Experimental investigation into the disease, produced by the inoculation of the bacillus pyocyaneus / by M. Armand Ruffer.

EXPERIMENTAL INVESTIGATION

INTO THE

Nature of the Disease, produced by the Inoculation of the Bacillus Pyocyaneus.

BY

M. ARMAND RUFFER, M.A., M.D.

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TO

CHARLES BOUCHARD,

MEMBER OF THE INSTITUTE OF FRANCE.

THIS PAPER IS DEDICATED

AS A MARK OF GRATITUDE AND ESTEEM

BY HIS FRIEND AND PUPIL,

THE AUTHOR.

EXPERIMENTAL INVESTIGATION

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Nature of the Disease Produced by the Inoculation of the Bacillus Pyocyaneus.

GENTLEMEN,—During the last thirty years, the knowledge of the true nature of infectious diseases has made considerable progress. Up to a few years ago, what medical men knew of infectious diseases was based on facts gathered at the bedside, or on observations taken on animals, but not on data derived from experiments. The huge mass of evidence thus collected was of the greatest value to the practitioner; but, nevertheless, it may safely be said that clinical study threw no or very little light on the true causes and on the nature of such diseases. For, to take an example, although the course of the temperature in cases of pneumonia or typhoid fever had been accurately noted, the symptoms recorded, and some of the pathological appearances minutely described, yet little was known regarding the cause of this rise of temperature, or regarding the true nature of the morbid process.

A new impetus was given to the study of infectious diseases through Davaine's, Pasteur's, and Koch's re-

searches ; and, after the discovery of specific germs in many of the specific affections of lower animals, it was assumed that the infectious fevers of man were due to similar organisms. The time soon came when the labours of Koch, Bouchard, Capitan and Charrin, Eberth, Löffler and Fränkel proved these suppositions to be correct.

But although the discovery of germs explained the origin, *i.e.*, the specific cause of infectious diseases, the mode of action of micro organisms remained obscure. The mere presence of lower forms of life in the putrefying fluid of a wound did not account for the intensity of the fever and the *severe general symptoms* following on a *strictly localised septic process*. It was again assumed that some of the symptoms produced by the growth of micro-organisms in the animal body were due to the absorption of poisonous substances secreted by them—a supposition based on fact, as Panum, Bergmann, Koch, Brieger and others afterwards showed.

Although the deleterious action of the poisons secreted by the agents of putrefaction had thus been experimentally demonstrated, little was known as to the physiological action of the toxic substances, the products of the life of true pathogenic organisms—and by pathogenic organism I mean one which will always, when inoculated into certain animals, give rise to a definite characteristic disease.

The study of these poisons is surrounded by many difficulties ; for, in order to gain accurate knowledge of their action they must be separated from the microbes secreting

them—a task not so easy as it might at first appear to be. The two methods used for their isolation are heat and filtration : the first often completely altering the nature of the toxic substances, or even destroying them, and the use of the second method of separation being open to the objection that some of the poisons may remain on the filter. A filtered culture of the anthrax bacillus, for instance (Tiegel and Zahn), gives rise to no symptoms when injected into the venous system of an animal ; and yet Chauveau has shown that large doses of the non-filtered culture of the same micro-organism injected into the veins of animals on which the anthrax bacillus has normally no effect, produce severe general symptoms—a fact evidently due to the soluble poisons contained in the culture having, in Tiegel and Zahn's experiments, been retained by the filter.

In 1880 Pasteur published a fact showing that some of the symptoms of infectious disease were due to chemical poisons. He filtered through porcelain 120 ccs. of a culture of the bacillus of fowl-cholera, evaporated the filtered fluid to dryness, redissolved the residue in 2 ccs. of distilled water, and injected this extract into a fowl. This animal showed some of the symptoms following on the subcutaneous introduction of the bacillus.

In November, 1884, Professor Bouchard showed that if the urine of a patient suffering from cholera be first sterilised by heat and filtration, and then injected into the veins of a rabbit, symptoms are produced greatly resemb-

ling those of cholera: namely, cyanosis of the mucous membranes, lowering of temperature, cramps of the hind limbs, diarrhœa with shedding of the intestinal epithelium, absence of bile in the intestine associated with distension of the gall-bladder, albuminuria, anuria and death three or four days after. It was not cholera he produced, but an intoxication greatly resembling it. The existence of a cholera poison was thus demonstrated, but Professor Bouchard would not commit himself to say whether this poison was secreted by the micro-organism of cholera or by the cells of the patient.

In 1885 Brieger extracted from the cultures of the bacillus of typhoid fever a body which he named typho-toxin, and which, when injected into animals, gives rise to some of the symptoms of typhoid fever as seen in the human subject.

In 1883 Dr. Charrin discovered the interesting fact that the bacillus which, when growing on the dressings of a wound, gives to those dressings a blue colour—the *bacillus pyocyaneus*—will, when injected into the veins of a rabbit, produce a characteristic disease ending fatally in from twenty-four to sixty hours; the duration of life varying to some extent with the number of micro-organisms injected.

Dr. Charrin then showed that after all the micro-organisms growing in a pure culture of the *bacillus pyocyaneus* have been destroyed by being exposed to a temperature of 115°C, and removed by filtration, small doses (10-20

ccs.) of this sterilised fluid produce no marked symptoms. These animals, however, are *vaccinated*, inasmuch as the injection of the living *bacillus pyocyaneus* has little or no effect on them. In 1887 Dr. Charrin published some further interesting facts concerning the effects produced by the injection of the filtered culture of the same bacillus, facts to which I must refer presently. During the years 1888-89 Dr. Charrin and I, in a series of papers read before the *Académie des Sciences* and the *Société de Biologie* of Paris have shown that *almost all the symptoms* and pathological appearances of the *pyocyanic disease* may be reproduced by the injection into animals of the soluble chemical poisons secreted by the *bacillus pyocyaneus* in the cultures in which it has lived. We have further found some of these substances in the blood of animals afflicted with the *pyocyanic disease* and shown that the poisons were secreted in the tissues by the bacilli, and not by the cells of the animal. Professor Bouchard has traced some of these poisons into the urine of *pyocyanic animals*, and we have confirmed and extended the facts published by our teacher. It is of the investigations all carried out under the direction and in the laboratory of Professor Bouchard that I propose to give you an account to-night.

I.

The *bacillus pyocyaneus*, examined with a high power under the microscope, is a small organism about 1 μ. long and 0.2 μ. thick. It is easily separated from other micro-

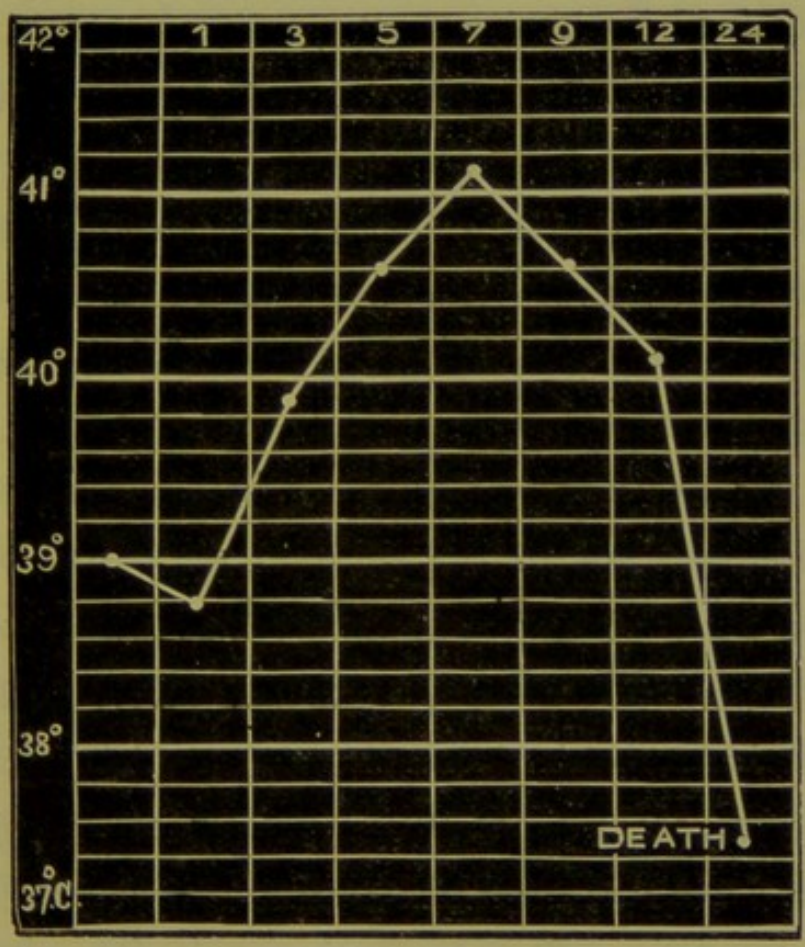
organisms when grown on gelatine, and is efficiently cultivated in agar-agar, gelatine, or beef broth. When cultivated in peptonised beef broth it gives to the fluid in which it grows a light bluish-green colour. The same colour it also produces when living in gelatine or agar-agar. If the flask containing the bacillus be forcibly shaken up in the presence of air, the colour passes into a light Cambridge blue. Pouring now a little of this liquid into a test tube, I render it alkaline with dilute ammonia, add chloroform to it, and shake up the whole. You perceive now that the chloroform takes up almost the whole of the colouring matter, and assumes a beautiful Cambridge blue colouration. I now add a drop of dilute H_2SO_4, shake up the tube again, and the colouring matter passes into the supernatent fluid, which assumes a pinkish tint. I now again add some ammonia until the fluid is alkaline, shake up the tube, and you perceive that the chloroform again takes up the colouring matter, which again passes into blue. This chemical test is extremely useful, for if it is doubtful whether the bacillus be present in the tissues or fluids of an animal, all that need be done to make sure of its being present, is to put a small piece of the suspected organ or to pour a drop of the fluid into some cultivating medium, place the flask in a warm chamber, and in twenty-four hours the presence or absence of this colouring matter with its characteristic reactions will settle all doubts (Gessard). Experiment has shown us that the bacillus will grow and secrete its characteristic colouring matter even when living

in the same flask with other micro-organisms, with the anthrax bacillus or the agents of putrefaction, for instance.

If a rabbit, weighing from 1kil. 500gr. to 2 kil. 500gr., be inoculated with 0cc., 25 of this culture in one of the veins of the ear, the animal dies in from twenty-four to sixty hours,

CURVE No. I.

Number of hours after inoculation.



White rabbit weighing 2kg. 270gr., inoculated with 0cc., 25 of a culture of *bacillus pyocyaneus.*

after presenting the following symptoms. During the first ten or twelve hours after the injection the latter's effects are not apparent, for the rabbit runs about, eats, and appears to be in its normal health. Even at this time however, the thermometer shows a considerable rise in the

animal's temperature, as the curve which I place before your eyes shows. Ten to twelve hours after the inoculation, the rabbit retires into a corner of its cage, appears drowsy, sitting with eyes closed, often somewhat unsteady in its movements. The temperature, which up to that time had been rising, now begins to fall, and will continue to do so steadily up to the time of the animal's death. If the urine be examined, it will almost always contain a small quantity of albumen and the specific bacillus. Diarrhœa freqently sets in at that period. The temperature now falls steadily, the stools become more frequent and more liquid, the sleepiness increases, and the animal perishes without showing any signs of paralysis, though convulsions sometimes make their appearance towards the end of life.

At the *post-mortem* examination, the only marked characteristics are all the signs of the gravest enteritis. The small and large intestines are filled with a yellowish-green fluid, and contain epithelial *débris* and mucus. Hæmorrhages in the kidneys and petechial spots in the heart-substance are not rare, but the favourite situations for these extravasations of blood are the cœcum and the large intestine. The hæmorrhages in the cœcum are usually underneath the mucous membrane, and it is but seldom that the blood forces its way into the cavity of the intestine. The spleen, as a rule, is very red, but not markedly enlarged.

Microscopically there are no very typical pathological appearances in any of the organs. In the kidneys, how-

ever, after the action of osmic acid, the epithelium cells of the tubuli contorti, of the descending and ascending tubes, are seen to be stained by the acid somewhat darker than usual. Moreover, instead of being sharply separated from each other, the cells seem fused together and have lost their normal striation. Some of the tubes are filled with a homogeneous material representing a hyaline cast. The glomeruli appear healthy but, in some of them, an albuminous fluid has exuded between the vascular bunch and the outer membrane, this fluid staining dark brown with osmic acid. Small hæmorrhagic spots are often present in the cortical substance of the kidney, but the remainder of the organ shows no naked-eye or histologic changes.

The specific bacillus is easily demonstrated by the microscope, or even better by cultures, in all the fluids and tissues of the body; in the blood, urine, liver, kidneys, spleen, heart, lungs, nervous centres and muscles. Notice particularly its constant presence in urine, for the knowledge of this fact is not without some practical interest. All that need be done to demonstrate this, is to take, with all antiseptic precautions, a drop of the animal's urine from the animal's bladder, to pour it into a small flask containing bouillon, and, in twenty-four hours, the blue colour of this fluid will show conclusively that the bacillus has successfully passed the epithelial layer lining the tubes of the kidneys. I cannot help thinking that if the presence of pathogenic organisms in urine of patients suffer-

ing from infectious disease has often been denied, it is owing to the difficulties attending this research, for Dr. Charrin and I have found that another bacillus, which likewise produces a characteristic colouring matter in the cultures in which it grows—*bacillus prodigiosus*—passes with great rapidity in the urine when injected into an animal. The practical interest lies in the fact that if pathogenic organisms pass easily through the kidneys, urine which, as a rule is not disinfected either in private or hospital practice, may become a fruitful source of contamination and infection.

After death, the bacillus is always present in the blood, and the same may be said to be the case during life also, but every drop of blood does not contain it. In several cases the beef broth, into which a drop of blood had been poured, remained sterile, whilst a drop of urine of the same animal taken at the same time contained the *bacillus pyocyaneus*. The micro-organism is therefore constantly being excreted by the kidneys.

If, instead of injecting the bacilli into the venous system, they be introduced subcutaneously, or if an old culture be used, or if the animal be imperfectly vaccinated so that its life be prolonged to five days or more, a new symptom makes its appearance, namely, a kind of spastic paresis. The paresis attacks first the posterior limbs, both as a rule, though occasionally one limb only is affected in the first place, or it may even remain limited to one. In very rare cases, the loss of power spreads to the anterior

limbs. On holding up this animal by the ears, and after its struggles have subsided, you notice that the hind legs do not hang down straight, as in the typical paralysis of rabies, or after the section of the sciatic nerve, but that the affected limbs are drawn upwards, the thigh being flexed on the pelvis and the leg on the thigh. If the leg be now forcibly extended, it returns to its former flexed condition as soon as the hand is withdrawn, and in many cases this manipulation gives the animal some pain. The limbs are therefore in a state of *painful contracture*, which however disappears under chloroform (Charrin and Babinski). The reflexes are exaggerated and, on palpation, peculiar tremors are felt in the muscles. The animals generally remain sitting on their haunches, and it is only during the last few hours of life that they become quite unable to move. The electrical reactions remain normal, and there is an apparent but no real wasting of the muscles. The *post-mortem* examination shows the muscles, nervous centres, nerves, to be perfectly healthy; at least, that is the result Babinski, Charrin, and I arrived at after repeated and independent examinations of nerves hardened in various ways, and of others in the fresh state. One point to be noticed is that, at the *post-mortem* examination, the bladder is always found enormously distended, sometimes reaching up to the liver, a fact evidently due to some abnormal action of the spinal cord.

At the same time the animals waste rapidly and lose, in one week sometimes, as much as one-quarter of their

weight. In animals dying from three to ten days after the inoculation, the kidneys frequently show the most typical infarcts, undoubtedly due to the blocking up of small vessels by plugs of bacilli. The same infarcts are sometimes present in the stomach also, where they are followed by minute ulcerations. More rarely they are met with in other organs. (See Charrin and M.A.R. in Bullet. Soc. Anatom., January, 1889.)

The symptoms of the disease have been thus insisted on, because they will always be referred to in this paper. Bear in mind therefore, that the six cardinal symptoms characterising the *pyocyanic disease* are :—

1. Fever.
2. Albuminuria.
3. Paralysis.
4. Hæmorrhages.
5. Diarrhœa.
6. Wasting.

I will try and show you now how these symptoms are produced.

II.

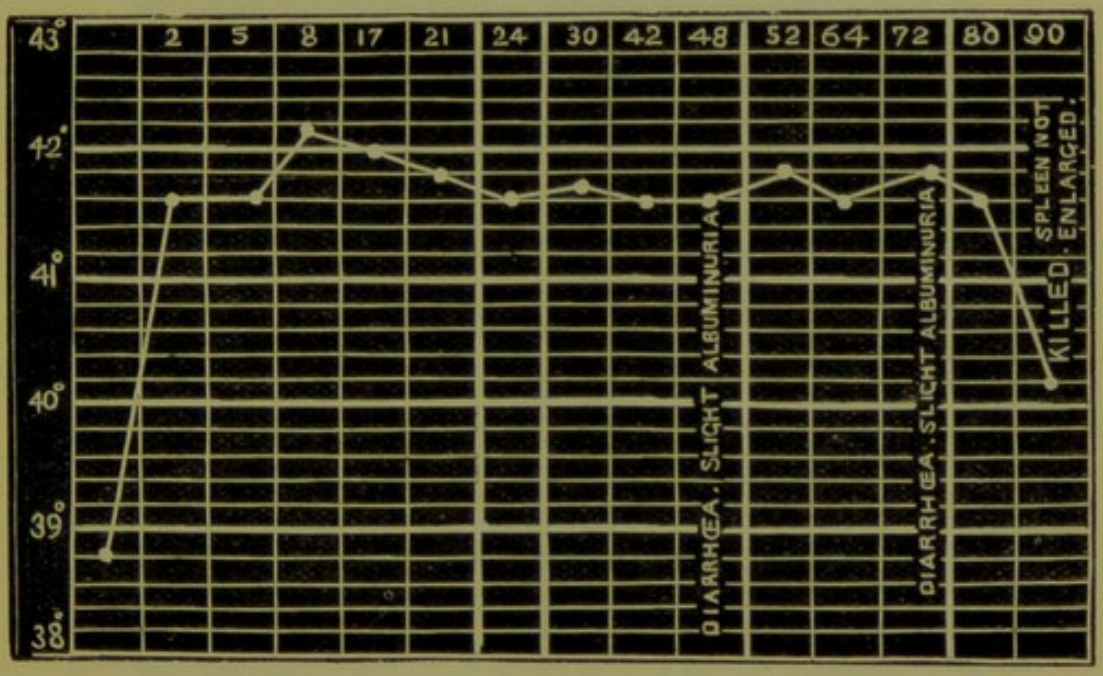
If the mouth of a flask containing a culture of *bacillus pyocyaneus* be securely plugged with sterilised cotton wool, and if it be heated to 115° C. under pressure, the contents of the flask, even if placed afterwards in the warm chamber at 38° C. for an unlimited space of time, will remain

perfectly sterile. This fluid contains the substances which may be considered as the products of the life of the *bacillus pyocyaneus*, but no living bacilli. If this heated culture be now filtered on porcelain, all the dead bacilli will remain on the filter, the fluid remaining perfectly clear.

What will be the effect of the injection of this sterilised fluid on the temperature of an animal?

CURVE NO. II.

Number of hours after injection.



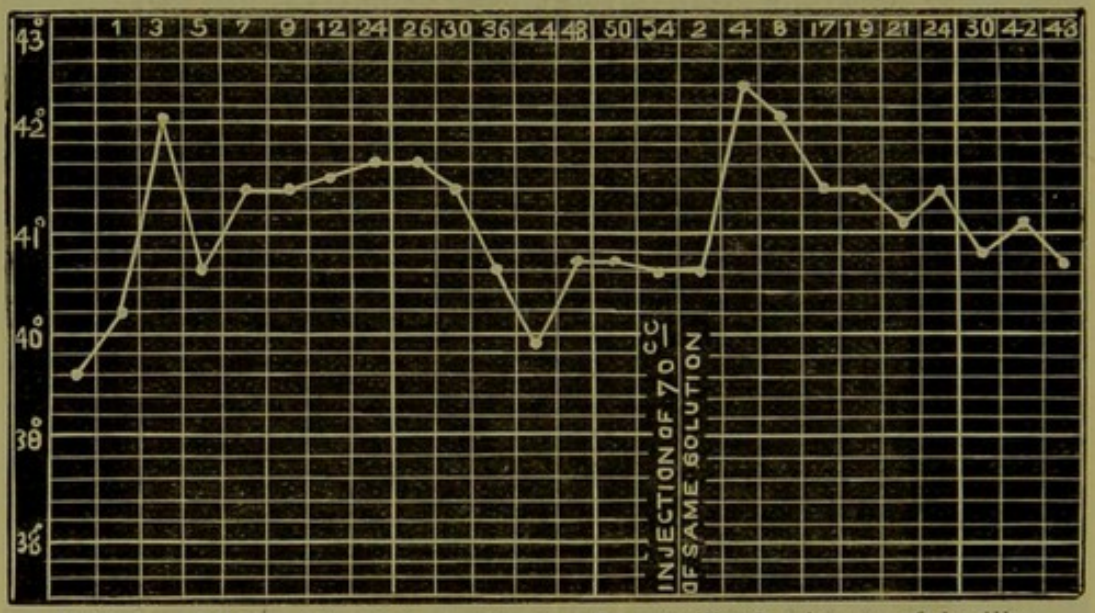
Rabbit weighing 1 kg. 620 grs. 50 cc. of sterilized culture of *bacillus pyocyaneus* injected under the skin.

Looking at the temperature-curves placed before you notice that after the injection of large doses of this filtered sterilised fluid into a rabbit's veins or under its skin, the temperature, after a preliminary fall, rises suddenly and reaches its maximum in from three to eight hours. The temperature of the animal was of course always taken immediately before each experiment. This elevation

of temperature persists for some time, so that twenty-four hours after the injection (see Curve II.) it is still 2°C.8 above normal, and ninety hours elapse before the temperature falls nearly to its proper level. In the other animal (Curve III.) the temperature reached its maximum (2°.4) in three hours, fell slightly, but was still raised 1°C. above the normal, fifty hours after the injection. It may

CURVE NO. III.

Number of hours after injection.



Rabbit weighing 2 kg. 50 gr. 50 cc. of a sterilized mixture of *bacillus pyocyaneus* heated to 11° injected subcute.

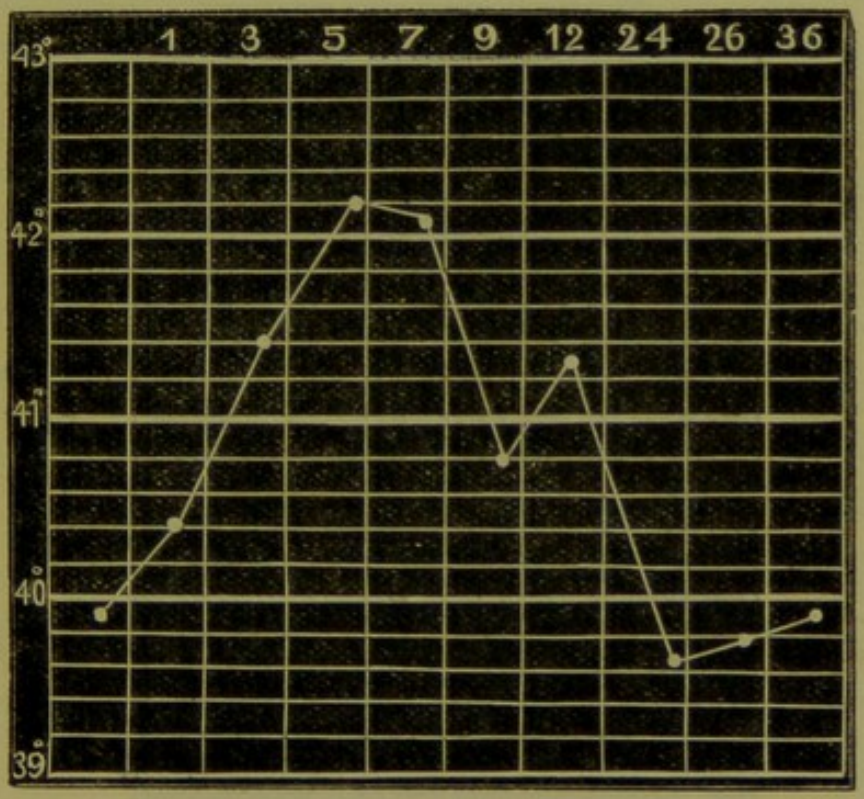
appear strange that the temperature should remain high for so long a period, but there is evidence to show that these poisons are excreted out of the system with exceeding slowness. Notice also that a more or less marked diarrhœa is a prominent symptom in most cases, setting in after two or three days. This latter symptom may perhaps in itself be sufficient to account for the persistence of the high tempera-

ture, though not for the initial rise. Albuminuria followed the inoculation of the *bacillus pyocyaneus*, and is a symptom which may also be reproduced by the injection of the sterilised culture.

Fever, albuminuria and diarrhœa were characteristic symptoms produced by the injection of the living bacillus

CURVE NO. IV.

Number of hours after injection.



White rabbit weighing 2 kg. 80 grs. 50 cc. of sterilized bouillon injected under skin.

into the animal's body, and also follow on the injection of the soluble poisons contained in the sterilised cultures of the same bacillus.

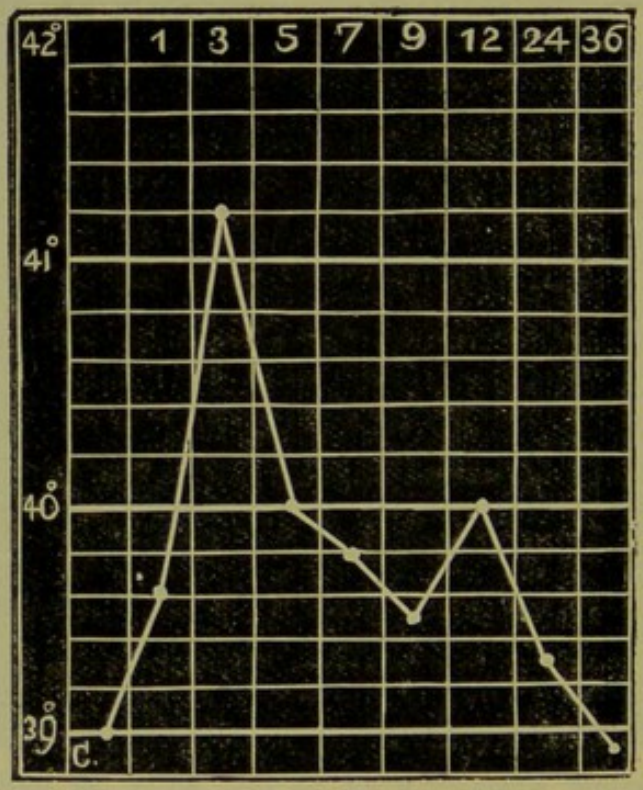
But before attaching any value to these facts it was necessary to show that pure bouillon did not cause a

similar rise of temperature, together with albuminuria and diarrhœa.

In order to settle this point, like quantities of freshly prepared sterilised beef broth were injected into rabbits, and it was found that the temperature of the animal after the injection certainly rose, sometimes as much as 2°C.3 but that this rise was of short duration only, the temperature

CURVE NO. V.

Number of hours after injection.



Grey rabbit weighing 2 kg. 250 gr. 35 cc. of sterilized bouillon injected into the venous system through lateral vein of ear.

returning to normal in eight or nine hours. Compare the curves, that is the curves obtained by the injection of the fresh pure beef broth with that of the filtered and heated culture, and the differences between the two must at once be noticed.

Thus our experiments are, I think, not uninteresting, for they show the fallacy of Gamaleïa's theory of the origin of fever. He supposed it to be due to the action of certain peculiar cells of the animal body on dead or living micro-organisms. In our experiments the filtered fluid alone was injected—that is a fluid quite free from either living or dead micro-organisms. In another series of experiments, instead of filtering the culture before using it, we sterilised it by heat only, and we noticed that its introduction into an animal's body raised its temperature slightly more than the introduction of the filtered fluid—a fact which may probably be explained by the supposition that some of the pyretogenic material remained on the filter, adhering to the dead bacilli. It is important to note also that the fresh pure bouillon raises the temperature, for, bearing this fact in mind, the theory that fever is always due to the action of micro-organisms falls to the ground. Moreover, numerous clinical as well as experimental facts could be brought forward against this latter view.

If an animal to which one or two large doses (50 to 60 ccs.) of a sterilized culture of the *bacillus pyocyaneus* have been injected be carefully watched, it appears, after two or three days, to have recovered its pristine vigour, looks perfectly normal or may even gain in weight. After a period, varying from fourteen days to two months, a peculiar train of symptoms makes its appearance.

The first noticeable point is that when placed on the floor, the rabbit seems disinclined to run away or to move. When

turned over on its side it regains its sitting posture with great difficulty only. Shortly—that is, in two to seven days after the first onset of symptoms—it shows all the signs of the typical spastic paresis already described. The same paresis will also make its appearance if, instead of injecting large doses, small ones be introduced every day or several times in a week. One such experiment may be conveniently related here, to show the other points of interest.

Experiment X.—*White rabbit weighing* 1 *kg.* 520 *grs., June* 21, 1888—15 ccs. of a culture of *bacillus pyocyaneus.* which had been previously heated to 115° C. and filtered on porcelain, are injected under the animal's skin. *June* 22—Injection repeated. *June* 23—Very slight albuminuria, 7 ccs. injected to-day. *June* 24—Weight of animal 1 kg. 500 grs.; no albumen in urine, 10 ccs. again injected. *June* 25—No albumen in urine, same injection as yesterday. *June* 27—No albuminuria, 15 ccs. injected under the skin. *June* 29—No albuminuria, no paralysis. *July* 1—To-day, on placing the animal on the ground, it is noticed to be almost unable to move. When placed on its back, it can regain the sitting posture after many ineffectual attempts only. Slight albuminuria. Nothing more was injected, but the animal lost weight steadily, diarrhœa set in, albuminuria increased slightly, and the animal died paralysed, on July 10th, weighing only 1 kg. 50 grs. *It had lost therefore one-third of its weight nearly.*

Compare the symptoms produced by the injection of the

filtered culture—*i.e.*, by the soluble poisons secreted by the *bacillus pyocyaneus*—with those the bacillus itself gave rise to when introduced into the system, and notice that all the symptoms have been reproduced by the injection of these chemical poisons—all except the hæmorrhages in the cœcum and the pathological changes in the kidneys.

Dr. Charrin and I have been able to reproduce these hæmorrhages also.

Thus, after injecting 20 ccs. of a culture of *bacillus pyocyaneus* sterilised in the usual way into the peritoneum of a rabbit, and killing the animal the next day by a blow on the head, a submucous hæmorrhage the size of one shilling, and several small extravasations of blood were found in the cœcum of that animal, together with smali petechiæ in the heart's substance. Similar appearances were discovered in another rabbit which was killed twenty-four hours after a subcutaneous injection of 15 ccs. of the sterilised culture.

The pathological specimens, which are placed before you to-night have been obtained in a similar manner. Lastly, in the kidneys of animals dying after repeated injections of the sterilised cultures, we have found the same appearances as in the animals inoculated with the bacillus itself.

All the symptoms, and most of the pathological appearances of the disease have been reproduced through the injection of the poisons contained in the sterilised cultures. They are, however, not reproduced with equal facility. Fever, paralysis and diarrhœa never fail to follow the injec-

tion of large doses of sterilised cultures into rabbits, whereas albuminuria and hæmorrhages are more rarely noticed.

The infarcts were not reproduced by the injection of these chemical substances, but it is of course impossible to reproduce, with a filtered liquid, lesions which are clearly due to solid particles contained in that fluid before filtration. The most important fact of all, namely the vaccinating action of these soluble poisons, has not been mentioned as yet.

If two rabbits, *a* and *b*, be taken, and if to the first (*a*) 20 ccs. or more of the sterilised culture be injected, and if then, two or three days afterwards, both rabbits be inoculated at the same time with an equal dose of the same culture of the *bacillus pyocyaneus*, it will be found that *a* has been vaccinated, that it will recover, whereas *b* will die within twenty-four hours.

To Dr. Charrin belongs the credit of having first demonstrated the vaccinating and paralysing power of chemical substances. Since then this fact has been demonstrated to be true for other diseases by Roux and Chamberland, Chantemesse and Widal and other observers.

In the media in which it grows, therefore, the *bacillus pyocyaneus* secretes chemical substances, which, when injected into an animal:

1stly, Vaccinate it against a further attack of the disease.

2ndly, Produce

a, Fever.

b, Paralysis.

c, Enteritis.

d, Albuminuria.

e, Wasting.

f, Hæmorrhages.

In one word, these chemical substances reproduce all the symptoms, and most of the pathological appearances to which the bacillus itself gave rise when inoculated. Let us see now whether these substances are also found in the tissues of infected animals.

II.

The blood is naturally the first fluid which ought to have been examined for these poisons, and it is their presence in that fluid which will be studied here first. It is only fair to state however, that Professor Bouchard's researches on the presence of vaccinating substances in the urine had preceded our own on the blood.

The following method was used to demonstate the presence of vaccinating substances in the blood of infected animals. One or two rabbits were inoculated with a culture of the living *bacillus pyocyaneus* in the afternoon of one day, and the next morning, that is about eighteen hours after the inoculation, these animals always exhibited the characteristic signs of the *pyocyanic* disease. The animals were then tied down on the table and bled to death from the jugular vein, or from the femoral artery. An equal quantity of water was added to the blood thus collected (from 40 to 100ccs.), and this mix-

ture at once heated to 110° C., the albumen being thus coagulated and all the bacilli killed. The magma formed was first filtered through a fine linen cloth, then placed under Claude Bernard's screw-press and all the fluid pressed out. The collected fluids were then filtered on paper, again heated to 110°C. in a flask plugged with cotton wool, and proved after that to be free from any living micro-organisms. Most of the chemical poisons which the bacillus might have secreted in the blood were probably to be found in that sterilised fluid, but no living bacilli.

To demonstate the presence of the soluble chemical vaccinating substances in the blood of infected animals, the fluids pressed out of 90ccs. of blood were divided into four doses, and two rabbits were inoculated subcutaneously during two consecutive days with 22ccs. of this fluid. Two days after the last injection 1cc. of culture of the *bacillus pyocyaneus* was injected into the veins of the ear of these two rabbits and of two other animals of nearly the same weight, but it was found that the duration of life was not in the least prolonged in the animals previously subjected to the injection of the sterilised blood.

Though this result was not encouraging we determined to persevere, and accordingly each of two other rabbits was inoculated with one half of the heated and filtered fluid extract of 160ccs. of the sterilised blood of two other *pyocyanic* animals.

Injecting 1cc. of a culture of *bacillus pyocyaneus* into

the venous circulation of each of these two rabbits and of two other non-vaccinated animals, it was found that the two latter animals died within twenty-four hours, whereas the two other rabbits are alive to this day, more than two months after the inoculation.

Not satisfied with this result, we tried to see whether the vaccination was perfect and lasting. Accordingly, nine days afterwards, we reinoculated the same two rabbits with the most virulent culture at our disposal, and they again came safely through the ordeal, another non-vaccinated animal inoculated at the same time, with the same dose of the same culture, dying in less than twenty-four hours.

Since then these experiments have been repeated by us on fourteen other rabbits, with the result that the animals previously subjected to the injections of the filtered and heated extract of blood of *pyocyanic* animals resisted the introduction of the *bacillus pyocyaneus* into their system; whereas the other rabbits—and we always inoculated another rabbit at the same time and with the same culture—died within forty-eight hours.

It must, however, not be thought that these animals were efficiently vaccinated. The fact is, that although the rabbits previously subjected to the injection of the sterilised blood did not die within forty-eight hours of the injection of the bacillus as did the non-vaccinated animals, they most of them perished seven to fourteen days after the inoculation of the bacillus—all except three alive now,

more than three months after the final inoculation. Their resistance was increased, but not perfect. Hence we concluded our paper (Charrin and M.A.R., C.R. Soc. de Biol., 22nd Feb., 1889) by saying that the blood of *pyocyanic* animals contained some amount of vaccinating substance, for we had animals under observation alive two months after having been thus vaccinated and inoculated with the living bacillus, the non-vaccinated animals inoculated at the same time and with the same culture having died two months before. But, we added, it is evident also that these soluble poisons are only present in the blood temporarily, and vary in amount according to the animals, possibly according to the time at which the blood is collected. The cultures and the urine—as we shall see presently—contain a larger amount of these poisons than the blood. The toxic substances pass through the circulatory system, are carried to the kidneys to be excreted by these organs; a point to which Professor Bouchard, who made some remarks on our papers, drew special attention (Ch. Bouchard, C.R. Soc. de Biol., 22nd Feb., 1889).

The researches on the presence of the paralysing chemical substances in the blood are not finished as yet, but their existence in large quantities in the urine renders their presence in the blood more than probable.

III.

The physiologist studying the action of a poison which he has introduced into an animal's body is not satisfied

with knowing on what system this agent exerts its chief influence, or in what organs it is stored up, but he traces it into the excretions of the body and determines, as accurately as he is able, how the toxic agent is excreted out of the animal's body.

Following the physiologist's example let us try and see what becomes of the chemical substances secreted by the *bacillus pyocyaneus* in the body of an animal. Do these substances stay there stored up for ever in some organ or organs, like particles of carmine or even like mercury or lead salts, or are they eliminated by some of the excretory organs? And if so, by what organs?

Of all the excretory organs the kidneys take the greatest share in the elimination of any poisons circulating in the blood, and, accordingly, it was the urine which attracted Professor Bouchard's attention, when he first investigated the mode of excretion of vaccinating substances. Rabbit's urine, however, even when filtered and sterilised, is intensely poisonous, and this toxic action is chiefly due to the large amount of potash salts present in it—green vegetable matter, on which rabbits are chiefly fed, containing large quantities of potash salts. This fact having been ascertained, Professor Bouchard got over the difficulty easily by placing the rabbits, whose urine was to be used, on a strict milk diet. The animals so fed passed a large (150 to 200 ccs.) quantity of pale clear urine, which by experiment was found to be less poisonous than distilled water, if somewhat more so than artificial serum.

The experiments were performed in the following manner:—Two or more rabbits were placed on milk diet and then inoculated with a virulent culture of the *bacillus pyocyaneus*. The animals were then placed in a cage, the sloping floor of which was pierced with numerous small holes. The urine, as soon as it was passed, fell through these holes into a reservoir underneath, and, from thence, through a single opening into a tube, in which it was first filtered on paper and afterwards through porcelain. After passing through porcelain, the fluid was placed in the warm chamber at 38° C., to see if all life was extinct in it, and, when this had been proved to be the case, it was ready for injection. The following, which is simply a repetition of one of Professor Bouchard's experiments, will illustrate the mode of procedure.

Experiment, *Feb.* 3, 1889.—Rabbit weighing 2 kgs., fed on milk only.

0cc., 6 of a culture of *bacillus pyocyaneus* injected into the lateral vein of the ear.

Feb. 4. — Animal dying fast. Had passed 140 ccs. of urine in that time. Urine sterilised in the usual way.

Feb. 5.—35 ccs. of the urine injected *subcutaneously* to a grey rabbit (*a*).

Feb. 6.—Injection repeated.

Feb. 7. — Rabbit (*a*) and a new rabbit (*b*) inoculated through lateral vein of the ear with 0.6 cc. of a *bacillus pyocyaneus*. The rabbit (*b*) died in twenty-four hours.

The other one (*a*) is alive now—one month after the last inoculation.

This experiment proves that 70 ccs. of the urine of a *pyocyanic* rabbit contain enough chemical vaccinating matter to vaccinate another animal. It is, however, unnecessary to use such large doses, for Professor Bouchard has succeeded in vaccinating with 10 ccs., 16 ccs., 30 ccs. of urine of *pyocyanic* animals.

In order to give a further proof of the truth of Professor Bouchard's views, and to show that it is the bacillus itself and not the cells of the animal body which produce these substances, the following experiments were performed :—

500 ccs. of a culture of the *bacillus pyocyaneus* were taken and sterilised in the usual manner by heat and filtration. Two rabbits (*a* and *b*) being then placed on milk-diet, 22 ccs. of this sterilised fluid were injected under the skin of each of them every day, during three consecutive days. Their urine was collected in the usual way during these three days, sterilised by heat and filtration, and injected under the skin of two other rabbits (*c* and *d*), until each of them had absorbed 45 ccs. of this fluid per kilogramme of its weight. 0 cc., 25 of a culture of *bacillus pyocyaneus* being then injected into the veins of *c* and *d*, and also of two other non-vaccinated rabbits, it was found that *c* and *d* felt no appreciable evil effects after the inoculation, whereas the two other animals died within twenty-four hours.

These experiments were repeated on six other animals and the same result was obtained each time. Hence we thought we were justified in concluding our paper (see

Charrin and M.A.R., C.R.Soc. de Biol. 13 Oct., 1888) by the following remarks :—" We may conclude from our experiments that in the *pyocyanic* disease the chemical vaccinating substances secreted in the cultures pass through the animal's body and are excreted in the urine without losing their specific property. We have proved further that the vaccinating power of the chemical substances formed in the tissues of rabbits inoculated with the *bacillus pyocyaneus* are, for the most part at least, secreted by the bacillus itself, and not by the animal's cells, as the sterilised cultures as well as the urine of the animals to which they have been injected possess that property."

The eminent physiologist, Professor Chauveau, who presided on the day we read our paper to the Société de Biologie, asked at once, with characteristic readiness, whether, as the chemical vaccinating substances passed into the urine, the paralysing poisons were not likewise excreted by the kidneys? Our experiments on that point were not sufficiently advanced to enable us to answer that question at once, but, the very next week, we were able to convince Professor Chauveau and the other members of that learned society that the paralysing chemical substances also passed in the urine, for we showed them a rabbit, which after having been submitted to the injection of 50 ccs. (per kilo.) of the urine of animals in the veins of the sterilised cultures of the bacillus pyocyaneus had been introduced, showed a marked monoplegia affecting the left hind leg. This experiment has since been repeatedly performed, and

this animal shows a typical monoplegia produced in a similar manner.

It is probable that these substances do not pass into the urine *en bloc*, but slowly and gradually, for vaccination may sometimes be accomplished successfully with the urine of animals to which a large quantity of the sterilised culture had been injected subcutaneously fourteen days beforehand.

There is evidence to show that the fever-producing substances also pass in the urine, but these experiments (Charrin and M.A.R.) have not been published as yet, although Professor Bouchard said a few words concerning them in his paper in the *Gazette Hebdomadaire* on the 22nd Feb., 1889.

Conclusions.

1st. With the chemical poisons contained in the sterilised cultures of the *bacillus pyocyaneus*, all the symptoms, and most of the pathological appearances characteristic of the *pyocyanic* disease may be reproduced.

2nd. The *bacillus pyocyaneus* growing in an inert medium, or in an animal's body, secretes chemical substances which, injected into an animal, vaccinate it against a further attack of the disease.

3rd. The chemical vaccinating, paralysing and fever-producing substances are ultimately excreted in the urine.

Our knowledge of the chemical characteristics of these poisons is at present so small that it is better not to make any statements concerning them.

IV.

These researches throw some light on the genesis of the symptoms and of the pathological appearances of infectious diseases.

The *bacillus pyocyaneus* when injected into a rabbit's venous system produces its effects in two ways.

It gives rise to *mechanical lesions*, such as emboli in the kidneys, stomach, &c. The fatal result following its injection, however, is due more to the action of the soluble chemical poisons secreted by the bacillus, than to the mechanical lesions. It has been shown conclusively that the injection of the chemical substances alone is sufficient to account for most of the symptoms and lesions of the disease.

To Dr. Charrin belongs the credit of having proved that micro-organisms, when growing in artificial media secrete substances which have the property of increasing the resistance of that animal towards that particular bacillus, provided these substances are introduced in the animal's body some time before the inoculation of the bacillus itself. Similar vaccinating and paralysing substances are secreted by the same bacilli in the blood of infected animals and constantly excreted by the kidneys, as we have shown.

And now the following question may be raised. Is vaccination in the *pyocyanic* disease of rabbits due to the presence in the blood of a soluble vaccinating matter? or, does an animal resist, because, through the injection of

vaccine matter, its cells and fluids have undergone a peculiar modification, rendering them more able to resist the attack of the specific bacillus?

Vaccination cannot be due to the mere presence of chemical vaccinating substances in the blood, because, in the first place, an animal is still vaccinated even after all trace of this chemical substance has left its body. In the second place, this chemical matter may be present in the blood in large quantities, even without vaccinating the animal. In a rabbit dying of the *pyocyanic* disease, enough chemical vaccinating matter was found to vaccinate another animal. Moreover, that animal passed 150ccs. urine in the twenty-four hours; and remember 30ccs. vaccinate—it excreted therefore enough to vaccinate five other rabbits at least. In spite of that the animal died of the *pyocyanic* disease.

Another direct proof of the fallacy of this view may be given. Inject, as has frequently been done in Professor Bouchard's laboratory, large quantities of the sterilised culture into the blood; then immediately afterwards inoculate the same animal with the living bacillus. The animal will die even faster than one to which the bacillus alone has been injected. Inject the sterilised culture on one day, the bacillus two days afterwards, and the animal will resist.

Is there, then, any evidence to show that the life of the animal's cells can be modified in any way by the injection of soluble chemical poisons secreted by micro-organisms?

To answer that question it is necessary to be reminded of how the typical spastic paralysis may be produced in the *pyocyanic* disease. After the injection of 50 ccs. or more the animal becomes feverish, very ill, and then to all appearances recovers. But fourteen days to two months afterwards the paralysis sets in, wasting and diarrhœa make their appearance, and the animal dies long after all the soluble poisons have been excreted out of its body.

Are there not many like instances in human pathology? The evil effects of alcohol do not cease even after the alcoholic individual has given up his drinking habits, when every drop of alcohol has left his body. A morbid action in the arteries, in the cells of the liver, kidneys or nerves, has been set up, and this process once started follows its own independent course, often causing the death of the patient.

Syphilis is another good example of the permanent changes which may be set up in the animal economy by an infectious illness. In the tertiary stage of syphilis the patient is probably unable to infect other people, and yet the nutrition of his cells is undoubtedly changed. Further, a syphilitic patient may be cured, and ten, fifteen, twenty years afterwards locomotor ataxy sets in. And iodide of potassium in drachm doses or mercury to salivation will not be of any use to him, for locomotor ataxy, though often following in the wake of syphilis, as Dr. Gowers has proved, is not a syphilitic disease either in its clinical course or pathological appearances. Here an infectious disease has started in the spinal cord a morbid process which, once started follows its own course.

The etiological relation of the diseases of the heart and of the spinal cord to infectious diseases is often conspicuous. Do not Mr. Horsley's observations on the chorea of dogs illustrate almost as plainly as actual experiment what may be the late consequences of an infectious disorder? If the cells of the animal body can be thus changed for the worse, by some of the poisons secreted by micro-organisms, I cannot see why other poisons should not act in another way and confer on the cells of an animal a power they did not possess before, namely, that of resisting in some way the introduction of specific micro-organisms.

Another important point in the experiments is that they illustrate the fact that the cells of the kidneys seem to have the peculiar power of selecting these poisonous substances out of the circulating plasma, and of excreting them. Their presence in the urine is not due merely to a force of filtration, for we have seen that if 80 ccs. of blood just vaccinate 20 ccs. or 30 ccs. of urine do so with perfect certainty. There is therefore two to four times as much of this chemical substance in the urine as there is in the blood. The cells of the kidney pick out these toxic substances just as they do urea.

I know that there are many more problems to be worked out connected with that disease, and we hope to be able to give a satisfactory answer to some of them soon.

The etiology of infectious fevers, the causation of their symptoms, have attracted universal attention for centuries but if asked what share clinical medicine has had up to the

present in answering these questions, the answer ought to be that its share has been very small as compared with that taken by experimental medicine during the last thirty years. And it is to experiments on animals that the future progress in pathology will be chiefly due.