

rate of variation with the temperature of its thermal conductivity is very probably much above the average.

To reduce the results of the present paper to the C.G.S. system of units, it is only necessary to divide them by 60.

“On Rabies.” By G. F. DOWDESWELL, M.A., F.L.S., F.C.S.  
Communicated by Professor VICTOR HORSLEY, F.R.S. Received May 9,—Read June 16, 1887.

[PLATE 1.]

CONTENTS.

	Page
I. Introduction .....	48
II. Methods of preparation and inoculation with virus.....	49
III. Symptoms and post-mortem appearances .....	50
IV. Seat of virus and results of inoculation .....	58
V. Occurrence of infectivity in tissues.....	64
VI. Duration of incubation period.....	66
VII. Preservation and modification of virus.....	68
VIII. Protective inoculation .....	70
IX. Action of drugs .....	75
X. Nature of virus .....	83
XI. Conclusions.....	85

Numerous as are the communications upon the subject of rabies, the paucity of experimental investigation is remarkable; the disease has remained for upwards of 2000 years, since the first recorded mention of it by Aristotle, exceedingly obscure in many essential points. The unparalleled and variable length of its incubation period has offered the greatest obstacle to systematic examination; in the words of John Hunter in the last century, “It has defied alike scientific investigation as to its intimate nature, and all remedial measures for its successful treatment.”

Lately, however, the results announced to have been attained by M. Pasteur, have promised to remove these obstacles, and encouraged research by new methods and with fresh views.

This investigation was commenced early in 1885, during the prevalence of rabies in and around London. Two well-marked cases in dogs were obtained, and inoculations with their saliva, taken both during life and shortly after death, were made into the subcutaneous tissue of other animals, but failed to produce infection.

At that time I was not sufficiently conversant with the results of M. Pasteur's investigations to place reliance upon his methods of intracranial inoculation with the cerebro-spinal substance of a rabid animal, and I must admit that his statements seemed to me to be im-

probable and inconsistent with the facts which were previously well established in this disease.

The outbreak of the epizooty shortly afterwards subsiding, I was unable to resume experiments till the summer of 1886, when it had become necessary to examine the results said to have been attained by M. Pasteur. His statements, now widely known, communicated to the Academy of Sciences, Paris, from time to time, and published in their 'Comptes Rendus,' are essentially these: (1) That the virus of rabies and hydrophobia resides in the cerebro-spinal tissues, and is not confined to the salivary glands as hitherto supposed. (2) That by inoculation of this substance upon the brain of another animal by trephining, or by intravenous injection, infection follows infallibly and much more quickly than by subcutaneous inoculation. (3) That the virus from a rabid dog by passing through a series of animals of a different species is modified in virulence; in monkeys it is attenuated and ultimately lost, in rabbits on the contrary, it is intensified, and after a certain number of inoculations in these animals reaches a maximum, which it maintains unaltered; these modifications of activity being shown by the duration of the incubation period following inoculation. (4) That by successive inoculations with virus, the activity of which is progressively diminished either by passing through a series of monkeys, or by the action of dry air upon the spinal cords which contain it, it is possible to confer upon dogs and other animals, together with man, immunity against subsequent infection with the most active lyssic virus.

In reference to these statements, the first points for investigation were now, the effects produced by inoculation with the cerebro-spinal substance of a rabid animal upon the brain of another, and whether the symptoms stated to be produced thereby were those of infective rabies—lyssa—or, as some contended, merely a neurosis resulting from the injection of foreign matter.

In the methods adopted in these experiments, I have followed those described by M. Pasteur in his published statements, but for imparting to me many details of manipulation, I am greatly indebted to Professor Horsley, F.R.S., who learned them from M. Pasteur himself in Paris.

## II. *Methods of Preparation and Inoculation with Virus.*

The animal from which it is desired to inoculate having died or been killed, a part of the spinal cord is exposed, and the portion desired removed, with precautions against contamination, the requisite instruments, vessels, and other apparatus having been previously sterilised by the recognised methods; the medulla is then carefully ground up to a homogeneous pulp in a glass mortar, and triturated with the proper proportion of sterilised beef-bouillon, as prescribed by

M. Pasteur. Salt solution or any other indifferent fluid would no doubt answer as well, but bouillon has the great advantage of showing at once the occurrence of any septic change in the fluid, by the turbidity which it occasions.

In order that the conditions of experiment might be strictly similar, I have myself always used definite proportions of cord and bouillon; as 1 inch of the former, of an average-sized rabbit, weighs about 0·8 gramme, I have mixed or “diluted” this quantity with four times its weight or bulk—their specific gravity being very nearly the same—viz., 3·2 c.c. of bouillon.

In order to free the infusion or “mash” thus prepared, from any portion of the membranes investing it, or grosser particles of its substance unreduced, it is strained through fine muslin, sterilised by passing over the flame of a spirit-lamp.

In the earlier experiments with rabbits, the animal to be inoculated was anæsthetised by æther; it was soon found, however, that this was unnecessary, inducing a great mortality, and being productive of pain to the animal, whilst coming under and recovering from the influence of the drug. I then used a solution of cocaine as a local anæsthetic, with apparently satisfactory results, but ultimately found that nothing whatever is required beyond the 5 per cent. solution of carbolic acid, with which, after clipping the hair closely, the head is washed, as an antiseptic; if this is rubbed in for a short time, complete anæsthesia is produced locally, the animal in the large majority of cases remaining perfectly quiet, frequently with its eyes closed, during the slight operation of trephining and inoculation, and not requiring confinement or restraint in any way, save by a hand lightly laid upon it.

The bone is then trephined in the usual manner, a small incision being made in the skin and periosteum, a little behind the coronal suture, and on one side of the median line. The virus is injected with a Pravaz syringe either between the bone of the skull and the dura mater, or by perforating the latter with the curved point of the needle the requisite quantity is injected into the sub-dural lymph space.

The effect of either method is much the same, but by the former the incubation period is slightly but appreciably longer than by the latter, the difference being, with intensified virus, one or two days.

### III. *Symptoms and Post-mortem Appearances of Rabies.*

1. *In the Dog.*—These in the dog have been described by numerous writers from the time of Cælius Aurelianus,\* nevertheless considerable misapprehension still generally prevails upon some points. The

\* ‘De Morbis Acutis’ (Amsterdam, 1722). His account is short, but accurate in most points, and is the earliest extant. The period at which he lived is uncertain.

symptoms will vary in some respects in an animal kept quietly in confinement from those found in the mad dog of the streets that has lost its home and been hunted about.

In most cases the first change observed is a dulness and sullenness, with an indisposition to move, the animal lying crouched up in a corner; probably this is invariably the first symptom, though, especially in dogs at large, it may be overlooked, and the symptoms, or as sometimes termed, stage, next following may be the first to attract notice. In this, a shy and suspicious or threatening look is a most characteristic feature; the previous dulness is succeeded by irritability and constant restlessness, with, usually, a disposition to fly at any strange object and bite. A depraved appetite is frequently noticeable in the early stages, natural food being most usually rejected, hay and straw, bits of cloth, wood or cinder frequently being eaten. This is one of the most constant and best recognised symptoms, though not absolutely invariable.

Hydrophobia, or dread of water, is never present in the dog; there is sometimes increased thirst, but in dogs in confinement this is not generally marked: there is often inability to swallow from paralysis of the muscles of deglutition and those of the lower jaw, which in an early stage is usually observed drooping, with inability to close it, though the extent and duration of this is variable, and it passes off at a later period.

Excessive salivation is not usual; when observed it is in the hot weather, and occurs from loss of the power of deglutition. One of the most characteristic and best recognised symptoms, occurring generally in an early stage, is a remarkable change in the voice, the bark becomes a hollow howl, commencing with a short low note and ending in a higher one prolonged; it has always a peculiar metallic ring, which once heard cannot be mistaken.

The further symptoms developed depend chiefly upon the temperament of the animal, modified somewhat as above remarked, by its external conditions; an aggressive disposition is usually found, but is not invariably present, though in an irascible savage animal it may realise the popular idea of furious rabies, attacking and tearing everything; in confinement, however, this extreme is not usual, and some dogs are with difficulty induced to bite anything presented to them, even a rabbit or another dog, and the fury said to be excited by the sight of the latter is not generally found in confinement.

The last stage is that of paralysis, which, more or less developed, is invariable; it commences in the hind limbs, its first indication often is the animal standing with its hind legs wide apart; when it moves it is unsteady, swaying from side to side, as this progresses it becomes unable to stand, is ultimately completely paralysed, and comatose in the large majority of cases. The tail in confinement is never carried

depressed between the legs, as is described by some of the earlier writers as a character of this disease, if it occurs in a street dog it is the result of exhaustion.

It has been usual to describe rabies in the dog as of two forms, the furious and the dumb, or paralytic. Fleming, however, and others of the best authorities recognise that there is no real distinction between the two, every case of rabies probably, if permitted to run its course and terminate naturally in death, develops symptoms, more or less marked, of paresis; there is no constant distinction between the two forms, the difference consists merely in one or the other class of symptoms, rage or paresis, being the more preponderant, according to the part of the cerebro-spinal system which is principally affected.

Whatever the disposition of the animal may be, it almost invariably recognises its master or attendant, and is in some degree amenable to his control until completely paralysed and unconscious. There is a danger in this feature that not being well known it may occasion the presence of a virulent disease to be overlooked or mistaken, as indeed frequently does happen.

The post-mortem appearances of rabies in the dog have frequently been described as mainly negative, characterised by the absence of any distinct lesions; this, however, is only very exceptionally correct. In animals which die naturally at the termination of the disease, the appearances are in the majority of cases sufficiently diagnostic; in those killed at an earlier stage, as necessarily occurs in the large majority of cases of "street rabies," the condition of the stomach as to its contents may be the only diagnostic character.

The general condition is frequently wasted, to an extent dependent upon the duration of the symptoms and the inability to feed.

The brain and spinal cord being now recognised as the essential seat of the virus, it is to the appearances they present that attention is first directed. In most cases the dura mater of both is distinctly congested, occasionally intensely so; I have seen one case at least of a street dog killed in an advanced stage of the disease, where this membrane in a portion of the spinal cord received, was most intensely congested and livid in colour. This, however, is exceptional. The pia mater of the hemispheres is likewise most frequently injected, and in the greater number of cases, capillary congestion is apparent in the cortex in microscopical sections, with extravasation of lymph cells through the walls of the vessels and perivascular lymph spaces into the surrounding tissues. In the cerebellum this occurs to a more limited extent. It is by no means confined to the floor of the 4th ventricle as has been sometimes stated. Throughout the medulla oblongata it is constant, and often occurs in the spinal cord. In the latter, extravasation of red corpuscles or minute hæmorrhages are frequent; in some cases these are of large size and quite apparent to the unaided eye. In

one case of street rabies, in a part of the cervical portion of the cord I found this so extensive as to obliterate nearly the whole of the gray substance for some length of the cord, the hæmorrhage becoming distinctly organised, with the formation of vessels or channels; I have found similar appearances in other parts of the cord in different cases, but none so extensive as this. These seem to originate in the vessels running in the anterior fissure, in which clots forming thrombi are often apparent, presenting the appearances figured by Gowers ('*Pathol. Soc. Trans.*,' vol. 28, 1876-77, p. 10, &c.), though frequently of greater extent proportionately to the size of the vessel. Extensive extravasation and, in stained preparations, much granular matter is always apparent, the formation presenting every appearance of being caused by microparasites: in the majority of cases I have been unable to demonstrate their presence, from causes mentioned below, but in some few sections, as described, I have clearly found the microbes in the pericellular and perivascular lymph spaces, accompanied by appearances of embolism, and extravasation in the capillaries of their immediate neighbourhood. The occurrence of these hæmorrhages in different situations, involving the roots of different sets of spinal nerves, will obviously affect the symptoms of paralysis according to the muscles supplied by the nerves involved.

In the alimentary canal and respiratory organs conspicuous changes are constantly present. The tongue is generally dry and discoloured, often brown; the epiglottis is frequently conspicuously injected, the lower part of the larynx so deeply congested as to appear crimson. This often involves the greater portion of the trachea and extends to the bronchi. the lungs are generally congested, though to a variable extent, most usually they are bright red, with portions deeply injected, very frequently on the margins of the lower lobes; parts of them sometimes are consolidated and livid. Though some of these changes may be due to causes independent of rabies, congestion however is most usually present: oedema I have not found.

The pharynx and œsophagus less frequently show congestion than the trachea, but the stomach, as generally remarked, shows very constant and typical changes; in every case, excepting one, in the dog, I have found it devoid of solid food; in that case, as in every other excepting one, it has contained some hay; in the greater number of the cases of street rabies there have also been found other foreign substances—cinders, coal, wood, cloth, &c. In cases, however, of experimental inoculation, a dog confined in a cage throughout the course of the disease can have but little opportunity of eating any indigestible substances, excepting hay and straw.

The stomach frequently contains a thick, dark-brown fluid, which is also found in the duodenum; the mucous membrane if not discoloured by the fluid present, is usually redder than normally; con-

gestion of the veins is generally very apparent on the exterior or serous surface, being most marked towards the cardia, and on their ramifications are apparent hæmorrhages or ecchymoses of very variable size and number, from the most minute up to usually 2, 4, or 6 mm. in width or more. When very minute they are more easily distinguished on the interior or mucous coat where they appear as black specks or spots prominent upon the surface, generally on the summits of the rugæ: they are in fact small clots. They have been described by previous writers,\* and are figured by Fleming in a coloured drawing of a stomach with a portion of the mucous membrane exposed; I have, however, usually found them more distinct and clearly defined than those there shown, not having observed the mucous membrane as highly coloured as in his drawing, the contrast consequently being greater. They are, too, correctly described by Youatt ('The Dog,' p. 143) as "effusions of bloody matter, or spots of ecchymosis on the summits of the rugæ," and regarded by him as very pathognomonic.

I have found these present in nearly all the dogs I have examined in which the disease has run its course; in those that are killed during its progress they are necessarily less developed.

Their appearance is very diagnostic; they occur in some few other cases,† but then only, I believe, of small size; when large or well defined, as above described, in conjunction with the presence of foreign bodies, cinders, wood, cloth, &c., in the stomach, no doubt of the nature of the case can exist.

The presence of hay alone, though suspicious, is not of itself conclusive, for in the "Brown Institution" it has lately been found in dogs not rabid and apparently healthy, in some cases entirely filling the stomach; this may be accounted for by the inability of these dogs in confinement to get grass, which when at large they constantly eat. I may add that the pylorus is invariably hyperæmic, sometimes intensely so; this is best observed in the serous coats.

The appearance of the liver is variable, usually it is very dark and congested; the spleen I have found normal in all cases except one, when it was somewhat enlarged, but unchanged in other respects.

The salivary glands have hitherto been regarded as the seat of the virus, and received much attention, but they do not present any constant pathognomonic appearances; in one case I found the sub-maxillary gland somewhat hypertrophied and vascular, with the

\* They have been described by some as "hæmorrhagic erosions;" the term is not appropriate, though "erosions" may apply to the appearances of post-mortem digestion, which are sometimes observed, but not constantly.

† Viz., in swine fever (Dr. Klein), in some cases of experimental tuberculosis, and in anthrax in rabbits caused by "capillary embolism by masses of bacteria," as recorded by M. Feltz ('Comptes Rendus,' vol. 95, 1882, p. 859).

parotid normal; but this is not constantly the case, and as often as not these glands are normal both to the eye and in microscopical sections.

The kidneys are frequently but not invariably congested; the urinary bladder is generally so, and in the dog is frequently empty or contains a small quantity of urine.

The blood is always very dark coloured; in about half the cases it is fluid without any, or with very little clot; its reaction very shortly after death and within the vessels is neutral. No changes are apparent in the tissues of the heart; it is generally moderately distended with blood, whether fluid or clotted. In the morphological elements of the blood no alteration can be detected by the microscope, excepting in some cases an increase in the number of leucocytes. In the microscopical appearances of the other organs or tissues the changes which may occur, as has been described by some writers (*e.g.*, the granular appearance of the liver cells, by Bollinger), are to my observation by no means constant, nor can they be regarded as pathognomonic.

2. *In the Rabbit*.—The occurrence of rabies in this animal has till recently been a matter of some doubt. The first authentically recorded case of the successful transmission of rabies to the rabbit is where Mr. Simonds (22nd April, 1838) at the Royal Veterinary College\* inoculated two rabbits subcutaneously behind the ears with the saliva of a rabid sheep. After an incubation period of four days, they showed symptoms of infection, being found dull, hanging their heads and inclining them to one side; one shortly afterwards showed excitement; they then became comatose and died. The occurrence of paraplegia is not recorded. The incubation period is unusually short, but there appears no doubt that it was true rabies that was developed.

In the rabbit, as in the dog, infection is very uncertainly produced by inoculation even with active cerebro-spinal substance into the subcutaneous tissues, and, when it does occur in the former animal, the symptoms are materially different from those previously regarded as typical in the dog, and nothing can be less appropriate than the application of the term rabies or lyssa to them.

\* Reported in the 'Proceedings of the Veterinary Medical Association' for 1838-39, p. 369, and in 'The Veterinary Record' for 1845; also in the 'Veterinarian' for March, 1881, p. 189; and referred to by Fleming in the appendix to his work, before mentioned. Youatt also (*op. cit.*, p. 149) refers to cases of asserted rabies in the rabbit, mentioned in evidence before a Royal Commission on that subject, but considers them doubtful.

In 1879 M. Galtier ('Paris, Acad. Méd. Bull.,' vol. 8, p. 1114) inoculated a rabbit with the saliva of a case of hydrophobia in man, producing rabies with great excitement. From its submaxillary gland two other rabbits were inoculated, and became paraplegic. In the same year ('Comptes Rendus,' vol. 89, p. 444) in twenty-five cases he transmitted rabies from a dog to rabbits, the incubation period being from four to forty-three days, the average in twenty-five cases being eighteen.



The results of intracranial inoculation with virulent medulla, or with the secretion of the salivary glands, of either rabid dog or rabbit, are, as before stated, in all essential respects identical with those that follow subcutaneous inoculation of the same matter—in the small proportion of instances where this is successful—or with those induced experimentally by the bite of a rabid dog, though in these, as described below, the incubation period is of very variable and uncertain duration and much prolonged.

The first symptom of infection in rabbits is usually, as in the dog, dulness; the animal sits up with its eyes closed, its head frequently thrown back and inclined to one side. In some few cases, though exceptionally, and not exceeding 3 or 4 per cent., the animal is restless and excitable, running round and round its cage, and altogether hyperæsthetic; still more rarely is it aggressive, in one case and one only out of upwards of 200, have I seen a disposition to bite, and in two or three others an inclination to butt. This as in dogs depends no doubt on the disposition of the animal; tame rabbits are usually quiet and familiar enough, but those used to the care of them state that occasionally a normal rabbit in confinement will attempt to bite a hand put into its cage.

Concurrently with this change, there is a rise of the rectal temperature of about  $1^{\circ}$  C. from  $39.2$ — $39.8^{\circ}$ , the normal, to between  $40^{\circ}$  and  $41^{\circ}$  C., seldom exceeding the latter. This rise is, I believe, invariable, in the regular course of the disease, that is, if not influenced by the action of drugs or other circumstances; it is very transient, and may occur during the night and easily be unobserved. Usually it lasts about twenty-four hours and then begins to fall more or less quickly, *pari passu* with the progress of paresis, which is the essential feature of this disease in the rabbit. At first the animal moves slowly and with reluctance, its gait becomes unsteady, the loss of power usually commencing in the hind limbs; it then entirely loses the use of them; they are dragged after it if it moves, scrambling along by its fore-legs; it lies on its side with its hind-legs stretched out; respiration which was at first accelerated becomes slow and feeble, the muscles of the trunk and those of the fore limbs are successively paralysed, lastly those of the head and neck, the animal continuing to feed to the very last, frequently dying with hay in its mouth and between its teeth. The motor nerves alone appear to be affected in the rabbit, the reflexes remaining unimpaired to the last. A comatose state always precedes death, which is very gradual and imperceptible, the temperature continuously falling to a very low point. The immediate cause of death appears to be paralysis of the respiration, in those animals of which I have witnessed the death. I have found the heart continue to contract for some time afterwards, in one case for nearly half an hour.

The post-mortem appearances in the rabbit are better marked and more constant than in the dog. The brain and medulla are more frequently hyperæmic; in the majority of cases they are materially softened, which is not altogether dependent upon the duration of the symptoms; sometimes the spinal cord especially is so soft that it is difficult to detach a portion of it entire. The microscopical appearances are similar to those described in the case of the dog, but hæmorrhages in the substance of the cord, so frequent in the latter animal, are not found in the rabbit.

Continuing to feed till the very last, the stomach is usually full of partially digested food, as is frequently the gullet, in this differing markedly from the dog. The stomach constantly shows congestion, with hæmorrhagic spots in almost every case; they may be minute and very few in number, only two or three, but are always present unless in those exceptional cases where death has followed very shortly after the appearance of the first symptoms. These hæmorrhages are similar to those in the dog, occurring in the same situation, viz., chiefly towards the cardia and on the greater curvature, but are usually more conspicuous, attaining a larger size and sometimes becoming confluent, covering a large portion of the wall of the stomach.\*

The small and large intestines are generally normal, the fæces in the lower bowel being firm; in summer, however, diarrhœa is sometimes present, though this is probably due to other concurrent causes, and not to the specific action of the virus. I have never observed its occurrence during the winter months. The same remark as to its cause applies also to the loss of condition and emaciation that is sometimes found.

The subcutaneous tissue is generally very vascular, and small patches of congestion are found, which to superficial observation appear as red spots of variable extent.

The larynx and trachea are almost invariably hyperæmic, frequently intensely so; the lungs are as frequently congested to a variable extent, the margin of the lower lobe being usually the seat of the greatest changes; sometimes portions may be found consolidated or cyanotic, though this not unfrequently occurs in tame rabbits kept in confinement, and independently of experiment.

The liver is frequently enlarged, almost invariably congested, and often engorged with dark blood; in only two cases out of upwards of 100 noted have I observed it perfectly unchanged and healthy.

The spleen in nearly one-third of the cases is small. I have never observed it materially enlarged or softened.

The kidneys are frequently congested, and the urinary bladder is

\* These are correctly represented in the accompanying drawing, fig. 3, of a very well marked case.

always very vascular, generally distended with urine, frequently to an enormous extent. In one case I observed it of nearly the size of an ordinary soda-water bottle, filling and distending the abdominal cavity; the urine is strongly acid, and in other respects normal.

The blood, as in the dog, is always dark coloured, sometimes fluid, but as often clotted, and I have observed in several instances that the clot in the cavities of the heart was colourless and hyaline. Its morphological characters are unchanged, and as in the dog its reaction is neutral.

#### IV. *Seat of the Virus and Results of Inoculation.*

In July last, having obtained a rabid "street dog," upon its death another dog and a rabbit were inoculated by intracranial injection of a portion of its medulla, prepared as above described.

The dog was unaffected till the seventh day, when it was found dull, lying crouched up in a corner of the cage; the next day evident symptoms of rabies were apparent, the animal being restless and irritable, flying at and biting anything presented to it, with commencing paresis of the hind limbs; it was never heard to bark, and died on the following day, the 9th. As this was Sunday no further changes had been noted.

Examined the next day, the stomach and small intestine were found devoid of solid contents, containing only a dark-brown fluid; congestion was apparent in the outer wall of the stomach; the appearances of the other organs were characteristic of rabies, as hereinbefore described, but less marked than in many other cases, owing to the very rapid course of the disease, of the nature of which there could be no doubt.

To prove this further, from its medulla a rabbit was inoculated intracranially, this animal showed an incubation period of only four days, when with a scarcely appreciable rise of temperature paresis commenced, and was complete on the sixth day, the animal being found dead on the morning of the seventh.

The brain and spinal cord were found much softened, and with their membranes distinctly congested; the lungs presented typical appearances; the stomach was highly vascular but showed no hæmorrhages, in accordance with the rapid course of the disease, which, with the remarkably short incubation period, confirmed the view of the very active character of the virus, which the previous cases had suggested.

From the medulla of the first above-mentioned case of street rabies, the rabbit (narcotised by chloral hydrate) which was inoculated intracranially, similarly to the dog, on the fifth day showed commencing paraplegia; this continued for the next two or three days; the animal

then partially recovered. It however ultimately relapsed and died on the 23rd day.

On post-mortem examination the appearances were found to be very distinctly marked and diagnostic, the brain and spinal cord congested and softened, the stomach showing moderately large and distinct hæmorrhagic spots (ecchymoses); the condition of the other organs, too, was typical.

In this case, as in the dog, the incubation period was remarkably short. The temporary recovery is unparalleled in my observations, but is recorded by M. Pasteur as sometimes occurring. Other inoculations were made in the same manner intracranially with virus from different sources, all with similar results as to infection and the symptoms produced, varying only in the length of the incubation period.

These cases in dogs and rabbits proved sufficiently that by cerebral inoculation of a healthy animal with portions of the medulla of a rabid street dog, or an animal infected from it, paralytic rabies is produced, which in the dog does not differ in its essential characters from ordinary street rabies; in the rabbit, however, its occurrence was not so well recognised previous to M. Pasteur's experiments, and the symptoms are different from those in the dog.

In order, therefore, to meet the objection that these symptoms are not those of infective rabies or lyssa, subcutaneous inoculations with infective medulla were practised.

With this object a young healthy dog was injected under the skin of the back with half a c.c. of the mashed cord of a rabbit that had just died with the usual symptoms of paralytic rabies.

The dog, beyond at times an apparently increased irritability and disposition to bite, which may have been merely the result of confinement, showed no appreciable change until the thirty-ninth day after inoculation, when it was observed to be markedly snappish and irritable; on the following day it was very dull and indisposed to move or notice anything; this increased, and it became paralysed in the hind limbs, lying on its side; there appeared constant irritation of the skin, at which it was perpetually scratching, with continued twitching of the muscles of the neck and trunk; it frequently uttered a short yelp, altered in tone and characteristically metallic, but not the typical prolonged howl of rabies.

It died during the night of the forty-second day, with post-mortem appearances that were sufficiently characteristic; it was clearly a case of rabies with tetanic symptoms more pronounced than usual, but in its essential characters did not differ from street rabies in the same animal.

In similar manner rabbits were inoculated subcutaneously; many experiments failed to produce infection, as did also one in another

dog. In some cases the rabbits died of sapræmia (septic intoxication), to which these animals are extremely liable.

One rabbit, however, inoculated October 18th, 1886, subcutaneously with virulent medulla, on the twelfth day showed symptoms of infection, with weakness of the hind limbs, the temperature being below the normal and falling. On the fifteenth day it was completely paralysed and died in the afternoon, the post-mortem appearances being highly characteristic, the stomach showing numerous large well-defined hæmorrhages, before described, conspicuous from the serous surface as well as on the mucous membrane.

In another rabbit inoculated in similar manner subcutaneously, the result was precisely the same, excepting that the incubation period was shorter.

The results of these inoculations in both dogs and rabbits showed conclusively that rabies is produced in both animals, alike by subcutaneous and by intracranial inoculation of infective medulla of both dogs and rabbits, confirming M. Pasteur's statements in this respect.

It was subsequently found that in the rabbits and dogs thus inoculated subcutaneously without producing infection, no protection was afforded against the effect of subsequent intracranial inoculation, which in every instance produced fatal infection.

Still further to dispose of the objection that the symptoms following intracranial inoculation are not due to specific infection, a rabbit was inoculated sub-durally by trephining with the usual quantity (0.1 c.c.) of mashed spinal cord of a healthy rabbit. The animal remained perfectly unaffected in any way for upwards of a month; it was then again inoculated intracranially with virulent medulla, by which it was infected, and died after a short incubation period with the usual symptoms and post-mortem appearances.

I have also made several other inoculations intracranially in rabbits, employing two or three animals at the same time, with medulla of suspected cases of rabies in different animals. In many of these specific infection and death, with typical symptoms and appearances, have followed, but in those cases where the material used has not been specifically infective, the injections have been perfectly innocuous, the animals being in no wise affected by the operation.

I have used for intracranial and sub-dural inoculation quantities of medulla, mashed and diluted in bouillon, of from 1 to 10 minims,\* with the same results, without, in the large majority of cases, any disturbance, previous to or beyond the regular symptoms of infection, following the operation, and without any perceptible difference in the incubation period. Some few animals, especially during the hot weather of August and September, died from accidental causes,

\* The latter quantity, however, is much greater than it is necessary or desirable to use.

parasitical, lung disease, or other ailments, such as diarrhœa, and an epizootic form of nasal catarrh, invariably and rapidly fatal, to which rabbits in confinement seem to be very liable.

For the sake of uniformity I have latterly always used in these intracranial inoculations 0·1 c.c., or one minim and a half of the mashed medulla.

Another experiment was as follows:—Two dogs were inoculated, 18/9/86, from the medulla of a rabbit of short incubation period. The one a rough terrier, D 8, intracranially by trephining, the other a smooth terrier, D 9, by injecting half a c.c. of mashed medulla into the tibial vein.

Two rabbits were inoculated intracranially from the same cord; they both died infected, with typical symptoms and appearances, after an incubation period of seven days.

Both the dogs, however, D 8 and D 9, remained unaffected. The one, D 8, after the lapse of four months was then bitten sharply by a rabid dog in several places on the fore-leg, which had been previously shaved; but again in upwards of two months more has shown no symptoms of infection, though some rabbits bitten by the same dog were infected and died in the usual manner. The other dog, D 9, after the lapse of some months was again injected in the tibial vein with half a syringe of virulent rabbit's medulla, but it also up to the present time (five months after inoculation) has shown no disturbance, though two rabbits inoculated intracranially from the same virus died infected in the usual course.

This result was quite unexpected, both from my own experiments with rabbits and from the statements of others; it shows how very much more strongly refractory to the infection with the virus of rabbit rabies dogs are than are rabbits themselves, in which, by intracranial inoculation, infection is produced almost invariably.

All immunity from, or refractoriness to, infection is relative, as in the original case of vaccination against variola, and also in rabies, as shown conclusively long ago by Hertwig (*loc. cit.*, *infra*), also by Chauveau in the refractoriness of Algerian sheep to anthrax ('Comptes Rendus,' vol. 90, 1880, p. 1525), and stated in express terms by Pasteur himself in reference to the general theory of protection, (*ibid.*, vol. 90, 1880, p. 953). Its bearing upon testing the results of inoculation in dogs, with the object of prophylaxis, is referred to below.

To examine the infectivity of the peripheral nerves, I took a portion of the sciatic nerve of a rabbit recently dead, one of a series of six or seven days' incubation period, and triturated it with bouillon in the usual manner, but as it was more viscid or tenacious than the medullary substance, I was obliged to dilute it more than in the usual proportion, in order to render it sufficiently fluid to inject.

With it I inoculated three rabbits intracranially by trephining; all three showed a rise of temperature towards the end of the sixth day, and died with typical symptoms and appearances of infection shortly afterwards.

As I had diluted the nerve substance about twice as much as I usually did medulla, and only injected the same quantities, producing infection without any variation in the incubation period, it is shown to be fully as virulent as the cerebro-spinal substance; and we may conclude that the tissues both of the central and peripheral nervous systems are equally the seat of the virus.\*

At an early stage of the investigation I made a series of experiments upon the relative activity of the virus of the spinal cord and medulla oblongata of the same animals, and I found that as shown by the duration of the incubation period, there was no appreciable difference whatever in the infective virulence of the two.

I have tried the infectivity of the tissues of the salivary glands of a rabid dog, or the secretion expressed from them, taking portions of the parotid and submaxillary, crushing each in a mortar, adding a small portion of bouillon, with which it was macerated for a time, the fluid then being injected intracranially in rabbits.

Of two rabbits inoculated from the submaxillary gland one died on the 2nd day apparently from an accidental cause, the other was found dead in the morning of the 4th day, no symptoms of infection having been apparent nor any pathognomonic appearances on post-mortem examination. Two other animals were inoculated intracranially from its medulla and both remained unaffected. The two rabbits inoculated from the parotid both developed symptoms of infection on the 17th day, and died during the 20th with typical appearances, showing apparently that the tissues or secretion of the parotid gland are infective, but much less actively so than the medulla.

\* Hertwig (*op. cit.*, *infra*, *vide* p. 65) failed to produce infection in six dogs inoculated with the crural and sympathetic nerves of other rabid animals.

Rossi, of Turin ('Torino, Accad. Sci. Mem.,' 1805-1808, p. 94) asserts that he produced rabies in a dog after an incubation period of eighteen days by inoculating it in the tail with a portion of the crural nerve of a rabid cat just killed. This cat, however, was one of "several" which he states that he rendered rabid by confining in a room without food or drink; then killing them, he inoculated the different fluids of the body to ascertain which besides the saliva most readily induced rabies, and found that only that secretion *and the nerves while warm*, did so. In a subsequent communication ('Memorie,' &c., vol. 30, 1826, p. 22) he further states that two dogs became rabid by being bitten by a cat confined as described, but that by similar means he was unable to excite rabies in dogs.

His experiments appear to have been numerous, but his statements on this point are difficult to understand if his own conclusion be rejected, that rabies in the cat is spontaneous and may be produced experimentally.

The infectivity of the blood of rabid animals has been a moot question, some accounts having asserted infection to have been produced by inoculation with it, the experiments having, of course, been made by subcutaneous inoculation.

I have taken blood from the heart of a rabbit recently dead of rabies, defibrinated it by whipping with a sterilised glass rod, and injected portions of 0·1 c.c. subdurally in rabbits, but have not succeeded in producing infection, the animals as long as observed being unaffected in any way.

It seems probable that, as in other analogous cases, this fluid is but exceptionally infective, or only so in large quantities, as it is not the primary seat of the virus, which we now know to be principally in the tissues of the central nervous system.

---

The value of these methods of intracranial inoculation with rabbits, from the greatly curtailed incubation period and practically certain resulting infection, for the purpose of determining whether a suspected case is one of true rabies or not, is obvious.

In one instance a dog was destroyed at Caterham in a state, apparently, of furious rabies, after having bitten several persons and other dogs; as it was very desirable to ascertain positively the nature of the case, I inoculated from a portion of its medulla a rabbit, which after sixteen days developed symptoms of infection, and died shortly afterwards of paralytic rabies.

In another instance I received a portion of the spinal cord of a dog that had bitten several persons at Grantham, but which, as was stated, showed no symptoms of rabies; from the cord I inoculated a rabbit by trephining, and after nineteen days symptoms of infection appeared, the animal dying in the usual manner, leaving no doubt as to the dog having been rabid.

The duration of the incubation period, too, being proportionate to the activity of the virus, which varies from different sources, may in case of death from hydrophobia, afford a means of determining the source whether, *e.g.*, infection arose from the bite of a rabid street dog, or was caused by inoculation with rabbit virus.

This must, however, be received with some reservation, the incubation period resulting from inoculation with the virus of street rabies in some cases, though very exceptionally, being even shorter than that of the Pasteurian or constant rabbit virus, as shown in two of my experiments (*supra*, p. 58) where in a dog and rabbit inoculated subdurally from the cord of a street dog, this period was respectively seven and five days, and in another rabbit similarly inoculated from the last-mentioned dog, it was only four days.



*V. On the Occurrence of Infectivity in the Tissues after Inoculation.*

The period at which the tissues of an animal inoculated may become virulent, or the bite of a dog be infective, is of importance, and as yet there are no observations on record to enable us to form an opinion on this point.

I had found in numerous experiments that if a rabbit was killed upon the termination of the incubation period, on the appearance of the first appreciable symptoms, its medulla was as actively infective as that of an animal which had died after the disease had run its full course.

To determine at what period this infectivity is developed I inoculated five rabbits, A, B, C, D, and E, intracranially in the usual manner from a medulla of six to seven days' incubation period.

Of these A was killed towards the termination of the 2nd day, about 44 hours after inoculation, and from its cord two others, A 2 and 3, were also inoculated intracranially. Another animal, B, was killed at the expiration of the 4th day, and B 2 and 3 were similarly inoculated. A third, C, first showed symptoms of infection towards the close of the 7th day, about 164 hours after inoculation; it was thereupon killed, and C 2 and 3 were inoculated intracranially. D and E, which developed symptoms during the same day as C, were allowed to die in the regular course of the disease; the one was found dead on the 10th, the other died during the 11th day, with typical symptoms and appearances. From the medulla of this latter two other animals, E 2 and 3, were inoculated.

Of these rabbits, A 2 and 3 as well as B 2 and 3 were altogether unaffected, with the exception of a slight and transient rise of temperature on the 5th day in A 2, which was probably accidental.

C 2 and 3 developed symptoms by the 7th day, which took their usual course. No. 2 was found dead on the morning of the 12th day, No. 3 dying during the same day.

D 2 and 3 showed an incubation period of six to seven days, and died shortly afterwards.

From this it appears that the spinal cord of an infected animal is not itself in anywise virulent till towards the close of the incubation period, concurrently with the appearance of the first symptoms of constitutional disturbance. I think we may conclude from this that the virus is latent at the site of inoculation till this period, when, somewhat suddenly, it bursts forth and pervades the tissues. In the case of bites from rabid animals this seems to suggest the possible utility of excising or deeply cauterising the wound, even at a subsequent period, and throws great doubt upon the authenticity of those cases where hydrophobia has been said to have been occasioned by the bite of an animal, which itself remained unaffected for a consider-

able time afterwards; and I should be disposed to conclude that if such an animal developed no symptoms of rabies for some days after having bitten a person or other animal, the latter would be safe from any danger of infection.\*

\* The most extensive and important observations and experiments upon this subject ever recorded before M. Pasteur's are those of Hertwig, made in the Veterinary School of Berlin between the years 1823 and 1827. They are published in Hufeland and E. Osann's 'Journal f. prakt. Heilkunde,' Berlin, vol. 67, 1828, (Beit. z. nähern Kenntniss d. Wuthkrankheit, oder Tollheit d. Hunde, von Dr. Hertwig). They have been but little noticed by English writers, important as they are. Their chief results are:—

(1.) Of 16 dogs inoculated with the saliva of others rabid, by puncturing the skin of the head, 6 died infected.

(2.) Of 7 similarly inoculated with the secretion expressed from the parotid glands, 1 was infected.

(3.) Of 2 inoculated with the crural and 4 with the sympathetic nerve, no infection resulted.

(4.) Saliva put in the mouth of more than 20 dogs in no case produced infection.

(5.) Of 11 dogs inoculated with the blood of others rabid, taken during life and shortly after death, no specific infection occurred.

(6.) Of 15 caused to be bitten by others rabid, 5 died infected; but of 137 apparently brought to the infirmary bitten by others rabid or (qy.) supposed to be so, only 6 died infected.

His own pug was inoculated nine times during three years and resisted infection, but succumbed to a subsequent trial. He remarks (*op. cit.*, p. 172) that of the other dogs that died after inoculation, some withstood infection two, three, or four times, and one died at first, clearly showing how variable is the degree of refractoriness possessed by different animals.

His observations upon the symptoms and appearances in upwards of 200 cases that he examined are carefully recorded, and his statement that he compared the latter with those of healthy animals shows the scrupulous care with which they were made. Their description is fully given in his later work 'Die Krankheiten d. Hunde u. deren Heilung' (Berlin, 1853).

A series of observations made upon even a larger number—375 cases of street rabies, in the Veterinary Institute of Vienna, during twenty years—is that of Bruckmüller, recorded in his 'Lehrbuch d. Pathol. Anatomie der Hausthiere' (Wien 1869). He found a morbid appearance in the stomach in 254 cases, or nearly 70 per cent., with the presence of foreign substances in it in 199 cases, or 55 per cent.; it was "inflamed" in 125, or 33 per cent.

It appears probable, however, that some at least of these cases may have been destroyed during the progress of the disease, and not improbably some of them may not have been cases of true rabies, which circumstance would materially affect the proportions of pathognomonic appearances observable.

The best work extant on this subject, of both literary merit and scientific accuracy, is the well-known 'Rabies and Hydrophobia,' by George Fleming, LL.D., Principal Veterinary Surgeon to the Army (London, 1872), which gives a complete and excellent account of the disease in all its relations, with a notice of the principal publications up to that time. Of these the best in the English language are those of William Youatt, M.R.C.V.S. ('The Dog,' London, 1845, and 'On Canine Madness,' London, 1830), in which the account of this disease in the lower animals is given from the numerous cases observed in his own extensive practice.

VI. *Duration of the Incubation Period.*

The great variability of the incubation period and the extreme length to which it may extend after the bite of a rabid animal, is well known, and constitutes the most unaccountable feature of this disease. It is well established that both in man and the lower animals it may extend to at least several months, and even periods of some years have been recorded upon apparently good evidence. Subcutaneous inoculation with saliva taken from the mouth in all the experiments which I made failed. In intracranial inoculations with the secretion expressed from the parotid gland, as previously described, the incubation period was seventeen days.

By subcutaneous inoculation with the medulla of street rabies, it is uncertain and generally prolonged, both in dogs and rabbits, but by intracranial inoculation it is much shortened and more regular. I have had two cases above recorded in rabbits, where the first symptoms appeared on the fourth and seventh days respectively, after intracranial inoculation, but this is most unusual with virus from this source, *i.e.*,

The most important recent work upon the subject in English is the article by Bollinger, in the American translation of Ziemssen's 'Cyclopædia of the Medical Sciences,' which gives a good account of the ætiology of the disease, its symptoms, and other features, both in man and the lower animals, with a notice of the previous literature.

A copious bibliography is contained in the 'Dictionnaire Encyclopédique des Sciences Médicales,' ed. by A. Dechambre, Paris, 1874, article "Rage." The list is brought up to the date of the commencement of Pasteur's investigation and the inauguration of the present views upon the subject in the 'Nouveau Dictionnaire de Médecine et de Chirurgie Pratique,' by Dr. Jaccoud, vol. 30, Paris, 1881.

The most complete account of the literature of the subject, however, is that given in the invaluable 'Index Catalogue of the Surgeon-General's Office U.S. Army,' Washington, 1885, vol. 6. Art. "Hydrophobia."

The communications to the Royal Society upon rabies or hydrophobia have not been numerous or important; they are mainly records of cases in man and reports of asserted cures. One of these by Dr. James has been above referred to.

Amongst the more recent publications upon this subject may be mentioned that of M. Bourrel, a veterinary surgeon, formerly in the French Army, and director of the Institution for the Study of Canine Pathology in Paris. His observations ('*Traité complet de la Rage, chez le Chien et chez le Chat, Moyen de s'en préserver, &c.*,' Paris, 1874) are important on account of the very large number of cases in the dog which he had the opportunity of observing.

Between the years 1859 and 1872, as he states, out of 18,531 dogs admitted to the establishment 1219 were rabid. He advocates the prompt application of caustic to the bite of a rabid animal, and admits that the enforced muzzling of dogs had been beneficial; but his specific to abolish all risk to man from this disease is by filing down the points and sharp edges of the canine teeth and incisors of all dogs.

Since the publication of M. Pasteur's results the only independent investigations as yet recorded are those, before referred to, of Professor Frisch, in Vienna; and, more recently, in the 'Annales de l'Institut Pasteur,' Paris, March, 1887, those of Dr. Bardach, of Odessa, noticed below.

rabid street dogs. In other cases the period has been from seventeen to nineteen days, which appears to be about the average, and agrees nearly with that given by M. Pasteur and Prof. Frisch.

When the virus of street rabies is passed through a sufficient number of rabbits the period is further reduced to six or seven days, and becomes markedly constant. In a period of about six months I have carried this virus, originally obtained from M. Pasteur's Laboratory, through a series of twenty rabbits, inoculating two or more of each series. In the large majority of cases the first symptoms of infection have appeared between the sixth and seventh days, exceptionally not till the eighth day, in a few instances till the ninth. Latterly I have observed two cases in which the latent period was only four days.

In one case quite recently, two rabbits were inoculated intracranially from one of a Pasteurian series, that had died after a very unusually short incubation period, with characteristic symptoms, but of only some hours' duration, on the fourth day after inoculation. One of these so inoculated died on the third day, apparently from accidental causes, the other remained unaffected and healthy till the fortieth day, when it was observed to be paralysed, and was found dead the following morning, with post-mortem appearances that were very well marked and characteristic; the hæmorrhages in the stomach, though not perceptible on the serous coats, were larger on the mucous surface, though few in number, than any other I have observed, almost resembling, as has been described, "crushed currants." This duration of the incubation period is quite exceptional.

From the medulla of this case two other rabbits were inoculated by trephining; they both showed an incubation period of the usual length—six to seven days—with well-marked symptoms, thus proving that the remarkably protracted incubation period in the above case was due to some accidental cause, and that the virus had undergone no permanent modification.

I have had the opportunity of inoculating intracranially from the medulla of a rabid horse, in this case with an incubation period of seventeen days, and from a rabid ox, as also from a case in man, in all of which it was about the same, and the symptoms of infection and post-mortem appearances were identically similar to those following inoculation from the dog or rabbit.

I have also inoculated rabbits from the medulla of a furiously rabid cat,\* which had been itself inoculated from a street dog. In this case the incubation period—in the rabbits—was between seventeen and eighteen days, with the regular symptoms and post-mortem appearances.

That in inoculating rabbits intracranially, the duration of the incubation period is usually determined by the activity of the virus,

\* The same animal that bit the man, Joseph Smith, hereinafter mentioned.

and only very exceptionally by any reaction of the animal inoculated, is shown by the circumstance that, however many animals, of the same species, are inoculated from the same source, this period generally shows no variation at all in them, though the duration of the disease, and consequent occurrence of death, is evidently dependent mainly upon the age, condition, and vigour of the subject.

## VII. *Preservation of the Virus and Methods of Modification.*

On the occurrence of septic decomposition in the medulla the virus is destroyed, but M. Pasteur has stated that by removing portions of virulent medulla with precautions against contamination, and suspending them in an atmosphere of pure carbonic acid, their infectivity is retained unimpaired for some weeks.

I have not, however, found this to be the case; I have in several experiments carefully removed a portion of the cord of a rabid rabbit, passing it and the ligature to which it was attached through the flame of a spirit-lamp and suspended it in a vessel, previously disinfected, plugged with sterilised cotton-wool, and kept saturated by a constant current of  $\text{CO}_2$ , filtered through cotton-wool.

In every case I found that within a few days the virus was materially modified, and soon completely destroyed, the rapidity of the change probably depending upon the temperature. In summer on the third day this diminution in virulence is apparent in the results of inoculating rabbits with it. Septic microbes, however, do not develop in the medullas, as long as kept in this manner.

I consequently find this method unsuitable for preservation of the virus for even the shortest period.

The basis of M. Pasteur's present methods of protective inoculation consists in the asserted progressive modification and ultimate extinction of the virus which is produced by suspending a portion of infective medulla in a current of dry air. The methods adopted are to take out a portion of the spinal cord of a rabid rabbit soon after death, then passing it lightly through the flame of a spirit-lamp,\* in order to destroy any septic germs which may have fallen upon its surface, to suspend it by a ligature similarly flamed, in a previously sterilised bottle, with tubulature at top and bottom, plugged with cotton-wool, and containing a quantity of caustic potash.

Thus prepared the cords are gradually dried; the potash, absorbing all moisture, prevents any development of septic organisms quite effectually. A portion of a cord dried for the length of time required for inoculation is then triturated with bouillon, strained as before described, and injected subcutaneously.

\* The sterilisation however is superfluous, inasmuch as saprophytes do not develop in dry air.

Pasteur states ('Comptes Rendus,' vol. 101, 1885, p. 770) that the progressive modification of virulence in cords thus preserved is attested by the increasing length of the incubation period in rabbits inoculated intracranially with them, and that the duration of this period increases regularly with those dried up to seven days, but that from and after that period they are not virulent.

I have myself in several experiments invariably found the latter part of this statement correct, and that cords dried for seven days or more are absolutely inert, as are frequently those of six and of five days.

But I have not by any means found the progressive prolongation of the incubation period, with cords dried for a shorter time, as regular as he records, but on the contrary I have found it usually the same as with fresh cords, unaltered up to and including the fourth day; in one case only when inoculating from one dried four days, did the first symptoms of infection, which were not well marked, appear to be deferred till between the ninth and tenth day, the animal dying on the thirteenth.

As in cords thus treated the virus certainly becomes altogether extinguished, *teste* Pasteur, and, as I have myself found, somewhat suddenly, by the seventh day, it appears doubtful what benefit can result by inoculating subcutaneously with those of a longer period, with the object of prophylaxis.

The preservation of cords by this method, in an atmosphere kept perfectly dry by caustic potash, entirely prevents the occurrence of microbial putrefaction; its absence is plainly evinced by no fœtor being perceptible in them, as saprophytes are unable to develop in a perfectly dry atmosphere, as well as in one of carbonic acid.

I have too examined with the microscope portions of cords thus preserved for different periods, but have never recognised any microphytes; if they had been present in any numbers they could not have escaped observation.

To prove their absence certainly, I took part of a cord preserved as described for five days, and snipping the outer surface, which was dry and firm, I plunged a sterilised platinum needle into its substance, which was moist and viscid, adhering in very appreciable quantities to the needle. With this I inoculated a tube of agar-agar bouillon peptone, and performed this operation three times; the tubes thus inoculated were placed in the incubator at 38° C., no organisms developed, and their contents remained altogether unchanged until they ultimately dried up, showing the total absence of septic microbes.

I may add that in a room of any ordinary laboratory it would, I believe, be practically impossible to remove any number of cords and transfer them to the requisite vessels, without some germs of septic organisms falling upon them during the operation; and that con-

sequently the only reliable means of preserving them from septic changes is by keeping them under conditions where saprophytes cannot develop, such as that adopted by M. Pasteur and here followed. Another method would be to keep them at a very low temperature.

The result of attempting to protect rabbits by subcutaneous injections with medullas treated as above, is in accordance with these observations. Here, as recorded below, in the first series of experiments, where large quantities were injected, death shortly followed from sapræmia in every one of the animals inoculated; in the second series, using smaller quantities for injection, fewer deaths from the same cause occurred, illustrating the distinction between infection with a specific bacterial virus and intoxication by a chemical poison, viz., that in the former case within certain limits, the result is independent of the quantity inoculated, one viable germ producing the same effect as an immeasurably greater number; but in the other case—the action of a soluble or chemical ferment or poison—the effect is directly and obviously proportionate to the quantity used for inoculation.

#### VIII. *Protective Inoculation.*

In the first series of experiments upon rabbits, five were taken and inoculated daily after M. Pasteur's original methods with half a Pravaz syringeful—about 0·7 c.c.—of mashed spinal cord, commencing with that dried as just described for 15 days; on the third day with one of 13 days; on the fifth with one of 11 days; the sixth with one of 10; and so on daily, or as often as a cord of the requisite age was available, till the thirteenth and last inoculation was made with a cord dried one day only, and as several previous experiments had shown, of unmitigated virulence, at least for rabbits.

Three of the rabbits, however, had died during the course of the inoculations; one, the youngest of the batch, which died first, apparently from accidental causes, the two others from sapræmia; but two remained for the concluding inoculation, and these both died a few days after it was made. None of them, however, showed any symptoms of infection with rabies, they were those of sapræmia or septic intoxication. The series of experiments was inconclusive therefore in its results, and it seemed possible that the quantity of matter injected was too large.

In a subsequent communication ('Comptes Rendus,' 2nd Nov., 1886) M. Pasteur, objecting to the results of similar experiments published by Professor Frisch, of Vienna ('Wiener Med. Wochenschr.,' referred to below), promulgated a new "rapid" or "intensive" method of treatment, which appeared likely to be more successful with rabbits, liable as these animals are to septic poisoning by inoculation with any foreign matter. Accordingly six rabbits were taken, all apparently

healthy, and were inoculated in the following manner, with medullas of progressively increasing virulence, 0·15 c.c. of the mash prepared as above described being used in each subcutaneous inoculation.

On the 1st day, morning, cord dried 13 days.

"	"	"	evening,	"	11	"
"	2nd	"		"	9	"
"	3rd	"	morning	"	6	"
"	"	"	evening,	"	4	"
"	4th	"	morning	"	3	"
"	"	"	evening,	"	2	"
"	5th	"		"	1	"

Of the rabbits thus inoculated, one was found much wasted and partially paralysed behind, with a falling temperature, on the 6th day after the concluding inoculation, it died on the 9th day with well marked and unmistakable post-mortem appearances.

A second animal died on the 11th day after the last inoculation with symptoms and appearances that clearly showed infection. A third was first affected on the 19th day, and died on the 22nd, clearly of paralytic rabies.

Two others died some days after the completion of the inoculations with appearances of sapræmia. One remained in good condition and unaffected; this on the 24th day after the last inoculation, was injected intracranially with 0·1 c.c. of mashed medulla of a rabid rabbit just dead. On the 6th day following, the temperature, previously normal, rose to 40° C., and the following day was the same, with commencing paresis. The symptoms followed the usual course, and the rabbit was found dead on the morning of the 11th day; the duration of the disease—between four and five days—showed the animal to be very robust and healthy, consequently a most favourable subject for protection, but the shortness of the incubation period—the test rightly applied by M. Pasteur to the activity of the virus—proves that it was not in any wise modified by any refractoriness induced in the animal by the previous inoculation; and I think it must be concluded from these experiments that the method followed, essentially in accordance with M. Pasteur's last published rapid method, is, as far as rabbits are concerned, inefficient to confer any immunity against subsequent infection, and dangerous as likely to produce it.

It must, however, be understood that M. Pasteur has not asserted in his communications to the Parisian Academy, that rabbits are capable of being protected. He has confined his statement to dogs.

*Protective Inoculation in the Dog.*—The dog should be a far better subject for these experiments than the rabbit, being far more resistant to septicæmia and sapræmia, and much less liable to those



accidental affections, parasitical and others, by which the latter animal is constantly attacked; moreover, it is rightly regarded as the typical subject, the *fons et origo* of the malady here under investigation.

Two dogs were taken for this trial, the one a mongrel hound of medium size, Pr. No. 1, the other a rough white terrier, Pr. No. 2, and treated as follows:

1886, October 4. Both were injected under the skin of the back with half a Pravaz syringeful (about 0·7 c.c.) of mashed medulla of a rabbit of a Pasteurian series, dried thirteen days.

On the 5th October 0·4 c.c. of a similar cord dried 11 days.

„ 6th	„	0·5	„	„	9	„
„ 7th	„	„	„	„	8	„
„ 8th	„	„	„	„	7	„
„ 11th	„	„	„	„	6	„
„ 12th	„	„	„	„	5	„
„ 13th	„	„	„	„	4	„
„ 16th	„	„	„	„	3	„
„ 18th	„	„	„	„	2	„
„ 2nd Nov.	„	„	„	„	1	„

These dogs both remained unaffected in any way whatever, and on the 6th November the first, Pr. No. 1, had the fore-leg shaved, and was bitten by a rabid street dog, the teeth of which penetrated the skin in several places, drawing blood, the saliva also was evident upon the leg, and was spread with a scalpel over the marks of the teeth, and where the skin had been cut in shaving.

This dog remained perfectly unaffected, lively, and good-tempered for more than four months after being bitten; it was then again inoculated by injection into the tibial vein of half a c.c. of active virus, again without showing any symptoms of infection up to this present time (twenty days after inoculation).\*

At the same time a fresh dog, a rough white terrier, D 10, was similarly inoculated in the tibial vein with the same virus; this animal also remains unaffected, though rabbits inoculated intracranially from the same cord died infected in the usual course. This animal is obviously strongly refractory to infection.

The second dog inoculated for protection, the rough terrier, Pr. No. 2, was kept under observation without showing any disturbance till the 24th January, 1887, when it was inoculated intracranially under æther, with a full quantity of the mashed spinal cord of a rabbit of the Pasteurian series recently dead.

Two rabbits were similarly inoculated with the same virus, they

\* P.S.—And for three months subsequently. 29/7/87.

both died with usual symptoms of infection, after an incubation period of 6—7 days.

The dog, however, after recovering from the effects of the anæsthetic, remained perfectly well and unaffected in any way, and appears, as the first, to be completely refractory to infection by the most active method of inoculation.

From these two cases I should have concluded that the methods of protective inoculation introduced by M. Pasteur were successful and efficient in dogs, but the cases of the three unprotected animals described (*viz.*, D 8 and D 9 *supra*, and D 10), which are equally refractory to infection, do not support this conclusion as a result of the limited number of my experiments upon this point. The virus of rabbit rabies, almost invariably infective by intracranial inoculation to animals of that species, would appear to be less certain in its action upon dogs, and it is only by the results of a series of numerous comparative experiments that a final conclusion can be formed, whether these methods have or have not any effect in increasing the constitutional refractoriness of the dog to infection with rabies. I would add, however, that it appears to me from the more numerous recorded experiments of others upon dogs, *viz.*, those of Professor Frisch, of Vienna, and those of Professor Horsley at the "Brown Institution," exclusive of those on an extended scale by M. Pasteur himself, in all of which infection seems to have been invariably produced by intracranial inoculation, that the principle of protection is established, and that in some cases at least, judged by the results of comparative experiments, increased refractoriness to infection in the dog is produced by the methods indicated, which is as much as could be expected or hoped for, immunity, as above remarked, being always merely relative.

With regard to the protective inoculation of man, the end and object of M. Pasteur's work, this cannot be conclusively judged by the result of experiments upon the lower animals of widely different constitution; for in the rabbit and the dog its effect is very dissimilar; all that this can do is to establish or disprove the principle of the method. It is by the statistics of the treatment in man that it must be judged. These will no doubt be examined exhaustively by the Parliamentary Commission now sitting:

Taking, however, the accounts last published ('Comptes Rendus,' 24th January, 1887) in which the number of patients treated is stated at 2682, and the deaths amongst them from all causes 31, or only 1.15 per cent., it appears probable that the treatment has been successful in at least some cases, since all published statistics, widely as they vary, give a mortality from the bites of rabid dogs much in excess of this.

But beyond this, inasmuch as the last injection in each course is made by Pasteur with virus dried for one day only and not materially

or at all modified, this would presumably be infective in a considerable proportion of cases unless the patients were protected by the preceding inoculations.

I cannot, however, as above stated, avoid the conclusion that the rapid method of inoculation is dangerous. This opinion is confirmed by the experiments of Professor Frisch, of Vienna, the only independent investigation of these methods yet recorded.\*

The statistics of his treatment must very shortly show whether the mortality amongst his patients has or has not increased since the practice of his intensive methods.

Within the last few days, too, since the above was written, this opinion of the danger of infection from the intensive or rapid method of treatment is strengthened, by a report published in the current number of the 'Annales' of the Pasteur Institute, by Dr.

\* His first report ('Wiener Med. Wochenschr.,' 1886, April 24th, No. 17) in the main confirmed the results of M. Pasteur; twelve dogs protected by the original method resisted intravenous injection of acute virus, while three out of six control animals were infected.

In a second report (*ib.*, 7th August, 1886, No. 32), of sixteen rabbits inoculated by trephining, with fifteen of which preventive inoculations were commenced immediately afterwards, and continued daily for eighteen days, all save two died infected.

M. Pasteur having attributed this unfavourable result to the inoculations having followed one another too slowly, and recommended his rapid or intensive method ('Comptes Rendus,' 2nd November, 1886), Professor Frisch repeated his experiments on a larger scale, following the new method of inoculation, with almost uniform failure, and consequently concludes decidedly that this is dangerous.

Nor can I overlook a case in man, all the stages of which, both before and after his treatment by the rapid method of M. Pasteur, were within my own observation. It is that of Joseph Smith, or Goffi, of this Institution, already noticed in different journals. He was bitten sharply on the hand by the furiously rabid cat above mentioned; within a few minutes the wounds were well washed under the tap, sucked by himself, and then, together with his mouth, washed with a strong solution of permanganate of potash, again with water, and then thoroughly treated with anhydrous carbolic acid—absolute phenol. Shortly afterwards the parts bitten were excised under chloroform. The same night he was taken to Paris, and the following day his treatment by M. Pasteur's intensive method commenced.

Shortly after the completion of the course and his return home, he developed symptoms of spinal paralysis and died under circumstances which suggested the probability of his having been infected by rabbit virus. A report of the case will, I believe, shortly be published by those who had charge of it.

The wounds caused by the bite were thoroughly canterised so shortly afterwards, that there was certainly every prospect of his escaping infection from that source. The symptoms developed and other circumstances seem to point clearly to the circumstance of his having been infected by subsequent inoculation, and not by the original bite. The incubation period in the rabbits which were, I believe, inoculated from his medulla, will settle this conclusively. It must, however, be remarked that he was debilitated during the treatment in Paris by the effects of intemperance, and consequently, no doubt, rendered more susceptible to infection by the inoculations to which he was subjected there.

Bardach, Director of the Bacteriological Institute at Odessa, where of 15 dogs inoculated intracranially with lyssic virus from different sources, and immediately afterwards subjected to protective inoculation by M. Pasteur's intensive method, 6 developed rabies, 9 surviving. Of 6 control animals, similarly infected, all died. Of the 6 protected animals that died, 3, as is shown, were infected with paralytic rabbit rabies, the result of the subcutaneous inoculations; again showing the dangers of this method. The proportion, too, of the survivors, 9 out of 15, or 60 per cent. is not favourable.

#### IX. *The Action of some Drugs upon Infection.*

The various substances and measures that have been tried as remedies for rabies are innumerable, from viper's venom to plain water; from time to time certain cases of cure have been announced, but a large proportion of these may obviously be accounted for by the absence of infection; others in which distinct symptoms of the disease are recorded are more difficult to dispose of, though some of them in man probably were not true hydrophobia or lyssa, but a nervous or hysterical affection simulating its symptoms,—lyssophobia.

In investigating the action of drugs upon animals infected with this virus, it appeared to me that two methods of treatment might be followed, the one to endeavour to destroy the virus, almost certainly a micro-parasite, by the administration of a germicide; the other, to treat the symptoms developed with appropriate remedies, and by the use of tonics and stimulants to enable the animal to survive the attack, when, as in other cases, the virus would have exhausted itself and died out. The explicit statement of M. Pasteur (*vide infra*), that spontaneous recovery in dogs does sometimes occur, seemed to offer some prospect of success by this method.

I naturally commenced with bichloride of mercury, as being not only the most powerful germicide known, but also almost equally active as an antizymotic, in the combination of these two qualities standing quite alone; it has, too, lately been stated that Dr. Theodore Cash had found it a prophylactic against infection with anthrax inoculated subsequently to its use. I had thus some hopes of its efficacy in rabies.

I found that 6 to 7 tenths of a milligram was about the maximum dose that could be safely given to a medium-sized rabbit; consequently I inoculated one, 26/9/86, intracranially with active virus, and three hours afterwards injected subcutaneously 2 minims of  $\frac{1}{2}$  per cent. solution of bichloride of mercury; this was continued daily, with the interval of one Sunday. The animal was unaffected in any way till the 9th day, when the temperature rose to 41.2° C.,

paresis with the usual symptoms of infection was observed, and it died on the 12th day with characteristic appearances.

In the control animal, infected in similar manner, the incubation period was of exactly the same length, and it died on the same day as the one treated with sublimate, which had been therefore obviously inoperative in this case to destroy or in any wise modify the action of the virus. Had it only prolonged the incubation for a few hours it would have encouraged further trial, but with the result here obtained I saw no object in this.

Benzoic acid is recognised as a powerful germicide: Graham Brown ('Archiv Exper. Pathol.,' vol. 8, p. 144) found its soda-salt remarkably destructive to the virus of diphtheria. Rabbits will take considerable quantities of this—benzoate of soda—continued for several days, without any ill effects.

A rabbit inoculated intracranially with rabies, 15/9/86, one hour afterwards received by subcutaneous injection 1 c.c. of a saturated solution of the salt, which was repeated daily; on the seventh day the animal, much wasted, showed symptoms of infection, with paresis and rise of temperature; it died on the afternoon of the 9th day with well-marked characteristic appearances. In a control rabbit similarly inoculated the incubation period was one day longer than in the animal treated with the benzoate, and it died about twelve hours later. Here, too, the drug obviously had no beneficial action, and even seemed to tend to shorten the incubation period, and assist the activity of the virus.

I next tried iodine, as an active germicide, dissolving it in a solution of potassic iodide. I found subcutaneous injections of 2 cgrms. of iodine were borne well, which is a materially larger quantity relatively to their weight than the established dose for man. Accordingly a rabbit was inoculated with active lyssic virus, 25/10/86, and an hour afterwards 1 cgrm. of iodine in solution was injected subcutaneously; this was repeated on the three following days, when the quantity was increased to 2 cgrms. On the afternoon of the 7th day, however, paresis appeared, and the temperature rose to 40·7° C., and on the morning of the 10th day the animal was found dead, with post-mortem appearances that were quite characteristic. In a control animal inoculated at the same time the incubation period was similar, and it died about eighteen hours after the first.

Thus iodine appeared as inert as the substances previously tried in its action on the virus.

The next remedy that suggested itself was chloral hydrate. This is not only a powerful germicide but has been often recommended as having a specific action upon the symptoms in rabies, acting directly upon the brain and spinal cord. Rabbits will take enormous doses of this; 4 grammes even, in an average rabbit, frequently producing

but partial narcosis, and after a few hours no disturbance whatever.

A rabbit infected in the usual manner, 30/10/86, one hour afterwards was injected with 1 grm. chloral hydrate; this quantity was repeated daily till, on the 7th day, the animal was found to be paralysed, but most unusually, the fore limbs were affected more strongly than the hind; the usual rise of temperature was absent or escaped observation; it was found dead on the following morning, the 9th day. A control rabbit similarly inoculated, after an incubation period of between eight or nine days, died on the 12th day; another with an incubation period of eight days died on the 11th.

This result, though not favourable to the protective action of chloral hydrate, yet seemed to point to a modifying action on the virus in some respects. I had also observed the results of previous experiments which seemed to lead to the same conclusion. On the 31st July, 1886, a strong gray rabbit that had been partially narcotised by the subcutaneous injection of about 3 grms. chloral hydrate, was inoculated intracranially with infective medulla; this animal remained quite unaffected till the 28th October, when it was found to be partially paralysed with a falling temperature; it died on the 30th October; the post-mortem appearances were well marked and unmistakable.

Again, in an experiment previously referred to, a large rabbit was narcotised by the injection of 3 grms. of the same salt, and then inoculated intracranially from the medulla of a rabid street dog. On the 5th day partial paraplegia was apparent, but no rise in temperature, which, however, may have occurred previously and fallen again; the animal continued to feed well, and towards the 10th day appeared to be recovering, which it gradually did, and remained well till the 22nd day, when it was found dead—any previous recurrence of symptoms not being observed. The post-mortem appearances were remarkably distinct and diagnostic; there could be no doubt that it died of paralytic rabies.

As these were the only anomalous cases with intracranial inoculations of intensified rabbit virus, as regards the incubation period, that I had had up to this time out of upwards of sixty cases, it appeared to me that the results described in two instances could not be due to mere chance, and must be owing to the action of the drug previously administered. I therefore continued experiments with it.

To another rabbit inoculated intracranially with active virus, 1 gramme of chloral hydrate in solution was injected daily for seven days; general paralysis was then observed, but again the rise of temperature, usually one of the first symptoms, was not noticed, being probably inhibited by the action of the drug; the animal died on the following, the 8th day.

Two other rabbits similarly inoculated for control showed incubation periods of rather longer duration—eight to nine days, living till the 12th and 15th days respectively; the drug in this case had no effect in prolonging the incubation period or in modifying the symptoms. Again, to another rabbit infected in the usual manner, a smaller quantity of chloral—half a gramme—was given by injection daily till the 9th day, when, as in former cases, incipient paresis appeared, but not as usual, commencing in the hind limbs, the fore limbs being first strongly affected, in marked contrast to the regular course of the symptoms; there was again no rise of temperature; the animal gradually wasted, general paresis became complete, and it died on the 11th day.

Two control rabbits similarly inoculated showed incubation periods respectively of between four and five and seven and eight days, dying on the 6th and 11th days.

In this case the action of the drug certainly modified the symptoms and possibly delayed their development and fatal termination; another rabbit, therefore, inoculated intracranially from a rabid dog was, from the second day after inoculation, treated daily with chloral hydrate in quantities of from half a gramme up to 3 grammes till the 7th day; on the 9th day the animal was weak and losing condition, but without any symptoms of specific infection, and there was no rise of temperature. It now received 1·5 grm. bisulphate of quinine without any obvious effect. On the following day, morning and evening, 0·2 mgrm. bisulphate of strychnia, and subsequently 0·3 mgrm. was given till the 12th day, when paresis commenced, and the animal was obviously sinking, but without showing the usual course of temperature; it died on the 17th day with very well-marked post-mortem appearances. The control animals similarly inoculated showed incubation periods of 17 and 20 days, dying both on the 21st day; so that here again the action of the drug was unfavourable, and I was forced to conclude that, whatever effect it may have when administered previously to inoculation, when given subsequently it has no beneficial action at all.

Terebine is highly extolled as an antiseptic and as a remedy in many virulent diseases. Mixed 1 part with 4 of olive oil, it may be given to rabbits by subcutaneous injection of even 1 c.c. without disturbance. Accordingly after infection I gave a rabbit daily, morning and evening, 0·5 c.c. of terebine in olive oil. On the 10th day, however, it was found paralysed, with a fall in temperature, and died during the 11th day.

A control animal showed no symptoms till the 12th day, and lived till the 15th.

I tried this drug again with another rabbit, using larger doses, but with a similarly unsatisfactory result.

I next tried curari. The action of this on healthy rabbits is somewhat uncertain, a quantity of the same solution that at one time is borne without disturbance, at another being rapidly fatal. I found that about 0.3 mgrm. of the sample I had and of the solution as I made it was the maximum quantity that could be safely employed. To a rabbit inoculated sub-durally with infective medulla I injected subcutaneously 0.2 mgrm. on the 5th day, and subsequently 0.3 mgrm. daily; the animal was unaffected till the morning of the 14th day, when it was found weak in the hind limbs, the bodily temperature having fallen to 35.4° C. It died on the same day shortly after injection of 0.3 mgrm. curari. A control animal showed an incubation period of only nine days, and was found dead on the morning of the 12th.

In this case, the curari appeared to protract the incubation period and prolong the life of the animal; I therefore repeated the experiment with the drug, giving smaller quantities administered more frequently.

To a rabbit inoculated 4th December, 1886, 0.1 mgrm. curari was injected on the 4th day. On the 5th morning and evening, 0.15 mgrm., and subsequently 0.15 mgrm. till the 10th day, when the temperature had fallen to 38° C. and paresis was apparent, but confined to the fore limbs. Injection of the same quantity of curari—0.15 mgrm.—which hitherto had been without any appreciable effect on the animal, now greatly depressed it, within a few minutes of administration; the next day it was completely paralysed and died towards the middle of the 12th day.

In two control animals similarly inoculated the incubation period was in each 9 days; the one was then killed for another experiment, the other died on the 11th day. In this case the drug given more frequently, but in the same aggregate quantity daily, had, if any, but a very slight effect on the action of the virus in prolonging the incubation period or its fatal termination, and did not appear to warrant further experiments with it, the more especially as I found that even smaller quantities of curari than those I had given were dangerous, two rabbits of average size having been killed, the one by injections twice in the day of 0.13 mgrm., the other by a single injection of 0.100 mgrm.

I used the drug in 1 per cent. solution, freshly made by carefully triturating it with cold water only.

Salol, salicylate of phenol, is a drug recently introduced, which from its constitution should be a powerful germicide. Dissolved in olive oil, 1 part in 5, and injected subcutaneously, I found it was borne very well in moderate quantities by rabbits. I consequently treated a rabbit, inoculated with the virus of rabies, by giving it 0.2 gm. of salol twice daily during the incubation period, but as



compared with a control animal I found no benefit resulting from its use. I tried it again in another case in much increased quantities, but with no better results.

I had thus tried divers agents, and the most powerful germicides with which I am acquainted, without the effects of infection being counteracted or modified, and could see no prospect of protection by their use. The other method proposed above to counteract or enable the animal to resist the result of infection, was by the administration of general tonics, or specific therapeutical agents.

I found that quinine in comparatively large doses (0.3 grm.) frequently repeated, had no appreciable tonic action, and in fact, was inert upon rabbits. Strychnia does seem so to act to some extent, in minute doses, which, however, must be continued for several days to produce any beneficial effect; its stimulating action upon the spinal cord, and its specific effect in spinal paralysis, is well established, and recommended it for the treatment of rabies in the rabbit, in which the stage of excitement is very slight and transient.

I found that cocaine acts very markedly and quickly as a general tonic in the rabbit; an animal to which the hydrochlorate is given frequently, in quantities of from  $\frac{1}{2}$  grain to 1 grain or more, within a few days improves much in condition, with an increase of several per cent. of body weight, and an apparently increased appetite; even the smaller quantity, however, sometimes, but uncertainly, produced temporary excitement and general hyperæsthesia.

After preliminary trials I gave a rabbit, on the 4th day after infection, 5 minims of a 10 per cent. aqueous solution of cocaine hydrochlorate, equal to about 0.04 grm. of the salt, and subsequently the same quantity morning and evening; between the 9th and the 10th days symptoms of infection appeared, and the animal was found dead on the 11th day, the length of the incubation period and the time of death being precisely the same as in two control animals inoculated at the same time.

To another rabbit similarly inoculated, I also gave on the 4th day about the same quantity (0.04 grm.) of this salt, repeating it subsequently twice daily till the 10th day, when in the control animals similarly infected the first symptoms had appeared; the cocaine was then alternated with 0.2 mgrm. of strychnia bisulphate, but without effect, and the animal died at the same time as its companion. Another case in which I gave strychnia for a longer period was as follows: a rabbit inoculated intracranially from the medulla of a rabid dog, 9th November, 1887, received daily from the 7th day,  $\frac{1}{2}$  to 3 grammes chloral hydrate; on the 9th day 0.1 mgrm. quinine bisulphate, and from the following day, twice daily, 0.05 to 0.075 mgrm. strychnia bisulphate. The access of the first symptoms was not well marked either in this animal or in two others similarly inoculated,

but the former died on the morning of the 18th day, and both the others on the 21st, so that here again there was no benefit from the action of the drug, but apparently the reverse.

Allyl alcohol has been suggested as a powerful germicide; I therefore tried its action upon rabbits, but I found it so rapidly and fatally toxic, even in the most minute quantities, that no benefit could be expected from its action.

Urethan (carbamate of ethyl) has been recommended for its action on the spinal cord; I therefore tried it, giving it subsequently to infection, but the result was equally negative.

Rabbits are singularly tolerant of atropine, even 1 gramme of the sulphate given subcutaneously often having no apparent action upon them. It could not therefore be expected to modify the symptoms. Moreover, Youatt\* had tried the effect of belladonna extensively upon dogs infected with rabies, and though at first he had hopes of its efficacy, these were disappointed, and he ultimately found it useless.

I have also tried the action of arsenic upon rabbits. In the dog, given as arsenite of potash, it is a well-known and active tonic alterative. In man, too, and the horse it is used in some countries, with the result of increasing strength and endurance. In the rabbit, however, I could perceive no beneficial result from its administration, though the animal is very tolerant of it, and it takes large quantities proportionately to its weight without showing any symptoms of disturbance; I have not consequently tried its effect upon the virus of rabies.

In order to ascertain conclusively whether the bichloride of mercury, chloral, benzoate of soda or iodine had any toxic or inhibitory action upon the virus itself, though not modifying the symptoms it produces, other rabbits were inoculated intracranially from the medullas of the animals that had been subjected to their influence; in every instance they died infected, without any modification of the symptoms or the length of the incubation period, showing that these drugs had no action at all upon the virus.

Thus germicides, the most active tonics that I could find for the animal experimented upon, together with drugs acting specifically upon the spinal cord, were one and all inert materially to inhibit or modify the result of infection; but though none may be found that can do so in the rabbit, this, however, may not apply to other species very differently constituted, and it appears to me that of the many asserted cases of cure or recovery from this disease both in man and the dog, many of which rest apparently upon the best authority, some at least are authentic.

To take one such instance in man, the case of Offenbergh which he treated by curari (reported in the 'Med. Times and Gazette,' 6th

\* 'On Canine Madness,' by William Youatt, M.R.C.V.S., London, 1830.

October, 1877), where a country girl, 21 years of age, bitten by a dog suspected of rabies 28th July, 1874, admitted into the hospital at Wickrath in Rhenish Prussia, on the 80th day developed symptoms of hydrophobia, spasms in attempting to drink, followed by the usual course of symptoms. She was excited by light, with hyperæsthesia of the senses of smell and touch. Morphia and chloroform were without effect; she was then treated with frequent subcutaneous injections of curari, to the point of commencing general paralysis of the voluntary muscles; after being for two hours under the influence of the drug the symptoms of hydrophobia gradually disappeared and the patient ultimately recovered.

It is not probable that in this case the symptoms were merely simulative or hysterical (lyssophobic). The photophobia and hyperæsthesia of the sense of smell and touch do not favour that view; the patient, a peasant girl, was very unlikely to have heard of the occurrence of these symptoms, or to have been apprehensive of them.

This is one case out of several in which it does not seem to me that there is reason to doubt the fact of recovery, though it may well be that a method of treatment successful in one case would fail in another, or very possibly even aggravate the symptoms, owing to their great diversity.

With regard to dogs, the records of cure or recovery are very numerous. To take one instance;\* rabies having broken out in a pack of hounds, Dr. James, relying on the action of mercury, treated two hounds which had both developed symptoms of infection, with turpeth mineral (the yellow subsulphate of mercury). The one recovered, the other died. It was also, he states, successfully employed in other cases, both in man and dogs.

Here it was improbable that the symptoms and nature of the outbreak could have been mistaken; misrepresentation, too, is precluded by the fact that in a pack of hounds all the circumstances affecting them would be perfectly well known.

The statements of M. Pasteur, too, which in a matter of fact may be implicitly relied upon, appear to me conclusive upon this point. He states distinctly ('Comptes Rendus,' vol. 95, p. 1187) that he has seen *some* cases of "spontaneous" recovery in dogs, after the first symptoms have appeared,† though never after the severe symptoms, and (*loc. cit.*, 25th February, 1884) that recovery is *frequent* in fowls.

From this it appears to me that this disease is not necessarily incurable in man and the dog, though the symptoms are so different

\* 'Phil. Trans.,' vol. 39 (No. 441, 1736), p. 244.

† He adds that he has *also* seen cases of partial recovery and *subsequent relapse* after some months, followed by death.

in different cases that it may well be that treatment which in one is successful would fail in, or even aggravate another; and it seems to me very desirable that the effect of various therapeutical agents upon the dog should be investigated by those who have the opportunity and inducement to do so, though with this animal it obviously requires special methods, appliances, and precautions.

I cannot conclude this portion of the subject without expressing my strong opinion that for us in our insular position, remedial measures ought to be entirely unnecessary; to stamp out rabies and hydrophobia throughout England nothing more is required than an order by the Privy Council, rigorously enforced, for the muzzling of all dogs throughout the country for a sufficient period. Of the efficacy of this there can be no doubt.

In the Metropolitan district we see its effects in the disappearance from the streets of rabies, and of the cases of hydrophobia from the hospitals, lately so prevalent and calamitous; unfortunately, however, this can only be temporary, as, under existing conditions, the disease will, sooner or later, be again introduced from other parts where these regulations have not been enforced.\*

#### X. *Nature of the Virus.*

Though nothing can be said to be positively known of the intimate nature of the virus of rabies, it has been considered by many observers† that it must be a micro-organism. Its evident powers of multiplication and reproduction, with the extreme length of its incubation period, alone go far to prove this. It is impossible to conceive that a soluble or chemical poison, or ferment, should remain latent and unaltered in the animal body for so long a period, then at once becoming active should multiply itself throughout the tissues, rendering them infective to other animals in the most minute quantities.

In the supposed discoveries of a specific microbe in the saliva of rabid animals, it has merely been one of the many saprophytes always present therein, but which was not familiar to the observer; and it is probable, from the uncertain result of inoculation with this secretion, that the virus is present in it in very small quantities, and consequently, though particulate, would be exceedingly difficult of observation with the microscope.

\* P.S.—Already even—7/5/87—since the above was written this apprehension is realised; the police reports for April, just issued, showing a recrudescence of street rabies in the district.

† As by Hallier, 'Zeitschr. f. Parasitenkunde,' vol. 1, p. 301; and by Klebs, 'Aertzl. Correspondenzblatt,' No. 11, 1874; abstract in 'Archives Gén. de Méd.,' vol. 20, 1872, p. 352, &c.

In the asserted discoveries of a microbe in the tissues of the cerebro-spinal system, since the publication of M. Pasteur's statement that this is the seat of the virus, in some instances these have obviously resulted from mistaking the morphological elements of these tissues for micro-organisms. In the case of the statements of M. Fol,\* that he has found a microbe in the ganglion cells, and within Schwann's sheath of the nerve fibres, of the encephalon and spinal cord, though these have received the qualified support of M. Pasteur, I have no doubt that the appearances which he describes as microbes are due to alterations in the cells and nerve fibres, produced by the strange modifications of the method of Weigert which he has adopted for preservation and staining. The appearances of stained granules which he describes, can always be produced by methods similar to those he has employed. I may add, that in very numerous experiments, by inoculating from infective medulla the material in which he asserted that he cultivated the microbe, viz., infusion of sheep's brain, I have never obtained the development of any form of vegetation whatever.

I have myself, as previously stated ('Lancet,' 1886, vol. 1, p. 1112), found a micrococcus in the cerebro-spinal tissues in some cases of rabies. It occurs chiefly in and around the central canal of the medulla spinalis and oblongata, and in the perivascular and pericellular lymph channels, but it is exceedingly difficult to stain, and I have not discovered any reagent by which this can be done with certainty, for I found in sections in which it was undoubtedly present—from their being portions contiguous to others in which it was demonstrated in vast numbers—that it was impossible to recognise it by any means whatever, with the best microscopical appliances, and though mounted in media of widely different refractive indices.

I have not been able to cultivate it constantly, but I did obtain some growths in agar-agar bouillon peptone, though never in any fluid medium, and from the second series of cultivations of these, with a minute portion of its scanty development, I inoculated one rabbit subcutaneously. The animal was unaffected for three months. It was then re-inoculated intracranially with a portion of medulla of intensified virulence; here also it remained unaffected for upwards of two months, when, being again inoculated, it died on the second or third day from accidental causes.

This, which up to that time was the only case I had had of the failure of infection after intracranial inoculation in upwards of sixty cases, could not have been merely accidental, and was presumably due to a protective or inhibitory action of the cultivation; but, as I have not been able to demonstrate the presence of or cultivate the microbe constantly, a final conclusion upon its functions must await further observations.

\* 'Archives Sci. Phys. Nat.,' vol. 10, 1886, p. 327.

*XI. Conclusions.*

By these experiments it is shown :—

(1.) That the virus of rabies in the lower animals, and of hydrophobia in man, resides principally in the cerebro-spinal substance and in the peripheral nerves, as well as in the salivary glands, in accordance with the fundamental statement of M. Pasteur.

(2.) That inoculation of this substance upon the brain of an animal, by trephining, produces infective rabies in rabbits almost infallibly, and with a much shorter and less variable incubation period than after subcutaneous inoculation.

(3.) That in an infected animal, the tissues do not become virulent till towards the close of the incubation period.

(4.) That rabies, however produced, in both dogs and rabbits, is essentially a paralytic affection, the same disease in both animals, and that there is no constant distinction between the so-termed dumb and furious rabies in the dog.

(5.) That the activity of the virus of street rabies generally is increased, and becomes remarkably constant, by passing through a series of rabbits.

(6.) That the activity of the virus is shown by the duration of the incubation period, to which it is inversely proportionate, and that this circumstance may afford a means of determining the source of infection in case of death from rabies or hydrophobia.

(7.) That of numerous drugs of different classes tried on the rabbit, none have any constant effect upon the result of infection.

(8.) That by subcutaneous inoculations with modified virus, as practised by M. Pasteur, it is not practicable to confer immunity, even against subsequent infection, upon rabbits; and that with these animals the intensive or rapid method of inoculation is very liable itself to produce infection; that the constitutional refractoriness of the dog to infection with rabies by any method of inoculation, renders it extremely difficult to judge of the results of remedial or prophylactic measures with this animal, from a limited number of experiments; and that it is by the statistics of the treatment that the results in man must be judged.

---

Finally, I must state that my experiments were not undertaken with primary reference to M. Pasteur's statements, but that the fundamental importance of these so greatly modified and subverted previous views upon this disease, that it necessitated my investigating them, with the result of confirming the conclusions of their author in many essential points; and that it is to his notable discovery of the chief seat of the virus, with the constant and rapid effects, in the

rabbit, of the methods of inoculation which he has introduced, that we are indebted for the means of investigating with ease and certainty the phenomena of this disease, which previously had been most difficult and inconclusive.

These experiments were performed at the Brown Institution, and I must express my hearty thanks to Professor Horsley, F.R.S., for the facilities and assistance he has so kindly afforded me, in this and other investigations.

A considerable portion of the cost of material for this investigation was defrayed by a grant from the Association for the Advancement of Medicine by Research.

#### EXPLANATION OF PLATE.

Fig. 1. Encephalon of rabid rabbit, intensely and unusually congested, the dura mater removed. The site of inoculation is perceptible at *x*, by slightly increased congestion.

Fig. 2. Tongue, larynx, and part of trachea, of the same rabbit, showing deep congestion.

Fig. 3. Stomach of a similar rabbit, showing the veins of the serous coats much distended, together with numerous and moderately large hæmorrhagic spots, distinctly marked in a typical manner, as described in text.

“A Further Minute Analysis, by Electric Stimulation, of the so-called Motor Region of the Cortex Cerebri in the Monkey (*Macacus sinicus*).” By CHARLES E. BEEVOR, M.D., M.R.C.P., and VICTOR HORSLEY, B.S., F.R.C.S., F.R.S. Received June 16, 1887.\* (From the Laboratory of the Brown Institution.)

(Abstract.)

The present research, of which the following is a brief abstract, is in continuation of an investigation which we commenced two years ago, the first part of which is about to be published in the ‘Philosophical Transactions.’

In our former paper we described the results of a minute analysis, obtained by electrical excitation, of that part of the cortex in which Professor Ferrier had previously shown that the movements of the upper limb were chiefly represented.

In the present paper the same mode of analysis has been employed for the investigation of the parts of the cortex grouped around the before-mentioned area.

*Mode of Excitation.*—The mode of excitation was the same, with a slight alteration, as that which we previously adopted.

\* Received and read June 16th in abstract only. Full paper received August 12, 1887.