Experiment V b.—Salt Solution Washings of Glycerine Extract heated at 56° C. Incubated.

13 m. only.	13 m. + Normal Serum.	13 m. + Heated Serum.
Marked haemolysis	Marked haemolysis	Marked haemolysis

It would seem that both amboceptor and complement were carried through the filter by adding a small quantity of salt solution.

Experiment VI.

5 per cent. washed human red corpuscles, 2 c.cm. in each tube. The venom used was from ninety fish mixed with 90 m. glycerine, and filtered through a Berkefeld filter. (The original mixture gave marked haemolysis with washed red cells alone.) The second filtrate gave with heated serum and red cells slight haemolysis, and with normal serum *nil*.

One drachm of glycerine was then added to the filter.

Ten drops of this with red cells only gave faint haemolysis.

Ten drops of this with red cells + heated serum gave marked haemolysis.

Ten drops of this with red cells + normal serum gave *no* haemolysis.

We are now in a position to review the results of these experiments, and in order to make the results quite clear I have tabulated them and arranged the tables to show the results first of all with venom glycerinated and filtered through filter paper and then the results obtained by filtering through a Berkefeld filter.

SUMMARY OF RESULTS.

Washed human red cells, 2 c.cm. Incubated 2 hours at 37°C., and allowed to stand in the cold. Serum was heated from 56°C. to 60°C. for $\frac{1}{2}$ to 1 hour in different experiments.

Signs.—Faint haemolysis is denoted by f.h.; slight haemolysis by h.; marked haemolysis by H.; almost complete haemolysis by Hc.; complete haemolysis by HC.

Filtered Glycerinated Venom (Filter Paper).

Experiment.		Only.	+ Normal Serum.	+ Heated Serum.	+ Lecithin.	
I.	m. j	h.	f.h.	Hc.	—	100 weavers + 30 m. glycerine. Record lost.
II.	—	H.	Hc.	HC.	—	
III.	m. v	h.	h.	H.	H.	60 weavers + 60 m. glycerine.

Filtered Glycerinated Venom (Berkefeld Filter).

Experiment.		Only.	+ Normal Serum.	+ Heated Serum.	+ Lecithin.	
IV.	m. j	0	0	f.h.	0	100 weavers + 100 m. glycerine.
	m. j	0	0	h.	0	
	m. v	h.	0	H.	H.	
	m. v	h.	0	H.	H.	
VI.	m. ij	0	0	h.	—	90 weavers + 90 m. glycerine.
	m. v	f.h.	0	H.	H.	

An analysis of the results of these experiments shows the broad facts that the admixture of glycerine and filtration alters the character of the venom, first, as to its virulence, and secondly, as to its character.

With filtration through filter paper, we find that, contrary to what happens with unfiltered venom, heated serum increases the amount of haemolysis in Experiments I, II, and III, and that in Experiment I normal serum diminishes haemolysis, in Experiment III it remains the same, and in Experiment II it appears to have increased it. At any rate we have the uniform definite result of diminished virulence and of an increase of haemolysis by the addition of heated serum.

With filtration through a Berkefeld filter, we found diminished virulence and an *absence of haemolysis* with normal serum, that is to say, a more definite loss of haemolytic power in the presence of normal serum when filtered through a Berkefeld filter than through filter paper. On the other hand, there is a more marked increase of haemolytic power with heated serum when filtered through a Berkefeld filter, as shown in Experiments IV and VI, where we get either slight haemolysis or marked haemolysis.

It would be possible to explain these results by assuming the presence of a complement-like body in addition to an amboceptor in fresh venom. The complement appears to pass through the filter less readily than the amboceptor,

and its passage can be encouraged by adding salt solution as shown in Experiments IV and V.

It was also observed that lecithin was able to complement the venom amboceptor in the cases in which heated serum increased the haemolysis.

The results of these observations confirm my original contention concerning the action of fresh venom; they explain how the difference in Briot's results and my own may have been produced, and they also show the possibility of the existence of a hitherto unsuspected body in weaver venom—namely, a complementary body that allows haemolysis to take place without the addition of heated serum or lecithin. This complementary body is, however, not a true complement, as it is able to withstand heating for half an hour to 56° C.

If the venom extracted by a syringe be dried *in vacuo*, and, after keeping several days, be dissolved in normal saline and filtered, a solution is obtained capable of dissolving washed red corpuscles without the addition of serum, normal or heated.

In conclusion, it will have been observed that throughout this paper the terminology of Ehrlich's school has been used, and the experiments have been made in the light of the studies on venoms by Preston Kyes, Sachs, Flexner and Noguchi and others. In view, however, of the recent work on the colloidal nature of antigens and their antibodies, especially the researches on haemolysis by colloidal silicic acid by Jagic and Landsteiner,⁴ it would seem possible that my results may be readily explained according to this newer theory.

REFERENCES.

- 1 Briot: *Comptes rendus Soc. de Biologie de Paris*, 1902 and 1904.
- 2 Calmette: *Les venins*.
- 3 Preston Kyes, in Ehrlich's *Collected Studies on Immunity*.
- 4 Landsteiner and Jagic: *Wien. klin. Woch.*, vol. xvii, p. 63.
- 5 Muir Evans: *Transactions, Pathological Society of London*, vol. lvi, Part I, 1907; and *BRITISH MEDICAL JOURNAL*, 1907.

A NOTE ON COWPOX IN MAN ;

WITH A REPORT OF A RECENT CASE.

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THE first distinct description of cowpox was given by Jenner when he published *An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, and known by the Name of the Cow Pox* (London, 1798). The disease is described as consisting of "irregular pustules on teats, of a palish blue colour surrounded by an erysipelatous inflammation, and characterized by a tendency to degenerate into phagedaenic sores." The communication of the disease to man was no doubt fairly common for a considerable period before the above treatise was written.

The gossip among cowherds of the vague, obscure, but popular belief regarding the possible communication of this disease to the milkers, and the protection from small-pox which this conferred, was the source which first attracted Jenner's attention.

One finds that Jenner cited a number of instances of accidental inoculation occurring on the fingers, hands, wrists, arms, and on the lips. Pearson¹ also cites cases, mostly on hands and arms. One of his cases is, I think, of special interest.

Annie Francis had pustules on her hands from milking cows affected. These pustules soon became scabs, which, falling off, discovered ulcerating and painful sores, which were long in healing. Some milk from one of the diseased cows having spurted on the cheek of her sister and on the breast of her mistress, produced, on these parts of both persons, pustules and sores similar to her own on her hands.

The two latter cases seem to me to be open to the question whether the milk from the animal suffering from cowpox was the infecting source, or whether the fingers of the originally infected case were the immediate inoculating factor.

I was talking to a cowman in a large dairy farm about cowpox recently, and he informed me that some years ago he was himself infected, chiefly about his finger-nails, and that during the course of the disease (to put it in his own words) "he was acting about with his missus one day, and accidentally scratched her forehead." About a week later the disease appeared at the site of the scratch; papule, pustule, and scab followed, with con-

siderable constitutional disturbance. The woman bears a large white, silvery cicatrix at the present time. Cases were reported, and a careful description of the disease in the cow given, by Ceely² in 1838, 1840, 1841, 1845. Other outbreaks were discovered in 1857, a full description of them being given in Professor E. M. Crookshank's book, *Bacteriology and Infective Disease*. Mention is also made of outbreaks in Italy, France, Germany, Holland, Denmark, Russia, and in North and South America. In December, 1887, in Wiltshire, Professor Crookshank investigated and described several cases, one being somewhat similar to the case shown in the photograph— inoculation of right cheek in a male milker, occurring in November, 1887. In June, 1888, Mr. Forty reported cases in Gloucestershire, and in 1894 Mr. Bucknill reported a case in a milkman.

On November 12th, 1909, I was consulted by a farmer in North Berkshire, aged 38, for swelling and irritation of the right lower eyelid. He said that two days previously he had noticed irritation and redness, with slight swelling. He presented a small, slightly elevated papule, about the size of a split pea, markedly hard on palpation; it merged into considerable swelling and infiltrated hardness of the areolar tissue of the whole lower lid. The condition puzzled me, and I asked a question I often put in vague cases of skin affections in farm labourers, etc., "Have you any disease prevalent among your cattle?" His reply was

that some of his cows had "breakings out on their teats." On November 14th the papule had increased in size, was more elevated, and had a flattish top of dark rose colour. The infiltration of the lower lid had increased, was markedly hard (did not pit on pressure), and there was slight swelling of the adjacent parts of the face. When I saw him on November 16th he said that on the previous day (probably the seventh of the disease) he felt "as if a bad cold were coming on" (headache, pain in back and limbs, with shivering; appetite bad and feeling of nausea). The papule had become of a bluish-grey slate colour on its flattened top, very slightly umbilicated, and gave the impression of little fluid contents, much less actual vesiculation, and less apparent fluid therein than in ordinary vaccination insertion at its height in the arm of a child. Round the bluish-grey central area on the summit of the papule was a ring of about 1½ mm. wide, of a somewhat lighter bluish-grey colour, showing minute venules, crossing and recrossing in a diagonal pattern. I believe that the slight apparent umbilication was due to the somewhat lighter colour of this marginal ring contrasted with the darker shade in the centre, and that the summit of the papule at this stage was practically flat. There was no oozing of lymph. The surrounding swelling was much increased, red, and hot. The sclerotic and conjunctiva of the right eye were injected and oedematous, giving the cornea the appearance of being sunk in a pit, with walls of oedematous sclerotic tissue. The general swelling extended over the whole right side of the face, down to and just under the chin, backwards to the auricle, and over the angle of the jaw. The lymphatic glands in front of the auricle were enlarged and tender, and he complained of pain and stiffness on moving the jaw.

On November 18th the swelling of the face had considerably subsided. The tissues of the eyelid were still much infiltrated and hard, but the conjunctiva was less oedematous. The central area of the vesiculo-papule was becoming brownish in colour, and now showed some umbilication, the surface of depression having a dryish appearance. The narrow bluish-grey ring had widened to about 3 mm., and was of fainter colour; the venules had disappeared. The skin just beyond was scaly, as if desquamating.

On November 20th the swelling was less in the lower lid, but still peculiarly hard. The scab was now blackish in colour, furnished, and the umbilication well marked. The marginal ring was disappearing into the desquamating area surrounding it. There was still no oozing or detachment of the scab. At this period the scab reached its maximum size; its length was fully 2 cm., and breadth at widest fully 1 cm.

The photograph was taken on November 22nd. Seropurulent, thickish fluid was then exuding from under the upper and inner margin of the scab. This was probably hastened by the scab being raised by a medical friend to whom I showed the

case. On exposure to the air and drying this exudation formed a granular deposit like particles of fine Demerara sugar.

On December 1st the scab was somewhat shrunken, the concavity (umbilication) was markedly increased, the edges of the scab were thicker, discharge was still present, and induration marked in the immediately surrounding part of the lower lid. The area of induration was slowly lessened in extent on December 4th. The scab was apparently becoming thickened by successive layers solidifying around and under the original layers of the scab first formed, giving the margin the appearance of concentric superimposed layers. The scab separated spontaneously on December 10th, leaving a small excoriated surface showing healthy granulation tissue. Slight surrounding discoloration and thickening were still present.

On January 8th, 1910, the sore had completely healed, but the skin over the seat of inoculation showed slight swelling and faint redness. The man had only been vaccinated as an infant (thirty-seven years before), and had never been revaccinated. On this date I vaccinated him with reliable vaccine lymph on the upper right arm, making two insertions. The skin was cleansed with soap and water, and afterwards with rectified spirit. The lymph was applied and scratched through with a sterile lancet. The part was covered with sterile gauze and fixed in position with strapping. At the same time the man's infant, aged 3½ months, was vaccinated with lymph from the same tube, four insertions being made. When I inspected the cases on January 16th there was absolutely no reaction in the man; the minute scarifications were visible, having healed apparently by first intention. In the case of the infant four typical vaccination vesicles were present, showing moderate areola and the usual infiltration of surrounding tissues. Three other infants were vaccinated with the same series of lymph, and all showed four good vaccination vesicles at the points of insertion.

Fig. 1.—Showing appearance of case about fourteenth day of disease. The small, irregular-looking part extending from inner and upper margin towards the edge of lower lid due to dried exudate from under scab.

It is interesting to remark that the outbreak of cow-pox occurred in a neighbouring county to Gloucestershire, where Jenner found his early cases. The farmer informed me that the outbreak among the herd of cows in North Berkshire in November, 1909, had been going on for about a month before I saw them. Out of 30 cows, 25 showed evidence of the disease on the teats and the base of the udder. Inquiry since showed that all the milch cows were affected. The pocks were multiple, and some, where the scabs had been knocked off, showed much inflammatory reaction, with sloughing and ulceration. The most marked differences in the cow and man were that in the vesicular stage the unbroken vesicles, of which I only found one or two, gave the idea of more fluid contents in the cow than in the man; most were broken, evidently from milking, the same slate-blue colour was evident, and they were discharging a sanguineous fluid.

The marked umbilication shown in the man in the scabbing stage was generally absent in the cow, and the scab was slightly convex to the surface of the teat; but there was the same formation of superimposed layers. According to the statements of the farmer and cowman, little constitutional disturbance was noticeable in the cows, and the secretion of milk was but slightly reduced. These remarks are only impressions roughly formed by inspecting a few of the cows on two occasions. The cattle being in the fields, only the more docile cows could be approached and examined. The photograph (taken on November 22nd) shows the udder and teats of one of the affected animals. I was unable to trace any other possible infections in man in this epidemic.

The farmer thought the first case occurred in a recently purchased cow. In reply to questions, he said that no disease was present among his farm or other horses (grease). He had changed his cowman and a milker shortly before, but neither had any sore places on their hands or elsewhere, as far as he knew.

The disease is said to be rare, but I am inclined to believe, from inquiries among a few dairy farmers and cowmen in my neighbourhood, that most have had out-

breaks of the disease in their herds during the last ten or fifteen years. I also found that several men, in all probability, contracted the disease on their fingers or wrists, and in one case it was communicated by a cowman to his wife as before mentioned.

I am also impressed by the fact that though the results produced by accidental inoculation direct from a case of cowpox to the human being broadly go through the same stages as those produced by vaccine lymph, yet they differ in the appearances at the various stages, and probably run a more severe and protracted course. This may possibly be due to accidental introduction of organisms producing more intense inflammatory reactions, or the preparation of the lymph for vaccination may modify the activity, and thereby subdue the intensity of results obtained.

As this disease chiefly prevails in milk cows, and usually affects a whole herd, it should, I think, be made notifiable. For in process of milking the scabs are knocked off, and the contents of

Fig. 2.—Showing appearance of cow's teats about twentieth day of disease.

the vesicles are sure to contaminate the milk; the scabs and products of secondary inflammation and sloughing must also to some extent find their way into the milk, and the result of drinking such milk, should any small abrasion be present in the mucous membrane of the upper alimentary tract, raises some speculation. Dairy farmers' knowledge of this may perhaps partly account for the apparent rareness of the disease. If notification were made compulsory, and if the disease was confirmed by competent veterinary inspectors as likely injuriously to affect the use of the milk for human food, surely its sale ought to be stopped until danger had passed. As the disease is rare, probably insurance against its occurrence could be made at a trifling cost.

REFERENCES.

¹ *An Inquiry into the History of Cow Pox*, 1798. ² *Transactions of Provincial Medical Society*. Further Observations on the Variola Vaccinae. Robert Ceely, Esq., Worcester. 1842.

ON MALARIAL FEVERS AND QUININE.*

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As all observers of malarial fevers know, quotidian fever and double and triple fever, and irregular and remittent, may be caused by any of the three well-known parasites, it is supposed by several different broods developing independently. There may of course be mixed infection also. As a matter of fact parasites in different stages will be found in any malarial blood at the same time, but there is generally a preponderance of organisms of the same size. Haemolysis, or breaking down of the blood discs, is more

or less rapidly going on during an attack, most rapidly when the sporulation is most complete. But the fever only manifests itself when enough of the poison manufactured by the parasites in the red cells is set free in the blood plasma to cause its physiological action on the nervous system. This poison is probably a protein derivative, and the dose required to cause fever manifestations is large but varies with the individual. We know as a case grows worse the fever anticipates, more cells break down, and more poison is set free. As an attack grows less the fever is postponed, fewer cells break down, and less poison is set free. The development of antibodies is but a small factor in the cure of malarial patients. Immunity to the disease, as we all know, is a thing of very slow growth and very many attacks.

In two or three days, according to the nature of the organisms, the asexual parasites sporulate, but doubtless very many infected cells break down before their uninvited guests reach this stage. The parasites doubtless, too, die prematurely from many causes. Why should they alone in Nature be free from accident and disease?

We know that in malaria there are profound changes in the quality and composition of the blood. (1) There is a great diminution of the red cells, much greater than can be accounted for by the ratio of cells infected. (2) The haemoglobin content of the discs is much reduced. It has been known to fall as much as 50 per cent. (3) The resistance of the discs is lowered. In normal blood nothing happens to the discs when added to a 0.41 per cent. salt solution, whereas malarial blood discs haemolyse at once. (4) There is a leucopenia, but as pointed out by Christophers and Stephens, there is both a relative and an actual increase of the large mononuclear cells. The meaning of this is unknown.

I think we may take the lowering of the resistance of the blood discs to be Nature's method of cure. If the infected discs break down before the asexual parasites can sporulate, a permanent cure ensues, unless, indeed, Schaudinn's theory be correct, namely, that the gametes may revert under favourable conditions to the asexual type. In other words, he supposed that the gametes are not only sexually perfect parasites but may also act the part of resting spores. It is a plausible theory, but there is no evidence to support it. It is much more likely that some spores may become encysted in certain internal organs and await the favourable turn of the tide when the human body becomes again a suitable habitat.

We come now to the action of quinine. Intimately connected with malignant malaria in the tropics is blackwater fever—acute haemoglobinuria. As pointed out by Koch, it nearly always occurs in patients taking quinine. Quinine lowers the resistance of the blood discs, and so does malaria, but we do not give it to our patients on the principle of *similia similibus curantur*. This action of quinine can easily be demonstrated *in vitro*. The amount of neutral quinine hydrochloride required to haemolyse 1 c.cm. of red cells at 37° C. is from 0.001 to 0.0008 gram. In blackwater fever during an attack it was only 0.0008 (Stephens and Christophers). Doubtless the proportion required to cause lysis of inhabited blood discs is very much less than in the case of uninhabited. When quinine is given in large doses for a considerable time to a person the resistance of whose blood has been greatly lowered by frequent attacks of malignant malaria, a time comes when, in spite of the natural safeguards of the system, an explosive breakdown of the blood discs will take place. But when quinine is judiciously given in malarial fever it causes the gradual premature breakdown of infected cells before their guests can sporulate. Thus the cycle of Golgi is cut short, and a cure is effected as far as the patient is concerned, for the time being at any rate. It is doubtful if the drug has any effect in cutting short the cycle of Ross, so that it is possible a patient may be cured as far as he himself is concerned, but still for a time be able to infect mosquitos. In other words, quinine cures malarial fevers by destroying the homes and food of the *Plasmodia malariae* before they can sporulate asexually; but yet the "cured" patient may remain a source of infection to his neighbours for some time. All observers admit that the gametes are practically unaffected by quinine. I have seen crescents very quickly disappear under moderate injections of soamin.

* Being extracts from a paper read before the Cardiff Division of the British Medical Association on December 15th, 1909.