THE PHAGOCYTIC BASIS OF ACQUIRED RESISTANCE TO INFECTION WITH *DERMATOPHILUS CONGOLENSIS*

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In the scarified skin of normal rabbits, resistance to infection by *Dermatophilus congolensis* depends on cellular infiltration of the affected area. The resistance is due partly to the phagocytosis of zoospores, which are thus prevented from infecting the broken edges of the epidermis, and partly to the inhibitory effect of the layer of granulocytes that forms beneath the scarification and prevents invasion of the dermis proper by hyphae derived from zoospores that escape phagocytosis. The natural resistance of rabbits is abolished by depletion of circulating granulocytes with nitrogen mustard (Roberts, 1965*c*).

It is shown here that sheep and guinea-pigs are normally almost as susceptible as agranulocytic rabbits but that, after vaccination, they are as resistant as normal rabbits. This acquired resistance is inactive against infection of the unbroken skin, where there is no opportunity for phagocytosis, and is dependent on enhanced destruction of ingested zoospores, a process that is ineffective without induced antibody within the phagocytes of sheep and guinea-pigs.

In animals in the state of delayed hypersensitivity to *D. congolensis* the extent of infection is not decreased but the ensuing invasion of follicle sheath tissue is inhibited ; circulating antibody has no inhibitory effect (Roberts, 1966).

MATERIALS AND METHODS

Organisms.—Strains of *D. congolensis* were isolated from naturally infected sheep in Australia. Strain 66 was used for both vaccination and test infection except where otherwise indicated.

Vaccination.—Intramuscular injections of vaccine in complete Freund's adjuvant were followed by intravenous zoospores as previously described (Roberts, 1966).

Measurement of resistance to infection.—Ten-fold dilutions of zoospore suspensions were applied to areas of skin prepared in one of several ways. In the case of scarification the highest dilution to produce confluent infection of at least 2 out of 3 scratches was taken as the end-point (Roberts, 1965*c*). In sheep, the highest dilution to infect at least one wool follicle per sq. cm., in an area depilated by plucking, was taken as the end-point and, in areas de-fatted with light petroleum, the highest to produce at least 4 focal lesions per sq. cm.

Agglutination tests.—Somatic and flagellar agglutination tests were conducted as described by Roberts (1965*a* and 1966). Rabbit sera were heated at 70° for 10 min., to decrease the natural antibody titre. The strength of antisera is expressed in terms of the reciprocal of the somatic agglutinin titre.

Immediate cutaneous anaphylaxis.—Tests for active cutaneous anaphylaxis (Ovary, 1958) were performed as previously described (Roberts, 1966).

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Serum bactericidal tests.—Sera were freshly collected from normal and vaccinated guinea-pigs and half of each sample was heated at 56° for 30 min. to inactivate heat-labile components. The fresh and inactivated portions were then added in 1·8 ml. volumes to 0·2 ml. of a suspension of zoospores in saline, in tubes of 8 mm. int. diam. The tubes were continually agitated for 3·5 hr. in a water bath at 37°. Samples of 0·1 ml. undiluted or diluted 10-fold were then spread on 10 per cent serum agar plates and colony counts made after 2 days' incubation in 10 per cent CO_2 at 37°.

Depletion of granulocytes with nitrogen mustard.—" Mustine Hydrochloride " (Boots), 2·0 mg. per kg., was injected into rabbits as previously described (Roberts, 1965*c*).

Phagocytosis in vivo.—At 2, 4 or 6 hr. after the application of suspensions of $1–5 \times 10^8$ zoospores per ml. to areas of scarified skin, guinea-pigs and rabbits were killed and the affected areas immediately excised and fixed in Bouin's solution. Paraffin sections were stained with Giemsa.

Phagocytosis in vitro.—Films of leucocytes were prepared by the method of Lachmann (1961). Drops of blood from the ear veins of normal animals were allowed to clot for 30 min. on glass slides in moist chambers at 37°. The clots were lifted away and the residual red cells washed from the adherent leucocytes with the suspensions of spores that were to be used in the tests. Suspensions of $1–5 \times 10^8$ spores per ml. in whole normal serum or antiserum were added to the films of leucocytes and cover-glasses applied. After very light blotting the slides were returned to the moist chambers and incubated in 10 per cent CO_2 at 37°. After the required period, the cover-glasses were removed and the films air-dried and stained with Giemsa. To avoid effects due to flagellar agglutinins in the antisera, spores of the non-motile variant of strain 47 were used.

Leucocidal activity of D. congolensis.—Preparations were made as for the study of phagocytosis in normal serum, but with typical motile strains of the organism. After incubation for 1 hr., the cover-glasses were removed and the suspensions of organisms washed off and replaced with 0·5 per cent trypan blue in equal parts of normal guinea-pig serum and isotonic buffered saline (9 volumes 0·85 per cent NaCl to 1 volume isotonic phosphate buffer, final pH 7·4). Incubation was then continued with hourly microscopic examination to estimate the viability of the leucocytes as indicated by their inability to take up the dye.

Tests of contingency.—Fisher's (1954) exact test was applied to 2×2 contingency tables.

RESULTS

Specific immunity to infection of broken skin surfaces

Vaccination.—Vaccinated sheep and guinea-pigs were partly protected against infection of the broken skin surface (Tables I and II). The average concentration of zoospores required to produce confluent infection of scarifications was increased between 6- and 80-fold by vaccination in guinea-pigs, and between 7- and 32-fold in sheep. In sheep depilated by plucking, the concentration required to infect at least one wool follicle per sq. cm. was increased 22-fold.

In terms of infecting dose normal rabbits proved as resistant to the infection of scarified skin as vaccinated sheep and guinea-pigs, but resistance was not altered by vaccination (Table III).

Previous infection.—Guinea-pigs infected once, 2 weeks previously, were as susceptible as normal controls (Table I). The fact that a single infection induces delayed hypersensitivity without detectable circulating antibody in guinea-pigs (Roberts, 1966) suggests that the increased resistance of vaccinated animals is associated with circulating antibody rather than delayed hypersensitivity.

Passive immunization with antiserum.—That the protective effect of vaccination is due to antibody is evident from the result of testing guinea-pigs one day after an intravenous injection of 10 ml. guinea-pig antiserum with a somatic agglutinin titre of 2000 ; their resistance was increased 6-fold (Table I).

TABLE I.—*Resistance to Infection in Guinea-Pigs Previously Infected, Vaccinated or Passively Immunized*

Experiment:	1			2			3		4		
Group	Control	Infected once	Vaccinated	Control	Infected once	Vaccinated	Control	Infected twice	Control	Passively immunized	Vaccinated
Mean somatic agglutinin titre	<25	<25	100	<25	<25	200	<25	<25	<25	200	400
Mean dose (log_{10} zoospores per ml.) to to produce confluent infection	5·0	5·25	6·9	5·2	5·2	6·5	5·5	5·3	5·0	5·8	6·3
Number with confluent infection/number in group at the dose stated	8/8	8/8	2/8	5/6	5/6	0/4	7/8	6/6	10/15	3/11	1/6
Dose (per ml.)		10^6			10^5			10^6		10^5	
P, comparison with controls by χ^2 test		—	0·004		—	0·03		—	—	0·02 < P < 0·05	

TABLE II.—*Resistance to Infection in Vaccinated Sheep*

Experiment:	1		2				3				4			
Group	Controls	Vaccinated	Controls		Vaccinated		Controls		Vaccinated		Controls		Vaccinated	
Mean somatic agglutinin titre	800	40,000	400		6,400		400		8,000		400		4,000	
Method of skin preparation	Wool plucked	Wool plucked	Defatted	Scarified	Defatted	Scarified	Defatted	Scarified	Defatted	Scarified	Defatted	Scarified	Defatted	Scarified
Mean dose (log_{10} zoospores per ml.) producing specified degree of infection (see p. 2)	4·9	6·25	4·3	4·8	4·5	6·3	5·0	4·5	5·2	5·8	6·8	5·2	6·5	6·0
Number with specified degree of infection/number in group, at dose 10^5/ml.	5/6	0/6	6/6	5/6	5/6	0/6	4/6	5/6	2/6	2/6	1/6	5/6	0/6	0/6
P, comparison with controls	—	0·008		—		0·008		—				—		0·008

TABLE III.—*Effect of Vaccination or Antiserum in Normal Rabbits or Rabbits Depleted of Granulocytes*

Experiment :	1		2		3				4		
Circulating granulocytes	Normal		Normal		Normal		Depleted		Normal	Depleted	
Immunization	Nil	Vaccinated	Nil	Vaccinated	Nil	Vaccinated	Nil	1 Vaccinated* 2 given antiserum	Nil	Nil	Vaccinated
Mean somatic agglutinin titre	50	400	25	800	50	800	50	200	Not tested	Not tested	800
Mean dose (log_{10} per ml.) producing confluent infection	6·5	6·75	6·25	6·25	6·25	6·25	4	4	5·25	4·25	4·25
Number with confluent infection/number in group, at the dose stated	3/4	2/4	3/4	2/4	0/4	0/4	2/3	3/3	0/4	3/4	3/4
Dose (per ml.)	10^6		10^6				10^4			10^4	
P, for association between confluence and depletion of granulocytes	—		—				0·003			0·03	

* The vaccinated rabbit and the 2 injected with antiserum gave identical results in the susceptibility test.

Specific immunity to infection of unbroken skin surfaces

When the sebaceous wax is removed from areas of woolled skin with light petroleum, the application of zoospore suspensions results in intense hyphal invasion of the undamaged epidermis (Roberts, 1963). Resistance to infection of this kind was not affected by vaccination (Table II). In the 3 experiments the infective dose was increased on the average by a factor of only 1·1. This suggests that the factors increasing resistance in broken skin do not operate in the intact epidermis.

The role of antibodies and hypersensitivity

Immediate-type anaphylactic hypersensitivity.—Immediate-type hypersensitivity is well developed in guinea-pigs that have been infected twice (Roberts, 1966), but there was no increase in their resistance to infection of the scarified skin (Table I). Moreover there was no reaction of the immediate type to infection even in animals made anaphylactic by vaccination. That is, there was no increased vascular permeability detectable in the skin around needle punctures or scratches swabbed with 10^9 zoospores per ml. up to 8 hr. before the intravenous injection of dye, although the reaction to intradermally injected antigen was positive.

Delayed-type hypersensitivity.—The absence of increased resistance from animals hypersensitized by previous infection indicate that the increased resistance of vaccinated animals is not due to a reaction of the delayed type.

Induced flagellar agglutinins.—The flagellar agglutinin response is well developed in guinea-pigs that have been infected twice (Roberts, 1966) but there was no increase in their resistance to infection. In Experiment 2 (Table I), the guinea-pigs were vaccinated with strain 66 and their resistance tested with strain 18, whose flagella have only a minor antigenic relation to those of strain 66. The 20-fold increase in resistance of the vaccinated group was very close to the average for the homologously vaccinated groups, suggesting that the protective effect of vaccination is due to antibody other than flagellar agglutinin, and to a reaction other than the rapid immobilization of zoospores by homologous flagellar antibody observable *in vitro* (Roberts, 1964).

The relevance of motility to infectivity was further tested with strain 47 and a non-flagellated non-motile variant of it. The infectivity of spore suspensions of the two strains, of approximately equal viability, was compared on opposite sides of 6 normal guinea-pigs. In each animal, the minimal dose producing confluent infection of scarified skin was the same for both strains, averaging 10^5 per ml. It follows that motility does not contribute to infectivity under the test conditions, and that the immobilization of zoospores is unlikely to be responsible for any of the increased resistance associated with circulating antibody. The increased resistance is therefore attributable to somatic antibody, although not necessarily to somatic agglutinin.

Natural somatic agglutinins.—Natural somatic agglutinin was present in all three species tested. Normal guinea-pigs had titres of 5–25, rabbits 100–400, and sheep 200–1600. These values were obtained with animals which, being reared indoors, were unlikely to have been infected with *D. congolensis*. In any event infection is a very poor stimulant of somatic agglutinin. Only in the rabbit was the natural antibody distinguishable from induced antibody by its instability at 70°.

Natural antibody appears to be irrelevant to natural resistance to infection because sheep, with the highest titres, are the most susceptible of the three species, and rabbits, with titres only 1–2 dilutions lower, are the most resistant (Table V).

Induced somatic agglutinins.—In guinea-pigs appreciable amounts of somatic agglutinin were induced by vaccination, but not by infection on 1 or 2 occasions. Increased resistance to infection was similarly inducible by vaccination only (Table I) indicating an association between induced somatic agglutinin and increased resistance to infection.

The absence of antibacterial factors from normal sera and antisera

The rapid growth of *D. congolensis* in fresh normal serum or plasma (Roberts, 1965*c*) was also observed in fresh sheep and rabbit antisera to the homologous strain. Moreover when organisms from old cultures were used as the inoculum there was a lag of about 24 hr. before growth began in normal serum, but no apparent lag in antiserum probably as the result of a concentration of intermediate metabolites (Hinshelwood, 1946) within clumps of agglutinated organisms. Antibody is therefore unlikely to inhibit the germination of zoospores or the growth of their hyphae.

To test the ability of antiserum to decrease the viable count of a suspension, zoospores were added to 6 replicate samples of fresh or heated guinea-pig normal sera or antisera at a final concentration of 10^5 per ml., and the suspensions plated after 3·5 hr. incubation. The number of colonies from the antisera was about 30 per cent less than from normal sera. The difference is statistically significant ($t = 4{\cdot}1$ with 10 degrees of freedom ; $P < 0{\cdot}01$), but is too small to explain the 6- to 80-fold increase in resistance after vaccination. The decrease in numbers was probably due not to a bactericidal effect but to a low degree of agglutination which would also explain the similar but smaller difference between the heated sera.

TABLE IV.—*The Survival of Zoospores after 3·5 hr. in Fresh or Heat-inactivated Sera from Normal or Vaccinated Guinea-pigs*

	Mean and S.E. number of colonies (6 plates) from serum containing 1000 zoospores	
	Normal sera	Antisera
Fresh, unheated	134±6	97±6
Heated 56° 30 min.	91±7	82±11

The higher rate of survival in the unheated antisera indicates that heat-labile antibacterial factors of the kind described by Skarnes and Watson (1957) did not contribute to the decrease in count. On the other hand, the effect of heat-inactivation, especially in the case of normal serum ($t = 4{\cdot}4$ with 10 degrees of freedom ; $P < 0{\cdot}01$) suggests that serum contains a heat-labile factor that helps zoospores to maintain their viability. The presence of such a factor was also suggested by the appreciably greater size of the 24 hr. old colonies on plates sown with organisms from the unheated sera. The difference in colony size is not attributable to the small amount of serum in the inoculum because the agar contained 10 per cent unheated serum.

It is therefore very unlikely that the decrease of infection in the presence of

antibody is due to either a reduction in the number of infective particles, or an inhibition of invasion, by serum antibacterial factors alone.

Phagocytosis and circulating antibody

In vivo.—Vaccination does not affect the intense proliferation of hyphae in the epidermis and dermis in rabbits depleted of granulocytes with nitrogen mustard before infection (Roberts, 1966). Neither vaccination nor passive immunization with antiserum modified the very high susceptibility of depleted rabbits to infection (Table III). This suggests that the protective effect of circulating somatic antibody in the naturally more susceptible species, sheep and guinea-pigs, is dependent upon the presence of granulocytes, and that the failure of vaccination to increase the resistance to infection of unbroken skin in sheep may be due to the absence of granulocytes from the unbroken skin surface. It appears therefore that the protective effect of circulating antibody in sheep and guinea-pigs might depend on an enhancement of the phagocytosis of infecting zoospores by the granulocytes.

In the normal rabbit a large proportion of the zoospores in suspensions applied to scarified skin are ingested by granulocytes that destroy them or inhibit their growth (Fig. 1 and 2). The natural resistance of rabbits is due largely to this process (Roberts, 1965*c*). The normal guinea-pig was found to be less resistant to infection than the normal rabbit (Table V). The difference in resistance was associated with very inefficient phagocytosis *in vivo*, apparent not in the low rate of ingestion of zoospores but in the failure of the phagocytes to inhibit germination and hyphal growth. There was almost no evidence of intraphagocytic growth at 2 hr. but numerous budded forms were evident within phagocytes at 4 hr., their hyphae often protruding from the cells (Fig. 3 and 4). By 6 hr. most phagocytes were no longer recognizable.

The capacity of phagocytes in the scarified skin of guinea-pigs to kill or inhibit ingested zoospores was greatly increased by vaccination or the intraperitoneal injection of 5 ml. sheep antiserum with a somatic agglutinin titre of 4,000, 24 hr.

EXPLANATION OF PLATES

FIGS. 1.–6.—Photomicrographs of sections of skin removed at different intervals after scarification and the application of *D. congolensis* zoospores. The sections were made at right angles to the scarifications and stained with Giemsa.

FIGS. 1. and 2.—Phagocytes in the depths of lesions in the skin of normal rabbits at 4 hr. Hyphae have not developed from the ingested zoospores. ×1250.

FIGS. 3 and 4.—Phagocytes in the skin of normal guinea-pigs at 4 hr. Hyphae from the ingested zoospores have grown out into the surrounding tissues. ×625.

FIG. 5.—A phagocyte in the lesion of a vaccinated guinea-pig at 4 hr. Hyphae have not developed from the phagocytosed zoospores. ×1250.

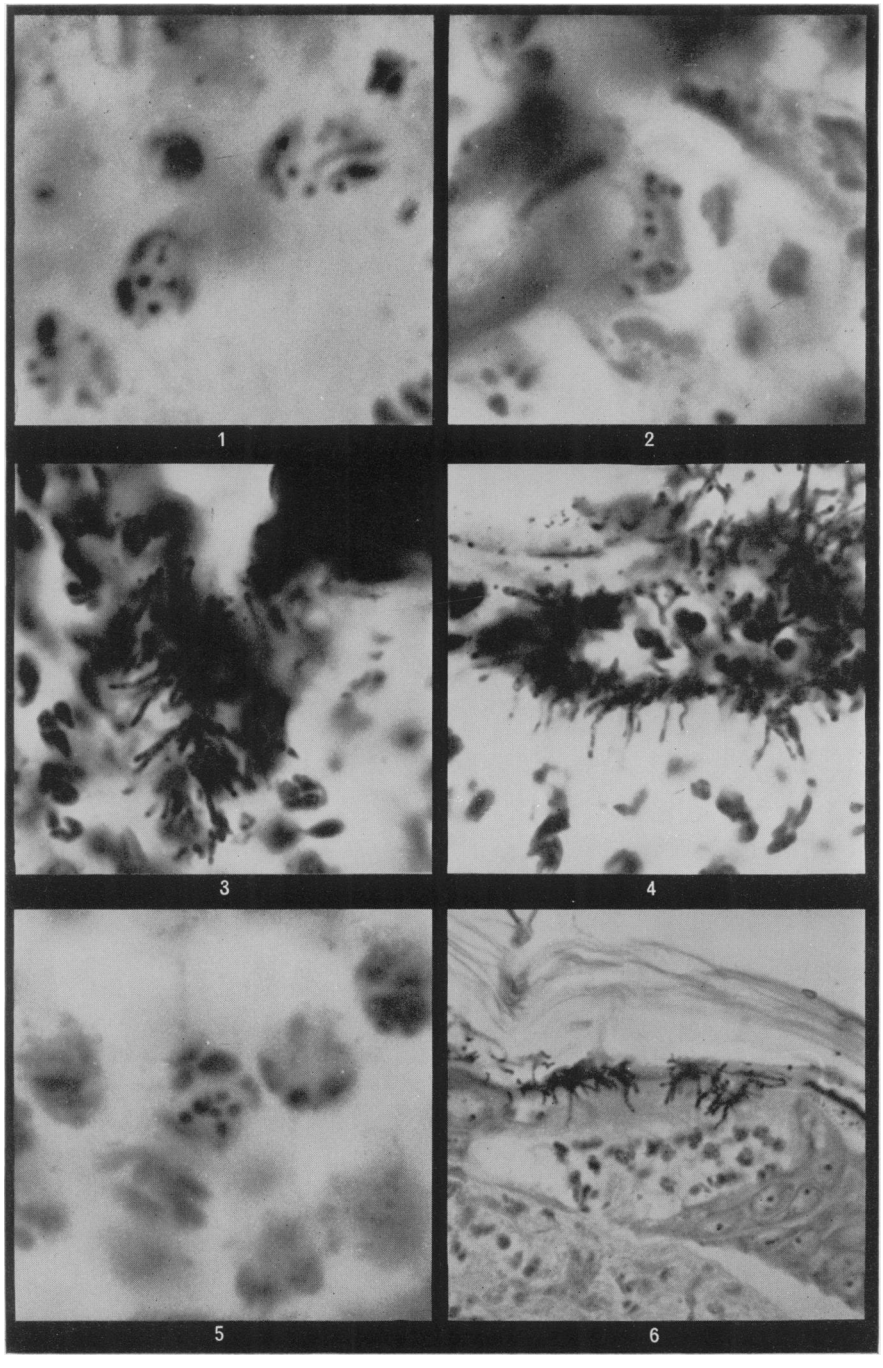
FIG. 6.—Intense hyphal invasion of epidermis that has not been broken sufficiently to allow contact between organisms and phagocytes, in a vaccinated guinea-pig at 6 hr. ×250.

FIGS. 7–12. *In vitro* phagocytic preparations air-dried and stained with Giemsa after incubation for 4 hr.

FIGS. 7 and 8.—Rabbit phagocytes in normal sheep serum. The ingested organisms, most of which had begun to form hyphae, have failed to develop further and have almost completely lost their affinity for the stain. ×1250.

FIGS. 9 and 10.—Guinea-pig phagocytes in normal sheep serum. The organisms are developing freely and staining intensely. ×1250.

FIGS. 11 and 12.—Guinea-pig phagocytes in sheep antiserum. The inhibition and destruction of ingested organisms appears to be nearly as efficient as in the rabbit cells in normal serum (Figs. 7 and 8). ×1250.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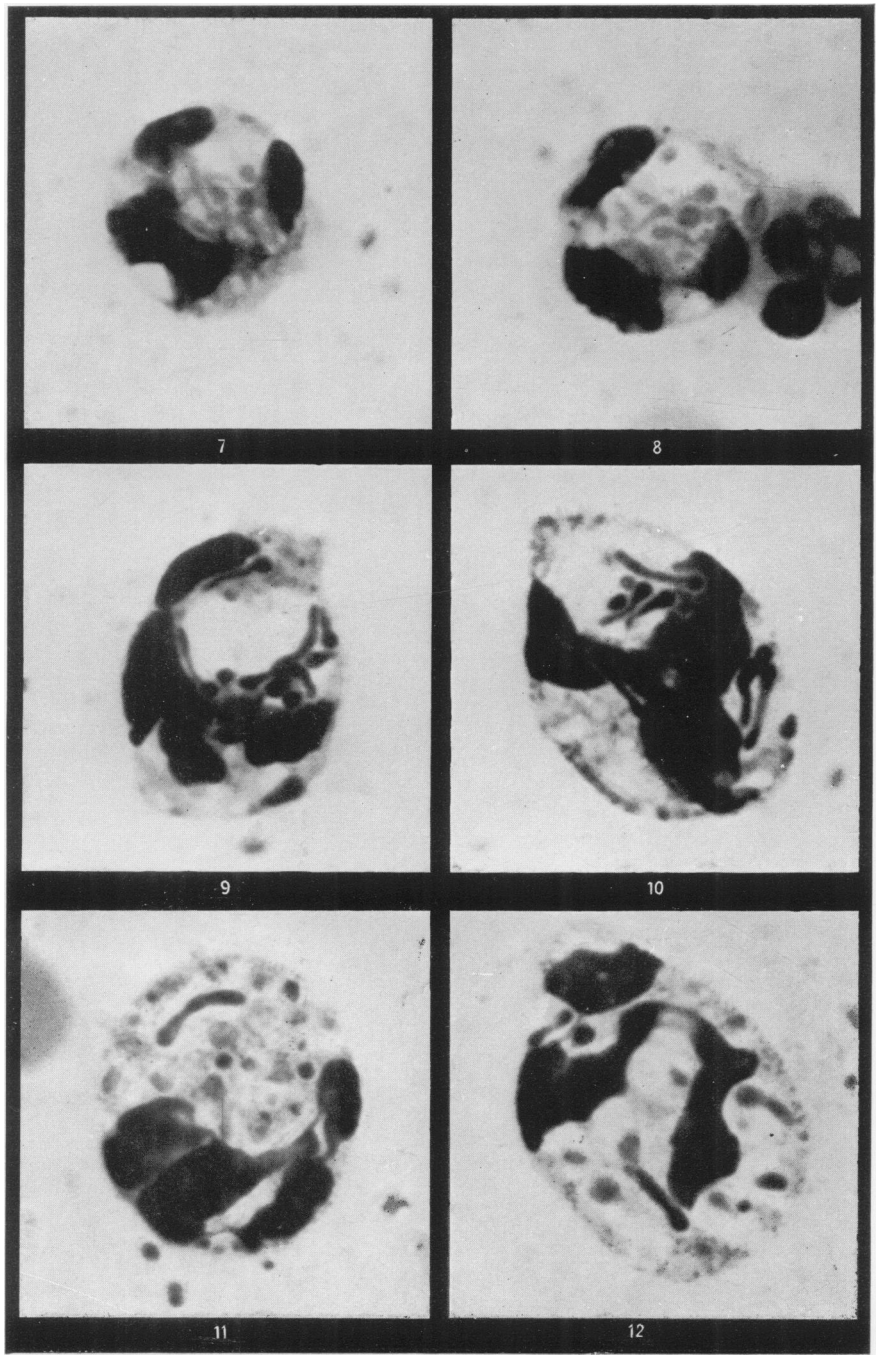
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TABLE V.—*The Association between Resistance to Infection by Zoospores and the Efficiency of Phagocytes* In Vivo *and* In Vitro

Species :	Rabbit		Guinea-Pig			Sheep	
				Present			
Induced antibody	Nil	Present	Nil	Vaccinated	Passive	Nil	Present
Mean dose (log_{10} per ml.) producing confluent infection of scratches*	6·0	6·4	5·2	6·6	—	4·8	6·0
Percentage of scratches showing intraphagocytic hyphal growth (mean and S.E.)	0	0	82±9	9±5	10±3	Not tested	Not tested
Difference compared to controls (mean and S.E.)		0		73±10	72±9		
Significance by *t*-test				$P<0·001$	$P<0·001$		
Percentage of ingested organisms growing in phagocytes *in vitro* (mean and S.E.)	7·5±0·5	10·3±2·7	60±4	23±7		60±7	14±2
Difference compared to controls (mean and S.E.)		−2·8±2·7		37±8		46±7	
Significance by *t*-test		$0·3<P<0·5$		$0·01<P<0·02$		$0·002<P<0·01$	

* Average of the mean values in Tables I, II and III.

earlier. In both cases phagocytosis was nearly as efficient as in the normal rabbit (Fig. 5). The effect was measured in sections of skin taken at 4 hr., by determining the percentage of scarifications in which hyphal growth was visible within phagocytes. The percentages for normal rabbits and guinea-pigs and for immunized guinea-pigs are shown in Table V. Scarifications were omitted from the count if the needle had not penetrated the epidermis sufficiently to allow contact between zoospores and phagocytes, or if the epidermis had been torn away so as to expose the dermal tissue to desiccation.

The failure of antibody to increase the resistance of the defatted, unbroken skin of sheep, was also evident in sections of guinea-pig skin in areas where the needle had damaged the stratum corneum but not the living cells beneath. Hyphal invasion of these areas was equally intense in vaccinated, passively immunized, and control guinea-pigs, supporting the view that increased resistance requires contact between zoospores and phagocytes (Fig. 6).

In vitro.—Observations were made after 4 hr. incubation, this being the period for maximum phagocytosis *in vivo*. In each preparation each ingested spore was classified either as "growing", *i.e.* staining normally after having formed a bud, or as "inhibited or destroyed" (Fig. 7 to 12), and (Table V) differential counts were made with sheep, guinea-pig, and rabbit cells, in sheep antiserum (somatic agglutinin titre, 4000 to 6000) or normal serum (titre, 400 to 800 due to natural antibody).

With the cells of rabbits and guinea-pigs the results obtained *in vitro* were very similar to those *in vivo*. The phagocytes of rabbits dealt effectively with ingested spores, even in the absence of induced somatic antibody (Fig. 7 and 8), whereas the phagocytes of guinea-pigs were quite ineffective against ingested organisms except in the presence of antibody, which made them as efficient as

rabbit cells (Fig. 9 to 12). In the case of sheep, which resemble guinea-pigs in their low resistance to infection in the absence of induced antibody, the cells were very similar in behaviour to the guinea-pig cells.

Normal sheep sera with titres of 400–800 did not promote the inhibition or destruction of ingested spores by sheep or guinea-pig phagocytes, suggesting that antibody does not assist phagocytosis in these species. The rabbit cells, by natural contrast, were virtually as efficient in dealing with spores in normal sheep serum as they were *in vivo*, indicating that the serum was not inhibitory to phagocytosis.

Absence of anti-leucocytic factors in D. congolensis

After exposure *in vitro* either to zoospore suspensions in rabbit, sheep, or guinea-pig serum, or to overnight cultures of *D. congolensis* in guinea-pig serum, granulocytes did not become permeable to trypan blue or lose structure in numbers greater than in controls with serum alone. At 6 hr., when about half the granulocyte population was swollen and permeable to the dye, cells engaged in phagocytosis of zoospores or hyphae were very rarely so affected. *D. congolensis* therefore probably has no leucocidal activity.

DISCUSSION

High titres of induced, as distinct from natural, antibody to *D. congolensis* are associated with an increase of resistance to infection of scarified skin by zoospores averaging 16-fold in both sheep and guinea-pigs (Table V). The increase of resistance after antiserum injection and its absence after a previous infection, which induces delayed hypersensitivity but no appreciable circulating antibody, indicate that the increase is due to circulating antibody.

Although the rapid immobilization of zoospores by homologous antiserum containing flagellar antibody is readily demonstrable *in vitro* (Roberts, 1964), it apparently does not contribute to the increase in resistance to infection. This is evident both in the cross-protection provided by vaccination with a strain of different flagellar antigen, and in the finding that a non-motile variant is as infective as the motile parent strain.

An immediate cutaneous anaphylactic response to infection might increase resistance by increasing or accelerating the local exudative reaction. Increased resistance, however, is not regularly associated with the presence of immediate-type hypersensitivity, and there was no immediate reaction to the artificial infection detectable in vaccinated animals that were anaphylactically hypersensitive.

The absence of any bactericidal or bacteriostatic activity in fresh undiluted antiserum, and the failure of antibody to restore any of the natural resistance of rabbits depleted of granulocytes, indicate that the increase of resistance due to somatic antibody does not depend on the neutralization of noxious products of the organism or on any other mechanism not involving the granulocytes—a finding consistent with the failure of antibody to increase resistance where the epidermis is not sufficiently damaged to permit contact between infecting organisms and infiltrating leucocytes, as in the de-fatted skin of sheep and in the skin of guinea-pigs where scarification has not completely penetrated the living epidermis.

The relatively high natural resistance of rabbits to infection of scarified skin and its dependence on circulating granulocytes were established by the demon-

stration of effective phagocytosis of zoospores by granulocytes both in scarified skin and *in vitro*. The failure of antiserum to increase the efficiency of the process *in vitro* was consistent with the finding that vaccination of rabbits, though it stimulates somatic antibody formation, does not increase resistance.

The low natural resistance of sheep and guinea-pigs, and the higher resistance induced in these species by vaccination, are also explicable in terms of phagocytosis. Numerous zoospores are taken up by the phagocytes of sheep and guinea-pigs in the absence of induced somatic antibody but are not killed inside the cells—as indicated by their rapid intracellular growth. In the scarified skin of normal guinea-pigs the hyphae from ingested zoospores penetrate the phagocytic cell membrane and invade the surrounding tissues. In the presence of somatic antibody however, the phagocytes of sheep and guinea-pigs become almost as effective as rabbit phagocytes in killing ingested organisms.

The difference in natural resistance between the rabbit on one hand and the sheep and guinea-pig on the other is therefore attributable to the superior ability of the granulocytes of rabbits to complete the process of phagocytosis. The failure of vaccination to increase the natural resistance of the rabbit is apparently a result of the naturally high destructive power of its phagocytes, which is not further improved by antibody, and suggests that antibody does not contribute to resistance through any other mechanism. By contrast, the poor capacity of sheep and guinea-pig granulocytes to deal with ingested zoospores is augmented by antibody, and as a result resistance to infection is increased by vaccination. The dependence of the acquired resistance on phagocytosis in these two species is confirmed by the finding that antibody increases resistance only where there is sufficient epidermal damage for the zoospores to come into contact with phagocytes. Its dependence on circulating antibody, rather than on a form of cellular immunity, is confirmed by the phagocytic competence of the cells of normal sheep and guinea-pigs in the presence of antiserum both *in vivo* and *in vitro*.

Natural somatic agglutinin to the zoospores of *D. congolensis* may be present in large amounts, particularly in sheep, but its presence is quite unrelated to natural resistance. Moreover, sheep and guinea-pig phagocytes do not inhibit or destroy ingested zoospores in the presence of a high concentration of natural agglutinin of the sheep. It is possible, though not proven, that natural antibody may facilitate the ingestion of zoospores in each of the species, and in the rabbit may contribute to their destruction, but it apparently lacks the capacity of induced antibody to enhance the destruction of intraphagocytic zoospores in the sheep and guinea-pig.

In sheep and guinea-pigs the immunity to infection of scarified skin is by no means solid ; even in specifically immunized animals with high titres of induced antibody, resistance is increased only about 16-fold. This is probably because some zoospores infect superficial parts of the broken epidermis where they do not come into contact with phagocytes. In addition, there is no increase in the resistance of unbroken skin, which in sheep appears to be infected frequently under natural conditions (Roberts, 1963). Vaccination of sheep therefore seems unlikely to be of great value in decreasing the incidence or severity of primary acute infection, though it may prove to be of value in preventing acute infections from becoming chronic (Roberts, 1966).

It appears from *in vitro* experiments that *D. congolensis* has no leucocidal or other adverse action on phagocytes. This supports the finding that *D. congolensis*

produces neither overt toxins nor diffusible substances that contribute to the pathogenesis of infection (Roberts, 1965*b*).

SUMMARY

Resistance to infection with *Dermatophilus congolensis* was measured in terms of the dose of zoospores required to induce a given density of infection in suitably prepared areas of skin. Vaccination increased by about 16-fold the resistance of scarified skin in sheep and guinea-pigs and of sheep skin depilated by plucking, but did not increase the resistance of sheep to infection of skin defatted without further damage to the epidermis. Vaccination did not increase the resistance of scarified skin in rabbits but this animal has a high natural resistance, equal to that of the vaccinated sheep or guinea-pig.

The acquired resistance is associated with circulating somatic antibody. It is not associated with the presence of flagellar agglutinin or antibody sensitizing the animal to local cutaneous anaphylaxis; nor have any of the antibodies any antibacterial action, either *in vivo* or *in vitro*, in the absence of granulocytes.

Acquired resistance is demonstrable only where the epidermis is damaged sufficiently for the infecting zoospores to come into contact with phagocytes. The relatively high natural resistance of the rabbit is associated with the possession of phagocytes that are fully competent to inhibit and destroy zoospores, both in the scarified skin and *in vitro*. By contrast, the phagocytes of sheep and guinea-pigs readily ingest the zoospores but neither destroy them nor inhibit their subsequent growth. Acquired resistance in sheep and guinea-pigs is associated with the capacity of somatic antibody to increase the destructive power of their phagocytes, *in vivo* and *in vitro*, so that they become almost as competent to deal with ingested zoospores as are the phagocytes of the normal rabbit. Natural somatic agglutinin to *D. congolensis*, which may be present in large amounts, particularly in sheep, does not affect the inhibition or destruction of zoospores within the phagocytes of sheep or guinea-pigs.

The observation that *D. congolensis* has no leucocidal action is consistent with the earlier finding that the organism is without toxins.

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