Asthmatic Deaths
Role of the Mast Cell

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The number of mast cells in the bronchial walls of patients who died of asthma was compared to that in walls of nonasthmatic patients. Mast cells are normal components of bronchial wall and contain chemical mediators which can cause changes in bronchial muscle tone and glandular secretion. Physiological amounts of mediator released by normal stimuli may constitute a local homeostatic mechanism for control of some bronchial functions. Unphysiological quantities released by abnormal stimuli could cause bronchospasm, secretion, and edema leading to obstruction typical of that found in asthma. Degranulated mast cells cannot be identified in tissue sections. There were fewer mast cells in bronchi of patients who died of asthma than in those of nonasthmatic patients, which suggests that marked degranulation of mast cells occurred, and is one mechanism associated with the pathophysiology of asthma.

Death occurring during severe attacks of asthma is due to bronchial obstruction and suffocation. Edema of the bronchial wall, bronchospasm, and thick tenacious secretions within the lumen combine to produce the obstruction. These events represent the final gross pathological state; initiating mechanisms are not known.

In many instances, the etiology of asthma is an allergic reaction. An antigen-antibody complex triggers primary mechanisms which in turn induce bronchial obstruction. However, in the majority of patients who die during asthmatic attacks, specific allergens cannot be identified. This latter type of patient poses a problem, not only as to the etiology of the asthma, but also regarding mechanisms which produce the pathological condition.

I reviewed the clinical records and autopsy findings of 21 patients in whom asthma was the primary or contributing cause of death. The findings were compared with those observed for 39 patients who died of causes other than asthma. Patients who had asthma terminally differed markedly from nonasthmatics in both the types and the number of cells present in the bronchial walls.

Materials and Methods

Four groups of patients were studied: (1) 12 patients who died in status asthmaticus, (2) 9 patients who died of causes unrelated to asthma, but in whom asthma was present at the time of death, (3) 7 patients who each had a history of asthma but whose actual cause of death was unrelated to asthma, and who did not have asthma at the time of death, and (4) 32 randomly selected patients who never had asthma and who died of other causes. The youngest patient who died of status asthmaticus was 45 years of age; the oldest was 86. The age range in the other three groups was similar.

Records and autopsy specimens of the asthmatic patients were obtained from three New York city hospitals, while patients who died of causes other than asthma and who had never had asthma were randomly selected from Roosevelt Hospital records. The asthmatic patients died during the period of 1954 to 1967; the deaths of the patients who died of causes other than asthma occurred during the years 1958 to 1968.

The study was retrospective. The type, quantity and reliability of information contained in the case reports varied greatly. Frequently, a history and findings from diagnostic studies related to allergy were not obtained.
Retrospective studies based on autopsy records introduce a serious sampling problem. One can question whether autopsied patients are a representative sample of all patients who die of the specified causes.

Significant Clinical Data Related to Status Asthmaticus Group.—The etiology of asthma in the 12 patients who died in status asthmaticus was unknown. Five had been skin-tested at some time during life, and in four, at least some of the skin tests were positive. In only one of the four with positive skin tests was the presence of an allergen which might have precipitated the terminal attack suggested.

Respiratory or sinus infection was associated with the onset of asthma in five patients. In two of these five, the agent precipitating terminal status asthmaticus may have been an inhaled chemical. One patient, employed in a photographic-developing concern, noted that his symptoms of asthma were aggravated at work when he was near the chemicals used in processing film. The terminal attack of the other patient began when the patient spent several days painting the inside of his house.

The paucity of data which might explain the etiology of asthma in these 12 patients is related to several factors. Frequently the patients were first seen when they were critically ill, and thorough allergic work-ups were not feasible. Even more unfortunate was the fact that asthma was attributed to emotional upset or recurrent asthma was treated with corticosteroids during this period. Of the other nine patients, five were not treated with steroids; one patient had been receiving steroid therapy for two years, which was inadvertently stopped four days prior to death because of medication error; one patient received 5 mg of prednisone orally, and two received 100 mg of hydrocortisone sodium succinate (Solu-cortef) intravenously in the final 24 hours of life.

Histological Examinations.—All of the pulmonary specimens obtained at autopsy were fixed in formalin. After fixation, they were cut in sections 4 μ thick, and the sections were mounted on slides and stained with Giemsa stain.

Enumeration of mast cells proved to be extremely important in this study. It is commonly stated that water-containing fixatives such as formalin are not recommended for mast cell identification in man, and that the best fixative varies for different species. On the contrary, Parish and Nepriakhin both state that formalin is an excellent fixa-

1. Left, Mast cells (dots) in status asthmaticus. Right, Mast cells in normal bronchus.

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2. Mast cell counts compared to cause of death and use of corticosteroids. Adequate doses of corticosteroids administered during the last 24 hours of life (dot). Inadequate or no corticosteroid therapy during last 24 hours of life (circle). Corticosteroid administration was not recorded for nonasthmatic patients whose cause of death is listed as “other.”

3. Mast cell count in patients with nonasthmatic pulmonary pathological condition or no pulmonary pathological condition.

The mast cell averages approximately 15 μ in diameter. This statistic was of little practical value in identifying the cell, since cellular configurations were so variable and the thin sections often contained only fragments of some cells. The cellular configurations varied from round, to stellate, to elongated. When seen, the nucleus of the cell was usually round. More often, its shape was obscured by the overlying dense, metachromatically stained granules. The nucleus of some cells was absent because only a portion of the cell was contained in the thin tissue sections. The most striking feature of the mast cell is the large, metachromatically staining granules. Identification was based solely on the presence of these characteristic granules.

To quantitate the number of mast cells in various regions of the respiratory airways, it was necessary to measure exactly the area of tissue examined. To do this, the stained tissue section was placed in a photographic enlarger and the image projected at ×10 magnification onto graph paper. Salient features of the magnified image were drawn on the graph paper. The tissue section was then examined at ×1,000 magnification, and the location and number of mast cells seen were plotted on the previously prepared graph. This technique provided a method for easily determining mast cell concentration per square millimeter. The count per square millimeter multiplied by a conversion factor of 80 (derived from tissue thickness and mast cell size) gave the number of mast cells per cubic millimeter.

Since the study was retrospective, I had no choice in selecting the bronchial tissue removed at post-mortem examinations. Consequently...
ly, tissue available for the study varied from case to case. Mast cell counts were made for at least one bronchus from each autopsy, and at least 5 sq mm were examined from each case, and up to 50 sq mm if tissue was available. A variation was often seen in mast cell counts of different parts of the same bronchial section of patients who died status asthmaticus (Fig 1, left).

To determine what the normal mast cell count was in various parts of the lower airways, I obtained specimens of trachea, main stem bronchus, and a bronchus 2 to 5 mm in diameter from ten cadavers whose airways were essentially normal at the time of death. For each cadaver, mast cell counts for these different-sized airways were similar, and varied from 1,500 to 4,000 mast cells per cubic millimeter of tissue.

One disadvantage of cell counts per unit of tissue examined when the tissue is "solid," such as the bronchial wall, is that such counts are influenced by the presence of edema. I have no way of evaluating the effect of edema on this study.

Results

The microscopic pathological findings in the bronchi of those patients who died in status asthmaticus were the same as those described in previous studies.1,4,5 Edema of the tunica propria was common. A marked mixed infiltrate, consisting predominantly of eosinophils, plasma, and round cells, filled the tunica propria. Plasma and round cells were more numerous in deeper portions of the bronchial wall. Polymorphonuclear leukocytes were rare in 11 of the patients, and their number was moderately increased in one. Hypertrophy of bronchial muscle, commonly noted in asthma, was difficult for me to substantiate because of edema and differences in bronchial size. Many bronchi contained an exudate of mucus and eosinophils. Sometimes the exudate and infiltrate completely occluded the lumen, and a Cur- schmann's spiral was found in one bronchus of one patient. Thus, the patients with a clinical diagnosis of status asthmaticus had microscopic findings corroborating that diagnosis.

The mast cell is the most prominent and colorful cell seen in bronchial tissue when the specimens are stained with Giemsa and examined under ×1,000 magnification. It is often overlooked unless specifically searched for, because the granules do not stain with hematoxylin and eosin routinely used in histopathological studies, and because the mast cell occurs in such small numbers relative to the other cells present. The mast

4. Degree of eosinophilia of bronchial wall vs mast cell count.

5. Eosinophilia vs mast cells vs diagnosis. Slight-to-marked mast cells (dot). No eosinophilia (circle).
cell was found most often at the ends of cartilaginous rings, between the cartilage and muscle and external to the basement membrane of the glands. It was seen less frequently in the tunica propria. Mast cells were not found in the epithelium nor in the lumen.

The most significant finding in this study was the variation in the numbers of mast cells among the four groups of patients studied (Fig 2). In ten of the 12 patients who died in status asthmaticus, the mast cell count varied from 10 to 400/cu mm of bronchial tissue. In the patient who had been receiving steroids for two years and in whom this therapy was inadvertently stopped four days prior to death, the mast cell count was only 100 cu mm. The mast cell counts in the three patients who died in status asthmaticus but who received adequate doses of steroids during their terminal illness were 300, 800, and 1,000 per cubic millimeter.

Mast cell counts in the nine patients who had asthma terminally, but who died of other causes, varied from 400 to 2,000/cu mm. However, six of these nine had counts of 400 to 800/cu mm, which were only slightly higher than those of patients who died in status asthmaticus.

For the seven patients who had a history of asthma but did not have asthma during their last illness, the counts were higher, and for six varied from 800 to 2,700/cu mm. In the seventh patient, the count was only 200/cu mm. This patient had chronic pulmonary insufficiency and respiratory wheezing during his terminal illness. Quite possibly, asthma played a role in his disease, although his physicians made no record of this diagnosis. Further evidence suggesting that this patient may have had asthma was a moderate eosinophilic infiltrate that was found in the bronchial wall.

The number of mast cells varied widely, 100 to 3,800/cu mm, in the 32 patients who never had asthma. However, only six of the 32 in this group had counts of 500 or less per cubic millimeter, which was the upper level of the mast cell count in patients who died of asthma and did not receive steroids. The diagnosis was lupus erythematosus for two of these six, and carcinoma of the lung, carcinoma of the pancreas, malignant lymphoma, and myocardial infarction with acute pulmonary edema for the other four.

Figure 1 shows two bronchial sections; one from a patient who died in status asthmaticus (Fig 1, left) and the other from a patient without asthma who died of carcinoma of the breast (Fig 1, right). Besides demonstrating the usual difference in quantity of mast cell between asthma patients and controls, it illustrates the variability which may be found within the same bronchus of a patient who has an asthmatic death. In Fig 1 (left), one large section of the bronchus which measures 15 sq mm contains 50 mast cells, or 3.3/sq mm. In the other section, which measures 20 sq mm, no mast cells were found. Figure 1 (right) illustrates findings in a patient without asthma, in whom the distribution of mast cells is quite uniform throughout, 30 mast cells per square millimeter.

Pathological findings were absent in the lungs of 17 of the 32 patients who never had asthma. Fifteen had significant pulmonary disease, which in some cases contributed to or caused death. The abnormalities in these latter patients included pulmonary malignancy (primary or metastatic), congestive heart failure and pulmonary edema, pneumonitis, emphysema, and pulmonary emboli. Although there was a marked variation in mast cell counts in these nonasthmatic individuals, the variation could not be correlated to the presence or absence of pulmonary pathological findings (Fig 3).

I observed an inverse relationship between the eosinophilic infiltrate and the mast cell content of the bronchial wall; the greater the number of eosinophils, the fewer the number of mast cells (Fig 4).

Figure 5 shows slight-to-marked eosinophilia in relation to the cause of death and mast cell count. Of the 32 nonasthmatic patients, only four had slight, and two had moderate infiltrates of eosinophils in the bronchial wall. An explanation for the eosinophilic infiltrates was not apparent from the clinical histories of these patients. Infiltrates of eosinophils were slight-to-marked in 19 of the 21 patients with terminal asthma; in only two were eosinophils absent.

Comment

Mast cells occur in many tissues as a normal cellular component. The mast cell is readily identified by its characteristic metachromatically stained granules. Unfortunately, these granules are soluble in a number of common fixatives. They are also difficult to identify in routine sections stained with hematoxylin and eosin preparations. Furthermore, the number of mast cells seen in any tissue is small and belies their physiological importance. For these reasons, mast cells are rarely noted in routine pathological examinations.

I made a concerted effort to determine as accurately as possible
the mast cell count in bronchial tissue. A fixative which preserved the granules and Giemsa stain which made them readily visible were used. Tissue sections were cut at a thickness of 4 μ and the area of tissue examined microscopically was accurately measured.

In the nonasthmatic group, mast cells were always found in the wall of the trachea, in main-stem bronchi, and in smaller bronchi. From these findings, it may be concluded that mast cells are normally present in the lower respiratory airways. The number of mast cells found in these airways varied widely, but two thirds of the counts were in the range of 500 to 2,500/ cu mm. Since this range was calculated for a group of patients who died, even it cannot be considered normal. For reasons to be discussed below, changes in mast cells caused by some diseases would more often result in a decrease rather than in an increase in the count. Therefore, a more reasonable figure for normal mast cell concentration would be 1,000 to 3,000/ cu mm. This figure has yet to be determined experimentally.

The nonasthmatic group of patients is also important in another way, since some of them had a significant pulmonary pathological condition. It is possible that pulmonary disease itself could cause lower mast cell counts. This was not the case, however, since high or normal counts were found as frequently in patients with a nonasthmatic pulmonary pathological condition as in those with normal lungs. The findings indicate that pulmonary disease in general did not cause a decrease of mast cells.

A marked difference in mast cell counts was found in the four groups studied. Counts were decreased in patients who died in status asthmaticus. They were highest in patients who never had asthma, or who had only a past history of asthma. Intermediate values were found in those patients who died of causes other than asthma but who had asthma during their terminal illness. Since pulmonary pathological conditions alone did not cause low mast cell counts, these observations demonstrate that asthma is associated with a decrease of mast cells. The findings are in agreement with those of Savalato who biopsied the bronchial wall of asthmatic patients before and during an asthmatic attack. He found significantly lower mast cell counts in biopsy material removed from patients during an episode of asthma than in biopsy material obtained from the same patients during symptom-free periods.

That the number of mast cells in bronchial walls was different for the four groups studied is of major significance, since it suggests a pathological mechanism which may explain the bronchial obstruction which occurs in asthma. The granules of the mast cells of man are known to contain histamine and heparin. Animal mast cells, in addition to histamine and heparin, contain serotonin and slow reacting substance. In animals, Riley demonstrated that the release of histamine from mast cells is accompanied by loss of cell granules. Since I identified mast cells by the presence of their specific granules, degranulation of the cells could account for lower than normal counts in bronchial tissue; degranulated cells would not be recognized as mast cells. When released into adjacent tissue, histamine or other chemical mediators from mast cells could cause bronchial muscle contraction, glandular secretion, and edema. Anatomical relationships are also compatible with this hypothesis, since the mast cell is in close proximity to bronchial muscle and glands in man. In addition, I studied mast cell distribution in guinea-pig tracheal rings. In sections of guinea-pig trachea, the muscle between the free ends of the cartilaginous rings is anatomically discrete (Fig 6). Seventy percent to 90% of the mast cells were found in the area immediately surrounding this muscle, again illustrating the close proximity of muscle to mast cells. Thus, the pharmacological and histological evidence suggest that mast cell mediators may be one mechanism involved in the pathophysiological condition of asthma.

One additional question must be answered. Is the amount of mediator present in the mast cell sufficient to produce the pathological changes seen in asthma? It has been estimated that one mast cell contains approximately \(1 \times 10^{-9}\) mg of histamine. I found that \(1 \times 10^{-7}\) mg of histamine injected intradermally in humans caused a positive skin test in most individuals. This relationship shows that the histamine content of mast cells in the bronchi is physiologically more than adequate to produce the pathological condition of asthma. The use of histamine as an example is not meant to imply that histamine is the only, or even the significant, mediator in asthma. In this case, it is employed only to illustrate quantitatively the potent store of mediator that is present in the mast cell.

The accumulated evidence suggests the following hypothesis: Mast cells are a normal constituent of the lower respiratory airways and are intimately associated with bronchial muscle and glands. They contain potent pharmacological agents. Under normal conditions, one function of the mast cells may be the maintenance of homeostasis by the release of controlled amounts of chemical mediators. The chemical mediators would in turn stimulate bronchial muscles and glands. Physiological stimuli activating granular release could be local changes in pH, carbon dioxide or oxygen tension, or changes in the humidity or temperature of in-
spired air.

Immunological complexes and a variety of dissimilar chemicals, such as polyomine, codeine, and dextran, can cause uncontrolled degranulation and mediator release from mast cells in vivo and in vitro. Therefore, these agents or others causing mast cell degranulation could cause severe bronchospasm, hypersecretion, and edema. The pathological findings in asthma. The results in this study and those of Salvato, of markedly decreased mast cell counts in patients with asthma are compatible with this hypothesis and suggest that massive uncontrolled degranulation occurred in status asthmaticus.

Many of the agents cited as being capable of causing mast cell degranulation are routinely encountered by the general population without ill effects. Because of this, it is apparent that other factors in addition to immunological reactants or chemicals must be operative before degranulation occurs. The other factors, which are presently unknown, could take the form of cellular intermediary metabolic derangements, abnormalities in the mast cells themselves, or a variety of adjuvant effects.

Since mast cell degranulation can be initiated by immunological and nonimmunological mechanisms, it follows that the etiology of asthma can be either allergic or nonallergic. Of the 12 patients who died during status asthmaticus, an allergic etiology for asthma seemed probable in one, because of positive skin tests and environmental exposure to a specific antigen. Inhalation of chemicals may have initiated the terminal attack in two others, in which the mechanism was nonallergic. In the remaining nine patients, the cause of asthma was not determined, and no assessment of mechanisms could be made.

As was mentioned previously, mast cell counts in six of the patients in the nonasthmatic group were low, 400 or less per cubic millimeter. In the diseases encountered in these six patients, lupus erythematosus, malignant lymphoma, and carcinoma, immunological abnormalities have been demonstrated or are suspected. It is possible that the immunological abnormalities or even the drugs used in the treatment of these diseases caused the low mast cell counts.

The manner in which corticosteroids clinically moderate some cases of asthma is not known. It has been suggested that corticoids stabilize the cell wall of the mast cell, making them more resistant to degranulation. On the other hand, Asboe-Hansen demonstrated degranulation of mast cells after adrenocortical steroid administration, which suggested to him that these cells are the end organs in the mediation of the effect of these hormones. The type of data collected in the present study cannot be used to confirm or to detract from either argument. Furthermore, all patients in the study, who were treated with corticoids, died and therefore they may not reflect the normal mechanisms associated with corticoid treatment.

Patients with terminal asthma almost always had an eosinophilic infiltrate in the bronchial wall and eosinophils in the bronchial lumen. In the nonasthmatic patients, an eosinophilic infiltrate was rare. Three known mechanisms can account for infiltrates of eosinophils. First, antigen-antibody complexes appear to be eosinotactic; the eosinophils are thought to phagocytize the complexes. Therefore, an allergic or immunological reaction is compatible with the presence of infiltrates of eosinophils. Second, Cohen et al demonstrated, in animals rendered immunologically incompetent by radiation, that foreign or altered proteins may be eosinotactic. Their evidence and my previous report suggest that eosinophilic infiltrates can be present in the absence of an allergic reaction. Third, since infiltrates of eosinophils were prominent when mast cell counts were low, it is possible that mediators released by mast cell degranulation are eosinotactic. Thus, in asthma, a number of possibilities could explain eosinophilic infiltrates, such as antigen-antibody complexes, foreign or altered proteins, and eosinotactic substances released from mast cells. These explanations indicate that the presence of eosinophils is compatible with both allergic and nonallergic causes of asthma.

In summary, the mast cell is a normal constituent of the lower respiratory airways; its normal function may be to contribute to homeostasis. Patients who died in status asthmaticus had lower mast cell counts in their bronchi than a nonasthmatic group. The mast cell contains chemical mediators which, if released in abnormal quantities, could cause the signs and symptoms of asthma. Immunological complexes and some chemicals are known to degranulate mast cells, which suggests that allergic and nonallergic mechanisms may cause asthma. Bronchial eosinophilic infiltrates occurred more frequently when mast cell counts were decreased. The presence of eosinophilic infiltrates may also be explained by means of allergic and nonallergic mechanisms.

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References

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one of the leading physiologists in the 19th century, was born in Liegnitz, Prussia, into a well-to-do family with scholarly interests. He completed his general education in Liegnitz, studied medicine in Berlin and Heidelberg and received his MD degree from Berlin in 1863 where he presented an inaugural dissertation on muscle fatigue. Intending to pursue the practice of medicine he shared his time as an assistant to Traube in medicine with studies in physiological chemistry under Kühne. In 1876 he was called to Berlin as head of the experimental physiological division in an institute recently organized by DuBois-Reymond. Seven years later he accepted the professorship of physiology at the University of Bern. The creation of new laboratory procedures and the design of new pieces of physiological apparatus were probably his greatest contributions to teaching and investigation.