RABIES IN IRAK, AND ITS TREATMENT BY CARBOLIZED VACCINE.

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During the Great War and the subsequent rebellion in Irak considerable loss of service and expense to Government were caused by the lack of local facilities for the treatment of rabies. Between two and three hundred men had to be sent every year to the Pasteur Institute at Kasauli for antirabic vaccination. Moreover, the unavoidable delays in the long journey from up-country stations in Irak resulted in the death from hydrophobia of a number of soldiers who—arriving in India too late for effective treatment—succumbed to this disease en route to Kasauli, or soon after arrival there. Now in 1910, Colonel Sir David Semple, R.A.M.C., [1] anticipating the needs of a large field force operating in the East, initiated and perfected a simple method of preparing a safe and efficient antirabic vaccine that any competent bacteriologist could carry out for the treatment of rabies locally, at a base or central laboratory.

The vaccine is prepared by emulsifying the brains of rabbits, that have died from inoculation with "fixed" rabies virus, in a dilution of carbolic acid of sufficient strength to kill the virus, but insufficient to destroy the antibodies present in the affected nerve tissue or to destroy its immunizing properties. This carbolized vaccine has stood the test of statistical examination and animal experiment by many investigators working independently, and has been used for many years as the standard antirabic treatment in all Pasteur Institutes throughout the Empire [2]. It is
proposed to record here a few general remarks on the incidence of rabies in Iraq, and to give in some detail the method adopted in working a Pasteur Institute in Baghdad, for the information of those who in future may find the necessity of establishing a similar institute under like conditions.

THE OCCURRENCE OF RABIES IN IRAQ.

Rabies in dogs and hydrophobia in man were recognized by medical practitioners in Baghdad long before the British occupation. For many years Arab "Hakims" in country districts have been acquainted with "madness" in dogs and have regarded their bites, when mad, as fatal to mankind. In like manner, they deem a bite from a wolf or a jackal to be especially dangerous. Their knowledge of the pathology of rabies is, however, chimerical.

Early in the late war canine rabies was detected by the Military Medical and Veterinary Services in Iraq [2]. As the Army, with its following of pet dogs, accumulated and it became known throughout the Force that a dog-bite, real or spurious, would elicit a passport from the Infernal Regions of Iraq to the Delectable Mountains of India, so the Military Establishment became depleted by an increasing stream of personnel passed over to Kasauli for antirabic treatment. There was undoubtedly a good deal of malingering, but since deaths from hydrophobia had actually occurred in the country, no medical officer could undertake the responsibility of denying antirabic treatment to any patient who presented a wound alleged to have been caused by a bite of dog or jackal that might have been rabid.

During 1919 a remarkable series of cases of hydrophobia occurred in a refugee camp at Bacuba, wherein forty-six persons were bitten by a rabid jackal which ran amok in the camp. Twenty-eight of the men bitten were sent to Kasauli for antirabic treatment. They arrived fifteen days too late, and five of them died of hydrophobia during, or shortly after, treatment.

Of the eighteen people—some of whom were women and children—who were not sent to Kasauli, eleven of them died of hydrophobia in the Military Hospital at Bacuba. The death-rate amongst the treated was 17·9 per cent. The death-rate amongst the untreated was 61·1 per cent. The figures are taken from official records and I have verified them from correspondence in the Central Laboratory, Baghdad, where the diagnosis of four of the fatal cases at Bacuba was confirmed by histological investigation.

In the year 1920, 50 Europeans and 212 Indians were despatched to Kasauli for antirabic treatment, and in the first six months of 1921, 58 Europeans and 167 Indians were sent to Kasauli. Of these 487 patients, 3 British soldiers and 3 Indians arrived too late for effective treatment and died of hydrophobia.
It is difficult to obtain trustworthy evidence regarding the prevalence of hydrophobia amongst the civil population. The Director of the Civil Hospital, Bagdad, informed me that he had seen four cases in that city during the last three years, and he was good enough to send me the brain of an Arab who recently died of rabies in Bagdad, and the diagnosis was confirmed by the finding of numerous Negri bodies.

The disastrous effects of a rabid animal running amok in a native village, and the beneficial effects of early vaccine treatment, are exemplified by the following well-authenticated case, details of which were kindly given to me by the Director of Health Services, Irak, and by Dr. Corner, the Civil Surgeon at Kirkuk, who personally investigated the cause of death of the victims.

In November last, an important Sheik and his daughter attended the Central Laboratory, Bagdad, for antirabic treatment. The man was severely bitten on the hand by a wolf, the girl also was dangerously bitten on the face. The Civil Surgeon at Kirkuk, who sent the patients for treatment, reported that eight other persons in the village had been bitten by the same wolf, but refused to attend for treatment. I communicated with Dr. Corner requesting him to keep an eye on all the people who were bitten. Four months afterwards he reported as follows:

"The Sheik of Kifri and his daughter are alive and well. Of the other 8 persons bitten, 2 women and 6 men developed hydrophobia and died; also 3 donkeys bitten by the same wolf have died. A cow that was bitten likewise sickened of the disease but was slaughtered and eaten by the natives."

The following figures show the incidence of canine rabies in Bagdad for the last three years:

<table>
<thead>
<tr>
<th></th>
<th>1919</th>
<th>1920</th>
<th>1921</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brains of dogs and jackals examined for Negative bodies</td>
<td>4(^{1})</td>
<td>6</td>
<td>11(^{1})</td>
</tr>
<tr>
<td>Dogs certified rabid by veterinary officer</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
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</table>

\(^1\) One Jackal included.

In addition, one dog in which no Negri bodies could be found was proved to have been rabid by the biological test. Also a cavalry horse, said to have been bitten by a wolf, or Jackal, was certified by a veterinary officer to have died of rabies some weeks afterwards. The brain of this animal was sent to the Central Laboratory in fresh condition. No Negri bodies or other abnormality could be found. The case seemed to be unusual, and to exclude the possibility of tetanus the biological test was carried out. The rabbit died with typical rabid symptoms on the seventeenth day after subdural inoculation with emulsion of the horse's brain substance.

This strain of virus was subsequently "passaged" through other rabbits but was lost before "fixation" occurred.
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THE DIAGNOSIS OF CANINE RABIES.

1. Clinical.
2. In the Laboratory.

Human rabies, or hydrophobia, is amply described in leading medical text books, and I will refer the reader to a classical description of a typical case by the late Colonel Sir P. J. Freyer, K.C.B., I.M.S. [3].

The Army doctor in the East is not infrequently required to give an opinion on cases of suspected rabies in dogs: a disease which is best described in veterinary text books not always accessible abroad. I therefore culled from a leading authority [4] the following account of the clinical signs and symptoms of canine rabies:

"At the initial stage of the disease the animals show a peculiar change in behaviour, they become capricious, irritable and gloomy, hiding in dark places under the furniture, or in dark corners of the room, and are sluggish in obeying the call of their masters; or they may show a restless uneasiness, constantly moving here and there, stopping suddenly and without cause, barking and biting at the air as though catching imaginary flies. The reflex excitability is decidedly increased and is shown by the fact that the animals when approached in a friendly way will become excited and snap at the caressing hand, or they will be startled and jump up at the slightest external cause, such as strong light or a sudden noise. At the same time the animals will disregard their ordinary food, which they will let drop out of their mouths. They show a perverted appetite for all sorts of rubbish and will chew up paper, sticks, and eat their own excrement. It will be noticed that they have difficulty in swallowing and the onset of pharyngeal spasm is further shown by dribbling of saliva.

"After one to three days the stage of unrest and excitement will increase and may pass into violent rage in which they leave their homes and wander aimlessly about, furiously snapping at and biting every creature within reach. If tied up at this stage, they will savagely bite their chain or the bars of their cage and tear up and swallow earth and stones. In this stage the symptoms of pharyngeal paralysis soon appear, as shown by a peculiar change in the tone of the bark which becomes hoarse and accompanied by long drawn-out howls. Water or food cannot be taken, and attempts to swallow cause paroxysms of respiratory spasm.

"After three or four days the rage subsides and is followed by symptoms of insensibility and dullness. The existing paralysis becomes more conspicuous, especially in the muscles of the jaw, tongue and eyes. The jaw droops, the tongue hangs out of the mouth, and long threads of viscid saliva flow from the lips. Paralysis of the hind limbs follows and the animal is seen to stagger and fall on attempting to run; and later the paralysed hind limbs are dragged along the ground. This condition is rapidly followed by extension of the paralysis, and death which, be it noted, invariably occurs within ten days.

"It sometimes happens that the stage of irritation and excitement is so
brief or transient as to pass unnoticed; the paralysis of the jaw and throat being the first symptom to attract attention. The owner, thinking that the dog has a bone in its throat may attempt to extract it, and in so doing infect himself with hydrophobia. Paralysis of the dog's hind limbs rapidly follows and it always dies within three or four days. This form of the disease, during the course of which the animals are, from the beginning, too weak to bark or bite is known as 'dumb rabies'; in contrast to the typical violent rabies."

(2) The Laboratory diagnosis of Rabies depends upon the finding of Negri bodies within the pyramidal cells of the brain. These cells are most readily found in considerable numbers in smears and sections taken from the hippocampus major. Negri bodies are found in ninety-seven per cent of cases of "street" rabies. Whether the bodies mark deposits of the causal organism, or represent merely cellular changes or activities brought about by the stimulus of the virus (Hæmatophagy, Hæmatoboly) is a matter of opinion—which the recent work of Woodcock has enlightened [5]. It is generally accepted, however, that the presence of Negri bodies within the pyramidal cells or in the cells of Purkinje is conclusive proof of rabies; but unfortunately if they are absent one cannot infer the contrary. In removing the brain of a dog suspected of rabies great care should be taken, for it must remembered that the brain tissue and the saliva may be infective and must not come in contact with the hands; it is therefore best to wear an old pair of riding gloves when performing this operation.

The head of the dog should first be washed in some antiseptic solution. If post-mortem instruments are available, it is quicker and neater to saw off the calvarium in the ordinary way; or a hammer may be taken and with a few sharp blows through the intact skin, the top and sides of the brain cavity can be broken into several pieces. Lateral flaps of the skin are then turned back, the fractured pieces of the skull removed and the brain exposed. Incise the membranes, remove the brain intact and put into a Petri dish. Divide the brain through the corpus callosum into two longitudinal halves. Put one half into a wide-mouthed jar previously padded inside with a layer of cotton wool at the bottom, and fill up to the top with methylated spirit or ninety per cent. alcohol. Seal up the jar and, if necessary, it can then be sent to a distant laboratory for section cutting.

To expose the hippocampus major: Take the other half of the brain and with a sharp razor shave successive layers off the top, until the lateral ventricle is exposed. The choroid plexus will be seen lying on the optic thalamus. The choroid plexus, being taken as a guide, should be traced backwards and downwards to where it descends into the depths of the middle or descending cornu of the ventricle which can be cut away along its outer side, exposing the hippocampus major as a prominent convex fold, chiefly of grey matter, occupying the inner side of the cavity.

With a fine pair of scissors snip through the hippocampus and remove a segment about the size of a split pea and put into ten per cent formalin.
solution, for fixing and subsequent section cutting if Negri bodies cannot be found in smear preparations. These should be made as follows:—

Snip another very small and thin portion off the hippocampus and press it out into a thin film between two perfectly clean microscope slides and, without separating the opposing surfaces of the two slides, draw them apart quickly so that a thin even smear of brain substance is drawn out along the two slides, which should be dropped, whilst the smears are still wet, into a jar of methyl alcohol used for fixing. Whilst the films are fixing, make up fresh the following stain [6] which I have found to be the most convenient and satisfactory of all the numerous staining methods recommended for Negri bodies. It is just as good for sections as for films.

Put fifty cubic centimetres of distilled water in a measure glass—add three drops of saturated aqueous solution of methylene blue, shake up and then add four drops of saturated alcoholic solution of basic fuchsin (if permanent section preparations are required make the stain up in fifty cubic centimetres of a five per cent solution of carbolic acid instead of water).

The slides should now be taken out of the methyl alcohol, which should be allowed to dry off—then flood the smear with the stain for five minutes, heating gently until steam arises. Examine under a low power objective and look for pyramidal cells which will often be found clumped together in one part of the film. They should be stained light blue; if densely stained soak the slide in water and control the decolorizing under 4-inch objective.

Preparations thus obtained have the advantage of presenting various depths of staining, some parts being too heavily stained, others too lightly, whilst in the intervening parts Negri bodies appear under the 1/2-inch objective as brilliant pink dots, globules or oval bodies in a light blue mounting formed by the pyramidal cells. Careful focusing will reveal in the depths of the Negri body a few dull bluish points or granules. If the stain is made up with five per cent carbolic solution instead of water, the Negri bodies take a bright ruby red colour in contrast to the cell nuclei which stain deep purple or chestnut. Sections after staining as above described should be dehydrated rapidly as follows:—

(1) Wash in water; (2) place in distilled water for five minutes; (3) place in rectified spirit for one second and dry with blotting paper; (4) clear with xylol and mount.

Negri bodies may be so numerous as to be present in nearly every pyramidal cell, or they may be so scarce as to require prolonged search through many slides before a typical and indisputable specimen can be found. In canine and jackal rabies, however, they are generally found at once in smear preparations, and an immediate diagnosis can be made without the necessity of cutting sections. If the finding in smears is difficult or uncertain, stained sections should always be examined before pronouncing an opinion.
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In cases in which the finding of Negri bodies was controlled by biological test, the rabbits died of rabies between the sixteenth and nineteenth day after subdural inoculation with 0.2 cubic centimetre of emulsion of the cerebellum of the suspected animal. In one case in which prolonged search failed to reveal Negri bodies, but in which the clinical evidence was convincing, the biological test proved positive.

I consider it important that the first case or two that occurs in a district should be biologically proved; for if the diagnosis is unquestionably rabies, then more cases may be expected and due precautions should be enforced without delay. In cases clinically suspicious of rabies, but negative on investigation for Negri bodies, the biological test should be done.

The necessary subdural inoculation of a couple of rabbits or guinea-pigs can easily be done without any special instruments. A cork-borer of about 3-inch calibre does very well instead of a trephine; forceps, a scalpel and a little surgical handicraft are presumably always available.

**THE MANUFACTURE OF ANTIRABIC VACCINE.**

The vaccine is made, as will be described presently, from the fresh brains of rabbits that have died as a result of subdural inoculation with "fixed rabies" virus. A local strain of fixed virus may be acquired from the original "wild" virus as found in the fresh brain of a naturally infected rabid dog, wolf or jackal and commonly termed "street" virus. This "street" virus, when inoculated subdurally upon a rabbit's brain, has a variable incubation period of fourteen to thirty days preceding the onset of symptoms.

If a strain of this virus is carried on from rabbit to rabbit by subdural inoculations of brain substance, the virulence of the poison becomes curiously altered in that the incubation period diminishes—possibly because of more rapid proliferation of the virus by successive "passages" through rabbits—until after some thirty or more of such inoculations we find the period of incubation of the disease has become reduced to a fixed limit of about seven days, followed by death not later than the tenth day. In contrast to the original "street" virus, however, the fixed virus—when injected subcutaneously—appears to be incapable of penetrating to the higher nerve centres and causing symptoms. It would be interesting, however, to have more experimental data on this matter, the explanation of which seems obscure.

Since the preparation of fixed virus is tedious and expensive in animals, it is convenient to obtain the strain of fixed virus from one of the Pasteur Institutes already established. It must be remembered that the virus is extremely delicate, and in hot weather would probably be killed during transmission through the post. Aeroplane transport would solve this difficulty—but in Bagdad it was necessary to get the virus sent over from Kasauli in live rabbits, relays of which were subinoculated as required during the journey.
Having obtained the fixed virus killing on the tenth day—in a portion of brain preserved in glycerine—a piece of the infected brain about the size of a pea is snipped off with sterile scissors and dropped into a sterile conical glass. Now wash off the glycerine in a little distilled water, pour off the excess of water and pound the bit of brain thoroughly with a sterile glass rod until it is a smooth homogeneous pulp; then add, drop by drop, one cubic centimetre of sterile distilled water from a sterile one cubic centimetre syringe, mixing thoroughly with the rod to form an even emulsion. Draw this up into the one cubic centimetre syringe, turn down the screw top of the syringe so that it will eject 0·2 cubic centimetre. Place the syringe and its contents in a sterile Petri dish and lay aside in the ice chest until wanted. Now take a full grown healthy rabbit. Snip the hair off the top of its head between eyes and ears, and swab the clipped area with iodine. Place the rabbit on a table and slip four loops of tape over the legs and tie each extended leg to nails conveniently fixed in the table. Anæsthetize the rabbit by pouring ether on a pad of wool held over the rabbit's nose, and then wash its head in one in twenty carbolic. The assistant giving the ether should now place one thumb over each of the rabbit's eyes and retract the skin when the incision is made. Make a longitudinal incision down to the bone, one inch long, and with its mid-point opposite the posterior margin of the orbit. The assistant retracts the edges and slides the wound slightly to the left of the middle line. Apply a trephine (0·5 centimetre in diameter) mounted on a hand drill, and drill a hole through the skull, exposing the dura just to the left of sagittal suture and longitudinal sinus. Now take up the syringe containing the fixed virus in brain emulsion already prepared, and push the needle point under the dura, passing it forwards towards the rabbit's nose as far as it will go, keeping it as near as possible parallel to the under surface of the dura; then withdraw the syringe very slowly, injecting meanwhile 0·2 cubic centimetre of the brain emulsion along the needle track. Having withdrawn the needle, close the skin wound with a stitch or a Michael's clip. No dressing is required. If the anaesthetic has been skilfully given and the operation dexterously performed, the rabbit should have recovered normal liveliness by the time it is untied and replaced in its cage, when within a few minutes after the operation it should be sitting up drinking the water and eating the grain provided. Finally, the details of the inoculation, number of the rabbit, etc., are entered up in a book, and the cage labelled with the number and date.

On the sixth or seventh day after the operation it will be observed, on disturbing the rabbit, that it has lost the power of judging the distance and the muscular effort required in jumping from one side to the other of its cage. The animal takes too forcible a spring and bangs its nose up against the wall of the cage towards which it jumps. This very early and, I believe, characteristic symptom of fixed virus infection should be noted. Within twenty-four hours of this first symptom appearing, a very fine tremor of
the ears and head will be observed, and the next day, if taken out of its cage and allowed to run about, the creature will be seen to have a reeling gait, as though intoxicated. On the ninth or tenth day it will be dead or dying. If it dies on the ninth day, its brain can be used for the preparation of vaccine only. If it survives until the tenth day, and is then obviously moribund, it can be used for subinoculation or "passage" and for the preparation of vaccine also. If the rabbit has died before the ninth day, its death has been caused by some intercurrent malady, and the animal should be discarded. During a period of intense heat in Bagdad, the virus seemed to lose its strength and did not kill until the twelfth or thirteenth day after inoculation. One passage of the virus through a guinea-pig, however, was followed by the restoration of its original lethal effect on rabbits, in which it remained stable, killing on the tenth day, until last summer, when, I am informed, it had to be "restored" again—this time by passage through a monkey. The stock virus should be maintained by passage from rabbits dying only on the tenth day. Occasionally a rabbit escapes and does not die. Such an event is probably due to a defect in technique, and will be of very rare occurrence after a little practice and careful attention to the details above given. Assuming that the inoculated rabbit is dead or dying on the tenth day (if moribund it can readily be dispatched by injecting a syringe full of air into its auricular vein), it should be disinected by immersion in a pail of cresol for a couple of minutes. The floor of the room and the operating table should be swabbed with cresol; the dead rabbit laid out with its head resting on the edge of the table and its legs tied out behind. Whilst an assistant grasps the rabbit's muzzle with lion bone forceps, take a sterile knife and make a medium incision extending from the nape of the neck to half way down the nose. Reflect the skin on either side of the roots of the ears and the edge of the orbit without opening either. Swab the whole flesh surface with 1 in 20 carbolic and sear with a hot iron the site of the trephine hole in the skull. A culture from the rabbit's brain can now be taken by pushing a platinum wire through the seared trephine hole and, on withdrawal, inoculating an agar slope to make sure that there is no secondary infection of the brain with pyogenic organisms. Now sever the head from the vertebral column by inserting the point of a sterile knife between the occiput and atlas. Take the bone forceps, put one blade in the occipital foramen and nibble away the top of the skull as far forward as the frontal lobe. The whole upper surface of the rabbit's brain will now be exposed. It should be normal in appearance, and there should be no indication of inflammatory reaction in the surrounding tissues.

Before proceeding further, snip off a portion of the cerebellum about the size of a pea and put it into a sterile test tube half filled with equal parts of glycerine and distilled water—not saline solution, for it would weaken the virus. This is the reserve supply of the virus, which should be sealed up in the tube and kept on the ice in the ice-chest to be used in the event of any mishap to the rabbit about to be inoculated.
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Snip off another portion of the cerebellum and drop it into a sterile conical glass, cover up and put aside in the ice chest for a while.

Now proceed to remove, with sterile forceps and scissors, the brain intact from before backwards. Place it on a sterile watch glass which has been previously weighed; weigh again on a chemical balance. It is sufficient to weigh to the nearest centigramme.

Suppose the weight of the glass plus the brain equals 15.22 grammes
weight of the glass only " 8-12 "
net weight of brain " 7-1 "

Now in making the vaccine the first step is to prepare an emulsion of this brain with one per cent solution of carbolie acid in the proportion of one of brain to fifty of the solution. Hence the carbolic solution required will be:

\[ 7-1 \times 50 = 355 \text{ c.c.} \]

Having made up 355 cubic centimetres of one per cent carbolic solution and put it into a sterile graduated glass cylinder with delivery nozzle such as is used for delivering intravenous medication; take the brain and put it into a sterile earthenware mortar about three to four inches in diameter. Pound it up thoroughly until of paste-like consistency, and whilst braying up the brain in the mortar, see the carbolic solution tested by an assistant, who will put a little of it into a test tube and add a few drops of perchloride of iron: a dirty blue colour appears if the solution is correct. When the brain has been comminuted and ground into a stiff paste, add the carbolic solution very slowly—grinding in the meantime, to make a good emulsion.

In the dusty atmosphere of Bagdad, it was necessary to carry out this operation under a sterile bell-jar in a perfectly still room previously swabbed with cresol to lay the dust.

As the mortar is filled, decant the contents through a layer of fine muslin stretched over an ordinary tea-strainer supported on the rim of a conical glass (urine specimen glasses do very well)—everything of course having been previously sterilized.

After all the liquid has been strained, decanted into conical glasses and covered with the lids of Petri dishes, the muslin strainers, with shreds of brain tissue entangled therein, are placed in the mortar and brayed with carbolic solution, which takes up in suspension the remaining brain substance. The washings of the strainers are then added to the bulk of the emulsion. We have now got in emulsion all the brain matter, without the vascular and connective tissue, which remains behind entangled in the muslin.

Pour the contents of the conical glasses into a sterile litre flask, and add carbolic solution until the whole amount of 355 cubic centimetres has been added. Replace the plug of wool in the flask and write on it the number and amount in cubic centimetres of the "brew," put it in the 37\(^\circ\) C. incubator and leave for twenty-four hours. Next day the
emulsion is further diluted by the addition of 355 cubic centimetres of normal saline solution. The whole, being well mixed, is now poured into a sterile glass cylinder or funnel on a stand, from which the vaccine is run off into 30 cubic centimetres sterile bottles that can be capped with sterile rubber caps, which should be tied on and hermetically sealed by dipping into hot melted wax.

Sterility tests should be made by running out the last few cubic centimetres of vaccine from the delivery tube into broth, on an agar slope, and into anaerobic broth culture media, which after incubation for seven days at 37°C should show no growth. When the vaccine has been stored for some weeks the sterility tests should be repeated on every bottle a few days before use. This additional precaution may be unnecessary in cool hills stations.

If the first test showed the vaccine was contaminated in bulk the whole "brew" must be thrown away and any bottle found contaminated at the second test should be discarded.

During the hot weather in Bagdad, when sandstorms were prevalent, contaminations with spore-bearing organisms were not infrequent. With improvement in the technique, however, these accidents became uncommon. It must be remembered that only by the most careful supervision and fastidious attention to details of sterilization throughout the whole process of manufacture can sterility be ensured, and unpleasant and possibly discreditable effects of treatment be prevented.

Now to go back to the removal of the brain from the rabbit’s skull. The portion of the cerebellum placed in the glycerine can be kept in the ice chest for several weeks to inoculate more rabbits in case of accident to those inoculated forthwith. With the second portion of cerebellum that was laid aside in the ice chest we should now proceed to inoculate a couple of rabbits for the maintenance of the fixed virus. In Bagdad it was customary to inoculate two fresh rabbits every tenth day.

Rabbits dying on the ninth day may be used for vaccine but not for "passage." No portion of brain from a ninth day rabbit need be preserved in glycerine.

Indications for Antirabic Treatment.

The decision as to whether a person has or has not been subjected to the risk of hydrophobia when bitten by an apparently healthy dog in a rabies-infected country depends upon the following points:

1. A dog cannot have been infective for more than ten days prior to the onset of its symptoms.
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(2) The virus cannot penetrate unbroken surfaces, whether of skin or of mucous membrane.

(3) From a practical point of view it is wise to assume rabies to be present if an undiagnosed disease in a dog is of short duration and ends in death: and in cases of unprovoked attack, especially if the owner of the dog or a number of persons have been bitten.

(4) If a jackal attacks a human being without provocation it is almost certainly rabid.

The procedure that should be adopted in practice is as follows:—

If possible keep the suspected animal under observation for ten days and keep the patient waiting until the period of observation has passed—unless the bite is on the face or otherwise near the brain, in which case it is not safe to risk any delay in immunizing the patient. If ten days after inflicting the bite the animal is alive and well, then it cannot have conveyed infection and the bitten person is free from risk. If, however, the animal shows symptoms of sickness or dies or escapes during the ten-day period, treatment by vaccine should be commenced without delay, and on no account should it be postponed until a microscopical examination of the dead dog's brain is made, for though a positive result would be decisive, it must be remembered that a negative examination does not exclude the presence of rabies.

LIMITATIONS OF ANTRABIC TREATMENT.

The object aimed at in antirabic treatment is to confer an active immunity against rabies before the virus in the saliva of the animal which inflicted the bite reaches the nerve centres; when this has been accomplished the failures rarely exceed 0.8 per cent. On the other hand, should the virus have already reached the nerve centres by the time the patient has arrived for treatment, there will be no symptoms to show that this has taken place, but hydrophobia will set in fourteen days or so afterwards, irrespective of whether he receives treatment or not. Suppose for instance the virus reaches the brain of a bitten person one day before the course of treatment is completed, the patient will develop hydrophobia fourteen days afterwards and during this interval he will not have a single symptom to show that the object of treatment was defeated one day before completion. The explanation of such a case is clear; since we know that street virus planted direct on the brain of a rabbit has an incubation period of at least fourteen days, and that no treatment subsequent to direct inoculation of the virus on the nerve centres will prevent the onset of rabies. Now antirabic treatment extends over a period of fourteen days, and the time occupied by the virus in growing up the nerves to the nerve centres is variable and mainly dependent on the proximity of the bite to the brain. The importance of early treatment is evident, for we have to set going a race between the growth of a disease and the progress of immunity in which immunity is handicapped by disease having a considerable start. If the
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disease wins and reaches the vital nerve centres before their defence is organized, the patient will be in the same hopeless condition as a rabbit would be, had it been inoculated directly upon the brain with street virus.

METHODS OF TREATMENT.

(1) Local.—Cauterisation of the wound when thoroughly carried out within half an hour of infliction of the bite will prevent hydrophobia in some cases, but not in all. It is probably not much good after three or four hours, and certainly quite useless after twenty four. Local treatment at best can only reduce the chance of infection by diminishing the virus in the wound and prolonging the incubation period of the disease, so that the vaccine treatment is made easier and more certain of success. Undiluted carbolic acid is the best caustic to use and it should be well swabbed into the depths of every tooth mark.

(2) Administration of vaccine.—Not a day or even an hour should be unnecessarily wasted in sending persons exposed to the risk of hydrophobia to the Pasteur Institute for treatment. If this is impossible, the vaccine, packed in ice in a thermos flask, can be sent to the patient and injected by a local doctor. It is more satisfactory however that the patient should attend the Pasteur Institute daily as an out-patient during the fourteen days of treatment.

The following details of each case should be recorded.

(1) Particulars of patient.
(2) Station where bitten.
(3) (a) Whether bitten or licked by animal proved to be rabid (i.e., Negri bodies found).
   (b) Whether bitten or licked by animal certified to be rabid (by Vet. or M.O.).
   (c) Whether bitten or licked by animal suspected to be rabid.
(4) Number and position of wounds.
(5) Description of wounds; whether punctures, abrasions or through clothing, and if cauterized?
(6) Position of wounds and interval between date of bite and the commencement of treatment.

Whilst these details are being booked, sterilize a large size Roux syringe by filling and expelling from it eight or nine times, hot oil maintained at a temperature of 140° C. Then push the needle of the syringe through the waxed rubber cap of a bottle of vaccine and withdraw two cubic centimetres of the vaccine for each patient to be treated. The amount of vaccine withdrawn should be expelled into a sterile gallipot; an equal amount of sterile 0'85 per cent salt solution is now taken up in the syringe and thoroughly mixed with the vaccine in the gallipot.

We have then a suspension of 0'5 per cent brain substance, 0'25 per cent phenol, and 0'85 per cent salt.
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Each patient, however severely bitten, receives daily doses of five cubic centimetres of this suspension for a period of fourteen days. Two and a half cubic centimetres are inoculated on either side of the middle line of the abdomen by inserting the point of the needle at an acute angle between the superficial and deep layers of the skin and pressing out the vaccine between the epidermis and dermis, i.e., intracutaneously rather than subcutaneously. It is usual to dab a little tincture of iodine on the skin before inserting the needle. The total amount of brain substance injected is about 0.25 gramme for each patient. Roux syringes holding five cubic centimetres are most suitable for injecting the vaccine. The needle should be sterilized by dipping it in hot oil between inoculation of each patient. The inoculation causes no pain, no general reaction and no local reaction beyond a little redness and itching of the skin. Patients should be advised against taking alcohol or indulging in any unnecessary physical exertion during, and for ten days after completion of, treatment. At the conclusion of the course of inoculations each patient is given a stamped addressed post card and requested to inform the Director of the Institute of the state of his health three months after treatment.

Hydrophobia usually develops before the eighth week after infection in those who arrive too late for treatment. Patients reported to be alive three months after completion of treatment are recorded as having been successfully vaccinated.

Results of Antirabic Treatment in Bagdad.

During the last six months of 1921, 137 patients attended the Central Laboratory for antirabic treatment by carbolized vaccine. Up to the time I left Iraq in May, 1922, no case of hydrophobia nor any unpleasant after-effects of the treatment had occurred.

Analysis of the records shows that sixteen per cent of the patients were bitten by animals proved by the laboratory investigation (i.e., by biological test or by the finding of Negri bodies) to have been rabid at the time of biting, and 10.9 per cent were bitten by dogs not examined in the laboratory, but certified by veterinary medical officers to have been rabid. In 26.9 per cent of the cases treated there was evidence of rabies in the biting animals, though evidence on clinical grounds only cannot always be regarded as conclusive.

Cost of Antirabic Treatment.

Provided that there is a well-equipped laboratory already established for general bacteriological work, the additional outlay required is trivial, and any extra allowances that may be granted to the personnel would probably be more than covered by fees obtainable in payment for treatment of Civil Servants, etc.; money that, presumably, would be claimed by the Military Financial Authorities, as was the case in Iraq.
The cost is roughly estimated as follows:

**Initial Expenditure.**

- Outlay for instruments and apparatus: Rs. 620
- Cost of rabbits imported from Kasauli: Rs. 208
- Cost of rabbit hutches, etc.: Rs. 360

**Recurring Expenditure.**

- Salary of 1 Assistant Surgeon I.M.D., at say Rs. 1,188 per month
- 1 R.A.M.C. Laboratory Attendant: Rs. 450
- 1 extra Sweeper: Rs. 200
- Charge allowance for the responsible Medical Officer: Rs. 1,188

The cost of rabbit food is difficult to estimate and is not included. Cut grass was supplied daily from the military grass farm and crushed oats and gram was supplied in bulk by the R.A.S.C. in Bagdad.

The successful breeding of rabbits is an essential item in the maintenance of a Pasteur institute. A note on the care of these animals is appended.

In conclusion, I desire to acknowledge my indebtedness to the Committee of the Pasteur Institute of India for their assistance in enabling me to establish antirabic treatment in Bagdad, and my gratitude is especially due to Major John Morison, I.M.S., the Director of the Pasteur Institute at Kasauli, to whom I am beholden for the strain of fixed virus, rabbits, etc., also for invaluable advice and precise technical details which I have embodied in the compilation of this paper.

To Lieutenant-Colonel J. D. Graham, C.I.E., I.M.S., the Director of Health Services, Irak, who first initiated and proposed the scheme for a Pasteur institute in Bagdad, I am deeply indebted for much kindly assistance. Also to Colonel A. H. Morris, C.B.E., C.I.E., A.M.S., D.D.M.S., Iraq, who pushed the scheme through official channels and encouraged the enterprise in every way—my grateful acknowledgments are due.

Finally, I express my appreciation of the loyal assistance and good services rendered by Assistant Surgeon J. Dewey, I.M.D., of the Pasteur Institute, Kasauli, and the commendable devotion to duty with which my laboratory assistant, Corporal R. H. Welch, R.A.M.C., carried on his extra work during an exceptionally trying hot weather.

**APPENDIX.**

**POINTS IN RABBIT BREEDING.**

In a climate like that of Irak rabbits will not pair during the hot weather. The stock required for the whole year must be bred between the months from October to April.

The breeding stock should comprise about 30 does, which should be kept in separate hutches provided with dark nesting apartments, and 6 or 8 bucks which should also be kept separate or they will fight and kill each other. A working
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stock of about 150 animals should be maintained. They thrive best if allowed the run of fairly large cool basements, cellars or mud huts.

The following conditions regarding the breeding of rabbits should be observed:

1. Does and bucks not under six months, but under three years, must be selected for breeding.
2. All does and bucks will be numbered, and a register showing particulars will be maintained.
3. Does for mating will be placed and left in the same cage as the buck. Success or failure, i.e., accepts or refuses, is known within a minute or less.
4. In the event of a success the doe will be tested on the tenth day with the same buck; if she refuses it means that pregnancy has occurred, and in which case the doe is not to be put to a buck again.
5. In the case of a failure, the doe will be taken to the same buck the next day and if she refuses the second time without sufficient cause, she should be tried with a different buck at an interval of a day. If she refuses for a third time, she must be left alone for a fortnight.
6. If does are seen carrying grass into the next compartment, the nest must not on any account be disturbed. The period of gestation is thirty days.
7. Particular attention must be paid to the feeding and watering of the does which have littered, for if they are not fed well they are apt to kill their young.
8. As the mothers kill the young which have been handled, the young must not be touched before they leave the nesting compartment, which is usually between second and third weeks. If the nesting compartment has to be opened for removal of a young one which has died, all manipulations must be done with a stick.
9. The young are to be separated from the mother after six weeks, and let loose in a common run. Bucks and does to be kept apart when they are three months old.
10. Does are not to be put to bucks before seven weeks after the date of last litter.
11. Bucks are not to be used more often than three times a week, successes only counting.

REFERENCES.

[7] HUTYKA and MAREK. Ibid. No. 4, p. 511 (the quantities of ingredients for making up the stain are not quite the same as given in this paper).