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The Passive Transfer of Delayed Hypersensitivity in the Guinea-Pig

I. THE SYNERGIC EFFECT OF IMMUNE CELLS AND IMMUNE SERUM ON THE 24-HOUR SKIN REACTION AND A STUDY OF THE HISTOLOGY

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Summary. Donor guinea-pigs were immunized with antigen in Freund's complete adjuvant. Three weeks later these donor guinea-pigs showed strong delayed hypersensitivity to the antigen. Peritoneal exudate cells (macrophages) and serum were then transferred intravenously from the immunized donors to normal recipients. Good 24-hour skin reactions were obtained in the recipients which had received cells and serum from donors immunized with bovine serum albumin, γ-globulin, ovalbumin and haemocyanin. The reaction transferred by immune cells and serum was greater than the reaction transferred by either singly. This synergy was not found with PPD or blood group substance as antigen. Good transfers of 24-hour skin reactions to PPD were obtained with peritoneal exudate cells both alone and with serum. Good transfers could not be obtained with blood group substance.

The synergic action of cells and serum in the passive transfer of 24-hour skin reactions was specific. The ability of peritoneal exudate cells to transfer 24-hour skin reactions was present at 1 week and greater at 3 weeks. In contrast the ability of serum to enhance passive transfers was absent at 1 week and present at 2 and 3 weeks.

The histology of reactions passively transferred by spleen, lymph node and peritoneal exudate cells in the presence and absence of serum was studied. Serum alone produced a predominantly polymorphonuclear infiltrate which was maximal at 18–24 hours and almost absent at 48 hours. Cells alone produced only a very slight reaction. The combination of cells and serum produced lesions at 4 and 18 hours resembling those caused by serum alone. At 24 hours however the histiocytes and lymphocytes were more numerous than in the pure serum reaction and by 48 hours the infiltrate consisted of histiocytes and lymphocytes.

It was concluded that, with certain antigens, there is a synergic effect of immune cells and serum in the passive transfer of 24-hour skin reactions and reasons are given for considering that these passively transferred 24-hour skin reactions are indeed delayed hypersensitivity reactions.

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INTRODUCTION

Guinea-pigs immunized with protein antigens, such as bovine γ-globulin in Freund's complete adjuvant, give strong delayed hypersensitivity reactions when tested by the intradermal injection of bovine γ-globulin. These guinea-pigs also show delayed hypersensitivity to purified protein derivative of tuberculin (PPD) by virtue of their immunization with *M. tuberculosis* bacilli contained in Freund's complete adjuvant.

Delayed hypersensitivity to PPD can be passively transferred to other guinea-pigs by the intravenous injection of peritoneal exudate, lymph node or spleen cells. The reactions are erythematous, indurated and well defined. In contrast, the reactions to bovine γ-globulin in the recipient guinea-pigs are usually faint, flat and ill defined and are unsuitable for the study of factors which influence the passive transfer of delayed hypersensitivity.

It was found, however, that excellent passive transfer of delayed hypersensitivity to bovine γ-globulin and other antigens was obtained when both immune cells and serum were transferred from the immunized donor to the recipient. This paper gives a description of this synergic effect and the histology of the passively transferred lesions.

MATERIALS AND METHODS

See Asherson and Stone (1965).

Immunization of donors

Female guinea pigs of 200–300 g were immunized with 0·05 ml of adjuvant mixture into each of the four footpads. Each millilitre contained 1 mg of heat killed human tubercule bacilli and 250 μg of antigen. The donors were usually used 3–5 weeks later.

Passive transfer

Preparation of cells and serum. The donors were anaesthetized with ether and given 0·1 ml of 0·5 per cent heparin by intracardiac injection. The vessels of the neck were severed and the blood pooled. Plasma was obtained by centrifugation and was sometimes recentrifuged. This material is referred to as serum in the text.

Peritoneal exudate cells were obtained by injecting 10 ml of light liquid paraffin into the peritoneal cavity 4 days before death. The cells were removed by injecting 40 ml of Hanks's balanced buffered saline containing 5 units of heparin per ml. After massaging the abdomen the fluid was poured out. The fluid from several animals was pooled. The cells were recovered by centrifugation, filtered through bolting cloth and then washed once or twice. In the later experiments 1 per cent inactivated normal guinea-pig serum was added to Hanks's solution. These procedures were done with ordinary glassware at room temperature.

The cells were finally made up to a volume of about 5 ml for every two donors and kept at 4°.

Lymph node and spleen cells were similarly handled. The cell suspension was made by forcing the chopped tissue through a sieve.

Transfer of cells and serum. The serum was injected first and the cells as soon as convenient afterwards, which was usually within 2 hours. Both injections were intravenous.

Skin testing. The donors were shaved with electric clippers and depilated with 'Buto'. This facilitated the accurate placing of intradermal injections. Antigen in 0·1 ml was

injected usually within half an hour of the injection of cells. The arithmetic mean diameter of the skin reactions was recorded in millimetres and the increase of skin thickness was measured with a Quicktest dial calliper gauge.

Histology

The point of injection was marked. The tissue was excised under anaesthesia or at death, pinned out on cork and placed in 10 per cent formol saline which had been neutralized with calcium carbonate.

Nomenclature

The phrase 24-hour skin reaction is used in the text to describe reactions in the recipients observed 24 hours after the intradermal injection of antigen. It is thought that when cells or cells and serum are transferred that the transferred reaction is, at least in part, a delayed hypersensitivity reaction.

RESULTS

THE SYNERGIC EFFECT OF IMMUNE CELLS AND SERUM ON THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTIONS (DELAYED HYPERSENSITIVITY)

Twenty-four-hour skin reactions may be passively transferred with peritoneal exudate cells. With certain antigens the passively transferred 24-hour skin reactions are increased if both cells and serum are used. In the first experiment donor guinea-pigs were immunized with 50 μg of bovine serum albumin in Freund's complete adjuvant. Three weeks later, peritoneal exudate cells and serum were prepared and transferred to three groups

TABLE 1

THE EFFECT OF IMMUNE SERUM ON THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTIONS TO BOVINE SERUM ALBUMIN IN GUINEA-PIGS

Recipient given	Mean diameter and range (mm) of the 24-hour skin reaction	
Peritoneal exudate cells only	3·2	3–3·5
Serum only	5·0	4·5–6
Peritoneal exudate cells and serum	14·3	12–16

Three millilitres of serum and/or $4{\cdot}6 \times 10^8$ peritoneal exudate cells transferred to the three recipients of each group. Skin tested with 100 μg of bovine serum albumin in 0·1 ml. Donors immunized with 50 μg of bovine serum albumin in Freund's complete adjuvant 3 weeks previously. The skins of these guinea-pigs were taken for histology.

of normal guinea-pigs. Two groups received either cells or serum only, while the third group received both cells and serum. The recipient guinea-pigs were then skin tested with bovine serum albumin and PPD. Table 1 shows that at 24 hours there were only slight reactions in the guinea-pigs which had received cells or serum only and the mean diameter of the reaction in these two groups was 3·2 and 5·0 mm. However, much stronger reactions occurred in the guinea-pigs which had received both cells and serum and the mean diameter of the skin reactions was 14·3 mm.

TABLE 2

EFFECT OF IMMUNE SERUM ON THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTION TO OVALBUMIN IN GUINEA-PIGS

Recipients given	Mean diameter and range of 24-hour skin reactions (mm)	Average increase in skin thickness of 24-hour skin reactions (mm)
Peritoneal exudate cells only	6·8 (3–11)	0
Serum only	6·3 (6–7)	0·59
Peritoneal exudate cells and serum	14·4 (12–18)	1·35

Four millilitres of serum and/or $1{\cdot}5 \times 10^8$ peritoneal exudate cells transferred to five recipients in each group, except serum only group which consisted of three animals. Donors immunized with 50 μg ovalbumin in complete adjuvant 3 weeks earlier. Skin tests with 50 μg ovalbumin in 0·1 ml.

Similar results were obtained with ovalbumin. Table 2 shows that the mean diameter of the 24-hour skin reactions in the guinea-pigs which had received cells or serum only was 6·8 and 6·3 mm. However the mean diameter in the guinea-pigs which had received both cells and serum was 14·4 mm. The synergic effect of cells and serum was also shown by the measurement of induration with a dial micrometer. It was concluded that with both bovine serum albumin and ovalbumin the transfer of cells and serum produced bigger 24-hour reactions than the transfer of cells alone.

This synergic effect occurs with some but not all antigens. Cells and serum together give better 24-hour transfers than cells or serum alone when bovine serum albumin, ovalbumin, bovine γ-globulin (Table 3) and haemocyanin (Asherson, unpublished) are used as antigens. This is not true, however, for PPD or blood group substance.

In numerous experiments on guinea-pigs immunized with various antigens in Freund's complete adjuvant the size of the 24-hour skin reaction to PPD transferred by peritoneal exudate cells was unaffected by the injection of serum. A similar absence of synergy was found in one experiment with human blood group substances, which have been used as antigens by Holborow and Loewi (1962), and by Loewi, Holborow and Temple (1966). Eight guinea-pigs received $1–1{\cdot}5 \times 10^8$ peritoneal exudate cells from guinea-pigs immunized with blood group A or Le^a substance in Freund's complete adjuvant. Another three guinea-pigs received cells together with 5–7 ml of serum. The 24-hour skin reactions to test doses of 10–50 μg of blood group substance did not exceed 5 mm and showed no induration.

TABLE 3

SPECIFICITY OF THE EFFECT OF IMMUNE SERUM ON THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTIONS TO BOVINE γ-GLOBULIN

Recipients given		Diameter of 24-hour skin reactions (mm)		
Cells from	Serum from			
BGG donor	BGG donor	20*	20	19
BGG donor	None	8	6	3
BGG donor	Adjuvant donor	11·5	9	6·3
None	BGG donor	9	5	
Adjuvant donor	BGG donor	17	12·5	7·5
Eluate from spleen of BGG donor	BGG donor	9·5	7·5	6

Seven millilitres of serum from guinea-pigs immunized with bovine γ-globulin and 11 ml of serum from donors immunized with Freund's complete adjuvant used. One to one transfers. Tested with 100 μg of bovine γ-globulin in 0·1 ml. Spleen eluate prepared by washing spleen cells from nine donors twice, heating at 56° for 40 minutes and centrifuging at 56°. Seven millilitres of eluate corresponding to three spleens injected into each recipient.

* Figures from individual recipients.

SPECIFICITY OF THE SYNERGIC EFFECT OF CELLS AND SERUM

The question arises whether the synergic effect of serum on the passive transfer of 24-hour skin reactions to bovine γ-globulin is specific, i.e. will serum from a donor immunized with Freund's complete adjuvant alone enhance 24-hour skin reactions transferred by peritoneal exudate cells from a donor immunized with bovine γ-globulin? Conversely, what is the effect of transferring cells from a donor immunized with Freund's complete adjuvant and serum from a donor immunized with bovine γ-globulin? Table 3 shows that the effect of serum is specific. The suggestion of a non-specific action of cells was not confirmed in other experiments (Asherson, unpublished).

The reactions transferred by cells and serum were present at 48 hours and had a mean diameter of 11·6 mm while the reactions transferred by either singly had faded.

TIME COURSE OF PASSIVELY TRANSFERRED SKIN REACTIONS

In the recipients given immune serum alone the oedema and often the haemorrhage of the Arthus reaction is seen at 4 hours. By 24 hours most of the oedema has subsided but there is slight erythema and sometimes discoloration due to haemorrhage. Usually, however, the haemorrhage is only visible from the underside of the skin. By 48 hours the skin appears normal. After the transfer of cells alone no reaction is seen at 4 hours. At 24 hours the reactions are usually flat, pale and small and they fade almost entirely by 48 hours. The lesions produced by serum and cells together resemble those of serum alone at 4 hours. At 24 hours the lesions are erythematous, raised, indurated and well defined and

TABLE 4

THE EFFECT OF SERUM ON THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTIONS BY SPLEEN, LYMPH NODE AND PERITONEAL EXUDATE CELLS FROM SENSITIZED DONORS

Source of cells	Presence of serum	Time after immunization (days)	Diameter (mm) of skin reaction at			Increase (0·1 mm) in skin thickness at		
			4 hr	24 hr	48 hr	4 hr	24 hr	48 hr
Spleen	Serum	21	4·5*	6	2·5	10	2	2
	No serum	21	0	4	2·5	2	0	0
	Serum	34	17†	10	12	18·5	11·5	15·5
	No serum	34	0	2·5	—	3	3	—
Lymph node cells	Serum	21	6·0*	11	9	11	4	5
	No serum	21	0	11	2·5	3	1	0
	Serum	34	12·5†	11	8·5	15	14	11
	No serum	34	0	5·5	4	5	6	5
Peritoneal exudate cells	Serum	21	4·0*	10	9·5	9	0·5	5·5
	No serum	21	0	6·0	2·5	4	2	0
	Serum	34	17·5†	11·5	10·5	16	19	23
	No serum	34	0	9·0	5	6	5·5	3
No cells	Serum	21	8·0*	4·5	0	15	0	0
	Serum	34	14·5†	8·5	6·5	16	9	4

The guinea-pigs, which received cells from donors 21 days after immunization, were given 8 ml of serum, and $5·4 \times 10^8$, $6·5 \times 10^8$ and $2·2 \times 10^8$ spleen, lymph node and peritoneal exudate cells. Those receiving cells from donors 34 days after immunization were given 12 ml of serum and 1·6, 7·2 and $2·5 \times 10^8$ of the three types of cells 20 μg of bovine γ-globulin were used for the skin tests. Each figure is based on one guinea-pig. The skin of these guinea-pigs was taken for histology.

* Diameter of haemorrhage.
† Diameter of oedema.

sometimes, but not always, retain these characteristics at 48 hours. The time course is illustrated in Table 4.

In this and certain other experiments the skin reactions transferred by immune cells and serum show considerable swelling which lasts for 48 hours and can be detected with a gauge micrometer. Boyden (1957) regarded this prolonged time course of swelling characteristic of delayed hypersensitivity. In contrast immune cells alone cause little swelling at 24 hours while the swelling from serum alone, although present at 24 hours, has almost gone at 48 hours.

TIME COURSE OF APPEARANCE OF THE CELL AND SERUM FACTORS INVOLVED IN THE PASSIVE TRANSFER OF 24-HOUR SKIN REACTIONS

In most experiments the cell and serum pools used for passive transfer were taken from donors 3–5 weeks after immunization. In the present experiment cells and serum were taken from guinea-pigs at different times after immunization.

Donor guinea-pigs were immunized with bovine γ-globulin 1 and 3 weeks before transfer. The transfers were undertaken with all the possible combinations of cells alone, serum alone, and cells and serum together taken 1 and 3 weeks after immunization. Table 5 shows the results. The ability of peritoneal exudate cells to transfer 24-hour skin reactions to both PPD and bovine γ-globulin is present at 7 days but greater 21 days after immunization. Thus the mean diameter of the 24-hour reaction transferred by cells taken at 1 week is 5·8 mm; that transferred by cells taken at 3 weeks is 9·7 mm. This shows that cells taken at 1 week have only 60 per cent of the ability of cells taken 3 weeks after immunization to transfer 24-hour skin reactions to PPD. This is based on the ratio of the diameter of the skin reactions and this ratio may only apply to the particular cell and test antigen

TABLE 5

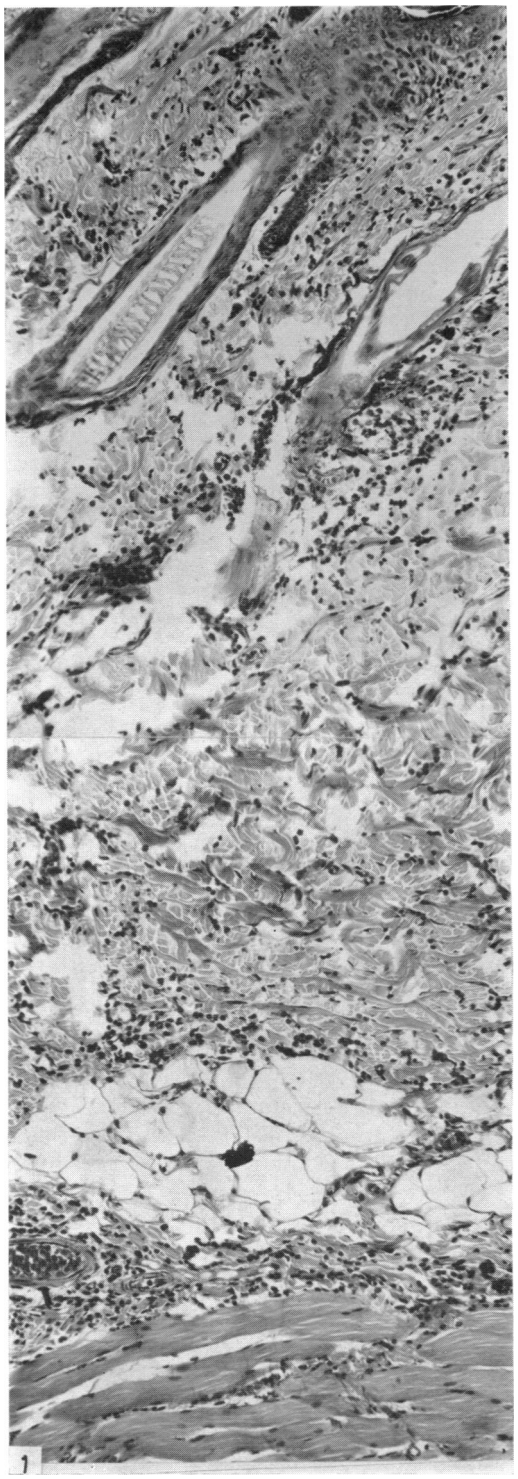
24-HOUR SKIN REACTIONS IN RECIPIENT GUINEA-PIGS GIVEN PERITONEAL EXUDATE CELLS AND SERUM FROM DONOR GUINEA-PIGS TAKEN 1 OR 3 WEEKS AFTER IMMUNIZATION

Time after immunization at which the cells and serum were obtained		Diameter (mm) of the 24-hour skin reaction to:	
Cells	Serum	PPD	BGG
1 week	No serum	6·6 (4–9·5)	6·0 (4–7·5)
1 week	1 week	5·0 (4–6)	5·5 (0–9·5)
1 week	3 weeks	5·9 (4·5–8·5)	11·75 (8–17·5)
3 weeks	No serum	11·0 (6–14·5)	9 (6–19)
3 weeks	1 week	9·0 (7–10·5)	7·75 (5–10·5)
3 weeks	3 weeks	9·1 (6·5–13)	18·0 (15·5–19)
No cells*	1 week	0	8·3 (0–25)
No cells*	3 weeks	0	6·6 (0–7)
2 weeks†	No serum	14·2 (11·5–16)	13·6 (13–14)
2 weeks†	2 weeks	15·3 (13–19)	17·8 (13·5–22·5)
No cells	2 weeks	0	0

The recipient guinea-pigs were given $2{\cdot}6 \times 10^8$ of peritoneal exudate cells taken 3 weeks after immunization or $3{\cdot}2 \times 10^8$ cells taken 1 week after immunization. The serum dose was 4 ml. The reaction to PPD and 25 μg of bovine γ-globulin is recorded by the mean and range of the diameter of the 24 hour reactions in each group of four guinea pigs.

* Only three guinea-pigs in this group.

† Separate experiment.



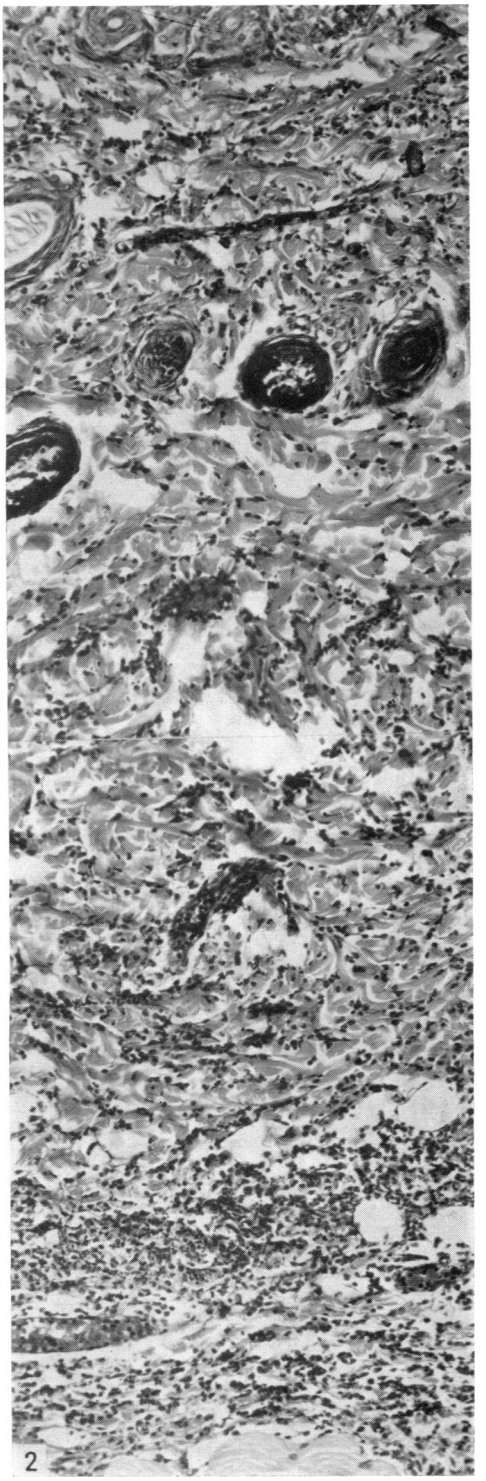


FIG. 1. Fifteen-hour skin reaction to 20 μg bovine γ-globulin, following transfer of 8 ml serum and $2{\cdot}2 \times 10^8$ macrophages from animal sensitized with bovine γ-globulin.

FIG. 2. Twenty-four-hour skin reaction. Transfer and test as for Fig. 1.

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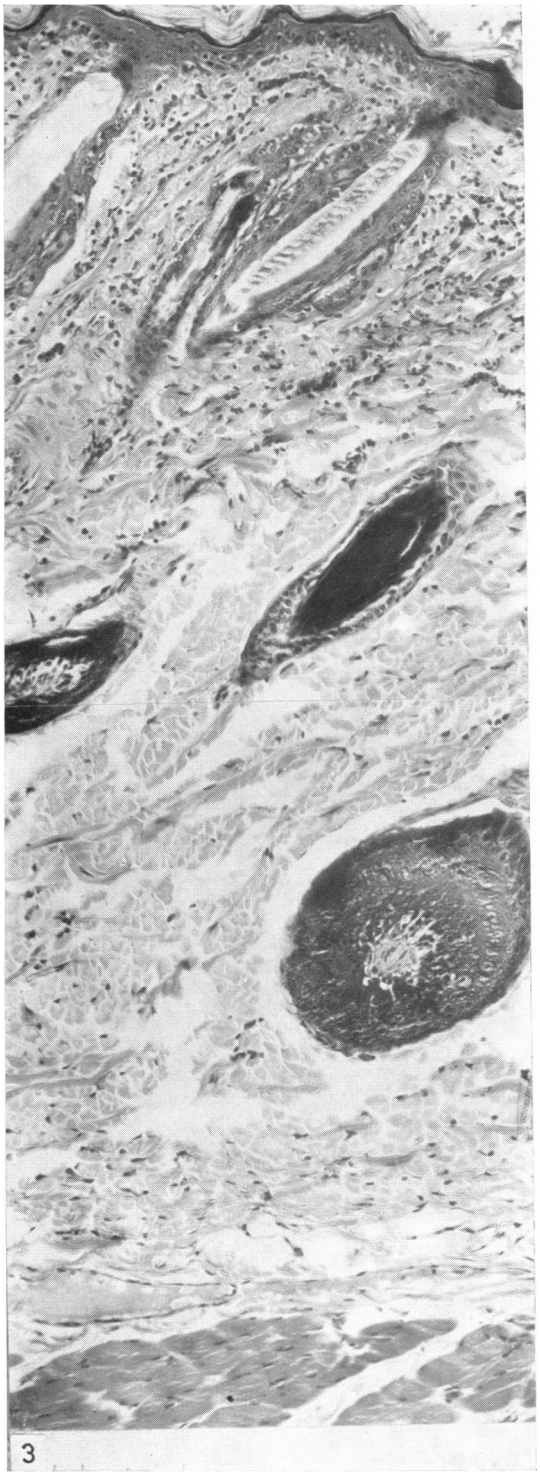

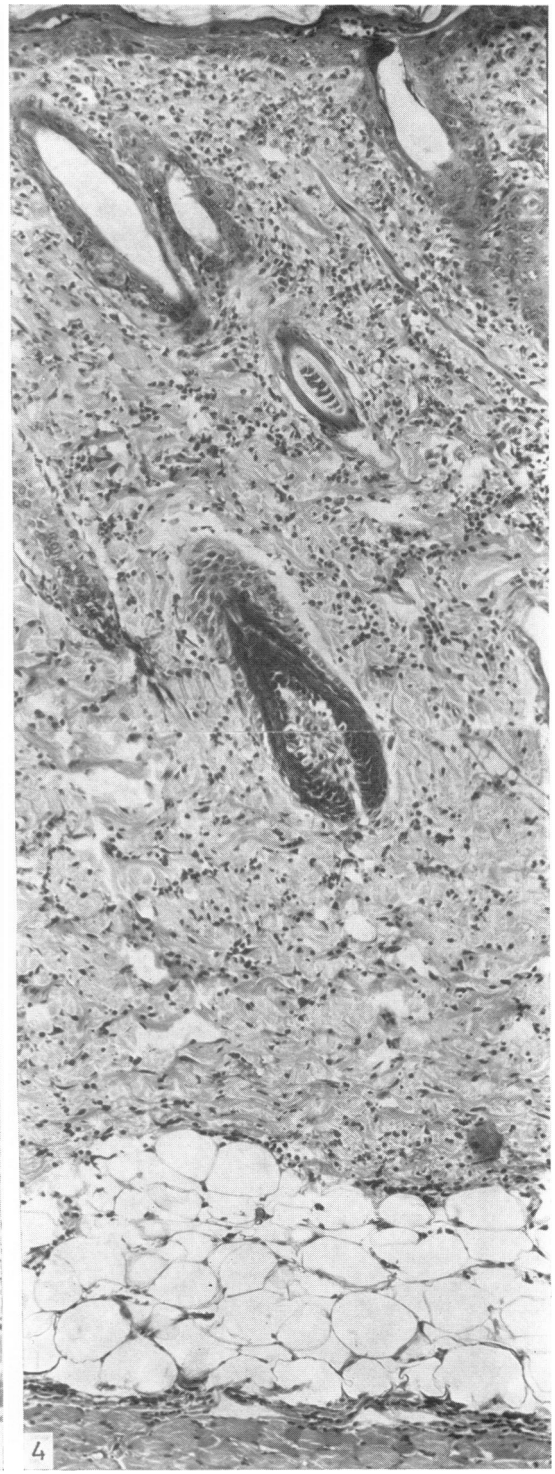

FIG. 3. Twenty-four-hour skin reaction to 20 μg bovine γ-globulin, following transfer of $5{\cdot}4 \times 10^8$ spleen cells from guinea-pigs sensitized with bovine γ-globulin.

FIG. 4. Forty-eight-hour skin reaction to 20 μg bovine γ-globulin, following transfer of 12 ml serum without cells, from guinea-pigs sensitized with bovine γ-globulin (second set, No. 4).

FIG. 5. Forty-eight-hour skin reaction to 20 μg bovine γ-globulin, following transfer of 8 ml serum and $5{\cdot}4 \times 10^8$ spleen cells from guinea-pigs sensitized with bovine γ-globulin.

dose used in the experiment. Likewise cells taken at 1 week have 63–75 per cent of the ability of cells taken at 3 weeks to transfer 24-hour skin reactions to bovine γ-globulin.

The time course of the development of the serum factor involved in passive transfer of 24-hour skin reactions is different. This factor is absent at 1 week but present at 3 weeks. In a separate experiment (Table 5) the serum factor was demonstrated at 2 weeks.

It was concluded that the cell factor in synergy was present 1 week after immunization and stronger at 3 weeks; while the serum factor was absent at 1 week but present at 2 and 3 weeks.

HISTOLOGICAL APPEARANCE OF SKIN REACTIONS

These are based on the passive transfer of skin reactions to bovine serum albumin by peritoneal exudate cells (Table 1) and the passive transfer of skin reactions to bovine γ-globulin by spleen, lymph node and peritoneal exudate cells (Table 4). No studies were made of skin reactions in the donor animals.

Four hours following the injection of a test dose of antigen, excised skin sites showed cellular exudates consisting almost entirely of polymorphonuclear cells. Cells were present in the form of a band with concentrations immediately beneath the epidermis and near the panniculus carnosus, especially in the neighbourhood of small congested veins. No distinction could be made between recipients of serum with or without cells. No reaction was seen where only cells had been transferred.

At 15 hours, heavy infiltration with cells was seen in the dermis but with additional small numbers of cells deep to the panniculus. At this stage, polymorphs predominated but some histiocytes and fibroblasts were also present (Fig. 1). No differences were noted between reactions of animals which had received serum and others which had received serum and cells from any of the three sources (spleen, lymph nodes, peritoneal exudate cells). Where only cells had been transferred, either no reaction or only a very small number of cells was seen.

At 24 hours, recipients of serum showed exudate consisting predominantly of polymorphs, some showing nuclear fragmentation. Recipients of serum with cells, whether from spleen, lymph nodes or peritoneal macrophages, showed moderate to heavy infiltrates of polymorphs, histiocytes and fibroblasts (Fig. 2), extending from the epidermis to the panniculus carnosus. Some animals that had only received cells showed very small numbers of polymorphs and histiocytes, mostly near the deep limit of the epidermis (Fig. 3); others showed no cellular reaction.

At 48 hours, variable numbers of polymorphs still predominated in reactions of serum recipients (Fig. 4). Guinea-pigs which had received cells and serum had moderate to heavy infiltrates of polymorphs, histiocytes and fibroblasts (Fig. 5), while recipients of cells showed only small numbers of cells, mostly consisting of histiocytes and fibroblasts.

Reactions with cells and serum or with serum only were slight at 72 hours. Those with cells and serum showed small numbers of histiocytes with a few polymorphs spread diffusely in a zone bordering the epidermis with only a slight histiocytic reaction in the septa between fat cells in the lower dermis. With serum transfer, there were fewer cells superficially and very few deeply. Only very small numbers of histiocytes were seen at this stage, when only cells had been transferred.

In summary, extensive cellular reactions were seen at test sites of recipients of serum or cells and serum, while recipients of cells showed only sparse infiltrates. Cells changed from an early polymorphonuclear exudate to a later mixed population of polymorphs, histiocytes

and lymphocytes, irrespective of whether the cells transferred with serum were spleen, lymph node or peritoneal macrophages. The proportion of polymorphs to histiocytes seemed to be relatively greater in serum recipients than in animals which had been given both serum and cells.

DISCUSSION

These results show that there is a synergic effect of immune cells and immune serum in the passive transfer of 24-hour skin reactions, i.e. larger 24-hour skin reactions are transferred by cells and serum together than by either singly. The synergy is specific, that is to say the cells and serum must both come from donors immunized against the test antigen. It is difficult to be certain that the delayed hypersensitivity component of the 24-hour skin reaction is increased. Delayed hypersensitivity may be regarded as a reaction which cannot be passively transferred without cells and is characterized by histiocyte and lymphocyte infiltration at 24 and 48 hours. By these criteria there was indeed a synergic effect of immune serum and cells in the passive transfer of delayed hypersensitivity. This synergic action may be analogous to the synergic action of isoantibody and immune cells in graft rejection described by Batchelor, Boyse and Gorer (1960).

The observation that serum taken at 1 week has no synergic actions suggests that the early macroglobulin antibody is unimportant. The finding that cells taken at 3 weeks give stronger delayed hypersensitivity than cells taken at 1 week indicates that the cellular factor in delayed hypersensitivity is increasing during that time.

The morphological study of the role of cells and serum in the passive transfer of reactions showed that skin reactions due to serum alone underwent the classical evolution of the Arthus reaction with early infiltration with polymorphonuclear cells followed later, at 24 hours, by a mixed exudate consisting predominantly of polymorphonuclear cells but also containing a few histiocytes and lymphocytes (see Gell, 1959). By 48 hours these lesions were much reduced.

In contrast, the reaction to cells alone was very slight, and even at its peak at 24 hours there was only slight infiltration with histiocytes and lymphocytes and virtually no residual lesion at 48 hours.

When cells and serum were transferred together the appearances resembled those of a simple Arthus reaction up to 18 hours. By 24 hours, however, the mixed reaction showed more histiocytes and lymphocytes than the corresponding pure serum reaction and by 48 hours the reaction infiltrate consisted almost entirely of histiocytes and lymphocytes.

These results show that the synergic effect of cells and serum seen by inspection in the passive transfer of 24-hours skin lesions has a histological counterpart. This suggests that cells and serum are synergic in the passive transfer of lesions with the histology of delayed hypersensitivity. This does not imply, however, that a serum factor is a necessary element in the passive transfer of delayed hypersensitivity.

The lesions at 18 hours were very similar whether cells and serum or serum alone were transferred. The infiltrate at this stage is mainly polymorphonuclear with a few histiocytes and lymphocytes. This pinpoints the problem of the histological criteria of delayed hypersensitivity.

The microscopic appearance of the delayed hypersensitivity transferred by peritoneal exudate cells, spleen or lymph nodes is similar and the extent of histiocyte infiltration

comparable. This is evidence against the view that different cell populations are able to transfer different types of delayed hypersensitivity.

The synergy of immune cells and serum in the production of delayed hypersensitivity suggests that the extent of tissue damage in some disease states may depend on both factors. The key question about this synergy is whether it is due to a trivial mechanism (such as an inflammatory response caused by antibody–antigen reaction or the local retention of antigen at the site of the test injection) or whether it is closely related to the (unknown) mechanism of delayed hypersensitivity.

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REFERENCES

Asherson, G. L. and Stone, S. H. (1965). 'Selective and specific inhibition of 24-hour skin reactions in the guinea-pig I. Immune Deviation: description of the phenomenon and the effect of splenectomy.' *Immunology*, **9**, 205.

Batchelor, J. R., Boyse, E. A. and Gorer, P. A. (1960). 'Synergic action between isoantibody and immune cells in graft rejection.' *Transplant. Bull.*, **26**, 449.

Boyden, S. V. (1957). 'The immunological response to antigens of mycobacterium tuberculosis in the guinea pig.' *Int. Arch. Allergy*, **10**, 65.

Gell, P. G. H. (1959). 'Cytologic events in hypersensitivity reactions.' *Cellular and Humoral Aspects of the Hypersensitive States* (Ed. by H. S. Lawrence), p. 43. Cassell, London.

Holborow, E. J. and Loewi, G. (1962). 'The immune response to blood group substances.' *Immunology*, **5**, 278.

Loewi, G., Holborow, E. J. and Temple, A. (1966). 'Inhibition of delayed hypersensitivity by pre-immunization without complete adjuvant.' *Immunology*, **10**, 4.