Passive Transfer of Delayed Hypersensitivity to 2,4-Dinitrochlorobenzene in Guinea Pigs with Leucocytic Extracts.* (21064)

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(Introduced by J. R. Porter.)

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The passive transfer of delayed type skin sensitivity to simple chemical substances(1) and to tuberculin(2-4) has been successful only when mediated by viable leucocytes. Cells that are frozen or heated lose not only their viability but also their ability to transfer passive sensitization. The possibility exists, however, that these methods alter the chemical composition of cellular components as well as the morphological integrity of the cells and that a gentler physical method of treatment might spare the substance responsible for transfer. Sonic vibration would seem to be such a method of cellular disruption, since it has been used effectively by microbiologists and other workers in the preparation of enzymatic extracts from bacteria with a minimum of untoward consequences of various other physical methods. This method should yield preparations showing minimal denaturation, requiring a brief preparative interval, and having less likelihood of thermal alteration of the substance or substances responsible for passive transfer.

This paper summarizes experiments showing passive transfer of delayed type sensitivity to 2,4-dinitrochlorobenzene with extracts of exudative leucocytes disrupted by sonic vibration.

Methods. Guinea pigs weighing 350-500 g were sensitized to 2,4-dinitrochlorobenzene by painting a 2% solution of the chemical in absolute ethyl alcohol on the skin each day for 7 days. Details of sensitization have been described previously(5). Following a latent period of a week a peritoneal exudate was evoked by the injection of 30 ml of light mineral oil. Two days later the exudative leucocytes were collected in heparinized Locke's solution containing 0.2% gelatin and sedimented by centrifugation at 2000 RPM for 15 minutes. Cells from 8-15 donor animals were resuspended in 20 ml of gelatin-Locke's solution. Total leucocyte counts and differential counts on supravital stains were done after the cell suspension was divided into 2 equal portions. Total counts are noted in each experiment. The differential counts were consistent, showing approximately 60% monocytes, 25% lymphocytes, 14% neutrophils, and 1% eosinophils. Half the cell suspension was injected into a control recipient animal, and the other half was subjected to sonic vibration in a 9 KC Raytheon Magnetostriction Oscillator for 5-15 minutes. The temperature of the water jacket was maintained at 30°C ±2. Following this treatment the cellular extract was either injected into a recipient animal or centrifuged at 5,000 RPM for 10 minutes. Centrifugation gave rise to 3 layers: 1) sediment; 2) aqueous supernatant fluid; and 3) surface lipid. The aqueous supernatant layer was injected into a second recipient animal and the combined resuspended sediment and lipid portions into a third. All injections were made by the intraabdominal route. Total leucocyte counts and Giemsa-stained smears were made on both supernatant fluid and sediment to ascertain the degree of cellular disruption. Recipient animals were tested 48 hours after injection by painting with a 1% solution of 2,4-dinitrochlorobenzene in olive oil on the back. The hair on the test area was clipped and the area cleaned with depilatory cream prior to testing. Reactions were observed at 24 and 48 hours following testing and recorded as: 0, no reaction; +, slight erythema; 2+, patchy erythema; 3+, homogeneous erythema; and 4+, marked homogeneous erythema.

Results. The first experiments were de-

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TABLE I. Passive Transfer after Injection of the Whole Product of Sonic Vibration in a Single Recipient Animal.

No. of donors	Time of vibration (min.)	WBC/aliquot*	Reactions 24 hr Vib.	24 hr Cells	48 hr Vib.	48 hr Cells
		×1000				
8	10	1197900	+++	++	+++	++
10	10	1684375	+++	+	+++	++
10	15	1610000	+	+	+	+
15	7.5	1726300	+++	+++	++++	++++
16	6.5	2096300	+	+	++	+++

* Equal numbers of exudative leucocytes were treated with sonic vibration or used intact for control.

0 = no reaction. + = slight erythema. ++ = patchy erythema. +++ = homogeneous erythema. ++++ = marked homogeneous erythema.

Vib. = Animals receiving vibrated cells. Cells = Animals receiving intact cells.

signed to show whether the capability of passive sensitization was retained by cells after treatment with sonic vibration. Leucocytes were collected from 8-16 donors. Half the cells were treated, the other half injected as whole cells for control. The treated cells were injected into a single recipient animal in each experiment. Table I shows that vibrated material is at least as effective as whole cells in the transfer of activity. Smears and total counts of these preparations showed that the method effects complete disruption of leucocytes.

Although no residual intact cells could be seen microscopically, the question of possible transfer by cells still intact after treatment could be obviated by centrifugation of the sonic lysate. In addition the source of transfer activity might be detected by injecting the products of centrifugation separately. In the next group of experiments one recipient animal received the sediment plus the lipoidal fraction, while a second received the aqueous supernatant fluid alone. A third animal served as a control on the intact cells. Table II shows that passive transfer was accomplished in 7 experiments with intact cells and the aqueous supernatant fluid after centrifugation. In only one instance did the sediment possess activity, and this was minimal. Smears and total counts on both the aqueous supernatant fluid and sediment demonstrated that 7.5 minutes was effective in completely disrupting the cells.

Discussion. Owing to the variable nature of passive cellular transfer of hypersensitivity to simple chemical compounds, a control on intact cells from sensitive animals was included in each experiment. In several experiments neither the untreated nor the vibrated cells accomplished transfer. We are at a loss to explain such negative experiments except on the basis of either inadequate donor sensitivity which was not detectable or the heterogeneity of sources of animals used. Such completely negative experiments tend to rule out the possibility that the observed reactions are the result of irritative qualities of the chemical itself. It is significant to note, however, that in cases where sensitivity was transferable with intact cells, it was likewise transferable with extracts.

These results do not preclude the possibility that whole cells involved in transfer may be actively engaged in the production of a sensitizing factor after transfer to a recipient. On the contrary, such productive activity could be the explanation for the lesser intensity of some of the reactions seen in animals receiving fractions of cellular extracts than in those receiving intact cells. However, the exudative cells doubtless have accumulated sufficient amounts of the transfer factor to allow successful passive sensitization.

It is not possible to characterize the factor responsible for transfer at this time. The supernatant fluid after centrifugation at 5,000 RPM for 10 minutes contains particulate matter in addition to soluble constituents. Centrifugation under these conditions could be expected to sediment particles the size of many mitochondria or most bacteria. There-

TABLE II. Passive Transfer after Injection of Separated Portions of Sonic Vibrated Leucocytes into Recipient Animals.

No. of donors	Time of vibration (min.)	WBC/aliquot*	Reactions 24 hr Sup.	24 hr Sed.	24 hr Cells	48 hr Sup.	48 hr Sed.	48 hr Cells
		×1000						
15	6.5	1653000	++	+	++++	++	+	++++
15	7.5	1440827	+	0	+	+	0	++
14	7.5	1285920	+	0	++	++	0	+++
13	7.5	1151702	++	0	++	+++	0	++++
10	7.5	875448	+	0	+++	+	0	+++
10	7.5	625000	+	0	+	+	0	+
10	10	1341316	+	0	+	+	0	+

* Equal numbers of exudative leucocytes were treated with sonic vibration or injected intact for control.

0 = no reaction. + = slight erythema. ++ = patchy erythema. +++ = homogeneous erythema. ++++ = marked homogeneous erythema.

Sup. = Animals receiving aqueous supernatant fluid. Sed. = Animals receiving sediment + lipid fractions. Cells = Animals receiving intact cells.

fore, any particles remaining would be under a size of 0.5 μ.

Attempts to extend this study to other delayed systems have been unsuccessful. In several experiments using tuberculin sensitive donors results were questionable. This may be due to a fundamental difference in the mechanisms of simple chemical and tuberculin sensitivity. However, in none of the tuberculin experiments was an exquisite donor sensitivity achieved, and the degree of sensitivity transferred in control animals was minimal. In experiments in progress we hope to achieve a higher quality of donor sensitivity to enable us to determine whether transfer of tuberculin sensitivity can be accomplished with cellular extracts.

Summary. Passive transfer of delayed cutaneous hypersensitivity to 2,4-dinitrochlorobenzene in guinea pigs has been accomplished with extracts of peritoneal exudative leucocytes disrupted by sonic vibration. Crude extracts of cells treated and subsequently centrifuged were effective as transfer media. Sedimented material was essentially ineffective.

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Hematological Changes Influenced by Short and Long Exposure to Cold. (21065)

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Because of the present interest in studies involving stress and resultant adaptive changes we wish to report our findings with short and long exposures of mice to cold. Selye's adaptation syndrome explanation(1) has met with both acceptance and criticism. Various forms of stress are commonly used, including heat and cold, since these stimuli are easily varied quantitatively. The duration as well as the severity of the stress is undoubtedly important for adaptation may not be a simple linear function. Emphasis at