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INHIBITION OF MACROPHAGE MIGRATION IN *SALMONELLA* IMMUNITY

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ABSTRACT

CAMERON, C. M. & VAN RENSBURG, J. J., 1975. Inhibition of macrophage migration in *Salmonella* immunity. *Onderstepoort J. vet. Res.*, 42 (1) 15–24 (1975).

Protein antigens were prepared from rough strains of *Salmonella typhimurium* and *S. dublin* by phenol and veronal-buffer extraction. It was shown that the *in vitro* migration of peritoneal exudate cells from guinea pigs that were immunized with rough avirulent mutants could be inhibited effectively with these antigens.

The cells obtained from *S. typhimurium*-immunized guinea pigs were also sensitive to *S. dublin* antigens and *vice versa*.

A degree of sensitivity and inhibition could be demonstrated consistently in a group of immunized guinea pigs. However, the variation in samples, even from among individual animals that had survived challenge, was so great that it precludes the use of the macrophage migration technique as a routine standard assay procedure for immunity.

INTRODUCTION

The mechanism of immunity to *Salmonella* infections is a complex problem and has been intensively investigated and reviewed (Jenkin & Rowley, 1963). In a series of publications it has been shown that the immunity that is associated with a carrier state (Jenkin, Rowley & Auzins, 1964) is due to the production of specific antibody (Turner, Jenkin & Rowley, 1964) and that this particular antibody is attached to supposedly 'immune' cells (Rowley, Turner & Jenkin, 1964). The role of humoral antibodies in protection against infection has also been stressed by other workers such as Jonas & Pulford (1970), Venneman & Berry (1971a) and particularly by Kenny & Herzberg (1967, 1968).

Conversely, other investigations have led to the conclusion that true cellular immunity is the primary mediator of protection against salmonellosis, especially when living vaccines are employed (Blanden, Mackaness & Collins, 1966; Osawa, Kawakami, Kurashige & Mitshuashi, 1967; Venneman & Berry, 1971b). It has also been shown that this cellular immunity is associated with the development of delayed hypersensitivity (Collins & Mackaness, 1968).

It would appear, however, that neither humoral nor cellular mechanism operates separately, but that in fact both mechanisms are active simultaneously (Collins, 1971; Venneman & Berry, 1971c). Moreover, appreciable evidence has been accumulated which indicates that the phagocytic activity of macrophages is mediated by cytophilic antibodies (Kurashige, Osawa, Kawakami & Mitsuhashi, 1967; Rowley, Auzins & Jenkin, 1968; Hsu & Mayo, 1973).

Consequently it is obvious that an assay of humoral antibodies alone would not be an acceptable measure for immunity to *Salmonella* infections. Therefore, since the macrophage migration technique is an accepted *in vitro* measure for delayed hypersensitivity and possibly also other manifestations of cellular immunity (Melnick, 1971; Bloom, Landy & Lawrence, 1973; David, 1973), experiments were conducted to determine whether this technique could be applied as a measure of immunity against *Salmonella*.

MATERIALS AND METHODS

Experimental animals

Albino guinea pigs obtained from a known *Salmonella*-free source were used throughout. They were fed a balanced pelleted ration supplemented by fresh lucerne.

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Bacterial strains

The virulent smooth *S. typhimurium* strain 2656V as well as the avirulent rough mutants used in this study are described by Cameron & Fuls (1974). *S. dublin* strains 5765 and 5565 are avirulent mutants (Cameron & Fuls, unpublished data, 1974). The rough *S. dublin* strain 1/17 was described by Botes (1964) and the virulent *S. dublin* strain 2652V was isolated from a case of calf paratyphoid.

Preparation of vaccine and immunization of guinea pigs

The preparation of fresh live vaccines of the various mutants as well as the formalin inactivated vaccine composed of the virulent smooth strain of *S. dublin* is described by Cameron & Fuls (1974). The live vaccines were nephelometrically standardized to contain approximately 3×10^8 bacteria/ml while the inactivated vaccine contained 0,5% packed cells.

Guinea pigs were immunized by administering a single subcutaneous injection of 1,0 ml of either of the vaccines.

Challenge of immunized animals

Bacteria used for challenge purposes were lyophilized and reconstituted in tryptone water prior to use (Cameron & Fuls, 1974). The suspension was diluted to contain approximately 3×10^8 bacteria/ml and guinea pigs were challenged by the intraperitoneal injection of 1,0 ml of the suspension. Deaths were recorded for 14 days.

Preparation of antigens

Large quantities of bacteria were produced in D15 broth (Schlecht & Westphal, 1966) shake cultures. The cells were harvested by centrifugation and either frozen at —20° or lyophilized. Antigens were prepared from rough *S. typhimurium* 2656 34 × 0 by the following procedures:

pH 11 extract: The pH 11 extract was prepared as described by Kawakami, Osawa & Mitsuhashi (1966).

Sodium dodecyl sulphate extract (SDS): SDS extracts were prepared according to the method of Jenkin & Rowley (1965).

Phenol extract: A phenol: water extract of bacteria was prepared as described by Sutherland & Wilkinson (1971) and the protein precipitated from the phenol phase with ethanol as described by Tauber & Garson (1959). The precipitate was washed with ethanol, suspended in distilled water and lyophilized.

Veronal extract: The procedure used was adapted from the method employed by Barber, Eylan & Keydar (1968) and Barber & Eylan (1972).

In order to remove as much of the flagellar proteins as possible the bacteria were first treated with ethanol and acetone as follows:

Washed wet cells (40 g) were suspended in distilled water to give a dense suspension which was mixed with 9 volumes of 95% ethanol and stirred at 37 °C for 4 h. The cells were sedimented by centrifugation and washed twice with acetone. They were resuspended in a small volume of acetone, placed in a petri dish and allowed to dry at 37 °C for 18 h.

The dry bacteria (5% m/v) were suspended in 0,05 M veronal buffer pH 8,4 and stirred at 37 °C for 48 h. The cells were removed by centrifugation and trichloracetic acid crystals added carefully to the supernatant fluid to give a final concentration of 20% (m/v). The precipitated protein was collected by centrifugation and dissolved in 0,1 N NaOH. This solution was exhaustively dialysed against distilled water at 4 °C and lyophilized.

Phenol and veronal extracts were prepared similarly from *S. dublin* strain 5765.

Macrophage migration

The technique employed was essentially as described by David, Al-Askari, Lawrence & Thomas (1964) with minor modifications.

Peritoneal exudate cells were collected from either immune or non-immune guinea pigs 4 days after intraperitoneal injection of 10 ml of a light mineral oil*. They were exsanguinated, the abdominal skin removed and approximately 40 ml of warm Hank's solution containing 10 units of heparin/ml injected intraperitoneally. After gentle massage the abdomen was opened and the dense cell suspension aspirated by means of a 50 ml syringe fitted with a 17 gauge needle. The oil was allowed to separate for a few minutes and the suspension placed in siliconized 10 ml conical centrifuge tubes. The cells were deposited by centrifugation at 800 × *g* for 5 min. The supernatant fluid was decanted and a 50% suspension of the cells prepared in Eagle's medium. Capillary tubes (1,0 mm ID) were filled, sealed at one end with paraffin wax or by melting a tip of the tube and centrifuged at 800 × *g* for 5 min. The tubes were cut at the interface of the cells and fluid and duplicate tubes mounted in each well of a Mackaness-type culture chamber. The wells were filled with tissue culture medium. The medium consisted of Eagle's medium containing 20% fresh guinea pig serum and 32 units of penicillin/ml. No other antibiotics were added. The medium was used either without antigen as control or contained 100; 50; 25 or 24; 12,5 or 12; 6,25 or 6,0 μg/ml antigen, depending on the requirements and design of a particular experiment.

The chambers were incubated at 37 °C in 5% CO_2 for 24 h after which the degree of migration was recorded.

* White oil A grade, Caltex, P.B. 680, Silverton

Measurement of migration and migration inhibition

The extent of migration was measured by means of a microscope fitted with a calibrated eyepiece. The diameter of the area of migration from each of the 2 tubes in a well were measured at right angles. The average of the 4 diameter readings obtained was used to calculate the surface area of outgrowth and arbitrary units.

Since the antigens were slightly toxic this had to be taken into account when the true % inhibition of migration in the presence of antigen was calculated. The following formula was therefore employed:

$$\text{Percentage of migration inhibition} = 100 - \frac{\text{area of migration of immune cells with antigen}}{\text{area of migration of immune cells without antigen}} \times 100$$

minus

$$100 - \frac{\text{area of migration of normal cells with antigen}}{\text{area of migration of normal cells without antigen}} \times 100$$

Each bar in the histograms represents the results obtained from a single guinea pig.

Experimental design

The first experiments were designed to find soluble antigen preparations of *S. typhimurium* and *S. dublin* that would not cause non-specific inhibition of macrophage migration due to their inherent toxicity. For this purpose different concentrations of the 4 antigen extracts were included in tissue culture medium. The migration of normal peritoneal cells in the presence of antigen was compared with their migration in the absence of antigen (Fig. 2 and Fig. 3).

Once suitable antigens had been found, their ability to cause specific inhibition of migration of peritoneal cells from immunized guinea pigs was investigated.

Initially the cells from guinea pigs that had first been immunized with avirulent strains and then challenged with virulent bacteria were tested. Once it was established that these cells were sensitive to antigen, the cells from guinea pigs that had only been immunized were tested. This procedure was followed for guinea pigs immunized with *S. typhimurium* and with *S. dublin*.

In both instances the reactivity of the cells was tested using *S. typhimurium* and *S. dublin* antigens.

Groups of 6, 8 or 12 guinea pigs were used in all the experiments.

Migration assays were done 14 days after either immunization or challenge. In some of the experiments it was impossible to assay all the guinea pigs simultaneously. In such instances 2 guinea pigs from each group were assayed at 3 or 4 day intervals.

RESULTS

Macrophage migration

An example of 'immune' macrophages which have migrated from a capillary tube in the absence of antigen is shown in Fig. 1a. The migration of these macrophages is inhibited, however, in the presence of antigen (Fig. 1b). Macrophages from normal guinea pigs migrate equally well both in presence or absence of antigen.

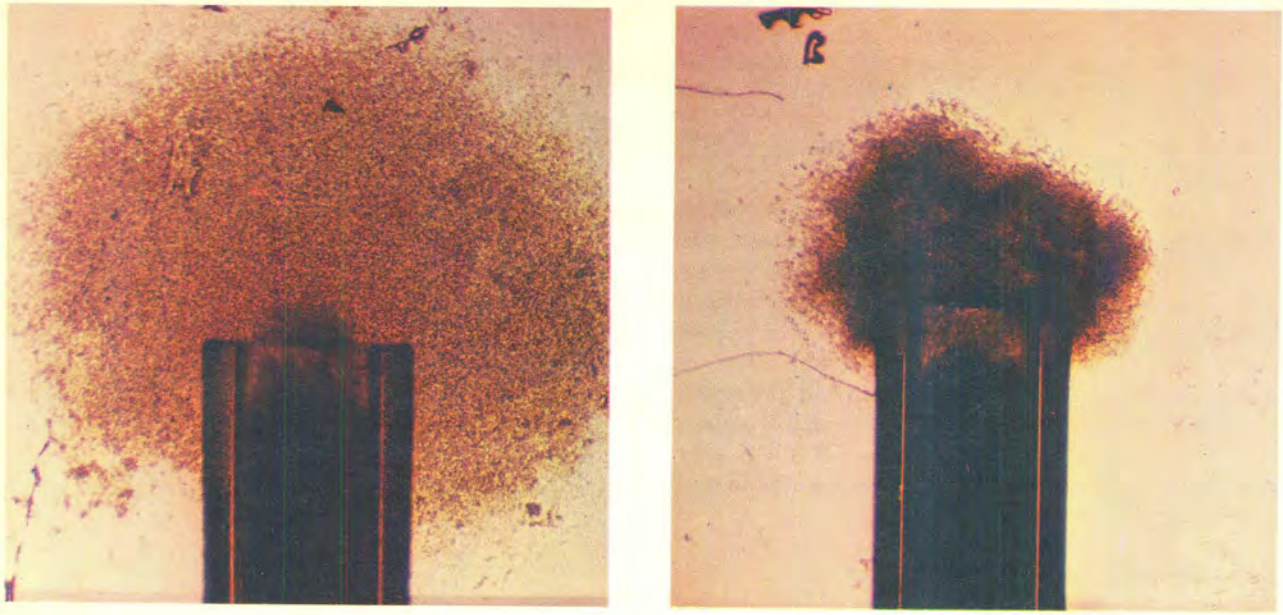

FIG. 1 Example of migration of 'immune' macrophages in the absence of antigen (a) compared with inhibition of migration in the presence of antigen (b)

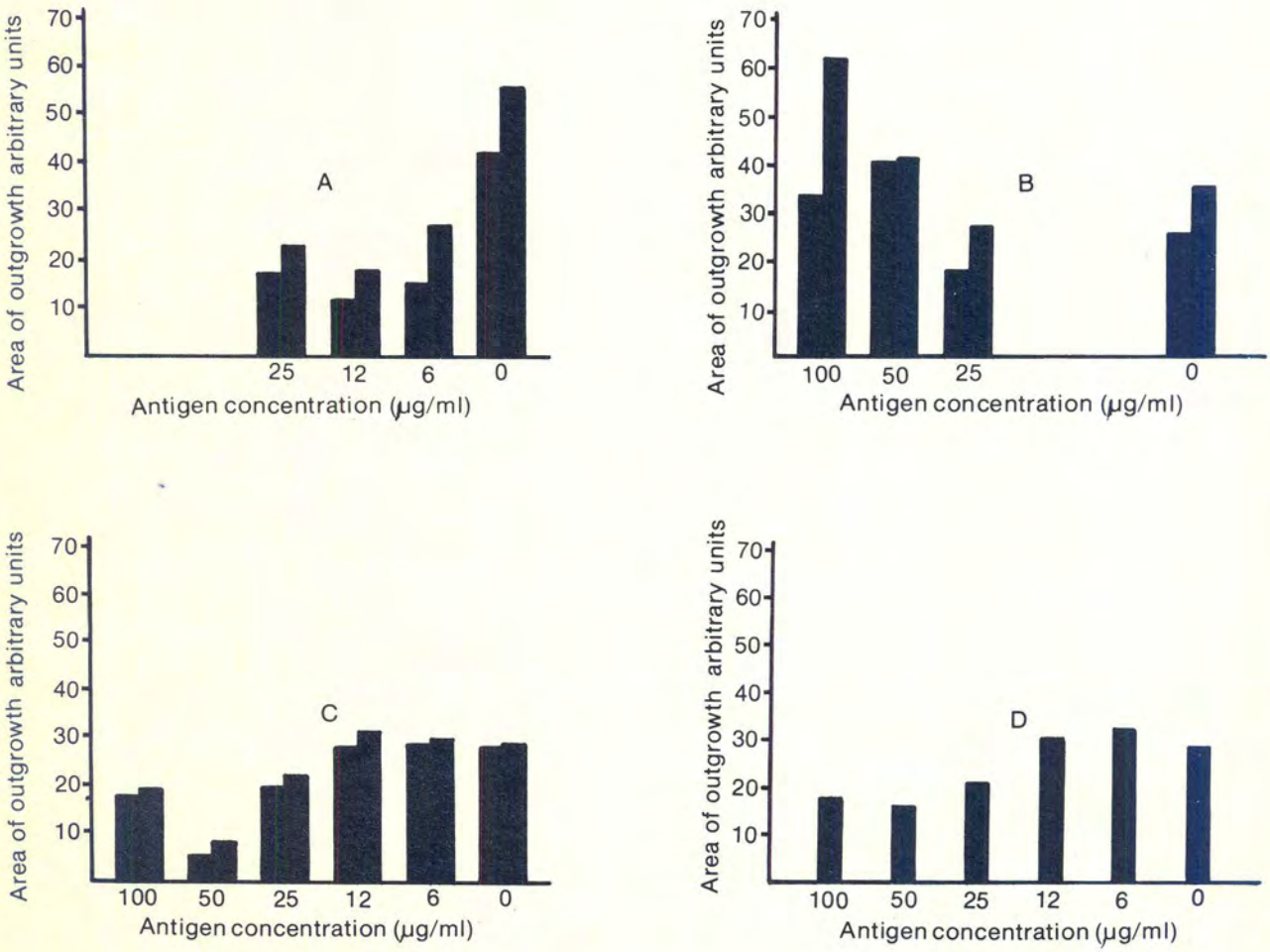

FIG. 2 Non-specific inhibitory effect of various *S. typhimurium* extracts for normal guinea pig peritoneal exudate cells:
A = pH 11 extract
B = SDS extract
C = Phenol extract
D = Veronal extract

Toxicity of antigen preparations

Before commencing this study it was necessary to find non-toxic antigens that would not cause non-specific inhibition of migration and thus give false results.

Toxicity of S. typhimurium *antigens prepared from* S. typhimurium 2652*V* 34 × 0

The effect of the 4 antigens on cells from normal guinea pigs is shown in Fig. 2. The pH 11 extract was extremely toxic even at 6μg/ml while the SDS extract had a stimulatory effect, consequently these 2 antigen preparations were not studied any further. The phenol extract and the veronal extract were also toxic at concentrations of 25 μg/ml and higher but this effect was lost at concentrations of 12 μg/ml and 6 μg/ml. The phenol extract was poorly soluble and was used at 12 μg/ml in the subsequent experiments while the veronal extract was used at 6 μg/ml.

Toxicity of S. dublin *antigens prepared from* S. dublin 2652

The toxicity of the 2 *S. dublin* antigens was similarly tested and they were found to be less toxic than the *S. typhimurium* antigens (Fig. 3). Although higher concentrations would probably also have been satisfactory they were used at 6 μg/ml for comparative purposes.

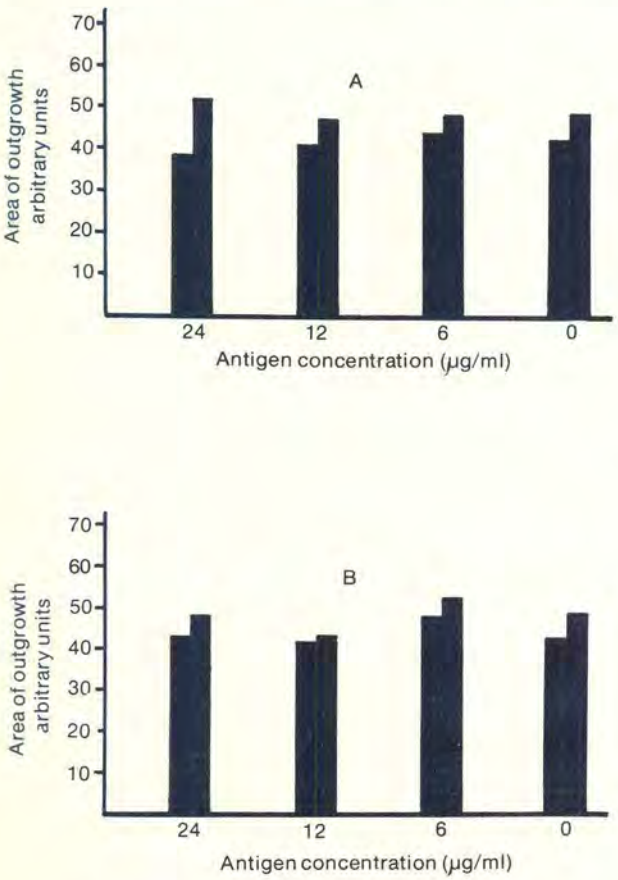

FIG. 3 Non-specific inhibitory effect of two *S. dublin* extracts for normal guinea pig peritoneal exudate cells

A = Phenol extract
B = Veronal extract

Immunity of guinea pigs immunized with S. typhimurium *live vaccines*

In order to select the best strains for immunization of guinea pigs, 6 animals were immunized with live vaccines prepared from *S. typhimurium* strains R0 R4, R9, R10, M206, T34–59, G30D, ILJ–15, DHI and 2656 34 × 0 and challenged with *S. typhimurium* 2656V. The results are shown in Table 1.

TABLE 1 Immunity produced by various rough *S. tyhpimurium* mutants in guinea pigs against *S. tyhpimurium* 2656V

Strain	Survivors/6
R0	2
R4	1
R9	3
R10	3
M206	4
T34–59	4
G30D	3
ILJ–15	5
DHI	1
2656 34 × 0	0
Controls	0

The degree of immunity afforded by the various strains was variable and only strains M206, T34–59, and ILJ–15 gave an immunity of 50% or more.

Migration inhibition of cells from guinea pigs immunized with live S. typhimurium *vaccines and challenged with* S. typhimurium 2656V

The cells from 2 guinea pigs that were immunized with *S. typhimurium* strains ILJ and M 206 and subsequently survived challenge with *S. typhimurium* strain 2656 were tested for sensitivity to *S. typhimurium* and *S. dublin* antigens.

As demonstrated in Fig. 4 the cells from all 4 guinea pigs were clearly inhibited by *S. typhimurium* antigen while they varied considerably with respect to inhibition by *S. dublin* antigens.

Migration inhibition of cells from guinea pigs immunized with rough strains of S. typhimurium *but not challenged*

In the light of the promising results obtained in the previous experiments, groups of 6 guinea pigs were immunized with *S. typhimurium* strain R0, R9, R10, ILJ–15, DHI and M206 and the sensitivity of their cells assayed. From the results shown in Fig. 5 it is apparent that migration of the cells from all the groups were inhibited by antigen but that there is a great difference with respect to individual guinea pigs within a group. While the cells of 1 or 2 guinea pigs within a group show 50–60% inhibition other individuals show only 10% inhibition or less.

In general the veronal extract gave slightly better inhibition than the phenol extracts.

There is no particular correlation between the degree of migration inhibition and the actual immunity afforded by the strains that were tested (Table 1).

The above findings were confirmed in a similar experiment using guinea pigs immunized with *S. typhimurium* strains M206 and ILJ–15 (Fig. 6). In this experiment it was also shown that the cells from *S. typhimurium*-immunized guinea pigs were also sensitive to *S. dublin* antigens.

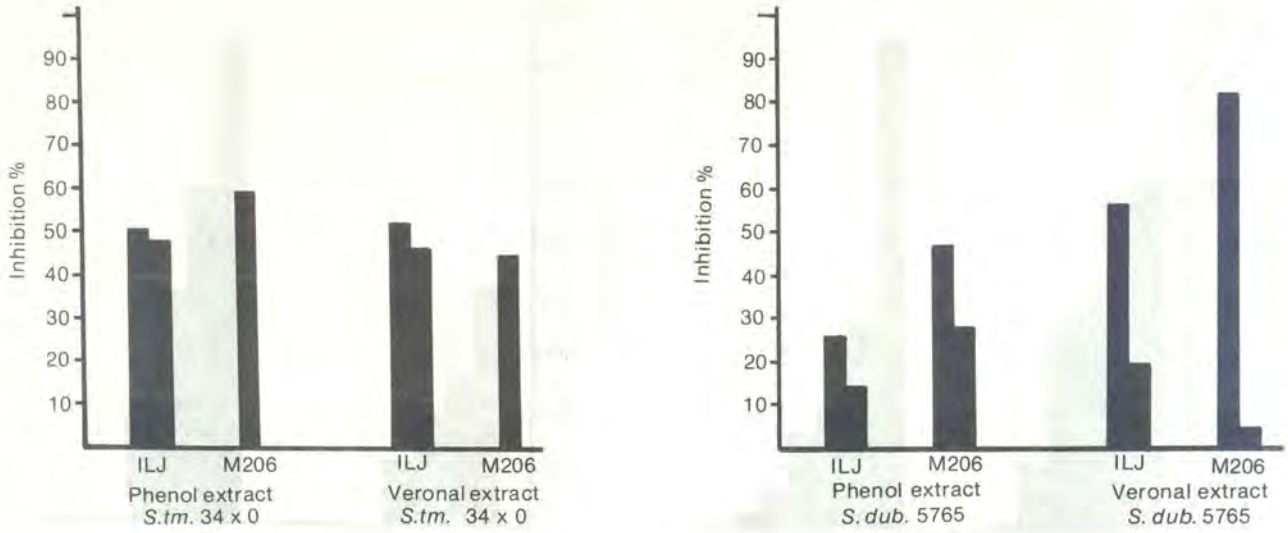

FIG. 4 Migration inhibition of peritoneal exudate cells obtained from guinea pigs immunized with either *S. typhimurium* strains ILJ or M206, and challenged with *S. typhimurium* 2656V. Sensitivity of the cells was tested with *S. typhimurium* as well as *S. dublin* extracts.

Migration inhibition of cells from guinea pigs immunized with S. dublin *rough strains and challenged with* S. dublin *strain* 2652V

Groups of 8 guinea pigs were immunized with *S. dublin* strains HB1/17, 5765 and 5565 respectively and challenged. The results are shown in Table 2.

TABLE 2 Immunity to *S. dublin* produced in guinea pigs by immunization with rough *S. dublin* mutants

Strain	Survivors/8
HB 1/17	4
5765	3
5565	1
Controls	1

The cells from 4 survivors of vaccine HB1/17, 2 of vaccine 5765 and 1 of vaccine 5565 were assayed and the results are shown in Fig. 7. As in the case of the *S. typhimurium* experiments, the cells of the guinea pigs were clearly sensitive to antigen when viewed as a group, but even in these animals that survived challenge and should have been fully immune, there was a pronounced variation among individuals.

Migration inhibition of cells from guinea pigs immunized with S. dublin *but not challenged*

Fig. 8 shows the results of migration inhibition assays on cells of groups of guinea pigs immunized with *S. dublin* strains HB1/17 and 5765. There was little difference between the two immunizing strains but once again the variation among individuals was pronounced. As in the case of *S. typhimurium* the veronal extract gave better results than the phenol extract antigen.

The cells were also at least equally sensitive to *S. typhimurium* antigens indicating an appreciable cross reactivity.

Discussion

The phenomenon of "cellular" immunity as measured by the macrophage migration technique has been very well documented in a disease such as tuberculosis, in which delayed hypersensitivity is a prominent feature (Heise, Han & Weiser, 1968; Kostiala & Kosunen, 1972; Neiburger & Youmans, 1973). It has also been recorded in other diseases of animals such as anaplasmosis (Buening, 1973), mycoplasmosis (Biberfeld, 1973) as well as *Streptococcus* (Seravalli & Taranta, 1973; Heymer, Bültmann, Schachenmayer, Spanel, Haferkamp & Schmidt, 1973) and *Pasteurella haemolytica* infections (Tizard & Elliot, 1974). The results presented in this paper show that inhibition of macrophage migration is also demonstrable in *S. typhimurium* and *S. dublin* immunity in guinea pigs, but that there is an appreciable variation in the degree to which cells from individual animals exhibit the phenomenon.

Whether inhibition of macrophage migration may be regarded as a true measure of immunity is still a point of contention (Neiburger, Youmans & Youmans, 1973; Bloom *et al.*, 1973; David, 1973) and although promising results have been obtained (Yamamoto, Anacker & Ribi, 1970; Simon & Sheagren, 1971), much more information is required before any final conclusion can be drawn. This problem is further complicated by the variations in results which can be ascribed to technical inaccuracies and variations (Bendixen & Sϕborg, 1970).

The issue is also clouded by the fact that in order to conduct meaningful assays the correct antigen preparation should be used. Protein preparations were employed because of their low toxicity but there is no evidence that they necessarily contain the most important immunizing antigens.

As indicated earlier, the probable role of cytophilic antibodies as mediators of *Salmonella* immunity also complicates the relevance of the migration inhibition technique as a measure of immunity, since the precise role of these antibodies as receptors on lymphocytes or macrophages is unknown. Although some attempts to elucidate the situation have been undertaken, the results are inconclusive and much more information and penetrating investigation are required (Lokaj, 1971; Tizard, 1971; Kostiala & Kosunen, 1972).

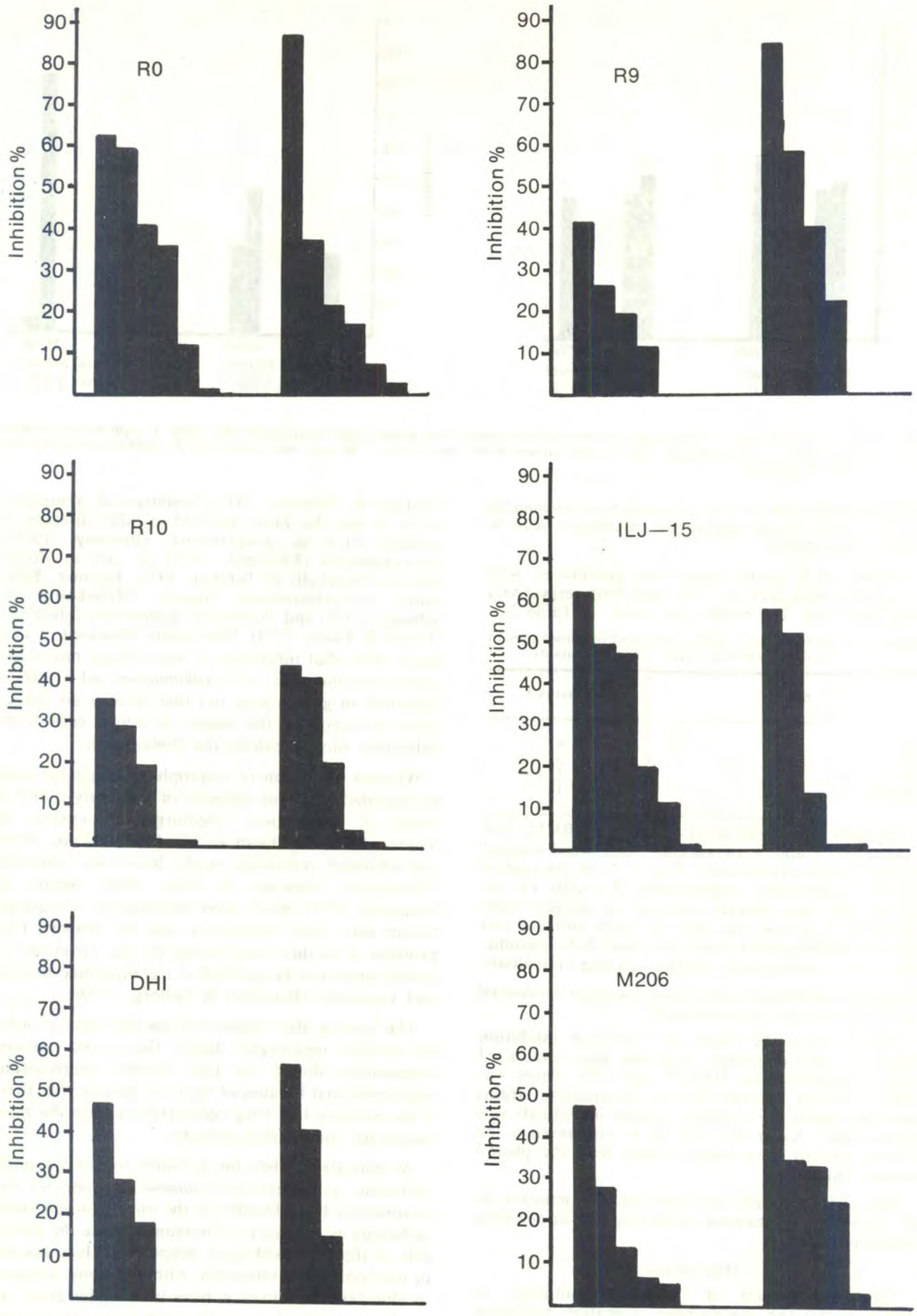

FIG. 5 Migration inhibition of peritoneal exudate cells, obtained from guinea pigs immunized with *S. typhimurium* strains R0; R9; R10; ILJ–15; DHI; or M206, by phenol extracts (left) or veronal extracts (right) prepared from *S. typhimurium* strain 2656 34 × 0

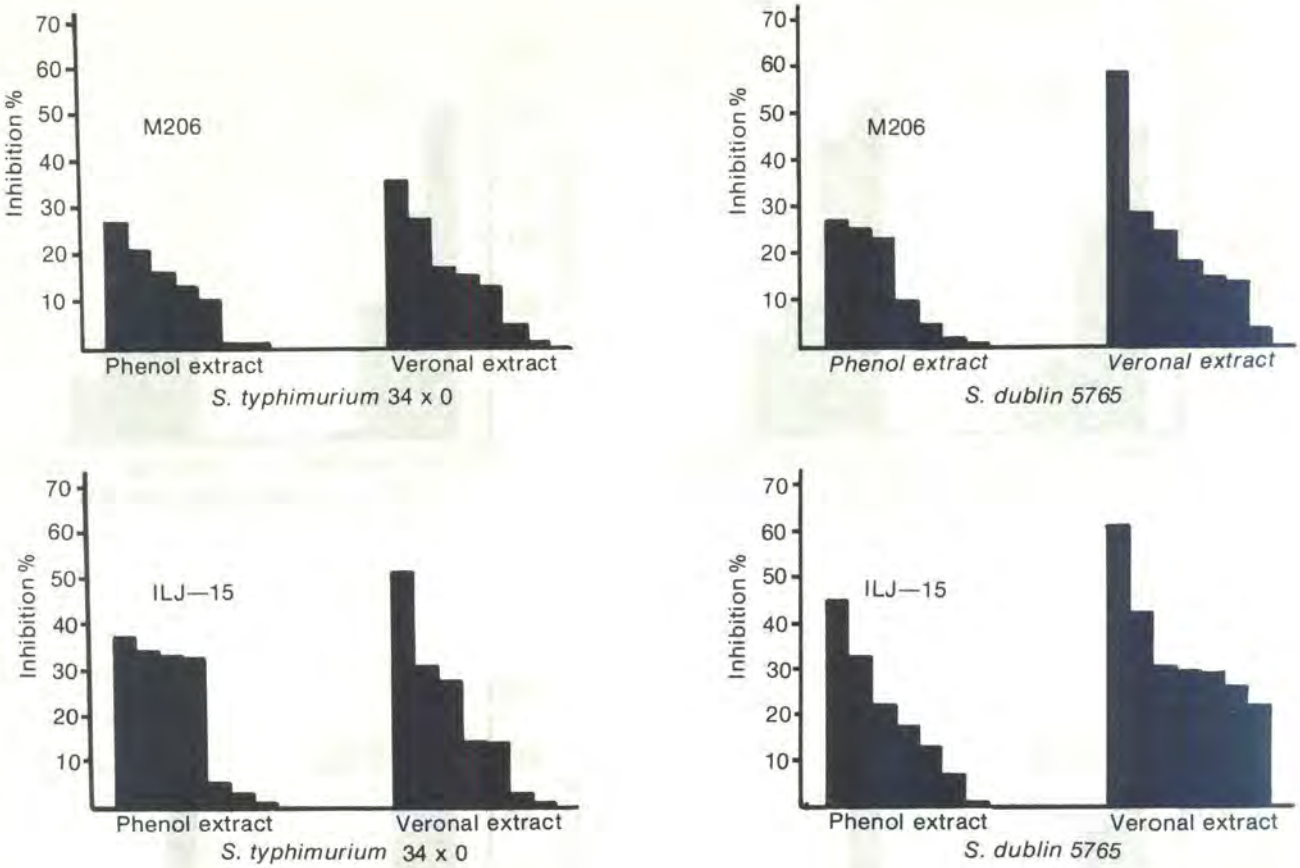

FIG. 6 Migration inhibition of peritoneal exudate cells obtained from guinea pigs which were immunized either with *S. typhimurium* strain M206 or ILJ–15 and tested with phenol and veronal extracts of *S. typhimurium* strain 34 × 0 and *S. dublin* strain 5765

Finally it should be pointed out that the macrophage migration assay is highly specific (David, Lawrence & Thomas, 1964), but that an appreciable degree of cross protection may exist between various microbial parasites (Mackaness, 1964) which might not be reflected by macrophage sensitivity (Simon & Sheagren, 1972). This discrepancy was also observed in our work, though in reverse. Whereas we have been unable to demonstrate protection against *S. dublin* after immunization with *S. typhimurium* (Cameron & Fulls, 1974) there is virtually complete reciprocity with respect to macrophage migration inhibition with protein antigens of the 2 species. The fact that mice were used for the immunity experiments while guinea pigs were used in the migration assays might, however, account for the conflicting results. Alternatively the protein extracts used in the assays may contain common antigens which are not important with respect to the induction of protection against infection.

From the foregoing it is abundantly evident that although the peritoneal cells from *Salmonella*-immunized guinea pigs are sensitive to antigen the validity of the macrophage migration inhibition assay as a measure of protection is unproven and requires more intensive study. For this purpose a more suitable model such as brucellosis would be desirable.

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References

BARBER, C. & EYLAN, E., 1972. Immunochimie de *Salmonella typhimurium*. Variations de la fraction proteique de l'antigéne somatique. *Rev. Immun. Paris* 36, 1–13.

BARBER, C., EYLAN, E. & KEYDAR, Y., 1968. Contributions à l'étude des *Salmonelles*. Protéines communes des *Salmonelles* appartenant aux groupes B, C, D et E et leur, relation avec *Citrobacter ballerup*. *Path. Microbiol.* 31 165–174.

BENDIXEN, G. & SØBORG, M., 1970. Comments on the leucocyte migration technique as an *in vitro* method for demonstrating cellular hypersensitivity in man. *J. Immunol.* 104, 1551–1552.

BIBERFELD, G., 1973. Macrophage migration inhibition in response to experimental *Mycoplasma pneumoniae* infection in the hamster. *J. Immunol.* 110, 1146–1150.

BLANDEN, R. V., MACKANESS, G. B. & COLLINS, F. M., 1966. Mechanisms of acquired resistance in mouse typhoid. *J. exp. Med.*, 124, 585–600.

BLOOM, B. R., LANDY, M. & LAWRENCE, H. S., 1973. *In vitro* methods in cell-mediated immunity: A progress report. *Cell. Immun.* 6, 331–347.

BOTES, H J. W., 1964. Calf paratyhpoid immunity: Evaluation of formol-killed and live attenuated vaccines. *Bull. Off. int. Epiz.* 62, 581–605.

BUENING, G. M., 1973. Cell-mediated immune responses in calves with anaplasmosis. *Am. J. vet. Res.* 34, 757–763.

CAMERON, C. M. & FULS, W. J. P., 1974. A comparative study of the immunogenicity of live and inactivated *Salmonella typhimurium* vaccines in mice. *Onderstepoort J. vet. Res.* 41 (3), 81–92.

COLLINS, F. M. & MACKANESS, G. B., 1968. Delayed hypersensitivity and arthus reactivity in relation to host resistance in *Salmonella*-infected mice. *J. Immunol.* 101, 830–845.

COLLINS, F. M., 1971 Mechanisms of antimicrobial immunity. *J. ret. endoth. Soc.* 10, 58–99.

DAVID, J. R., 1973. Lymphocyte mediators and cellular hypersensitivity. *New Engl. J. Med.* 288, 143–149.

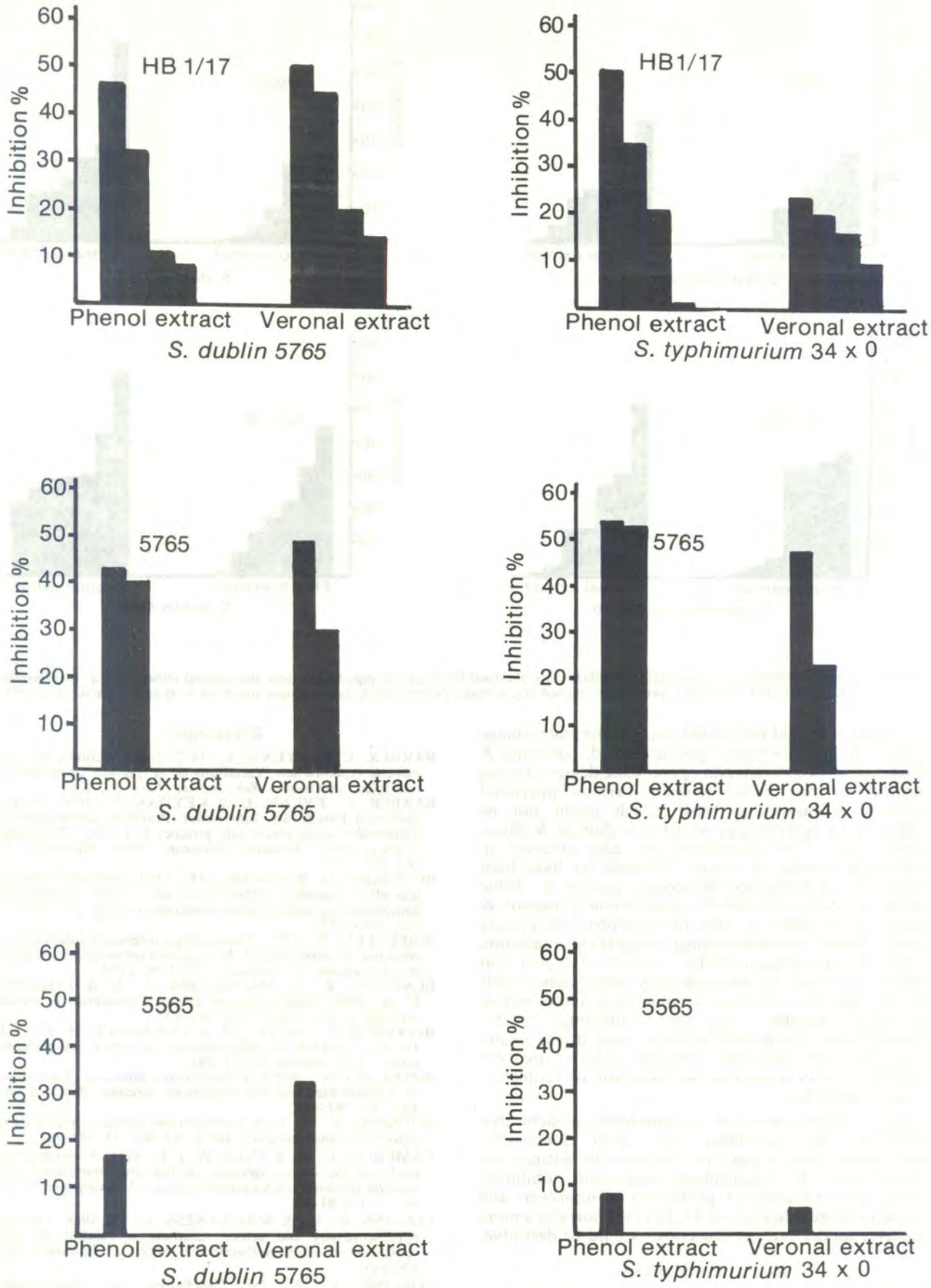

FIG. 7 Migration inhibition of peritoneal exudate cells obtained from guinea pigs which were immunized with *S. dublin* strain HB 1/17; 5765 or 5565 and challenged with *S. dublin* 2656. The sensitivity of the cells was tested with both *S. typhimurium* and *S. dublin* extracts

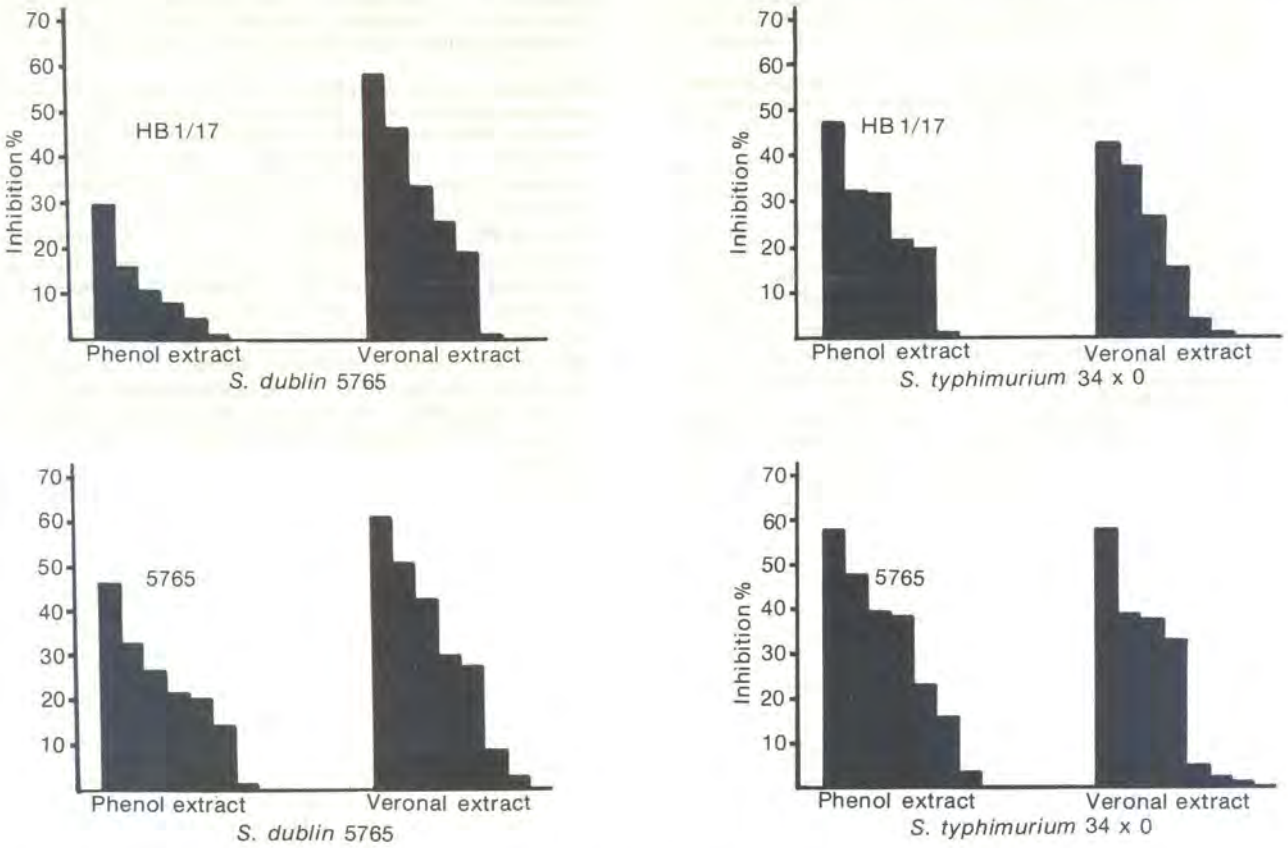

FIG. 8 Migration inhibition of peritoneal exudate cells obtained from guinea pigs which were immunized with *S. dublin* strain HB 1/17 or 5765 by phenol and veronal extracts of *S. dublin* strain 5765 and *S. typhimurium* strain 34 × 0.

DAVID, J. R., AL-ASKARI, S., LAWRENCE, H. A. & THOMAS, L., 1964. Delayed hypersensitivity *in vitro*. I. The specificity of inhibition of cell migration by antigens. *J. Immunol.* 93, 264–273.

DAVID, J. R., LAWRENCE, H. S. & THOMAS, L., 1964. Delayed hypersensitivity *in vitro*. III. The specificity of hapten-protein conjugates in the inhibition of cell migration. *J. Immunol.* 93, 279–282.

HEISE, E. R., HAN, S. & WEISER, R. S., 1968. *In vitro* studies on the mechanism of macrophage migration inhibition in tuberculin sensitivity. *J. Immunol.* 5, 1004–1015.

HEYMER, B., BULTMANN, B., SCHACHENMAYER, W., SPANEL, R., HAFEKAMP, O. & SCHMIDT, W. C. 1973. Migration inhibition of rat peritoneal cells induced by streptococcal mucopeptides. Characteristics of the reaction and properties of the mucopeptide preparations. *J. Immunol.* 116, 1743–1754.

HSU, H. S. & MAYO, D. R., 1973. Interactions between macrophages of guinea pigs and *Salmonellae*. *Infect. & Immunity* 8, 165–172.

JENKIN, C. R. & ROWLEY, D., 1963. Basis for immunity to typhoid in mice and the question of "Cellular immunity". *Bact. Rev.* 27, 391–404.

JENKIN, C. R. & ROWLEY, D., 1965. Partial purification of the protective antigen of *Salmonella typhimurium* and its distribution among various strains of bacteria. *Aust. J. exp. Biol. med. Sci* 43, 65–78.

JENKIN, C. R., ROWLEY, D. & AUZINS, I., 1964. The basis for immunity to mouse typhoid. 1. The carrier state. *Aust. J. exp. Biol. med. Sci.* 42, 215–228.

JONAS, W. E. & PULFORD, H. D., 1970. Studies on the immunological aspects of salmonellosis of mice: Passive immunity. *N.Z. vet. J.* 18, 205–208.

KAWAKAMI, M., OSAWA, N. & MITSUHASHI, S., 1966. Experimental salmonellosis. VII Comparison of the immunizing effect of live vaccine and materials extracted from *Salmonella enteritidis*. *J. Bact.* 92, 1585–1589.

KENNY, KATHRYN & HERZBERG, M., 1967. Early antibody response in mice to either infection or immunization with *Salmonella typhimurium*. *J. Bact.* 93, 773–778.

KENNY, KATHRYN & HERZBERG, M., 1968. Antibody response and protection induced by immunization with smooth and rough strains in experimental salmonellosis. *J. Bact.* 95, 406–417.

KOSTIALA, ANJA, A. I. & KOSUNEN, T. U., 1972. Delayed hypersensitivity in the guinea pig immunized with killed tubercle bacilli in adjuvant. 3. Peritoneal cell migration inhibition and macrophage cytophylic antibodies to tuberculin purified protein derivative. *Acta path. microbiol. scand. Section* B, 80, 1–9.

KURASHIGE, S., OSAWA, N., KAWAKAMI, M. & MITSUHASHI, S., 1967. Experimental salmonellosis. X. Cellular immunity and its antibody in mouse mononuclear phagocytes. *J. Bact.* 94, 902–906.

LOKAJ, J., 1971. Die cytophile Aktivität in Eluaten aus passiv sensibiliserten und nicht sensibilisierten Macrophagen von Meerschweinchen. *Z. Immun. Forsch.* 142, 347–356.

MACKANESS, G. B., 1964. The immunological basis of acquired cellular resistance. *J. exp. Med.* 120, 105–119.

MELNICK, H. D., 1971. Inhibition of macrophage migration and the mediation of cellular immunity: A review. *Ann. Allerg.* 29, 195–208.

NEIBURGER, R. G. & YOUMANS, G. P., 1973. Inhibition of migration of mouse macrophages by tuberculin-sensitive mouse lymphocytes and by mouse migration inhibitory factor. *Infect. & Immunity* 7, 190–195.

NEIBURGER, G., YOUMANS, G. P. & YOUMANS, ANNE, S., 1973. Relationship between tuberculin hypersensitivity and cellular immunity to infection in mice vaccinated with viable attenuated mycobacterial cells or with mycobacterial ribonucleic acid preparations. *Infect. & Immunity* 8, 42–47.

OSAWA, N., KAWAKAMI, M., KURASHIGE, S. & MITSUHASHI, S., 1967. Experimental salmonellosis. VIII. Post-infective immunity and its significance for conferring cellular immunity. *J. Bact.* 93, 1534–1540.

ROWLEY, D., TURNER, K. J. & JENKIN, C. R., 1964. The basis for immunity to mouse typhoid. 3. Cell-bound antibody. *Aust. J. exp. Biol. med. Sci.* 42, 237–248.

ROWLEY, D., AUZINS, I. & JENKIN, C. R., 1968. Further studies regarding the question of cellular immunity in mouse typhoid. *Aust. J. exp. Biol. med. Sci.* 46, 447–463.

SCHLECHT, S. & WESTPHAL, O., 1966. Wachstum und Lipopolysaccharid (O-Antigen)—gehalt von *Salmonellen* bei zuchtung auf Agarnahrböden. *Zbl. Bakt. I. Orig.* 200, 241–259.

SERAVALLI, E. & TARANTA, A., 1973. Release of macrophage migration inhibitory factor(s) from lymphocytes stimulated by streptococcal preparations. *Cell. Immun.* 8, 40–54.

SIMON, H. B. & SHEAGREN, J. N., 1971. Cellular immunity *in vitro*. I. Immunologically mediated enhancement of macrophage bactericidal capacity. *J. exp. Med.* 133, 1377–1389.

SIMON, H. B. & SHEAGREN, J. N., 1972. Migration inhibitory factor and macrophage bactericidal function. *Infect. & Immunity* 6, 101–103.

SUTHERLAND, I. W. & WILKINSON, J. F., 1971. Chemical extraction methods of microbial cells. *In* NORRIS, J. R. & RIBBONS, D. W. (Ed.), 1971. Methods in Microbiology, Vol. 5B, 345–383. London & New York: Academic Press.

TAUBER, H. & GARSON, W., 1959. Isolation of lipopolysaccharide endotoxin. *J. biol. Chem.* 234, 1391–1393.

TIZARD, I. R., 1971. Macrophage-cytophilic antibodies and the functions of macrophage-bound immunoglobulins. *Bact. Rev.* 35, 365–389.

TIZARD, I. R. & ELLIOT, D. I., 1974. Cell mediated hypersensitivity to *Pasteurella haemolytica* in normal and cold-stressed mice. *Can. J. comp. Med.* 38, 44–48.

TURNER, K. J., JENKIN, C. R. & ROWLEY, D., 1964. The basis for immunity to mouse typhoid. 2. Antibody formation during the carrier state. *Aust. J. exp. Biol. med. Sci* 42, 229–236.

VENNEMAN, M. R. & BERRY, L. J., 1971a. Serum mediated resistance induced with immunogenic preparations of *Salmonella typhimurium*. *Infect. & Immunity* 4, 374–380.

VENNEMAN, M. R. & BERRY, L. J., 1971b. Cell mediated resistance induced with immunogenic preparations of *Salmonella typhimurium Infect. & Immunity* 4, 381–387.

VENNEMAN, M. R. & BERRY, L. J., 1971c. Experimental salmonellosis: Differential passive transfer of immunity with serum and cells obtained from ribosomal and ribonucleic acid-immunized mice. *J. ret. endoth. Soc.* 9, 491–502.

YAMAMOTO, K., ANACKER, R. L. & RIBI, E., 1970. Macrophage migration inhibition studies with cell walls of *Mycobacterium bovis* BCG: Relationship between inhibitory activity of lung cells and resistance to airborne challenge with *Mycobacterium tuberculosis* H37RV. *Infect. & Immunity* 6, 595–599.

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