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Received July 28, 1966. P.S.E.B.M., 1967, v124.

Subcellular Distribution of Histamine in Human Leucocytes.* (31665)

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Histamine is released from washed leucocytes of allergic individuals during incubation with specific antigen(1,2). We have shown (3) that combination of antigen with antibody on the washed sensitive cell is an important rate limiting step in the histamine release process. In addition, this phase of the reaction can be separated from the subsequent series of events leading to the appearance of histamine in the medium. The intracellular events initiated by the combination of added antigen and antibody bound to leucocytes is unknown. To investigate the nature of certain of these events, the intracellular distribution of histamine and of beta glucuronidase, a representative hydrolytic enzyme, was studied. Mobilization of these substances during specific immunologic histamine release and under nonspecific conditions was studied.

Materials and methods. Human leucocytes. Whole blood was obtained from a volunteer with a clinical history of rhinitis and asthma due to mold spores and with a marked immediate cutaneous wheel and erythema reactivity to mold antigen. Control blood samples were obtained from nonsensitive subjects with no cutaneous reactivity to any of a variety of antigens.

Antigen. Dry mold antigens (a mixture of alternaria, hormodendrum, helminthosporium, penicillium, monilia, aspergillus, rhizopus, chaetomium, fusarium and phoma) were extracted with buffer(4). This antigen extract resulted in release of histamine from leucocytes of mold sensitive individuals, but not of nonallergic controls.

Histamine release. Leucocyte preparations were obtained from whole blood by sedimentation and washing and the cellular histamine was released by incubation with antigen as previously described(1,2). The histamine was determined fluorometrically by the method of Shore *et al*(5). Tris ACM medium, used for suspension of leucocytes, is a salt solution containing Ca^{++}, Mg^{++} and human albumin and is buffered at pH 7.4 using Tris-hydroxymethylaminomethane.

Leucocyte fractions. Washed human leucocytes were disrupted and separated into subcellular fractions by the method of Hirschhorn and Weissmann(6). The fractions were designated as follows: *nuclear,* particulate matter sedimented at 2,000 *g* for 10 minutes; *granular,* sedimented at 27,000 *g* for 20 min-

* This investigation was supported in part by USPHS Research Grant A106139-03 from Nat. Inst. Allergy & Infect. Dis. and the Ernest S. Bazley Asthmatic Research Fund, Chicago Wesley Memorial Hospital.

† Career Development Investigator Awardee, USPHS Research Grant 5-K3-19, 460-02.

TABLE I. Distribution of Histamine and β-Glucuronidase in Subcellular Fractions of Human Leucocytes.

Fraction	Percent of total histamine				Percent of total β-glucuronidase			
	Donor A		Donor B		Donor A		Donor B	
Supernatant	22	15*	15	18	11	15	18	13
Nuclear	22	22	20	20	25	25	28	26
Granular	56	63	65	62	64	62	54	61

* Two values for each donor represent duplicate samples obtained for each experiment.

utes; *supernatant,* not sedimented at 27,000 *g* for 20 minutes.

Hydrolytic enzyme assay. Assay of beta glucuronidase activity was performed on 0.2 ml aliquots of supernatant fraction or of distilled water lysates of the particulate nuclear and granular fractions. The fractions were first brought to 5 ml volume which was the original volume of the leucocyte suspensions obtained in the processing of 5 ml of whole blood. The samples were added to a mixture of 0.4 ml 0.08 M acetate buffer pH 5, 0.3 ml distilled water and 0.1 ml 0.1 M phenolphthalein glucuronate. After incubation for 48 hours at 37°C, 5 ml of 0.2 M glycine buffer pH 10.4 was added and the phenolphthalein liberated was determined spectrophotometrically at 540 mμ. Activity was measured in optical density units.

Results. Washed leucocytes from 2 nonallergic control subjects were lysed in sucrose medium and fractionated by differential centrifugation into supernatant, nuclear and granular fractions. Two separate aliquots of washed cells from each donor were disrupted. The percentage of the total histamine and of the beta glucuronidase present in each of the fractions of the duplicate samples of cells is shown in Table I. Similar proportions of histamine and beta glucuronidase were present in respective fractions of each of the aliquots. The variations that did occur were as great among duplicate samples from the same donor as between aliquots from separate donors.

The distribution of beta glucuronidase and of histamine was determined using cells from an allergic donor incubated either in the presence or in the absence of specific antigen. Incubation was carried out at 37°C for 45 minutes in each instance. Following incubation, cells were separated from the incubation medium by centrifugation at 110 *g* for 10 minutes. The cells were then lysed in sucrose and fractionated. Histamine and beta glucuronidase activity was determined in each of the fractions. The percentage distribution is recorded in Table II. Histamine was released from the cells and into the incubation medium which contained specific antigen but not where antigen was absent. In contrast, the beta glucuronidase activity was essentially the same in corresponding fractions of both aliquots of cells. These results were confirmed by repetition with cells from the same and from 2 other donors. The histamine released into the medium varied from 62 to 95 percent depending on the sensitivity of the donors, but the intracellular distribution of histamine was proportional in all instances. The beta glucuronidase activities were no more variable than those of Table I.

An attempt was made to find a means of releasing hydrolytic enzymes from human leucocytes without the release of histamine. Leucocytes were suspended in Tris ACM medium containing 10 mg potato starch and incubated at 37°C for 45 minutes with intermittent shaking. A control aliquot of

TABLE II. Distribution of Histamine and β-Glucuronidase Subcellular Fractions from Leucocytes of Allergic Subjects Incubated With or Without Specific Antigen.

Incubation	Fraction	Percent of histamine	Percent of total β-glucuronidase
Antigen	Medium*	86	7
	Supernatant	3	14
	Nuclear	4	15
	Granular	7	64
No antigen	Medium	6	7
	Supernatant	10	11
	Nuclear	22	28
	Granular	62	54

* Incubation medium after removal of cells by centrifugation at 110 *g*.

TABLE III. Distribution of Histamine and β-Glucuronidase in Subcellular Fractions of Human Leucocytes Incubated in Presence or Absence of Starch.

Incubation	Fraction	Percent of histamine	Percent of total β-glucuronidase
Starch	Medium*	10	31
	Supernatant	16	14
	Nuclear	9	9
	Granular	65	46
No starch	Medium	8	14
	Supernatant	13	13
	Nuclear	12	15
	Granular	67	58

* Incubation medium after removal of cells by centrifugation at 110 *g*.

cells was incubated without starch for 45 minutes with intermittent shaking at the same time and in same manner as the experimental sample. The results obtained in the first series are presented in Table III. Histamine release from cells during incubation was small in both experiments. The beta glucuronidase activity found in the incubation medium was considerably higher in the presence of starch than in its absence. The appearance of enzyme activity in the medium was correlated with a proportionate loss in the enzyme activity of the granules. A second experiment was performed with cells from the same donor at another time. One and one-half times more of both histamine and beta glucuronidase were released than in the first experiment.

The results have been expressed in terms of percentages in order to emphasize distribution. The total amounts of histamine and beta glucuronidase were very similar for aliquots of cells from one individual regardless of the conditions employed in any experiment described in this study. This indicated that these substances were preformed in the cell and were not produced rapidly in response to the various experimental conditions.

Discussion. Both histamine and β-glucuronidase have similar intracellular distributions when fractions produced by differential centrifugation of disrupted cells are compared. Approximately 20% is present in the supernatant fraction which represents cell fluid. Sixty percent appears in a fraction sedimented only by high speed centrifugation and corresponds to the granular or lysosomal fraction. Whether the remaining 20% of the histamine is part of the nuclear fraction or is associated with granules that are attached to this fraction has not been determined. In either case, at least 80% of both of these physiologically active substances is associated with particulate matter in the cells.

In the guinea pig mast cell, histamine has been localized to granules(7) which have been shown to become greatly reduced in number during the reaction of sensitized cells with specific antigen. In this process, histamine is released extracellularly. Histamine that is present in human peripheral leucocytes is reported to be divided almost entirely among the basophils, neutrophils and eosinophils(8), but no intracellular localization has been described previously. The results obtained in this study suggest that histamine of human leucocytes is also present largely in granules and that during a specific reaction of sensitive cells from allergic donors with antigen it is released from these granules to the extracellular medium. The granules containing histamine are apparently different from those containing hydrolytic enzymes, the lysosomes, since histamine was released with minimal concomitant loss of beta glucuronidase. In addition, under other conditions such as incubation with starch powder, a proportionately greater release of enzyme than of histamine occurred.

Since immunologically mediated histamine release has been considered to be the result of the action of hydrolytic enzymes(9), it is of some significance that beta glucuronidase was not mobilized during this specific reaction. Although beta glucuronidase is considered to be a lysomal marker, it may be that lysosomes are a very heterogeneous group of granules and that only certain proteolytic enzyme containing granules are rapidly and selectively disrupted by an appropriate antigen antibody reaction at the surface of the cell. This would result in the release of histamine without release of beta glucuronidase.

Summary. Histamine and β-glucuronidase are present mostly in a granular fraction of human leucocytes. Histamine was released preferentially from the cells of allergic donors incubated with specific antigen, and beta

glucuronidase was preferentially discharged during incubation with starch granules.

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Received August 20, 1966. P.S.E.B.M., 1967, v124.

Transmission of Marek's Disease with Oral Washings and Feces From Infected Chickens. (31666)

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Marek's disease (MD) is a disease of the avian leukosis complex that causes severe mortality and economic loss in young chickens. The etiological agent of MD is unrelated to viruses causing lymphoid leukosis, another major disease of the avian leukosis complex (1). Also, MD has been observed in chickens known to be free of lymphoid leukosis virus infection(2).

The modes of natural transmission of MD are poorly documented. Under natural and experimental conditions the disease appears highly contagious and Sevoian(3) has shown the agent to be airborne. Certain beetles may be carriers of the infectious agent and can transmit the disease when eaten by susceptible chickens(4). Early attempts at transmission with feces or oral washings were apparently successful in some cases(5,6,7) but were negative in others(8,9). Since horizontal transmission by both direct and indirect contact appeared to be highly important in the epizootiology of MD, studies were undertaken to explain further the mechanisms involved. This preliminary report describes the presence of the infectious agent in oral washings and feces from chickens experimentally infected with the JM strain of MD.

Materials and methods. Marek's disease agent. The JM strain of MD, isolated by Sevoian *et al*(10), was used in this trial. This strain has been maintained at this laboratory by serial passage in chickens at 4- to 6-week intervals with heparinized whole blood or tumor suspension. No change in potency or pathologic characteristics has occurred with passage.

Chickens. White Leghorn chickens of line 7, an inbred line developed at this laboratory, were used in these studies. Line 7 chickens are highly susceptible to MD but are genetically resistant to infection with common strains of lymphoid leukosis viruses(11).

Inocula. Oral swabs were obtained by massaging the oral cavity of each donor bird with sterile cotton swabs. Since 5 or 6 such swabs were prepared from each donor, an attempt was made to insure that each swab received approximately equal quantities of fluid and mucus. The swabs immediately were placed in individual sterile glass tubes.

Oral washings were collected 2 hours after swabs were taken to permit the oral fluids to be replenished. The oral and nasal cavities of each donor bird were repeatedly irrigated with 5 ml of cell culture media consisting of medium 199 with 2% bovine fetal serum, 10% tryptose phosphate broth and penicillin and streptomycin at 10,000 units and 10 mg per ml respectively. The washings were pooled and centrifuged at 4 C for 5 minutes at 400 $\times$ *g*. About 7/8 of the supernatant fluid was carefully drawn off and used for inoculation.