A SPECTRUM OF IMMUNE RESPONSES AND PATHOLOGICAL CONDITIONS BETWEEN CERTAIN ANIMAL SPECIES TO EXPERIMENTAL *MYCOBACTERIUM BOVIS* INFECTION

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Summary.—Guinea-pigs, rabbits, rats, ferrets and hedgehogs were infected with a recent field isolate of *Mycobacterium bovis*. The cell-mediated and antibody responses were studied up to 8 weeks after infection at which time the animals were killed and pathological, histological and bacteriological examinations were carried out.

Guinea-pigs and rabbits produced an intense cell-mediated response and strong tissue reactions around the lesions. This appears, in part, to be responsible for the susceptibility of these animals to *M. bovis*. The strong cell-mediated response was also related to the small numbers of organisms in the tissues.

Ferrets produced very little cell-mediated response and only minor tissue reactions. The lack of any cell-mediated response was related to the large numbers of organisms in the tissues which produced an acute disseminated disease.

The antibody response produced by ferrets, rabbits and guinea-pigs was variable within and between the species and could not be related to numbers of organisms in the tissues.

In rats and hedgehogs a specific cell-mediated and humoral response was difficult to detect but the growth of the organism was controlled by the host resulting in a persistent subclinical infection with no mortality.

FOR SOME YEARS it has been realized that there are spectra of immune responses and pathological conditions in many chronic human infections, such as leprosy, syphilis and tuberculosis (Godal, 1974; Lenzini, Rottoli and Rottoli, 1977; Mac-Kaness, 1968; Turk and Bryceson, 1971; W.H.O. Scientific Group Report, 1973).

Resistance to mycobacterial infections involves primarily the cell-mediated immunity (CMI) of the host (MacKaness, 1971). In tuberculosis of man a spectrum of clinical conditions is associated with variations in the patients' levels of cell-mediated immunity, often with an inverse relationship to concurrent antibody response (Lenzini *et al.*, 1977).

Although there is little evidence of a spectrum of clinical and pathological conditions within animal species, Koch (1884) recognized that tuberculosis exhibits a spectrum between species. Francis (1958) grouped certain animal species by their reactions to *M. bovis*. He noted that there was an inverse relationship between the number of bacilli occurring in the lesions and the degree of allergy (measured by the intradermal delayed hypersensitivity test) and the amount of caseation in the lesions. At one end of the spectrum were species producing a strong allergic response to *M. bovis*, including monkeys, guinea-pigs and rabbits. At the opposite end of the spectrum were species that produced a very weak allergic response such as rats, mice, ferrets and hamsters.

Rook and Stanford (1979) have recently described and redefined the various types of CMI responses produced against mycobacterial infections in man and some

animal species. These responses have been referred to as the Jones–Mote reaction (Jones and Mote, 1934), Listeria-type reaction (MacKaness, 1968) and the Koch-type reaction (Koch, 1891). This paper describes these cell-mediated and antibody responses to *M. bovis* in guinea-pigs (*Cavia porcellus*), rabbits (*Oryctolagus cuniculus*), rats (*Rattus rattus*), ferrets (*Mustela furo L.*) and hedgehogs (*Erinaceus europaeus*) and discusses the results in relation to a spectrum of pathological conditions in animals.

MATERIALS AND METHODS

Experimental design

Experimental animals of different species were each divided into 4 groups containing 3 animals per group. All the animals were inoculated with *M. bovis* except the control group. Tuberculin tests were performed on different groups at 7, 15 or 36 days after inoculation and in all the groups 4 weeks before inoculation. Blood was taken before infection and at weekly intervals thereafter for 8 weeks or until animals died. Between 4 and 6 inoculated animals of each species were examined *post mortem* 40–60 days after inoculation.

Mycobacterium bovis *strain*

A recently isolated field strain (AF 117/79) from a bovine lung was grown in 4 parallel cultures of 7H11 Tween 80 broth for 11 days. The opacity of each culture was determined by nephelometry and viable counts performed in triplicate for each culture using the method of Miles–Misra on Stonebrink medium (Lesslie, 1959). The mean of the values were then recorded.

Experimental infection

1 ml aliquots of strain AF117/79 were added to 7H11 Tween 80 medium and cultured for 11 days, after which the opacity was measured and the viable count determined.

Experimental animals were inoculated i.p. with 0·5 ml of *M. bovis* strain AF117/79 containing between 10^3 and 10^4 organisms per ml. Control animals were inoculated with 0·5 ml of 7H11 Tween 80 broth.

Experimental animals

Rabbits.—Laboratory bred New Zealand Whites $2\frac{1}{2}$–3 kg in weight were used. Blood was obtained from the marginal ear vein.

Guinea-pigs.—Female Duncan/Hartley albino

animals 200–250 g in weight were used. Blood was obtained by cardiac puncture.

Rats.—The inbred F344 strain from the laboratory colony of rats 5 weeks of age were used and blood was obtained by cardiac puncture.

Ferrets.—8–10-week-old newly weaned ferret/polecats were obtained from a registered supplier. Blood was obtained by cardiac puncture.

Hedgehogs.—Wild hedgehogs were trapped in May/June around the Guildford–Worplesdon area in Surrey. They were housed in $18'' \times 12''$ rat cages with solid floors and sawdust. They were fed daily on bread and sterilized milk diluted with water. Blood was obtained by cardiac puncture.

Necropsy procedures

Post mortem examinations were carried out between 40 and 60 days after inoculation. Various tissues were fixed in 10% buffered formalin and embedded in paraffin wax, routinely processed and sections cut and stained with haematoxylin and eosin (H & E) and Ziehl–Neelsen's (ZN) stains. Mycobacterial isolation was attempted by direct culture and guinea-pig biological tests (Table V).

Intradermal tuberculin test

Bovine purified protein derivative (PPD) Batch 291 from *M. bovis* (Central Veterinary Laboratory, Weybridge) was used at 10, 50 and 100 μg/ml in saline. Animals were inoculated intradermally with 0·1 ml of PPD in the right flank or, in the case of hedgehogs, along the midline of the abdomen.

Groups of animals were skin-tested once only at 7, 15 or 36 days after inoculation and the reactions were evaluated at the site of inoculation by measuring the diameter of induration and erythema at various times between 16 and 70 h.

Lymphocyte transformation test

The test was based on the methods used by Morris *et al.* (1978). Medium 199 (Wellcome Laboratories), which contained 5% sodium bicarbonate, 10% foetal calf serum (Flow Laboratories) with added streptomycin (100 μg/ml) and penicillin (200 u/ml), was used as the lymphocyte culture medium. Immediately after preparation, the medium was passed through a 0·2 μm membrane filter and stored in tight-capped universals at 4°. The leucocytes were isolated from heparinized blood by the addition of 3 ml of a 3% w/v aqueous solution of dextran sulphate. The mixture was allowed to separate at 37° for 1–2 h. The separated leucocytes were washed 3 × in Hanks' solution containing 5 u/ml

heparin and resuspended in Medium 199 to 5×10^6/ml of viable cells.

Tests were performed in flat-bottomed micro-titre trays with separate lids (Sterilin products, Middlesex) in quadruplicate; 200 μl of cell suspension was added to the microtitre wells. Purified phytohaemagglutinin (PHA, Wellcome) at concentrations from 1 to 10 μg/ml of culture was used. Bovine PPD was used between 1 and 20 μg/ml of culture and sterile saline used for the unstimulated controls. The tests were incubated for 48 h at 37° in an atmosphere of 5% carbon dioxide in air. Tests were then pulsed with 0·5 μCi of tritiated thymidine (Radiochemical Centre, Amersham, s.p. act. 5 Ci/m mole) in 20 μl and incubated for a further 18 h. The cells were harvested on to glass-fibre discs using an automatic cell harvester (MASH II, Dynatech) and transferred to plastic mini vials (Koch-Light) and 3 ml of scintillation fluid (0·4% 2, 5-diphenyloxazole and 0·01% 1,4-di-(5-phenyloxazolyl)-benzene in toluene) The cells were counted for 5 min each on a Tracerlab Corumatic 200 liquid scintillation counter.

Geometric means were calculated from the quadruplicate samples and the stimulation index (SI) reported as:

$$SI = \frac{\text{Geometric mean count per min (ct/min) of stimulated cultures}}{\text{Geometric mean ct/min of unstimulated cultures.}}$$

Complement fixation test (CFT)

The test was carried out in round-bottomed microtitre plates (Sterilin Products, Middlesex), using four 50% haemolytic doses of complement (Tissue Culture Services, Slough). Washed $M.$ $bovis$ strain C13, heated at 60° for 30 min and standardized for the optimum concentration against a homologous high-titre serum, was used as antigen. The serum-complement antibody mixture was incubated for 3 h at 37° and the haemolytic system added; the test was read after a further 30 min at 37°. The haemolytic system was 1·5% sheep red blood cells (SRBC) sensitized with 5 minimal haemolytic doses (MHD) of horse haemolysin (Burroughs Well-come) for 1 h at 37°.

Passive haemagglutination test (PHT)

The tests were done in round-bottomed microtitre plates using 2% SRBC in PBS sensitized with equal volumes of 0·01% tannic acid at 37° for 15 min. Equal volumes of tanned red cells and antigen (bovine PPD at 50 μg/ml) were incubated at 37° for 30 min. All test serum dilutions were performed in phosphate-buffered saline (PBS) containing 1% normal rabbit serum (NRS) and incubated at 37° for 1 h followed by 2 h at 4°. The end-point was the highest dilution of serum causing complete haemagglutination.

Agar immunodiffusion test (ID)

Double diffusion in 1% tris barbital/sodium barbital agar pH 8·6 was performed on 4″ × 4″ glass slides. Bovine PPD 291 at 1 and 5 mg/ml
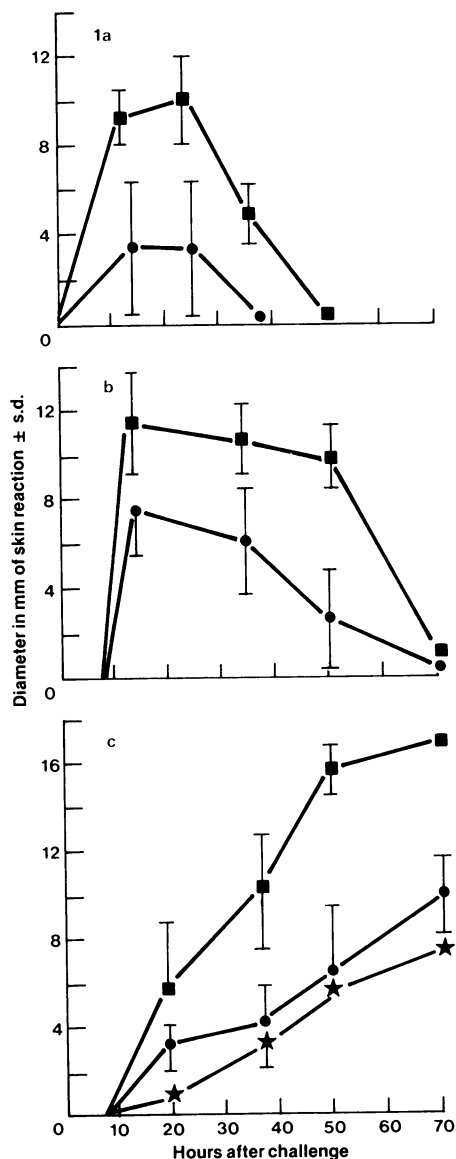

FIG. 1.—The time course of the skin reaction following challenge with 5 μg of $M.$ $bovis$ PPD 291 in guinea-pigs (■), rabbits (●) and ferrets (★), 7 days (a), 15 days (b) and 36 days (c) after infection with $M.$ $bovis$.

was used. The tests were incubated in a moist chamber at 4° for 48 h before reading.

RESULTS

Tuberculin test

The results are presented in Figure 1.

At 7 days after inoculation, rabbits and guinea-pigs produced a non-necrotic reaction comprising induration and erythema. The reaction was most marked between 16 and 24 h and then disappeared within 48 h with all the concentrations of bovine PPD used (Fig. 1a). This reaction closely resembled the Jones–Mote reaction previously described in man and guinea-pigs. Ferrets, rats and hedgehogs failed to produce a response with any concentration of bovine PPD 7 days after inoculation.

At 15 days after inoculation, rabbits and guinea-pigs produced a response similar to the 7-day reaction which was most marked between 20 and 36 h. This reaction was still present at 48 h but disappeared within 70 h (Fig. 1b) and resembled the Listeria-type reaction described in mice.

TABLE I.—*Lymphocyte transformation tests on lymphocytes from animals infected with* M. bovis

Animal species	Time tested	No. examined	(Stimulation index (SI) of cultures)		
			PPD (μg/ml)		PHA (μg/ml)
			10	5	5
Rabbit	A	9	0·45	0·23	8·68[a]
	B	9	5·27[a]	5·13[a]	15·36[a]
	C	—	NT	NT	NT
	D	6	1·62	1·92[a]	3·65[a]
Guinea-pig	A	9	0·51	0·81	4·74[a]
	B	9	0·70	0·53	4·18[a]
	C	—	NT	NT	NT
	D	6	1·88[a]	2·04[a]	5·37[a]
Ferret	A	9	0·80	0·91	19·31[a]
	B	9	1·59	1·58	2·0[a]
	C	9	0·78	0·88	3·34[a]
	D	9	1·06	0·85	7·59[a]
Rat	A	9	NT	NT	NT
	B	9	1·20	1·40	5·40[a]
	C	9	0·94	1·40	8·20[a]
	D	9	1·23	0·80	6·40[a]
Hedgehog	A	9	1·63	1·26	12·20[a]
	B	9	0·70	0·39	4·98[a]
	C	9	1·55	0·92	3·01[a]
	D	6	0·94	0·63	2·43

[a] Mean SI significantly different from control mean $P < 0·05$
A Before inoculation
B 7 days after inoculation (PI)
C 15 days PI
D 36 days PI
NT Not tested

TABLE II.—*Summary of immune response in different animals inoculated with* M. bovis

Animal species	Antibody response	7 days	CMI response 15 days	36 days	Bacilli in lesions
Guinea-pig	+	+	+	+	+
Rabbit	+ +	+	+	+	+
Ferret	+ +	—	—	+	+ + +
Rat	±	—	—	—[a]	±
Hedgehog	—	—	—	—	±

[a] Detectable after 42 days

Ferrets, rats and hedgehogs failed to produce any response 15 days after inoculation.

At 36 days after inoculation, rabbits, guinea-pigs and ferrets produced a reaction which was most marked at 70 h (Fig. 1c). Guinea-pigs and rabbits produced severe necrotic reactions as well as erythema and induration characteristic of the Koch-type reaction. Ferrets, however, exhibited less severe reactions without any necrosis.

Rats showed no reactions at 36 days. When re-tested at 42 days after inoculation, an atypical reaction which was most marked at 48 h was produced which con-sisted of diffuse erythema with little induration and no necrosis.

Hedgehogs failed to show any response up to 56 days after inoculation.

Lymphocyte transformation test

Only lymphocytes from rabbits and guinea-pigs responded significantly to the bovine PPD antigen used at 10 and 5 μg/ml ($P < 0.05$). Rabbit lymphocytes responded significantly at 7 and 36 days after inoculation whereas lymphocytes from guinea-pigs responded significantly at 36 days only (Table I). Results were not obtained from animals 15 days after inoculation.

Lymphocytes from all the species res-ponded significantly ($P < 0.05$) to the non-specific mitogen PHA (Table 1) through-out the duration of the experiment, although there was decreased lymphocyte transformation by PHA in inoculated ferrets and hedgehogs as compared with the pre-inoculation control group (Table I). Lymphocytes from the pre-inoculation control groups responded only to PHA.

Table III summarizes the CMI res-ponses of the animals inoculated with *M. bovis*. Guinea-pigs and rabbits pro-duced a CMI response as early as 7 days after inoculation and this continued to 36 days. No CMI response could be detected in ferrets and rats until 36 and 42 days respectively. No CMI response of any kind could be detected in hedgehogs throughout the duration of the experiment.

Antibody response

Figure 2 shows the PHT and CFT titres of sera from rabbits, guinea-pigs and ferrets inoculated with *M. bovis*. The immunodiffusion (ID) test was negative to all sera tested.

Table II summarizes the antibody response to *M. bovis* of the species examined. Rabbits and ferrets produced antibodies that reacted in CFT and PHT. Antibodies from guinea-pigs could only be detected in the PHT. Antibodies from rats were detected in the CFT but could only be detected after 42 days after inocula-
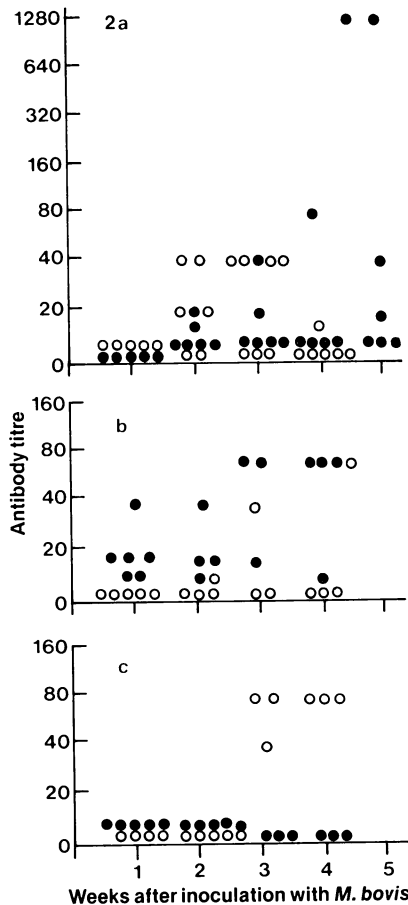

FIG. 2.—Antibody response of ferrets (a), rabbits (b) and guinea-pigs (c) following inoculation with *M. bovis* as detected by CFT (●) and PHT (○).

TABLE III.—*Size and distribution of macroscopic lesions in animals 6 weeks after inoculation with* M. bovis

Animal species	No. examined	Lung	Liver	Kidney	Spleen	Mesenteric lymph nodes and mesentery
Guinea-pig	5	L (2–5)	L (2–5)	NVL	L (3–5)	L (2–5)
Rabbit	5	D (2–4)	L (2–4)	L (3–5)	L (2–4)	D (1–4)
Ferret	6	D (0·5–1)	D (1)	L (1–2)	D (1)	D (1)
Rat	4	L (2)	NVL	NVL	NVL	NVL
Hedgehog	5	NVL	NVL	NVL	NVL	NVL

L Localized
D Diffuse
( ) Range of lesion diameter in mm
NVL No visible lesions

TABLE IV.—*Typical pathological findings in animals 6 weeks after inoculation with* M. bovis

Animal species	No. examined	Caseation	Lymphocytic infiltration	Epithelioid cells	Fibrous encapsulation
Guinea-pig	5	+	+	+ + +	+
Rabbit	5	+	+	+ + +[a]	+
Ferret	6	—	+ + +	+	—
Rat	4	—	+ +	+ + +	—
Hedgehog	5	—	±	+	—

[a] Giant cells of the Langhans type present

tion. Antibodies from hedgehogs could not be detected by any of the tests used.

Pathological findings

The size and distribution of macroscopic lesions are presented in Table III. The lesions in the guinea-pig were localized and ranged from 2 to 5 mm in diameter. Most lesions in rabbits were also localized but in the lung, mesenteric lymph nodes and mesentery the majority were diffuse and smaller in size (1–4 mm). Only lesions in guinea-pigs and rabbits were caseous and encapsulated. The lesions in ferrets were miliary and diffuse, and in many cases the minute soft white lesions coalesced to form foci of diameter 2–4 mm. Two of the rats examined were found to have greenish gritty lesions confined to the lung (1–3 per lung). No macroscopical lesions were found in the hedgehogs.

The histopathological findings are shown in Table IV. A granulomatous cellular response, including giant cells of the Langhans type and fibrous encapsulation, was seen in lesions in guinea-pigs and rabbits (Fig. 3). These lesions showed small numbers of acid-fast bacilli stained by Ziehl–Neelsen's method. Tuberculous lesions from ferrets were of a progressive type in which diffuse lymphocytic infiltration predominated. A clear granulomatous reaction was not evident. There was necrosis of the inflammatory cells with many nuclei showing pyknosis or karyorrhexis (Fig. 4). Ziehl–Neelsen staining of the sections revealed large numbers of acid-fast bacilli in the tissues and many of these were extracellular (Fig. 5).

The lesions in the rat resembled lesions in ferrets but more epithelioid cells were evident and very few intact acid-fast bacilli were seen.

In hedgehogs a few microscopical lesions were seen confined to the kidneys, they had a small, poorly defined focus comprising a few necrotic cells including polymorphonuclear leucocytes, bordered by small numbers of epithelioid cells (Fig. 6). Acid-fast bacilli were occasionally seen in tissues without accompanying pathological change both in the kidney and other organs examined.
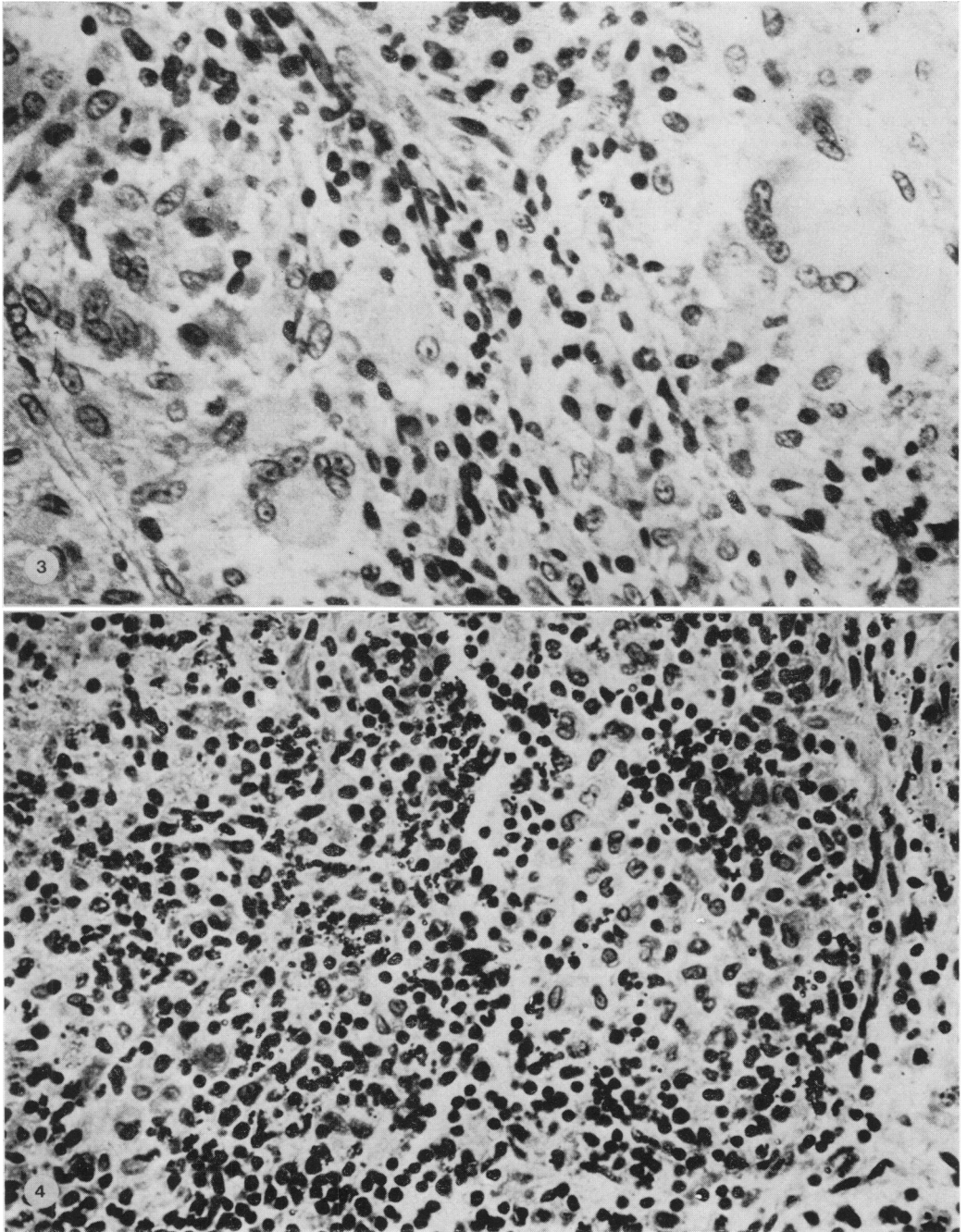
FIG. 3.—Lesion in a rabbit lung 6 weeks after inoculation with *M. bovis*. Giant cells, epithelioid cells and fibroblasts can be seen. H. & E. × 576.

FIG. 4.—Lesion in a ferret liver 6 weeks after inoculation with *M. bovis*. Intense lymphocytosis and necrosis of the inflammatory cells is present. H. & E. × 576.

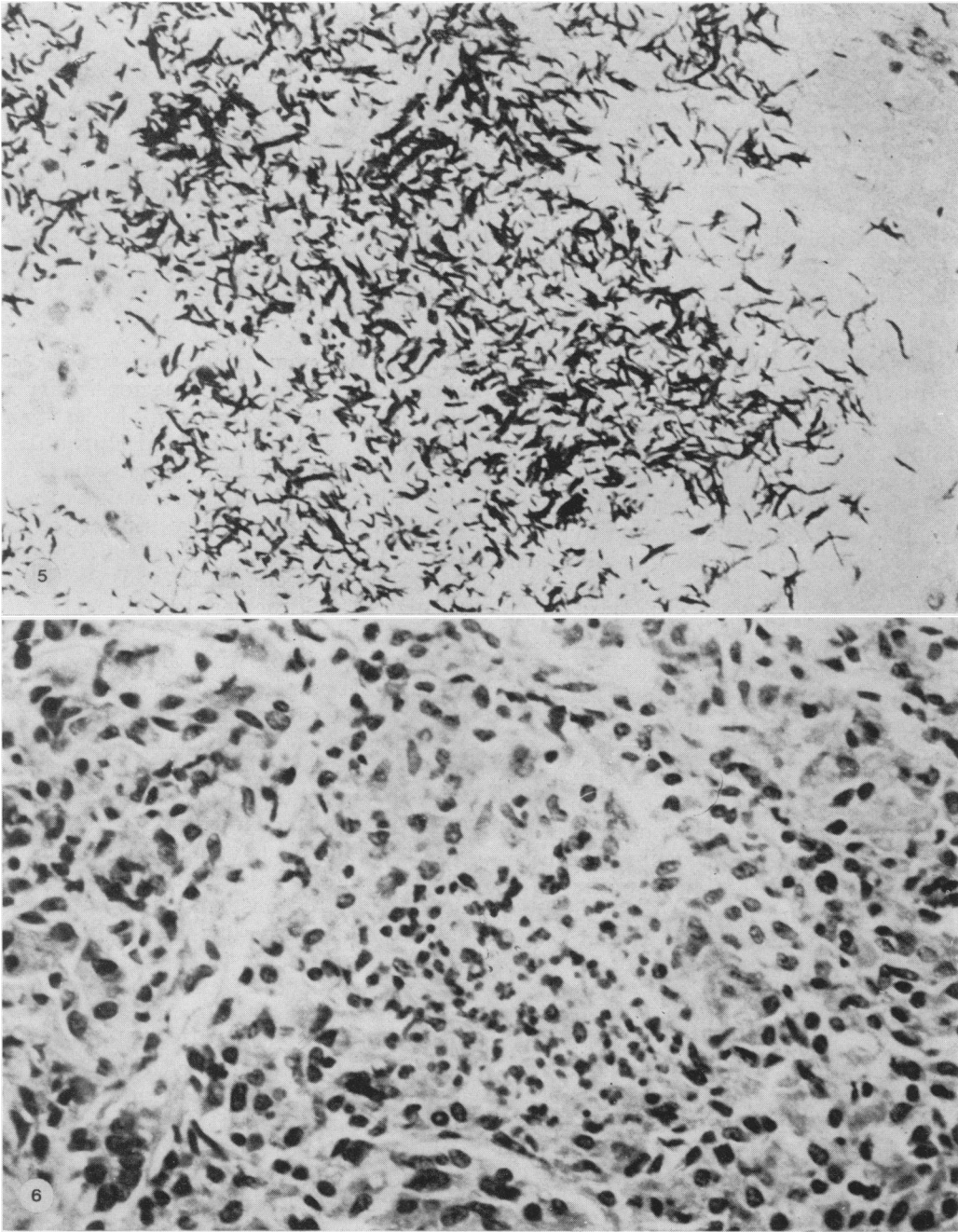FIG. 5.—Liver lesion in a ferret showing large numbes of acid-fast organisms. Z.N. × 576.
FIG. 6.—Kidney lesion in hedgehog 8 weeks after inoculation with *M. bovis*. Small numbers of polymorphonuclear leucocytes, lymphocytes and epithelioid cells can be seen. H. & E. × 576.

TABLE V.—*Distribution of acid-fast bacilli in organs 6 weeks after inoculation with* M. bovis

Animal species	Lung	Kidney	Liver	Spleen	Mesenteric lymph nodes
Guinea-pig	+ +	—	+ +	+ +	+ +
Rabbit	+ +	+ +	+ +	+ +	+ +
Ferret	+ + +	+ +	+ + +	+ + +	+ + +
Rat	+ +	—	—	—	—
Hedgehog	+	±	±	+	—

± Bacilli isolated only after guinea-pig had been inoculated with tissues
+ Culture + Ziehl-Neelsen–
+ + Culture + Ziehl-Neelsen +
+ + + Culture + + Ziehl-Neelsen + +

Distribution of acid-fast bacilli in the organs of inoculated animals

Table V summarizes the bacteriological findings. All the tissues examined from ferrets contained many acid-fast bacilli and only in kidneys was there a slight reduction in numbers, estimated by direct culture and Ziehl–Neelsen staining. The distribution and numbers of acid-fast bacilli in rabbits and guinea-pigs was similar although there were no detectable bacilli in the kidneys of guinea-pigs. Inoculated rats had acid-fast bacilli in their lungs only.

Small numbers of acid-fast bacilli, showing the typical cultural and morphological appearance of *M. bovis*, were isolated from the lungs, kidneys livers and spleen of inoculated hedgehogs. Mesenteric lymph nodes did not appear to be affected. In many instances the numbers were so small they could only be detected by guinea-pig biological tests.

DISCUSSION

Francis (1958) first postulated a relationship between the CMI and the number of bacilli occurring in the lesions of animals infected with *M. bovis*. This study provides experimental evidence for this relationship.

The species of animals chosen were carefully selected to represent, as far as possible, a wide variation of pathological conditions.

The in *vitro* transformation of lymphocytes from animals sensitized to myco- bacteria is now considered to be an expression of delayed hypersensitivity to that organism (Pearmain, Lycett and Fitzgerald, 1963; Seeger and Oppenheim, 1970; Rosenstreich and Rosenthal, 1974). It is also considered that mainly the T lymphocytes are directly stimulated by PHA in the transformation test (Greaves, Janossy and Doenhoff, 1974; Weksler and Kuntz, 1976; Brochier, 1977).

In the present study guinea-pigs and rabbits inoculated with *M. bovis* elicited all the CMI responses so far described: *i.e.* response measured at 7 days, equivalent to the Jones–Mote reaction; that measured at 15 days, equivalent to the Listeria-type reaction; and the response measured at 36 days, the Koch-type reaction. In *M. bovis*-infected guinea-pigs, lymphokines are not produced at the time of the Jones–Mote response and the lymphocyte transformation is low (Himeno *et al.*, 1977). The present study has also shown that lymphocyte transformation using specific antigens was absent in guinea-pigs 7 days after infection. In rabbits, on the other hand, lymphocyte transformation could be detected at this time. Thus it is important not to extrapolate from one animal species to another.

Ferrets and rats inoculated with *M. bovis* produced an unusual skin test reaction, which was only evident 30 days after inoculation. In both cases the time-course of the skin test indicated a Koch-type response, but no necrosis was evident and the lymphocyte transformation tests were negative throughout. Further work

is required to determine whether ferrets and rats produce specific suppressor cells capable of modulating the cell-mediated responses, thereby affecting the delayed type hypersensitivity reactions. The *in vitro* transformation of T lymphocytes from infected ferrets using PHA was markedly lower than the non-infected control animals, indicating a nonspecific suppression of T-cell function. There have been no previous reports on cell-mediated responses of ferrets infected with *M. bovis*, but mink, which are closely related to ferrets, do not react to tuberculin even when diagnosed as tuberculous (Pulling, 1952). Similarly, rats produce an "atypical" tuberculin response (Wessels, 1941) which is probably different from the Koch type described previously in guinea-pigs (Koch, 1891).

Hedgehogs inoculated with *M. bovis* failed to show any cell-mediated immune responses as detected by the skin test and lymphocyte transformation test. However, the nonspecific stimulation of T lymphocytes was lower in inoculated hedgehogs than in the controls. It is possible that suppressor lymphocytes are produced very early in the infection and they inhibit the cell-mediated immune responses throughout the infection.

When guinea-pigs, rabbits and ferrets were infected with *M. bovis*, all 3 species develop a generalized disease which usually killed the host. Strong necrotic Koch-type reactions were a major factor in tissue damage and disease in rabbits and guinea-pigs whereas high concentrations of organisms in the tissues of ferrets was probably an important factor in disease in this species. High numbers of *M. bovis* organisms inhibit lymphocyte transformation *in vitro* and are cytotoxic for ferret lymphocytes (Thorns, unpublished results). The numbers of organisms necessary to achieve this *in vitro* could well be present in many tissues from infected ferrets.

It appears that in some animals there is an inverse relationship between CMI and the number of organisms in tissues. In one group of animals, exemplified by ungulates and primates, the host's CMI response hyper-reacts and produces tissue damage, while in another, notably marsupials (Julian, 1977) and mustelids, little or no CMI occurs, allowing unrestricted multiplication of the bacilli. The relationship does not seem to be true for infected rats or hedgehogs because they harbour small numbers of the organism in their tissues while producing very little or undetectable CMI.

The reasons why some groups of animals react differently to others after infection with *M. bovis* are complex. However, variations in the genetics of the immune response and the processing of antigen by macrophages thereby affecting the type of CMI produced may be important. It is probable that rats and other species that are more resistant to the disease are able to modulate the cell-mediated immune response so that the protective Listeria-like reaction is not overwhelmed by the more harmful necrotic Koch-type reaction. It may be significant that degraded acid-fast bacilli were seen associated with macrophages in many sections of infected rat tissues (Thorns, unpublished results).

It seems that hedgehogs are unable to mount detectable CMI to *M. bovis* infection and yet they do not develop clinical disease. Probably other nonspecific factors are involved that restrict multiplication of the organism in the tissues of these animals. The body temperature of hedgehogs varies between 33° and 38° and is a few degrees lower during hibernation. *M. bovis* grows very slowly at 34° *in vitro* and this may be one such factor.

The variability of the antibody response in the animals studied suggests that current serological tests have no apparent practical value in the diagnosis of tuberculosis which is almost certainly modulated by T cells (MacKaness, 1971; North, 1974). However, recent reports on the blocking of some cell-mediated responses by products present in serum of anergic patients should not be dismissed (Roupe

and Strannegard, 1972; Dreschage, Blomberg and Flier, 1980) and may constitute the basis of a test for the diagnosis of tuberculosis in certain humans and animals that otherwise might prove difficult.

Koch's original statement, almost 100 years ago, on the diversity of tuberculosis in animals is still very true today. It is possible that tuberculosis manifests itself in more ways and in a greater variety of animal species than any other infectious disease known to man. It is therefore extremely important when describing certain aspects of the disease that comments should be confined to the animal species being examined and that generalizations should not be made between species.

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