very low temperatures (0°–8° C.) their life may be prolonged to over two months. Both excessive moisture and excessive dryness are inimical, but a certain amount of moisture is beneficial. Darkness does not prolong their life, for those which were kept in bright daylight lived, on an average, longer than those kept in the shade or in darkness.

It was further found that fleas may remain in the larval and pupal stages for much longer periods than normal under certain circumstances. Several experiments were made to determine this point. In the first, material containing fleas was removed from a rat cage and isolated. It was observed to remain infected with fleas for a period of over five months. A second experiment continued to produce fleas for a period of eleven months. In a third experiment this period was extended to fourteen months. Some fleas in this experiment at the end of a year were placed along with a young rat, and in three months they had multiplied very considerably.

From these experiments it is evident that the larval and pupal stages of the rat flea, which normally last from two to six weeks, may be prolonged to over a year. As seen in the first set of experiments, the length of life of an adult flea under these conditions is generally under a month, and no evidence was obtained that the fleas multiplied during the period, although this may be possible; so that it may be concluded that the appearance of adult fleas after such a long period as a year was due to the larval and pupal stages being prolonged.

The importance of these observations to preventive medicine lies in the fact that as fleas are carriers of disease, it may be—and is—of considerable moment to determine exactly how long they can exist under various conditions. A full account of these observations is being prepared for publication.

THE OPSONIC INDEX IN PLAGUE VACCINATION.

By RALPH ST. JOHN BROOKS, M.B., D.P.H.,
RESEARCH SCHOLAR.

[ABSTRACT.]

THE production of immunity in rats vaccinated against plague, is correlated with the production of immune opsonin in the serum of such animals.

Various anti-bodies appear in the serum of animals inoculated with plague vaccines, but the development of agglutinins and bactericidal substances, for example, do not lend themselves to satisfactory quantitative study, in the case of pest. The opsonic response has been studied in man by Douglas,* who found a good response after inoculation with Haffkine's Prophylatic. It, therefore, seemed advisable to correlate the development of opsonin with the absolute immunity of some susceptible animal in order to see whether the amount of opsonin produced could be used as evidence of the development of immunity in man.

In most of the following experiments the results obtained with a single dose of vaccine are recorded in relation to the opsonic index; in others the results of repeated dosage are shown.

* Private communication.

The scope of the enquiry resolved itself into the following points:—

1. Is there any correlation between immunity and opsonic production in plague infection?
2. What is the influence of previous vaccination in the experimental animal?
3. What is the source of opsonin in immune plague serum?

METHODS OF EXPERIMENT.

In all cases the dilution method of Klien (1) was employed. A strain of avirulent plague bacillus killed in 50 % formalin solution was employed in the opsonic technique.

The nucleo-protein used as a vaccine was prepared in the manner described by Rowland in the *Journal of Hygiene* (2).

The bacterial emulsions were prepared from 24 hour agar slopes.

The pooled serum of ten rats were used in each experiment.

EXPERIMENTS ON RATS.

The following is a summary of the results obtained on estimating the opsonic indices of the various pooled sera from day to day (see table at foot of page).

By plotting out curves based on these figures, it will be seen that those obtained with 1/10 mg. whole bacillus vaccine, and 1/100 mg. nucleo-protein are very similar, though in the former case the maximum is displaced somewhat to the right. The doses correspond, as it has been shown that the nucleo-protein contained in the bacillus is about 1/10 the weight of the whole organism. The smallest response is elicited with the smallest dose (1/12000 mg. nucleo-protein) and the largest response with the greatest dose (1 mg.). No response is elicited with the bodies of the bacilli washed free from nucleo-protein, and used as a vaccine, or with nucleo-protein solution which had been hydrolysing for several months, though the hydrolysing process has no effect on the immunising property of the nucleo-protein.† The general conclusions from these experiments would seem to show, as found by S. Rowland, by directly determining the protection aroused in rats to a subsequent fatal dose of living pest, that the soluble nucleo-protein contained in the plague bacillus is the responsible agent in the production of opsonin. The production of opsonin as first rises with the immunity. In the first two series on the table the maximum response is reached by the first day, by which time full immunity is established with this dose of vaccine.

HUMAN EXPERIMENTS.

The dose used in each case was 1/10 mg. of hydrolysed nucleo-protein, which is about the quantity of nucleo-protein contained in 1 cc. of Haffkine's Prophylatic. The first experiment was conducted on myself (R. St. J. B.), the second on five persons who had been inoculated, like myself, some time previously with Haffkine's Prophylatic (P. I.), and the third on

† In the latter experiment the absence of opsonin is somewhat remarkable and may possibly be due to hydrolysis having gone too far, or to the carbolic added as a preservative interfering in some way with the opsonic response. It was subsequently shown that rats, inoculated with this particular vaccine, did not acquire immunity.

DOSE AND NATURE OF VACCINE.	HIGHEST DILUTION OF SERUM WHICH SHOWS A GREATER PHAGOCYTIC INDEX THAN SALINE CONTROL.											
	Before in- oculation.	10 hours.	22 hours.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.
1/100 mg. nucleo-protein ..	1/16	1/2048	1/4096	1/2048	—	1/1024	1/1024	—	1/256	on 18th	day.	
1/10 mg. whole bacillus ..	1/16	1/1024	1/4096	1/4096	1/2048	1/1024	—	1/1024	1/64	on 21st	day.	
1/12,000 mg. nucleo-protein	—	—	1/512	1/1024	1/2048	1/2048	1/1024	1/828	—	1/512	—	—
1 mg. nucleo-protein ..	—	1/4096	1/6044	1/4096	1/6044	1/2048	1/2048	—	—	1/1536	—	—
1/10 mg. whole bacillus (re- peated dose after 35 days)	—	1/4096	1/16384	1/24476	1/24476	1/16384	—	—	1/4096	—	—	—
1/10 mg. nucleo-protein (hy- drolysing for 8 months)			No	response.								
9/10 mg. of washed bodies of bacilli			No	response.								

five normal persons (P. II.) The following Table shows the highest serum dilutions which gave an index higher than normal saline.

	Highest dilution of Serum which shows a greater phagocytic index than normal saline.									
	Before Inoculation.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.	
R. St. J. B.	—	1/1024	1/768	1/1024	1/1536	1/512	—	1/512	—	
P. I.	—	1/512	1/512	1/1024	1/1024	1/512	1/512	1/256	—	
P. II.	less than 1/32	1/128	1/128	1/256	1/384	1/256	—	—	1/192	

Except in two cases in the third series, the effects, on inoculation, were very slight and in no case was any increase in pulse rate or temperature observed. In the third series the opsonic response is not as great as in series I. or II. In the case of the two groups of pooled sera the number of persons in each group is so small that one would hesitate to draw any definite conclusions from these results, but as far as they go, they tend to show that pre-existing sensibility has an influence on the opsonic index in human plague vaccination.

CONCLUSIONS.

1. The substance which produces a rise in the opsonic index in immune pest serum is the nucleo-protein contained in the bodies of the bacilli.
2. The washed bodies of the bacilli when used as a vaccine do not cause any increase in the opsonic index.
3. Within limits, the larger the dose the greater the opsonic response.
4. A repeated dose of vaccine raises the index above the maximum of the first inoculation, even after the curve has fallen to its normal level.
5. In the early stages, in rats, the opsonic response and the degree of protection aroused rise together.
6. In human beings the maximum response is observed to be much later than in the case of laboratory rats, and the response in a series of previously sensitised persons is somewhat greater than in a series of persons not previously vaccinated.
7. The local and constitutional effects of nucleo-protein vaccination compare very favourably with those observed in other methods of protective inoculation.

I desire to express my thanks to Dr. C. J. Martin, F.R.S., Director of the Lister Institute, and to Dr. J. C. G. Ledingham, Chief Bacteriologist, Lister Institute, for valuable advice and suggestions throughout the enquiry, and particularly to Dr. Sydney Rowland, of the Yersin Laboratory, Elstree, Herts, without whose constant advice and help the experiments could not have been carried out.

REFERENCES.

1. Klien: *Johns Hopkins Hosp. Bulletin*, xviii., p. 245.
2. Rowland: *Journal of Hygiene*, Vol. 10, No. 3, p. 536.

THE DESTRUCTION OF ALKALOIDS BY EMULSIONS OF THE BODY TISSUES.

By A. J. CLARK, M.B., M.R.C.P.,

BRITISH MEDICAL ASSOCIATION RESEARCH SCHOLAR.

(From the Pharmacological Laboratory, King's College, London, W.C.)

DURING recent years many workers have investigated both the natural tolerance to drugs, that occurs in certain animals, and also the tolerance that is acquired when certain drugs are administered repeatedly. We now know that the protective mechanism, which causes tolerance, differs in the case of different drugs, and that, with the same drug, tolerance may be produced by different mechanisms in different animals. This evidence has been recently reviewed by Dixon⁽¹⁾; it appears that tolerance may be established by the following mechanisms: (1) Diminished absorption. (2) Increased rapidity of excretion. (3) Increased resistance of the tissues upon which the drug acts. (4) Linkage of the drug with other bodies to form an inert substance. (5) Absorption of the drug by organs upon which it has no action. (6) Destruction of the drug by the body tissues. All of these six mechanisms, except the third, have been shown to exist in mammals, the third has been demonstrated chiefly in protozoa and may depend upon physical causes. The sixth mechanism, the destruction

of the drug by the body tissues, has been studied in various ways, both in the intact animal, in isolated surviving organs, and in emulsions made of excised organs; the present paper is concerned only with results obtained by the last method.

1. HISTORY.

Numerous investigators have examined the power of emulsions of the tissues to destroy alkaloids, and the method employed has usually been the following: An organ is excised from a freshly killed animal, and made into a fine emulsion with normal saline, a small quantity of alkaloid is added, and the mixture incubated for some time at body temperature, in the presence of oxygen; after incubation the alkaloid is extracted and estimated. Using this method Buys⁽²⁾ showed that the livers of the frog and the rabbit could destroy hyoscyamine, but that the frog's blood, and the dog's liver did not do so. Cloetta^(3 and 4) showed that the rabbit's liver could destroy atropine, he also considered that the rabbit's brain, and the cat's liver and brain could destroy atropine. Fleischmann^(5 and 6), and Metzner⁽⁷⁾ both showed that the rabbit's serum could destroy atropine, but that this power varied greatly according to the locality from which the rabbit came; they noted particularly that the serum of rabbits suffering from goitre did not destroy atropine. Fleischmann moreover showed that normal human serum did not destroy atropine, but that the serum of patients suffering from exophthalmic goitre could do so. Cloetta and Fischer⁽⁸⁾ showed that neither the dog's liver, brain, nor heart could destroy digitoxin.

Several investigators have compared the action, which emulsions of the organs of normal animals have upon a drug, with the action of emulsions of the organs of animals made tolerant to the drug. Albanese⁽⁹⁾ showed that liver emulsions from normal dogs did not destroy morphine, but that liver emulsions from dogs made tolerant to morphine could destroy considerable quantities of the drug. Dixon⁽¹⁾ showed that the liver of normal rabbits possessed a slight power of destroying nicotine, and that this power was increased in rabbits in which tolerance to the drug had been induced. Cloetta⁽⁴⁾ compared the destruction of atropine, produced by the livers of normal cats and rabbits, with that produced by the livers of cats and rabbits made tolerant to atropine; he showed that the tolerant rabbits possessed increased powers of destruction, but that there was no such increase in the tolerant cats.

2. METHOD.

The method adopted by the writer was similar to that already outlined. A freshly excised organ was made into a fine emulsion by pounding in a mortar with sand and normal saline, and then filtering through muslin: a few drops of toluol were added to prevent sepsis, a minute quantity of the alkaloid to be tested was next added, and the mixture well shaken, and then incubated for a few hours at body temperature; after incubation the alkaloid was extracted by the Staas method, and the amount recovered estimated. It was found by experiment that heating to 60°C. completely removed the power of emulsions to destroy alkaloids; control experiments were therefore made by always taking two equal portions of emulsion, and heating one portion to 60°C. for 30 minutes before adding the alkaloid; the two portions were then treated in an exactly similar manner, and the amount of alkaloid recovered from each at the end was compared. In all cases small quantities of alkaloid (usually less than 1 mgm.) were added, and destruction of the alkaloid was only considered to have been proved, when, firstly the unheated portion was found to contain either no alkaloid, or at most a trace, and secondly there was a large difference between the amount recovered from the heated and the unheated portions; in this manner errors due to the extraction and estimation of such minute amounts of alkaloid were eliminated as far as possible.

3. MODE OF DESTRUCTION OF ATROPINE BY THE TISSUES.

The livers of frogs and rabbits were used, since emulsions of these organs were known to possess the power of destroying atropine (Buys⁽²⁾). The atropine was estimated by Vitali's test; the extracted alkaloid being diluted until Vitali's test was only just positive. When 1 c.c. of fluid was taken I found that 0.003 mgm. of sulphate gave a distinct violet colour, and that 0.002 mgm. gave a very feeble violet colour; smaller quantities showed a red colour, but no violet colour. The sensitiveness of the test depends upon the fuming nitric acid used, and upon the freshness of the alcoholic solution of caustic potash. Cloetta⁽⁴⁾ found the end point with this method to lie between 0.005 and 0.01 mgm. of atropine, but the end point is so indistinct that no two observers are likely to obtain exactly similar results.