rothera test, though not Gerhard's, was positive. At 6 p.m. he was again catheterized, 31 oz. of urine being withdrawn.

July 27th.—The secondary rise in the temperature chart, so characteristic of yellow fever, now occurred (see chart). The pulse remained slow, and did not appreciably increase with the subsequent rises in temperature (Faget's sign); generally the rate varied between 50 and 65 beats per minute. The blood pressure was still well maintained, the systolic being 115 and the diastolic 70, while the urinary findings were similar to those seen on July 26th. The conjunctival vessels were somewhat injected, and an icteroid tinge of the skin was present. During the day he complained of headache, and slept on and off. He passed 20 oz. of urine with difficulty at 10 p.m., and after that slept well from 11 p.m. to 6.30 a.m.

July 28th.—The jaundice now appeared less, but abdominal discomfort persisted, necessitating hot fomentations. During the day the temperature varied from 100° to 101.4°, and the pulse from 60 to 65 beats per minute. The respirations remained stationary at 20 per minute. The patient slept from 9.30 p.m. to midnight, and then complained of inability to micturate. After hot fomentations 20 oz. of urine were passed.

July 29th.—The eyes were still very injected, the face looked flushed, and the patient complained of headache. Throughout the day the temperature varied between 99.2° and 101.6°, and the pulse between 58 and 64 per minute. The respirations remained unaltered at 20 per minute. The urine showed a fair amount of both albumin and urobilin, but no bile.

July 30th.—The patient passed 18 oz. of urine during the morning, but only after hot fomentations had been applied to the hypogastrium. A delayed direct van den Bergh reaction and an indirect reaction of 2.5 units were recorded. Clinically, jaundice was now more evident than at any previous time. The urine showed albumin and bile pigments, but not bile salts.

July 31st.—In the early hours of the morning the temperature fell to normal, varying from 97° to 98.4° throughout the day; the pulse remained slow, varying from 52 to 60 beats per minute. Albumin, urobilin, and bile pigments were still present in the urine, and next day bile salts were also detected. The subsequent course of the illness was uneventful, except that for the next five days rises of temperature of from 99° to 100.4° were noted. Bradycardia also persisted, the pulse remaining between 49 and 68 beats per minute. Throughout this period the tongue was somewhat furred, and on occasions there were attacks of headache, restlessness, and excessive perspiration.

August 5th.—The urine contained a trace of albumin, but no bile salts or pigment.

August 6th.—The diet was now reinforced with junket, chicken broth, and toast; later, eggs, sago pudding, and chicken were added to the menu. After this steady improvement followed.

August 23rd.—The patient was now transferred to a convalescent home, and later, after a month's holiday, he returned to duty in normal health.

Commentary

Points of interest in this particular case are the definite incubation period of ten days, and the proved persistence of virus for at least 89½ hours in the circulating blood. The classical "saddle back" temperature and bradycardia are well illustrated in the accompanying chart.

CASE III

On July 24th, before the establishment of a definite diagnosis, a laboratory technician, aged 21, had performed a blood count on the second patient, and for this purpose had collected the blood himself. Until August 2nd he was at work as usual in the laboratory.

August 3rd, 1930.—At 2 a.m. he awoke with chill and sweating. Headache, backache, and epigastric pain soon developed, and rapidly became worse. He was admitted to the Hospital for Tropical Diseases at 11 a.m. that day, complaining of shivering, headache, and abdominal pain. The temperature was then 99.8°, the pulse 136, and the respirations 28 per minute. Shortly after admission he vomited a small quantity of bile-stained fluid speckled with brown.

Examination revealed a young man of good physical development, with a flushed face and slightly injected conjunctivae. He was very restless, the breathing was rapid and laboured, there was a dry cough, and occasional attempts

at vomiting were evident. The skin was warm, dry, and not jaundiced. The tongue was dry and the throat congested. The pulse was full and bounding, its rate being 136 per minute; the systolic pressure was 120, and the diastolic 80. No abnormal physical signs were detected in the chest.

On examining the abdomen there was no rigidity, though tenderness was marked in the epigastrium, the right and left hypochondrium, and the flanks. The spleen was also tender and just palpable, but the liver showed no demonstrable enlargement. Active reflexes and photophobia were present. The urine was alkaline, and contained no albumin or bile; 16 oz. were passed soon after admission.

By 9 p.m. the headache was less severe, but the general condition remained unaltered. About 24 c.cm. of convalescent yellow fever serum from Case I was then administered subcutaneously, and 10 grains of calcium lactate three times a day was prescribed. The general line of treatment followed that outlined in the preceding cases, fluids and glucose being given freely. During the night the patient slept for short intervals, but restlessness was marked, the skin was hot and dry, and headache and painful micturition were present.

August 4th.—The tongue was dry, with central fur; the temperature was 104°, the pulse 120, and the respirations 26 per minute. The blood pressure was well sustained, the systolic being 124 and the diastolic 80. Urine was passed naturally, the specific gravity being 1022; there was an amphoter reaction and a fair quantity of albumin. Urobilin was present, but not bile salts or pigment, sugar, or acetone bodies. The total quantity passed in the twenty-four hours was 34 oz. During the afternoon the patient again complained of headache, and appeared dazed, but he slept for short intervals. At 9 p.m. sighing, irregular respirations were first noted, and later incontinence of faeces developed, several loose stools being passed throughout the night.

August 5th.—In the morning the patient was obviously worse; he was very restless, at times delirious, and vomited

a small amount of yellowish brown fluid. The temperature was 104°, the pulse 100, and the blood pressure had risen to 132 systolic and 96 diastolic. The face was flushed, the skin was definitely jaundiced, and the motions were loose and dark. Epistaxis occurred. During the day 54 oz. of urine were passed. Examination showed a specific gravity of 1016, slight acidity, and a moderate amount of albumin (25 mg. per cent.). Urobilin and a trace of bile salts were present, while the deposit contained numerous granular tube casts and epithelial cells.

August 6th.—There was no improvement, though the temperature was somewhat lower (102°). The patient was very restless and delirious, and vomited several times before 11 a.m. Retching was troublesome, and the typical "black vomit" now appeared. Yawning and sighing respirations also ensued, and later an attack of hiccups occurred, lasting one hour. The urine findings were similar to those on August 5th, except that pus cells appeared for the first time in the centrifugized deposit. Again 54 oz. were passed in the

Charts illustrating the incubation period in hospital infections with yellow fever.

Commentary

This patient undoubtedly contracted his infection while either taking the blood or performing a blood count on Case II. The incubation period was ten days. From the onset he was severely ill, and the pulse, though slower than at first, failed to show the characteristic bradycardia met with in the other cases. There was no evidence that the convalescent serum administered on the first day of illness decreased the intensity of the toxæmia, or that calcium lactate had materially modified the tendency to hæmorrhage, as Hindle and Findlay (1930) had found in experimentally infected monkeys.

General Discussion

Until 1928 investigations on yellow fever were invariably conducted in countries where it was endemic, and for this reason when members of the medical, laboratory, and nursing staff developed the disease, infection was attributed as a matter of course to the bite of the natural vector, *Aedes argenteus* (*Stegomyia fasciata*). The tragic ends of Guillet, Adrian Stokes, Young, and Noguchi on the West Coast of Africa dramatically directed attention to the dangers of direct infection, while, as Hindle (1930) points out, the frequency of laboratory infections in Europe and America during the past two years has proved beyond all doubt the danger to man of direct virus transmission from contact with the blood of infected monkeys during life, or with their organs at necropsy. As a result of the death of Adrian Stokes, who had not been working with infected mosquitos for several months, Bauer and Hudson (1928) investigated the passage of virus through the skin of monkeys. They showed that yellow fever resulted if infected blood was merely rubbed on the normal intact skin of the abdomen of *Macacus rhesus*, but they stress the delicacy of the dermis in this situation, and point out that these experimental results did not determine whether the virus was capable of penetrating the normal unimpaired human skin of the hands, which is of thicker and denser texture. In the case of Adrian Stokes several abrasions were recorded as being present, but in our patients this was not so. Cases II and III illustrate the important fact that, in man, yellow fever virus is capable of penetrating the intact skin of the hands.

In countries where yellow fever is endemic it must frequently be necessary to take blood films, to make blood counts, and to perform routine tests of a similar nature on patients during the incubation period and febrile stages of this disease prior to its diagnosis. Our experience shows conclusively the very great danger associated with such procedures—at least during the first four days of fever. In the laboratory of the Hospital for Tropical Diseases antiseptic lotions were used for the hands, and all pipettes, syringes, and other glassware were unfailingly sterilized after coming into contact with infective blood, yet despite these precautions two of our staff were directly infected, as detailed in the text. Gloves, however, were not worn. This measure appears to be the only satisfactory one for preventing direct infection, for several others of us who employed gloves in collecting and handling infective blood did not acquire the disease. The virus content of human blood, during both the incubation period and the febrile stage, is not known as accurately as in monkeys, where it is recorded that 1 c.cm. of blood on the first day of fever commonly contains from one to ten million certainly lethal doses. Microscopic quantities of blood will probably infect, and our recent experience convinces us that in yellow fever countries many members of the medical, laboratory, and nursing staffs must in the past have acquired the disease in so simple a procedure as preparing blood slides, through not realizing its extraordinary infectivity and the capacity of the virus to penetrate unbroken skin.

The black vomitus, urine, and faeces from human sources are generally regarded as non-infective, and the organs at necropsy are held to be innocuous, owing to the disappearance of the virus by the time the patient succumbs. Davis and Burke (1929), however, infected a monkey from kidney tissue in a patient dying after three and a half days of fever, while in one of our cases human blood was proved to be infective 89½ hours after the illness commenced. It should not be too lightly assumed, therefore, that post-mortem examination in man is entirely free from the risks of direct infection, and in all patients dying before the fifth day of the disease adequate precautions should be taken.

The subcutaneous administration of convalescent serum derived from patients who had recovered from yellow fever did not appear to modify the course of the disease in our patients. This was especially evident in Case III, death occurring after less than five days' illness, despite the injection of 24 c.cm. of convalescent serum within nineteen hours of the onset of the fever. Similarly, in Case II, though the patient recovered, the blood was found to be infective 89½ hours after onset, despite the fact that serum had been injected 15½ hours previously. In monkeys serum is of no therapeutic value once the disease is established, but Stokes, Bauer, and Hudson (1928) showed that as small a quantity as 0.1 c.cm. of immune serum would afford absolute protection for a monkey against subsequent inoculation with virus. Such findings suggest the advisability of immediate prophylactic inoculation whenever man has been exposed to contact with infected blood, while the long incubation period of ten days in the present series indicates the feasibility of this procedure.

Summary and Conclusions

1. Three cases of direct infection with a Brazilian strain of yellow fever are recorded in laboratory workers in London.
2. Two of these patients never came into contact, either directly or indirectly, with infected monkeys; they acquired the disease during routine hospital examinations, one in making blood slides and performing a white cell count, the other in doing routine biochemistry on a specimen of blood sent to the laboratory.
3. The incubation period in both cases was ten days.
4. Monkey inoculation showed that the blood of one case was infective for at least 89½ hours after the onset of fever, despite the earlier injection of immune convalescent serum.
5. The hitherto little-appreciated danger of routine blood examination in cases of yellow fever during the first four days' illness, and probably also in the incubation period, is emphasized.
6. It is strongly urged that in endemic areas when yellow fever is prevalent gloves should invariably be worn when taking blood, whether the case is suspected of being yellow fever or not.

Our best thanks are due to the nursing staff of the hospital and to the resident medical officers, Dr. K. Lindsay and Dr. C. J. Hackett, for their tireless devotion to these cases during their illness.

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