Brigham et al found that histamine infusion caused a dose-related, reversible increase in lung vascular permeability to protein in unanesthetized sheep. He later showed that the H₁ receptor inhibitor diphenhydramine infused with histamine phosphate prevented the histamine-induced pulmonary edema. Metiamide, an H₂ receptor inhibitor, infused with histamine did not prevent the increased lung vascular permeability. Morphologic studies suggest that histamine causes large gaps to appear between venular endothelial cells, perhaps resulting from endothelial cell contraction.

In contrast to the release of preformed histamine, slow-reacting substances of anaphylaxis (SRS-A) is not present in the cell in the preformed state and the kinetics of its release are different. On a weight basis, it is at least as potent as histamine as a smooth muscle contractor. Its presence in tracheal secretions and its release are different. On a weight basis, it is at least as potent as histamine as a smooth muscle muscle contractor. Its presence in tracheal secretions and the kinetics of its release are different. On a weight basis, it is at least as potent as histamine as a smooth muscle muscle contractor. Its presence in tracheal secretions and the kinetics of its release are different.

In summary, our studies show that histamine and a smooth muscle contracting substance are released after acid-induced injury in the dog. These vasoactive mediators may be important in pulmonary edema from increased microvascular permeability. Since antagonists to histamine and SRS-A are available, their true role in increased microvascular permeability and alter pulmonary physiology.

**REFERENCES**


**DISCUSSION**

**Dr. Petty:** Your model is appealing because it has the same degree of compliance abnormality and rapidly developing hypoxemia that is found in humans, but I think that there's one thing missing: the latent period between the injury and the development of this syndrome.

**Dr. Bone:** We were using such large doses of hydrochloric acid that it produced death in the animals. However, if we use smaller doses, we don't have the lethal reactions, and we may be able to see a latent period.

**Dr. Turner-Warwick:** With the amounts of histamine and SRS-A which you see in the secretions, would you expect an effect on the airways?

**Dr. Bone:** Although the predominant abnormality which we see in this syndrome is decrease in compliance, Drazan and others have shown airway resistance changes after mediator infusion. We did have a change in airway resistance, but compliance changes more.

**Dr. Lakshminarayan:** There seems to be a lag between the rises in the levels of histamine and the physiologic changes.

**Dr. Bone:** That's correct. The greatest physiologic changes seemed to better parallel the finding of slow-reacting substance than histamine.

**Dr. Chester:** Hypoxemia alone produces a number of changes in mediator release. Do you have any information whether hypoxemia alone may increase histamine?

**Dr. Bone:** The saline treated animals were also hypoxemic, so hypoxemia alone could not have produced the mediator changes.

**Dr. Brody:** Dr. Bone, in the animal model using acid, you'd expect a lot of damage to type I and II alveolar lining cells. Can you separate the effects of histamine on vascular permeability from the effects of damaged alveolar epithelial cells? Is this a problem in your model?

**Dr. Bone:** I don't think that it's a problem. Studies by Pietra showed that histamine increased pores in the bronchial vasculature before the alveolar-capillary membrane. For that reason, people are thinking about changes in the small airways as well as the alveolar-capillary membrane in this syndrome.

**Lymphocytic Acid-Hydrolases and Response to Mitogens in Cystic Fibrosis**

Jack Lieberman, M.D., F.C.C.P., and W. Kaneshiro, B.S.

Marchi et al were the first to report that lymphocytes were hyporesponsive to phytohemagglutinin

*From the UCLA San Fernando Valley Medical Program, and the Veterans Administration Hospital, Sepulveda, California. Supported by Grant no. HL 21158 from the U.S. Public Health Service, and the Medical Research Service of the Veterans Administration.

Reprint requests: Dr. Lieberman, VA Hospital, Sepulveda, California 91343
(PHA) when cultured in the presence of serum from patients with cystic fibrosis (CF). Normally, β-glucuronidase activity increases in lymphocytes that are stimulated to transform by PHA, but when serum from CF homozygotes or heterozygotes is present in the culture media, the increase in β-glucuronidase activity is significantly reduced.

We have confirmed Marchi's observation, but have found that the differences observed between CF and controls are enhanced when enzyme activity is calculated per number of lymphocytes rather than per mg of lymphocytic protein, as Marchi had done. This was due to the fact that both lymphocytic total-protein and β-glucuronidase activity increase when stimulated by PHA, and both levels decrease when serum from CF subjects was introduced into the culture media. Thus, the ratio of β-glucuronidase to total-protein fluctuates minimally, if at all, with either normal or CF specimens.

In the present study, we 1) compared the levels of a series of cultured lymphocytic acid-hydrolase enzymes in CF homozygotes, heterozygotes and controls; 2) compared the effects of three different mitogens on these levels, and 3) studied the effect of CF vs control serum in the culture medium. Our observations provide an entirely new interpretation for the apparent changes occurring in lymphocytic enzymes during culture of lymphocytes with mitogens, and suggest that a serum factor is present in cystic fibrosis serum that mimics mitogens in this regard.

Lymphocytes were separated by a Ficoll-Hypaque gradient from blood anticoagulated with EDTA. Platelets were removed by differential centrifugation, and monocytes were eliminated by their characteristic adhesiveness to the walls of plastic culture flasks. The lymphocytes were then cultured in MEM media containing 20 percent by volume of autologous serum with or without added mitogen. After 48 hours, the cells were harvested, washed, counted in a hemacytometer and subsequently lysed in distilled water for enzyme and protein analysis.

The acid-hydrolases that we assayed included: β-N-acetyl glucosaminidase, acid-phosphatase, β-glucuronidase, α-mannosidase, α-arabinosidase, α and β-galactosidase, β-fucosidase and β-xilosidase (the latter two activities could not be detected in lymphocytes). All assays were performed by a fluorescent method with 4-methyl umbelliferyl substrates at pH 4.4, and total protein was measured with a fluorescamine (fluorescent Fluram) reagent technique.

Figure 1 shows the mean concentration and 1 SD for three enzyme activities and total-protein in lymphocytes cultured for 48 hours with autologous serum but without mitogen stimulation. Both CF heterozygotes and homozygotes had significantly higher levels than the healthy controls. Activity in CF patients was approximately twice that in controls, and heterozygotes had intermediate levels. All nine enzymes assayed in this fashion had higher levels of activity in both CF heterozygous and homozygous cultured lymphocytes than in the controls, though some of the differences were not statistically significant. Marchi et al. and Antonowicz et al. also found that β-glucuronidase and α-glucosidase respec-
tively were elevated in unstimulated cultured lymphocytes from CF patients, but they did not find the other acid-hydrolases to be elevated when calculated per mg protein.

When both PHA and autologous serum were added to the lymphocyte cultures, we observed striking differences in response between the controls and the CF patients and carriers. In the 12 healthy controls, all mean enzyme activities increased averaging 42.4 ± 24 percent. Among the CF heterozygotes the mean change was a decrease of 31.8 percent, and in the CF homozygotes a decrease of 35.6 percent. The most consistent assays for showing differences between CF and controls were β-N-acetyl glucosaminidase and acid-phosphatase.

We also compared the effects of three different mitogens on lymphocytic total-protein and acid-hydrolases in the controls and subjects with the CF gene. Concanavalin A and PHA are said to both stimulate T cells, whereas pokeweed mitogen stimulates T and B cells. The three mitogens caused similar responses within each group of subjects tested and on all enzymes assayed. Healthy controls showed a mean increase of approximately 50 percent with each mitogen, whereas the CF specimens (heterozygote and homozygote) showed a decrease of approximately 20 percent for each mitogen. Individually, PHA was the most consistent mitogen to stimulate a "normal" response in the controls, whereas all three mitogens consistently showed abnormal responses with lymphocytes and autologous serum from subjects with a CF gene. Thus, PHA appears to be most reliable for distinguishing CF carriers from normal subjects.

It is possible that lymphocytic protein and enzyme content are already maximally stimulated by a CF mitogen-like, serum factor, so that subsequent responsiveness to mitogen is blunted. This is suggested by the higher levels of lymphocytic protein and acid-hydrolases in lymphocytes from CF subjects cultured without mitogen. A CF serum factor, thus, could act to increase cellular protein concentration. This hypothesis was explored by culturing an individual's lymphocytes without mitogen, but in the presence of normal autologous serum vs donor-cystic fibrosis serum; both sera had been heated at 56°C for a half hour to deactivate complement. We found that the levels of total-protein and acid-hydrolases were all higher in lymphocytes cultured in the presence of CF serum than in those cultured with normal autologous serum, indicating that the type of serum in the culture medium does affect the protein content of the cultured lymphocytes.

Another explanation of these findings could be that lymphocytes may normally lose cell protein during culture without mitogens, but that a CF serum factor or a mitogen may prevent this loss resulting in higher protein levels with the cultured cells. This premise is supported by an experiment in which measurements were made on lymphocytes that were freshly isolated from blood, and compared to lymphocytes that had been cultured for 48 hours without a mitogen, but with either normal serum or donor-CF-serum in the media. We found that the enzyme and protein levels in the lymphocytes all decreased during culture with normal serum, but when CF serum was present the drop in concentration was less for either total-protein or acid-phosphatase, and the levels of β-glucuronidase and β-N-acetyl glucosaminidase actually increased (Fig 2). Thus, both CF serum and mitogens reduce the loss of cellular proteins during lymphocyte culture.

This phenomenon was explored further by measuring protein levels in the culture media to see if protein was in fact escaping from the cultured cells during the 48-hour interval. Total protein concentration was calculated per 10¹¹ cells inoculated into the media. In seven controls and in five of seven heterozygotes, the concentration of protein was greater in media from lymphocytes cultured without mitogen than from cells cultured with mitogen. In two of the heterozygotes more protein was lost with mitogen than without, and lymphocytes from two CF homozygotes showed minimal protein loss into the media either with or without added mitogen, suggesting that the presence of CF serum acts like a mitogen in reducing protein loss from the cells. (The proportion of inoculated cells actually harvested after 48 hours was similar for all samples.) These data confirm that mitogens normally reduce the loss of cellular protein into the culture media, and suggest that homozygous CF serum acts in a similar fashion. Serum from CF heterozygotes was variable in this regard, however.

In conclusion, lymphocytic acid-hydrolases and total-protein are present in higher concentration in lymphocytes cultured for 48 hours without mitogen when CF homozygous or heterozygous serum is present in the culture media. This appears to result from a CF serum factor that has mitogen-like activity and prevents loss of cell protein during culture. Responsiveness of the cellular proteins to mitogens is blunted when CF serum is present in the culture media, either because the cell proteins are already maximally stimulated by the CF serum factor, or the factor blocks or modifies the action.

![Figure 2. Variations in total-protein and acid-hydrolases of lymphocytes cultured with normal as compared to CF serum added to culture media.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21018/)
of mitogens on lymphocyte protein content. This phenomenon is useful for detection of CF heterozygosity, and appears to reflect the genetic abnormality causing cystic fibrosis.

REFERENCES

DISCUSSION
Dr. Gleich: Have you pursued the physico-chemical nature of the factor?
Dr. Lieberman: It's heat resistant at 56 °C X 3 hr, which distinguishes it from the ciliary dyskinesia factor which is heat labile. It does not pass through a dialysis membrane. We don't know the molecular weight yet.
Dr. Reynolds: Since many patients with cystic fibrosis are colonized with Pseudomonas, I presume that sera from these patients might contain lipopolysaccharides such as endotoxin. Have your sera been screened for endotoxin?
Dr. Lieberman: No. But don’t forget that the same phenomenon occurs with the heterozygous sera. These are obtained from relatives of patients so I don’t think that infection causes this factor to appear.

Mediators of Immediate Type Hypersensitivity in Sputum from Symptomatic Young Cigarette Smokers*

A. Gordon Leitch, M.B., Ch.B. and A. Barry Kay, M.B., Ch.B.

In a previous study we showed that the sputum from patients with chronic bronchitis contained histamine, SRS-A, IgE and eosinophils, suggesting that some of these patients had a local immediate type hypersensitivity for at least some of the time (Turnbull et al: Mediators of immediate-type hypersensitivity in sputum from patients with chronic bronchitis and asthma. Lancet 2, 528-529, 1977).

We have now studied sputum from 23 smokers under the age of 35 whose only complaints were persistent cough and morning sputum. All had normal values for FEV1.0 and FVC. Findings in this group are compared with those in a group of 11 young lifelong nonsmokers who were producing sputum in association with an upper respiratory tract infection.

*From the Departments of Respiratory Diseases and Pathology, University of Edinburgh, Scotland.

Dr. Schwartz: Did you do skin tests on your chronic bronchitis patients?
Dr. Leitch: Ten percent were skin-test positive, which is the pattern in the general population in our area.
Dr. Lyons: What were the races and occupations of your subjects?
Dr. Leitch: Our subjects were white clerical and industrial workers.
Dr. Campbell: Were there any other differences between the groups with bronchitis who did or did not have mediators in their sputum?
Dr. Leitch: We were not aware of any other differences.
Dr. Gleich: Dr. Leitch, what do you consider to be the trigger for immediate hypersensitivity in chronic bronchi-