Experimental Studies on the Local Passive Transfer of Tuberculin Hypersensitivity

Report II. Tuberculin hypersensitivity induced in normal animal skin by injection of heterologous cells mixed with antigen

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INTRODUCTION

In the previous paper[1] it was demonstrated that the typical immediate type skin reaction is induced in the homologous recipient, by using mainly peritoneal exudate cells which are obtained from sensitized guinea pigs or rabbits, and mixed with antigen *in vitro*. This may indicate that tuberculin skin reaction is a secondary non-specific mesenchymal inflammatory reaction, induced by a certain active principle of the substance released from the primary antigen-antibody reaction.

It might therefore be considered to be possible to transfer tuberculin hypersensitivity passively in the heterologous as well as in the homologous animal, by using cells plus antigen mixture.

As yet, there have been few investigations[2-3] which show exact evidence of heterologous passive transfer of tuberculin hypersensitivity.

In the present paper, therefore, an experiment performed in the attempt to induce heterologous local passive transfer of tuberculin hypersensitivity will be reported.

As it has been generally considered that the rat is less sensitive to allergic and anaphylactic stimuli by various antigens, this species has been used first. And then, interspecies local passive transfer between two of the following three animal species : the guinea pig, rabbit and rat, was carried out.

Except when the rat was used as a recipient, heterologous local passive transfer always induced a typical delayed type reaction.

MATERIALS AND METHODS

Animals :

Male albino rats of the Wistar strain, each weighing from 150 to 200g., were

fed with chow and fresh cabbage daily in our laboratory for about one month before the beginning of the experiment and confirmed to be completely healthy. Albino rabbits and guinea pigs of both sexes, weighing 2.5 to 3 kg. and 300 to 400g., respectively, were also used as donors and recipients.

Sensitization :

Each donor animal received a subcutaneous injection of 10 mg. of heat-killed H37Rv strain of tubercle bacilli suspended in lanolin oil and liquid-praffin mixture in the thigh once a week for two or three weeks. At approximately six weeks after the last injection, rabbits and guinea pigs were tested for skin sensitivity with 0.1 ml. of ten-fold dilution of old tuberculin. All the animals had a very marked skin reaction with erythema, induration, and central blanching or necrosis.

Preparation of cell-materials :

The methods have already been described in detail in the previous paper[1]. In brief, they were as follows. Sterile liquid-paraffin oil, 10 ml. for the rat, 20 ml. for the guinea pig and 100 ml. for the rabbit was injected into the peritoneal cavity.

After three days, the donors were sacrificed by heart puncture. The peritoneal cavity was then lavaged with chilled heparinized Tyrode's solution. A suspension of the exudate cells was centrifuged for five minutes at 1.000 r.p.m. After the supernatant fluid was discarded, the packed cells were resuspended in fresh Tyrode's solution.

Homogenated cell-materials of other visceral organs were also prepared using the same method described in the previous paper.

Antigen :

Old tuberculin (O.T) was generously supplied by The National Institute of Health in Tokyo City.

In vitro treatment of test-materials :

Cell-materials were mixed with an equal volume of old tuberculin diluted in physiological saline solution in a small test-tube. In one experiment, they were incubated for various lengths of time and at different temperatures before being injected into the recipient. In the control experiments, cell-materials were injected without incubation, after being mixed either with antigen or with glycerin-bouillon.

Injection of test-materials and observation of skin reaction :

A 0.2 ml. aliquot of each test-material containing about 0.05 to 0.08 ml. of

exudate cells was injected into the shaved skin of both flanks of the recipient. At an appropriate time ; occasionally 2, 4 and 6 hours, usually 12, 24, 48, 72 and 96 hours after the injection, the size of the skin reaction was measured in the manner described in the previous paper[1].

RESULTS

1) Experiment in which rats' peritoneal exudate cells were injected into the skin of guinea pigs.

It has been generally considered, with a few exceptions,[4] that the rat is incapable of developing a tuberculin skin reaction.

And, it has not yet been determined whether the cells of sensitized rats fail to transfer passively tuberculin hypersensitivity because they cannot produce antibody or because of some other unknown factor. It may be that lack of tuberculin skin reaction in the rat is due to the lower skin reactivity of this

Table 1. Skin reaction induced in normal guinea pig after injection of peritoneal exudate cells of sensitized rat : Effect of previous treatment *in vitro* on cell-antigen mixture.

Incubation Temperature	Incubation Period (hr)	Cell	Antigen	Skin Reaction (mm) 12	24	48	72	96 hrs
—	—	S	O·T	18×18	24×20	31×25	26×18	22×17
			G·B	16×15	18×15	15×13	13×13	12×12
		N	O·T	10×10	12×12	17×20	12×14	9×13
			G·B	12×11	12×11	9×8	9×10	10×9
4°C	3	S	O·T	18×18	25×22	32×28	26×25	20×18
		N		7×9	10×8	12×12	10×12	9×10
			G·B	6×8	8×5	7×7	7×7	7×6
37°C	1	S		15×15	16×13	11×13	12×12	12×10
			O·T	18×18	23×21	28×24	20×18	18×18
		N		7×7	8×8	10×13	7×8	6×8
	3	S		15×16	17×15	18×18	14×14	10×10
70°C	1		O·T	8×8	8×7	8×6	5×4	5×5
100°C	30min			8×8	8×8	8×8	8×8	8×6
		N		4×4	4×3	3×3	3×2	2×2

O·T=Old Tuberculin
G·B=Glycerine-bouillon
S=Sensitized
N=Normal

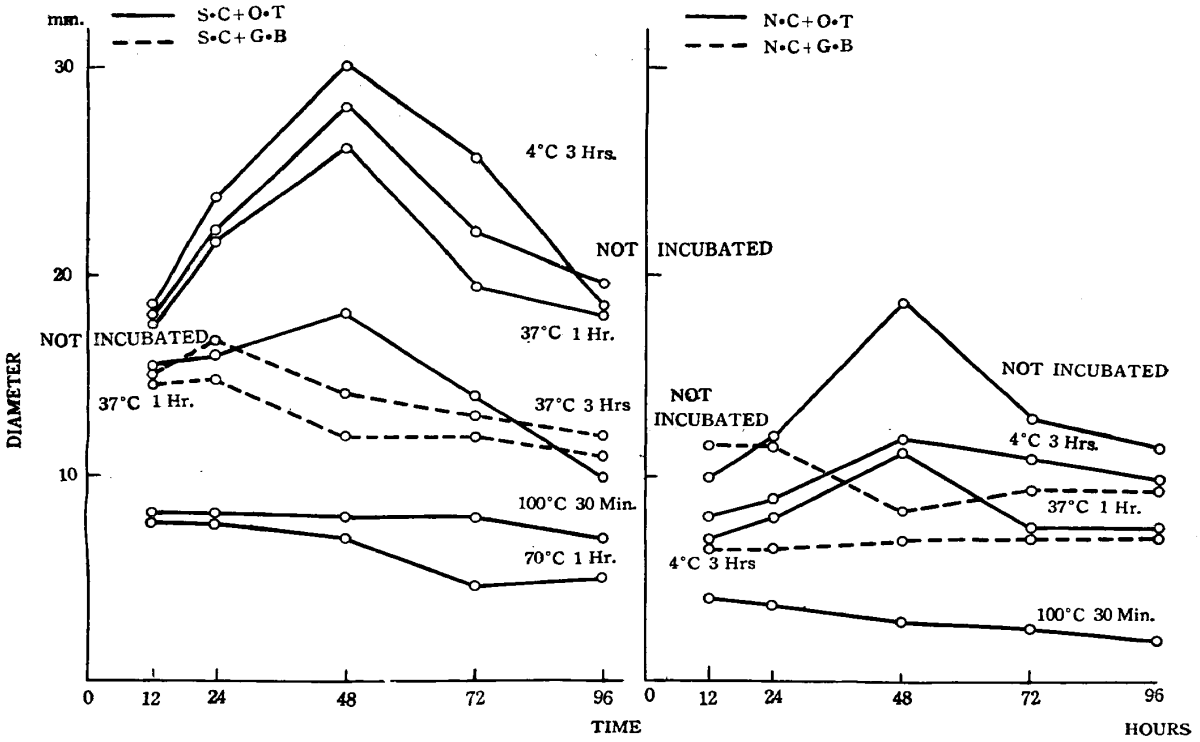

Fig. 1. Skin reaction induced in guinea pigs by incubated rat cells plus antigen mixture.

species than other laboratory animals, such as the guinea pig and rabbit.

In order to find the real cause of this insensitivity of the rat, rats' peritoneal exudate cells mixed with antigen were injected into the skin of normal guinea pigs.

Before the injection, some samples of the test-materials were incubated at 4°C for 3 hours, and at 37°C for one or three hours. And other samples were heated at 70°C for one hour or at 100°C for half an hour in a water bath. The results are summarized in table 1. Typical and very strong delayed type reactions were induced in the guinea pig by using sensitized cells from the rat. These reactions reached their maximum about 48 hours after the injection, differing from the immediate type reaction of homologous passive transfer.

Often these reactions showed an induration of about 30 mm. in size and were accompanied with slight or moderate central necrosis. Heated cells lost their activity completely.

2) Experiment in which rats' visceral cells were injected into the skin of guinea pigs.

An experiment was carried out using cells from the homogenated liver, spleen and lung of the rat. The data are shown in table 2. Small but well-defined delayed type reactions were observed.

3) Experiment in which guinea pig cells were injected into the skin of rats.

Table 2. Skin reaction induced in the guinea pig after injection of rats' visceral cells mixed with antigen.

No. of Recipient	Orgen	Cell	Skin Reaction (mm) 12	24	36	48	72	96 hrs
No. 22	Spleen	S	10×11	14×13	20×16	18×18	8×10	8×9
		N	8×10	8×10	7×7	6×7	6×7	5×7
No. 23	Liver	S	3×4	5×5	5×6	4×4	4×3	3×2
		N	3×4	4×5	5×5	5×4	3×2	3×2
No. 24	Lung	S	8×7	13×10	8×10	8×8	6×9	5×7
		N	7×6	8×7	6×7	6×6	5×7	6×7

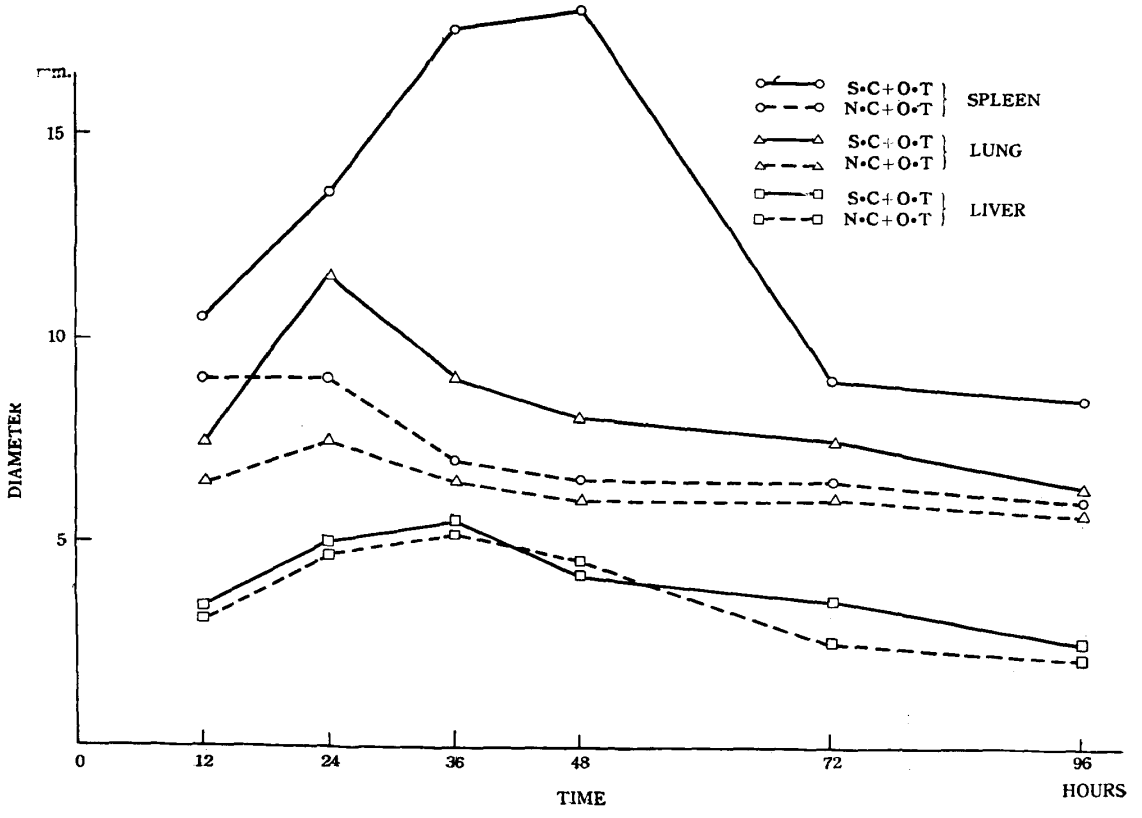

Fig. 2. Skin reaction induced in guinea pigs by rats' visceral cells plus antigen mixture.

Peritoneal exudate cells from sensitized guinea pigs mixed with dilute old tuberculin showed neither erythema nor induration in the skin of the normal rat, but only a slight swelling.

It may be of interest that cells from the sensitized rat, which usually reveals no skin reaction to old tuberculin, can induce a strong reaction, when the normal guinea pig is used as a recipient.

From these experimental findings, it may be inferred that lack of skin sensitivity in the rat is related rather to the nature of the skin than to the incapability of antibody production of this animal.

It is not clear, however, why the delayed type reaction has been induced in the rat-guinea pig system, differing from the immediate type reaction which is observed in homologous passive transfer. The following experiments were per-

Table 3. Skin reaction induced in the rabbit after injection of rats' peritoneal exudate cells mixed with various concentration of antigen.

Cell	No. of Recipient	Concentration of O·T	Skin Reaction (mm) 6	12	24	36	48	60	72	96 hrs
S	No. 71	1 : 1	6×5	9×9	19×10	11×12	13×13	8×9	8×8	8×7
		1 : 5	3×4	9×7	9×8	9×10	10×10	7×7	6×6	5×5
		1 : 10	3×3	6×6	7×6	7×6	5×5	5×5	4×4	4×4
		1 : 20	0×0	6×6	7×7	6×6	5×5	5×5	4×4	4×4
		1 : 40	0×0	5×6	5×6	5×5	5×4	5×5	4×4	4×3
		1 : 80	0×0	5×4	5×5	5×5	5×4	5×4	4×4	3×4
		1 : 160	0×0	5×4	5×4	5×5	5×4	5×4	4×4	3×3
		1 : 320	0×0	5×6	5×6	5×5	5×4	4×3	3×4	3×3
		1 : 640	0×0	5×5	5×5	5×4	4×4	3×2	3×2	0×0
	No. 72	1 : 1	6×6	9×9	9×9	10×11	13×13	10×10	8×8	7×8
		1 : 5	4×5	7×6	7×6	7×7	10×9	8×6	5×5	4×5
		1 : 10	0×0	5×4	5×4	6×6	6×6	5×5	4×4	4×4
		1 : 20	0×0	5×4	5×5	6×6	5×5	5×5	4×4	4×4
		1 : 40	0×0	4×4	5×5	5×5	5×5	5×4	3×4	3×4
		1 : 80	0×0	5×4	5×5	5×5	5×4	4×4	3×4	3×3
		1 : 160	0×0	3×4	4×5	4×4	4×4	4×4	3×4	3×3
		1 : 320	0×0	4×4	4×4	4×4	4×4	3×3	3×3	3×4
		1 : 640	0×0	3×3	3×3	3×3	3×3	3×3	3×2	0×0
N	No. 73	1 : 1	0×0	5×2	5×4	5×5	5×6	4×5	4×5	2×1
		1 : 5	0×0	2×2	5×4	4×4	5×4	5×4	2×2	2×1
		1 : 10	0×0	2×2	4×4	5×4	5×4	5×4	2×2	1×1
		1 : 20	0×0	0×0	2×1	0×0	0×0	0×0	0×0	0×0
		1 : 640	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0

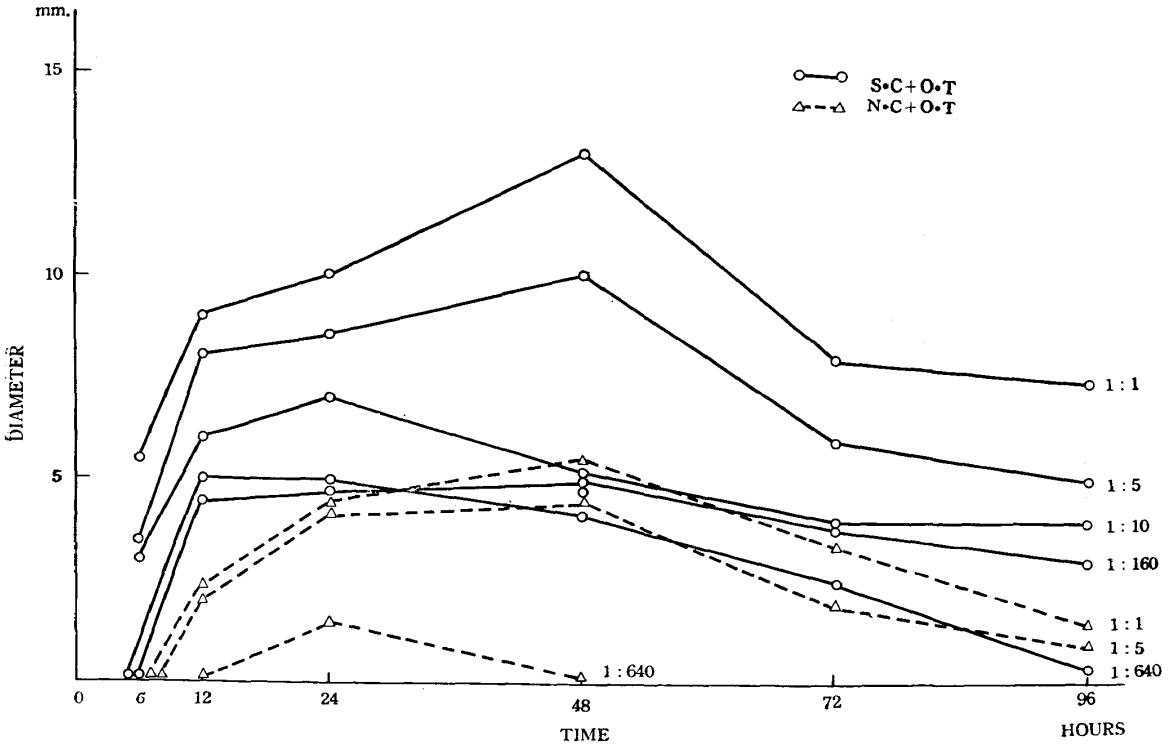

Fig. 3. Skin reaction induced in the rabbit by rat cells mixed with various concentration of O.T.

formed to determine whether or not this delayed type reaction appeared particularly when the rat was the donor and the guinea pig the recipient.

4) Experiment in which rat or guinea pig cells were injected into the skin

Table 4. Skin reaction indsced in the rabbit after injection of guines pigs' peritoneal exudate cells mixed with various concentrations of antigen.

Cell	No. of Recipient	Concentration of O•T	Skin Reaction (mm) 6	12	24	36	48	60	72	96 hrs
S	No. 81	1 : 1	0×0	5×5	6×7	8×9	10×10	8×9	7×7	7×7
		1 : 5	0×0	5×4	6×8	7×8	8×8	7×7	5×5	5×5
		1 : 10	0×0	3×3	6×6	6×6	6×6	5×6	5×4	4×4
		1 : 20	0×0	3×4	6×6	6×6	6×6	5×5	4×4	4×4
		1 : 40	0×0	0×0	6×5	5×5	5×5	5×4	4×4	4×3
		1 : 80	0×0	0×0	6×5	5×5	5×4	4×4	3×4	4×3
		1 : 160	0×0	0×0	5×5	4×4	4×4	3×3	3×2	3×2
		1 : 320	0×0	0×0	5×5	4×4	4×4	3×3	3×2	2×2
		1 : 640	0×0	0×0	5×4	4×4	3×4	3×2	0×0	0×0

S	No. 82	1 : 1	0×0	4×4	6×5	8×8	10×9	8×8	8×7	8×7
		1 : 5	0×0	4×4	5×5	7×8	8×8	7×6	7×5	6×5
		1 : 10	0×0	0×0	6×5	6×5	5×5	5×5	4×4	4×4
		1 : 20	0×0	0×0	4×4	4×5	5×5	5×5	4×5	4×4
		1 : 40	0×0	0×0	5×4	5×4	4×4	4×5	4×5	4×4
		1 : 80	0×0	0×0	5×5	5×5	4×4	4×4	4×4	4×3
		1 : 160	0×0	0×0	5×4	4×5	4×4	4×4	4×4	3×3
		1 : 320	0×0	0×0	4×4	4×3	3×3	3×3	3×3	3×3
		1 : 640	0×0	0×0	4×4	4×4	3×3	3×3	3×2	0×0
N	No. 83	1 : 1	0×0	0×0	2×2	0×0	0×0	0×0	0×0	0×0
		1 : 5	0×0	0×0	2×1	0×0	0×0	0×0	0×0	0×0
		1 : 10	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
		1 : 20	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
		1 : 640	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0

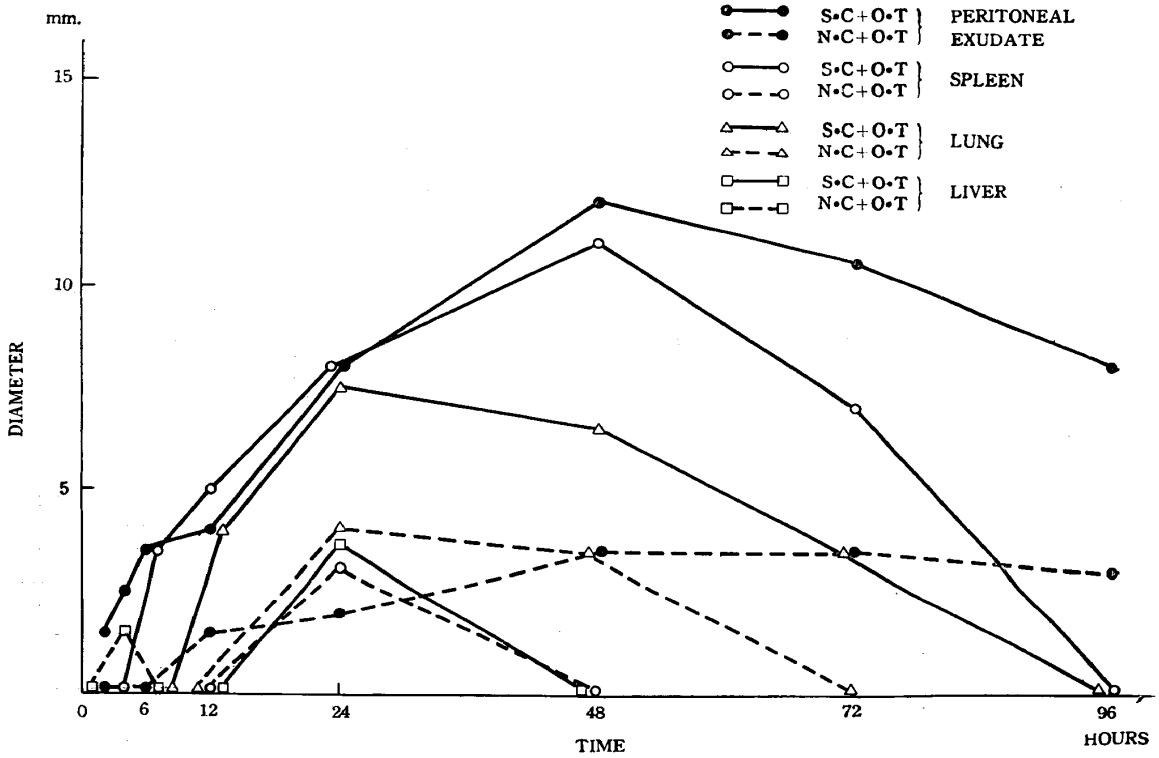

Fig. 4. Skin reaction induced in guinea pigs by rabbit cells plus antigen mixture.

of the rabbit.

Experiments were performed using either rats or guinea pigs as donors and rabbits as recipients. Results are shown in table 3 and 4.

It may be of interest that delayed type reactions similar to those in the experiment described above were observed in these experiments. The reactions, however, could be induced only by using a comparatively high concentration of old tuberculin.

Table 5. Skin reaction induced in the guinea pig after injection of rabbit cells mixed with antigen.

No. of Recipient	Organ	Cell	Skin Reaction (mm) 2	4	6	12	24	48	72	96 hrs
No. 25	Peritoneal Exudate	S	1×2	3×3	3×4	3×5	8×8	12×12	10×11	8×8
		N	0×0	0×0	0×0	1×2	2×2	3×4	3×4	3×3
No. 26	Spleen	S	0×0	0×0	3×4	5×5	8×8	10×12	8×6	0×0
		N	0×0	0×0	0×0	0×0	3×3	0×0	0×0	0×0
No. 27	Liver	S	0×0	0×0	0×0	0×0	3×4	0×0	0×0	0×0
		N	0×0	2×1	0×0	0×0	0×0	0×0	0×0	0×0
No. 28	Lung	S	0×0	0×0	0×0	3×5	7×8	5×8	3×4	0×0
		N	0×0	0×0	0×0	0×0	3×5	4×3	0×0	0×0

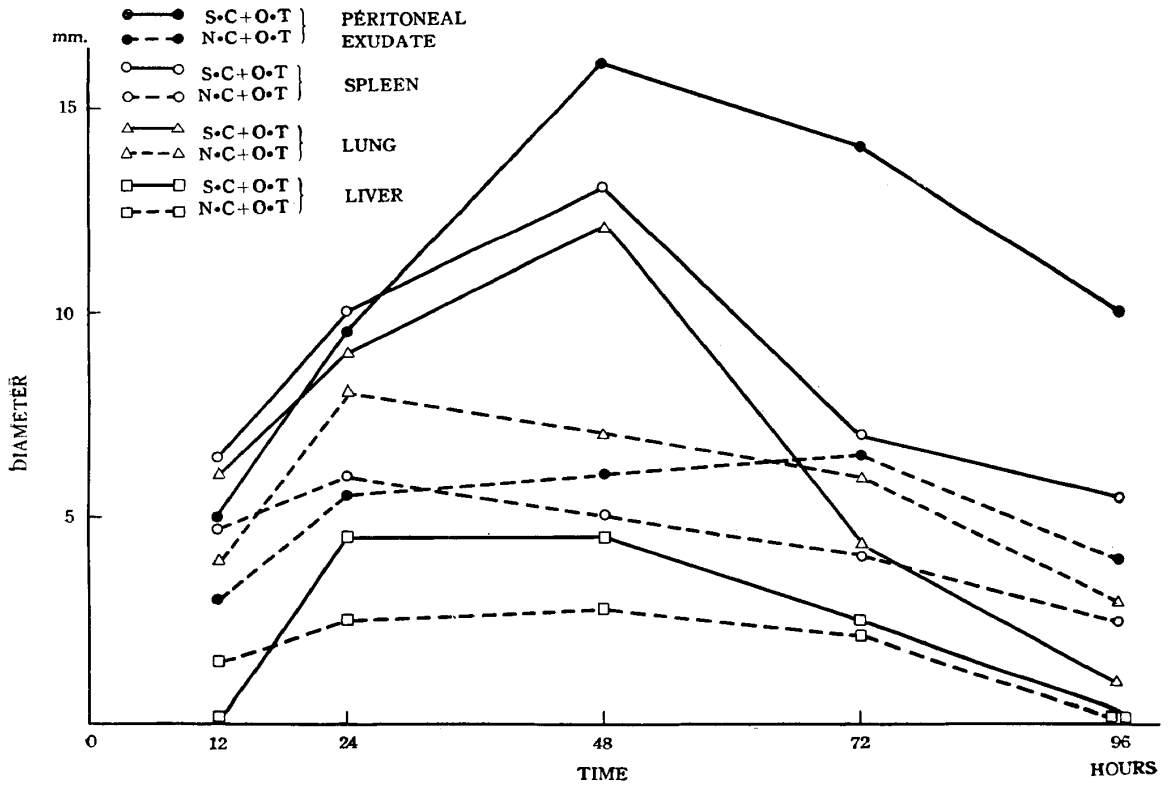

Fig. 5. Skin reaction induced in rabbits by guinea pig cells plus antigen mixture.

5) Experiment in which rabbit's cells were injected into the skin of the guinea pig or *vice versa*.

The delayed type skin reactions were also obtained in experiments using the rabbit as donor and the guinea pig as recipient or *vice versa*.

Results are shown in table 5 and 6.

Table 6. Skin reaction induced in the rabbit after injection of guinea pig cells mixed with antigen.

No. of Recipient	Organ	Cell	Skin Reaction (mm) 12	24	48	72	96 hrs
No. 29	Peritoneal Exudate	S	5×5	9×10	16×16	15×13	10×10
		N	3×3	5×6	6×6	6×7	4×4
No. 30	Spleen	S	6×7	10×10	13×13	7×7	5×6
		N	5×5	6×6	5×5	4×4	2×3
No. 31	Liver	S	0×0	4×5	4×5	2×3	0×0
		N	1×2	2×3	2×3	2×2	0×0
No. 32	Lung	S	6×6	9×9	11×13	5×3	1×1
		N	4×4	8×8	7×7	6×6	3×3

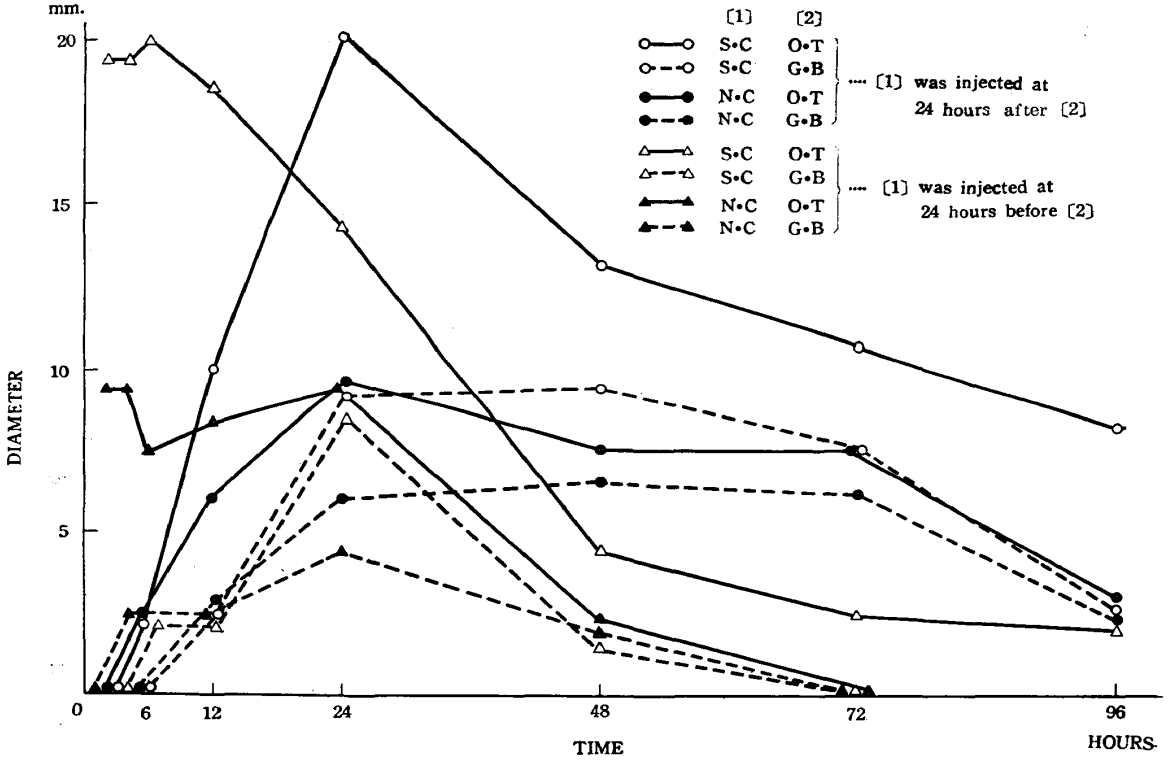

Fig. 6. Skin reaction induced in the guinea pig after injection of rat cells and O.T seperately.

The results obtained in the above experiments are summarized according to the type of reaction in table 7.

Table 7. Interrelationship of the type of reaction induced between guinea pig, rat and rabbit.

Recipient \ Donor	Guinea Pig	Rat	Rabbit
Guinea Pig	Immediate Type	Typical Delayed Type	Delayed Type
Rat	No Reaction	No Reaction	No Reaction
Rabbit	Delayed Type	Delayed Type	Immediate Type

Immediate type reactions in homologous and delayed type reactions in heterologous animals were regularily induced.

6) Experiment in which antigen and cell-material were injected into the recipient separately.

Intracutaneous injection of rat cells and old tuberculin in the guinea pig was performed separately with an interval of 24 hours, first antigen then cells and *vice verse*. The data are shown in table 8.

Table 8. Skin reaction induced by rat cells injected into the skin of the guinea pig 24 hours before or after the injection of antigen.

Injection of Antigen	Cell	Antigen	Skin Reaction (mm) 2	4	6	12	24	48	72	96 hrs
24 hrs before Cell-injection	S	O·T*	0×0	0×0	2×2	10×10	10×21	13×13	9×12	8×8
		G·B	0×0	0×0	0×0	2×3	8×10	9×10	7×8	2×3
	N	O·T	0×0	0×0	2×3	6×6	9×10	7×8	7×8	3×3
		G·B	0×0	0×0	0×0	3×3	6×6	6×7	6×6	2×3
24 hrs after Cell-injection	S	O·T	19×20	19×20	20×20	18×19	14×14	4×5	2×3	2×2
		G·B	0×0	0×0	2×2	2×2	8×9	1×2	0×0	0×0
	N	O·T	9×10	9×10	7×8	8×9	9×10	2×3	0×0	0×0
		G·B	0×0	0×0	2×3	2×3	4×5	1×3	0×0	0×0

* 0.2 ml of 1 : 5 dilution of O·T was injected into skin site

When antigen was injected before cell-material, the maximum reaction was accelerated to 24 hours, as compared with 48 hours in the previous expriments. And, when antigen was injected after cell-material, the maximum reaction was

altered to an immediate type without latent period.

DISCUSSION

It has been generally considered that the rat occupies a peculiar position among many laboratory animals in regard to sensitivity to various antigens.

Opie[5] reported that it was more difficult to induce the Arthus phenomenon in the skin of the rat, than in other animals.

Ovary[6] found that much more antibody was necessary in the rat than in the guinea pig in order to transfer passively the Arthus type hypersensitivity.

From the point of view of tuberculous allergy, too, the rat, mouse and hamster are different from the guinea pig and rabbit.

Wessels[4] observed a slight skin reaction which was induced by injection of tuberculin in the sensitized rat, and Gray[7] found also a positive reaction in the foot pad of the mouse. However, Kumashiro[8] could not confirm the results described by Wessls in the rat. And, Tao[9] demonstrated a negative tuberculin skin reaction in the golden hamster also. Anyway, it is usually considered that these animals reveal no definite skin reaction to any tuberculous antigens and that these animals are peculiar species having poor allergic responses.

On the other hand, the possibility of systemic shock reactions induced by the injection of a large amount of tuberculous antigen in the sensitized rat or mouse has been reported by several investigators[10-11].

In recent years, experimental allergic encephalomyelitis in the mouse[12-13] and rat[14-15] was shown to develop after intracutaneous injection of tissuse of either the brain or the spinal cord.

In addition, successful passive transfer of this encephalomyelitis in the rat was accomplished by using parabiotic technique[16].

Recently Yasuhira and his co-workers[17] succeeded in producing allergy-like tuberculous foci in the lung of the rat and mouse, using Yamamura's method[18]. Tuberculous foci were also produced in the lungs of guinea pigs and rabbits.

In the present paper, the author has also reported on the typical delayed type skin reaction in the guinea pig and rabbit by local passive transfer using intracutaneous injection of sensitized rat cells.

These experimental facts suggest the possibility of antibody production even in the rat and mouse, in which no skin reactions are usually shown.

The reasons for lack of tuberculin skin reaction in the rat are complicated and indefinite. However, it may be that one of these reasons is the insensitivity of the skin against stimuli in general.

In 1926, Zinsser[19] reported a specific skin reaction in the normal guinea pig, by using macerated tissues of human lung mixed with diltue old tuberculin.

Recently Wallace[20] described another piece of evidence concerning interspecies transfer of delayed hypersensitivity. In his paper, he demonstrated that intracutaneous injection of cells, which were obtained from rats infected with murine leprosy bacilli and suspended in dilute old tuberculin or M. leprae murium antigen, induced a typical delayed type reaction in the normal guinea pig.

Chase,[21] Metaxas and Metaxas-Bühler,[22] and Wallace[20] showed that this activity of cells was destroyed by heating at 48～45°C for 15 to 30 minutes.

In the present paper, inactivation of the sensitized cells by heating also be demonstrated, but only at somewhat higher temperatures than they mentioned.

Waksman[23] pointed out the toxic action of very high concentrations of old tuberculin on the sensitized cells in tissue culture. But such an action was not confirmed by the present investigation.

In some cases in the present investigation, the skin sites of injection were excised, when the reaction had reached its maximum. These specimens were fixed in ten per cent formalin solution and stained with Hematoxylin and Eosin.

The histopathological examinations of these skin specimens, in which either the immediate or the delayed type reaction had been induced, always showed the picture of non-specific inflammatory reaction, with a dominance of polymorphonuclear leucocytes.

Diens and Mallory,[24] and Laport[25] reported that monocytes dominate generally in the inflammatory foci of tuberculin reaction, in comparison with the Arthus reaction. Later this dominance of monocytes in the tuberculin reaction was denied by several investigators[26-28].

In the Arthus reaction Gell and Hinde[29] observed no dominace of any kind of cells. There were both acute exudative (leucocytic reaction) and chronic productive (monocytic reaction) responses in all stages of the reaction.

In conclusion, there is no characteristic morphology in the tuberculin reaction.

Therefore the present finding, that the reaction induced by the injection of heterologous cells plus old tuberculin shows only a picture of non-specific inflammatory reaction is not an indication that there is no antigen-antibody reaction.

From this point of view, it can be suggested that a certain active chemical substance produced in the antigen-antibody reaction, in the hetrologous as well as in the homologous animals described in the previous paper, may be postulated.

It may be difficult to explain the fact that the delayed type skin reaction in the heterologous animal is changed to the immediate type reaction, when antigen and cell-material are injected separately with a 24 hour interval.

It seems that the latent period of skin reaction is greatly influenced by many factors participating in the reaction.

Problems with reference to this latent period of skin reaction will be the

subjects of further investigation.

SUMMARY

The heterologous local passive transfer of tuberculin hypersensitivity was accomplished by injection of cell-antigen mixture obtained from sensitized rats, guinea pigs and rabbits. From these experiments, the following results were obtained :

1) Delayed type skin reactions were obtained by heterologous local passive transfer using peritoneal exudate cells from sensitized animals. But no reactions were obtained when the rat was used as the recipient.

2) Cells from homogenated visceral organs, especially splenic cells, also caused typical delayed skin reactions.

3) The lack of tuberculin skin reaction in the rat may be due to its low skin reactivity rather than to deficiency of antibody production.

4) Histopathological pictures at the skin site of injection always showed a dominance of polymorphonuclear leucocytes.

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