number of fits was progressive from 25 to 48. At this stage—namely, November, 1907—the administration of the bromide salt was begun, and the results are seen in the following table:

TABLE II.

No. of Men.	Date.		Fits.	Decrease.
	From	To		
9	Nov. 29, 1907	Dec. 27, 1907	70	43 from Sept.
9	Jan. 3, 1908	Feb. 22, 1908	25	88 " "
9	Feb. 23, "	Mar. 21, "	30	
9	Mar. 22, "	Mar. 31, "	15	(In 9 days)
9	Apr. 1, "	Apr. 28, "	59*	
10	Apr. 26, "	May 23, "	13	100 from Sept.
10	May 23, "	June 20, "	40*	
8	June 21, "	July 18, "	21	
8	July 19, "	Aug. 15, "	19	
8	Aug. 16, "	Sept. 12, "	9	104 " "
8	Sep. 13, "	Oct. 10, "	4	109 " "
7	Oct. 11, "	Nov. 7, "	5	
6	Nov. 8, "	Nov. 30, "	7	
In 12 months 317—Nov. 1907 to Nov. 1908. Less fits by 581 than 1907.				

It is to be noted that in April and in May-June the number of fits largely increased—this was due to the omission of the salt by the attendant; this man was admonished, but his carelessness was repeated, and he was replaced by another who has proved himself thoroughly reliable, with the result seen at the end of the table. It is also to be noted that the number of epileptics treated fell from 10 to 8, and ultimately to 6, 1 having been removed to hospital for an abdominal operation, but the general and almost continuous fall in the number of fits from 113 a month in October, 1907, to October, 1908, when there were only 4 in the month, sufficiently proves the efficacy of the treatment. The greatest number of attacks, 113, occurred in October, 1907, increasing from the previous July, when only 9 instead of 10 men were under treatment, whereas in Table II when 10 men were again under treatment, that is, from April 26th, 1908, to May 23rd, 1908, the number of fits fell 100 below those recorded from September 29th to October 26th, 1907, before any treatment was begun.

In observing the effects of the bromide salt treatment, it is found that when the epileptics come under its full influence they become rather listless, stupid, and drowsy; this is the expected result, and a result to be attained; it indicates the point of saturation of the system by the salt, the point when the fits become controlled; the dose daily can then be diminished one half, but not altogether discontinued. The drowsy, listless, symptoms soon pass off and the patient is fit again for work. It should be said that the administration of the salt is not confined to giving a definite amount with the three daily meals, but their bread is specially baked for them, the amount of common salt usually mixed with the dough is excluded and is replaced by an equivalent quantity of the bromide salt.

Some eight or ten years ago two French physicians, MM. Toulouse and Richet, discovered that the desired effects from sodium bromide were interfered with when sodium chloride was allowed to be used in the food. When the common salt was excluded from the food, the good effects of the bromide immediately became pronounced; the cause of antagonism was supposed to be decomposition of the salts in the body when taken together. The results were published in the BRITISH MEDICAL JOURNAL, and Dr. Eurich read a paper on the same results in his own practice before a meeting of the Bradford Medico-Chirurgical Society. I immediately thereafter tested the treatment in my private practice, with most satisfactory effects in several cases.

The amount of bromide given by experts to patients largely exceeds the doses given by general practitioners,

Dr. McCallum of Kendal, the medical officer of the Starnthwaite colliery and school for epileptic boys, with whom I corresponded after my visit to Starnthwaite, gives 1 to 2 drachms or even more in a day. Our dose may have been a third or sixth of this in mixture, but now the bromide salt we give at meals and with the bread approaches more to his, as he believes, necessary standard. He pushes the doses to saturation of the system, indicated by the diminution or ceasing of the fits, and some degree of drowsiness and stupidity of the patient; but he alleges that this condition does not in any way impair the ultimate intellectual capacity, which returns when the doses are reduced and the fits subside, but he continues the bromide until assured that the attacks are subdued. It is no use trifling with epilepsy if any permanent results are to be achieved; the treatment must be strong, continuous, and well watched. In some cases the salt produces an eruption, but this can be met by cleanliness and appropriate applications; better the rash a hundred times than the epileptic nerve storm and convulsive spasms of the epileptic fit. There is no proof that the mere place of residence has any influence on the ultimate cure of the disease; change of place does diminish the fits for a time, but they ultimately recur and continue as bad as or worse than before; what is wanted is adequate treatment, not change of place.

ON THE FLAGELLATION OF LYMPHOCYTES IN THE PRESENCE OF EXCITANTS BOTH ARTIFICIAL AND CANCEROUS.

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PRELIMINARY NOTICE.

In a recent paper¹ we have described how the plasmata of patients suffering from carcinoma will excite amoeboid movements in the leucocytes of healthy persons. In another paper,² which will be published shortly, one of us has shown how leucocytes can also be excited by placing a drop of blood on a film of agar jelly containing a mixture of methylene blue and atropine. In yet another paper³ experiments are detailed which show how this atropine excitant can be so modified as to reduce its poisonous effects, and also that by omitting the agar jelly it can be prepared in a liquid form which enables it, still remaining effective, to be mixed with blood. In this last paper several formulæ for making the excitant are given, and among them is one prepared from the following solutions:

1. A volume of Unna's polychrome methylene blue (Grübler) is diluted with an equal volume of water.

2. A neutral solution containing 4.5 per cent. sodium citrate, 1.5 per cent. sodium chloride, and 0.225 per cent. atropine sulphate.

3. A 5 per cent. solution of bicarbonate of sodium.

Of these solutions mix together in a test tube 1 c.cm. of the diluted stain, 4 c.cm. of the citrated solution, 1 c.cm. of water, and 0.2 c.cm. of the sodium bicarbonate solution.

Into a capillary tube draw up a volume of this mixture, and add to it an equal volume of the blood to be examined, freshly drawn from the finger. Incubate the tube at 37° C. for half an hour, and then place a drop of the mixture on to a slide, and cover with a cover-glass, which may be gently pressed down if the blood will not spread. The slide should be examined at a temperature of 20° C.

The granules, but not the nuclei, of the leucocytes are well stained. The polymorphonuclear cells can usually be distinguished by the arrangement of the granules which outline the many-lobed nucleus. The eosinophiles of course have large granules which stain well.

In from a quarter to half an hour these cells will extrude long snake-like pseudopodia identical with those seen when agar jelly is employed, because they have absorbed the excitant with the stain while they were in the incubator.

In the first paper to which reference has been made,¹ we have shown that when a cancer patient's blood is mixed with an equal volume of a certain solution of sodium

citrate in a capillary tube, and its plasma, obtained by centrifugalization, mixed with one-eighth of its volume of a healthy person's blood, and then incubated for half an hour, and the slides prepared and examined as above, some of the leucocytes will show the same exaggerated movements. The blood plasma of apparently healthy people occasionally causes the leucocytes of another healthy person to throw out pseudopodia, but if the effects of the cancer plasma be compared with those of the healthy, it will be seen that the movements caused by the former are exaggerated as compared with those produced by the latter. We are therefore of opinion that the plasma of cancer patients contains some excitant for leucocytes.

In the present paper we desire to direct attention to the behaviour of the lymphocytes of healthy people in the presence of the excitant, whether it be cancerous or that composed of methylene blue and atropine. If the slide, prepared according to the stain-containing formula which has just been given, be examined, the small mononuclears will readily be recognized by their size, which is much less than that of the other leucocytes. By this method of *in-vitro* staining (for the granules of the colourless cells become stained) the lymphocytes present rather unusual characters. Most of the textbooks state that they are either non-granular or only contain very small granules, and in fixed films when the cells are killed this usually appears to be the case. When, however,

Sketch of two flagellated lymphocytes and one polymorphonuclear leucocyte, showing exaggerated amoebic movements.

they are living and resting in the stain-containing excitant, it will be seen that the cytoplasm of the small mononuclear cells contains a few deeply-stained granules arranged round the nucleus. These granules may be, and generally are, larger than the granules of the polymorphonuclear cells, though slightly smaller than those of the eosinophiles, and they are further differentiated by the very bright way in which they stain, so that they appear of a brilliant scarlet colour compared with the granules of other cells. It will be noted also that the granules will appear to collect in masses in the cytoplasm round the nucleus.

If the specimen is passed rapidly under the objective (preferably $\frac{1}{2}$ in.), and all the leucocytes examined in turn, suddenly the eye will be arrested by a larger cell which also contains the same sort of bright scarlet granules as are seen in the small mononuclears. We believe these cells to be the large mononuclears or large lymphocytes. Their granules are larger than those of the polymorphs, and slightly smaller than those of the eosinophiles, but they are fewer in number and usually more discrete, and may be collected in masses, as in the case of the smaller cells. By careful observation the outline of the single nucleus may be made out, for it will not be stained, the cells being alive. If the nuclei are stained the cells are dead,⁴ and the excitant has been wrongly prepared. The specimen should be searched and all the lymphocytes carefully examined, the other cells, although they will probably be showing exaggerated movements, being neglected.

We will now describe how these lymphocytes may become flagellated, though it must not be supposed that

flagellation always occurs, for sometimes we see it in every specimen and at other times numerous preparations may be examined without its being observed although the same exciting solution is employed.

Suppose the specimen has been prepared with the methylene-blue formula and the slide is being carefully searched for cells large and small (for lymphocytes of both sizes may become flagellated), which have bright discrete granules, suddenly one meets with a cell, which may or may not be extruding pseudopodia, probably not, and which appears to have a granule which is dancing about, as though by Brownian movement, just outside its cell wall. A close scrutiny will show that the granule is attached to the cell by a thin, hair-like filament. Sometimes this flagellation begins immediately after the slide has been prepared, at other times it may take an hour before it begins, and, as we have stated, it may not occur at all. The cell should be watched and it may be seen that the flagellum is slowly extruded until it may become longer than the width of the cell. Occasionally cells will be found with several flagella extruded, all waving about in the surrounding plasma. We have seen a cell almost surrounded by twenty such flagella. They nearly always have a dot or granule at the distal end and may have others at intervals in the length of the filament. On one occasion we have seen these flagella exhibiting what appeared to be lashing movements. They must not be mistaken for the pseudopodia thrown out by the cells in the presence of the excitant; the pseudopodia, which are much thicker, may continue to be slowly extruded and retracted for some hours, and they generally contain no granules. The flagella, unlike pseudopodia so far as our experience goes, never become retracted, but usually break off in a short time. They must not be mistaken for fibrin, which is much thicker than a flagellum, and it must be observed that if the excitant is correctly prepared, no fibrin will be present as coagulation is prevented. They are quite characteristic and we do not think they will be mistaken once they are seen.

Up to the present we have only seen flagellation occur in those cells, large and small, which have bright staining granules, and as we shall show later, we are of opinion that they are lymphocytes, though when we first saw them we thought they were a variety of eosinophile owing to the size of their granules.

The phenomenon of flagellation was seen by one of us more than a year ago, when he had made the excitant in liquid form, but it was not until more than six months later that one of us suggested that there might be a similar excitant in cancer. The reasons for this suggestion have been fully given in a former paper, together with the steps taken to demonstrate it. The flagellation-producing properties of the blood plasma in cases of carcinoma were observed in one of the early cases which we investigated (a case of cancer of the pylorus). In this case, as in the others, eight volumes of the citrated plasma of the patient were mixed with one volume of the blood of one of us, and having incubated the mixture in a capillary tube and made the slide in the usual way, we found that some of the lymphocytes had become flagellated in precisely the same manner as had so often been seen when the artificial methylene blue excitant was used, though in this experiment there was neither methylene blue nor atropine present.

Since this case we have found that the plasmata of several cancer patients have caused healthy people's lymphocytes to become flagellated, but we have not succeeded in demonstrating this phenomenon in every cancer case. On the other hand, we have never yet seen it occur with the plasma of persons other than cancer patients unless artificial excitant was added. In stating this it must be remembered that we have not seen flagellation in many cases, and we do not wish to be dogmatic, for it is quite possible that we may yet find it produced by the plasma of other persons.

We wish to take this opportunity of pointing out that we have recently found excited movements in the leucocytes of healthy persons produced by the plasmata of persons who are not apparently suffering from cancer, though the movements so produced are not so marked as those caused by cancer plasma, and the two can be differentiated. We are experimenting with a modification of the citrate solution with which we mix the blood, as we

find that a 3 per cent. solution of sodium citrate sometimes itself excites the production of short pseudopodia. By this means we hope to get a better contrast between cancer and other plasmata. It will be necessary to make a large number of experiments before we can satisfy ourselves that other persons' plasmata never excite amoeboid movements to the same extent as we have found to be characteristic of those produced by the cancer plasmata, or cause lymphocytes to become flagellated.

The next question is: Is flagellation a living phenomenon or is it a *post-mortem* effect? We consider that it must be a living phenomenon, because not only can the extrusion be watched, but we have also seen a flagellated cell extruding and retracting long snake-like pseudopodia. Moreover, the cover-glass has been carefully removed from a slide in which flagellated cells were seen, and was then dropped on to an agar film which contained excitant, when all the cells immediately showed excited movements even if they were not doing it in the first slide.

We have stated that the cells with bright staining granules or masses in the cytoplasm, which appear to be the only ones which become flagellated, are the mononuclear leucocytes or lymphocytes. The identity of the small lymphocytes is conclusive owing to their small size, and in order to determine the class of the larger cell with brightly-stained granules in the cytoplasm we succeeded in staining their nuclei and so clearing up their nature by the following experiment:

A specimen was made with artificial excitant, as described in the early part of this paper. Some flagellated cells were seen, and the appearance of the brightly stained granules noted. An agar film was prepared which contained a sufficiency of stain and alkali to stain the nuclei of lymphocytes at 37° C. in ten minutes.⁴ The cover-glass was carefully removed from the first slide, and without allowing the adherent leucocytes to dry, was dropped on to the agar film. A field was found which contained a cell with brightly stained granules. The slide was then incubated at 37° C. for ten minutes. Since the agar film contained sufficient stain and alkali to stain the nuclei in ten minutes, it was found on removal from the incubator and examination of the same field that the cells which had brightly-stained granules contained large single nuclei, which showed them to be lymphocytes. This experiment has been repeated many times.

Up to the present, therefore, we feel justified in stating that it is only the lymphocytes which present brightly staining masses or granules in their cytoplasm, and that it is only these cells which become flagellated.*

There is yet another reason which shows that these cells are the ones commonly known as lymphocytes; we mean the almost invariable presence in them of centrosomes. If blood is placed on a jelly which contains sufficient stain and alkali, and is kept at such a temperature as to cause the granules of the leucocytes to stain deeply, every lymphocyte will be seen to contain one, two, or three brightly stained small round or oval rings lying in the cytoplasm. (These have been illustrated in a former paper.) We presume that these bodies are centrosomes, but as far as can be seen the lymphocyte is the only leucocyte which contains them. They can always be seen on the jelly which excites amoeboid movement in leucocytes. They may be small rings or ovals, and sometimes appear thickened on one side like a signet ring, or they may be semilunar or horse-shoe shaped. They are always present, and can be readily found with a $\frac{1}{2}$ in. objective.

Professor Ronald Ross, who invented the jelly method of *in vitro* staining, has frequently seen these rings. He thought that they might be what are commonly known as nucleoli because they appeared to be within the nucleus. When the cell is spread out between the jelly and the cover-glass, as the nucleus is so large the centrosomes will generally appear as though they were within the nucleus, whereas in reality they are situated outside it, that is, above or below it. One of us who has seen them a large number of times also thought they were nucleoli until he saw them lying in the cytoplasm beyond the edge of the nucleus. Moreover, they become stained long before the nucleus,

and cells will live and be very active with their centrosomes stained, but die if the nuclei are stained. The centrosomes must not be mistaken for "red spots,"⁵ which begin as small points, and attain a large size, whereas a centrosome (if such it be) is always the same size, and never appears to alter. A red spot is homogeneously coloured, whereas a centrosome is ring-shaped, and only its circumference will colour. As stated above, we have never seen a similar centrosome in any blood cell except a lymphocyte, and if the cells with brightly staining masses or granules in their cytoplasm are placed on stained agar, they will generally exhibit one, two, or three of these rings.

We wish to take this opportunity of stating that, although we have examined a very large number of fresh blood cells by this *in vitro* method of staining, we have never yet seen any sign of a mitotic phase in a cell, though of course we have only examined blood from the peripheral circulation.

At this stage we have no right to speculate as to the nature of these flagella; but perhaps we may be permitted to suggest an hypothesis which we have discussed with several of our friends who have seen this phenomenon. Anyone repeating the technique for demonstrating the excitant for leucocytes in cancer plasma is practically certain, sooner or later, to come across these flagellated cells, so that the advancement of a theory as to their nature, although it may be based on few facts, may not be amiss. The flagella usually separate off and progress through the plasma nodule first. Whether this progression is due to a living movement or to streams in the plasma is difficult to determine. Sometimes they look very much as if they had the power of movement. They can easily be seen to break off, and can be followed if the microscope has a mechanical stage. They usually either stick to a red cell or get lost among them.

About a year ago a lymphocyte which had become flagellated owing to artificial excitant was shown to Dr. E. H. Ross, of the Egyptian Public Health Department, Port Said, and he remarked upon the resemblance which it presented—though a possible association between flagellation and cancer was then quite unthought of—to the gametocyte of malaria. When he saw the separation he also suggested that the flagella were spermatozoa.

Now this is a suggestion which may be of the greatest importance, an importance which is greatly increased if flagellation is produced by a possible excitant in the plasma of persons suffering from carcinoma. We are aware, and Dr. E. H. Ross was also aware, of certain cytological difficulties in the way of the suggestion. But these difficulties are by no means insuperable, and do not render the suggestion impossible. In this paper we shall not go into these cytological problems, as our original intention was merely to record the facts as seen by us. Yet we will draw attention to the possibility of the brightly staining masses and granules in the cytoplasm being chromatin, and to the dot or nodule at the ends of the flagella being composed of one of these granules. Hermann, quoted by Walker,⁶ has described chromatin bodies in the cytoplasm of cells, deriving them from the nucleus, and it has been suggested that this may be the extrusion of the male element. If the flagella are spermatozoa, and if they are produced by some excitant in the plasma of cancer patients, they may play an important part in the causation of the disease. It is impossible to say what cells may be fertilized by these spermatozoa, if they are such, and we do not propose to speculate further.

In conclusion, we may add that we have not yet been able to fix a flagellated cell on to a slide, because the filaments break off on being handled. If a specimen containing cells with brightly staining granules in the cytoplasm is fixed on to a slide and restained, the brightly staining granules and masses seem to have disappeared in the process of fixation.

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* Dr. Stevens has informed one of us that he has in the tropics seen red cells apparently become flagellated, but he has never seen a leucocyte appear like the one he saw in our laboratory. This is referred to in the *Practical Study of Malaria and Other Blood Parasites*, second edition, by Dr. Stevens.