INVESTIGATIONS INTO THE CAUSE OF MICROFILARIAL PERIODICITY

BY

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In 1879 Manson described in man the phenomenon of the periodicity of the larvae of the nematode parasite *Filaria nocturna* (*Wuchereria bancrofti*). He found that the larvae, or microfilariae, were largely absent from the blood of the host during the day, but in the evening they appeared in the blood in increasing numbers, reaching a maximum about midnight, and gradually decreasing again till morning. It is now known that in a single host—namely, man—there are species of filarial infection the microfilariae of which exhibit three types of periodicity. The first type was that described by Manson, in which the microfilariae show nocturnal periodicity; secondly, there is the complete reverse of this in *Loa loa* infections, in which the microfilariae have a diurnal periodicity; and, finally, there is the non-periodic variety, *W. bancrofti* var. *pacifica*, in which the microfilariae are present in the blood stream throughout the 24 hours with no regular fluctuation in their numbers. The cause of these phenomena is still unknown, but important observations have been made. McKenzie (1881) showed that the cycle is orientated to the waking and sleeping habits of the host; reversal of these produces reversal of the microfilarial periodicity. Manson (1899) made a post-mortem examination of a patient who died during the day from cyanide poisoning, and found that most of the microfilariae were in the lungs and great vessels at this time.

Hawking and Thurston (1951) took biopsies of the lungs during the day and during the night from filaria-infected monkeys and dogs having nocturnally periodic microfilariae, and showed that the increased number of microfilariae in the lungs during the day would account for the decrease in numbers in the blood during the same period. It is now clear that the periodic appearance of microfilariae in the peripheral blood is due to their migration backwards and forwards between this site and the small vessels of the lungs, and these migrations are stimulated by some obscure cyclic change or changes within the host. (Incidentally, although it is always assumed that the mechanism responsible for the periodicity in *Loa loa* is the same as that for *W. bancrofti*, very few investigations in support of this assumption have ever been carried out. Further, nothing is known of the distribution of the microfilariae of *L. loa* in the body; it is significant that while the periodicity of the microfilariae of *W. bancrofti* is reversed within a few days by reversal of the host's sleeping habits, Low and O'Driscoll (1921) failed to reverse the periodicity in a patient infected with *L. loa*, and the only record of a successful attempt is that by Külz (1914), who recorded that travelling round the globe for two months changed the peak of the microfilarial tide by nine hours.)

Factors Influencing the Microfilarial Cycle

The problem which now presents itself is to find a cyclic change within the host which can be shown to influence the microfilarial cycle. Many factors have been investigated in the past, from darkness to the inhalation of turpentine spray, but a significant influence has rarely been demon-

strated. Pyrexia can upset the cycle, and considerable numbers of microfilariae may be found in the blood during the day in *W. bancrofti* infections (Manson, 1882); but this finding is not supported by the present experiments. Sodium pentobarbitone injected intravenously increases the numbers of microfilariae in the blood to a varying degree (Hawking and Thurston, 1951).

Since the microfilariae of nocturnally periodic species are in the lungs during the day, much of the present investigation deals with the effects of varying the gas concentrations breathed by the host. According to the literature there is an increase in the partial pressure of carbon dioxide in the alveoli in the early morning (Bass and Herr, 1922), and during sleep there is an increase in the carbon dioxide in the blood (Endres, 1923) and a decrease in the oxygen saturation (Lundsgaard, 1918). Furthermore, a cycle with timing very like the microfilarial cycle is exhibited by the amount of phosphorus in the urine. According to Kleitman (1925): (1) Greater quantities of phosphorus are excreted per hour during sleep than during waking, independent of the volume of the urine. (2) On different dietary regimes and during fasting most persons excreted the greatest amount of phosphorus during the first half of the night. (3) The rate of phosphorus excretion during the day is greater in the afternoon than in the morning and there is a definite curve in some cases, with the excretion falling to its lowest point around noon. (4) The total acid in the urine does not always follow the phosphorus closely, but there appears to be some relationship.

Campbell and Webster (1922) reversed the normal routines of waking and sleeping for four days. At the end of that time the urinary cycle of the amounts of urine excreted between day and night remained the same, but there was an absolute increase in the phosphorus excreted during the day, so that more phosphorus was excreted then than during the night. They could dissociate the level of phosphorus excretion from every factor (including acidity of the urine) except sleep (confirmed by Kleitman, 1923).

Materials and Methods

The present investigations were carried out on African monkeys (*Cercopithecus aethiops johnstoni*) infected with a filarial nematode as yet undescribed. The adult worms live in the fascial planes of the hamstring muscles and give birth to unsheathed microfilariae which exhibit a nocturnal periodicity very similar to that of *W. bancrofti* (Hawking and Thurston, 1951).

To count the microfilariae, two or more quantities of 20 c.mm. of blood were taken from the ear and spread evenly on a slide, dried, dehaemoglobinized, fixed with methyl alcohol, and stained with Mayer's haemalum. All the counts were performed by one person, and the results are recorded as the average number of microfilariae per 20 c.mm. The monkeys were exposed to the various gas concentrations in a metal tank measuring approximately 3 by 2 by 2 ft. (91×61×61 cm.), with a lid fitting into a water moat, thus rendering it airtight. The gas under investigation was introduced through a hole at one side of the tank, and an outlet at the other side allowed the mixed gases to escape. A water manometer was attached to ensure that the pressure within the tank did not rise above normal air pressure.

Results

Increased Oxygen Concentration

A standard set of conditions was evolved in order to make the experiments reproducible. An unanaesthetized monkey in a cage was placed in the tank and oxygen administered at a rate of 4 litres a minute. Samples of the atmosphere within the tank were taken every five minutes for 30 minutes, and the concentration of oxygen was estimated with a Beckman oxygen analyser. At the end of 30 minutes the oxygen concentration had risen to 60%. Blood samples were taken from the ear before the

animal was put in the tank and after the lid was taken off at the end of 30 minutes. Three monkeys with filariasis were investigated in this way, each monkey being placed in the tank for 30 minutes around midday, when there are fewest microfilariae in the peripheral blood. Examples of the changes in the number of microfilariae in the blood are as follows (their blood at 10 p.m. contained 150–200 microfilariae per 20 c.mm.):

Monkey 185.—The number of microfilariae in the blood rose from 8 to 161 per 20 c.mm.

Monkey 187.—The number of microfilariae in the blood rose from 14 to 232 per 20 c.mm.

Monkey 192.—The number of microfilariae in the blood rose from 7 to 29 per 20 c.mm.

Detailed results of microfilariae counts on monkey 185 are given in Fig. 1.

To eliminate the possibility that some factor other than the increase in oxygen concentration might have been responsible for the increase in the number of microfilariae in the blood, control experiments were performed in which monkey 185

was put in the tank for 30 minutes and air administered at 4 litres a minute. It was then removed from the tank for blood samples to be taken, and later exposed to oxygen under the standard conditions. No increase occurred in the number of microfilariae after the monkey had been in the tank exposed to air, but there was a great increase after its exposure to oxygen.

Reduced Oxygen Concentration

Lowered concentrations of oxygen were produced in a similar manner to that in which increased oxygen concentrations were obtained, by introducing nitrogen into the tank at a rate of 4 litres a minute as described above. A standard set of readings of the oxygen concentrations was

FIG. 1.—Monkey 185. Showing the effect of increased oxygen concentration on the number of microfilariae in the capillary blood.

FIG. 2.—Monkey 185. Showing the effect of decreased oxygen concentration on the number of microfilariae in the capillary blood.

obtained with the Beckman oxygen analyser. It was found that a monkey could easily withstand this treatment for 30 minutes, with yawning as the only sign of anoxia. After 40 minutes the animal vomited and tended to faint. Thus again 30 minutes was selected as the time of exposure during which the oxygen concentration fell to 10%. Examples of the results obtained with the same three monkeys are as follows:

Monkey 185.—The number of microfilariae in the blood rose from 1 to 109 per 20 c.mm.

Monkey 187.—The number of microfilariae in the blood rose from 13 to 102 per 20 c.mm.

Monkey 192.—The number of microfilariae in the blood rose from 2 to 42 per 20 c.mm.

Details of the results obtained with monkey 185 are recorded in Fig. 2, which shows a much more rapid fall

in the number of microfilariae in the blood on re-exposure to the air than was recorded on re-exposure to the air after increased oxygen concentration. In the case of monkey 187 there was some delay in collecting the blood samples when it was removed from the atmosphere of low oxygen concentration; and this delay may account for the smaller rise in the microfilarial count on this occasion compared with that

FIG. 3.—Monkey 187. Showing the effect of increased oxygen concentration on the number of microfilariae in capillary blood and tail blood.

of the previous experiment, when the monkey had been exposed to high oxygen concentration.

In order to obtain blood samples while the oxygen concentrations were changing, further experiments were performed in which a monkey anaesthetized with intravenous sodium pentobarbitone was put in the tank with its tail through a hole in the side. To eliminate so far as possible the effects of the anaesthetic which might confuse the picture, the monkey was left for approximately an hour after it was anaesthetized; when this was done the number of microfilariae in the blood, which had increased with the anaesthetic, fell again to a reasonably steady level and so permitted the start of the experiment.

Blood obtained from the tail is usually arterial, and, as was expected, samples taken while the microfilariae were being liberated from the lungs contained greater numbers than capillary blood.

The result of one such experiment is depicted in Fig. 3. When the concentration of oxygen was 38% the count started to rise, and it reached its maximum when the concentration of oxygen was 70%. Fig. 4 shows the result of a similar experiment in which the oxygen tension was lowered. At a concentration of 17% oxygen the count had

FIG. 4.—Monkey 187. Showing the effect of decreased oxygen concentration on the number of microfilariae in capillary blood and tail blood.

not risen, but at a concentration of 13% it had risen by 200 per 20 c.mm. The last blood sample from the tail, just before bringing the monkey out of the tank, had 516 microfilariae per 20 c.mm.; at this point the blood was very blue. The next sample was taken four minutes later, after the blood had become oxygenated; the tail blood then contained only 176 microfilariae per 20 c.mm. This shows a very rapid removal of the microfilariae from the peripheral blood. The capillary blood contained approximately the number present before the oxygen tension was lowered, because the second blood sample was not taken until the blood was oxygenated.

Increased Concentration of Carbon Dioxide

For safety purposes these experiments were not performed in the tank. A monkey was anaesthetized with intravenous sodium pentobarbitone and left for approximately one hour to allow the first liberation of microfilariae from the lungs to settle. Then pure carbon dioxide was administered by a tube placed near the monkey's face. The concentration of carbon dioxide was not measured accurately, but the dosage was estimated by the increase in the respiratory rate, which was raised from 16 to 30–40 a minute for half an hour. This was repeated a number of times, with no significant alteration in the number of microfilariae in the blood.

Hyperventilation

Again a monkey was anaesthetized with intravenous sodium pentobarbitone, and a tracheal tube was inserted. After one hour hyperventilation—that is, decreased concentration of carbon dioxide in the alveolar air—was begun, using a pump which delivered 250 c.cm. of air 44 times a minute. The hyperventilation was continued for a period of one hour; on disconnecting the pump there was apnoea for two and a half minutes. Blood samples at intervals revealed no significant change in the number of microfilariae present.

Night Experiments

All the experiments described above were performed during the daytime, so as to study factors which might liberate the microfilariae from the lungs, since it is simpler to investigate the problem from this aspect than to investigate the factors which might remove the microfilariae from the blood at night-time. However, the experiments were repeated shortly before midnight, at an hour when the microfilariae were gradually increasing in number. Exposure to carbon dioxide had no effect upon the number of microfilariae in the blood, while exposure to increased or decreased oxygen concentrations caused an increase in their number just as they did in the daytime.

Possible Role of the Phosphate Cycle

It was shown above, in the review of the literature, that there is a 24-hour cycle in the excretion of phosphates in the urine which closely resembles the cycle of nocturnally periodic microfilariae. Moreover, the urinary phosphates parallel the blood phosphates; and certain changes in gas concentrations influence phosphate levels—namely, (1) increased concentration of carbon dioxide in the lungs increases the concentration of phosphates in the blood and urine; (2) hyperventilation decreases the concentration of phosphates in the blood and urine (Haldane, Wigglesworth, and Woodrow, 1924). Thus, if the phosphate and microfilarial cycles were directly related, increased carbon dioxide should increase the number of microfilariae during the day and it has been shown that this does not happen. Campbell and Webster (1922) showed that 35 to 40% oxygen concentration did not influence the urinary phosphates. To determine whether 60% oxygen influenced the acid-soluble phosphorus of the blood six uninfected monkeys were in turn placed in the tank and oxygen was administered in the standard manner. Samples of venous blood were taken before and after the animals were put in the tank and the concentration of acid-soluble phosphorus in the plasma was estimated. No significant changes were detected. Monkey

192 was transfused with a total of 621 mg. of phosphorus (94 mg. per kg.) in the form of neutral phosphates as described by Addis *et al.* (1925). This quantity was more than adequate to produce a change in the phosphorus blood level, but no change was produced in the number of microfilariae in the blood. From these findings there seems to be no direct relation between the phosphate cycle and the microfilarial cycle.

Effect of Vasodilatation

General vasodilatation can readily be produced by the injection of hexamethonium iodide. This was done in two filaria-infected monkeys, both of which were given slightly less than 1 mg. per kg. body weight intravenously. No significant change was recorded in the number of microfilariae in the blood.

Effect of Varying the Temperature, Pulse Rate, and Respiratory Rate

In a series of experiments the temperature of a monkey with filariasis was made to vary over the range of 92.3 to 104.6° F. (33.5 to 40.3° C.) (rectal) without producing any significant change in the number of microfilariae in the blood. Similarly, the pulse rate was made to vary from 88 to 180 a minute and the respiratory rate from 16 to 36 a minute by the administration of carbon dioxide, again without materially influencing the number of microfilariae in the blood. After one of the monkeys (192) had taken energetic exercise the blood was found to contain considerably more than the usual number of microfilariae. The monkey was then put into the tank exposed to air, and the number of microfilariae in the blood fell to the low figure usual at midday. Apparently the energetic exercise had promoted the migration of microfilariae into the peripheral blood.

Discussion

The characteristic cycle of microfilariae has been shown by Hawking and Thurston to be due to their migration into the vessels of the lungs during the daytime and into the peripheral blood at night. It has been shown above that both increased and decreased oxygen concentration repeatedly liberated the microfilariae into the peripheral blood during the day (in numbers comparable to the night counts in two out of three cases, with variable increases in the third case), while variation of the concentration of carbon dioxide had no significant effect.

One is unable to correlate these experimental findings with the changes in gas concentration known to occur in man in the waking and sleeping states. Further, of the many factors in the body which undergo cyclic changes, only the cycle of phosphate excretion closely parallels the microfilarial cycle, but it has been shown above that the two cycles are not directly related to one another. Further experimental investigations of the known cycles in man may reveal that one or more of them resemble the microfilarial cycle more closely than is thought at present, especially regarding a change occurring in the evening and reaching a maximum around midnight. It is more than likely, however, that in man there is a major cycle governing the changes in the waking and sleeping states. Further investigations of the periodicity of microfilariae may not only supply the solution to an interesting phenomenon but may also assist in the investigation of the still obscure phenomenon of sleep.

Summary

Investigations have been made into the effect of the variation of the concentration of inspired gases on the periodicity of nocturnally periodic microfilariae in monkeys.

Increase in the oxygen concentration to above 38% or decrease below 13%, both at atmospheric pressure, produced great increases in the number of microfilariae in the blood in the daytime, when the number is

normally low; the same stimuli also produced a small increase when applied at night, when the number of microfilariae in the blood is normally high. The mechanism of production of these effects is unknown.

Increase in the concentration of carbon dioxide in the alveolar air had no significant effect on the microfilarial count during either day or night.

Vasodilatation produced by the intravenous injection of hexamethonium iodide had no significant effect on the microfilarial count, but severe exercise in one monkey produced an increase during the daytime.

Variation of temperature, pulse rate, and respiratory rate had no effect.

There is much resemblance between the cyclic changes in the concentration of phosphates in the blood and urine (as recorded in the literature) and those in the number of microfilariae in the blood; but no direct relationship between the two could be demonstrated experimentally.

ADDENDUM.—Since this paper was written Stanbury and Thomson (1951) have shown that in the diurnal cycle of excretion of sodium, potassium, chloride, and bicarbonate ions, a maximum was reached at midday and a minimum during sleep, and in some cases the fall began in the afternoon. There were, however, no parallel changes in the plasma levels of these substances during the 24 hours.

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The first meeting took place recently of a working party set up in Scotland to consider a series of reports issued by the Advisory Council on Education in Scotland on education for handicapped children. These reports cover the needs of children suffering from handicaps in sight, hearing, and speech, physical and mental impairment, and maladjustment, as well as those whose deprivation lies more in the social field. The working party, under the chairmanship of Dr. John Jardine, will study these reports and see how best to produce a co-ordinated plan on a national basis, and how education, training, and care can be brought to every handicapped child no matter where he is situated. The reports of the Advisory Council show that the provision to meet the needs of handicapped children is far from adequate, and a great deal remains to be done, unfortunately at a time when resources of all kinds are depleted. Priorities will therefore have to be determined. The working party is anxious to hear of the results of recent inquiries and investigations bearing upon any aspect of its work. All relevant information of this kind will be received most gratefully by Mr. James B. Frizell, secretary to the working party, at the City Education Department, 12, St. Giles Street, Edinburgh, 1.

EFFECT OF HYPOCHROMASIA ON THE SPECIFIC GRAVITY AND SEDIMENTATION OF RED CELLS*

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As the red blood cells and haemoglobin content of the blood can be measured by relatively accurate methods, it would seem unquestionable that any variations of these should neither be measured by such a relatively inaccurate technique as the sedimentation rate nor be allowed to become a second active factor influencing the result that this test sets out to provide. The sedimentation rate is used normally as an indirect measure of change in the proportions or total quantity of the plasma proteins, especially of a rise in plasma fibrinogen or globulins; these plasma protein changes occur in pregnancy and in many diseases. The alteration in the plasma proteins is reflected by changes in the suspension stability of the red cells. Movement of the patient's red cells, suspended in his own plasma, is observed and recorded, and the red cells are the only indicators in the test. In no other laboratory experiment would the indicator be allowed to vary and provide a different result, unless the answer was corrected by the factor appropriate to the change in the indicator. There would appear to be no valid reason why an exception to this generalization on indicators should be made in the test known as the sedimentation rate.

Correction Charts

There are still conflicting opinions on the use of the correction charts employed in this test. One of the most recent criticisms is that of Terry (1950), who suggested that the small abnormal cells of hypochromic anaemia resist rouleaux formation, or that the plasma of anaemia is deficient in rouleaux-forming properties. The mean corpuscular haemoglobin concentration (M.C.H.C.) values of the bloods he examined were extremely low. His Tables I and III showed 38 bloods with M.C.H.C. values between 16 and 23%, and 10 between 24 and 29%.

Other workers have demonstrated that the blood from cases of "chronic anaemia" (hypochromic anaemia) sediments with abnormal slowness, and that the use of accepted correction charts provides abnormally low corrected rates.

Schuster (1938) remarked that certain cases of hypochromic anaemia had a slow sedimentation rate and the diminution of cells had but little accelerating effect. Gregg (1937) produced artificial anaemia in rabbits and observed that repeated bleedings, resulting in a "chronic anaemia," gave this slowing of the sedimentation rate as distinct from acute anaemias, which caused an acceleration proportional to the degree of anaemia. Smith (1938) noted that the blood in nutritional anaemia sedimented very slowly and did not "correct" either by Rourke and Ernstene's (1930) or by Wintrobe's (1946) chart. MacFarlane and O'Brien (1946) stated that in certain cases of chronic anaemia the sedimentation rate was comparatively slow despite the low packed cell volume, and that there appeared to be a natural compensation for the tendency of anaemic bloods to sediment quickly. Wintrobe and Landsberg (1935) stated that the specific gravity of the red cells had no effect on the sedimentation rate. Ham and Curtis (1938) quoted Neuberg and Snapper as having found a correlation between the colour index and the sedimentation rate, but in their own experi-

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