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A Note on the Survival of the Microfilariae of *Mansonella ozzardi*

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Various studies have been made on the period of survival of microfilariae in the blood. In 1892 Gruby and Delafond, after injecting blood containing *Microfilaria repens* into two healthy dogs, discovered that these embryos

were detectable for a period of three years. ^a Fülleborn ^b repeated these experiments and found microfilariae of the same species in a dog which had received the injection two years and nine months previously.

Hinman, Faust & De Bakey ^c injected blood containing *Dirofilaria immitis* into healthy dogs; microfilariae were found to be present during a period of six weeks. These investigators calculated that only 8% of the microfilariae injected appeared in the blood after the transfusion. Lane ^d endeavoured to explain the periodicity of the microfilariae of *Wuchereria bancrofti* and assumed that they live only a few hours, this being the reason why they disappear from the peripheral blood-stream during the day and are replaced by a fresh invasion of embryos, which are found in the blood the following night. On the basis of this theory various workers continued to carry out experiments mainly by injecting blood containing microfilariae into both animals and human beings.

Murgatroyd, ^e for example, injected citrated blood containing 720 000 microfilariae of *W. bancrofti*, but was never able to detect the microfilariae in the person receiving the blood. Knott ^f injected 175 ml of blood containing 80 microfilariae of *W. bancrofti* per 20 mm³ into a healthy subject and found that the microfilariae persisted, although decreasing in number, for up to 14 days. Rao ^g studied two patients with *W. bancrofti* filariasis, from whom he removed the adult worms, which were localized in a single lymphatic group. He concluded that the life of the microfilariae of this species is approximately 70 days. Gonnert ^h injected himself with 160 ml of human blood containing microfilariae of both *Dipetalonema perstans* and *Loa loa*. The latter disappeared completely by the fourth day after the transfusion, whereas the microfilariae of *D. perstans* remained for three years.

As can be seen from the above data, the object of all these experiments using man as a subject was to study the survival of *W. bancrofti*, *Loa loa* and *D. perstans*. Since no information existed on *Mansonella ozzardi*, we carried out a series of similar experiments in order to ascertain the life-span of this non-pathogenic species; the results are described in the present note.

We selected three patients whose peripheral blood-stream contained the microfilariae of *M. ozzardi*. Patient P. M. had 64 microfilariae per 20 mm³, patient E. P., 24 microfilariae per 20 mm³, and patient E. M., 6 microfilariae per 20 mm³.

The above patients, who were natives and residents of the town of Tenabo in the State of Campeche, were transferred in November 1952 to the city of Mérida (Yucatán), where three other experimental subjects were selected. These persons, who came originally from villages in the State of Yucatán where there is no filariasis, had been examined previously to ensure that their

^a Brumpt, E. (1936) *Précis de parasitologie*, 5th ed., Paris, p. 961

^b Fülleborn, F. (1912) *Zbl. Bakt., 1. Abt. Orig.*, 66, 255

^c Hinman, E. F., Faust, E. C. & De Bakey, M. E. (1934) *Proc. Soc. exp. Biol. (N.Y.)*, 31, 1043

^d Lane, C. (1929) *Lancet*, 1, 1291

^e Murgatroyd, F. (1933) *Lancet*, 1, 610

^f Knott, J. (1935) *Trans. roy. Soc. trop. Med. Hyg.*, 29, 59

^g Rao, S. S. (1933) *Indian med. Gaz.*, 68, 3

^h Gonnert, R. (1942) *Zbl. Bakt., 1. Abt. Orig.*, 149, 75

blood was free from microfilariae. The blood type was taken into account in selecting the donors and receptors.

Subject A., 19 years of age, received 250 ml of blood (containing approximately 800 000 microfilariae of *M. ozzardi*) from patient P. M. Subject F., 18 years of age, received 100 ml of blood (approximately 120 000 filariae) from patient E. P. Subject L., 40 years of age, received 150 ml of blood (approximately 45 000 microfilariae) from patient E. M.

In the first two tests, which were carried out 3 and 10 days respectively after the transfusion, 40 mm³ of blood were taken from each patient. The tests were positive only in the case of subject A., a microfilaria being detected on both occasions. We then used Knott's concentration method, carrying out the test with 1 ml of blood during the first six months and increasing the volume of blood examined until a 10-ml sample was taken during the final tests (see the following tabulated data).

Date	Volume of blood examined (ml)	Number of microfilariae found		
		subject A.	subject F.	subject L.
22.11.52	1	80	3	5
7.12.52	1	82	6	5
2. 1.53	1	84	7	4
1. 2.53	1	76	4	4
27. 2.53	1	80	6	—
7. 4.53	1	70	2	—
12. 5.53	1	74	3	—
15. 6.53	1	71	3	—
12. 8.53	2	—	3	—
17. 9.53	2	—	2	—
6.11.53	2	28	3	2
10.11.53	2	30	—	—
2. 1.54	2	26	3	1
1. 3.54	2	18	2	0
4. 5.54	2	19	2	0
7. 7.54	2	14	1	0
11.10.54	2	6	2	0
15.11.54	3	(died)	3	3
10. 1.55	3		2	1
12. 2.55	3		1	0
14. 3.55	3		0	0
12. 4.55	5		1	0
16. 5.55	5		0	0
17. 6.55	5		1	0
16. 5.55	5		0	0
17. 6.55	5		1	0
20. 7.55	5		0	0
11. 8.55	10		1	0
22. 8.55	10		0	0
12. 9.55	10		0	0
26. 9.55	10		0	0
14.10.55	10		0	0
15.10.55	10		0	0

Subject A. died of bacillary dysentery two years after the transfusion. In the first six months he showed about 80 microfilariae per ml—some

50% of the number that should have been present in view of the number of microfilariae injected.

In subject F. a decrease of 25% was evident even in the first tests made after the transfusion. On an average, only 6 microfilariae per ml were found, whereas theoretically there should have been 24. Microfilariae were present in this patient for two years and nine months after the transfusion.

Subject L. showed a reduction of approximately 50%, since, instead of the 9 microfilariae per ml which should have been present, an average of only 5 was found in the first four tests. Microfilariae were present for a period of two years and two months after the transfusion.

Each of these subjects weighed about 50 kg; they had a weak constitution and their blood volume, although assumed to be five litres, may have been somewhat less. This, coupled with the fact that the number of microfilariae in the blood of the donors was ascertained by taking an average of two samples of 20 mm³ each, whereas in the case of the persons receiving the transfusions Knott's concentration method had to be employed, may possibly explain why the reduction in the number of microfilariae was not as great as that obtained by other investigators using different filariae. In their experiments the number of microfilariae detected after the transfusion was approximately 10% of the number injected. On the other hand, it is possible that *M. ozzardi* microfilariae are more resistant than those of other species. This point needs to be clarified by means of fresh experiments, in which the embryo count in both donors and receptors would be made by the concentration method. The greater vitality displayed by these microfilariae may explain the less pronounced decrease which we obtained.

The period of survival of the microfilariae appeared to bear some relation to the number injected, as can be seen in the case of subjects F. and L. It may be assumed that if subject A., who showed a larger number of microfilariae than the other two patients, had survived, he would have retained the organisms in his blood for a longer time.

No reaction following transfusion was observed.

* * *

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Observations on Filariasis in Sarawak and Brunei

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In 1952 and 1953, the Sarawak Malaria Pilot Project carried out a general malaria survey of Sarawak and Brunei, during which a considerable amount of information was collected regarding the geographical distribution of filariasis in both territories. The observations made in 1953 and