EXPERIMENTAL BILHARZIASIS IN ANIMALS

VII.* EFFECT OF A LOW-PROTEIN DIET ON BILHARZIASIS IN WHITE MICE

BOTHA DE MEILLON and SHIRLEY PATERSON

Bilharzia Natural History Unit, South African Council for Scientific and Industrial Research and South African Institute for Medical Research, Johannesburg

For some time we have been interested in the possibility that a severer type of pathology can be induced by repeated reinfection or low protein intake. If this were so it would be of some importance to the Union because with the recent expansion and intensification of irrigation works in *S. mansoni* areas repeated reinfection in labourers would have to be reckoned with. Secondly, it is generally accepted that the diet of a proportion of the Bantu is low in protein. The effects of repeated reinfection have already been dealt with[1] and it was shown that in mice, at least, repeated reinfection does not produce a graver pathology but quite the reverse, namely a certain degree of immunity.

The studies on the effect of low protein intake reported here were completed some time ago but we did not recognize their significance at first as we were led to believe that the low percentage of egg passers in our experimental mice was due to poor infecting material. It was only after learning of unpublished work in America that the true significance of our results became evident. The American workers concluded that the diets of their experimental hosts had actually interfered with the reproductive development of the worms, an effect which we never suspected because we were looking for graver pathology and not for crippled schistosomes! In view of this unpublished information we re-examined our material and found that our mice on a low protein diet were not passing many ova because these were being held up in the tissues in the gut wall by a granulomatous reaction and hence were not appearing in the faeces. Further, the male worms at least, were not normal. In other words, our results confirmed the finding that host diet may have a rather drastic effect on the worms. In view of this we have withheld publication until after the appearance of the American work.[2]

The discovery that host diet has an influence on schistosomes is of more than passing interest. It means that a new technique for the study of the requirements of these worms is now available. This, plus promising *in vitro* studies,[3] might, amongst other things, eventually lead to a more rational approach to the synthesis of effective therapeutic substances.

TECHNIQUE

100 pairs of white mice, at the age of approximately 4 weeks, of a standard strain from the South African Institute for Medical Research were mated, 50 pairs receiving intraperi-

* Articles I-VI in this series appeared in the *South African Medical Journal*, 1952, **26**, 1005; 1953, **27**, 950; 1956, **30**, 79; 1956, **30**, 611; and 1957, **31**, 68 and 281.

TABLE I. COMPOSITION OF NORMAL AND LOW-PROTEIN BISCUITS GIVEN TO THE EXPERIMENTAL MICE

	Percentage protein in each constituent	Parts per 1,000 by weight	
		Normal mouse biscuits	Low-protein mouse biscuits
Fish meal	63	74·7	37·0
Monkey-nut meal	43	91·3	45·2
Carcase meal	40	93·0	46·0
Lucerne meal	17	10·0	4·9
Wheaten bran	14	66·4	32·9
Sussex ground oats	12	132·9	65·7
Mealie meal	10	446·9	221·2
Husk meal	4	—	493·2
Molasses	3	64·8	32·1
Fermavite	12	10·0	9·9
Salt	—	5·0	4·9
Di-calcium phosphate	—	—	2·5
Vitamin A (30,000 I.U. per g.)	—	0·08	0·09
Limestone	—	5·0	4·9
Percentage protein in whole	20·2		12·8

toneally 50 cercariae per mouse of an Egyptian strain of *Schistosoma mansoni*. On the same day these mice and 50 uninfected pairs were placed on a diet low in protein. The compositions of this diet and the normal comproid biscuits are given in Table I.

The mouse boxes, each containing a pair of mice, were examined daily, except on Sundays, for deaths. When the mice died, the gut wall was examined for ova and the adult worms were removed from the blood vessels of the liver and mesentery and preserved for comparison with worms from mice fed on a normal diet. The young born to each pair were noted and the size and weights of each litter were recorded.

The mice were weighed at mating and at weekly intervals therafter. The weight increase of the males was calculated at 9 and at 17 weeks after mating and compared.

The performance of mice uninfected, and infected with cercariae, on normal diet is taken from de Meillon and Paterson.[4]

Where results were analysed statistically, two populations were taken to be significantly different for a factor if the difference between the means was greater than 3 times the sum of the standard errors of the mean, except for assessment of the death rate where the X^2 test was used.

RESULTS

It was found that the low-protein diet, except in very few cases, caused sterility in mice whether infected with bilharzia or not (Table II).

TABLE II. REPRODUCTION OF INFECTED AND UNINFECTED MICE ON NORMAL AND LOW-PROTEIN DIETS UP TO 9 WEEKS AFTER MATING

	Normal diet		Low-protein diet	
	Uninfected	50 cercariae	Uninfected	50 cercariae
Number of litters	57	49	3	1
Number of young born	425	378	12	3
Average weight at birth	1·4 g.	1·3 g.	1·0 g.	0·7 g.
Number of young weaned (21 days)	403	298	9	3
Average weight at weaning	10·5 g.	7·9 g.	5·6 g.	3·3 g.

The mean weight increase in the protein-deficient mice was extremely low and very significantly different from infected and uninfected mice fed on a normal diet. The difference in mean weight increase between the infected and uninfected mice on the deficient diet was not significant (Table III).

TABLE III. MEAN WEIGHT INCREASES IN GRAMMES, OF FOUR GROUPS OF MICE, AND DIFFERENCES IN WEIGHT INCREASES BETWEEN GROUPS

Group of mice, and groups compared	Mean weight increase: From infection to 9 weeks later	From infection to 17 weeks later
Normal diet		
Uninfected (*a*)	15·5	17·5
50 cercariae (*b*)	12·6	15·2
Low-protein diet		
Uninfected (*c*)	1·6	2·0
50 cercariae (*d*)	2·6	0·6

Differential treatment	Differences in mean weight increase between groups: 9 weeks later	17 weeks later
(a)-(b) infection	2·9	2·3
(a)-(c) diet	13·9*	15·5*
(a)-(d) infection+diet	12·9*	16·9*
(b)-(c) diet—infection	11·0*	13·2*
(b)-(d) diet	10·0*	14·6*
(c)-(d) infection	−1·0	1·4

*= Significant differences.

Mortality amongst the protein-deficient mice was found to be much higher in the infected mice, the differences being significant after 9 weeks and increasingly more so after 17 weeks and 7 months. In uninfected mice protein deficiency resulted in significantly higher mortality than in mice on a normal diet from 9 weeks onwards. For infected mice protein deficiency increases the mortality rate significantly after 9 and 17 weeks (table IV).

TABLE IV. MORTALITY: NUMBERS OF MICE DEAD WITHIN GIVEN PERIODS AFTER MATING, AND SIGNIFICANCE OF DIFFERENCES BETWEEN GROUPS

Groups of mice, and groups compared	Percentage dead after: 9 weeks	17 weeks	30 weeks
Normal diet,			
uninfected (*a*)	0	1	—
50 cercariae (*b*)	1	23	—
Low protein diet,			
uninfected (*c*)	11	25	48
50 cercariae (*d*)	22	46	89

Differential treatment	For distribution of numbers dead and alive in 2×2 tables: 9 weeks	17 weeks	7 months
(*a*)-(*b*) infection	(*a*)	20·881†	not observed
(*a*)-(*c*) diet	9·620†	23·386†	
(*a*)-(*d*) infection+diet	22·523†	53·845†	
(*b*)-(*c*) diet—infection	7·181†	0·027	
(*b*)-(*d*) diet	19·651†	10·709†	
(*c*)-(*d*) infection	3·629*(*b*)	8·735†	12·222†

* significant at 5% level.
† significant at 1% level and beyond.
(*a*) cannot be applied here, but the difference is obviously insignificant.
(*b*) one-tailed only.
Yates' correction for continuity was applied throughout.

From the measurements of the adult worms it was found that the differences in length between the worms from the mice on the low-protein diet and those from mice on a normal

diet were significant in the males but not in the females (Table V). Adult worms from the protein-deficient mice were

TABLE V. LENGTH OF ADULT WORMS RECOVERED FROM MICE ON NORMAL AND LOW-PROTEIN DIETS

	Males	*Females*
Worms from mice on low-protein diet	3·0 mm. (71)*	4·9 mm. (25)
Worms from mice on normal diet	5·0 mm. (50)*	6·7 mm. (48)

In brackets number of worms measured. *=Significantly different.

sent to Dr. Gönnert of Farbenfabriken, Bayer, Germany, for examination. His impression was that the worms were comparatively small and in many cases the reproductive organs were smaller or relatively undeveloped. But his opinion was that the sample of material sent was too meagre for definite conclusions. Since then Dr. Gönnert has found similar lesions in a second batch of worms, from experimental mice, sent to him by us.

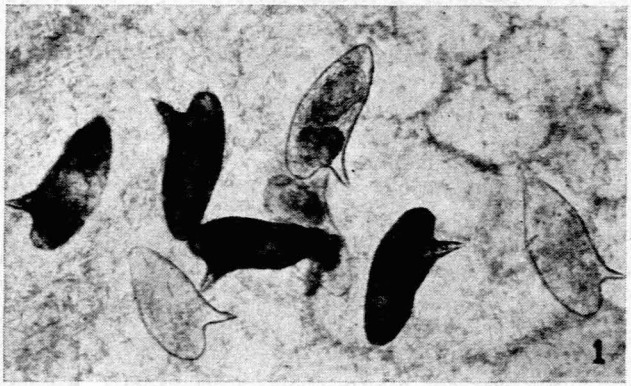

Fig. 1. Normal mouse: preparation of fresh gut wall showing dead and living eggs and the empty shells of hatched eggs. Note the absence of granulomata.

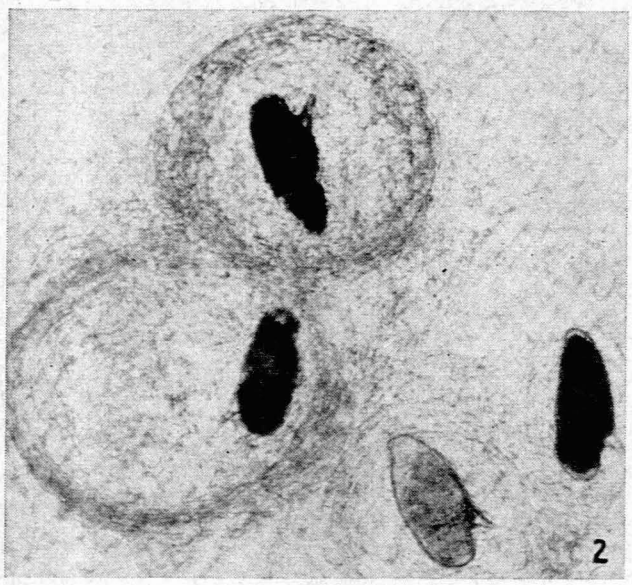

Fig. 2. Mouse on low protein diet: preparation of fresh gut wall showing dead eggs all in granulomata.

The mice on the low-protein diet passed less ova in the faeces than mice on normal food infected with the same number of cercariae. From examination of the gut wall of such mice it was noticed that in protein-deficient mice the ova were frequently incorporated in granulomata, whereas in mice on a normal diet they were usually lying free in the tissue (Figs. 1 and 2). It is of course highly probable that owing to the imperfect development of the worms less eggs were actually laid.

From these results it is shown that in the mice on the deficient diet the bilharzia infection resulted in no difference in reproduction and weight increase but had effect only on mortality. The small number of ova observed in the faeces, the difference in size of the male worms and the relative immaturity of some of the worms indicate that low protein intake may inhibit the full development of the schistosome. Certainly the action on the surviving mice was not more drastic as a result of the protein deficiency.

SUMMARY

1. The effects of an Egyptian strain of *S. mansoni* on mice kept on normal and low-protein diets were studied.

2. A low-protein diet causes infected mice to die earlier than uninfected ones. There is no difference between the rate of reproduction or weight gain of these two groups.

3. Evidence is presented that male worms recovered from mice on low-protein are smaller and it appears that their reproductive organs are abnormal as compared with worms from normal mice.

4. Mice on a low-protein diet pass less eggs in the faeces than mice on a normal diet. This may be because, being fertilized by imperfectly developed males, the females actually lay less eggs, and/or because the eggs resulting from such a union are abnormal and more readily stimulate a granulomatous reaction.

5. The relatively greater frequency and efficiency of absorption of eggs by mice on low-protein diet and hence their failure to appear in the faeces is more likely related to disturbances produced in the reproductive organs of the worms, with the consequent deposition of abnormal eggs, rather than an enhancement of defence mechanisms in the host produced by the low-protein diet.

We are very grateful to Dr. R. Gönnert, of Farbenfabriken, Bayer, Germany, for the trouble he has taken in examining our material. Our thanks are also due to Dr. H. Reuning, of the National Institute for Personnel Research, Council for Scientific and Industrial Research, for advice on the statistical procedures followed, to Mr. E. van Manen of Lion Bridge Milling Co. Ltd., Pretoria, for information regarding the composition of the diets, and to Miss T. Marcus and Miss V. Williams for technical assistance.

REFERENCES

1. Lurie, H. I. and de Meillon, B. (1957): S. Afr. Med. J., 31, 68.
2. de Witt, W. B. (1957): J. Parasit. 43, 119.
3. Senft, A. W. and Weller, T. H. (1956): Proc. Soc. Exp. Biol. (N.Y.), 93, 16.
4. de Meillon, B. and Paterson, S. (1957): S. Afr. Med. J., 31, 281.