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HYPEREOSINOPHILIA IN RATS WITH *TRICHINELLA SPIRALIS* INFECTIONS

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Summary.—Six LOU strain rats developed blood eosinophil counts of over 10×10^9/l. Six months previously they had been infected with 2 larvae/g of *Trichinella spiralis*, 2 days after being exposed to high ambient temperatures. The morphology of dividing and peripheral eosinophils was normal, but eosinophils in the blood and peritoneum had increased binding capacity for complexed IgG. Detailed studies were done on 2 rats with blood eosinophil counts of 24 and $32{\cdot}5 \times 10^9$/l. Their hearts were firm and contained adherent thrombus in both ventricles, but there were no histological signs of endomyocardial damage. There were extensive eosinophil infiltrates in lymphoid organs and lungs, and one of the rats had hepatic cirrhosis. Attempts to transfer the disease to syngeneic rats with tissues from affected rats, and to reproduce the disorder in other rats were unsuccessful. The ability of LOU strain rats to expel adult *T. spiralis* worms, and their eosinophil response in the early stages of infection were normal.

It is concluded that hypereosinophilia can sometimes occur in rats, and that this has some features which are also found in hypereosinophilic syndromes of man. It is suggested that the rats with hypereosinophilia had developed the disease as an unusual response to *T. spiralis* infection. Although the disorder has not been reproduced in other experiments, these findings are described in order to show that they can occur, as the development of an animal model of the hypereosinophilic state would be of value in the study of how the eosinophil response is regulated, and the mechanism of tissue damage that may be seen in association with persistently raised blood eosinophil counts.

PERSISTENTLY RAISED blood eosinophil counts in man of over 2×10^9/l (hypereosinophilia) are rare, but may occur in certain parasitic, fungal and neoplastic diseases (Hardy and Anderson, 1968). When no cause for the marked eosinophilia is found they are often considered to have the "hypereosinophilic syndrome" (Chusid *et al.*, 1975). In these patients eosinopoiesis is increased, and the blood eosinophil half-life may be prolonged (Spry, unpublished). Serious complications which occur in patients with hypereosinophilia include Löffler's endomyocardial disease, and lesions in the lungs, brain, kidneys and blood vessels (Olsen and Spry, 1979).

In rats, blood eosinophil counts are finely regulated by factors which control eosinophil production (Spry, 1970*a*) and distribution (Spry, 1970*b*). Even when eosinopoiesis was strongly stimulated by infection or i.v. injection of muscle-stage *Trichinella spiralis* larvae (Basten, Boyer and Beeson, 1970) blood eosinophil counts rarely rose above 1×10^9/l. The finding of blood eosinophil counts of over 10×10^9/l in a group of LOU rats with trichinosis was therefore of great interest, and these animals are the subject of this report.

The aims of the study were to see if eosinophils in these rats were abnormal, and whether they developed tissue lesions which were comparable to those seen in patients with hypereosinophilic states. In addition attempts were made to transfer

and induce the disease in other syngeneic rats, and to determine whether this strain had a defective immune response to *T. spiralis* infections.

MATERIAL AND METHODS

Animals.—The LOU rats which developed hypereosinophilia were bred from 4 litter-mate pairs from the Sir William Dunn School of Pathology, Oxford, and were originally derived from the strain developed by Dr H. Bazin in Louvain, Belgium. They were males, weighing 200–250 g, and were reared and kept in conventional, but not specific-pathogen-free (SPF) conditions. Other LOU rats, SD and PVG rats were from the SPF colony at the National Institute for Medical Research, Mill Hill, London.

Trichinella spiralis.—Muscle-stage larvae were digested with pepsin from the carcases of rats with trichinosis, washed, and 2 or 5 larvae/g body wt were given by tube into the stomach under ether anaesthesia. Muscle-stage larvae were counted by the method of Kagan (1960). Adult worm loads in the intestine were measured by the method of Love, Ogilvie and McLaren (1976).

Eosinophils.—Blood eosinophil counts were done by the method of Discombe (1946). Differential blood counts, measurements of marrow eosinophils and assessment of eosinophil morphology were done on smears stained with Leishman's or Giemsa's stain. Eosinophil membrane receptors for complexed IgG were measured by forming rosettes with rabbit-IgG-antibody-coated ox erythrocytes (EA rabbit) by the method of Tai and Spry (1976).

RESULTS

Hypereosinophilic rats

Six rats were exposed to temperatures up to 40° during a heatwave which lasted 2 days, and resulted in the death of one third of the rats in the part of the colony where they were being kept, despite provision of unrestricted water and food. Two days after the heatwave ended they were infected with 2 *T. spiralis* larvae/g body wt and kept in a different part of the animal house. Two months later their blood eosinophil counts were $0{\cdot}615 \pm 0{\cdot}293 \times 10^9$/l (6 rats) [mean ± s.d. (number of animals)], which was the expected level for rats with trichinosis (Basten *et al.*, 1970). Six months after infection 4 of these rats were bled from the aorta, and peritoneal cells were obtained by saline lavage. Their blood eosinophil counts were found to be extremely high [$14{\cdot}250 \pm 4{\cdot}524 \times 10^9$/l (in 4 rats)] and counts in the remaining 2 rats continued to rise during the following month (Table I).

TABLE I.—*Blood eosinophil counts in rats developing hypereosinophilia after infection with* T. spiralis

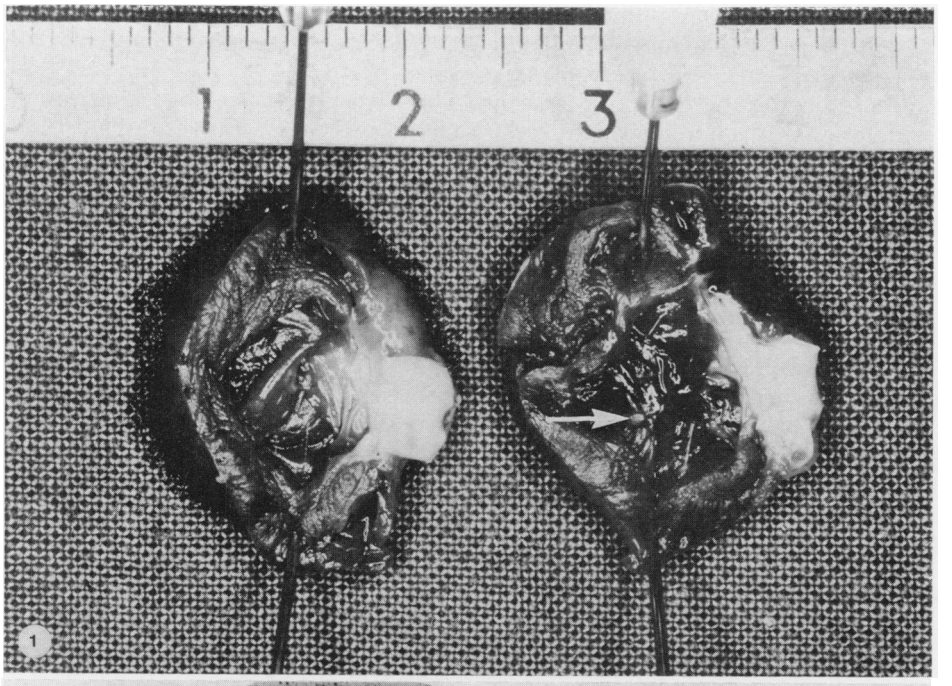
Weeks after infection	Blood eosinophil counts ($\times 10^9$/l) Mean ± s.d.		
	All rats	Rat A	Rat B
0	0·120 ± 0·016	—	—
4	0·310 ± 0·083	0·139	0·256
8	0·615 ± 0·297	0·583	0·844
12	1·320 ± 0·250	1·320	1·650
23	14·250 ± 4·524	15·000	11·600
24	—	21·900	28·500
25	—	24·000	30·000
26	—	1·116	29·900
27	—	—	32·300

The proportions of their blood and peritoneal eosinophils which were able to form rosettes with rabbit-IgG-coated ox erythrocytes were also much greater than in uninfected LOU rats or PVG rats which had been infected with an equal quantity of larvae (Table II). When rat antibody was used instead of rabbit antibody, no difference was found in the proportions of eosinophils which bound EA.

Two weeks later, when one of the remaining rats (Rat A) became cold, with ruffled fur and a hunched position in the corner of its cage, it was killed by ether

TABLE II.—*The proportion of eosinophils in blood and peritoneum from rats with hypereosinophilia and controls which bound EA. More blood eosinophils from hypereosinophilic rats bound EA than controls, but no differences were found between peritoneal eosinophils*

Rat group (Number of rats)	% eosinophils binding EA (mean ± s.d.)	
	Blood	Peritoneum
"Hypereosinophilic" LOU rats infected with 2 larvae/g (4)	59 ± 12	66 ± 8
Uninfected LOU rats (4)	15 ± 5	60 ± 8
PVG rats infected with 2 larvae/g (6)	16 ± 8	64 ± 6
	$P < 0{\cdot}001$	N.S.



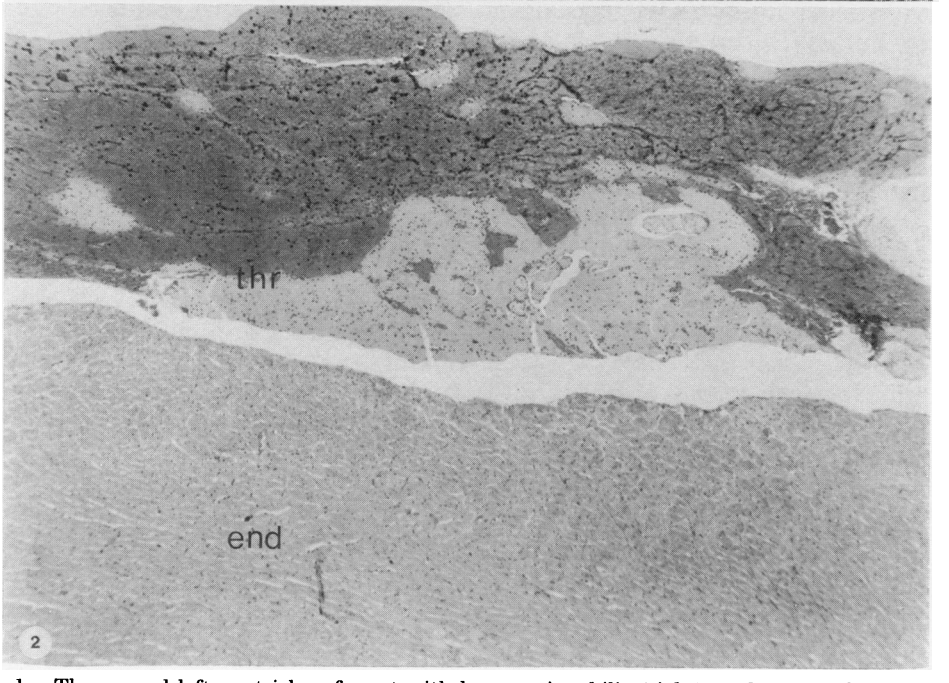


FIG. 1.—The opened left ventricles of a rat with hypereosinophilia (right), and a normal rat (left), showing densely adherent thrombus (arrow). Scale in cm.

FIG. 2.—Left ventricular endocardium (end) and adherent thrombus (thr), some of which is partially organized. There was no histological evidence of endomyocardial damage or fibrosis. H. & E., × 48.

inhalation. The blood eosinophil count had fallen to $1{\cdot}116 \times 10^9$/l. The lungs had small grey areas on their surfaces. The heart was firm and contained adherent thrombus in both ventricles, only some of which could be wiped off with a wet swab. The second surviving rat (Rat B) remained well, and was killed 7 months after infection, when its blood eosinophil count had reached $32{\cdot}3 \times 10^9$/l and 48% of bone marrow cells were eosinophils. The carcase contained only 300 larvae. Its spleen was enlarged, and the liver was large with a granular appearance. The heart was also firm, and again there was adherent thrombus in the ventricular cavities. In contrast thrombus was not found in the hearts of 3 normal LOU rats which were killed at the same time (Fig. 1).

Transfer of the disease

Attempts to transfer the disease from Rats A and B to other litter-mate LOU rats, which had also survived the heat exposure but which had not been infected with *T. spiralis* larvae, were unsuccessful. Tissues which were given i.v. or i.p. were: concentrated blood cells, lymph node, thymus and bone-marrow cells, whole blood and serum. Recipients showed no change in blood eosinophil counts during the following 6 months. As no further LOU rats from the original colony remained, it was necessary to continue with experiments on LOU rats which had been bred at the National Institute for Medical Research. However, further attempts with these rats to re-create the conditions which had preceded the development of the disease were also unsuccessful in inducing hypereosinophilia.

Immune responses

Studies were then done to see if LOU rats had a strain-specific defect in their ability to expel adult *T. spiralis* or mount an eosinophilia. Ten LOU rats and 10 matched SD rats (which share the same Ag-B2 or H-lw haplotype), were given 1,000 larvae orally. Seven days later the number of adult worms recovered in the intestines was 230 ± 84 (5 rats) and 289 ± 60 (5 rats) respectively, and at 2 weeks there were $7 \pm 9{\cdot}5$ (5), and $1{\cdot}6 \pm 1{\cdot}6$ (5). These results were not significantly different in the 2 strains, and no difference was detected in blood or bone-marrow eosinophil numbers at 1 and 2 weeks after infection. This showed that LOU rats were able to reject adult worms normally, and to mount a normal eosinophil response in the early stages of infection.

Histology

Tissues from Rats A and B were examined.

Hearts.—Adherent thrombus was found in the right atria and left ventricles, involving the mitral valves and apices. Adherent parts of the thrombi were organized, but the superficial bulk of the thrombi was fresh. No evidence of underlying endomyocardial disease was found, and the myocardium was normal (Fig. 2). There were excessive numbers of eosinophils in the gut, in lamina propria, muscle and mesentery.

Spleens.—Contained large numbers of eosinophils in the red pulp around the marginal zones.

Lungs.—The lungs of Rat A were affected by dense areas of eosinophil and mononuclear cell accumulations in peribronchial and perivascular sites. The media of some pulmonary arteries in these areas were damaged and contained inflammatory cell infiltrates (Fig. 3). The lungs of Rat B showed perivascular eosinophil infiltrates only.

Liver.—In Rat B the liver showed areas of necrosis and inflammatory-cell infiltrates consisting almost entirely of eosinophils in some areas, and associated regions of fibrous tissue replacement (Fig. 4). The liver in Rat A was normal.

Lymph nodes.—In Rat B one enlarged lymph node was found partly obscuring the thymus, which was normal. This node had marked sinus histiocytosis, with extensive germinal-centre formation and surrounding infiltrates of eosinophils. Nodes from other sites, and from Rat A

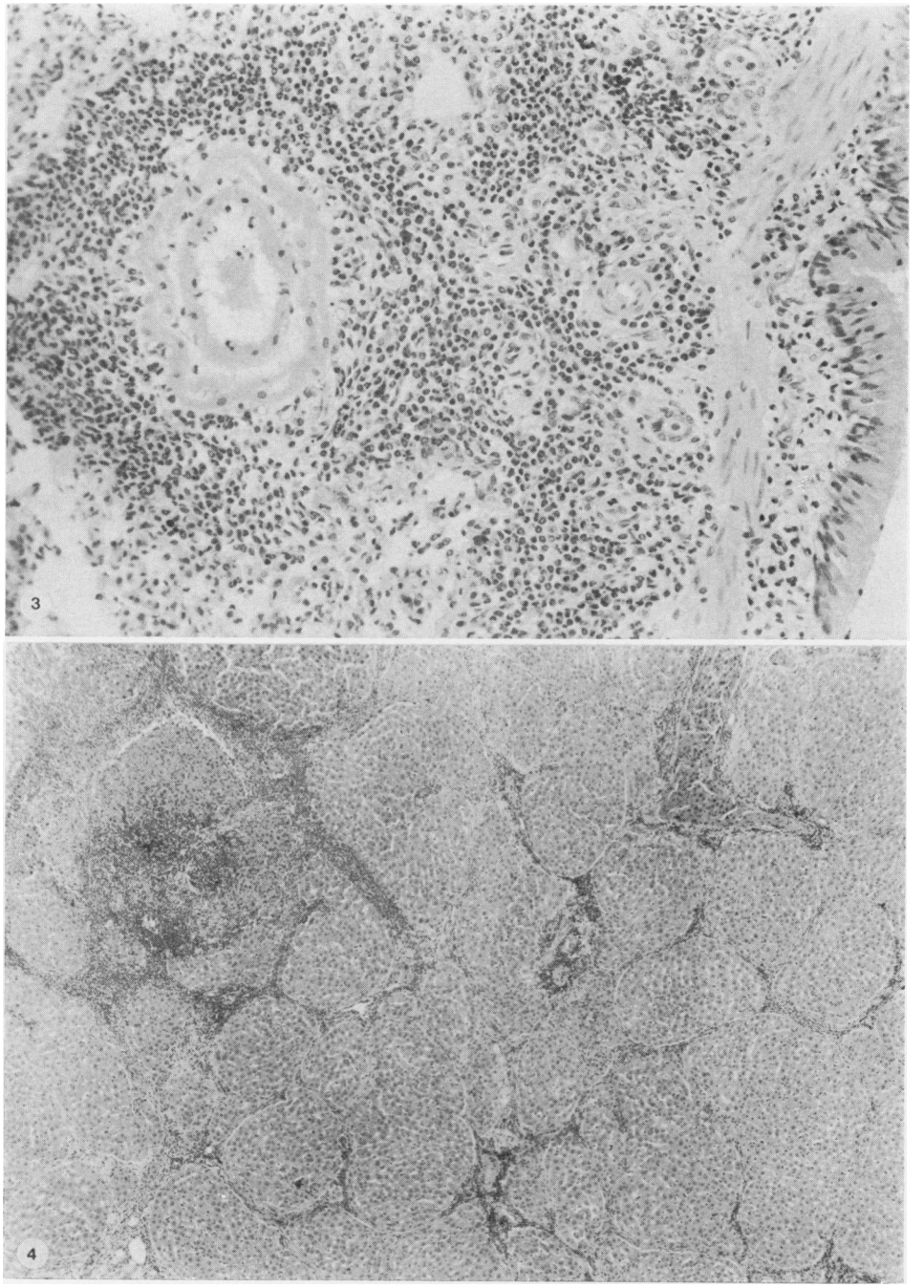


FIG. 3.—Rat lung showing perivascular infiltrate of inflammatory cells, most of which were eosinophils, and pulmonary arterial damage. H. & E., × 320.

FIG. 4.—Rat liver. There were cirrhosis and dense inflammatory cell infiltrates in some areas consisting largely of eosinophils. H. & E., × 48.

showed lesser degrees of eosinophil infiltration.

Parasites.—The inflammatory reaction around encysted larvae in skeletal muscle was normal in both rats and excessive numbers of eosinophils were not found in these sites. The worm burdens were judged to be light, and no other types of parasites were detected.

Eosinophil morphology

No abnormal forms were seen in the blood or bone marrow, where normal eosinopoiesis appeared to be accelerated, and no degranulated eosinophils were seen.

DISCUSSION

This report describes a disease in which 6 rats developed blood eosinophil counts between 10·1 and $32{\cdot}3 \times 10^9$/l 6 months after being infected with a small number of *T. spiralis* larvae. Although hypereosinophilia can occur occasionally in human trichinosis, both with heavy and light infections (Gould, 1970), we have been unable to find any descriptions of hypereosinophilia occurring in rats. Also during an 8-year period in which many experiments were done in rats with trichinosis, only one other rat was found with a markedly raised blood eosinophil count. This was a Wistar rat which was given 10,000 *T. spiralis* muscle-stage larvae i.v., and developed $6{\cdot}271 \times 10^9$/l blood eosinophils 11 days later. The count then returned to normal and when it was killed at 21 days, 101 living muscle-stage larvae were recovered.

Unfortunately experiments failed to find the cause of the hypereosinophilia. It was unlikely to have been an infectious disease or a malignant disorder affecting eosinophils, as it could not be transferred to normal syngeneic rats with serum or cells from a variety of tissues. The normal morphology of bone marrow and blood eosinophils also made it unlikely that there had been a malignant transformation in eosinophil precursors. It seemed more probable that there had been an abnormality in the regulation of the eosinophil response to the parasitic infection, possibly initiated in some way by the initial 2 days of marked heat exposure. Unfortunately the exact conditions for inducing the disease remain unknown, and attempts to reproduce them with syngeneic LOU rats have been unsuccessful.

The possibility that this disease had a strain-specific factor was considered. These LOU rats had been derived from a colony of Wistar rats with a high incidence of apparently spontaneous ileo-caecal immunocytomas (Bazin *et al.*, 1972). Although the animals which were used here had lost this property, they were still able to support the growth of these tumours. For this reason it is speculated that some as yet undefined defect had predisposed these rats to both types of disease. A second possibility, that they had an abnormality in their response to infection with *T. spiralis*, appears to be unlikely, as this strain was shown to expel adult worms from the gut within the normal period of 14 days and had a normal blood eosinophil response (Love *et al.*, 1976). In addition skeletal muscles of the hypereosinophilic rats contained normal numbers of encysted larvae surrounded by the expected local cellular reactions.

The proportion of blood eosinophils from the hypereosinophilic rats which were able to form rosettes with EA rabbit or rat was higher than normal. Similarly, blood eosinophils from patients with Löffler's endomyocardial disease (Tai and Spry, 1976), or onchocerciasis (Guerra-Caceres, unpublished) have also been shown to have an increased capacity to bind EA rabbit. Several lines of evidence suggest that this was due to the exposure of normally hidden Fc binding sites on the cell membrane (Tai and Spry, 1980). The cause of this alteration in these rat eosinophils, and the relevance of this finding to the tissue lesions which were found, are not known.

Important pathological features of the disease in these rats were the occurrence of thrombi in the heart, cirrhosis, pulmonary eosinophilia, and lymphoid-tissue involvement. These abnormalities could

have been due to the parasitic disease itself. But this is unlikely as these lesions were found many months after the rats had been infected, and the worm burden was light. Also in human *T. spiralis* infections severe tissue injury, including endocardial lesions with superimposed thrombi (Andy *et al.*, 1977) is confined to the early period of larva migration following heavy infections (Gould, 1970). Alternatively, the lesions could have developed as complications of the hypereosinophilic state. Similar tissue lesions have been found in patients with idiopathic hypereosinophilic syndromes (Chusid *et al.*, 1975), and hypereosinophilia associated with other forms of infective neoplastic or allergic diseases (Spry, unpublished). It has been suggested that eosinophils may induce tissue lesions in some patients with hypereosinophilia, and there is indirect evidence to support the possibility that eosinophils or their products cause endomyocardial necrosis and thrombosis in these patients (Olsen and Spry, 1979). The presence of ventricular mural thrombi in rats with hypereosinophilia supports this hypothesis.

Although the disorder could not be induced in other rats, the findings have been described in detail, in order to alert others to the possibility of producing an animal model of the hypereosinophilic syndromes. The development of a reproducible hypereosinophilic response in rats would be of great value in assessing the factors which control eosinopoiesis, and the development of tissue lesions in hypereosinophilic states. It is concluded that rats may occasionally develop an exaggerated eosinopoietic response to infection with *T. spiralis* larvae, which may be rat-strain-specific. The presence of endocardial thrombi and lesions in the liver, lymph nodes, spleen and lungs supports the possibility that certain types of tissue injury result from the presence of large numbers of eosinophils in the blood and tissues.

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