

Precipitin Reactions in Hypersensitivity Pneumonitides.* (32257)

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An increasing variety of inhaled organic materials has been implicated in the etiology of a hypersensitivity reaction occurring in susceptible individuals(1-4). Acute exposure to the offending agents is followed in 3 to 6 hours by general malaise, dyspnea, chills and fever. Prolonged exposure may be associated with the development of an interstitial pneumonitis. Furthermore, sera from affected patients contain precipitating antibodies against the inhaled antigens. Examples of this syndrome include farmers' lung(1,5), maple bark strippers' disease(2), pigeon breeders' disease(3) and bagassosis(4).

Because of the similarity of symptoms and immunologic reactions in this group of diseases, it was decided to investigate whether the various sensitizing materials might share antigenic determinants, suggesting the possibility of a common etiologic agent.

Materials and methods. Sera were obtained from patients with the clinical diagnosis of farmers' lung, maple bark strippers' disease, pigeon breeders' disease and bagassosis, and from patients without any respiratory illnesses. The antigenic material was prepared as a saline extract of 1. mouldy hay(6) 2. *Coniosporium corticale*, a fungus associated with maple bark strippers' disease(2) 3. Pigeon droppings(3) and 4. *Micromonospora vulgaris*, a thermophilic actinomycete believed to be one of the main sources of antigens of bagassosis(7). The sera were tested for the presence of precipitating antibodies against these antigenic materials by the Wadsworth modification(8) of the Ouchterlony agar-gel immuno diffusion technique(9).

Results. Examples of precipitin reactions between the tested sera and antigenic materials are shown in Fig. 1. All sera gave strong positive reactions against their primary anti-

gens, *i.e.*, the serum from the patients with bagassosis reacted with the extract of *M. vulgaris*; the serum from the patients with farmers' lung reacted with the extract of mouldy hay, etc. In addition, most of the sera reacted with the other antigenic materials (Table I). The sera from all patients with bagassosis and one half of the patients with pigeon breeders' disease reacted with all antigens. The sera from patients with farmers' lung gave precipitin reactions with the extracts of *C. corticale* and pigeon droppings but not with *M. vulgaris*. Serum from the patient with maple bark strippers' disease contained precipitins against antigens of *C. corticale* only. Two of the 4 sera from patients with pigeon breeders' disease did not contain precipitating antibodies against mouldy hay and *M. vulgaris* extracts. No precipitin reactions were obtained with the control sera.

Discussion. The immunologic reactions occurring in the sera of these patients may be explained by the possible presence of common antigenic determinants of mould origin in the tested extracts. Two of the extracts were obtained from fungi (*M. vulgaris* and *C. corticale*) grown from the crude organic material associated with the illness. The major source of the antigen of mouldy hay has been shown to be a thermophilic actinomycete(10). Pigeon droppings have been shown to contain *Cryptococcus neoformans*(11), in addition to other saprophytes such as *Candida albicans* (personal observations).

Not all sera from patients with pigeon breeders' disease contained precipitating antibodies against antigens of mouldy hay or against *M. vulgaris*. This may be the result of variation in immunologic responsiveness of the individual patient or may be a reflection of the fluctuation of titers of antibodies found in various stages of this illness (12).

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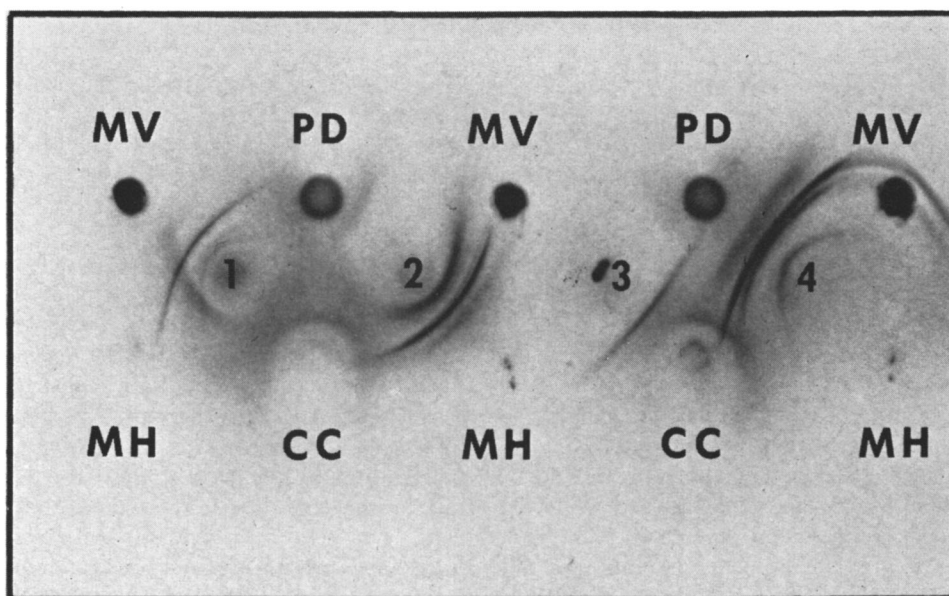


FIG. 1. Immuno diffusion patterns of sera from patients with hypersensitivity pneumonitides against various offending antigens. Peripheral wells contain extracts of: *Micromonospora vulgaris* (MV), pigeon droppings (PD), mouldy hay (MH), *Coniosporium corticale* (CC.). Inner wells contain serum from patients with bagassosis (1), farmers' lung (2), maple bark strippers' disease (3), pigeon breeders' disease (4).

TABLE I. Results of Immuno Diffusion Reactions.

Serum from patients with		Antigens			
		<i>Micromono- spora vulgaris</i>	Mouldy hay	<i>Coniosporium corticale</i>	Pigeon droppings
Bagassosis	(3)*	+	+	+	+
Farmers' lung	(2)	—	+	+	+
Maple bark strippers' disease	(1)	—	—	+	—
Pigeon breeders' disease	(4)	+†	+†	+	+
No respiratory illness	(5)	—	—	—	—

* No. of sera tested in parentheses.

† Only 2 sera contained precipitins against these antigens.

Because of the rare occurrence of maple bark strippers' disease, only one serum could be obtained. Additional sera will be necessary to provide firm conclusions regarding possible reactions with other antigen preparations.

The only antigens found to react with all sera were those of *C. corticale*. This finding suggests that this organism contains antigenic determinants common to the other tested extracts. At present there is, however, no evidence implicating this organism in the etiology of hypersensitivity illnesses other than maple bark strippers' disease.

Summary. Sera from patients with farmers' lung, pigeon breeders' disease, bagassosis, and

maple bark strippers' disease were examined for the presence of precipitating antibodies against the corresponding antigenic materials. All sera showed reactions with 3 or 4 of the antigens. The one exception was the serum from the patient with maple bark strippers' disease, which reacted only with the antigens thought to cause the disease.

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Incorporation and Release of H³-Catecholamines by Cultured Fetal Human Sympathetic Nerve Cells and Neuroblastoma Cells.* (32258)

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Children with neuroblastomas have an increased urinary excretion of dopa, dopamine, norepinephrine and the metabolites of these catecholamines(1-3). The excretion of the amines is not constant; there may be a variation in the quantities of the various amines and their metabolites as the disease progresses. The excretion of these amines has not been correlated with the Bright Field histology of the tumor cells(1). La Brosse and coworkers(4) reported that 3-O-methylated metabolites of H³-norepinephrine were found in media after adding H³-norepinephrine to cultures of neural crest tumor cells. Their studies indicated that these tumors contained enzymes which could rapidly degrade norepinephrine and other precursors of this amine.

The purpose of the present study was to compare the incorporation and retention of H³-dopamine and H³-norepinephrine by neuroblastoma cells and by human fetal

sympathetic neuroblasts growing in tissue culture.

Material and methods. Neuroblastoma cells from 8 children whose tumors were composed of immature, small neuroblasts were explanted on the surface of collagen coated cover slips in 30 mm plastic petri dishes. A dense suspension was made from the friable tumors in growth medium. One-tenth cc of the suspension, consisting of small clumps and many isolated tumor cells, was placed on the collagen. One and one-half cc of medium, composed of 30% calf serum and 70% 199 containing 200 units/ml of penicillin G, was added after the cultures had been incubated at 37°C for one hour in a humidified incubator gassed with 5% CO₂ and air.

The sympathetic chains were dissected from 10 human fetuses aborted between 12 and 14 weeks gestation. The chains from a fetus were cut into small fragments after removing the capsule of connective tissue and incubated with intermittent shaking at 37°C in 0.5% crude trypsin in Hanks' solution for 90 minutes. The nerve cells were dissociated by pipetting and the cells were centrifuged at 1,000 RPM for 10 minutes. The super-

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