intradermal inoculation and to prevent a severe septicæmia, but insufficient to prevent a local infection of the uterus; just as every grade of susceptibility to scarlet fever or tonsillitis is well recognized.

No skin tests on women actually suffering from puerperal fever have been made, but it is hoped to undertake these as opportunities arise. A fatal case of *S. pyogenes* septicæmia in a man, recently tested, proved vigorously toxin-sensitive. In a few instances toxins prepared from strains of *S. pyogenes* from puerperal sources have been tested simultaneously with the standard scarlatinal toxin, and so far with the same results, thus supporting Okell's findings.

It is realized that one hundred cases are insufficient to afford conclusive proof of the significance of the toxin test. The investigation is being pursued, therefore, and it is hoped soon to have further evidence. Finally, it may be stated that the possibilities of the toxin-sensitive test as a guide to preventive treatment against *S. pyogenes* infections have not been overlooked. If the conclusions reached receive support, the time may come when prophylactic immunization of toxin-sensitive women will be practised as a routine. But the indications for such immunization, the optimum time in relation to labour, the means employed—by active dosage with toxin or by passive immunization with antitoxic serum—have yet to be studied.

Summary.

1. Of 100 pregnant women, 27 were sensitive to a dose of 1/5 c.cm. of a dilution of 1 in 1,000 scarlatinal toxin injected intradermally.

2. Eight, or 30 per cent., of the "toxin-sensitive" women experienced morbid puerperia. From the cervixes of those examined a pure growth of *S. pyogenes* was obtained. Labour was altogether normal in 6 of these cases and normal in the remaining 2, except for slight post-partum hæmorrhage.

3. Of 100 pregnant women, 73 gave no reaction to scarlatinal toxin; two of these exhibited morbid puerperia (British Medical Association standard), but in neither case could streptococci be found in the cervix or blood.

4. Thirteen of the non-reacting women, or 19 per cent., sustained difficult labours but healthy puerperia; 6 of these were examined bacteriologically, but in no case was *S. pyogenes* found.

Conclusions.

1. The optimum dose of scarlatinal toxin for intradermal test is 1/5 c.cm. of a dilution of 1 in 1,000 strength.

2. Women who react positively to intradermal inoculation of scarlatinal toxin are more liable to develop puerperal sepsis than non-reactors.

3. Difficult labour, with or without laceration, predisposes to puerperal infection only in so far as the risk of introduction of *S. pyogenes* is thereby increased, provided an external source of infection be present.

The work was carried out during the second year of my tenure of the Lawrence Scholarship at St. Bartholomew's Hospital. My thanks are due to Dr. R. R. Armstrong for active collaboration and advice throughout the inquiry.

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A YELLOW FEVER VACCINE.

BY

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THE urgent necessity of some method of protective vaccination against yellow fever has been strikingly demonstrated by the occurrence of epidemics of increasing severity in West Africa, and also by the recent tragic deaths of three distinguished investigators from this disease. Consequently it seemed of interest to see whether a protective vaccine could be prepared by using any of the methods employed in the case of other diseases caused by filterable viruses, and in particular those recommended by Todd (1928) for fowl plague, Laidlaw and Dunkin (1928) for dog distemper, and Bedson, Maitland, and Burbury (1927) for foot-and-mouth disease.

The strain of yellow fever employed was isolated from a case at Dakar, and has been maintained by passage in rhesus monkeys and mosquitos until the present time (see Sellards and Hindle, 1928). Although the minimal dose of virus has not been estimated the subcutaneous inoculation of 0.0001 gram of the liver of an infected monkey produces death in four to five days, and sixteen monkeys have been inoculated with the virus, with fatal results in every case. The results of Stokes, Bauer, and Hudson (1928) in West Africa have also shown that the infection is almost invariably fatal in rhesus monkeys, and therefore in the following experiments the possibility of natural recovery from the disease need hardly be considered.

Formalinized Vaccine.

Part of the liver and spleen of monkey H 10, which had died from the disease, was ground up in a mortar with broken glass, and the resulting paste mixed with five parts its weight of normal saline, to which was added sufficient formalin to make a strength of 1 in 1,000 formaldehyde. The emulsion was filtered through muslin and kept in the ice chest at 0° to -3° C. until used. This vaccine has been tried on only one monkey, with the following results.

Rhesus H 13 was inoculated subcutaneously with 1 c.cm. of formalinized vaccine on April 19th, 1928. It showed a rise of temperature of two to three degrees on the fourth and fifth days, but no other obvious reaction. On April 27th it was inoculated subcutaneously with 0.2 gram of infected liver from monkey H 11, which had died from the

disease, and after an incubation period of three days had a mild attack of yellow fever, with four days of fever followed by a subnormal temperature and then a return to the normal. Two untreated control monkeys, inoculated subcutaneously at the same time, one with 0.001 gram and the other with 0.0001 gram of the liver of monkey H 11, died of yellow fever after five and five and a half days respectively.

This vaccinated monkey was again inoculated on May 24th with approximately 1 gram of liver material from an infected monkey, and again showed nothing more than a slight febrile reaction, which was probably the result of the large dose administered. Further experiments with this vaccine are in progress.

Phenol Glycerin Vaccine.

Part of the liver and spleen of the same monkey (H 10) used in the preparation of the above-described vaccine was treated in a different manner. The organs were cut up into small pieces and washed well with normal saline in order to remove all traces of blood. The tissue was then ground up in a mortar with broken glass, and the resulting paste mixed with four times its weight of the following mixture:

Glycerin	...	...	...	...	...	600 c.cm.
5 per cent. phenol	...	...	...	...	...	100 c.cm.
Distilled water	...	...	...	...	...	300 c.cm.

The emulsion was then filtered through muslin and kept at room temperature for seven days, after which it was placed in the ice chest. This vaccine has been tested on seven monkeys, with the following results.

Rhesus H 12. This monkey was inoculated subcutaneously on April 19th, 1928, with 1 c.cm. of phenol glycerin vaccine which had been kept in the ice chest for twenty-four hours, and, except for one day, when it had a poor appetite, the animal showed no reaction as a result of the inoculation. Eight days later it received a subcutaneous injection of 0.2 gram of infected liver material from monkey H 11, but showed no reaction. Six days later it was again inoculated with 1 gram of infected liver, and on May 24th, five weeks after vaccination, received yet another inoculation of 1 to 2 grams of liver material from two monkeys which had died from the disease.

This monkey, therefore, has been inoculated on three separate occasions with enormous doses of the virus, but in no case was any rise in temperature observed, and the animal has remained in perfect health.

These results were so favourable that it was decided to

put this vaccine to as severe a test as possible in order to see whether its use could be recommended for protection against yellow fever.

On May 15th six rhesus monkeys were each inoculated subcutaneously with 1 c.cm. of vaccine which had been kept in the ice chest for four weeks. None of these animals showed any febrile reaction, although, as usual, their temperatures were taken every morning and evening.

On May 24th these six monkeys, and also four untreated controls, were each inoculated with approximately 1 gram of infected liver material from two monkeys that had died of the disease, one only four days after inoculation. In order to eliminate any personal equation, the inoculations were kindly performed by Dr. C. M. Wenyon, who was unaware which of the monkeys had been vaccinated and which were controls.

All the controls died of yellow fever with typical symptoms, one after four days, two after four and a half days, and one after five days.

One of the vaccinated monkeys which had received a double dose of the virus—about 2 grams—also died of yellow fever, but the other five monkeys have remained uninfected.

In view of the enormous doses these animals received it is evident, therefore, that phenol glycerin vaccine, prepared from the liver and spleen of infected monkeys in the manner indicated above, is likely to afford a simple means of protection against yellow fever in human beings. Further work is in progress on the duration of the protection in monkeys, and also on the length of time the vaccine will maintain its efficiency.

In conclusion, I should like to express heartiest thanks to Dr. C. Todd for many helpful suggestions, and to Dr. C. M. Wenyon, F.R.S., for providing me with accommodation, and also for much valuable assistance.

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TWO CASES OF HAEMATURIA CAUSED BY INSULIN TREATMENT.

BY

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So few cases have been recorded in which haematuria has been produced by insulin injections, or at least occurred during insulin treatment, that the following two cases seem worthy of mention.

CASE I.

A youth, aged 19, developed mild diabetes in August, 1927. This was easily controlled by diet without insulin until he developed severe tonsillitis and pharyngitis on November 7th. He was admitted to hospital at midnight on November 9th in a precomatose condition. He received 120 units that day, which completely abolished the glycosuria and reduced the ketonuria to the merest trace. On November 10th and 11th he received 50 units a day. In the urine passed at noon and in the evening of the 11th a considerable amount of blood was present—smoky appearance and red deposit. The blood was much less on the morning of the 12th, had almost disappeared at noon, and in the evening not a single red blood cell could be found. Again three days later (November 15th) blood appeared in the noon and evening samples of urine, but had disappeared by the next morning, and never returned. On November 17th the insulin was gradually reduced, and was discontinued altogether a fortnight later.

Throughout his period in hospital the urine contained no other abnormal constituent, albumin and white blood corpuscles appearing only when the blood was present and in commensurate amounts. The kidney function tests were normal, and casts absent. There was no history of previous haemorrhages in the patient, nor a family diathesis. He had a perfectly compensated mitral lesion due to previous rheumatism, but no other disease complicating the diabetes. The pharyngitis (pneumococcal) was almost better when the first attack of haematuria occurred and

quite better at the date of the second. The insulin used was Burroughs Wellcome's, and was being used on other patients without producing haematuria. No drug which might have produced haematuria was given.

CASE II.

A boy of 18, a severe diabetic, was admitted to King's College Hospital on March 5th, 1928, very emaciated and in a condition of severe ketosis. Slight proteinuria was present, probably as a result of the ketosis, because it disappeared when the glycosuria and ketosis were cleared up by large doses of insulin. On the sixth day of insulin treatment (25 and 18 units morning and evening) an obvious haematuria occurred in the specimen of urine passed at noon, four hours after the morning dose. Slight proteinuria and a few white blood cells were present, strictly commensurate with the amount of blood, and no casts were seen. A few red cells were present in the urine passed at 6 p.m. After this no further haematuria or proteinuria occurred. The kidney function tests—blood urea and urea concentration tests—were entirely normal. At the time of the haematuria a slight ketonuria was present (negative ferric chloride test), but much less than on the few days following admission. No septic focus was discovered and no drugs other than insulin were given.

Only seven cases of haematuria occurring during insulin treatment seem to be recorded in the literature. The condition must therefore be very rare, as it is not likely to be overlooked in a diabetic. All except our own two cases have occurred during the earlier days of insulin treatment—in 1923 and 1924, when impurities in the insulin were more frequent, especially abroad, and might have accounted for the haematuria. Thomson¹ mentions two cases in 1923, but gives no details except that they were on large doses of insulin. Henderson² also records two cases in 1923, in diabetic men aged 32 and 59, with no evidence of kidney disease and no complications; and another in a boy admitted in coma, in which case a few hyaline casts were present. The haematuria lasted only one day. Gudemann³ reports two cases; one of a man aged 50, who, after having been on insulin continuously for some months, suddenly commenced to have attacks of haematuria, which disappeared when the insulin was discontinued and reappeared whenever insulin was administered. This case was complicated by tuberculosis of the lungs, and arterio-sclerosis was found in the kidneys at necropsy. His second case of haematuria was also complicated by tuberculosis. Haemorrhages from other parts of the body have been recorded⁴ as occurring from the use of insulin—intestinal haemorrhage, cerebral haemorrhage, and two cases of death in coma, in which meningeal and lung haemorrhages were found. In these latter cases, however, there is no real evidence that the haemorrhages were caused by the insulin and were not merely incidental to its administration.

An analysis of all the few cases of haematuria in which any details are published does not reveal any common factor which would explain the occurrence of the haematuria occasionally produced by insulin. These were all, however, on large doses of insulin, and no case seems to have arisen where only small doses of insulin were being used. Concurrent infections were present in some cases, but not in all. The records are not detailed enough to gather whether a heavy ketosis was present in all the cases—an important factor, because it is well known that the excretion of ketone bodies will irritate the kidney at least to the extent of causing some protein and casts to appear temporarily from an otherwise normal kidney. Under such conditions insulin might be more likely to produce haematuria. In our cases ketosis had been severe, but had almost disappeared by the time of the first attack of haematuria, and was completely absent at the time of the second. Curiously enough all the cases recorded so far have occurred in males. It seems to occur both in the old and the young, so that arterio-sclerotic changes in the kidney have probably little to do with the production of haematuria by insulin.

Most of these attacks of haematuria have been trivial and of short duration, and have caused no inconvenience, except some alarm, especially to the doctor. There seems no reason, therefore, from the practical point of view, for discontinuing insulin either temporarily or permanently where it has caused haematuria.

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