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Reagin-like Antibodies in Rats Infected with the Nematode Parasite *Nippostrongylus brasiliensis*

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Summary. The immune response to *Nippostrongylus brasiliensis* in rats is closely associated with the formation of antibodies resembling human reagins. These are detected in some rats immediately after acquiring resistance to the parasite after an initial infection and in all rats 1 week later. Further infections stimulate an anamnestic rise in 'reagin' production. 'Reagin' formation is due to the living worms, primarily the adult stage. 'Reagins' were detected by passive cutaneous anaphylaxis in the rat (homologous passive cutaneous anaphylaxis = PCA) with a 72-hour interval between intradermal injection of antibody and intravenous injection of antigen, which was a saline extract of adult worms.

'Reagin' production in rats vaccinated with worm extracts contrasts sharply with 'reagin' production in rats infected with living worms. Vaccination with worm extracts stimulates 'reagin' production in only some rats during the primary response only; after second and later vaccinations, 'reagins' were not detected in any rats. Prolonged vaccination induces the formation of 'blocking' antibodies and a further type of antibody capable of inducing 6-hour PCA, but not 72-hour PCA in rats. Even after prolonged vaccination, however, a subsequent infection stimulates an anamnestic rise in 'reagin' production in some rats. Vaccination did not induce resistance to infection in any rats.

Rats recovered from an initial infection become increasingly susceptible to anaphylactic shock when challenged with worm antigen, but the sensitivity of rats to systemic anaphylaxis is not closely correlated to the level of circulating 'reagins'. Heterologous anaphylactic reactions did not induce expulsion of worms from the intestine and anaphylaxis is not directly related to the ability of rats to resist infection.

INTRODUCTION

In a previous paper (Ogilvie, 1964b) it was shown that antibodies resembling human reagins are stimulated by living helminth infections, but vaccination with extracts of parasites does not provoke a similar immune response. The possible role of these antibodies in protective immunity to helminths is being investigated in this laboratory. The present report shows that 'reagins' are closely associated with both the primary and secondary immune response of rats to the nematode *Nippostrongylus brasiliensis* and that the stimulation of 'reagin' production is related to infection by living worms. Vaccination of rats with worm extracts stimulates 'reagin' production during the primary response only and the prolonged vaccination stimulates the formation of a blocking antibody. The latter antibody does not have the high affinity for homologous skin characteristic of 'reagins'

but it appears to react with the same antigen as the 'reagins'. One aspect of the possible role of the 'reagins' in the mechanism of host resistance to the parasite is investigated. This work has been briefly reported elsewhere (Ogilvie 1964a, b).

MATERIALS AND METHODS

Rats

Male and female albino Sprague Dawley rats bred at N.I.M.R., weight from 150 to 200 g, were used throughout. Female rats attained this weight at about 10 weeks of age, male rats at about 8–9 weeks. In these rats, a primary infection of *N. brasiliensis* terminated between the 11th and 14th day after infection as shown by egg counts, whereas an initial infection in the albino Wistar rats previously used did not terminate until the 18th to the 21st day. However, resistance to reinfection developed by Sprague Dawley rats was comparable to that previously reported for albino Wistar rats (Ogilvie, 1965a). Briefly, worms in a second infection migrating in rats previously infected by even a small number of worms pass no eggs and are expelled from the intestine between the 4th and 9th day after the second infection.

Parasite

The methods of maintaining this parasite, of infecting animals and of recovering worms, have been reported in a previous paper (Ogilvie, 1965a). Egg counts were made by a salt flotation technique using a McMaster slide (Whitlock, 1948).

Antigen

Antigen prepared from adult worms was used in all experiments. Worms were recovered from the small intestine of rats infected with 3000–4000 larvae, 7–10 days previously. These worms migrated actively from the split intestine placed on gauze covering the mouth of a large funnel containing warm water and they were collected in a tube attached to the end of the funnel. Little intestinal debris passed through the gauze with the worms, and most of this was removed by several decantations of water or saline. Preliminary experiments showed that a known number of worms can be related approximately to the volume occupied by the worms in a graduated centrifuge tube; the number of worms was therefore estimated from the volume (1·0 ml = 4000 worms). Because these worms characteristically intertwine and form large clumps it is difficult to estimate the numbers by a dilution count. The worms were crushed in a Potter–Elvehjem glass homogenizer in saline, and the supernatant obtained after centrifugation was diluted to contain approximately 1000 worm-equivalents/ml.

Estimation of antigen

Antigenicity was measured by comparing the ability of various dilutions of the antigen to induce maximal PCA reactions in a group of rats, all of which had been injected intradermally 3 days previously with serial dilutions of a serum with a maximum homologous PCA titre of 640. This serum had been taken from rats after four infections of *N. brasiliensis*. For this experiment, antigen was prepared from a suspension of worms which has been counted accurately. Each antigen dilution was tested in five rats. The results showed that 0·1 ml of antigen, i.e. 100 adult worm-equivalents, was the minimum volume of extract which gave maximum development of the PCA titre in all rats. When smaller amounts of antigen were used, the results varied from animal to animal, the maximum titre was not

reached, and the intensity of all reactions was greatly reduced. For routine homologous PCA, 0·2 ml of antigen was used.

Antisera

Rats were bled by heart puncture or by bleeding from the ophthalmic venous plexus under ether anaesthesia. The serum was stored at −20°. There were no indications of loss of PCA activity in any samples, even after repeated freezing and thawing. Titres are expressed as the reciprocals of the highest serum dilution still giving the reaction under test.

Homologous PCA

The method used for detecting the rat 'reagins' was essentially that described by Ovary (1964), except that a latent period of 72 hours was usual (Mota, 1963a). Sera were diluted serially in saline and each two-fold dilution was injected intradermally in 0·1ml amounts into the dorsal skin of shaved normal rats. Antigen mixed with 0·5 ml of a 0·5 per cent solution of Evan's blue was injected into the tail vein 3 days later. The test was read 30 minutes after antigen injection, and the rats were killed only in doubtful cases. Reading the reaction in the live animal was facilitated by wetting the skin surface. Each serum was tested in duplicate; duplicate titrations varied by no more than a two-fold dilution. The PCA titre was recorded as the reciprocal of the maximum serum dilution which gave a skin reaction. In most cases the titre was maximal by 6 hours after antibody injection, but in some animals the titre was not maximal until 2–3 days later. A preliminary experiment with a standard serum (PCA titre 640) showed there was no reduction in PCA titre when injection of antigen was delayed until 1 week after antibody injection. Thereafter the titre fell, and by day 15 the maximum titre was 10–20.

RESULTS

STABILITY OF THE ANTIGEN

Freshly prepared antigen, which gave a maximum PCA reaction in 0·1 ml amounts per rat, was divided into two aliquots and stored either at −20° or at +4° for 10 weeks without preservative. After this time, its activity in homologous PCA was reassessed, using the same serum pool and the method described above. There was no loss of activity in this test after storage either at −20° or at +4°, as 0·1 ml of the stored antigen was still capable of eliciting a maximal PCA reaction.

HETEROLOGOUS PCA

Mice and guinea-pigs were injected intradermally with a 1:10 dilution of a rat serum which gave a titre of 640 in homologous PCA. Antigen was injected with Evan's blue 6 hours or 3 days later. The test was negative in guinea-pigs throughout, but in mice a reaction could be demonstrated at 6 hours and 3 days. The PCA reaction with rat serum in mice was irregular and blotchy in appearance unlike the discrete round sometimes slightly raised spot found on the under surface of rat skin in homologous PCA.

EFFECT OF HEAT AT 56° ON REAGIN-LIKE ANTIBODIES

The homologous PCA activity of serum from rats infected with *N. brasiliensis* was diminished greatly by heating at 56°. The activity could not be restored by addition of

fresh normal serum. The time taken to destroy all PCA activity varied with the serum and appeared to be related to the initial titre. In all cases, previous heating for 30 minutes at 56° reduced the PCA titre of the sera, but some sera with titres of 640 or greater were heated for 2–3 hours at 56° before the titre was destroyed completely.

OCCURRENCE OF REAGINS AND SENSITIVITY TO SYSTEMIC ANAPHYLAXIS IN RATS AFTER A PRIMARY INFECTION WITH *N. brasiliensis*

In the following experiments, the development of resistance in rats after initial infection was measured primarily by decline in egg output, and was related to the appearance of

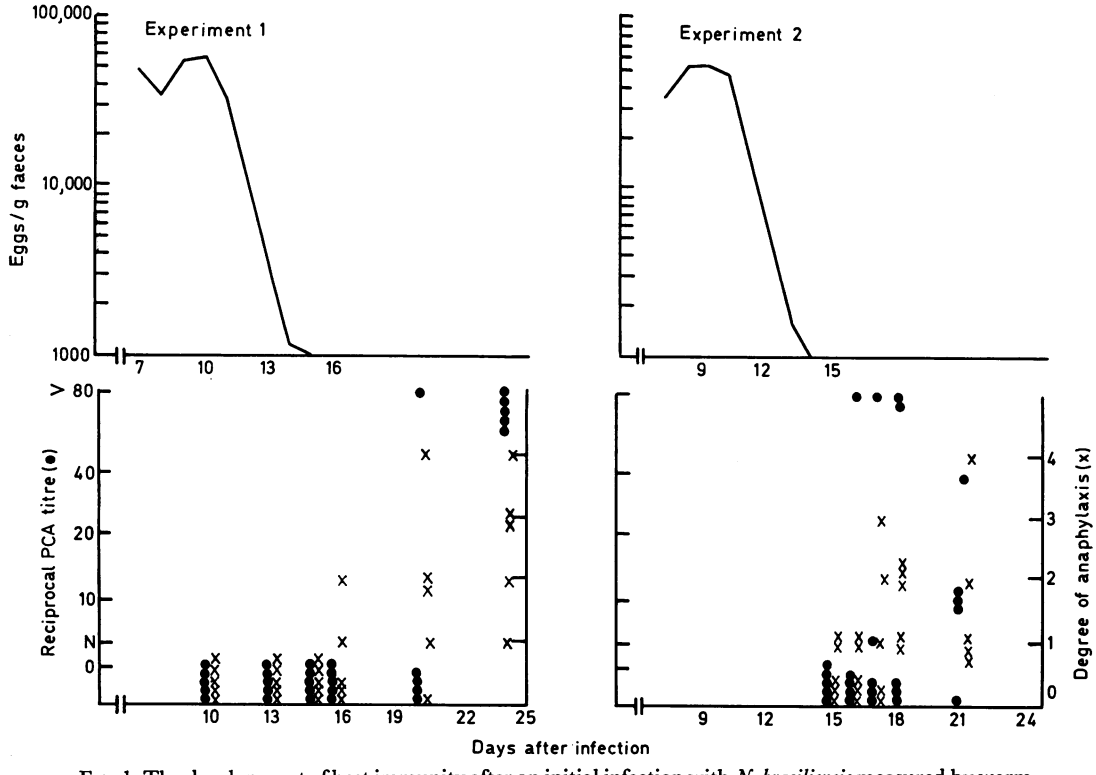

FIG. 1. The development of host immunity after an initial infection with *N. brasiliensis* measured by worm egg production, the occurrence of 'reagins' in the circulation and the degree of systemic anaphylaxis in the rats. 'Reagins' were measured by homologous PCA, anaphylaxis was classified arbitrarily when rats were killed 30 minutes after intravenous antigen injection, as follows: 0, No visible haemorrhage; 1, Haemorrhage along one-quarter or less of the intestine; 2, haemorrhage along one-half intestine; 3, haemorrhage the length of the intestine; 4, haemorrhage the length of the intestine and death within 30 minutes of antigen injection.

'reagins' in the circulation and also to the development of sensitivity to systemic anaphylactic shock induced by intravenous injection of 0·5 ml of antigen. In two experiments, rats were infected with approximately 3000 larvae and the course of the infection was followed by group faecal egg counts, which are a sensitive method for following the development of resistance (Ogilvie, 1965a) (Fig. 1). At intervals after the infection blood was taken from five rats which were then injected intravenously with antigen. The animals were watched for clinical signs of systemic anaphylaxis and after 30 minutes they were killed and their intestines were examined for gross pathological lesions. The two experiments

differed only in the time intervals chosen to examine groups of rats. The worms present in the intestines of the rats killed on day 15 and day 16 of experiment 2 were counted. On day 15 there was a mean of 439 worms; on day 16 only sixteen remained. The results of the homologous PCA test on each serum and the signs of anaphylaxis as shown by gut damage (arbitrarily classified from 0 to 4), are shown in Fig. 1. The egg counts of both experiments and the results of worm counts in the second experiment show that the rats had acquired resistance to this infection by the 16th day after infection. These worm counts, and unpublished observations, show that all rats expel an initial infection of this size by about the 16th day and that the time taken to expel worms varies only by 2–3 days between rats: there is thus a remarkable uniformity in the rate at which rats acquire resistance to this parasite. A similar uniformity in the production of circulating 'reagins'

TABLE 1

THE RELATIONSHIP BETWEEN CIRCULATING 'REAGINS', SYSTEMIC ANAPHYLAXIS AND WORM POPULATION IN RATS INFECTED ONCE OR TWICE WITH *N. brasiliensis*

Rat No.	PCA titre	Degree of anaphylaxis	Worm population
Rats infected once			
1	0	Killed	20
2	80	Killed	64
3	20	Killed	82
4	0	Killed	4
5	160	Killed	4
Rats infected twice			
6	40	Slight	73
7	20	Slight	11
8	20	Slight	7
9	10	Killed	12
10	320	Nil	104

and in the development of sensitivity to systemic anaphylaxis was not observed. Neither 'reagins' nor gross signs of anaphylaxis were detected until immediately after the infection had been terminated. If both experiments are considered together, 'reagins' were not detected until day 16 and then in only one of five rats, and thereafter in some but not all rats. Similarly, gross intestinal damage produced by systemic anaphylaxis could be detected in some animals from day 17. The number of rats sensitive to anaphylaxis and the number with detectable 'reagins' increased progressively from day 16 and both reactions were detectable in all animals 1 week after the infection had terminated. Although both 'reagins' and sensitivity to anaphylaxis occur after infection, their appearance was not closely correlated. Some rats died from systemic anaphylaxis with no detectable 'reagins' in the circulation, and some animals with a 'reagin' titre of 80 were unaffected by the attempt to induce anaphylaxis.

The relationship between 'reagins' and sensitivity to systemic anaphylaxis was next investigated by shocking rats either after an initial infection or after a second infection, by which time high levels of 'reagins' are found in all animals.

Ten rats were each infected with about 1200 larvae. Four weeks later five of these animals were each reinfected with approximately 4000 larvae. Twelve days later still, serum samples were taken and the two groups of rats, five infected once and five infected twice, were shocked and killed 90 minutes later. The results of the shock, the PCA tests on the serum and the number of worms recovered from the intestines are shown in Table 1.

All rats that had been infected only once 6 weeks prior to shock died in severe anaphylaxis within 1 hour of antigen injection although two animals had no detectable 'reagins'. On the other hand, among the rats which had been infected twice before infection only one died in anaphylaxis; the remaining four were affected hardly at all by the attempt to induce anaphylaxis although all animals had detectable 'reagins' in their serum immediately prior to shock. Clearly, there is no correlation between the occurrence of 'reagins' in the circulation, the sensitivity of rats to systemic anaphylaxis and the number of worms found in the intestine.

ANAMNESTIC PRODUCTION OF 'REAGINS' AFTER A SECOND INFECTION

Nine rats were each infected with approximately 2000 larvae. Five weeks later all the rats were bled and six rats were reinfected with approximately 3000 larvae. The remaining three rats were not infected again but served as controls for variations in 'reagin' level in the absence of a challenge infection. All the animals were bled from the heart on days 3, 6, 9, 15 and 27 after reinfection and the sera were tested by PCA (Table 2). The infected

TABLE 2

THE OCCURRENCE OF A SECONDARY RISE IN 'REAGIN' PRODUCTION IN INDIVIDUAL RATS AFTER A SECOND INFECTION WITH *N. brasiliensis* COMPARED WITH 'REAGIN' PRODUCTION IN CONTROL RATS GIVEN THE FIRST INFECTION ONLY

	PCA titres at given intervals (days) after second infection					
Rat No.	Day 0	Day 3	Day 6	Day 9	Day 15	Day 27
Infected twice						
1	10	10	2560	1280	20	160
2	0	0	2560	320	0	40
3	320	640	2560	1280	1280	160
4	0	0	320	20	80	80
5	20	80	1280	320	80	40
6	0	Undil.	320	640	10	40
Infected once only						
7	320	320	40	160	80	Dead*
8	20	10	40	20	40	20
9	20	10	80	160	20	Dead*

* Some animals died after bleeding from the heart.

rats were resistant to the second infection since egg counts were negative throughout. On the day of reinfection, six of the nine rats had detectable 'reagins' in their sera. In the absence of reinfection, the level of 'reagins' in the serum did not vary appreciably. In the reinfected rats, however, the 'reagin' titre rose sharply between the 3rd and 6th day after the second infection, but fell to a lower level by about the 15th day. From this it may be deduced that a second infection stimulated a secondary rise in 'reagin' production.

STIMULATION OF 'REAGIN' PRODUCTION BY A SMALL LARVAL INFECTION AND BY AN INFECTION CONFINED TO THE SMALL INTESTINE

The preceding experiments show that 'reagin' production occurs after one large infection with this parasite. Previous work has also shown that a small infection of larvae will stimulate host immunity and that the main source of antigens that stimulates

protective immunity is the adult worms in the small intestine (Ogilvie, 1965a). Accordingly, 'reagin' production was investigated after a small infection with third stage infective larvae and also after an infection of adult worms confined to the small intestine.

Seven rats were infected by transfer of about 600 adult worms directly into the intestine. Adult worms were recovered from the intestine of donor rats infected 8 days previously and injected directly through the wall of the duodenum exposed by laparotomy. It has been shown that infections established by this means stimulate a host immunity which is similar to the immunity resulting from a complete infection of a comparable size (Ogilvie, 1965a). A further ten rats were infected with about 100 infective larvae. Six weeks later, all rats were bled and reinfected with approximately 3000 larvae. Further serum samples were obtained on days 6, 9 and 15 after reinfection. The course of the second infection was followed daily by group faecal egg counts. No eggs were detected in the faeces of the two groups of rats previously infected either with 600 adult worms or with 100 larvae although control rats given the infection of 3000 larvae as an initial infection produced large numbers of eggs reaching 50,000 eggs/g faeces on the 10th day.

TABLE 3

'REAGIN' PRODUCTION IN SEVENTEEN RATS AFTER A SECOND INFECTION WITH ABOUT 3000 *N. brasiliensis* LARVAE

Rat No.	PCA titres of serial serum samples (days after infection)			
	0	6	9	15
Initial infection with 600 adults				
1	160	640	1280	640
2	320	640	1280	640
3	320	320	320	640
4	160	640	1280	640
5	640	640	1280	640
6	160	640	1280	160
7	640	160	1280	320
Initial infection with 100 larvae				
8	80	640	320	160
9	0	40	320	40
10	20	40	320	160
11	10	160	320	640
12	320	80	640	160
13	40	160	640	320
14	40	320	1280	320
15	40	640	1280	160
16	40	640	640	320
17	80	320	160	160

Ten rats were infected initially with about 100 larvae, seven rats with about 600 adult worms introduced directly into the small intestine.

The results of homologous PCA tests (Table 3) show that all rats in both groups had detectable 'reagins' in their sera 6 weeks after initial infection. After reinfection the level of 'reagins' rose irrespective of the type of initial infection in fourteen of the seventeen rats and in the remaining three, the same high level present before reinfection, was maintained. These results suggest that adult worms in the small intestine are the main source of stimulation of 'reagin' production as they are of immunity to reinfection (Ogilvie, 1965a), and that even a small infection stimulates production of 'reagins'.

STIMULATION OF 'REAGIN' PRODUCTION BY LIVING ADULT WORMS IN THE PERITONEAL CAVITY OF RATS

When living adult worms are introduced in to the peritoneal cavity of rats, they live for some days and stimulate some resistance to reinfection with third stage larvae, but the worms do not grow or reproduce (Chandler, 1936). In the experiment which follows, an examination was made of occurrence of 'reagin' production in rats given four intraperitoneal injections with living adult worms.

Living adult worms were injected into the peritoneal cavity of ten rats. Four injections were given, about 600 worms on day 0, 1000 on day 9, 1000 on day 14 and 600 on day 70. Twelve weeks after the beginning of the experiment these animals were bled and together with seven uninfected rats, were infected with about 700 infective larvae. The infection was followed by daily faecal egg counts (Table 4) and the rats were bled out 10 days after infection. 'Reagin' titres of the sera taken on the day of the larval infection and of the sera taken 10 days after infection are shown in Table 4.

TABLE 4

THE IMMUNE RESPONSE TO AN INFECTION OF ABOUT 700 LARVAE IN RATS PREVIOUSLY IMMUNIZED BY INJECTION OF LIVING ADULT WORMS INTRAPERITONEALLY AND IN PREVIOUSLY UNINFECTED CONTROL RATS

Days after larval infection	PCA titres in individual rats									
0	0	0	1	0	1	1	0	0	0	0
10	1	40	160	160	0	10	20	20	0	1

Days after larval infection	Group faecal egg counts (eggs/g faeces)	
	Rats previously infected with adult worms	Control rats
6	0	500
7	500	1200
8	400	4300
9	150	3300

Immune response estimated by group faecal egg counts in immunized and control rats and 'reagin' levels in the immunized group.

Three out of ten rats were producing detectable 'reagins' before the larval infection and by 10 days after this infection, the titre had increased in eight out of ten animals. A low level of 'reagin' production and a low level of resistance as shown by egg output compared with controls was induced by intraperitoneal infection with living adult worms.

EFFECT OF VACCINATION OF RATS WITH WORM ANTIGEN ON PRODUCTION OF 'REAGINS' AND RESISTANCE TO REINFECTION

Eight rats were vaccinated by intraperitoneal injection of worm antigen together with 2×10^{10} *H. pertussis* killed bacilli as adjuvant. Three injections with 0·75, 0·5 and 0·5 ml antigen respectively, of the antigen–adjuvant mixture were given on days 0, 23 and 40

of the experiment. Serum samples were taken on day 15 and day 23 after the first vaccination and on day 30, 1 week after the second vaccination. Fifty-two days from the beginning of the experiment the surviving rats together with seven control animals were infected with approximately 600 larvae. This infection was followed by group faecal egg counts on days 6, 7 and 8, and the worm population in individual rats was counted on the 8th day (Table 5).

TABLE 5

THE COURSE OF A CHALLENGE INFECTION IN VACCINATED AND CONTROL RATS MEASURED BY EGG PRODUCTION AND THE NUMBER OF WORMS RECOVERED FROM THE INTESTINE 8 DAYS AFTER THE CHALLENGE INFECTION

Days after infection	Eggs/g faeces	
	Rats vaccinated prior to infection	Controls
6	50	200
7	1100	3000
8	3600	2000

Vaccinated		Controls	
Rat No.	No. worms recovered 8 days after infection	Rat No.	No. worms recovered 8 days after infection
1	346	7	144
2	291	8	147
3	460	9	168
4	331	10	192
5	228	11	283
6	266	12	193
Mean	320 ± 73	Mean	186 ± 47

On day 15 and day 23 following the primary vaccination three out of eight and two out of eight rats had detectable serum 'reagins', but on day 30, 1 week after the second vaccination, no serum was positive in the PCA test. Nevertheless, two rats died with severe systemic anaphylaxis after the third vaccination and all rats died in severe shock when 0·3 ml antigen was injected intravenously 8 days after the infection. Vaccinated and control rats passed comparable numbers of eggs after infection but more worms were found in vaccinated rats than in controls. A similar finding occurred in a later experiment (Table 8), but the significance of these results is obscure. Nevertheless, in all vaccination experiments the number of worms recovered from vaccinated rats was never fewer than in controls.

It is clear from this experiment that vaccination of rats with worm extract mixed with *H. pertussis* adjuvant stimulates neither prolonged 'reagin' production nor resistance to a living infection. It is interesting that some rats vaccinated for the second time died in severe anaphylaxis when no circulating 'reagins' were present. Rats dying in anaphylaxis after infection may or may not have had detectable circulating 'reagins'. The production of 'reagins' by rats infected after vaccination is investigated in the next experiment.

PRODUCTION OF 'REAGINS' BY RATS VACCINATED WITH ANTIGEN BEFORE INFECTION

A group of nine rats was vaccinated by intraperitoneal injection of 0·8 ml freshly prepared antigen together with 2×10^{10} killed *H. pertussis* bacilli. Four weeks later, serum samples were taken from all rats, and six of the rats were given an infection of approximately 3000 larvae. The course of this infection in individual rats was followed by faecal egg counts beginning on day 6, the first day of patency (Table 6). The remaining three rats

TABLE 6

EGG PRODUCTION BY THE WORMS IN AN INITIAL INFECTION IN VACCINATED RATS AND THE 'REAGIN' LEVELS STIMULATED BY THIS INFECTION COMPARED WITH THE 'REAGIN' LEVEL IN UNINFECTED VACCINATED RATS

	PCA titre (days after infection)					
	0	3	6	9	15	27
Vaccinated with *H. pertussis* adjuvant and infected						
1	1	10	80	80	Dead*	
2	1	0	160	10	20	0
3	0	0	20	0	20	0
4	0	0	1	0	80	0
5	0	0	10	10	0	Dead*
6	0	0	0	0	Dead*	
Vaccinated but not infected						
7	0	0	1	0	0	0
8	0	0	0	0	10	0
9	0	0	0	0	0	0

Days after infection	Egg production (eggs/g faeces) by individual rats vaccinated and then infected					
	1	2	3	4	5	6
6	700	150	350	300	200	300
7	18,000	3000	3600	11,700	14,000	7,800
8	15,000	4000	7800	15,600	25,500	11,400
9	24,000	7000	5200	15,900	11,800	7,500
10	Dead*	3500	400	7,000	6,900	Dead*
13		700	0	1,600	0	
14		50	0	100	0	

* Rats were killed when bled from the heart.

were not infected but were kept to check the level of 'reagins' in the absence of the challenge infection. All the animals were bled from the heart on days 3, 6, 9, 15 and 27 after the infection. The sera were tested for 'reagins' by homologous PCA and the results are shown in Table 6.

Before the challenge infection seven out of nine animals did not have detectable 'reagins' in their serum, and two animals showed 'reagins' only in undiluted sera. In this experiment five of the six rats infected after vaccination produced a low level of 'reagins' from 6 days after infection. 'Reagins' were detected only twice in the sera of the three rats which were not infected after vaccination. All rats were fully susceptible to infection, since

they passed many eggs for a period corresponding to the usual patent period of a first infection in this strain of rat (see experiment 1).

This experiment has been repeated with variable results. In one experiment nine rats were vaccinated with 1·0 ml fresh antigen $+2 \times 10^{10}$ dead *H. pertussis* bacilli injected intraperitoneally. Two weeks later the sera from all rats were negative in homologous PCA. They were infected with approximately 3000 larvae 6 weeks after vaccination, and sera taken 6, 9 and 15 days later were all negative in homologous PCA.

It is concluded that intraperitoneal vaccination with antigen+*H. pertussis* adjuvant rarely stimulates the production of 'reagins' in rats since even a subsequent infection does not regularly stimulate an anamnestic production of this antibody. Vaccination did not stimulate resistance to a subsequent infection.

VACCINATION WITH FREUND'S COMPLETE (DIFCO) OR *Haemophilus pertussis* ADJUVANT

Nine rats were vaccinated with 0·5 ml antigen injected intraperitoneally after emulsification with an equal volume of Freund's (Difco) complete adjuvant. A second group of nine animals was given an intraperitoneal injection of 0·5 ml antigen mixed with 2×10^{10} killed *H. pertussis* bacilli (Glaxo) as adjuvant. Serum samples were taken on days 10, 15, 20, 27 and 34, and the rats were vaccinated again with 1·0 ml antigen+2×10^{10} *H. pertussis* intradermally and in the footpads on day 27. Sera from rats vaccinated initially with *H. pertussis* adjuvant were negative in the homologous PCA test throughout. All sera from rats vaccinated initially with Freund's adjuvant were negative in homologous PCA on day 10, but on day 15, three rats gave a positive PCA reaction; on day 20, five were positive and on day 27 all were positive. On the other hand, these sera reacted in homologous PCA only when undiluted and all sera taken 7 days after revaccination were negative in this test.

In this experiment, rats vaccinated with adult antigen plus *H. pertussis* adjuvant produced no 'reaginic' antibody at any time. All rats vaccinated with antigen+Freund's adjuvant produced a low level of 'reaginic' antibodies by the 27th day after primary vaccination, but not after a second injection of antigen. It appears that the use of Freund's adjuvant with this antigen results in the production of 'reagins' more consistently than the use of *H. pertussis* bacilli does.

STIMULATION OF 'BLOCKING' ANTIBODIES BY PROLONGED VACCINATION

Ten rats were given a prolonged course of vaccination with adult worm antigen. The initial vaccination was with 500 adult equivalents of uncentrifuged worm extract injected subcutaneously after emulsification with an equal volume of Freund's complete adjuvant (Difco). Subsequent vaccinations were made with the supernatant fraction of worm extract given intraperitoneally on day 10 (300 adult equivalents), on day 14 (500), on day 27 (300), on day 62 (1000) and on day 90 (600). Six rats were infected together with six controls 5 days after the last vaccination, and the remaining four rats were infected together with four challenge controls 8 weeks later. The course of these infections was followed by group faecal egg counts and individual worm counts were also made on each rat when the rats were bled out 9 days after each infection (Table 7). Sera taken on the day of infection were negative by homologous PCA in all cases. Some sera taken from vaccinated rats 9 days after infection were positive in the PCA test, though only when undiluted (Table 7). None of the vaccinated rats was immune to infection.

TABLE 7

THE COURSE OF CHALLENGE INFECTIONS IN VACCINATED AND CONTROL RATS MEASURED BY EGG PRODUCTION, THE NUMBER OF WORMS IN THE SMALL INTESTINE, AND THE OCCURRENCE OF CIRCULATING 'REAGINS'

1. *Results of infection* 5 *days after last vaccination*

Control rats			Vaccinated rats		
Rat No.	Worm count	72-hour PCA reaction	Rat No.	Worm count	72-hour PCA reaction
1	47	0	7	352	+
2	71	0	8	287	+
3	122	0	9	74	+
4	30	0	10	53	0
5	45	0	11	375	0
6	54	0	12	222	+
Mean	62 ± 41		Mean	227 ± 126	

	Egg counts (eggs/g faeces)	
Days after infection	Control rats	Vaccinated rats
6	50	0
7	1000	1200
8	1200	2200
9	600	1700

2. *Results of infection* 8 *weeks after last vaccination*

Control rats			Vaccinated rats		
Rat No.	Worm count	72-hour PCA reaction	Rat No.	Worm count	72-hour PCA reaction
13	613	0	17	527	0
14	344	0	18	405	+
15	278	0	19	414	+
16	175	0	20	246	0
Mean	353 ± 162		Mean	398 ± 100	

	Egg counts (eggs/g faeces)	
Days after infection	Control rats	Vaccinated rats
6	0	0
7	1300	3000
8	23000	8200
9	2000	3000

Sera taken from the vaccinated rats both before and after infection contained a 'blocking' antibody. This antibody was detected by its effect on the antigen used in the homologous PCA test as follows:

Serial dilutions of a serum with a titre of 640 by homologous PCA were injected into the skin of each animal in a group of nine rats. Three days later, the rats were divided into three groups and injected intravenously with 0·1 ml antigen which had been incubated during the previous 24 hours at 4° with 0·3 ml either of saline, normal serum or serum taken from the vaccinated rats. The PCA titre of the serum in rats injected with the antigen incubated either with saline or with normal serum was similar, but when the

PCA reaction was induced with antigen incubated with serum from the vaccinated rats, the test was negative or greatly reduced (Fig. 2).

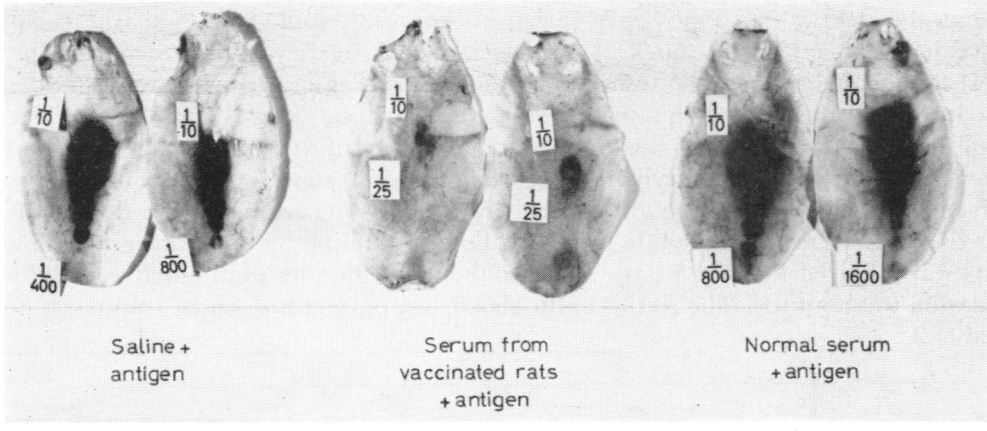

FIG. 2. Inhibition by PCA reaction by non-reaginic 'blocking' antibody. The rats from which these skins were taken were all injected intradermally with serial dilutions (1 : 10 to 1 : 640) of the same serum. Three days later, PCA reactions were induced with antigen which had been incubated either with saline, or with serum from normal rats, or with serum taken from rats vaccinated with worm antigen.

This blocking antibody, therefore, has the same antigenic specificity as do 'reagins', but blocking antibody does not attach to the skin of rats. In other experiments, antigen has been incubated before use in the PCA tests with pooled serum, PCA titre 640, taken from rats after three or four infections. Antigen incubated with these highly 'reaginic' sera was still able to elicit a maximal PCA reaction which suggests that the blocking activity of the sera from vaccinated rats could not have been explained by the presence of a low level of 'reagins' undetectable even by 72 hours PCA.

A further experiment has shown that rats vaccinated with 1·0 ml antigen and Freund's adjuvant injected all over the body, in the footpads, under the skin and into the peritoneal cavity, and revaccinated with a total of 2·5 ml antigen given subcutaneously three times, 3 weeks later, have detectable blocking antibody 3 weeks after the last vaccination. Sera taken from rats given a single vaccination, however, did not contain blocking antibody but this is probably because the technique for measuring blocking antibody is relatively insensitive. 'Blocking' sera from vaccinated animals which were not subsequently infected was tested in homologous PCA both at 72 hours and at 6 hours. At 72 hours, all sera were negative but some did produce a distinct reaction at 6 hours. This reaction was much less intense and more diffuse than the reaction which is found at 72 hours with 'reagins'.

Mota (1963b) reported that rats produce two types of antibodies that give homologous PCA: (1) 'reaginic' antibodies which remain attached to mast-cell-containing tissues for a long time, and (2) other antibodies found in sera with a high content of precipitating antibodies, which will give homologous PCA at 6 hours, but not at 72 hours.

EFFECT OF SEVERE SYSTEMIC ANAPHYLAXIS INDUCED BY AN UNRELATED ANAPHYLACTIC ANTIGEN–ANTIBODY INTERACTION ON ADULT WORMS IN THE INTESTINE OF RATS

Rats were immunized by intraperitoneal injection of 1·0 ml of a 1/1 mixture of raw egg white and saline injected with 2×10^{10} dead *H. pertussis* bacilli. One week later,

the rats together with non-immunized controls were infected with *N. brasiliensis* larvae. After a further week, the rats were given an intravenous injection with 0·5 ml of a 1 per cent (w/v) egg white solution. This injection induced systemic anaphylaxis in the immunized rats but the degree of anaphylaxis varied enormously; some rats died whereas others showed little or no signs of shock. Anaphylactic shock in the rat affects mainly the gut, so that the whole length of the intestine becomes grossly inflamed with resulting haemorrhage into the lumen and desquamation of the gut mucosa (Sanyal and West, 1958). The aim of the present experiment was to examine the effect of this gut damage on the ability of the worm population to survive and reproduce in the small intestine. The rats which survived this shock were therefore divided into groups according to the degree of anaphylaxis induced by the egg white injection. Egg production by the worms in each group of rats was followed for 2 or 3 days after shock before the rats were killed and the worm population was counted. The results of the shock, egg output and worm counts are shown in Table 8.

TABLE 8

EFFECT OF SYSTEMIC ANAPHYLAXIS INDUCED IN RATS BY AN UNRELATED ANTIGEN–ANTIBODY INTERACTION ON THE WORM POPULATION IN THE INTESTINE, MEASURED BY EGG PRODUCTION AND THE NUMBER OF WORMS PRESENT IN SHOCKED AND CONTROL GROUPS OF ANIMALS

	Degree of anaphylaxis in rats:		
	Severe	Moderate	Nil–controls
Shock induced on day 3 *after infection, during final larval moult*			
Egg production (eggs/g faeces) following shock			
Day 6	1,600	1300	2500
Day 7	23,000	4900	9000
Worms recovered from individual rats (day 7 after infection)	524, 135, 458	451, 96, 431 281, 373, 482	584, 42, 809 483, 598, 40
Shock induced on day 6 *after infection*			
Egg production (eggs/g faeces) following shock			
Day 7	8,200	5000	3200
Day 8	15,200	8000	5500
Worms recovered from individual rats (day 8 after infection)	619, 654	520, 370	406, 253, 498 540, 536
Shock induced on day 7 *after infection*			
Egg production (eggs/g faeces) following shock			
Day 8	9,600	10,800	8800
Day 9	21,500	7,900	4200
Day 10	7,600	6,200	7700
Worms recovered from individual rats (day 10 after infection)	550, 318, 281 456, 234	218, 653, 243	306, 440, 236 97, 466, 382

Classification of shock. Severe; Rats collapsed on cage floor within 10 minutes of antigen injection and did not recover for 30–60 minutes. These animals continued to show clinical signs of shock for at least 24 hours, i.e. ruffled fur, general lassitude and occasionally diarrhoea. Moderate; Some rats collapsed temporarily and all showed severe convulsive breathing, with cyanosis and ruffled fur. Rats showing little or no shock are not included.

These results show that even near-lethal systemic anaphylaxis induced in the rat by an unrelated antigen–antibody system had no effect on the population of adult worms in the gut. Egg production was unaffected and worms were not expelled from the intestine.

EFFECT OF HOMOLOGOUS SHOCK ON RESIDUAL WORM POPULATIONS

The majority of worms are expelled from the intestine of rats by the 16th day after initial infection, but a few parasites survive for a longer period. The following experiment investigated the possibility that worms which survived the expulsion of worms, mediated perhaps by a 'reagin'–allergen immunological reaction, may be expelled by a further violent anaphylactic reaction induced by injection of homologous worm antigen.

Forty rats were infected with approximately 2500 larvae. Four weeks later, twenty-six animals were shocked by intravenous injection with 0·3 ml antigen. Twelve animals died, five showed slight symptoms of shock and the remainder were severely or moderately shocked. The rats showing slight symptoms were discarded and 2 days after shock, the remaining rats were killed and the worms present in each rat were counted. The nine rats which were severely shocked harboured a mean of 12 ± 11 worms at autopsy, the fourteen unshocked animals a mean of 14 ± 17. It appears that the residual worm population is not affected by shock induced with homologous antigen.

DISCUSSION

In the investigations described above, antibodies resembling human reagins in some of their properties were found in the serum of rats infected with their natural nematode parasite *N. brasiliensis*. This antibody was detected by its ability to sensitize rat, but not guinea-pig skin, in passive cutaneous anaphylaxis; it was destroyed by heating at 56° for 30–180 minutes. Like human reagin, rat 'reagin' remained attached to homologous skin for a long time. The PCA titre was not diminished when a week elapsed between the intradermal injection of a high titred serum and intravenous injection of antigen. Thereafter a progressively weaker PCA reaction could be induced up to 2 weeks following intradermal injection of serum. This 'reaginic' antibody therefore seems to be similar in most of its biological properties to the antibody induced in rats by injection of defined antigens with *H. pertussis* or Freund's adjuvant (Mota, 1963a, b, 1964; Binaghi and Benacerraf, 1964; Binaghi, Benacerraf, Bloch and Kourilsky, 1964).

'Reagins' could be detected in the serum shortly after the rats had acquired resistance to an initial infection, as shown by the cessation of egg production by the worms and by the expulsion of the major part of the worm population from the small intestine. These antibodies were detected in the sera of some rats from the day following the termination of the infection and in the sera of most rats 1 week later. 'Reagins' continued to be produced in the absence of reinfection and have been detected up to 7 months after a single infection. After reinfection, there was a rapid rise in antibody production by the 6th day. Titres remained high for 3–6 days, and then fell to a lower level. Therefore, reagin production stimulated in rats by this parasite was not associated solely with the primary antibody response and further reinfections continued to re-stimulate high levels of 'reagins' in rats. In this respect 'reaginic' antibody stimulated by a living worm infection differs from the similar antibody described in rats by Mota (1963a, 1964) which was termed 'mast cell sensitizing antibody' and described also by Binaghi and Benacerraf (1964) who called

it the 'rat anaphylactic antibody'. These authors described this type of antibody in the rat following injection of defined antigens and associated it with the primary antibody response, because in their experiments reinjection of antigen not only failed to stimulate an anamnestic production of 'reagins', but also appeared to accelerate the disappearance of the antibody from the circulation. In other experiments not reported here, some animals given a small initial infection did not produce detectable reagins, but reinfection invariably stimulated an anamnestic rise in 'reagin' production. Thus, a negative PCA test for 'reagins' after initial infection did not necessarily prove that antibody was not being produced. It can be concluded that the occurrence of an anamnestic rise after a second infection was a more reliable guide to the stimulation of 'reagins' by first contact with antigen than was a positive PCA test after an initial infection. These antibodies appear to have such a high affinity for tissues containing mast cells, that the level in the circulation must reflect a surplus production of 'reagins'.

The antigen used in PCA was a saline extract of adult worms. This antigen was stable for at least 10 weeks *in vitro* at $+4°$ or $-20°$, and maximal PCA reactions could be induced with as little as 100 worm-equivalents/200 g rat. Antigen prepared from fourth stage larvae appeared to be equally effective in inducing PCA, but antigen prepared from third stage larvae was not so effective. A weak PCA reaction could be induced using an extract prepared from 0·5 ml of packed third stage larvae, both with serum from rats infected normally or with serum from rats infected solely with adult worms. It has been shown by Mota (1963b) that in the rat homologous PCA can be induced both by what we call 'reagins' here and by an antibody found in serum with high levels of precipitating antibody. These antibodies can be distinguished by allowing a 72-hour interval between serum and antigen injection, since the antibodies found in precipitating serum give PCA at 6 hours, but not at 72 hours. Therefore, in the present investigation antigen was injected 72 hours after intradermal serum injection in routine PCA tests.

When rats were vaccinated with the antigen used in the PCA test, 'reagins' were detected in most rats vaccinated with Freund's adjuvant and in some rats vaccinated with *H. pertussis* bacilli as adjuvant, but titres were low in all cases. As has been reported by Mota and by Binaghi and Benacerraf using defined antigens, further injections of worm antigen failed to stimulate an increase in 'reagin' production; in fact, 'reagins' were never detected after a second antigen injection. Furthermore, prolonged vaccination stimulated the production of a blocking antibody, which blocked the interaction of 'reagin' and antigen, but did not give the 72-hour PCA in the rat. Some of these sera which did not give a PCA reaction at 72 hours gave homologous PCA reactions at 6 hours, but whether the same component of the crude worm extract gave both reactions has yet to be determined.

Although 'reagins' were detected only during the primary response after vaccination, a subsequent infection in vaccinated animals stimulated a few rats to produce detectable 'reagins' by the 6th day after infection. This must have been an anamnestic rise in 'reagin' production because 'reagins' were not detected before the 16th day after initial infection in normal animals. This anamnestic production was detected even in rats which had been revaccinated several times over a period of 3 months before the infection. It is concluded that although continued vaccination failed to initiate production of the antibody at a level detectable by PCA, reagins were being produced in at least some rats prior to infection. Rats vaccinated with this antigen were never resistant to subsequent infection. Future investigations will aim to determine whether reagins found after living infections or stimulated by vaccination with dead worm material are directed against the same antigens.

The most interesting question arising from this work is why a living worm infection is such a potent inducer of reagin production. All helminth infections in the rats investigated so far stimulate reagin production specific to the infection concerned. Thus, reagins are found in the serum of rats infected with such diverse helminths as *Schistosoma mansoni* (Ogilvie, Smithers and Terry, 1966), *Litomosoides carinii* (Worms, unpublished) and *Trichinella spiralis* (Denham, personal communication). It is possible that continuous release of antigen into the host, which must occur in helminth infections, may be a factor in reagin production, although this seems unlikely since vaccination with Freund's adjuvant presumably results in continuous antigen release. Also preliminary investigations have failed to detect comparable antibodies with the protozoan parasites *Plasmodium berghei* and *Trypanosoma lewisi*, where continuous release of antigen into the host also occurs. A further possibility is that the site parasitized by the worms influences the type of immunoglobulin produced. In man, it is known that plasma cells producing γA-globulin predominate in the intestinal mucosa (Crabbé, Carbonara and Heremans, 1965) and reagins have been associated mainly with this serum fraction (Heremans and Vaermann, 1962, Fireman, Vannier and Goodman, 1963; Augustin, Connolly and Lloyd, 1964) although they occur in the γG and γM fractions in certain conditions (Terr and Bentz, 1965). Results presented here suggest that the main stimulus for 'reagin' production came from adult worms in the intestine. It is interesting that worm infections in other sites resulted in much lower levels of 'reagin' production. Infections with living *N. brasiliensis* in the peritoneal cavity and with *S. mansoni* where the worms are found in the blood vessels both resulted in low levels of 'reagin' production, even after a second or third infection. It would be interesting to determine the type of immunoglobulin found in plasma cells at these sites of parasitism. Whether the nature of the antigen is a determining factor in 'reagin' production cannot be ascertained until the antigen(s) have been isolated.

High levels of 'reagins' and a high degree of resistance to reinfection with *N. brasiliensis* are both stimulated only by living worm infections, but the relationship between the two is not clear-cut. Reagins do not appear in the circulation until the initial infection has been terminated and high titres are found by the 6th day after reinfection. Worms in a second infection are expelled from the host from the 4th day after reinfection (Ogilvie, 1965b). Also the level of circulating 'reagins' varies between rats within a group given the same infections, whereas the degree of resistance varies little. Finally, a few rats vaccinated before infection produce high levels of 'reagins' following infection, but are not resistant to the infection. This lack of complete correlation between the occurrence of circulating 'reagins' and resistance to infection is not surprising, for the crude worm extract used as antigen to detect 'reagin' does not itself stimulate protective immunity when rats are vaccinated with it. On the other hand, recent investigations have shown that there is some component of this crude antigen that inhibits passive transfer of immunity with serum taken from multiple infected rats (Ogilvie, to be published). Experiments now in progress aim to isolate this component and to compare its activity in PCA, its ability to inhibit passive transfer of immunity, and its capacity to induce systemic anaphylaxis.

Other authors have associated the occurrence of 'reagins' in rats with sensitivity to systemic anaphylaxis (Mota, 1963a, 1964; Binaghi and Benacerraf, 1964). It is known that rats immune to *N. brasiliensis* undergo severe anaphylaxis on intravenous injection of adult worm antigen (Urquhart, Mulligan, Eadie and Jennings, 1965). In the present report, the sensitivity of rats to systemic anaphylaxis during the time they develop resistance to an initial infection has been investigated in more detail. Whereas all rats expel the

worms within 2–3 days, the occurrence of 'reagins' and signs of systemic anaphylaxis varied greatly from rat to rat. Some rats were shocked or were producing 'reagins' immediately after the infection was terminated, but in most rats, 'reagins' and signs of systemic anaphylaxis were not detected until about a week after the termination of the infection. Thus within a group of rats, sensitivity to anaphylaxis and the occurrence of reagins were detected at the same time relative to the development of protective immunity; but when individual rats were considered, there was no relationship between systemic anaphylaxis and circulating 'reagins'. Within the same group, some rats died in severe anaphylaxis with no detectable 'reagins' in the serum; others with high levels of 'reagins' were unaffected by intravenous injection with the same quantity of antigen. Furthermore, some vaccinated rats were susceptible to anaphylaxis, but were not resistant to an infection. The same crude antigen was used to detect both, 'reagins' and anaphylactic sensitivity. The nature of the antigen(s) concerned and further investigations into this problem depend upon purification of the worm extract.

Urquhart *et al.* (1965) suggested that a local anaphylactic reaction was responsible for the elimination of worms from the gut at the termination of initial infection. The results of the experiment reported here show that this reaction must be worm-specific to result in worm expulsion; a severe anaphylactic reaction induced by an irrelevant antigen–antibody reaction had no detectable effect on the worm population in the shocked rats. Furthermore, when homologous shock was induced in rats by injection of worm antigen 28 days after initial infection, the residual worm population still found after the majority of worms had been expelled was unaffected. This result does not necessarily mean that a local worm-specific anaphylactic reaction fails to remove the major part of the worm population; worms which survive may do so because of their ability to endure such a reaction.

Further study of the relationship between 'reagins', systemic anaphylaxis and acquired resistance to the parasite depends upon an analysis of the worm antigen as well as the development of a technique capable of examining the relationship of these antigens to protective immunity.

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REFERENCES

AUGUSTIN, R., CONNOLLY, R. C. and LLOYD, G. M. (1964). 'Atopic reagins as a prototype of cytophylic antibodies.' *Protides of the Biological Fluids*, p.56 (Ed. by H. Peeters). Elsevier, Amsterdam.

BINAGHI, R. A. and BENACERRAF, B. (1964). 'The production of anaphylactic antibody in the rat.' *J. Immunol.*, **92**, 920.

BINAGHI, R. A., BENACERRAF, B., BLOCH, K. J. and KOURILSKY, F. M. (1964). 'Properties of rat anaphylactic antibody.' *J. Immunol.*, **92**, 927.

CHANDLER, A. C. (1936). 'Studies on the nature of immunity to intestinal helminths. IV. The interrelations between parental and intestinal immunity in rats infected with Nippostrongylus.' *Amer. J. Hyg.*, **24**, 129.

CRABBÉ, P. A., CARBONARA, A. O. and HEREMANS, J. F. (1965). 'The normal human intestinal mucosa as a major source of plasma cells containing γA-immunoglobulin.' *Lab. Invest.*, **14**, 235.

FIREMAN, P., VANNIER, W. E. and GOODMAN, H. C. (1963). 'The association of skin-sensitizing antibody with the β_2A-globulins in sera from ragweed-sensitive patients.' *J. exp. Med.*, **117**, 603.

HEREMANS, J. F. and VAERMAN, J. P. (1962). '$Beta_2A$-globulins as a possible carrier of allergic reaginic activity.' *Nature (Lond.)*, **193**, 1091.

MOTA, I. (1963a). 'Biological characterization of "mast cell sensitizing" antibodies.' *Life Sci.*, **1**, 465.

MOTA, I. (1963b). 'Passive cutaneous anaphylaxis induced with mast cell sensitizing antibody. The role of histamine and 5-hydroxytryptamine.' *Life Sci.*, **1**, 917.

MOTA, I. (1964). 'The mechanism of anaphylaxis. I. Production and biological properties of "mast cell sensitizing" antibody.' *Immunology*, **7**, 681.

OGILVIE, B. M. (1964a). 'Skin sensitizing (reaginic) antibodies in rats immune to *Nippostrongylus brasiliensis*'. *Proc. 1st int. Congr. Parasitol. Rome, September* 1964. (In press).

OGILVIE, B. M. (1964b). 'Reagin-like antibodies in animals immune to helminth parasites.' *Nature (Lond.)*, **204**, 91.

OGILVIE, B. M. (1965a). 'Role of adult worms in immunity of rats to *Nippostrongylus brasiliensis*.' *Parasitology*, **55**, 325.

OGILVIE, B. M. (1965b). 'Use of cortisone derivatives to inhibit resistance to *Nippostrongylus brasiliensis* and to study the fate of parasites in resistant hosts.' *Parasitology*, **55**, 723.

OGILVIE, B.M., SMITHERS, S. R. and TERRY, R. J. (1966). 'Reagin-like antibodies in experimental infections of *Schistosoma mansoni* and the passive transfer of resistance.' *Nature (Lond.)*, **209**, 1221.

OVARY, Z. (1964). 'Passive cutaneous anaphylaxis.' *C.I.O.M.S. Immunological Methods* (Ed. by J. F., Ackroyd), p. 259. Blackwell Scientific Publications Oxford.

SANYAL, R. K. and WEST, G. B. (1958). 'Anaphylactic shock in the albino rat.' *J. Physiol. (Lond.)*, **142**, 571.

TERR, A. I. and BENTZ, J. D. (1965). 'Skin sensitizing antibodies in serum sickness.' *J. Allergy*, **36**, 433.

URQUHART, C. M., MULLIGAN, W., EADIE, R. M. and JENNINGS, F. W. (1965). 'Immunological studies on *Nippostrongylus brasiliensis* infection in the rat: the role of local anaphylaxis.' *Exp. Parasit.*, **17**, 210.

WHITLOCK, H. V. (1948). 'Some modifications of the McMaster helminth egg-counting technique and apparatus.' *J. Counc. scient. ind. Res. Aust.*, **21**, 177.