Reports

Natural Occurrence of Malaria in Rhesus Monkeys

Abstract. A relatively high incidence of malarial infections has been noted in recent importations of rhesus monkeys from Pakistan. This finding, contraverting the widely held belief that rhesus monkeys are free of natively acquired malaria, is of substantial significance to those employing Pakistanian rhesus monkeys in their investigations.

This laboratory has pursued studies on the biology and therapy of primate malarias since 1945. Between this date and July 1960, some 5600 rhesus monkeys (*Macaca mulatta*) have been used in these investigations without evidence of the natural occurrence of malaria in any subject. In July 1960, one animal imported in December 1959 was found to be infected. With unwavering conviction that rhesus monkeys are not natively infected, we attributed the disease in this subject to an undefined "laboratory accident." Events of the past 2 months have made it necessary to re-evaluate this conclusion.

On 19 October 1960, 100 monkeys recently imported from Pakistan were introduced into the colony for conditioning purposes. In January 1961, prior to initiation of a malaria experiment, thick blood films were prepared on 79 of these animals, stained with Giemsa, and examined for the presence of blood parasites—a routine practice despite years of negative results. To our amazement, seven of these stock animals had positive thick films. This finding led immediately to examination of the animals of three later shipments, also imported from Pakistan. This resulted in findings of positive films for 13 of 65 animals imported on 23 November 1960, for one of 55 of a 15 December shipment, and for one of 29 in a 22 December shipment. In all, 22 of 228 monkeys from these four shipments were found to be naturally infected. The density of infection ranged from one to two parasites per entire thick film to 2×10^7 parasites per milliliter of blood. In the majority of subjects a single plasmodium appeared to be involved. In two cases there were evidences of multiple infections.

Identification of the plasmodial species is still in progress. Data acquired to date from thin-film studies, both on monkeys with naturally acquired infections and on others in whom disease was induced through inoculation of infected blood, suggest that the parasite found in the majority of cases is the morphologic counterpart of our stock strains of *Plasmodium inui*. In the two cases suggestive of multiple infections, a plasmodium morphologically similar to *P. cynomolgi* also appears to be involved. In both natural and induced infections the disease appears to follow a rather benign course. In the induced disease the parasitemia is intensified by splenectomy.

The observations reported here (*1*) are at variance with the widely held belief that the rhesus monkey is free of malaria in its native habitat. This belief cannot be dismissed lightly, since it is supported by some 30 years of careful study by investigators who have employed the rhesus monkey in work on the biology and therapy of simian malarias. It appears likely to us that the conflict between findings in earlier investigations, in which we participated, and the current observations is related to the area from which the animals were obtained. For many years the great majority of rhesus monkeys have come from areas adjacent to Lucknow. Monkeys in recent shipments from this region, like those of shipments in former years, have been free of malaria. On the other hand, the infected animals originated in East Pakistan, having been exported from Dacca. Although there is no absolute certainty in this matter, it is possible that they were obtained near the Burma-Assam border. If this is the case, the animals could have been in contact with *Macaca cynomolgus* or *M. nemestrina*, simian subjects frequently infected with *Plasmodium knowlesi, P. cynomolgi*, or *P. inui*. It is not too difficult to believe that the same vectors which transmit these plasmodia to the crab-eating and pig-tailed macaques could also transmit them to the rhesus monkey. There is evidence in the old literature on malaria that such an event does occur. Thus, in 1911, Mathis and Leger (*2*) found quartan infections (probably *P. inui*) in five of 40 rhesus monkeys collected in Tonkin. *Plasmodium inui* infections of *M. cynomolgus* are relatively common in this area.

The importance of these observations on the natural occurrence of malaria in Pakistanian rhesus monkeys to investigators of plasmodial diseases needs no comment. It is equally apparent that workers employing rhesus monkeys in other investigations should be alert to the existence and potential liabilities of concurrent malarial infections.

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Reference and Note

1. This study was supported in part by grant No. E-2372 from the National Institute of Allergy and Infectious Diseases, Bethesda, Md.
2. C. Mathis and M. Leger, *Ann. inst. Pasteur* **25**, 593 (1911).

16 February 1961

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

Stimulus Generalization of Conditioned Suppression

Abstract. A tone ending in unavoidable electric shock was periodically presented to pigeons while they pecked a key for food. When pecking was completely disrupted by tone, shock was disconnected, and the training tone and tones having new frequencies were presented. Initially the gradient of generalization was broad; as testing proceeded, however, the gradient narrowed severely.

When a stimulus has typically preceded a noxious event, subsequent presentation of that stimulus will often cause a reduction in the frequency of ongoing responses. This reduction is referred to as conditioned suppression. In the present research we sought to investigate the degree to which conditioned suppression could be mediated by stimuli which were like, but not identical to, the stimulus that was used in the original training. This latter phenomenon, the stimulus generalization of conditioned suppression, is of particular importance, for it represents a process by which the effects of aversive training may come to encompass large segments of an organism's behavior (*1*).

Two carneaux pigeons, deprived to 80 percent of their free-feeding body weight, were taught to peck a key in the wall of the experimental chamber. This behavior was maintained by occasionally reinforcing a key peck with food (VI-2). The noxious event was electrical shock which was administered to the birds by way of a pair of permanently fastened wing bands. A coiled wire connector was clipped onto the wing bands, and the other end was fastened to a shock power terminal at the top rear of the experimental chamber. After initial training to peck the key, the birds were always run with the connector in place. With the occurrence of high stable key-pecking rates, the tone which was to be used as the suppressing stimulus and the tones to be used in assessing stimulus generalization were presented without shock. This operation permitted the effects of novel stimulation to adapt out. The tones each lasted 48 seconds and were equated for intensity (80 db spl). There were seven tones selected such that their frequencies were equally spaced along a logarithmic scale. When the bird's rate of key pecking was indifferent to the presence of any of the tones, suppression training was initiated. During suppression training only the middle frequency in the sequence was presented (1000 cy/sec), and unavoidable shock was programmed during its final 8 seconds. The tone-shock combinations were presented at irregular intervals. Since the programming of the variable interval schedule of food reinforcement was independent of that of the tone-shock pairings, food reinforcement could occur at any time during the tone and during shock. Training was continued until the key-pecking rate during the initial 40 seconds of tone was minimal and the rate during nontone periods was normal.

With the achievement of stable suppression, the use of shock was permanently discontinued, and without altering the schedule of food reinforcement the tests for stimulus generalization were conducted. The test sessions were run daily and lasted 2 hours. During a given test session each of the seven tones was presented once with the order of presentation randomized by means of Latin squares.

The magnitude of suppression during a tone may be estimated by comparing the number of responses made during the initial 40 seconds of the tone period (tone R's) to the number of responses made in the 40-second period prior to the tone onset (pre-tone R's). The relationship is expressed by the equation: suppression ratio =

$$\frac{\text{Pre-tone R's} - \text{Tone R's}}{\text{Pre-tone R's}}$$

The generalization gradients at several stages in the tests are shown for pigeons No. 5 and No. 9 in Fig. 1. Each gradient for No. 5 is the average for five sessions, while for No. 9 each gradient is averaged over four sessions. As can be seen in Fig. 1, generalization was at first broad, and as testing proceeded the gradients narrowed. In that no shock occurred during the tests, the changes in the shape of the gradient (the sharpening) during testing must reflect differences in the extinction of the suppressing capacity of the several stimuli. In general, the larger the difference in frequency between a given stimulus and that of the tone used in training (1000 cy/sec), the more rapidly did that stimulus lose its capacity to suppress ongoing behavior.

In the one previous study of the generalization of conditioned suppression (*2*), discrimination training was used to cause rats to suppress bar presses to an 1800-cy/sec tone but not to a 200-cy/sec tone. It was found that tones with frequencies that were intermediate between these extremes caused intermediate degrees of suppression. Since, however, the original training involved the acquisition of a discrimination, and

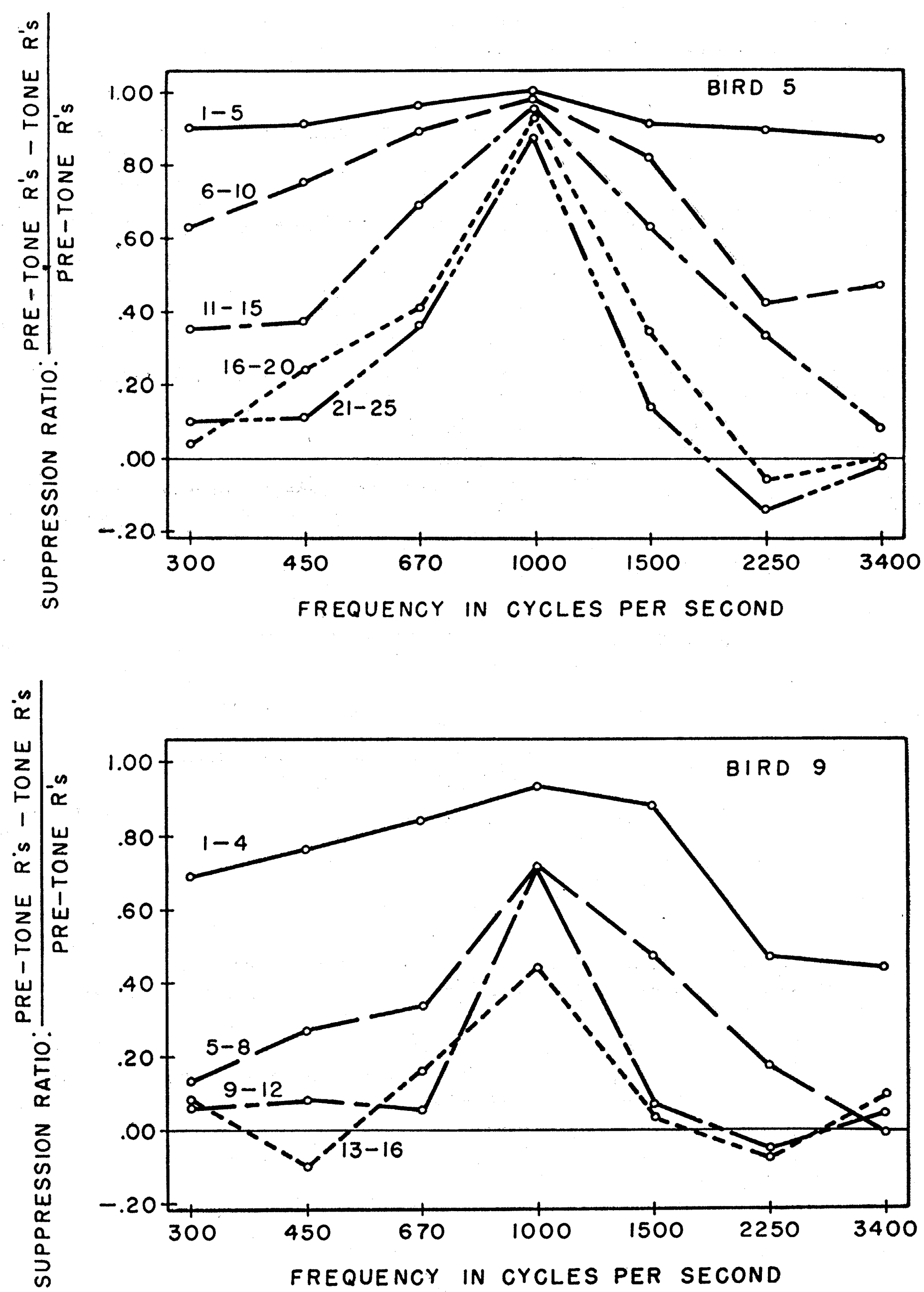

Fig. 1. Generalization gradients for birds No. 5 (top) and No. 9 (bottom). The numbers to the left of each gradient indicate the sessions included

since shock was used during the tests, the experiment provided no information about the nature of the gradient following training to a single stimulus; nor did it provide information about the changes in the shape of the gradient during the extinction of suppression.

The sharpening of the generalization gradient, during extinction, has also been reported in several studies involving the galvanic skin response (*3*), though in none were the effects as large as those herein described. It is perhaps of importance, however, that the methodology involved in the conditioned suppression paradigm and that involved in conditioning the galvanic skin response is similar. In both procedures, a noxious event is paired with a neutral stimulus.

Of the large number of other studies of stimulus generalization, only a few have reported data on changes in the gradient during extinction (*4*), and these indicate a variety of different results. At present the reasons for these differences are not clear (*5*).

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5. This research was supported by a U.S. Public Health Service grant (M-2433).

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Distribution of Strontium-90 in a 1959 Wheat Sample

Abstract. At least 22 percent of the strontium-90 found in a sample of wheat harvested in 1959 was due to direct deposition. Twenty-seven percent of the total strontium-90 content of this wheat sample was contained in the outermost bran layer.

Knowledge of the mechanism by which plants are contaminated by fallout is essential for the accurate prediction of future levels of Sr^{90} in foods. A particularly important fact that must be known is whether plants become contaminated principally from Sr^{90} that is absorbed from the soil or from Sr^{90} that results from the direct deposition of fallout on the plant surfaces. Studies presented here on the contamination of wheat may be of some help in resolving this problem.

Analyses, conducted by the Health and Safety Laboratory (*1*), of wheat and its milling products from 1958 and 1959 crops in the United States showed that the highest Sr^{90} concentration occurred in the bran fraction of wheat kernels. Similar results were reported by British investigators of the Agricultural Research Council Laboratory (*2*) for wheat grown in the United Kingdom and other countries in 1958.

The root uptake of Sr^{90} by wheat plants has been studied by C. C. Lee (*3*) in greenhouse experiments. He found that Sr^{90} absorbed from the soil is also concentrated in the bran.

Since the bran comprises the outer layers of the wheat kernel, it would be expected that much of the Sr^{90} found in this fraction is due to direct deposition. Lee's experiments indicate, however, that some of the bran contamination of field-grown wheat may result from root uptake.

Any directly deposited Sr^{90} that enters the wheat kernel must first pass through the outermost bran layer or epidermis. It is reasonable to expect, therefore, that a high proportion of the Sr^{90} in this layer is due to direct deposition. Experiments performed at the Health and Safety Laboratory on a sample of Ohio wheat harvested in 1958 showed that 25 percent of its Sr^{90} content was in the epidermis while this fraction constituted only 4 percent of the total weight of the wheat. To determine the origin of the Sr^{90} in the epidermal fraction of wheat kernels, further experiments measuring the ratio of Sr^{90} to stable strontium (specific activity) in the epidermis and in the remaining portion of wheat were conducted on a sample of Kansas wheat.

The separation of the epidermis was done by Theodore Earle (*4*) by a method, devised by him, which utilizes the friction produced in the controlled agitation of a mixture of grain and water to strip the epidermis from the wheat kernels.

Table 1 lists the results of analyses for Sr^{90}, stable strontium, and calcium performed on (i) a sample of hard red Kansas wheat, harvested in 1959, (ii) the epidermis stripped from the wheat, and (iii) the residual stripped wheat. The values tabulated are estimated to be accurate to within 10 percent.

Table 1. Distribution of strontium-90, stable strontium, and calcium in a sample of hard red Kansas wheat.

Per-cent of total	Strontium-90		Stable strontium		Calcium	
	$\mu\mu c$ in fraction	$\mu\mu c$/kg of fraction	mg in fraction	mg/kg of fraction	g in fraction	g/kg of fraction
			Original wheat kernel			
100.0	47.2	47.2	3.15	3.15	0.49	0.49
			Stripped wheat			
95.8	36.2	37.8	2.95	3.08	0.42	0.44
			Epidermis			
4.2	12.7	302.0	0.18	4.29	0.07	1.65
			Material balance			
	+1.7 (4%)		−0.02 (1%)		0.00 (0%)	

The material balances obtained indicate that the Sr^{90}, stable strontium, and calcium were not removed by the water used in the stripping operation.

Although strontium and calcium are chemically similar, they need not be physiologically identical. From data in Table 1, the ratio of stable strontium to calcium is found to be $0.18/0.07 = 2.6$ mg/g in the epidermis, while this ratio is $2.95/0.42 = 7.0$ mg/g in the stripped wheat. The difference in these ratios in indicative of dissimilar metabolic pathways for the soil-derived stable strontium and calcium. It is reasonable to conclude from these data that relatively little of the Sr^{90} found in the epidermis came from the soil.

Of the Sr^{90} found in the epidermis, that amount derived from root uptake may be estimated quantitatively by calculating the specific activity of the stripped wheat. If one assumes that the plant cannot distinguish stable strontium from Sr^{90} in the process of root absorption, the specific activity (Sr^{90} to stable strontium) of all parts of the wheat kernel should be the same. The specific activity of the stripped wheat is $36.2/2.95 = 12.3$ $\mu\mu c$ of Sr^{90} per milligram of stable strontium. This would be the specific activity of the epidermis, if there had been no direct deposition. The observed specific activity of the epidermis was $12.7/0.18 = 70.6$ $\mu\mu c$ of Sr^{90} per milligram of stable strontium; hence it appears that there must have been some direct deposition of Sr^{90}. The stable-strontium content of the epidermis was 0.18 mg; therefore, $12.3 \times 0.18 = 2.2$ $\mu\mu c$ of Sr^{90} must have resulted from root uptake. This is a maximum estimate since it has been assumed that all of the activity in the stripped wheat came from the