cirrhotic liver, congestion of the fourth stomach and intestines to a greater or less degree, and sometimes a bile-stained appearance of the tissues, there were no changes of sufficient severity to account for death.

Animals affected with hydatids often remain in apparent health and good condition, but those suffering from distomatosis are generally emaciated and anæmic, skin dry, and bowels irregular, and this state renders them more susceptible to the attack of any other disease.

APHTHA.

During the winter months a contagious form of aphtha frequently makes its appearance among the cattle kept at the Laboratory for experimental use. The infection is brought by the hill cattle coming from the parts some 60 to 80 miles further in the Himalayas. The disease affects the muzzle, angles of the mouth, mucous surface of gums, lips, under surface of the tongue, and floor of the mouth. It is contagious and spreads very rapidly among the whole herd. It causes no elevation of temperature, and only affects the animal in so far that the lesions of the mouth render feeding painful, with a consequent loss of condition. The sores heal and the effect of the disease passes over in about three weeks. In the first stage vesicles are formed which exude a serous fluid, and these change into ulcers which become covered by a crust and take several days to heal. Animals suffering from this form of aphtha are less resistant to an attack of rinderpest, owing to their debilitated state. It is also frequently difficult to distinguish between the apthous ulcers and those of rinderpest, when situated in the mucous membrane of the mouth. The ulcers of aphtha tend to run together into a circular lesion about the size of a threepenny piece or a sixpence. They are also covered with a necrotic crust. In rinderpest the ulcers are clear-cut and pin-point in appearance, do not run together, and are not covered with crust.

DESCRIPTION OF PLATE VIII.

- a.* Trypanosomata found in blood of cattle.
- b.* Forms of the same in smears from the spleen.
- c.* Piroplasmata in red blood corpuscles.

*Drawn with Zeiss oil-immersion apochromatic 2 mm. lens. Compensating ocular 8. Magnification 1000 diameters.

THE PROPHYLACTIC TREATMENT OF RINDERPEST BY MEANS OF PREVENTIVE INOCULATION, MORE ESPECIALLY CONSIDERED IN REGARD TO THE CONDITIONS PREVAILING IN INDIA.

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It may be well, at the outset, to say a few words with regard to the peculiar conditions appertaining to the treatment of cattle disease in India. It is a matter of fairly common knowledge that cattle are popularly regarded as sacred in that country. This assertion serves

to describe a condition of things very antagonistic to strong prophylactic measures. It may be said that the vast majority of the natives of India regard with great repugnance the slaughter of cattle in any shape or form. Frequently they object to the performance of *post-mortem* examinations, and in some cases to minor operations. As a rule they are very apathetic, and a visitation of cattle plague is regarded from a purely fatalistic point of view. In severe outbreaks, however, it may happen that some of the more intelligent are not content to remain indifferent. In these cases the native treatment usually takes the form of some weird religious ceremony, from which some religious mendicant materially benefits. Passive, and occasionally active, opposition is usually displayed if Western preventive methods are attempted.

In spite of all this there are signs that preventive inoculation, with serum alone, against rinderpest is becoming popular. In the Punjab, where there is a large Mahomedan element, a large number of cattle have been successfully inoculated in actual outbreaks, and in other parts of India, and also Burma, inoculations have been carried out. The success and growing popularity of vaccination against small-pox in human beings has been a factor in paving the way. In course of time natives may report outbreaks of disease and ask for assistance. This is occasionally done now, but as a rule outbreaks are concealed. European cattle-owners generally ask for help, as might be expected. Cattle owned by Europeans are in quite an insignificant proportion, and need not be considered when discussing methods of prevention applicable to India generally. It is always possible to deal with each outbreak on estates belonging to Europeans on its own merits, in consultation with the persons concerned.

Rinderpest, as it appears in India, differs in many respects from the disease as seen in Western Europe or South Africa. It has, no doubt, existed there from time immemorial, and it is said to be closely comparable to the disease as met with in the Steppes of Russia and in Turkey. So far as the actual symptoms are concerned, there is probably no great distinction to be drawn. It is worthy of note, perhaps, that in India skin lesions appear to be uncommon, and the lesions in the mouth are probably rarely so severe as those seen in Great Britain, if the models exhibited of the disease, as it appeared in the latter country, are accurate.

The disease in India is endemic, and in the plains the mortality among animals attacked is not large compared with that experienced in South Africa and Great Britain. From a large number of figures it is found that the average mortality in the plains of India is from 40 to 50 per cent., but in the Himalayas it is about 90 per cent.

Wide variations in mortality are met with in different parts of India, and even in neighbouring villages. The virulence of the disease and the immunity of the cattle vary to such a degree that it is practically impossible to fix doses of certain preventive materials, when such a proceeding is indicated, except after experiment upon the scene of the outbreak. This has rendered the application of certain methods of preventive inoculation a complicated and difficult matter.

The virulence of the disease is usually greatest in the cold season, and it is always most marked at the commencement of an outbreak. Nevertheless, outbreaks occur in the hills without any apparent

decrease in the virulence of the disease. It is obvious that this variation in virulence is explained to a great extent by the fact that the most susceptible animals are sure to be first attacked, but some observers are strongly of opinion that the virus has a tendency to become attenuated towards the end of an outbreak.

The few facts recounted above will show that in instituting a method of preventive inoculation for rinderpest in India many points had to be taken into consideration, and that there were many difficulties to be surmounted. In addition, many other objections to energetic measures existed. They were chiefly financial and political, and need not be discussed in this paper. Before inoculation work was instituted in India much had been done in South Africa and other places, and some of the methods could be regarded as having gone beyond the experimental stage. It was thought advisable, however, to make a series of experiments both in the laboratory and in the field, for reasons given above. Experimental work in India with regard to preventive inoculation against rinderpest dates from 1897, when Professor Koch gave a demonstration at Muktesar laboratory on his way back from South Africa.

From that date preparations were made for experiments on a large scale. Drs Lingard and Rogers are the principal names connected with the laboratory experiments, and they and certain officers of the Indian Civil Veterinary Department are responsible for the field operations.

The different methods of preventive inoculation will now be put forward in turn.

The routine of each method will be explained and its applicability to Indian conditions discussed. Methods of inoculation practised before 1896 will be passed over. They were crude, and consisted in direct transmission of the virus of rinderpest from animal to animal by various means. An exception to this statement may be made in regard to the experiments of Semmer in Russia in 1895. This observer ascertained that the blood of an animal that had recovered from rinderpest exercised an immunising influence if injected into a healthy animal, and Pitchford and Theiler confirmed this in South Africa in 1896. Severe losses from rinderpest in South Africa resulted in Professor Koch visiting that country in 1896, and protective inoculation on a large scale may be said to date from that time.

I.—KOCH'S BILE METHOD.

In a series of reports to the Secretary for Agriculture, Cape Town, Koch¹ described a number of experiments that he had conducted. The greatest prominence was given to a method of preventive inoculation with the bile from an animal suffering from rinderpest. It was one of the earliest methods instituted in the field, and, until superseded by others, popular, and considered hopeful. Koch does not seem to have stated anywhere why he tried bile as a protective agent. In his second report, however, when discussing a ready method of transmitting the virus of rinderpest from one animal to another, he says: "Experiments were also made with bile which was taken from animals that had succumbed to rinderpest, and which was injected in the subcutaneous tissue. The motive for

so doing was because a mixture of bile or other liquids was said to be sometimes used by Free State farmers, and also the circumstance that in the bile of most of the cases examined I have found, in pure cultivation, a bacterium which, according to the description published, is conformable with the microbe discovered by Dr Simpson in Calcutta, and declared to be the cause of rinderpest."

These experiments with bile had negative results, and Koch went on to say in the same report: "We are consequently justified in saying that bile does not contain the contagion of rinderpest, and that Simpson's bacterium cannot be considered to be the microbe of rinderpest."

The above statements are important in view of the question of the kind of immunity conferred by bile inoculation. Probably these experiments led Koch to try bile as a preventive. It was in his fourth report that he spoke of the use of bile in this way, and his method is best explained in his own words, which are: "One is able to render immune healthy cattle with the bile of such that have succumbed to rinderpest. In this case one hypodermic injection of 10 cc. is sufficient. This immunity sets in on the tenth day at latest, and is of such an extent that four weeks afterwards 40 cc. of rinderpest blood could be injected without any injurious result. I therefore conclude that the immunity produced in such manner is of an 'active' nature."

It will be seen from these statements that although Koch reported that bile did not contain the contagium of rinderpest, it was yet able to produce active immunity. As a matter of fact, it was later conclusively proved by Kolle² that rinderpest bile does contain the virus of the disease. The same observer also stated that bile of the third or fourth day may produce rinderpest.

It was found by Koch that only the bile from animals that had been ill for six days could be used for protective work. It may be accepted as a fact that all rinderpest biles do contain the virus, but that after the fifth day of the disease it is modified by the formation of some kind of protective substance formed within the bile.

Lingard³ says the passive immunity conferred by the injection of rinderpest bile is brought about by the action of the contained nucleo-proteid and lecithin, and the degree and duration of the immunity bear a direct ratio to the quantities of these bodies in the injected bile. He says that "the contagium of rinderpest only effects an entrance into the bile in the gall bladder on the fifth or sixth day of the disease," and "when a sixth day bile is utilised, with the *materies morbi* of rinderpest present, the contagium is more or less kept in check by the action of the nucleo-proteid present, and produces in the injected animal a very modified form of the disease, but nevertheless the result is a transitory *active* immunity, which may persist for a period of three to five months, according to the degree of virulence possessed by the contagium in the injected bile." The same observer claims to have conferred a passive immunity on plains cattle and buffaloes by successive doses of normal bovine bile containing nucleo-proteid and lecithin in sufficient quantities.

Koch used the biles of animals that had been inoculated with rinderpest and were killed on the sixth day of the disease. He stipulated that only biles with certain characteristics should be used.

They had to be clear, of a dark green colour, sweet smelling, and to give a white froth on being shaken up. They had to be collected aseptically in sterile flasks. Bile of this description could not be kept long, even in ice. In South Africa, to obtain bile answering to this description, 3 to 7 per cent. of herds were sacrificed. Rogers⁴ found in India that only 17 per cent. of biles from animals that died from rinderpest were fit for use by this method. Later, Turner and Kolle⁵ said that all biles not foul or bloody were safe, and these authorities recommended the mixing together of biles, and found that the great majority of biles obtained from animals slaughtered on the sixth day of the disease were fit for use by Koch's method. Animals that die rarely supply suitable bile for Koch's method. Hence, in order to obtain a large quantity of suitable bile slaughter is necessary. It is no doubt advisable, as Turner and Kolle⁵ recommended, to mix all apparently suitable biles together before use.

Although in India it was not found that biles of six days or upwards ever produced the disease, there was a good deal of rather conclusive evidence that in many cases in South Africa the disease was produced by bile inoculation. It is very unlikely that biles of suitable age, even if blood-stained, do so, because Koch himself and others found that bile and virulent blood, in equal proportions even, could be injected without producing disease.

It is possible that, in those cases not due to natural infection, young biles, *i.e.*, taken before the sixth day of the disease, were inadvertently used.

The injection of the bile is usually made in the dewlap, and a local swelling the size of a cricket ball results. The swelling is hard and painful, but gradually subsides. No systemic disturbances are usually seen. A local abscess may result if the bile is in a state of decomposition, but this does not seem to be detrimental to the process of immunisation. Larger doses than 10 cc. do not appear to increase the immunity conferred. An injection of bile does not surely produce immunity until ten days have elapsed since its injection, though immunity may in some cases be produced on the sixth day. Animals which may have contracted the disease before the injection, and which may be exposed to infection before ten days have elapsed, may die from rinderpest.

The duration of the immunity conferred in successful inoculations in South Africa was put down to average about four months. Occasionally it was only three weeks, but even in a single herd inoculated with the same bile the respective periods of immunity were found to vary. Whether preventive inoculation according to Koch's bile method produces an active or a passive immunity must be left an open question. The important point is that the immunity conferred is not a lasting one, and in some cases it is of very fleeting duration.

Results.—No doubt excellent results from this method were obtained in certain cases in South Africa. Turner and Kolle⁵ reported that in Basutoland 70,000 out of 100,000 animals were saved, of which 90 per cent. would have perished, if the disease had not been checked. However, it appears to have been practically discarded for the simultaneous method of Turner and Kolle, which is discussed later.

Nicolle and Adil Bey,⁶ working in Turkey, did not care to use bile if serum were available. The method does not appear to have gathered

adherents in Russia either. A number of experiments were made by this method by Rogers⁴ at Muktesar Laboratory in India. Both plains and hill cattle were experimented on. The former class are most unsatisfactory as experimental animals, owing to a large percentage being partially or wholly naturally immune. With regard to plains animals, it was found that those immunised by Koch's method were afforded an immunity of between three-and-a-half and five months—probable average four months.

These results agreed very closely with those obtained in South Africa, Russia, and Turkey. Raymond⁷ (Bengal) claimed to have produced immunity of a year's duration with bile taken from buffalo calves which had been inoculated with rinderpest, but the results appear to have been by no means conclusive.

With regard to the hill animals used by Rogers⁴ in bile experiments by Koch's method, it was found that little or no immunity was conferred. Animals tested at periods of less than four and three months, respectively, died of virulent rinderpest, and it was even found in one case that no immunity was 'existent on the tenth day after the bile inoculation.

It should be mentioned that the biles taken for Rogers' experiments were taken immediately after death. Death usually occurred in the experimental animals on the seventh or eighth day, and biles of this age were found as satisfactory as those which happened to be obtained on the sixth day.

Most of Rogers' experiments were, however, done with biles from animals that died on the sixth day. In some of his experiments the bile was used on the same day as obtained, whilst other biles were kept from three to five days in the cold room of the Laboratory. Again no difference was observed in the results obtained. Rogers confirmed the statement that biles taken on the third and fourth day of the disease conferred no immunity in hill cattle.

So far as field work in India is concerned, inoculation by this method was never general. Hagger⁸ inoculated 200 animals at the Government Cattle Farm at Hissar; 22 of these animals were attacked between the first and the tenth day, of which two died. He admitted that the disease was dying out at the time of inoculation, and said, moreover, that the bile used was neither clear nor of the stipulated colour. The serum method was preferred by this observer, hence few inoculations with bile were performed.

Raymond inoculated 261 animals belonging to a European planter in Bengal with buffalo bile with successful results. He found that in the large majority of cases immunity was conferred for an average period of five months. Later he⁷ found that some animals were still immune at the end of a year.

No other field work with the bile method in India appears to have been practised. There were many objections to it, as explained later, and other methods were preferred.

Advantages of Koch's Bile Method.—No systemic disturbance of importance in normal cases results, and, consequently, there is no loss of milk in cows, or fear of abortion. There is little fear of spreading rinderpest or other diseases by this method when operating on clean herds. This assertion is based on the assumption that bile suitable for injection purposes does not convey rinderpest. One inoculation

only is required, whereas in some of the other methods two or more are necessary.

Disadvantages of the Method.—The disadvantages appear to outweigh the advantages under any conditions, but in India the difficulties associated with this method are well nigh insurmountable. If the immunity conferred had proved to be a lengthy one, no doubt strenuous efforts would have been made to overcome the difficulties, because in one respect the method is peculiarly applicable to that country. That is, that blood is not brought into use at the time of inoculation. A great disadvantage is that immunity does not become absolute until ten days have elapsed after injection. This renders the inoculation of contact animals in diseased herds a delicate proceeding. Some animals would be sure to become diseased from natural infection within that period, and the ordinary Indian villager would put the worst possible construction on this. No doubt the method might have proved useful in healthy villages, but this plan would rarely be adopted, as the native believe in "leaving well alone," and if accidents occurred in this case the remedy would be much worse than the disease. The difficulty in obtaining bile is very real in India. For Koch's method bile must be fresh, and therefore it is practically necessary to obtain it on the spot. Slaughtering cattle in villages is out of the question. This difficulty might be got over by slaughtering cattle, or preferably buffaloes at certain depots, and no doubt the question of the preservation of the bile could be managed for at least a short period. A certain amount of bile can generally be obtained at the scene of an outbreak from dead cattle, but an immediate *post-mortem* when death takes place is indicated, and this might not always be convenient. In this case, also, it would be a difficult matter to judge how long the animal had been ill, and in virulent cases biles of the fourth and fifth day might come to be used. As a matter of fact, it is possible to take bile from the living animal fairly easily.

It was found by Rogers⁴ that from seventy-six cattle that died of rinderpest on the sixth, seventh, or eighth day only twenty biles were suitable for use by Koch's method. The average amount of bile collected from these twenty cattle was 190 cc. A simple calculation will show that seventy-six hill cattle would therefore only supply sufficient bile for 380, or 20 per cent. Plains cattle would no doubt furnish much more, as they are larger, but so many would be found to be immune from rinderpest that much disappointment would arise. This difficulty could be got over to some extent by using buffaloes, but these animals are much more expensive and a large percentage are immune. To sum up, Koch's bile method in India is practically only suitable for cattle kept under European management, and it is even then by no means ideal.

II.—GLYCERINE BILE METHOD OF EDINGTON.

It was found by Koch¹ that glycerine destroyed the virulence of rinderpest blood. Edington,¹⁰ therefore, took advantage of this fact to mix glycerine with bile in order to destroy the virus of rinderpest in that fluid. The belief that bile was capable of frequently causing fatal attacks of rinderpest, referred to above, was sufficient reason to

justify the employment of a modified bile incapable of spreading the disease. Although the immunising properties of this mixture were greatly modified, yet Edington found that glycerinated bile in doses of 15 cc. gave sufficient protection to allow of 0·1 cc. virulent blood being inoculated ten days afterwards.

Glycerinated bile (Edington) is prepared by mixing two parts of bile with one part of glycerine, and the mixture has to be kept for eight days before being used. It was also advised that, in addition to the blood inoculation on the tenth day, a further dose of 1 cc. of virulent blood should be inoculated to make the immunity conferred stronger still. Many herds in South Africa are said to have been protected by injecting glycerinated bile in doses from 15 to 20 cc., according to the size of the animal, followed ten days after by a small dose of virulent blood.

The immunity conferred is dependent upon a reaction occurring from the dose of virulent blood, but an important point is that if a too strong immunity is conferred by the glycerinated bile no reaction occurs. In the latter case immunity is very short and bad results may accrue.

It was reported that 393,777 animals were inoculated in South Africa with a loss of only 8·25 per cent. in twenty-three districts in which it was extensively used. Edington¹⁰ claimed that by this method more biles could be used than by Koch's method; but, with regard to the latter, later observers showed that more kinds of biles could be used than Koch originally allowed. Turner and Kolle⁶ admitted that glycerinated bile possessed feeble immunising powers owing to certain chemical substances contained in it. The bile is, however, deprived of all active immunising power, as the glycerine kills the virus contained in the gall.

Results.—In India Rogers⁴ inoculated a series of twelve plains cattle by this method, and in only four did any sign of rinderpest follow an inoculation of virulent blood ten days afterwards. No severe symptoms occurred in any of these animals. It may be allowed that they could claim to have attained a very strong immunity. Eight animals did not react, and consequently they were probably only protected for a short period. It is possible that in these cases some of the animals were naturally immune, a difficulty commonly met with in plains cattle.

Out of twelve hill animals inoculated by Rogers⁴ with glycerinated bile, nine died after the blood inoculation, and two were very ill. He therefore "regretfully admitted that the glycerine bile method not only fails to confer immunity on Kumaon hill cattle, but actually is fatal to them as frequently as is inoculated rinderpest."

No inoculations of any account were performed in the field in India by this method. Rogers⁴ mentions that Walker did three animals at Bareilly with satisfactory results.

Advantages of glycerinated bile.—Biles can be kept for a long period ready at hand; preserved in this way, it is claimed that they retain their properties for upwards of a year.

If a definite reaction is obtained after the blood inoculation an active immunity of long duration is conferred. A larger percentage of biles can be used than in Koch's method.

Disadvantages of glycerinated bile.—As in Koch's method, immunity

is not conferred for ten days after the inoculation has been performed. Many animals fail to react after the blood inoculation, and, consequently, immunity of short duration is conferred. The period is probably much less than with Koch's method. On the other hand, sometimes very severe reactions occur, a state of things which Indian cattle owners would not appreciate.

Much time is lost, viz., eight days, before the bile is ready for use. It was found in India that the method was dangerous in hill animals.

A double inoculation is necessary, which makes the method cumbersome, and certainly distasteful to cattle owners. An inoculation of blood is required, which entails the risk of spreading not only rinderpest but other diseases. To a great extent the same objections with regard to obtaining the bile in the method of Koch also apply here.

III.—OTHER METHODS ENTAILING THE USE OF ORDINARY OR MODIFIED BILES AND MIXTURES OF BILE AND OTHER FLUIDS.

In order to avoid risk of infection from bile, Rogers⁴ passed a *mixture* of biles through a porcelain filter. Other observers in Turkey and South Africa had previously found that filtered blood was no longer virulent. Rogers used all biles except those red with blood or light brown in colour. He inoculated with small doses of virulent blood after ten days had elapsed from the injection of bile, as in Edington's method. The results obtained were similar, but somewhat better than those in the glycerinated-bile method. The method practically possesses the same advantages and disadvantages as the latter. The time lost in the process of glycerinating the bile is saved, however. Biles sterilised by heat can be utilised in the same way, and they can be kept for a long time by adding half per cent carbolic acid.

The brothers Krause¹³ contended that simple rinderpest bile, as a preventive method, was superior to any other. They were convinced that it was quite capable of producing the disease, and that when it did so the period of incubation was eight days. If the immunising substances in the bile were sufficiently powerful no outbreak of disease would occur. They did not recommend the use of bile in herds already attacked with the disease. They advocated the use of serum as a curative if rinderpest appeared among cattle inoculated with bile.

They used all biles not red or foul, and made two inoculations. The first inoculation consisted of 8 cc. of bile three to seven days old, and twelve to fourteen days after a second inoculation of 15-20 cc. of bile twenty-four hours to five days old had to be given. Ten days after the last bile inoculation it was advised that 5 cc. of virulent blood be inoculated. They claimed for this method that it conferred an immunity of two months duration by the bile inoculations, and a longer period if 5 cc. of virulent was used. The method was complicated, and it seems never to have been used in the field to any appreciable extent.

The brothers Krause also considered that it was possible to prepare an artificial rinderpest bile.

Their formula was:—To an immunised serum add 5 per cent normal bile salts, or healthy bile and mucin. Add 10 per cent virulent rinderpest blood, and allow to stand for twenty-four hours.

Koch¹ made several experiments by mixing larger or smaller quantities of virulent rinderpest blood with rinderpest bile, and found that bile was able to make a considerable quantity of rinderpest blood innocuous. He obtained immunity after an injection of 5 cc. bile and 5 cc. virulent blood. It was thought that if bile were mixed with virulent blood it would be strengthened as a protective agent. This supposition does not seem to have been borne out by experiment.

Rogers⁴ performed a number of experiments on Indian cattle with mixtures of bile, blood, and fortified serum. His main idea was, if possible, to manufacture an artificial bile. In the first place, he found that in order to neutralise rinderpest blood, if injected into susceptible animals, about ten times as much serum as blood was necessary.

He then found that 1 cc. of a bile solution (made by dissolving the ox gall of the British Pharmacopœia in sterile water until the specific gravity was 1020.), neutralised 10 cc. of virulent blood. Varying mixtures of serum, blood, and normal bile were then used, but no success in conferring immunity was obtained. The same observer also tried mixing filtered bile and virulent blood. The experiments were not successful. In one animal a mixture of 20 cc. of virulent blood and 5 cc. of filtered bile did not cause the disease, yet the animal died on being inoculated with rinderpest blood ten days afterwards. Unsuccessful experiments were also made with mixtures of ordinary bile, filtered liver extract from an animal dead of rinderpest, and virulent blood.

If one sums up the different methods necessitating the injection of bile, modifications of bile, and mixtures of bile and other fluids, the following deductions seem indicated. Biles that answer to the characters prescribed by Koch in his method, and perhaps other biles to a less degree, contain substances, probably of an albuminoid nature, which inhibit the virus contained in such biles.

After inoculation, absorption of the virus, modified in some peculiar way, takes place, and immunity is conferred. In certain cases it is to be presumed that the virus enters the system in an unmodified form, and disease results. The peculiar modification of the virus in successful inoculations is such that no symptoms of the disease appear, and a passive immunity of rather long duration is produced. Lingard³ prefers to call this a transitory active immunity.

Artificial modifications of bile may render the operation of inoculation safe, in the sense that the disease itself is never conferred by them. They have, however, little protective influence as compared with Koch's unmodified bile. Lingard's experiments with normal bile appear to have been confined to plains cattle and buffaloes, many of which are found to be wholly, or partially immune.

With regard to mixtures of bile and other fluids, with the idea of preparing an artificial bile, no remarks seem necessary, as the experiments so far have failed.

A very striking point brought out, however, is the very large relative quantity of virulent blood that can be neutralised by mixing it with bile. It took ten times as much serum to neutralise virulent blood, but one part of normal bile neutralised ten parts of virulent blood (Rogers). No immunity was conferred, which points to the virus having been destroyed *in situ*. It may be mentioned that in these experiments large swellings were produced where the mixtures were injected. The local inflammation probably had a good deal to do with

the destruction of the virus. It is possible that the local swelling induced in Koch's method has more to do with the results attained than appears at first sight.

IV.—THE SERO-THERAPEUTICS OF RINDERPEST.

Semmer, in 1895, working in Russia, ascertained that the blood of an animal that had recovered from rinderpest possessed passive immunising qualities if injected into a healthy animal.

Pitchford and Theiler in Natal, and later Danysz and Bordet in the Transvaal, and Spreull and Campbell in Cape Colony, used the defibrinated blood of animals recovered from the disease to confer protection, with a considerable measure of success. The immunity conferred was, of course, only of a passive nature, but in many cases it was turned into an active one by infecting such protected animals with the disease by mixing them with diseased cattle, or by smearing the discharges of such animals on their noses.

At first, in South Africa, defibrinated blood collected on the spot was used, but later serum depôts were formed and serum supplied.

It is obvious that defibrinated blood collected in the field possessed many disadvantages, especially as it appears to have sometimes been obtained in a very crude manner. Defibrinated blood is weaker than serum in the relation of 30 cc. to 20 cc. (Turner and Kolle⁵). Koch¹ (fourth report) used the serum of salted animals in doses of 100 cc. to protect against experimentally inoculated rinderpest. He obtained the serum by the clotting method. In order to keep it, he recommended drying it in a vacuum apparatus. Defibrinated blood had to be used in doses of 100-200 cc., and its strength was very uncertain. Koch and others consequently preferred bile.

It was not until Turner and Kolle⁵ perfected a method of preparing a fortified or hyper-immunised serum that methods involving the use of serum became popular in South Africa. These observers claimed to manufacture a serum of such a strength that 20 cc. was efficacious as a cure in the early stages of the disease, and 50-100 cc. in the later. It had the additional advantage that it could be kept without losing its qualities. It was prepared by giving to a salted animal increasing doses of virulent blood up to 1000 cc. Successive doses of 100, 200, 500, and 1000 cc. were injected subcutaneously. The larger dose in each case was given after the reaction from the smaller one had subsided.

After the last injection the animal was bled once a week, for three successive weeks, and the fortifying process again resorted to in larger doses. Half per cent. carbolic acid was added to keep it.

By the method of Turner and Kolle some time is lost (nearly two months) in fortifying an animal. Nicolle and Adil Bey⁶ used what they termed the "brutal" method instead, and prepared fortified serum in fifteen days. They gave 4 litres of virulent blood and 25 cc. of serum by the simultaneous method (*see* later), and bled fifteen days after, *i.e.*, when the reaction had subsided. They bled animals fortified in this way every week for six weeks, and found that serum at the sixth bleeding was as strong as that of the first. Doses of 25 cc. were found to protect against a dose of virulent blood given a few days later, or from natural infection. Later they used as much as 8

litres of virulent blood at one injection to fortify, and found the serum still more active.

In India, serum was first prepared by Rogers⁴ according to the directions of Turner and Kolle. The "brutal" or rapid method is now usually employed, using both cattle and buffaloes. The blood is defibrinated in the flask and then centrifugalised. The serum is then carbolised and placed with other anti-rinderpest serum in bulk. When a quantity has been collected it is tested and bottled off. Serum-making animals have their temperatures taken night and morning, and unless they are perfectly healthy their blood is not employed. Lingard¹⁵ advised that animals under three years of age should not be used, as "they yield a serum the protective power of which may be five to eight times less than that yielded by the same breed of animal above three years of age." The same observer also said that: "By the rapid method large doses of virulent blood injected into the serum animal do not yield proportionate results in producing a high protective serum. For, it has been found that, if doses over 6000 cc. to 7000 cc. are injected at one time, a weak or attenuated form of serum is the result."

Nicolle and Adil Bey⁶ considered that the cattle of the steppes, being less susceptible to rinderpest, were most suitable for serum-making, as they would produce a stronger serum. This was not found to be the case in India, where the hill animals produced the strongest serum.

Lingard¹⁵ found that sera rapidly lost their qualities when exposed to the high temperatures registered in the plains of India during the hot season. At Muktesar laboratory, however, at an altitude of 7600 ft., the sera were found to retain their protective qualities for long periods. In preparing serum care should be taken to select good, strong, healthy animals, and the aim in view is to prepare as strong a serum as possible. Large doses of virulent blood should be injected with the object of obtaining good temperature reactions. Larger doses of virulent blood than are absolutely necessary are waste of material. It is important to see that the blood used is free from the virus of any other disease than rinderpest. It should be taken at the height of the fever, before diarrhoea commences. It should be defibrinated, strained through muslin, and injected as soon as possible, with due aseptic precautions. It is well not to inject too much blood in one place, or absorption is delayed and abscesses may result. The curative effects of even very strong serum are not very great. When once diarrhoea has set in no amount of serum appears to exert any restraining influence on the course of the disease. On the first or second day of the fever, before other symptoms have set in, fortified serum in doses of 100 to 200 cc. sometimes checks the course of the disease.

The Use of Serum Alone as a Preventive.

As mentioned above, both defibrinated blood and serum were used in South Africa by several workers. Danyasz and Bordet are said to have salted many animals in the Transvaal with serum alone. In South Africa it was given up almost entirely in favour of the simultaneous method of Turner and Kolle (referred to later). In Turkey

Nicolle and Adil Bey,⁶ and Nencki,⁴ in Russia, used fortified serum alone, and appear to have preferred it to any other method. The immunity conferred was said to last for several months, and the herds were at any rate protected through the particular outbreak to which they were exposed. The conditions of rinderpest in Russia closely resemble those in India, and, as it was considered that serum alone was an effectual method in that country, it was likely to suit Indian conditions also. With regard to the dose of serum required to protect, that must depend upon the strength of the serum, the size and breed of the animal, and the period that one wishes to protect for. In India the strength of the serum is tested according to the simultaneous method of Turner and Kolle. By this means it is found what quantity of serum will protect an animal from a severe attack of the disease, the disease having been given at the same time as the serum. The susceptibility of the different breeds varies so much in India that doses vary accordingly. Eighteen times more serum was found to be required for hill animals than for ordinary plains animals, and the dose is usually fixed in that ratio. With regard to the susceptibility of other breeds in different parts of the country, experiments are still being made to enable some sort of scale to be prepared. The serum is usually sent out at so much per animal of 600 lbs. as was the custom in South Africa. Fairly accurate estimates of the weight can be made by measurement, in the absence of weigh-bridges. It is not practicable, of course, to weigh or measure more than a few animals, nor is it necessary. The inoculator soon learns to give a fairly accurate guess at the weight of an animal at sight, and for the serum alone method great accuracy is not required. The more serum that can be given the better, within limits, but serum is rather a valuable commodity, and has to be used with discretion. In the serum alone method it is usual to give rather more than the dose specified as correct for the simultaneous operation, and a minimum of 5 cc. is the rule.

Working in India, on no account should sick animals be inoculated, as that might tend to destroy the confidence of natives. All contact animals that have not recovered, or that are not ill, should be inoculated and then all mixed together. Some animals may thus become sick and acquire an active immunity. Disinfection of the cattle sheds and surroundings is essential, or in course of time the inoculated animals may be re-infected. In India the hot sun is a great factor in this respect.

Results of Serum Alone in India.—As in other methods, experiments were first made in the laboratory, and then tried on cattle belonging to Government or public bodies before inoculations were applied generally. In the field Walker⁴ in 1899 inoculated thirty-one cows and thirty-eight milch buffaloes at the Bareilly Lunatic Asylum Dairy. This method was used instead of the simultaneous because it was desirable that the milk supply of the dairy should not be interfered with. No cases occurred after the inoculations, and the milk, which was measured, did not fall off in any appreciable degree. During 1900 Hagger¹⁰ inoculated over 1000 animals by this method with successful results. Since then the method has become fairly general in India. Between April 1902 and April 1903¹⁰ 24,457 animals were inoculated in actual outbreaks in the Punjab, out of

which only 2 died after inoculation. Villages in which inoculations took place were visited regularly for three months to watch results. In the same year 11,472¹⁷ animals were inoculated in Bengal, and the total for the whole of India and Burma came to 42,871, with 139 deaths. The uninoculated animals which died during the course of the outbreaks (as far as could be ascertained) came to 1074. These results speak for themselves, and although, no doubt, the method is far from ideal, it has been of great service in India, as it is peculiarly adapted to the conditions met with in that country.

Advantages of Serum Alone.—It affords immediate full immunity, and it may cure disease in the incubative period.

The material used can be stored and kept ready for use. It can be transported in bottles and used as *medicine*. This is an important point in India, where medicine is rather popular than otherwise. The patent use of body fluids would not be regarded with favour. The simplicity of the method is a great advantage, as most of the inoculations have to be done by the native staff. There is very little fear of disease being conveyed, or any septic infection being set up, local or otherwise. No great accuracy in determining the dose is required so long as a sufficiently large one is given.

One inoculation only is necessary. Animals can be worked or milked just as usual.

There is a practical certainty of getting good results (within a limited period) if the inoculations are carried out properly.

The cattle owners are put to little or no trouble themselves in the matter, as they would be if any segregation were necessary, or the animals became ill. The method inspires immediate confidence, being so harmless.

Disadvantages of Serum Alone.—These are practically summed up when one has stated that the immunity conferred is of rather a short duration. In most cases, however, if sufficient doses of serum are given, the immunity conferred is long enough to carry over the particular outbreak. Occasionally, if the disease is particularly virulent, or the serum particularly weak or scanty, unfortunate results may occur.

Serum Simultaneous Method.

This method was instituted by Turner and Kolle,⁵ and consists in injecting simultaneously a certain dose of serum on one side of the animal (usually behind the shoulder) and a dose of virulent blood on the other side, or in another part of the body. The object in view is to pass the animal through a slight attack of the disease, and thus confer an active immunity. The dose of serum has to be determined with some accuracy to bring about this result. If the amount injected be too small the animal is likely to get a severe, or even fatal, attack. On the other hand, if it be too large no reaction occurs, and simply a passive immunity results.

In South Africa the dose of serum could be determined with comparative ease, as all the animals were practically equally susceptible to the inoculated virus. The serum used was fortified serum, as first prepared by Turner and Kolle. It was tested at the *dépôt*, and at the time of injection the quantity to be given was regulated according to the weight of the animal. The weight had to

be determined by measurement. If a too severe reaction occurred more serum could be injected for its curative effect.

For this method fresh virulent blood is necessary. It can usually be obtained on the spot, but in India this is often rather a difficult proceeding, requiring great tact. Virulent blood should be drawn from an animal with a high temperature and suffering from rinderpest. It should not be taken if diarrhœa has commenced. It is important to be quite sure the disease is rinderpest, and the animal is not suffering from tick fever or any other disease as well. Rinderpest blood rapidly loses its activity—it cannot certainly be kept virulent more than three to four days at summer temperatures, or more than six to eight days in ice, and a high temperature or dessication destroys the virus rapidly.

Defibrinated or citrated blood ($\frac{3}{1000}$) in volume of 200 cc. remains virulent during twelve days at 15° to 18° C. (Nicolle and Adil Bey.⁶). Blood can be conveniently carried about in a live sheep inoculated with rinderpest. Turner and Kolle claimed that 90 per cent. of the animals inoculated in South Africa reacted. It is said that many thousands were treated, particularly in Rhodesia, with a loss of only 1-2 per cent.

Results of the Serum Simultaneous Method in India.—The laboratory experiments performed by Rogers⁴ at Muktesar proved that animals that reacted attained a very high degree of immunity. It was found that even if the reaction was purely a thermal one, and no other symptoms occurred, a very strong immunity was conferred. This was useful information, as it was not desirable to produce severe symptoms, on account of the ignorance of the cattle owners. In South Africa as severe an attack as possible consistent with safety was the object. In order to test serum, repeated inoculations were, and are, made with cattle at Muktesar by the simultaneous method. Many of the cattle are tested with virulent blood at long intervals afterwards, to see if they are still immune.

Although some of the plains animals inoculated by Rogers reacted, yet a great many failed to do so with relatively similar doses. The weights of the animals were accurately determined on the weighing machine. These uneven results were put down to the variation in susceptibility in these cattle.

Most irregular results were got with hill animals also by this observer, and he reported that the method was not reliable in this particular breed of animal. The reason was probably that at that time the enormous difference in susceptibility between these breeds of cattle had not been recognised. It was found later that fairly safe reactions could usually be obtained in hill cattle if sufficient serum was used. Lingard considers eighteen times the dose required for plains cattle necessary for hill cattle. Luckily the latter are generally small, but even then they often require a larger absolute dose than plains animals.

In 1899 Rogers, Hagger, Holmes, and Walker performed some inoculations in the field by this method, with, in most cases, very satisfactory results. In other cases reactions did not occur, or they were not detected. These inoculations were, of course, performed with great care, and under the direct superintendence of the officers themselves, as at that time the process was regarded as experimental.

Hagger,¹⁶ in 1900, however, had bad results in the Punjab, 24 animals dying out of 128 inoculated by one of his native assistants. This observer condemned the method for India as unsuitable, and recommended serum alone as more likely to give good results. The method is now rarely used in India, though there are occasions when it can be usefully employed. This is especially the case when one inoculates cattle belonging to persons who are capable of understanding its dangers, and are content to run some risk in order to obtain a distinct advantage.

In many ways the method is ideal, but, to be practised with success, a uniform scale of susceptibility in the cattle and a certain degree of intelligence in their owners are necessary.

Advantages of the Serum Simultaneous Method.—Immediate immunity is conferred. In successful inoculations—i.e., when good reactions occur without dangerous symptoms arising—the immunity conferred is active, and probably persists in most cases for the animal's life. Even when reactions do not occur the inoculation has not been without service, though it may be disappointing. Practically only one inoculation is required. The serum can be kept ready for use, though the blood is sometimes a difficulty.

Disadvantages of the Serum Simultaneous Method.—There is a certain amount of risk in the method, especially in countries where the cattle vary in susceptibility. It requires a good deal of skill and care to bring about good results in such countries. Consequently, in India only a selection from the native veterinary staff could be safely trusted with it on their own account. As a matter of fact, in that country, before inoculating any number of animals in a certain locality by this method, the serum should be tested on a few animals on the spot, to arrive at the correct dose. Even then the operator might be disappointed by irregular results, and valuable time would have been lost.

That virulent blood has to be used is rather a serious disadvantage in India. In the first place, it has to be obtained. In some cases this difficulty is only surmounted by taking the blood with one, and then it may not be fresh.

It is obvious to the most casual observer that blood is being used, and the ordinary Indian agriculturalist has scruples in this respect. They can usually be got over by judicious argument, but if any deaths do take place it is quite apparent to the owner that the inoculator caused it by injecting blood. It is possible to spread the disease in this method through the medium of the reacting animals.

Other Methods Entailing the Use of Serum.

Hutcheon¹² regarded the serum simultaneous method of Turner and Kolle as ideally perfect, but found it was difficult to invariably ensure the requisite reactions to justify that character. He therefore adopted the method of giving the virulent blood forty-eight hours after the serum, in the hope of ensuring more constant reactions. In many cases a second injection of serum was found necessary in this method, which made it complicated.

Rogers⁴ recommended a reinoculation of virulent blood in animals that did not react in the simultaneous method. The blood has to be given ten days after the double inoculation of blood and serum.

He used rather large doses of serum in the first inoculation to guard against mortality, and claimed that all animals that did not react at first would when reinoculated.

This method is also complicated, necessitating careful watch being made for reacting animals, and in many cases a second inoculation.

Koch¹ (fourth report) was in favour of a mixture of serum and virulent blood. He considered an inoculation of this mixture would confer active immunity, and used 99 parts of serum and 1 cc. of inoculated blood, injecting 20 cc. of the mixture. He claimed to obtain a certain basis of immunity, which he strengthened by means of repeated blood inoculations. Rogers⁴ found that mixtures of serum and virulent blood failed to confer immunity.

V.—EXPERIMENTS WITH MODIFIED BLOOD.

Edington found that citrated blood, kept for such a time as to ensure the death of the contagium, in doses of 20 cc. conferred a certain degree of immunity.

Koch¹ tried mixing virulent blood in varying concentrations with glycerine, phenol, distilled water, and normal salt solution, respectively, but no modified virus capable of being used for producing immunity was elaborated.

Rogers⁴ tried dried blood, sterilised blood, oxygenated blood, and hydrogenated blood, with similar results. The latter also tried blood from animals when reacting, and after reacting, to virulent blood in the process of serum-making. He considered that the disease was produced, slightly intensified, when the blood was taken during the temperature reaction. When the temperature was declining after injections of virulent blood into salted animals, the blood might, or might not, produce the disease. If it did produce it, it was in a severe and unmodified form. When no reaction occurred the disease was not produced in susceptible animals, and no immunity was conferred. Blood passed through a porcelain filter has been tried as a preventive by the writer and others with no success.

The above enumeration of the experiments conducted by different persons with blood in attempting to modify the virus for a useful purpose are interesting, but, as no practical results were obtained, they are not worth discussing.

VI.—PASSAGE THROUGH ANIMALS.

It may be said that ruminants only are susceptible to rinderpest. Nicolle and Adil Bey⁶ injected 5 cc. of virulent blood into the peritoneal cavities of rabbits and pigeons. From these animals they inoculated cattle on the fifth day. The result was—no disease and no immunity. Koch¹ carried rinderpest through goats and sheep to the seventh generation. He thought that the rinderpest virus after repeated passage through goats became slowly attenuated, but was inclined to believe that as far as sheep were concerned it became more virulent. Nicolle and Adil Bey⁶ did not think the virulence was augmented when passed through sheep, and that irregular results were obtained after passage through goats. The susceptibility of goats and sheep probably varies considerably. In India sheep become ill but rarely die. Passage through sheep did not appear to

modify or intensify the virulence of the disease to any appreciable degree. Goats generally die, but the disease in them runs rather a long course. They die of fever and progressive emaciation in from nine to sixty days. Females always abort. Passage through goats in India did not appear to alter the virulence of the virus.

Conclusion.

In conclusion it may be said that there is yet room for a safe and effectual method of inoculation against rinderpest. So far as India is concerned the serum alone method is doing a good deal of good, and is filling a gap. The expense incurred in preparing and using the serum is considerable, but it is probably more than covered by the great saving of cattle that without it would doubtless die of rinderpest. Cattle in India are almost the most important asset to agriculturalists, and the country practically exists by agriculture. It is hoped that a really efficient and economical method of preventive inoculation for rinderpest suitable to that country will eventually be evolved.

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