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Contact and Delayed Hypersensitivity in the Mouse

I. ACTIVE SENSITIZATION AND PASSIVE TRANSFER

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Summary. Ear swelling in mice was measured with a micrometer and used to quantify 24-hour skin reactions. Specific contact sensitivity occurred in mice immunized with picryl chloride, 2-phenyl-4-ethoxymethylene oxazolone, dinitrofluorobenzene and tetramethyl-*p*-phenylenediamine as shown by ear swelling 24 hours after challenge. In some mice sensitized with oxazolone significant swelling occurred 4 hours after challenge. It was possible to induce tolerance in adult mice and contact sensitivity to dinitrochlorobenzene was diminished by prior treatment with dinitrobenzenesulphonic acid.

There was some evidence of delayed hypersensitivity to protein antigens. Twenty-four-hour skin reactions to PPD (purified protein derivative of tuberculin) occurred in mice immunized with live BCG or dead tubercle bacilli in Freund's adjuvant. In most experiments the swelling at 24 hours was greater than at 4 hours. Similar reactions to egg albumin and bovine γ-globulin were seen in mice immunized with these antigens in Freund's complete adjuvant and one group of mice showed greater swelling at 24 hours after challenge than at 4 hours.

Contact sensitivity was readily transferred in inbred mice by peritoneal exudate cells when the challenge was undertaken immediately after transfer. Variable and usually inferior passive transfer was obtained with lymph node cells. Attempts to transfer contact sensitivity in outbred mice were unsuccessful.

INTRODUCTION

The mouse is the classical animal for studying certain types of cellular immunity. Transplantation and tumour rejection can be produced and transferred readily (Mitchison, 1953; Billingham, Brent and Medawar, 1954) and *in vitro* inhibition of migration of peritoneal exudate cells (Al-Askari, David, Lawrence and Thomas, 1965) and *in vitro* cytotoxicity (Wilson, 1965) have been demonstrated. Moreover, Fjelde and Turk (1965) found the characteristic lymph node changes associated with the induction of cellular immunity following exposure to contact sensitizing agents, and Munoz (1967) found a few mononuclear cells in skin tests in mice.

Despite this evidence and the work of Crowle (see Crowle and Hu, 1966), delayed and contact sensitivity are usually considered to be difficult to produce in the mouse, and there is only one report of their passive transfer (Crowle, 1959). There is also an account of the passive transfer of systemic tuberculin hypersensitivity (Han and Weiser, 1967). Vredevoe (1964) was unable to transfer delayed skin reaction to bovine serum albumin. This paper

describes contact sensitivity, 24-hour reactions to protein antigens and the successful passive transfer of contact sensitivity to oxazolone.

MATERIALS AND METHODS

Animals

Mice 6 weeks and older of various inbred and outbred strains were used. CBA mice were used for passive transfer. Mice were randomly assigned to experimental and control groups using a table of random numbers.

Immunization

Picryl chloride and other chemically reactive agents can be dispersed in saline by dissolving in alcohol followed by rapid pipetting into 19 or more volumes of saline. Freund's complete adjuvant was made with equal volumes of the aqueous and Arlacel A–paraffin oil (2:8) phase and contained 3 or 5 mg/ml final concentration of dead human tubercle bacilli.

Mice were immunized by antigen in Freund's complete adjuvant and/or by applying 0·1 ml of the agent in olive oil, alcohol or acetone to the skin of the clipped abdomen. In the later experiments 3 per cent 2-phenyl-4-ethoxymethylene oxazolone and 3 or 8 per cent picryl chloride in alcohol were used. The oxazolone was a generous gift from Dr J. A. Stock of the Chester Beatty Institute, London. Gloves were worn when handling it because it is a strong sensitizing agent. Burroughs Wellcome pertussis vaccine was used in Experiments 4–6 of Table 6.

Challenge and quantification

Mice were anaesthetized with ether or intraperitoneal tribromoethanol with amylene hydrate in *tert*-amyl alcohol (avertin). The thickness of the ear was measured with an ordinary engineer's micrometer and both sides of the ear were smeared with the agent in olive oil. In most experiments one drop from a 25 gauge needle was used on each side of the ear. In other experiments the agent in olive oil was applied with a plastic or glass rod. The ear was measured again at 24 hours and sometimes at 4 and 48 hours. Two measurements were made on each occasion and the results were expressed as the increase in thickness of the ear measured in units of 10^{-3} cm. The thickness of the normal mouse ear ranges from 24 to 34 units.

The concentration of the challenging agent was chosen so as to avoid excessive swelling in control mice. In most experiments 1 per cent picryl chloride and 2 per cent or sometimes 1 per cent oxazolone were used.

Induction of immune paralysis

Schneider mice were given 0·25 ml of 8 per cent dinitrobenzene sulphonic acid intraperitoneally on days 1, 2, 4, 6, 7 and 10 while control mice received saline. This treatment did not affect the weight of the mice. Some mice in the two groups were then given 0·25 ml Freund's complete adjuvant (1 mg/ml final concentration of human tubercle bacillus) intramuscularly in the thigh and 0·2 ml intradermally and subcutaneously on day 14. The mice were painted with 5 per cent dinitrofluorobenzene on days 34, 45, 85 and 92 and challenged on day 99 with 3 per cent dinitrochlorobenzene in olive oil.

Passive transfer

CBA mice were used 7 days after sensitization by a single painting with 3 per cent oxazolone or 8 per cent picryl chloride in alcohol. Four days before transfer 2 ml of heavy liquid paraffin were injected intraperitoneally. Peritoneal exudate cells were obtained by injecting 5 ml of Tyrode's solution intraperitoneally and washing out with a further 2 ml. Lymph node cells were excised and single cells obtained by pressing through a plastic tea strainer with the large end of the piston of a tuberculin syringe. In some experiments 10 per cent mouse serum was used for handling the lymph node cells. The cells were filtered through bolting cloth and injected intravenously. In some experiments immune serum was mixed with the cells and injected at the same time. The mice were challenged within 1 hour. Untreated mice were included in the transfer experiments. The results were read without knowledge of the group to which the mice belonged. Cell viability was measured by dye exclusion using 0·125 per cent eosin. Lymph node cells were 58–75 per cent viable and peritoneal exudate cells about 96 per cent viable by this test. The cell counts obtained using 1 per cent acetic acid as diluent were corrected to show the number of viable cells injected.

Statistics

The *t*-test or the Mann Whitney U test (Siegel, 1956) were used. The latter is applicable to data which do not follow a normal distribution, and can be performed more quickly than the *t*-test. Statistically significant refers to $P \leqslant 0{\cdot}05$ in a double tail test.

RESULTS

CONTACT SENSITIVITY IN MICE

Groups of five to eight female Schneider mice were immunized with chemically reactive haptens in Freund's complete adjuvant followed by skin painting. They were tested by applying a solution of the hapten to the skin of the ear and measuring the increase in thickness at 24 hours with a micrometer. Mice sensitized with another agent were used as controls. Table 1 shows that specific and statistically significant ear swelling occurred in mice sensitized with picryl chloride, oxazolone and tetramethyl-*p*-phenylenediamine. Swelling also occurred in mice sensitized with *p*-phenylenediamine and dinitrofluorobenzene and was statistically significant if a one-tailed *t*-test was employed. A number of other agents shown in Table 1 failed to sensitize.

Table 2 shows that contact sensitivity occurred 7 days after a single painting with picryl chloride and oxazolone in Schneider, Parkes and CBA mice. Balb/c mice were tested with picryl chloride and C57/Bl with oxazolone with positive results. It was concluded that contact sensitivity could be demonstrated following a single painting in several different strains of mice.

TIME COURSE OF CONTACT SENSITIVITY FOLLOWING IMMUNIZATION

Table 3 shows that there is considerable ear swelling on testing with picryl chloride at 6 but not at 13 days following sensitization by a single painting with picryl chloride. In contrast the contact sensitivity which follows immunization with picryl chloride in Freund's complete adjuvant is more long lasting. Different results were obtained in

Parkes mice (Allwood and Asherson, unpublished); after sensitization by painting with 8 per cent picryl chloride the increase in ear thickness at 24 hours was similar on testing at 6 and 13 days (30·8 and 32·2 units). Immunization with 50 μg of picryl chloride in 0·05 ml of Freund's complete adjuvant gave increments of 7 and 23 units at 6 and 13 days. It was concluded that the strain of mice and perhaps other factors influenced the time course.

TABLE 1

CONTACT SENSITIVITY IN MICE

Immunizing and test agent		Increase in ear thickness at 24 hours in units of 10^{-3} cm		
		Mean	Range	Significance
Picryl chloride	(7)	11·9	3·7–22·9	
Control	(8)	2·9	1·1– 3·9	$P<0·01$
2-Phenyl-4-ethoxymethylene oxazolone	(7)	9·6	7·9–11·8	
Control	(8)	3·2	2·3– 8·0	$P<0·01$
Tetramethyl-*p*-phenylenediamine hydrochloride	(5)	4·7	2·9– 6·3	
Control	(5)	2·3	1·3– 2·9	$0·05>P>0·02$
p-Phenylenediamine	(7)	3·3	−0·3– 9·6	
Control	(8)	1·6	−0·6– 6·4	$0·1>P>0·05$
Dinitrofluorobenzene	(5)	3·9	1·5–11·3	
Control	(6)	1·4	−0·7– 4·3	$0·1>P>0·05$
2:6 Dibromo-*p*-benzoquinone-4 chlorimine	(8)	2·0	0·6– 3·4	
Control	(8)	2·4	0·6– 3·7	$P>0·1$
4:4 Diamidinodiphenylamine dihydrochloride	(5)	2·0	1·5– 3·4	
Control	(6)	1·8	1·6– 2·0	$P>0·1$
2:4 Dinitroanilinomaleimide	(8)	1·6	−0·2– 4·1	
Control	(8)	1·5	0·1– 1·6	$P>0·1$

Mice were injected with 0·2 μM of various agents in 0·15 ml of Freund's complete adjuvant into the base of the tail, the nuchal area and thigh muscles. The clipped abdomen was painted at 1 and 2 weeks with 5 per cent solution of the agent in olive oil, olive oil–acetone mixture (1:1), or 10 per cent aqueous solution of Tween 80 and ear tests performed at 3 weeks. The solvents and concentrations of the agent used for testing in the order of the table were 2·5 per cent in olive oil, 3 per cent in olive oil, 2 per cent in aqueous 10 per cent Tween 80, saturated solution in olive oil–acetone mixture, 2 per cent in olive oil, saturated solution in olive oil–acetone mixture, 15 per cent in 10 per cent Tween 80 and 10 per cent in olive oil–acetone mixture. The number of mice in each group is shown in parentheses.

EFFECT OF REPEATED SKIN PAINTING

CBA mice developed good contact sensitivity on challenge 7 days after sensitization with 3 per cent oxazolone, and comparable results were obtained on testing 7 days after three weekly sensitizations. Ten mice were used in each group and the figures for the increase in swelling at 24 hours were $19·0\pm2·44$ (SD) and $17·0\pm4·04$ units, respectively. It was concluded that repeated skin painting did not increase ear swelling due to contact sensitivity to oxazolone in CBA mice.

TIME COURSE OF EAR SWELLING FOLLOWING CHALLENGE

Thirteen mice were immunized with oxazolone-treated sheep red cell stroma in Freund's complete adjuvant and skin painting, while eleven unimmunized mice served as a control. The mice were tested at 3 weeks. Experiment 1 of Table 4 shows that there

TABLE 2

INCREASE IN EAR THICKNESS AT 24 HOURS FOLLOWING CHALLENGE 7 DAYS AFTER A SINGLE PAINTING

Mouse strain	Group	Increase in ear thickness in absolute units and as percentage of initial thickness after painting with:	
		2% oxazolone	2% picryl chloride
Schneider	Exp.	15·2±5·6, 20·7±3·7* (60%, 80%)	5·8±3·2, 12·6±5·0* (24%, 52%)
	Con.	2·8±1·8, 2·8±1·3* (11%, 12%)	1·1±0·9, 4·0±2·2* (4%, 16%)
Parkes	Exp.	17·6±2·7, 28·2±3·8† (72%, 92%)	11·9±3·5, 19·7±3·1† (49%, 80%)
	Con.	2·4±1·7, 5·5±2·7† (8%, 20%)	6·6±1·6, 6·9±1·6† (27%)
CBA	Exp.	18·2±3·4, 20·7±4·5† (58%, 74%)	11·9±2·3, 15·7±1·8† (44%, 58%)
	Con.	3·8±1·1, 5·4±1·8† (13%, 18%)	1·2±0·7, 4·9±2·0† (5%, 18%)
C57/Bl	Exp.	8·4±1·8 (34%)	—
	Con.	0·0±0·9 (0%)	—

Each figure is based on at least ten mice.
* Two experiments.
† Extreme mean results from four experiments.

TABLE 3

INCREASE IN EAR THICKNESS 24 HOURS AFTER CHALLENGE WITH 1 PER CENT PICRYL CHLORIDE

Mode of immunization	Time after immunization	
	7 days	14 days
Nil (control)	0·8±0·1 (2·8%)	2·5±1·69 (9·5%)
Adjuvant only (control)	2·3±1·39 (8·7%)	—
Painting with 8 per cent picryl chloride	9·9±1·26 (36·8%)	2·2±0·97 (8·0%)
20 mg picryl chloride in Freund's complete adjuvant	8·3±3·53 (31·5%)	5·2±3·21 (20·5%)
1 mg picryl chloride in Freund's complete adjuvant	5·1±1·66 (19·5%)	3·9±1·79 (15·2%)

Each figure is based on five to eight Balb/c mice. The absolute and percentage increment of thickness and the standard deviation of the absolute increment are shown.

was significant ear swelling in the immunized mice as compared with controls at 4, 24 and 48 hours. This early swelling was seen in several other experiments in which oxazolone-treated stroma was used for sensitization. Experiment 2 of Table 4 shows that significant 4-hour swelling is seen in Schneider mice 7 days after sensitization by painting with 3 per cent oxazolone and testing with 2 per cent oxazolone. In one other experiment equivocal swelling occurred at 4 hours.

TABLE 4

INCREASE IN EAR THICKNESS AT VARIOUS TIMES AFTER CHALLENGE WITH 2 PER CENT OXAZOLONE

	Group	Time (hours) after challenge			
		4	8	24	48
Experiment 1	Immunized (13)	5·0	8·2	9·5	7·8
	Control (11)	1·8	2·4	2·1	1·9
Experiment 2	Immunized (9)	4·6		15·2	
	Control (10)	0·9		2·8	

In Experiment 1 mice were immunized with oxazolone-treated sheep red cell stroma in Freund's complete adjuvant (Turk and Asherson, 1962) and 0·02 ml of 5 per cent oxazolone in olive oil–acetone mixture (1:1) was applied to the clipped abdomen 14 days later. The mice were tested at 3 weeks with 1 per cent oxazolone. Control mice were left untreated. The details of Experiment 2 are in the text. The number of mice used is shown in parentheses.

TABLE 5

INCREASE IN EAR THICKNESS AFTER CHALLENGE WITH PPD

Group	Strain	Initial ear thickness	Increase in ear thickness at:		
			4 hours	24 hours	48 hours
Control	C57 (12)	27·4	6·15	1·52	
Immunized	C57 (12)	26·4	5·54	11·96	
Control	Schneider (6)	30·1	4·75	1·68	1·02
Immunized	Schneider (6)	28·6	5·46	7·62	6·6
Control	CBA (6)	25·9		2·1	
Immunized*	CBA (5+4)	27·2, 26·7		6·0, 6·7	
'Deviated'†	CBA (5+5)	31·8, 25·4		6·3, 6·9	

The number of mice is shown in parentheses.

* Five mice received no pretreatment while four received 200 mg alum precipitated bovine γ-globulin 1 week before immunization with Freund's complete adjuvant.

† Five mice received 200 μg alum precipitated PPD 1 week before immunization intravenously while another five mice were pretreated subcutaneously.

TWENTY-FOUR-HOUR REACTIONS TO PPD

Schneider, C57/Bl and CBA mice develop 24-hour skin reactions to PPD after immunization with Freund's complete adjuvant. Table 5 shows that there is no swelling at 4 hours and that the swelling persists at 48 hours. This time course suggests that the reactions are due to classical delayed hypersensitivity. However, in another experiment in Schneider mice there was comparable, significant swelling in the immunized mice at 4 and 24 hours. The skin reactions were not reduced by treatment with alum precipitated antigen before immunization with Freund's complete adjuvant (see Asherson and Stone, 1965).

TABLE 6

FOUR-, 24- AND 48-HOUR REACTIONS TO EGG ALBUMIN AND BOVINE γ-GLOBULIN IN MICE IMMUNIZED WITH THESE ANTIGENS

Experiment	Strain and number of mice	Group	Immunizing antigen	Mean and range of increment of ear swelling at:			Comment
				4 hours	24 hours	48 hours	
1a	Schneider (5)	Exp.	5 μg egg albumin	3·8 (1·8–5·7)	4·0 (1·0–6·3)		Tested day 21
	Schneider (5)	Con.	5 μg BGG	3·1 (2·7–5·1)	2·5 (0·63–5·3)		
1b	Schneider (5)	Exp.	5 μg egg albumin	16·1 (2·4–21·7)	13·8 (5·6–28·4)		Mice of experiment 1 re-tested day 27
	Schneider (5)	Con.	5 μg BGG	5·4 (3·2–8·9)	2·9 (1·9–4·6)		
1c	Schneider (4)	Exp.	5 μg egg albumin	3·8 (2·9–5·3)	5·5 (4·6–6·5)		Tested for first time day 27
	Schneider (3)	Con.	5 μg BGG	3·8 (2·3–5·5)	0·9 (0·3–2·0)		
2	Schneider (9)	Exp.	100 μg egg albumin	21·0 (16·4–24·8)	22·5 (17·4–27·1)	21·4 (14·4–30·1)	Tested day 12
	Schneider (9)	Con.	100 μg BGG	4·7 (1·3–10·0)	3·5 (2·0–4·0)	2·4 (1·0–3·7)	
3	Schneider (8)	Exp.	400 μg egg albumin	37·8 ± 3·8	43·0 ± 8·7		Tested day 35
	Schneider (8)	Con.	Adjuvant only	4·9 ± 1·6	3·0 ± 0·8		
4	CBA (10)	Exp.	400 μg egg albumin	6·9 ± 3·8	17·6 ± 7·1		Tested day 35
	CBA (10)	Con.	Adjuvant only	7·0 ± 4·1	9·1 ± 4·4		
5	Schneider (6)	Exp.	400 μg BGG	31·4 ± 10·3	25·3 ± 15·1		Tested day 35
	Schneider (12)	Con.	Adjuvant only	6·7 ± 2·9	4·7 ± 1·9		
6	CBA (10)	Exp.	400 μg BGG	31·3 ± 5·3	21·5 ± 8·6		Tested day 35
	CBA (10)	Con.	Adjuvant only	2·4 ± 1·6	3·8 ± 3·3		

Experiments 1a, 1b and 1c were performed concurrently. In these experiments the mice were sensitized once with antigen in Freund's complete adjuvant. In Experiments 2 and 3 the mice were boosted on day 28 with 200 μg antigen in Freund's complete adjuvant. In Experiments 4–6 the second injection was given in Freund's incomplete adjuvant emulsified with an equal volume of pertussis vaccine.

I am indebted to Dr R. J. W. Rees of the National Institute for Medical Research for the observation that mice infected with live BCG also develop 24-hour skin reaction to PPD. The mean ear thickness of infected mice after challenge was 23·8 units as compared to 15·6 in the control mice.

TWENTY-FOUR-HOUR REACTIONS TO EGG ALBUMIN AND BOVINE γ-GLOBULIN

Table 6 shows the results of ear tests in mice immunized with egg albumin and bovine γ-globulin in Freund's complete adjuvant. In the first experiment (1a–c) three groups of Schneider mice were sensitized with 5 μg egg albumin, while control mice received bovine γ-globulin. Little ear swelling was seen in the mice tested for the first time at 21 or 27 days. Testing at 21 days, however, increased the swelling seen after testing at 27 days and swelling of 16·1 and 13·8 units was seen at 4 and 24 hours in mice after the second test. A similar effect of the test injection is described by Munoz (1967).

Schneider mice sensitized with 100 μg egg albumin showed comparable swelling (21·0 units) at 4, 24 and 48 hours (Experiment 2). Schneider mice sensitized with 400 μg egg albumin showed greater swelling at 24 hours (43 units) than at 4 hours (Experiment 3). CBA mice sensitized with 400 μg egg albumin in Freund's complete adjuvant and boosted with antigen and pertussis vaccine in Freund's incomplete adjuvant (Experiment 4) showed no greater swelling than controls at 4 hours, but 17 units of swelling at 24 hours. In this experiment the time course provides some evidence that the 24-hour reactions were indeed due to delayed hypersensitivity. Nevertheless, 3→1 transfer of peritoneal exudate cells or lymph node cells or serum into groups of five mice were negative.

Swelling of 20–30 units was seen in mice similarly immunized with bovine γ-globulin, but this was less at 24 hours than at 4 hours (Experiment 6). Passive transfer with lymph node or peritoneal exudate cells was negative.

IMMUNOLOGICAL UNRESPONSIVENESS

In the guinea-pig, injection of dinitrobenzenesulphonic acid (DNBSA) prevents the contact sensitivity which otherwise follows sensitization with dinitrofluorobenzene (see Turk, 1967). Similar observations were made in two experiments in Schneider mice. One group received a series of injections with DNBSA while control mice received saline. Some, but not all, mice were then given Freund's complete adjuvant (without antigen). All the mice were then sensitized with dinitrofluorobenzene and finally tested 14 weeks after the beginning of the experiment. Table 7 shows the results. The first experiment provided statistically significant evidence that DNBSA reduces contact sensitivity caused by sensitization with dinitrofluorobenzene. The results of the second experiment were in the same direction but were not statistically significant. There was a suggestion that treatment with adjuvant at the end of the course of DNBSA diminished the ability of DNBSA to cause tolerance and decreased the sensitization caused by dinitrofluorobenzene.

Treatment with DNBSA also reduced antibody production to the dinitrophenyl group. Thirteen of twenty-three control mice died within 1 hour of the intravenous injection of 5 mg of dinitrophenylated casein, while only one out of the twenty-two mice treated with DNBSA died. It was concluded that DNBSA depressed antibody production and contact sensitivity which otherwise follow immunization with dinitrofluorobenzene.

PASSIVE TRANSFER OF DELAYED HYPERSENSITIVITY

Transfer by immune cells is an important criterion of cellular immunity. In the following experiments peritoneal exudate or other cells were obtained from mice 7 days after sensitization by a single painting with 3 per cent oxazolone. Cells and sometimes serum

TABLE 7

INHIBITION OF CONTACT SENSITIVITY TO DINITROFLUOROBENZENE BY PRETREATMENT WITH DINITROBENZENE SULPHONIC ACID

Treatment	Increase in ear thickness at 24 hours	
	Experiment 1	Experiment 2
DNBSA	2·2 (5)	2·5 (8)
Saline	10·0 (6)*	4·9 (9)
DNBSA followed by FCA	5·6 (6)	3·7 (4)
Saline followed by FCA	8·3 (6)	3·8 (3)

FCA = Freund's complete adjuvant; DNBSA = dinitrobenzene sulphonic acid. The details are given in 'Materials and methods'. The number of mice in each group is shown in parentheses.

* The swelling in Experiment 1 in mice treated with saline is significantly greater than in mice treated with DNBSA.

TABLE 8

THE EFFECT OF THE NUMBER OF PERITONEAL EXUDATE CELLS INJECTED ON THE PASSIVE TRANSFER OF CONTACT SENSITIVITY TO OXAZOLONE

No. of peritoneal exudate cells	Mean absolute and percentage increase of ear thickness at 24 hours
3→1 transfer $7{\cdot}2 \times 10^7$ cells	$11{\cdot}2 \pm 2{\cdot}04$ (43%)
2→1 transfer $4{\cdot}8 \times 10^7$ cells	$10{\cdot}7 \pm 2{\cdot}62$ (42%)
1→1 transfer $2{\cdot}4 \times 10^7$ cells	$6{\cdot}3 \pm 1{\cdot}54$ (24%)
½→1 transfer $1{\cdot}2 \times 10^7$ cells	$5{\cdot}8 \pm 2{\cdot}46$ (23%)
Serum (2 ml)	$3{\cdot}3 \pm 2{\cdot}15$ (12%)
Controls	$3{\cdot}7 \pm 0{\cdot}66$ (14%)

There were five recipients in each group.

were injected intravenously into recipients. These were challenged within 1 hour with 2 per cent oxazolone and the increase in swelling measured at 24 hours. All successful experiments were undertaken in CBA mice.

Table 8 shows that mice receiving pooled peritoneal exudate cells from three or two other mice showed swelling of about 11 units (42 per cent increase in thickness); those

receiving 1 to 1 or ½ to 1 transfers showed swelling of about 6 units. This was statistically significant ($P = 0{\cdot}016$) in the 1 to 1 transfer. Control mice or mice given 2 ml of immune serum showed swelling of about 3·4 units (13 per cent).

Table 9 shows that reproducible transfers were obtained in six consecutive experiments. Experiments 1 and 2 illustrate transfer by peritoneal exudate cells, lymph node cells and serum given together. Experiment 3 shows that peritoneal exudate cells alone give good transfers. In Experiment 4 cells and serum together transferred significantly greater swelling than either singly ($P \leqslant 0{\cdot}016$).

TABLE 9

PASSIVE TRANSFER OF CONTACT SENSITIVITY TO OXAZOLONE IN CBA MICE

Experiment	No. of peritoneal exudate cells	No. of lymph node cells	Serum (ml)	Mean absolute and percentage increase in thickness of ear	
				Experimental group	Control group
1	$3{\cdot}1 \times 10^7$	$1{\cdot}1 \times 10^8$	0·4	9·7 ± 1·69 (4) (40%)	3·5 ± 1·07 (4) (14%)
2	$2{\cdot}8 \times 10^7$	$7{\cdot}7 \times 10^7$	0·75	13·9 ± 3·40 (4) (44%)	8·8 ± 1·79 (5) (27%)
3	$1{\cdot}2 \times 10^8$	—	—	8·4 ± 1·59 (6) (33%)	4·4 ± 1·08 (7) (17%)
4A	$9{\cdot}3 \times 10^7$	$1{\cdot}0 \times 10^8$	—	10·6 ± 2·09 (5) (34%)	7·3 ± 2·06 (5) (23%)
4B	—	—	0·5	8·6 ± 1·55 (5) (27%)	
4C	$9{\cdot}3 \times 10^7$	$1{\cdot}0 \times 10^8$	0·5	15·9 ± 1·87 (5) (51%)	
5A	$3{\cdot}9 \times 10^7$	—	—	10·9 ± 1·71 (5) (42%)	5·8 ± 1·44 (5) (22%)
5B	—	$1{\cdot}3 \times 10^8$	—	7·6 ± 1·44 (5) (29%)	
6A	$2{\cdot}4 \times 10^7$	—	0·7	11·8 ± 1·95 (4) (38%)	3·7 ± 2·82 (8) (12%)
6B	—	$1{\cdot}0 \times 10^8$	0·7	5·9 ± 0·67 (4) (18%)	
6C	$2{\cdot}4 \times 10^7$	$1{\cdot}0 \times 10^8$	0·7	9·7 ± 2·69 (4) (32%)	

The number of recipient mice is shown in parentheses.

Experiments 5 and 6 show that peritoneal exudate cells transfer greater contact sensitivity than lymph node cells ($P = 0{\cdot}15$ and 0·028, respectively) even when the lymph node transfers are undertaken with four times as many cells as the peritoneal exudate cell transfers. This is a reproducible result and has been confirmed in two further experiments using donors sensitized three times over a 4-week period.

In contrast to these findings in inbred CBA mice, contact sensitivity could not be transferred in Parkes mice. The first experiment, using $4{\cdot}2 \times 10^7$ peritoneal exudate cells and 1 ml serum suggested that the serum produced 24-hour ear swelling (*single* tail Mann Whitney U test gave $P \simeq 0{\cdot}05$). Two further experiments were, however, entirely negative.

Negative results were also obtained in an 8 to 1 transfer of $1{\cdot}4 \times 10^8$ peritoneal exudate cells in Schneider mice.

DISCUSSION

This paper describes the passive transfer of contact sensitivity in mice and the production of 24-hour skin reactions to protein antigens. This confirms the work of Crowle (1959) and other workers who used the footpad (see Han and Weiser, 1967, for references) and thigh muscle tests (O'Grady, 1957). Quantification of the reactions was facilitated by using a micrometer to measure the thickness of the ear. This is a useful method of measuring inflammatory skin reactions and can be used even when there is no erythema. A similar method has been used in hamsters by Frenkel (1967).

The data show that specific 24-hour contact sensitivity reactions can be induced to picryl chloride, oxazolone and other chemically reactive haptens. Twenty-four-hour reactions can also be produced by PPD, egg albumin and bovine γ-globulin. The presumption is that these 24-hour reactions are due, at least in part, to cellular immunity.

Passive transfer by immune cells is one of the criteria of cellular immunity. In the present experiments contact sensitivity was transferred by peritoneal exudate cells. In the guinea-pig, 24-hour reactions to bovine γ-globulin and bovine serum albumin can be transferred by peritoneal exudate cells given intravenously. Much bigger reactions are seen if immune serum is also injected intravenously. There is some evidence that circulating antibody may play a role in the swelling seen at 24 hours in the mouse. For instance, polymorphs are the most frequent cell in the 24-hour reaction to picryl chloride and oxazolone in actively sensitized mice. Moreover, in one experiment, serum and cells transferred bigger reactions than cells alone.

Three factors are important in obtaining passive transfers. The first requirement is an adequate method of quantification. In the present experiments it was essential to use inbred mice. This parallels the findings of Turk and Leibowitz (1964) that delayed hypersensitivity could not be transferred between certain strains of guinea-pigs. It has also been found that peritoneal exudate cells are more effective than lymph node cells in transferring delayed hypersensitivity. Similar observations have been made in the rat (Asherson and Meyer, unpublished observations), and guinea-pig, and this is in keeping with the finding that peritoneal exudate cells are more active than lymph node cells in causing inhibition of migration of peritoneal exudate cells.

The current hypothesis about the mechanism of delayed hypersensitivity suggests that the first cells to arrive at the test site do so because of local inflammation. This implies that these are circulating cells which mediate delayed hypersensitivity and congregate in inflammatory lesions. It is not surprising, therefore, that a peritoneal exudate, produced as a result of inflammation caused by oil, contains many cells able to mediate delayed hypersensitivity. In contrast, many of the lymphocytes in lymph nodes must be concerned with the induction and perpetuation of immune responses and the number of cells mediating delayed hypersensitivity might be expected to be lower than in an inflammatory exudate. These considerations suggest that there are several functional populations of lymphocytes and raises the question of the origin of the peritoneal exudate cells that mediate delayed hypersensitivity.

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