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SELECTION OF A STRAIN OF ALBINO MICE  
REFRACTORY TO P. BERGHEI INFECTION

by

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1. Introduction

Studies over the past 60 years on the epidemiology of malaria in holoendemic areas of the world produced a number of reasoned assumptions or generally well-founded conjectures that in tropical Africa a degree of acquired humoral immunity to malaria is transmitted from the mother to her baby. The earliest and clearest reference to this will be found in the report of Stephens & Christophers (1900) on malaria in West Africa submitted to the Malaria Committee of the Royal Society. A review of the subject was prepared by Bruce-Chwatt (1961).

The presence of such congenital immunity was recently confirmed in a series of investigations which showed the relationship of the postulated antibodies to the high content of 7S gamma-globulin in the serum of inhabitants of West Africa (McGregor, 1960; Cohen, McGregor & Carrington, 1961; Gilles, 1961). Much promising work on the detection and quantitative estimation of such more or less specific antibodies present in the blood of West African populations has recently been carried out by Voller & Bray (1962) and Bray (1962).

While some features of the natural history of malaria in holoendemic areas can be explained by the presence of humoral antibodies, either transmitted to the offspring from the immune mother or acquired as a result of infection, there are aspects of malaria immunity which suggest the presence of a genetic factor. It has often been

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reported that Negroes show a higher innate degree of tolerance of some species of malaria parasites (Boyd & Stratman-Thomas, 1933). The subject of the genetic constitution of the host in relation to its susceptibility to malaria infection has been discussed by James & Ciuca (1938), Taliaferro (1949), Culbertson (1951), Sargent (1959), Swellengrebel (1950) and Simon (1960).

It is admitted that within a single species, individuals of one race or breed are sometimes more resistant to a given parasite than are individuals of other races or breeds. This difference has often been "explained" on the basis of a difference of genetic constitution of the host. And yet all immunity, whether acquired or not, is an expression of the genetic set-up of the animal conditioned by a particular environment in which the infectious disease occurs. It is virtually impossible to classify some immune responses as innate, and others as acquired, and one must admit the absence of a clear borderline.

There is in nature a constant process of selection operating against the highly susceptible host and the gradual formation of breeds of animals with increased tolerance of infective agents. This has been the crucial point of Theobald Smith's approach to the concept of infectious disease as a biological phenomenon tending to establish some sort of equilibrium between the parasite and the host.

The influence of genetic factors on bacterial immunity was experimentally studied by many authors, fully analysed by Bradford Hill (1934) and lucidly assessed by Wilson (1955). It is not surprising that owing to inherent difficulties in the study of immunity to protozoa generally and malaria parasites in particular much less work has been done in this field as compared to studies on immunity to bacteria and viruses.

Induced malaria of man provided some evidence of the immunological happenings while genetic studies on simian malaria are obviously difficult. Detailed experimental study of genetic aspects of immunity to malaria in mammals became possible as a result of the discovery of rodent plasmodia, viz. P. berghei and P. vinckei. Much work on various aspects of immunity to rodent malaria has been carried out during the past 15 years, but the attempts to produce by selection a strain of rodents with a high tolerance to a virulent parasite have been few (Kretschmar, 1962). The comparison of Kretschmar's (1961, 1962) work with our results will be mentioned in the later part of this report.

The present note gives an account of two such attempts - an early and not very successful one carried out in Nigeria and a later one, much more satisfactory, carried out in India.

The first trial was completed in 1957 at the Federal Malaria Service, Yaba-Lagos, Nigeria. Four generations of Swiss albino mice were raised from two parent couples who had been infected intraperitoneally with 50 000 parasites of a virulent strain of P. berghei and cured by chloroquine given at the mean total dose of 0.5 g. base per animal. The progeny of the first parents underwent at the age of approximately two months the same infection, followed by treatment, and the surviving mice of each generation were interbred. In the course of this experiment about 20% of each adult generation died and there was a high frequency of abortions or premature deaths of the litter. The total number of mice that survived and interbred through four generations was 86. The final, fifth, generation was composed of eight animals - five females and three males. These were given the "standard dose" of 50 000 parasites without subsequent treatment and all of them died. Nevertheless, the average survival time of this last lot was  $10.9 \pm 1.3$  days, as compared with  $6.3 \pm 0.3$  days of the nearly 1200 mice on whom the P. berghei strain was previously maintained for three years in the course of 182 subinoculations to groups of six mice.

Much more interesting results on the study of inheritance of immunity in mammals were obtained in the course of work carried out at the Malaria Institute of India<sup>1</sup> during the past four years. The investigation to determine the possibility of selecting a strain of albino mice refractory to P. berghei infection was repeated on a larger scale. Albino mice were specifically chosen as the host in the experimental model due to the fact that thousands of mice infected in the last few years at the Institute, as well as many other places in the world, invariably developed a 100% fatal infection with P. berghei, thereby showing that mice in general are extremely susceptible to this parasite. A second point that was particularly ensured in the planning was to inoculate the mice after the weaning period. This was to ensure that the passive immunity available to suckling mice through the infected mother's milk would be eliminated.

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## 2. Material and methods

Albino mice were used as the host animals and P. berghei,<sup>1</sup> Vincke & Lips (1948), as the parasite.

The mice were originally, in 1950, obtained from the Central Research Institute, Kassauli; Indian Veterinary Research Institute, Mukteshwar; Haffkine Institute, Bombay, and the Pasteur Institute of South India, Coonoor. Since then, they have all been interbred and subsequently inbred, resulting in an inbred strain of the Institute.

Fifty mice (25 males and 25 females) about six weeks (43 days) old were used for the first series, and each animal was kept separately in a cage. Each animal was given a single dose of one million parasites intraperitoneally. Blood films were taken and examined daily and the numbers of parasites per 10 000 erythrocytes (rbc) were counted.

When more than 2% of the host cells were found infected, the parasitaemia was controlled by intraperitoneal administration of 0.25 mg of chloroquine phosphate per animal. The drug was repeated as and when required so that parasitaemia was not allowed to go beyond 2% of host cells. Thus, the infection was allowed to run a mild course till it became subpatent. On the infection becoming subpatent, each animal was allowed to rest for 10 days. At the end of the rest period each animal was re-inoculated with one million parasites and the procedure outlined above was repeated as many times as required until the animals became totally refractory to the inoculation of one million parasites.

The males and females which became refractory to the re-inoculation of one million parasites were mated and the pregnant females were separated from the males. The first filial generation was weaned after four weeks and each animal was inoculated

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<sup>1</sup> The strain of P. berghei was obtained through the courtesy of Brigadier J. S. K. Boyd in 1952 and is being maintained in the albino rats by blood passage. The parasite had undergone 310 passages when used for the present experiment.

with 0.1 million parasites intraperitoneally on the twenty-ninth day after birth. The animals were examined and treated in the same way as the parent animals until some of them became refractory to the reinoculum of one million parasites. The animals refractory to the challenge dose were kept for mating and the procedure outlined for the parent generation was repeated for all subsequent generations.

It was intended to repeat the process for every filial generation until a stage was reached when it was possible to select males and females which were entirely refractory to the first inoculation of 0.1 million parasites.

The experiment was continued up to the sixth generation, after which it had to be abandoned as a large number of the refractory animals of the sixth generation failed to produce young ones. The number of animals in the seventh generation was not adequate for the continuation of the experiment.

### 3. Results

Table 1 shows the course of infection in different generations of mice.

TABLE 1. THE COURSE OF P. BERGHEI INFECTION IN SEVEN GENERATIONS OF MICE

Generation and number of mice in each	Time (in days) in which the animals became refractory to challenge infection		Number of doses of 0.25 mg chloroquine phosphate base administered		Duration (in days) of pre-patent period of the first infection	Duration (in days) of primary parasitaemia
	Range	Average	Range	Average		
0=Parent 50	69-138	105.6	2-21	8.8	1.2	92.5
1st 27	62-130	90.8	2-12	7.4	4.7	62.4
2nd 38	30-130	88.0	3-12	5.8	3.8	53.3
3rd 35	33-138	97.1	3-14	7.3	4.97	67.8
4th 29	30-127	73.2	2- 9	5.6	4.7	38.3
5th 37	36-117	85.2	5-14	7.5	4.9	52.2
6th 65	37-112	61.0	4-11	5.8	6.2	31.8
7th 4	82-107	83.5	1- 5	2.7	5.7	26.5

The preceding table shows the difference between the prepatent period of 1.2 days in the parent generation (where the first inoculation was one million parasites) and the 3.8 to 6.2 days in subsequent generations where the infection was carried out using one tenth of the above dose.

The primary parasitaemia lasted on an average 92.5 days in the case of the parent generation, whereas it showed a decreasing trend from 67.8 days to 31.8 days. Thus the period of primary parasitaemia in the sixth filial generation was significantly reduced in comparison with the previous five generations.

The proportion of mice in each generation refractory to the initial infection is shown in Table 2.

TABLE 2. PERCENTAGE OF MICE REFRACTORY TO INITIAL INFECTION IN SIX SUBSEQUENT GENERATIONS

Generation	Number of animals	Number of animals refractory to initial infection of 0.1 million parasites.	Percentage of animals refractory to initial infection of 0.1 million parasites
0=Parent	50	1*	2
1st	23	1	4.3
2nd	38	nil	-
3rd	34	nil	-
4th	29	nil	-
5th	37	nil	-
6th	63	12	19
7th	4	1	25

\* This animal was refractory to one million parasites.

This table indicates that only in the sixth generation was there an obvious increase of the proportion of refractory animals. The results in the last generation are not significant on account of the low numbers of mice inoculated.

#### 4. Discussion

The present results seem to indicate that in six generations of mice, comprising a total of 274 animals, there was evidence of a trend towards a gradual decrease of the susceptibility of each generation, particularly obvious in the shortening of the duration of primary parasitaemia and the lengthening (to a lesser extent) of the prepatent period. The sudden increase of the proportion of animals refractory to the infective dose of parasites seen in the sixth generation is suggestive.

It is a matter for regret that it was not possible to continue the experiment beyond the sixth generation as we were left with very few animals. Why most of the animals failed to produce further progeny is not clear. The present results (however preliminary) indicate that a gradual selection of a strain of mice less susceptible to an otherwise virulent infection with P. berghei is not impossible. Further work on larger numbers of animals is required to prove this working hypothesis and has been started.

On the other hand, it must be stressed that our results are different from those obtained by Kretschmar (1961), who worked with mice of the NMRI strain from Tübingen, which show a degree of innate resistance to P. berghei infections uniformly fatal to all other strains of mice. P. berghei infection in a high proportion of mice of this strain lasts for weeks or months seemingly without any pronounced effects on the individual (Kretschmar, 1962). According to this author, the low susceptibility of mice of the NMRI strain depends on the "genetic constitution" of the strain and is not further decreased by mating the survivors after the experimental infection and testing their progeny. It should be pointed out, however, that there are no reports of inbreeding of a susceptible strain and repeated subsequent passages of P. berghei infections. Nevertheless, Kretschmar (1962) agrees that the relative immunity of the NMRI strain to P. berghei is of a genetic character not confined to selected individuals but typical of the strain as such.

Although the present results of our work are not conclusive, they are not greatly different from a series of investigations on the inheritance of resistance to bacterial infections summarized by Hill (1934). Concluding his analysis, the latter author emphasized that even in large experimental samples there is considerable variation between strains so that the assessment of the genetic advantage is difficult. Within strains selective breeding from survivors leads generally to a decreased death-rate in the following generations, but this may be due to a combined action of genetic factors and parental acquired immunity transmitted to the progeny.

Wilson & Miles (1955) pointed out that in testing for resistance of each generation one invariably alters the innate response of the animal and this influences the progeny still further. This is unavoidable, however, and does not invalidate the conclusions of an experiment which tries to imitate the conditions occurring in highly endemic malarious areas. The present investigation suggests the possibility of selection of a strain of albino mice relatively refractory to an infection with P. berghei to which the original strain was highly susceptible, but much more work is needed on the assessment of the speed of the process and on the specificity of the genetic factors involved.

## 5. Summary

1. Six generations of albino mice originating from 50 parents fully susceptible to infection with P. berghei were interbred in such a way that each generation was exposed to an infection attenuated by chloroquine. The survivors were mated with the view of selecting a strain with a decreased susceptibility.
2. Twelve out of 63 animals (19%) of the sixth generation and one out of four in the seventh were able to resist the initial inoculation with 0.1 million parasites.
3. There was evidence of reduction of the period of primary parasitaemia and lengthening of the prepatent period, suggesting that some selection of animals with greater tolerance has occurred.
4. A decrease of fertility, particularly obvious in the seventh generation, limited the number of surviving animals to such an extent that the investigation came to an end.

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