Though sections of acari are to be seen in the canals upon and in the epithelium, I have not been fortunate enough to come across a portion of one in any of the canals in the corium immediately beneath the epidermis. That these acari burrow there can be no doubt, but during what stage of their life they occupy the sub-epithelial canals could not be guessed at in the sections I have examined. Nor can I say eggs are to be seen in the galleries beneath the epithelium; certainly oval shaped, granular, yellowish coloured bodies are seen in the canals in the epidermis, but I cannot recognise them either as eggs or sections of young acari. In Neumann's *Traité des Maladies Parasitaires des Animaux Domestiques* it is said that "they (the galleries) show at various points some deeper and wider portions where two or three eggs are deposited. At the extremity of these galleries the female acarus is found," etc. We have yet to come across these two points.

It may be desirable to draw attention to one or two practical points when manipulating pieces of skin containing acari.

The celloidin process of imbedding is to be preferred to freezing before section cutting is proceeded with, as it keeps the different component parts of epithelium, exudate, and acari *in situ*. Care is to be taken not to pierce the piece of skin with the needles. In some of the sections prepared by me there are straight deep canals or cavities, penetrating to the deeper hair-follicles and sudoriferous glands, containing a few granules and crystals only, and which are not margined by any extravasate whatever. These were caused by needle punctures during the fixation of the pieces of skin upon the dies when embedded in the celloidin.

EXPERIMENTS REGARDING TETANUS.¹

By Dr BEHRING.

IN an article published in common with Herr Kitasato in November 1890, I reported that with the blood of a rabbit rendered immune against tetanus we were able to protect mice from that disease, and to cure them when they had been previously affected.

The certainty of the cure and protection of even such animals as had received more than one hundred times the fatal dose of virus exceeded our most sanguine expectations. Provided only that no technical error was made in the treatment, failures in the result were altogether excluded.

The method of protection and cure here employed in the case of tetanus was suggested by observations which I had previously made in my studies regarding diphtheria, and its serviceability impressed us all the more because all former therapeutic and protective experiments which Herr Kitasato had made according to the methods known at the time, had entirely failed in their object. It will therefore be readily believed that we subsequently strove to develop the newly discovered method so as to make it available for larger animals than mice, and, above all, for human beings affected with tetanus.

Moreover, I had by further experiments convinced myself that we

¹ Translated from the *Zeitschrift für Hygiene u. Infektionskrankheiten*.

have here to do with a method that is applicable to various infectious diseases, but in none did the study of the mechanism of the curative and protective action of the blood—thanks to the admirable earlier work of Herr Kitasato—prove more fruitful than in tetanus, and in this there lay a further incentive to energetically pursue the investigation.

Herr Kitasato and I have therefore, while constantly keeping in touch with one another, independently multiplied our observations, and we have now, as we believe, made a notable step in the forward direction.

In the following I shall give a summary of the results of my own experiments.

Induced by the same considerations as led Wernicke and myself to select cultures treated with trichloride of iodine to confer immunity against diphtheria, which in that case yielded satisfactory results, I have also employed this method to obtain better and quicker protection against tetanus.

The experiments made in this direction with rabbits immediately gave positive results, and I can assert that the rendering of rabbits immune against tetanus, by previous treatment with tetanus *bouillon* cultures (or filtrates of the same) acted upon by trichloride of iodine, is one of the simplest tasks that can be submitted to a bacteriologist.

A necessary condition for the success of these experiments is an accurate knowledge of the degree of activity of the cultures or filtrates.

The cultures with which I worked were placed at my service by Herr Kitasato, for which I here tender my thanks; I cannot refrain from emphasising the fact that only through the aid of Herr Kitasato, who is incomparably preeminent in all special questions relating to the etiology of tetanus, has it been possible in a short time to advance the method of conferring immunity against and of curing tetanus in the manner disclosed in the following report.

In the course of the last months I have received eight different cultures. In order to determine the degree of activity of the same I tested them on mice and rabbits.

How I did that I will describe for the culture which I have most recently employed.

This had been prepared in meat extract *bouillon* on the 15th November 1891, and when I received it it had stood for ten days in the incubator.

On opening the stopper, which had been made tight by means of paraffin, there escaped some gases having a very powerful odour, characteristic of tetanus cultures but difficult to describe. The microscopic examination of the very turbid culture showed besides abundant bacilli also many spores.

Of this culture I had at my disposition 3 flasks of 200 ccm. each.

I now poured the contents of the 3 flasks together, mixed them well, and took of the mixture 30 ccm. to test the fresh unchanged culture. To the remainder I added carbolic acid in the proportion of 5 %.

Of the carbolised culture I now filtered the half through filter paper until the filtrate was clear, this being easily effected by pouring the

filtrate back again into the filter, the pores of which were gradually stopped with the insoluble constituents.

I now cut the filter paper into two parts; one of these I put into an incubator for 30 minutes at a temperature of 80° , in order to destroy the tetanus poison adhering to it. I spread the other out in a Petri's glass, and placed it in an exsiccator.

I had now to test the above-mentioned culture :

1. The same in the unaltered state,
2. With 0.5 % carbolic acid added,
3. The filtrate,
4. The residue, which
 - (a) could act only through infection, since all the poison is rendered inactive at 80° .
 - (b) contained spores, bacilli, and remains of the poison.

The examination gave the following result. Of the unaltered culture the minimum fatal dose for mice was 0.0002 grm. This was determined by preparing in the first place a dilution of the culture with 100 times its volume of water; after subcutaneous injection of 0.1 ccm of this 1 in 100 dilution, mice died from tetanus in less than 24 hours.

When diluted 500 times, 0.1 ccm. sufficed to determine death on the third day.

With a 1 in 1000 dilution mice died in 4-7 days after a subcutaneous injection of 0.1 ccm., and in 3-4 days after 0.2 ccm. As in the case of diphtheria, however, I regard as the certainly fatal minimum dose that which kills the animals after 3 to 4 days. Thus, for the present culture that is 0.0002 grm. or 0.0002 ccm.

This value altered in the culture preserved in the ice-chest, so that at the end of three weeks the minimum fatal dose was no longer 0.0002 ccm. but 0.001 ccm.

By a similar test I found that for mice the minimum fatal dose of the carbolised culture, which, moreover, was not kept in the ice-chest but at summer temperature, was 0.0003 grm.; after three weeks it was the same as that of the non-carbolised culture—0.001 grm. The minimum fatal dose of the filtrate was at first 0.005, and after 3 weeks 0.01.¹

For medium-sized rabbits the minimum dose certainly fatal within 3 to 4 days was—

Of the unaltered culture		0.6 ccm.
Of the carbolised culture		0.6 „
Of the filtrate		3.0 „

Four weeks later the minimum fatal dose of the carbolised and non-carbolised cultures was 0.8-1.0 ccm. The filtrate was not tested on rabbits.

As I have found in the case of other samples of culture, and as I could observe also with other species of animals, there does not exist any such relationship as will enable one to immediately infer, from the effect on mice, what will be the effect of cultures rich in bacilli, and others poor in or devoid of bacilli, on other animals. Nevertheless, by ascertaining the minimum fatal dose for mice, we obtain very valuable information regarding the degree of activity for rabbits also.

¹ The filtrate of another similarly treated culture was very much less active, and I expressly state that filtration, according to its special nature and duration, can exert a very varying influence on the poisonous action of tetanus cultures.

The residue after filtering, when heated to 80° , was very infectious for mice (placed under the skin with a fine strip of filter paper), but quite inactive for rabbits. That which had been dried in the exsiccator made 1 of 3 rabbits temporarily ill.

I then added to the carbolised fluid various quantities of trichloride of iodine, and after at least 36 hours' action of the trichloride I again determined the minimum fatal dose for mice. It was found to be as follows:—

ICl_3	0.05	per cent.	; dose for mice	0.005	ccm.	
"	0.1	"	"	0.05	"	
"	0.15	"	"	0.1	"	
"	0.175	"	"	0.3	"	
"	0.2	"	"	0.8	"	(uncertain)

For rabbits it was:—

ICl_3	0.05	per cent.	=	2.5	ccm.
"	0.1	"	=	0.6	"

Cultures with a higher proportion of ICl_3 I found to be no longer fatal in their action.

Those rabbits which had received such doses of the culture—either unaltered, carbolised, treated with trichloride of iodine, or filtered—or, lastly, of the residue remaining on the filter, as had not sufficed to determine their death, were subsequently tested as to whether they possessed an altered susceptibility for renewed infection.

It was found that such animals as did not die of tetanus, but showed slight tetanic symptoms, especially contraction of the muscles of the back, a higher rectal temperature, or such general symptoms of disease as emaciation, diminished appetite, awkwardness in moving, were without exception more sensitive to infection with tetanus or poisoning with filtered cultures than fresh animals as long as the disease lasted.

But those that had completely recovered from such illness bore more than the minimum fatal dose for control animals, and thus showed a certain degree of immunity, which through further treatment with culture fluid could always be raised higher.

If one now wished to employ methodically this method of conferring immunity, it would scarcely be possible to avoid the loss of a considerable proportion of the experimental animals in the act of conferring immunity. For if one keeps far below the minimum fatal dose the protective effect is very slight, and one would thus not attain the object; but if one approached the minimum dose previously ascertained to be fatal to one animal, the treatment might, owing to the not inconsiderable differences of different rabbits as regards susceptibility to tetanus, suffice to cause the death of another animal.

But, without any danger of losing animals, the condition of immunity may be quickly and certainly reached by ascending from quite unoffensive cultures to such as are more and more active. With this object one can begin with about the $\frac{1}{20}$ th part of the minimum fatal dose of the unaltered culture, or, still better, of the filtrate of the same, and then within 4 weeks ascend to double the fatal dose.

But one can also attain the object by making larger quantities of the culture less active by the addition of trichloride of iodine, and beginning the treatment with that.

I begin with the injection of 5 ccm. of 0.25 % trichloride of iodine, and at intervals of 3 to 5 days increase the dose, so as to inject next time 5 ccm. of a 0.2 % solution, then 5 ccm. of a 0.15 % solution, and so on. With this method of treatment the animals, as a rule, do not become ill at all, and I have never had to complain of any loss from it. Within from 4 to 6 weeks one can thus arrive at an immunity equal to 10 and over that (according to Ehrlich's reckoning). Just as in the case of diphtheria, the power of the blood to protect or cure other animals is in correspondence with the degree of immunity acquired, and one can therefore, even without infection with dangerous doses, ascertain the degree of immunity. In my rabbits the immunity of 10 (Ehrlich) corresponded to such a protective power of the blood that mice which had received by subcutaneous injection 0.05 ccm. of blood-serum no longer died of tetanus when they were subsequently infected with the minimum dose otherwise fatal to them. Taking 20 grms. as the weight of a mouse, that is equal to a proportion of 0.25 : 100, or 1 : 400.

While with rabbits one has the choice of both the before-described methods of conferring immunity, that is not the case when one wishes to protect mice against tetanus with culture fluids. In corroboration of Kitasato's statements, I can therefore simply report the negative results of my experiments in that direction.

On the contrary, it is possible to make mice immune according to the principles proved in the protection of rabbits; only, I begin in that case not with fluids containing 0.25 % ICl_3 , but with such as contain 0.4 %, and, gradually ascending, I inject each time 0.4 ccm. at intervals of 8 days. By that method I have lost about 40 % of the mice, but have ascertained that the rendering of even these animals is a practicable thing; and, with further development of the procedure, the losses would certainly be less.

I will not here enter into the grounds which possibly are to be taken into account in explaining the different action of the method of dilution, and that of attenuation by means of trichloride of iodine, but merely recall the fact that in the case of diphtheria also immunity of guinea-pigs has not hitherto been obtained by previous treatment with small and gradually increasing doses of the unaltered diphtheria poison or of living bacilli, whereas the use of culture fluids treated with trichloride of iodine is successful.

Having regard to these and the other above-mentioned experiments, I believed that I might now proceed to the protection of larger animals, in order to procure from these active blood in larger quantities than is possible from rabbits.

The opportunity to do this was afforded me through Professor Schütz of the Berlin Veterinary College. A representation having been made showing the position of matters regarding the conferring of immunity against tetanus, and the curative treatment of the same, he had placed at his disposal by the Board of Agriculture the means of testing the experimental results gained on small animals in the laboratory on such of the larger farm animals—the horse for example—as not rarely become affected with tetanus and die from that disease.

After the plan of experiments had been agreed upon at a conference presided over by Privy Councillor Koch on the 5th of October 1891,

the experiments were begun in the same month by Professor Schütz, Herr Kitasato, and myself. During the whole duration of the experiments Privy Councillor Koch has aided us with his advice.

The rendering of horses and then of sheep immune against tetanus was regarded as the first object; following upon that, it had to be ascertained whether, in these larger animals also, the increase in the degree of immunity was accompanied by such a change in the blood that one could with the latter protect and cure other animals. But the final purpose of these experiments was to obtain a specific therapeutic material, of such activity and in such quantity as to make it applicable for large animals, and ultimately even for horses. It was this plan of work that induced the Board of Agriculture to provide the money. But, of course, the chief incentive to the taking of these experiments in hand, at least for Kitasato and myself, was the hope that, by succeeding in them, the possibility of curing also human beings affected with tetanus would be brought nearer.

The greatest share of the work fell on Professor Schütz and his assistant Herr Casper. When subsequently the details are published in common with us from the careful record made by Professor Schütz, it will be seen how complicated, and in part unexpected, was the nature of the work that had to be done, and at the same time how valuable from a veterinary point of view is the experience that has been gained. Quite unknown to us, and indeed not yet recorded in veterinary literature, were the symptoms of tetanus, the peculiarity of its course in horses, and the highly interesting means employed to recognise the commencing disease even before the proper tetanic symptoms are observable; all these are things that, but for the experience of Professor Schütz, we should either have not paid any attention to at all, or wrongly interpreted.

For the rest, I worked according to a plan suggested by my experiments on laboratory animals, and Herr Kitasato after a plan which he thought the most promising on the ground of his own previous studies.

If I now in the following give a *resumé* of the results obtained by my method, I wish it to be expressly understood that, while these are reported completely so far as is necessary for the understanding and testing of the same, the detailed publication of the record of experiments will be made subsequently, and in common with Professor Schütz.

For my own experiments I had at my disposal 3 horses and 2 sheep. In these animals the attempts to confer immunity have had a positive result. Two of the horses and two of the sheep are still alive and healthy, and they are being further protected. The first horse submitted to treatment was an old animal with many defects, and from the outset regarded by Professor Schütz as one not likely to live long; it died from perforation of the bowel in consequence of very old well-advanced intestinal stenosis.

This horse (I.), in spite of the disturbing general disease, was so far rendered immune by a seven weeks' treatment that its blood had the power of protecting and curing other animals. The subcutaneous injection of 0.5 ccm. of serum from this horse sufficed to render a mouse immune against the otherwise fatal minimum dose. Expressed

as above in figures, the immunity-conferring strength of the serum was 2·5 : 100, or 1 : 40.

The results of the previously made examinations of this horse's blood are very important.

Treatment was begun on the 12th of October 1891. At this time 1 ccm. of the serum obtained from the blood was quite inactive when injected into the peritoneum of mice, but also quite harmless.

On the 20th of October the serum was, as before, quite inactive.

On the 30th October it contained toxic substances, several mice which had received 1 ccm. intra-peritoneally dying with symptoms like those of tetanus.

On the 10th of November an immunity-conferring action was found for the first time ; through 1 ccm. of serum death from tetanus was not absolutely prevented, but it was delayed for several days.

On the 21st of November 0·8 ccm. sufficed to render a mouse immune (1 : 25).

On the 30th November 0·5 ccm. (: 40).

The entire absence of immunity-conferring action at the outset, the appearance of the same with the progress of treatment, and its increase with the heightened toxic or infectious action of the dose of culture fluid which was still borne by treated animals—all this was so regularly repeated as to make it unnecessary to repeat the corresponding investigations in the case of the other animals.

In the four other animals the degree of tetanus immunity acquired at the time of concluding this article has been determined in a double way. Firstly as in the case of Horse I. On the 17th January 1892 Horse II. had in its serum an immunity-conferring action for mice equal to 1 : 5000 (one part serum was diluted with 49 parts of physiological chloride of sodium solution ; of this mixture 0·2 ccm. sufficed to prevent tetanus in full-grown mice).

Horse III.	.	.	.	= 1 : 500
Sheep I.	.	.	.	= 1 : 1000
Sheep II.	.	.	.	= 1 : 100

In the second place, in order to farther prove the immunity, all four animals were on the 16th December infected with fully virulent tetanus *bouillon* culture. The fatal minimal dose of this culture was 0·0005 ccm. for mice, and 0·8 ccm. for rabbits.

Of this culture there was injected on the 16th December 1891 :—

1. To a control horse 0·5 ccm. ; the animal showed the premonitory symptoms of tetanus on the 21st December, had quite typical symptoms on the 24th, and died on the 29th.
2. To a large old control sheep 44 ccm. ; the animal showed the first tetanic symptoms on the 18th December, and died on the 23rd.
3. To a young sheep 1·0 ccm. ; it became ill on the 20th December, and died on the 23rd.
4. To Horse II. 1·5 ccm., that is thrice as much as to the control horse.
5. To Horse III. 0·5 ccm.
6. To Sheep I. 8·0 ccm., that is 8 times as much as to the second control sheep.
7. To Sheep II. 1·0 ccm.

These previously treated animals, Nos. 4-7, did not at any time show any symptoms of disease, and meanwhile they are being further protected.

8. To a large rabbit 1'0 ccm. ; it showed the first distinct symptoms of tetanus after 30 hours, and died on the 19th of December.

No further commentary is required in order to prove the serviceability and certainty of my method of conferring immunity, especially when one regards the results in the horses, in which animals, according to the views hitherto held in veterinary circles, immunity is altogether impossible, since horses that have once recovered from tetanus (which, moreover, is an extremely rare occurrence) are not protected against further infection (Kitt).

In view of these results, I am justified in assuming that such a description of my method of conferring immunity as will make it possible to repeat it elsewhere will not be undesirable in interested circles.

For this purpose it is not necessary to give a detailed description of what is done to the single animals ; a general formula is quite sufficient, and I feel justified in drawing up that from the observations made at the Veterinary College.

When it is desired to confer immunity on a horse, one must procure a large quantity—at least 200 ccm.—of a tetanus *bouillon* culture of such a strength that 0'75 ccm. suffices to kill a full-grown rabbit with certainty in 3-4 days. (Through experiments on mice one may convince oneself that, even after 500 times dilution of such a culture, 0'1-0'2 ccm. suffices to kill every mouse with tetanus in three days at the latest.) In order to preserve these 200 ccm. of culture for a longer period, one adds to it carbolic acid in the proportion of 0'5 per cent.

The carbolised culture is then divided in different portions :—

1.	20 ccm.	remain without any further addition.
2.	40 ccm.	have ICl_3 added in the proportion of 1'25 per cent.
3.	60 ccm.	" " " 0'175 "
4.	80 ccm.	" " " 0'25 "

The horse is now first treated with the mixture No. 4. Of that it should receive first 10 ccm. ; after 8 days 20 ccm. ; after other 8 days, if, as is to be expected, a febrile period is meanwhile recovered from, again 20 ccm. ; and the remainder after other 3 days.

The mixture No. 3 is then injected in two doses of 30 ccm. each with intervals of 8 days.

The mixture No. 2 in two portions of 20 ccm.

Of the culture fluid without iodine trichloride one begins with 0'5 ccm., after it has previously been ascertained by taking blood and testing the serum that the latter has for mice an immunity-conferring power of at least 1 : 100 ; failing that one begins with 0'25 ccm.

At intervals of 5 days the doses of virulent culture by subcutaneous injection can then be doubled.

I am convinced that for those who are accustomed to these experiments the procedure for conferring immunity may be notably shortened. But in order to obtain a method of treatment devoid of danger, and yet leading with certainty to the object in view, I would meanwhile recommend this scheme as the best for the horse.

In sheep, which animals are not so very susceptible to tetanus as

horses, the object, as in the case of rabbits, may be obtained in various ways. By my method the steps of the treatment of sheep may be much more rapid than with the horse. After two injections of 10 ccm. 0.175 % ICl_3 with 5 days' interval, one can immediately proceed to mixture No. 2, beginning the treatment with the injection of 5 ccm., and doubling the dose at intervals of 5 days until one reaches 20 ccm. After other 5 days 1 ccm. of virulent culture may be immediately injected, and thereafter the dose may be doubled at intervals of 5 days. But if in the course of the treatment fever (temperature over 40°C.), colicky symptoms, or meteorism exists at a time when a new injection ought to be made, the further treatment must be stopped until these symptoms have completely disappeared; and subsequently, not the larger doses according to the scheme, but the last employed dose, is to be chosen.

It need hardly be said that this scheme must be varied according to the age of the animal and the occurrence of unforeseen circumstances.

It is very useful to acquire information regarding the progress of the protection process by drawing off blood at regular intervals, and testing the immunity-conferring action of the serum on mice during the different phases of the treatment. In our first experiments we regularly carried out these tests at intervals of ten days.

Apart from this summary report regarding the course of the experiments carried out at the Veterinary College according to my method of conferring immunity, I will report only one other observation which was there made, and which, as regards the principle involved, is of the greatest importance.

It is as follows. In selecting animals to furnish blood active for curative purposes, the question arises, should we choose such animals as are not very susceptible to tetanus, and which can in consequence, as we know from experience, be more easily and with less danger brought to a high degree of immunity; or is it better to choose very susceptible animals, which can be tided over the first phases of the immunity-conferring period without danger to life only by employing quite special measures of precaution?

Of the animals here tested I class sheep in the first category, and horses in the second.

Now, it has been found that with the same duration of treatment I can bring a sheep to such a point that it can without any danger whatever be infected with at least five times the quantity of virulent culture that a horse can tolerate after the same length of treatment.

At the first glance it might seem as if this decided the question raised above to the advantage of sheep, but the thing assumes another aspect when one keeps in mind the final object of the experiments, namely, the obtaining of active curative serum of the greatest possible strength. Then, without doubt, the making of horses immune must be given the preference. For, at the time when the horse No. I. had first withstood 15 ccm., and the sheep No. I., on the other hand, had twice within a short interval borne 8 ccm. of virulent culture without any reaction, the horse's serum was nevertheless distinctly more active than that from the sheep. Thus the horse's serum at the end of December had an immunity-conferring value of about 1 : 100, and that of the sheep a value of 1 : 80 at the highest.

It appears to me that thereby the observation made by me in the

experiments regarding diphtheria-immunity is definitely confirmed, namely, that the effect of an animal's blood in the way of conferring immunity does not depend upon the absolute degree of immunity possessed by that animal, but upon the extent of the difference between the original degree of resistance against an infectious disease and that to which it has subsequently been artificially raised; and I have from that formed the opinion that those changes which lead to acquired immunity transpire essentially in the cell-free blood plasma, and here can be brought to expression through transference of the immunity to other animals; that, on the contrary, the inherited immunity is a condition the determining factors of which are, as a rule, not to be found in the cell-free blood.

For the rest, I can only add further that, with the serum from horses and sheep, mice can now be, not simply protected, but even cured a long time after infection, and that in the same way as with mice we have to record curative effects in rabbits infected with tetanus.

MALPOSITIONS OF THE COLON.

By THOMAS WALLEY, M.R.C.V.S., Principal, Royal Veterinary College, Edinburgh.

To those who, like myself, have made the subject of malpositions of the colon a study, the articles published in the December issue of the Journal are of more than passing interest. Before alluding particularly to these, it may be as well that I should refer to my own position in connection with the matter. In the earliest days of my professional career the subject of bowel lesions in the horse was to me an interesting and fascinating one, but it was not until the year 1861—while acting as assistant to the late Mr Edward Stanley of Birmingham—that I formulated in my own mind any theory in reference to the nature of the serious, and so often fatal, bowel affections to which the horse was, *par excellence*, the subject. The views I formulated were the outcome of many hours' (by day and by night) patient watching, repeated rectal explorations, and numerous *post-mortem* examinations.

Practical expression of these views was first given by me in the year 1870, while assisting in the practice of Mr Lawson at Manchester, and that on the occasion of a *post-mortem* examination which I made on the body of a horse whose death was due to torsion of the colon. It so happened that at the autopsy in question several of the city practitioners were present, and the whole of them expressed the opinion that the lesions made manifest by opening the abdomen were those of enteritis. I was enabled to show, distinctly, that they were the result of complete torsion with vascular strangulation, and I then made the statement, often reiterated since, that not one in twenty of the so-called cases of enteritis were in reality of that nature, but, on the contrary, that they were cases of malposition or torsion of the colon.

In 1875 I read a paper on the subject at a meeting of the Liverpool Veterinary Medical Association, in which I pointed out that such lesions were of far more frequent occurrence than was enteritis, and that in many cases a successful diagnosis could be made. A few