Experimental Studies on the Local Passive Transfer of Tuberculin Hypersensitivity

Report I. Tuberculin hypersensitivity induced by intracutaneous injection of homologous cells, previously mixed with antigen

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INTRODUCTION

It has been generally considered that the delayed skin reaction of tuberculin hypersensitivity is induced by an interaction between antigen and sessile antibody in the sensitized cell. Although many studies have been made on this subject, no explanation has been found as to why the tuberculin reaction is of the delayed type rather than of the immediate type found in other allergic responses, except for contact allergy.

In 1945, Chase[1] reported the first successful passive transfer of tuberculin hypersensitivity in the normal guinea pig by using peritoneal exudate cells obtained from sensitized donors. Since that time several investigators have confirmed this experimental finding by using not only sensitized cells[2-11,17-19] but also extracts of these cells[12-16].

Recently, Metaxas and Metaxas-Bühler[20] reported that an immediate type of skin reaction without a latent period in the recipient is produced by the injection of donor cells containing antibody mixed with antigen.

Before seeing this report, this author had also been thinking that a certain substance might be formed and might act on the skin to induce the immediate type of reaction, when donor cells mixed with antigen were injected.

The author has confirmed the results of Metaxas and Metaxas-Bühler mentioned above, and, in addition has obtained a new finding concerning the relationship between heterologous cells and antigen[21].

MATERIALS AND METHODS

Animals :

Male or female albino guinea pigs weighing from 300 to 400 g. were used as donors and recipients. Male albino rabbits weighing from 2.5 to 3 kg. were

also used in some experiments.

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-paraffin mixture in the thigh once a week for two or three weeks.

Approximately six weeks after the last injection, all animals were tested for skin sensitivity with 0.1 ml. of 1 : 10 dilution of old tuberculin. All the animals developed a very marked skin reaction with erythema, induration, and central blanching or necrosis. The average diameter of these reactions was 25 to 30mm.

Preparation of cell-materials :

1) Exudate cells.

Each donor was injected with 10 to 50 ml. of sterile liquid-paraffin oil intraperitoneally.

After three days, the donor was sacrificed by heart puncture. An appropriate volume ranging from 20 to 100 ml. of chilled Tyrode's solution containing 1 : 20,000 dilution of heparin was introduced into the peritoneal cavity. After being kneaded for several minutes, the abdomen was punctured with a pipette and the peritoneal fluid was collected into sterile glass centrifuge-tubes. Exudate cells were treated twice by washing with Tyrode's solution and centrifuging for five minutes at 1000 r.p.m. The yield of packed cells was approximately 0.05 to 0.2 ml. per donor and 80 per cent being mononuclear cells and 20 per cent polymorphonuclear. Finally they were suspended in fresh Tyrode's solution.

2) Cells from other visceral organs.

The spleen, liver and lungs of the donor were simultaneously removed.

These organs were washed by repeated intravascular injections of fresh heparinized Tyrode's solution.

They were cut into small pieces and then homogenized in a potter's glass-homogenizer for five minutes in Tyrode's solution.

Throughout the preparation of cell-materials, manipulation was of course carried out aseptically.

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 obtained from sensitized or normal donors were mixed

with equal volumes of ten-fold dilutions of old tuberculin in a small test-tube. In some experiments, the test-materials were incubated at 37°C for various periods of time before being injected. Corresponding control-material was mixed with glycerine-bouillon in the place of old tuberculin.

Injection of test-materials and observation of skin reactions in recipients:

Healthy, tuberculin-negative animals were used as recipients in most of the experiments. But in one series tuberculin-positive guinea pigs similar to the donors of sensitized cells were also used as recipients. A 0.2 ml. aliquot of each test-material containing about 0.08 ml. of exudate cells was injected into the shaved skin of both sides flanks.

Induration was taken as an indication of skin reaction at an appropriate time.....usually 2, 4, 6, 12, 24, 48, 72 and 96 hours after the injection, by measuring the longest and the shortest diameters.

The average values of the measurements obtained at the two sites of injection will be shown in tables in the next chapter.

RESULTS

1) Experiments designed to determine whether or not the mixture of normal or sensitized cells and old tuberculin causes reactions in the skin of normal guinea pigs.

As control experiments, responses to glycerine-bouillon mixed with normal or sensitized cells and to the dilution of either old tuberculin or glycerine-bouillon alone were also examined.

One typical result among these several experiments is shown in table 1.

Table 1. Skin reaction in normal animals induced by normal or sensitized cells with or without antigen.

		Skin Reaction (mm)							
Cell	Antigen	2	4	6	8	12	24	36	48 hrs
S	O·T	10×13	12×13	12×14	11×13	11×12	10×12	10×10	7×9
	G·B	7×7	8×9	7×8	6×8	6×7	5×6	4×5	3×2
N	O·T	6×6	6×7	6×7	6×7	7×7	5×6	3×4	2×1
	G·B	5×5	5×5	6×7	6×7	5×5	5×5	4×4	2×1
—	O·T	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
	G·B	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0

O·T=Old Tuberculin (1 : 10) S=Sensitized
G·B=Glycerine-bouillon N=Normal

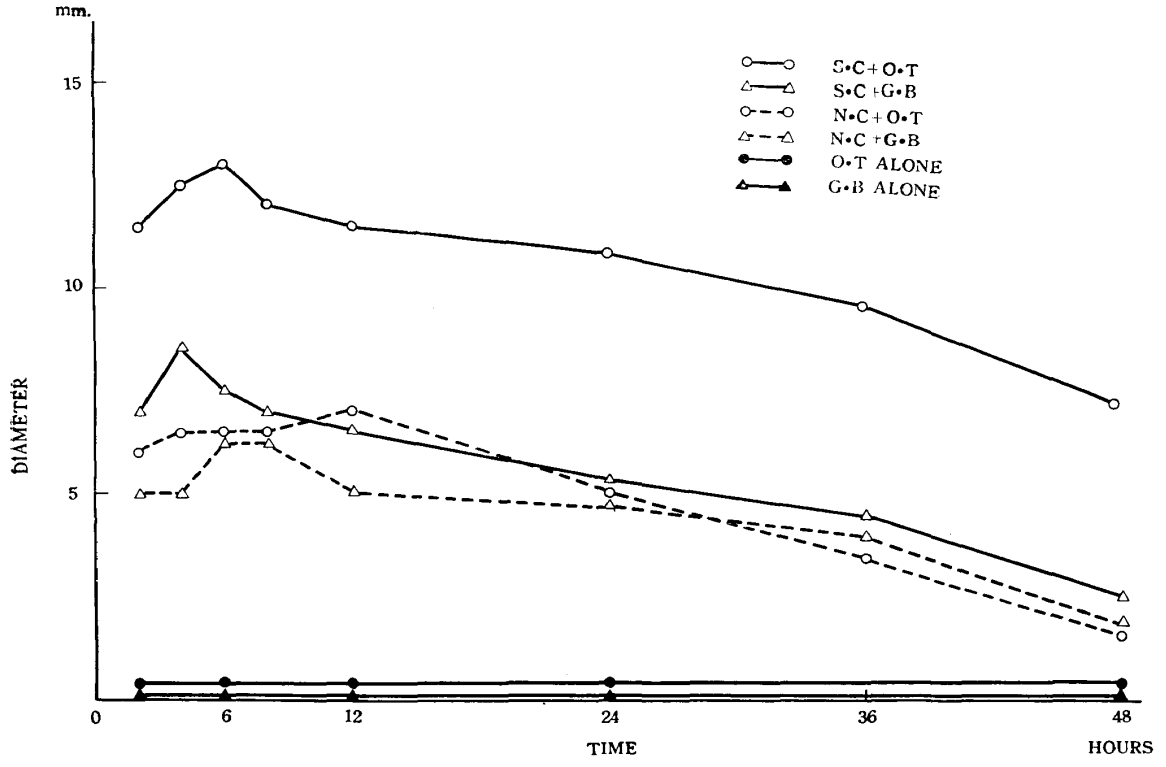

Fig. 1. Skin reaction of guinea pig to homologous cells with or without antigen.

The intracutaneous injection of sensitized cells mixed with 1 : 10 dilution of old tuberculin produced a marked reaction in a normal guinea pig. The reaction was more intense than that following injection of normal cells mixed with dilute old tuberculin or cells (either normal or sensitized) with glycerine-bouillon. These three control reactions were almost uniform and there were no significant differences. Erythema with slight induration appeared at about two hours, and developed to its maximum size at four to six hours after the injection. After that time, it decreased gradually.

No reactions were induced by injection of dilute old tuberculin or glycerine-bouillon alone.

2) The influence of incubation *in vitro* on cell-antigen mixtures

Before injection into the skin of recipients, the mixtures were incubated at 37°C for one, two, five and ten hours. As controls, the same mixtures were immediately injected into the skin without incubation.

Table 2. shows the effect of the incubation time *in vitro* on the ability of the peritoneal exudate cells mixed with antigen to produce reactions.

It seems that incubation of test-materials *in vitro* reduces their potency in producing skin reactions in the recipient.

The longer the incubation, the less was the reaction.

Almost no reaction was seen after incubation of five hours.

Table 2. Effect of incubation *in vitro* on the cell-antigen mixture.

Incubation Period (hr)	No. of Recipient	Cell	Skin Reaction (mm) 2	4	6	12	24	36	48	72	96hrs.
—	No. 71	S	13×13	13×16	13×18	10×13	11×11	11×11	10×11	10×10	9×10
	No. 61	N	5×7	7×9	8×9	7×9	8×8	8×8	7×8	6×8	7×7
1	No. 72	S	5×6	7×11	13×12	10×11	10×11	8×12	9×10	8×9	7×8
	No. 62	N	2×2	2×3	5×6	7×8	7×7	7×7	6×7	5×5	4×5
2	No. 73	S	6×6	10×10	11×10	10×10	10×9	8×8	8×8	7×8	7×8
	No. 63	N	5×6	7×10	7×11	7×7	6×7	6×6	6×6	5×6	5×5
5	No. 74	S	6×6	6×6	6×6	6×5	6×5	6×5	6×6	6×5	6×5
	No. 64	N	5×5	5×6	5×6	5×6	5×5	5×5	5×6	4×6	4×4
10	No. 75	S	6×7	7×7	7×7	7×5	7×5	7×5	5×6	4×4	3×4
	No. 65	N	4×5	4×6	4×6	4×6	4×5	4×5	4×5	4×4	4×4

Cells were mixed with 1 : 10 dilution of O·T and incubated at 37°C.

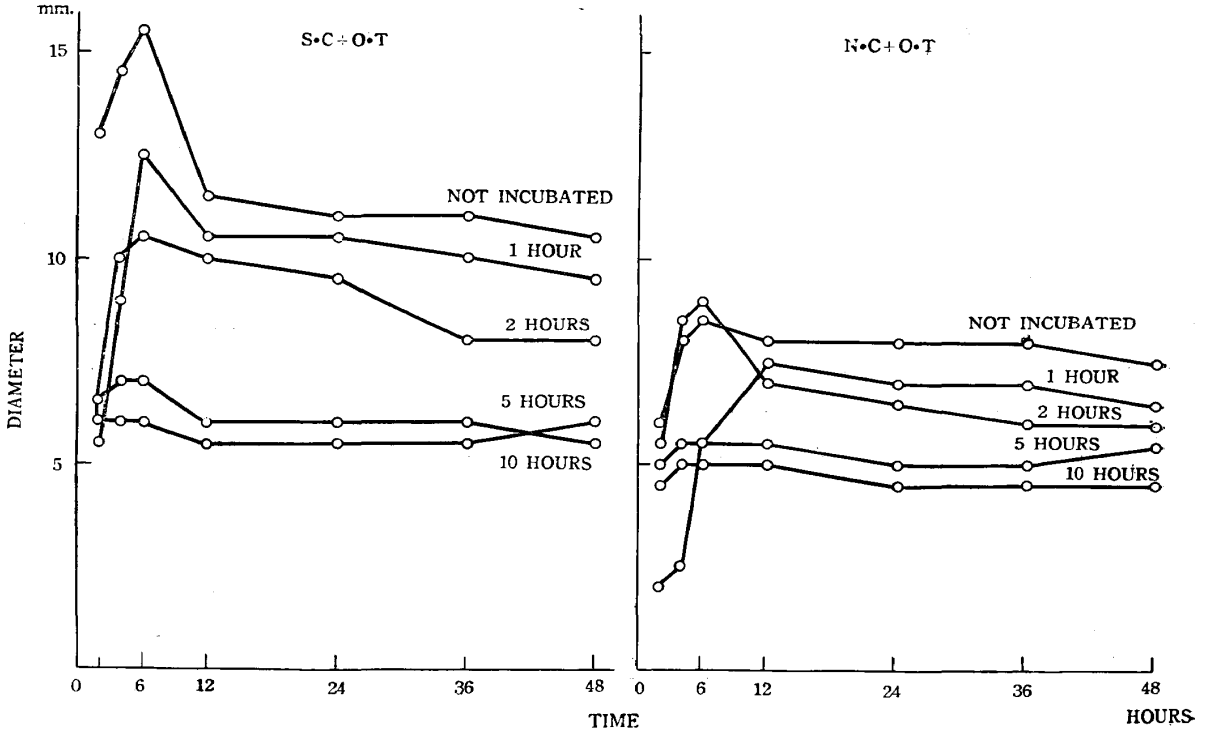

Fig. 2 Skin reaction induced by incubated cell-antigen mixture.

3) Experiment using both peritoneal exudate cells and visceral cells from rabbits

Similar experiments were also performed in rabbits by using peritoneal

exudate cells and cells from visceral organs ; the spleen, liver and lung.

The results are shown in table 3. The same patterns as seen in the guinea pig were obtained, while visceral cells were less reactive than the exudate cells in the following descending order ; spleen, lung, and liver.

4) Experiment concerning the influence of incubation *in vitro* on cells only.

Antigen was mixed with cell-materials at the end of the incubation period and was injected into the skin. The results are shown in table 4. It seems that cells incubated at 37°C for 30 minutes or more fail to induce skin reaction in the recipient, while the activity of the cells could be completely preserved at lower temperatures.

5) Experiment using the supernatant and sediment separated from the incubated cell-antigen mixture

A study was made as to whether or not any active principle of the substance would be produced after mixing sensitized cells and antigen *in vitro*. The

Table 3. Skin reaction induced in normal animals by peritoneal exudate cells and cells from visceral organs of the rabbit.

Incubation Period	Organ	Cell	Skin Reaction (mm) 2	4	6	12	24	48	72	96hrs.
—	Peritoneal Exudate	S	4×4	7×8	10×10	9×10	8×8	7×8	6×6	6×6
		N	0×0	0×0	1×1	1×2	1×1	1×1	0×0	0×0
	Spleen	S	5×5	5×5	5×6	5×6	3×4	2×1	0×0	0×0
		N	0×0	1×1	2×2	1×1	0×0	0×0	0×0	0×0
	Liver	S	3×2	2×2	3×2	2×2	0×0	0×0	0×0	0×0
		N	0×0	1×1	1×1	0×0	0×0	0×0	0×0	0×0
	Lung	S	5×5	6×5	5×5	4×4	1×2	1×2	1×2	0×0
		N	0×0	1×1	1×1	0×0	0×0	0×0	0×0	0×0
5hrs.	Peritoneal Exudate	S	3×3	4×4	4×4	3×4	3×4	2×2	2×1	2×1
		N	0×0	0×0	0×0	1×1	1×1	0×0	0×0	0×0
	Spleen	S	2×3	3×3	4×4	2×3	1×2	0×0	0×0	0×0
		N	0×0	0×0	1×1	1×1	0×0	0×0	0×0	0×0
	Liver	S	0×0	2×2	2×2	0×0	0×0	0×0	0×0	0×0
		N	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
	Lung	S	0×0	4×3	3×4	1×2	1×2	1×2	0×0	0×0
		N	0×0	0×0	1×1	1×1	0×0	0×0	0×0	0×0

Cell-antigen mixture was incubated at 37°C.

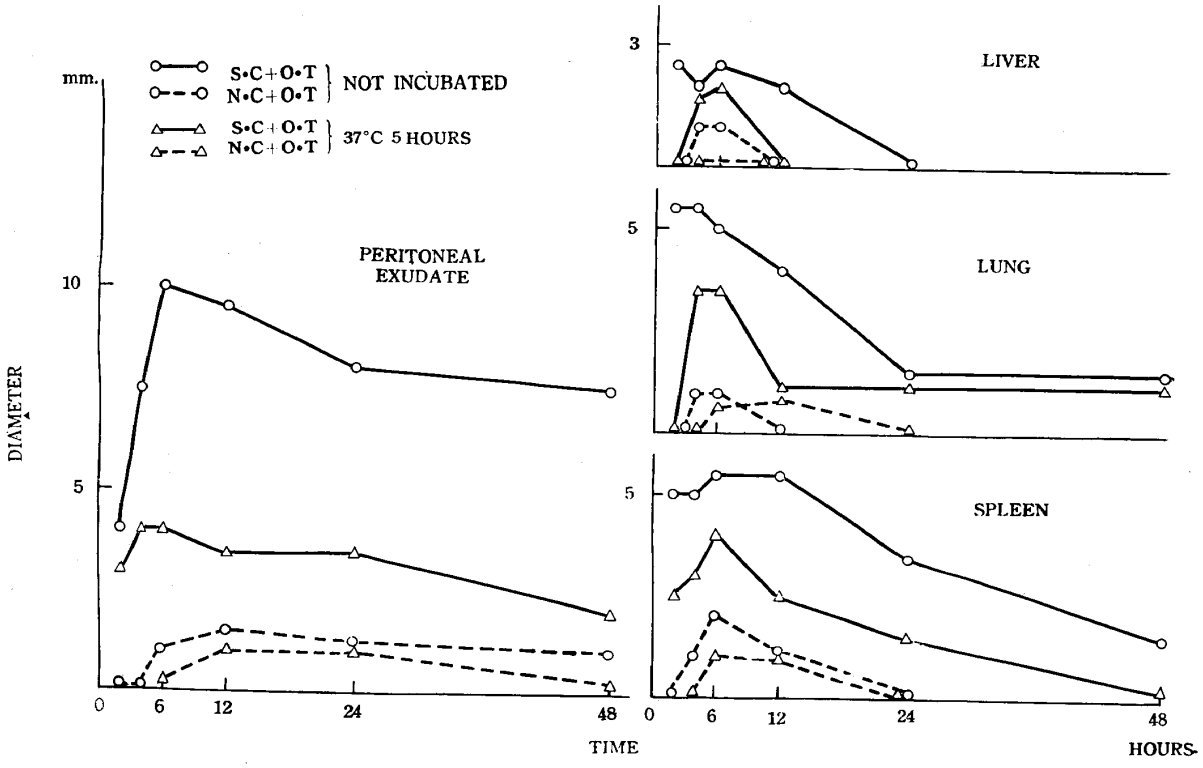

Fig. 3 Skin reaction induced in the rabbit by homologous cells plus antigen mixture.

Table 4. Skin reaction induced by cells mixed with antigen at the end of incubation period.

Incubation Temperature	Incubation Period (hrs)	Cell	Skin Reaction (mm) 2	4	6	12	24	48	72	96hrs.
—	—	S	6×7	8×9	10×8	9×8	8×8	4×4	3×4	0×0
		N	5×5	5×6	5×5	4×4	4×3	2×2	0×0	0×0
37°C	30min.	S	5×4	5×5	4×5	4×4	4×4	3×4	2×3	0×0
		N	3×3	5×5	5×5	4×4	4×4	2×3	0×0	0×0
	1	S	2×2	3×2	6×5	4×4	4×3	2×2	0×0	0×0
		N	0×0	3×2	4×2	3×4	3×2	0×0	0×0	0×0
	2	S	0×0	2×2	2×2	2×3	2×2	2×2	0×0	0×0
		N	0×0	0×0	3×2	2×3	2×2	2×2	0×0	0×0
	3	S	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
		N	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
	5	S	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0
		N	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0

4°C	3	S	3×3	9×5	8×10	8×9	8×6	3×4	2×2	0×0
		N	0×0	4×4	5×5	4×4	2×2	0×0	0×0	0×0
	5	S	7×9	8×9	9×9	8×9	8×8	4×4	2×2	0×0
		N	0×0	2×2	2×1	0×0	0×0	0×0	0×0	0×0
	24	S	5×4	3×4	3×3	2×1	0×0	0×0	0×0	0×0
		N	0×0	0×0	0×0	0×0	0×0	0×0	0×0	0×0

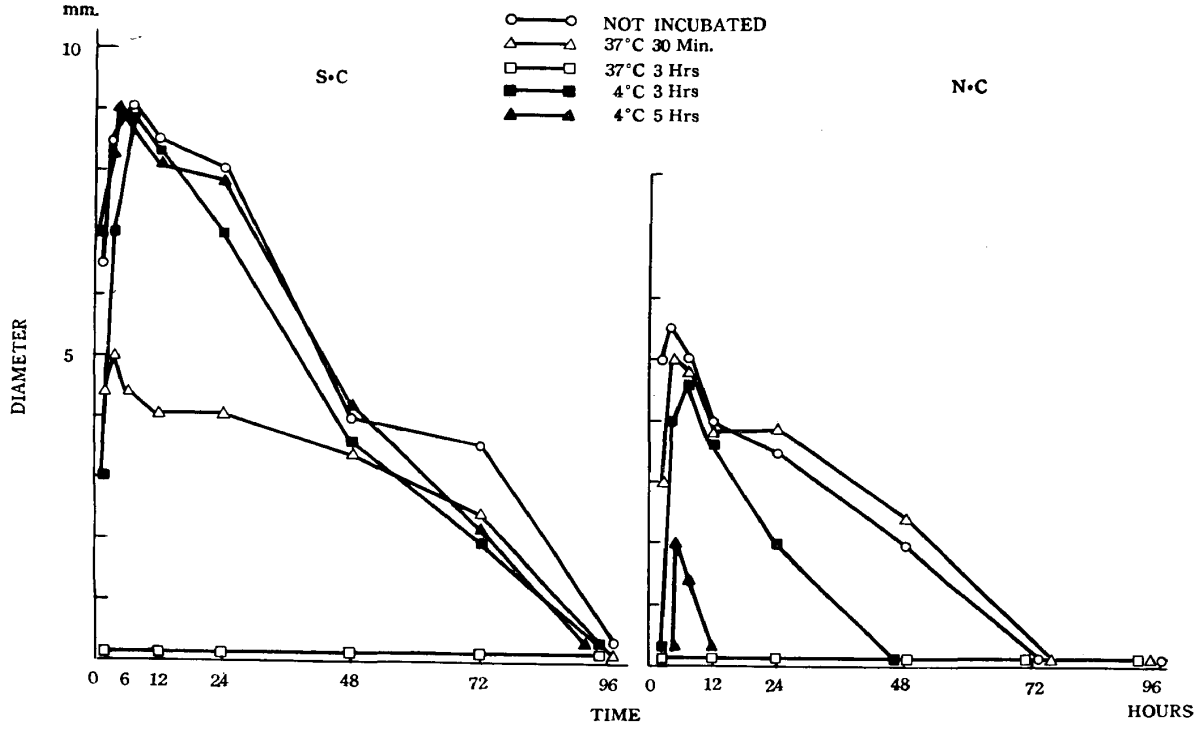

Fig. 4 Skin reaction induced by cells mixed with antigen after incubation.

mixtures were divided into supernatant and sediment by centrifugation after incubation and each part was injected into the skin separately. As shown in table 5, reactions were seen at the sites of injection of the sediment, but the supernatant caused no reaction.

6) Experiment using a hypersensitive animal as recipient.

Next, a sensitized guinea pig was used as a recipient. As demonstrated in table 6, an immediate type reaction was observed when sensitized cells plus O.T mixture were used, but a delayed type reaction with either normal cells plus O.T mixture or old tuberculin alone.

All the above results indicate that sensitized cells from guinea pigs and rabbits in combination with antigen react in the skin of the recipient without any latent period.

Table 5. Skin reaction induced by sediment or supernatant separated from incubated cell-antigen mixture.

Cell	Test-material	Incubation Period (hrs)	Skin Reaction (mm) 2	4	6	12	24	48hrs.
S	Sediment	—	8×10	8×11	6×9	6×8	6×7	6×6
		1	8×8	8×9	6×7	6×7	6×7	5×6
		3	7×8	6×8	6×7	6×7	5×7	3×3
	Supernatant	—	2×3	2×3	2×2	2×2	2×2	0×0
		1	5×5	3×4	3×4	3×3	2×3	2×3
		3	4×4	3×4	3×3	2×3	2×2	1×2
N	Sediment	—	4×5	5×5	5×5	4×3	2×3	2×2
		1	3×6	4×5	5×5	4×4	3×2	2×2
		3	3×4	4×4	4×4	2×4	2×2	1×1
	Supernatant	—	3×3	3×4	3×4	2×3	2×3	2×2
		1	3×3	3×2	3×3	2×2	1×2	1×2
		3	1×2	1×1	1×1	1×1	1×1	0×0

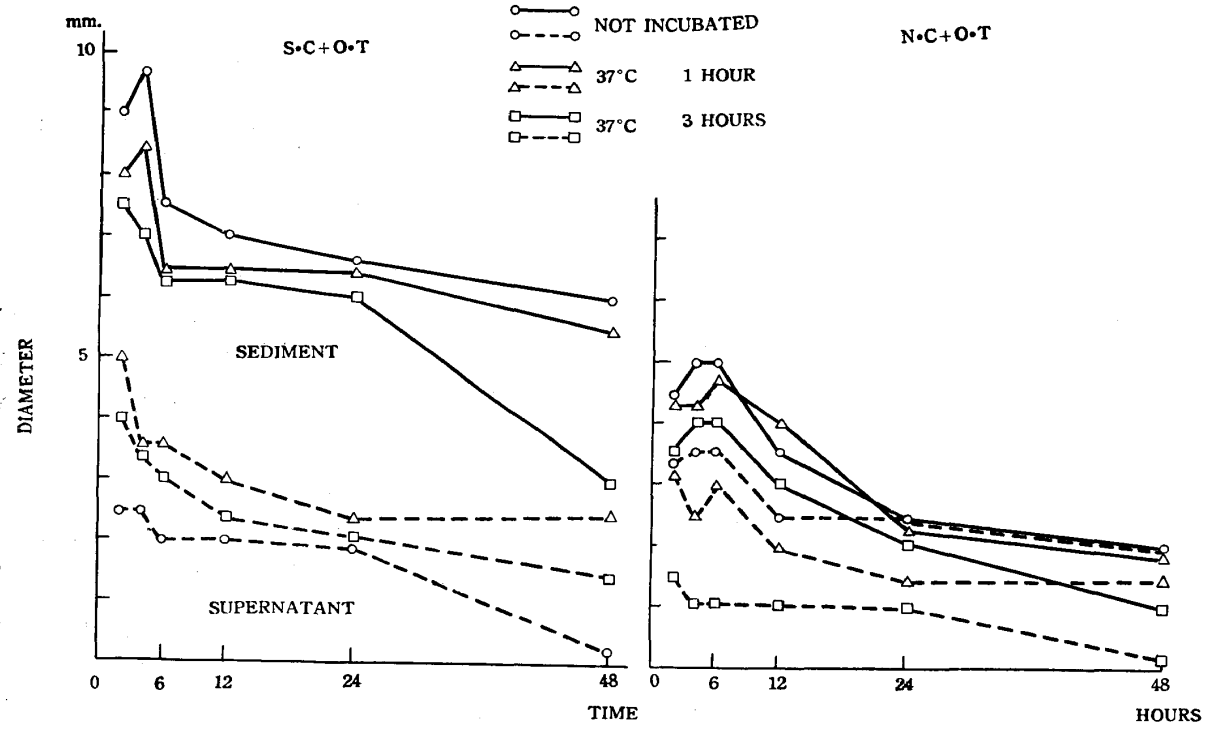

Fig. 5. Skin reaction induced by either sediment or supernatant of incubated mixture.

Table 6. Immediate type skin reaction in hypersensitive guinea pigs, after injection of cell-antigen mixture.

Incubation Period (hr)	No. of Recipient	Test-material	Skin Reaction (mm) 2	4	6	12	24	48	72	96hrs.
1	No. 31	S·C+O·T	2×2	7×8	12×16	15×25	17×26	12×14	8×10	6×5
		O·T	—	—	2×2	—	8×9	16×19	14×16	8×9
	No. 32	N·C+O·T	4×5	4×5	5×6	5×5	8×8	10×15	18×18	11×15
		O·T	0×0	3×4	4×5	4×4	5×8	10×10	16×17	13×13
10	No. 45	S·C+O·T	6×5	7×8	10×15	14×16	15×15	12×13	10×10	6×6
		O·T	—	—	2×2	—	8×8	18×20	17×17	10×10
	No. 46	N·C+O·T	3×3	4×4	4×6	4×5	10×10	10×12	12×12	10×10
		O·T	2×3	3×3	4×4	4×5	6×6	10×10	14×14	11×12

S·C = Sensitized cell N·C = Normal cell O·T = Old tuberculin

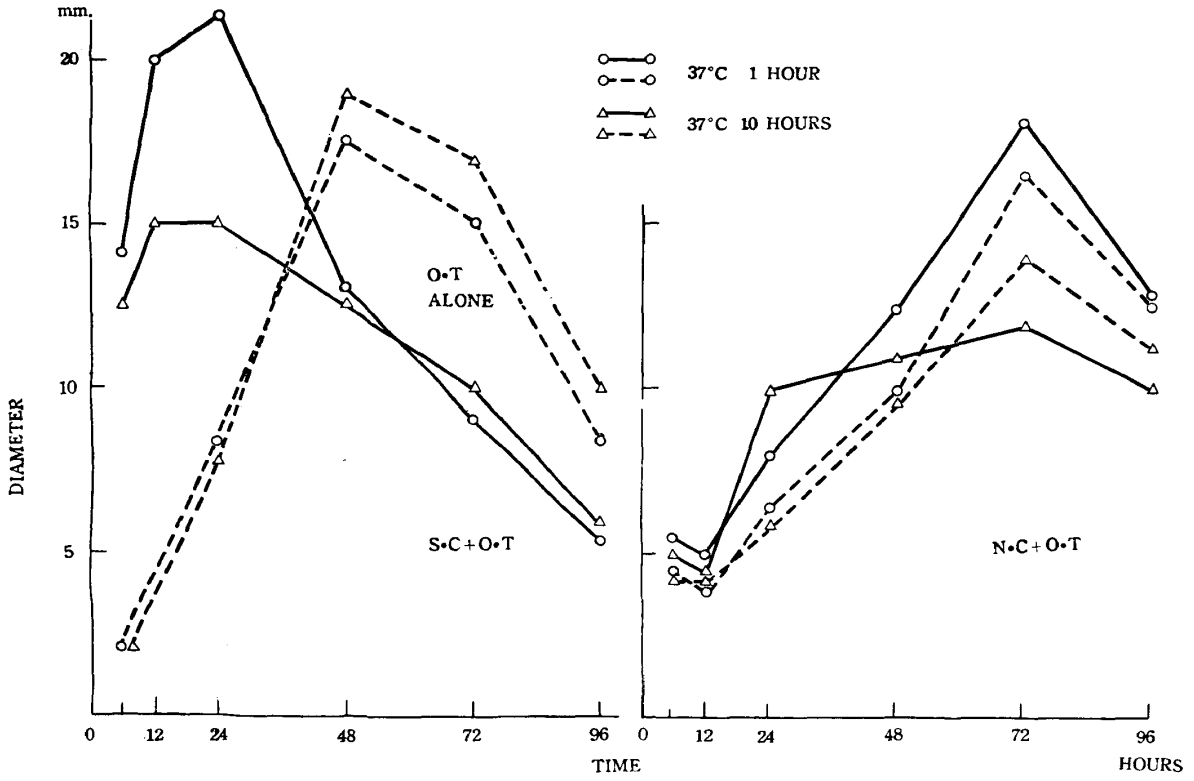

Fig. 6 Skin reaction induced in hypersensitive guinea pigs by cell-antigen mixture.

DISCUSSION

There has been no analysis of the mechanism of delay of the reaction in the tuberculin skin response.

In 1826, Zinsser and Tamiya[22] reported the experimental results that

macerated tissues of the lung obtained from tuberculous guinea pigs and mixed with old tuberculin, gave stronger reactions than control-materials in the skin of normal guinea pigs.

They also said, however, in their report that these reactions were often irregular.

In the present investigations similar experiments were repeated and extended using mainly peritoneal exudate cells mostly consisting of mononuclear cells, which are believed to retain sessile antibody. The author succeeded in inducing a specific immediate type skin reaction, which reached a maximum four to six hours after the injection of sensitized cells plus antigen mixture.

Kourilsky et Dercouix[23] carried out similar experiments using the same method, and reported positive but rather weak reactions. It may be that the cause of the weakness of the reactions observed by these investigators was their too late observation of the site of injection...24 or 48 hours after the injection, and that they would have observed as strong reactions as I did, if they had examined earlier.

Recently, Waksman and Matoltsy[24] also stated that the reactions were always stronger at 24 hours than at 48 hours after the injection of cells plus antigen mixture. This seems to agree with my opinion stated above.

Rich[25] observed, using a tissue culture method that the sensitized cells, for instance splenic cells, migrating cells from fragments of bone marrow and certain other mesenchymal cells, were killed by tuberculoprotein.

Waksman[26], however, in disagreement with Rich, reported that antigen could not kill cells, but stimulated the proliferation or the differentiation of sensitized cells.

Though the discrepancy in these reports may be partly due to the difference in experimental method, there may be another unknown reason. Anyway, it may be postulated that cells from sensitized animals are specifically influenced by antigen *in vitro*.

From the point of view of the postulation mentioned above, the following two explanations of the mechanism of delay of the tuberculin reaction may be possible :

1) A non-specific inflammatory reaction may be secondarily induced by certain chemical substances released from the primary antigen-antibody reaction at the skin site.

2) Though tissue cells may be damaged in the early stage of reaction, they may be stimulated in their proliferation or differentiation at a later stage, so the reaction is delayed.

According to Waksman[26], however, the proliferation or the differentiation of sensitized cells by antigen takes place after 72 to 96 hours in tissue cultures.

Furthermore Waksman[26] showed the early fall of the total cell count by tissue culture of exudate cells.

This may be regarded as the destruction of the cells by antigen.

In fact, reactions induced by cells plus antigen mixture are of the immediate type. Therefore the second explanation does not hold.

In conclusion, the first hypothesis that an immediate type skin reaction in the recipient is brought about by certain chemical substance released from the destroyed cells in the antigen-antibody reaction seems to be true.

The experimental fact that passive transfer of tuberculin hypersensitivity could not be induced by the supernatant fluid separated from the mixture of incubated cell and antigen indicates either that the active principle is fairly tightly fixed in the cell bodies and does not diffuse into the supernatant fluid or that though the active principle is capable of being liberated easily from the cell body, the substance may easily be destroyed *in vitro*.

Chase[1] also failed to extract any active principle from the sensitized cells, even by vigorous extraction.

Waksman[24] could not induce a skin reaction by using cells plus antigen mixture after long incubation.

These two experiments and the present experiment are not useful for determining whether the active substance is liberated *in vitro* or *in vivo*.

In conclusion, it may be considered that peritoneal exudate cells and some visceral cells from sensitized animals produce a certain active principle in consequence of contact with antigen, and that tuberculin skin reaction depends on the secondary non-specific inflammatory reaction induced by a certain active principle, which is produced by the primary reaction between antigen and antibody.

SUMMARY

Local passive transfers of tuberculin hypersensitivity were performed by using sensitized cells mixed with the dilution of old tuberculin.

And the following results were obtained.

1) A typical immediate type skin reaction was induced by injection of the peritoneal exudate cells from sensitized guinea pigs in both normal and hypersensitive animals.

2) Skin reaction was reduced by prolonged incubation of cells plus antigen mixture at 37°C

3) Tuberculin hypersensitivity could not be transferred passively by the injection of supernatant fluid separated from incubated cells plus antigen mixture.

4) Immediate type skin reactions were induced by injection of the peritoneal

exudate cells and cells from the spleen and lung of sensitized rabbits, but only slightly by liver cells.

5) From the point of view of these data, possible explanations of mechanism in tuberculin skin reaction were discussed.

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