populations of macrophages.

*Increased Blood Supply.* The number of capillaries supplying the BCG lesions was increased two to three times over the number in normal skin and remained at this level until the lesion healed.

REFERENCES
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DISCUSSION

*Dr. Henson:* I'd like to ask a couple of questions about the quantitative release of enzymes into the chamber. Could you not interpret the data as showing that as cells die all the enzymes are released, including lysozyme? And can you determine that the enzymes come from macrophages?

*Dr. Dannenberg:* When you study something *in vivo*, it is very complex. The cells are entering, dying (or leaving). In the chamber bed most of the cells are macrophages. Macrophage collagenase (which is secreted and *not* stored) would come from live (not dead) cells. However, lysozyme is both secreted and stored and much of the PMN collagenase is stored and released by regurgitation or cell death. The number of PMN decreased after 4 days, but the number of activated macrophages peaked at 11 and 18 days. At this time, the amount of collagenase in the chamber fluid also peaked. We therefore feel we were measuring macrophage (and not PMN) collagenase.

*Dr. Brooks:* In histologic studies reported, there are usually only a few organisms in the solid caseum. Do you have any information on the make-up of caseum and how it affects multiplication of the tubercle bacilli?

*Dr. Dannenberg:* Caseum is known to be inhibitory to the growth of tubercle bacilli, probably from some of the fatty acids and the anoxic condition that is present in it. However, bacilli grow well in caseous material after it liquefies. During liquefaction, enzymes partly hydrolyze the lipids, proteins and nucleic acids in caseous tissue, so that its osmotic pressure increases. As soon as that occurs, fluids rush in and probably oxygen, especially if a bronchus or bronchiolo is eroded. Since tubercle bacilli multiply readily in liquefied caseous material, the whole disease exacerbates.

*Dr. Davis:* Your very elegant histochemical stains raise the possibility of looking at different functional populations of macrophages, as well as different morphologic ones, and I wonder if time-course studies or any other studies allow you to predict whether these are sequences in the lifespan of the macrophage. In other words, are the same cells going through these functional states? Or do you view the secretory cell as being an end-stage macrophage of one type and the lysozome-rich macrophage as being an end-stage cell of a different type?

*Dr. Dannenberg:* Macrophages probably differentiate for different functions. Macrophage secreting factors which affect lymphocytes or secreting colony stimulating factors which affect bone marrow cells are probably not rich in all the digestive enzymes, but this remains to be shown. There may be markers by which you could identify different macrophage populations and it is possible that local factors make macrophages differentiate in different directions. In addition, some macrophages may *both* secrete and digest.

Comparison of the Alveolitis of Sarcoidosis and Idiopathic Pulmonary Fibrosis*


*Sarcoidosis and idiopathic pulmonary fibrosis (IPF) are associated with a parenchymal accumulation of inflammatory and immune effector cells (EC). Sarcoid, in contrast to IPF, is associated with parenchymal granulomata, suggesting that the EC present in the lung in sarcoid may differ from those in IPF. To evaluate this hypothesis, EC were isolated from the lung by bronchoalveolar lavage (BAL) (five sarcoid, five IPF, six normal subjects) and compared to EC in blood. To demonstrate that the EC present in BAL were representative of those present in the lung parenchyma, EC were also isolated from open lung biopsies (three sarcoid, three IPF). In IPF, the BAL was characterized by an increase in the percentage of neutrophils compared to normals (23 ± 10 vs 1 ± 1). In sarcoid, BAL was characterized by increased percentage of lymphocytes compared to normal subjects (57 ± 7 vs 7 ± 1, P < 0.001). In controls and IPF, the percentage of T-lymphocytes were similar in BAL and blood (P > 0.2 all comparisons). In marked contrast, sarcoid patients had a higher percentage T-lymphocytes in BAL compared to normals (23 ± 10 vs 1 ± 1).

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blood (80 ± 4 vs 57 ± 2, P < 0.001). Strikingly, the numbers of T-lymphocytes recovered by standard (100 ml) lavage of sarcoid patients were increased compared to controls (20.6 ± 4.8 × 10^6 vs 0.7 ± 0.2 × 10^6, P < 0.01), whereas the numbers of T-lymphocytes in blood of sarcoid were decreased compared to blood of normals (754 ± 184/mm³ vs 1874 ± 205/mm³, P < 0.01). In addition, the numbers of "activated" T-lymphocytes (T-lymphocytes forming rosettes with sheep red blood cells) were also increased in BAL of sarcoid patients compared to normals and IPF (P < 0.01). The validity of the BAL observations in sarcoid and IPF was confirmed by analysis of EC subpopulations isolated directly from the lung parenchyma (P > 0.2 all comparisons).

These observations suggest that the alveolitis of IPF is characterized by the presence of large numbers of neutrophils and normal lymphocyte subpopulations, whereas the alveolitis of sarcoid is characterized by the absence of neutrophils and by the presence of large numbers of T cells, many of which are activated; analysis of EC present within the lung, as opposed to blood, more accurately reflects local immune processes.

**DISCUSSION**

Dr. Turner-Warwick: These very beautiful studies are enormously valuable. We have done almost identical studies on fibrosing alveolitis both with regard to elution and lavage and with the rosetting techniques and differential counts. Our results are almost identical in every respect except for one small point that we still have to resolve. We get neutrophils, but the distribution among our cases is certainly very far from "normal." We get a few cases with considerably increased numbers of neutrophils and a lot that actually have no increase. We also agree completely about the increased numbers of eosinophils. Further, we agree on a point which you haven't made in this talk but I know you've published: in treated patients, the number of neutrophils is reduced.

Dr. Hunninghake: I think it's certainly true that certain patients have more polymorphonuclear leukocytes, not only in the lavage fluid, but in the biopsy cell suspensions.

Dr. Henson: I wonder if the selective increase in lymphocytes might in fact reflect an increase in susceptibility of some of the other cells—perhaps the macrophages—to the treatments, the teasing and so forth. Do you have any absolute figures or some measure of the recovery of cells you get from your biopsies?

Dr. Hunninghake: In the normal controls, the number of cells recovered routinely was about 14 million. In the patients with IPF, it was in the low 20's and this increase was almost totally accounted for by the number of polymorphonuclear leukocytes. In the patients with active sarcoidosis, however, the number of cells that were recovered was between 30 and 80 million. The number of T lymphocytes in lavage fluid of controls and patients with IPF is routinely less than 1 million cells per lavage. The number of T lymphocytes that are present in patients with sarcoidosis is around 20 million.

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**Models of Pulmonary Fibrosis**

**Misadventures and Ramifications***

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The search continues for an ideal animal model of human idiopathic interstitial pulmonary fibrosis, which has included a broad spectrum of interstitial lung diseases depending at least partly upon stages in the development of the disease. Ten years ago, Carrington proposed the following as requirements for an animal model: **anatomic changes** characterized by a mixed cellular exudate in the interstitium, protein exudate with or without cells in the air spaces, proliferation of lining epithelium, gradual progression to fibrosis with continuing activity and eventual honeycombing, and diffuse distribution with the possibility of skip zones.

Experimental interstitial lung diseases have been produced by airway administration of dusts, lipids, cadmium sulfate, foreign antigen, antilung antiserum, N-nitroso-N-methylurethane and most recently, bleomycin in Syrian hamsters and in baboons. Most but not all criteria have been met in several of these models. The most difficult criterion to meet has been gradual progression to fibrosis with continuing activity and eventual honeycombing, particularly after the initiating event has been withdrawn.

Our entrance into this arena was aglow with optimism initially because of success in the development of acute inhalational experimental immunologic lung injury, and the notion that interstitial fibrosis followed immunologic injury. What could be simpler than continuing the immunologic insult and observing the gradual development of interstitial fibrosis? The frustrations of these fruitless experiments have been published.

A second approach was based on the premise that an accumulation of macrophages is an essential prelude to pulmonary fibrosis. Therefore, keeping the interstitium packed with macrophages should eventually lead to fibrosis. Inspired by the work of Myrvik, Moore and associates, but instinctively uncomfortable, if human correlations are considered, with intravenous injections of mineral oil and intratracheal injections of solutions, we proceeded to immunize rabbits systemically with complete Freund's adjuvant via the toe pads and to follow this with repeated intravenous injections of killed BCG in saline solution. Marvelous accumulations of interstitial and alveolar macrophages (and other leukocytes) resulted and could be maintained by continuing the injections. Fibrosis was scanty and all healed when injections were stopped.

Having seen paraquat injury to the lungs in rabbits, we next reasoned that if macrophages contribute to

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