Pautard (*10*) has found, in *Spirostomum ambiguum*, a ciliated protozoan, intracellular deposition of apatite "bone" salts with an x-ray diffraction pattern indistinguishable from that of extracellular mesodermal hard tissue matrices.

Evidently, the "matrix" outside of the microorganisms represents the bulk of the calcified deposits; but it is not known to what extent the microorganisms contribute to the conversion of this initially amorphous substance, presumably salivary mucus, into a calcifiable framework. The nature of this matrix is basic to a fuller understanding of apatite nucleation in biological systems (*11*).

F. GONZALES*
R. F. SOGNNAES
Harvard School of Dental Medicine, Boston, Massachusetts

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* U.S. Public Health Service senior research fellow (SF-223) on leave from the University of Texas Dental Branch, Houston, as research associate in anatomy, Harvard School of Dental Medicine (1958–59).

8 August 1959

Application of the Network Model to Gas Diffusion in Moist Porous Media

Abstract. Millington's description of pore structure in porous media is compared with conclusions based on the network theory. Experimental gas-diffusion data for moist sand indicate that this porous material has a network structure in which each pore is connected to about 15 other pores.

Millington (*1*) has recently presented a theory from which he derives a relation between gas diffusion through moist porous media and the moisture content of the material. His theory is based on a consideration of the planar distribution of spherical pores and the interconnection of pores in two adjacent planes. Millington uses Taylor's experimental data (*2*) to establish the validity of his theory.

The purpose of this report is to point out that Taylor's experimental data can be used to support a different theoretical treatment of pore structure in porous media. This treatment, based on the network model of a porous material (*3*), gives more detailed information on pore structure than does Millington's theory.

Although Millington limits himself to a discussion of diffusion through the gas space in dry and moist porous media, the same theory and equations apply to electrical conduction through electrolyte-containing porous media (*4,5*). The following equivalences can be shown to exist because of the equivalence of Ohm's law and Fick's first law of diffusion. (i) Steady-state diffusion of gas through a dry porous medium is governed by the same type of equation as is electrical conduction through a porous medium saturated with electrolyte. (ii) Steady-state diffusion of gas through a moist porous medium is governed by the same type of equation as is electrical conduction through a porous medium which contains a nonconducting fluid (oil) as the wetting phase and an electrolyte as the nonwetting phase. A brief, but perhaps oversimplified, definition of wetting phase is that it is the fluid phase which is spread on the internal surface of the porous material; conversely, the nonwetting phase is the fluid phase which occupies only the central portion of each pore space. In moist sand or soil the nonwetting phase is air and the wetting phase is water. If the porous material is exposed to the vapors of an organosilane, its surface will become hydrophobic. Water, or an electrolyte, will then be the nonwetting phase, while gas or oil is the wetting phase.

I have previously shown (*3*) that by treating a porous medium as a network of interconnected tubes, a relation can be obtained between electrical conductance and fraction of pore space occupied by a nonwetting electrolyte. This relation was shown to be dependent on the extent of interconnection of the tubes (pores) in the network, but independent of the pore size distribution.

To facilitate comparison of Millington's theory, the network theory, and Taylor's experimental data, it was desirable to state the diffusion coefficient and the moisture content relative to the dry porous material. In Fig. 1, D_M/D_d is the effective diffusion coefficient for gas in moist porous sand divided by the effective diffusion coefficient in the same sand when dry. The air-filled-pore volume is given as a fraction of the total pore volume.

Figure 1 shows a comparison of Millington's theoretical results, Taylor's experimental data, and the results from network models in which there are ten, seven, and four pores, respectively, connected to each pore. It is apparent from Fig. 1 that the network model predicts a relation between effective diffusion coefficient and moisture content that is slightly better than the one obtained by Millington. The network model in which each pore is connected to ten others gives results that are in fair agreement with Taylor's experimental data. From the trend of the network-model results, it seems reasonable to expect that the relationship of the effective diffusion coefficient versus the moisture content from a network in which each pore is connected to about 15 other pores will be in almost perfect agreement with the experimental data.

This observation gives support to the conclusion I had reached previously (*3*) by comparing data on experimental wetting-phase electrical conductivity with predictions from the network model. From my earlier work I concluded that the characteristic shape of a plot of experimental electrical conductivity in the wetting phase versus moisture content was a result of the network structure of porous media. Furthermore, the numerical value of these experimental data, when compared to predictions from the network model, suggested that in materials such as sand there are seven to 25 pores connected to each pore. Both Taylor's

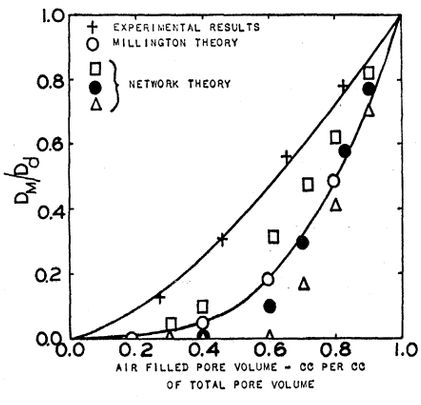

Fig. 1 Gas diffusion in a porous solid-liquid-gas system—a comparison of Taylor's experimental results (*2*), Millington's theory (*1*), and network theory (*3*). Number of pores connected to each pore in the network are: squares, 10; solid circles, 7; and triangles, 4.

experimental data and the previous experimental data on wetting-phase electrical conductivity can be interpreted in terms of the network model, and both suggest about the same degree of pore interconnection (*6*).

IRVING FATT
Department of Mineral Technology, University of California, Berkeley

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Effect of Stress on Skin Transplantation Immunity in Mice

Abstract. Chronic avoidance-learning stress was found to depress the immune reaction responsible for skin homograft rejection to a modest but significant degree. This effect was observed in a genetically uniform as well as a heterogeneous line of mice.

Although diverse types of systemic stress have long been known to modify the immunological responses of mammals (*1*), precise experimental investigations have recently elucidated the effect of such stress on particular immune reactions. Thus, mice subjected to a standardized avoidance-learning type of stress show an increased susceptibility to *Herpes simplex* virus infection (*2*) as well as a decreased susceptibility to passive anaphylaxis (*3*) and a depressed colloid-clearing capacity of the reticuloendothelial system.

The study discussed here was undertaken to determine the effect of controlled stress on skin-homograft rejection. The immunologic basis of the homograft reaction has been well established and shows the characteristics of a typical hypersensitivity of the delayed type (*4*).

The stressing procedure employed has been described in detail by Rasmussen *et al.* (*2*). The apparatus makes use of a shuttle box with wired floor each half of which is alternately electrified with a 20- to 30-volt current painful to the mouse. Alteration of current from one side to the other is preceded by signals from a light and buzzer. Mice soon learn to avoid the shocking current, which occurs at about 5-minute intervals. The animals are subjected to this stress 6 hours per day, 6 days per week. Such stress regularly engenders significant changes in the weights of organs: the weights of the thymus and spleen decrease, whereas those of the liver and adrenal increase. Moreover, a progressive leukopenia occurs as the stress is continued over several weeks. Randomly bred Swiss-Webster BRVS mice for which the stress parameters have been determined (*2*) were employed, along with highly inbred C57B1 and A-line mice. Single, orthotopic skin homografts were made by the technique of Billingham and Medawar (*5*) in two donor-recipient combinations—A→C57B1 and C57B1→Swiss. Thus, both inbred and genetically diverse recipients were tested. The Swiss recipients were all virgin females, whereas both sexes were represented in the C57B1's.

Mice about 5 weeks old were exposed to the standardized stress experience for 2 weeks before grafting. On the day after grafting, the mice were again subjected to stress until homograft rejection was complete. The control mice received grafts in the same manner but were not exposed to experimental stress. Protective bandages were removed for the initial inspection on the 8th day, and graft survival was scored daily thereafter. Intermediate stages of breakdown were estimated by gross inspection and confirmed in several instances by histologic examination of biopsy sections stained with hematoxylin and eosin. Zero survival end points were assessed on the basis of no surviving graft epithelium. Median survival times as well as tests for parallelism and reaction-time ratios, with their 95-percent confidence limits, were computed by the method of Litchfield (*6*).

The cumulative percentage of homografts destroyed in each experiment is plotted against days after grafting in Fig. 1. While it is apparent that the time-mortality distributions of grafts in the comparable groups of control mice and stressed mice are distinctive, the prolongation of skin homograft survival in the stressed mice was not extensive. Also, the figure reveals that the uniform C57B1 recipients showed a narrow range of graft-survival times, whereas the Swiss mice showed the broad distribution characteristic of genetically diverse recipients. The results are summarized in Table 1. When the data were subjected to the parallelism and reaction-time ratio tests of significance, the difference between stressed and control mice in both combinations is significant at the 95-percent level of probability.

Although the stress applied is known to induce profound physiological changes in mice, it appears probable that the observed inhibition of transplantation immunity in stressed mice is affected primarily by hypersecretion of adrenal corticosteroids. Indeed, the decrease in weight of the spleen and the progressive leukopenia in stressed mice can be duplicated by administration of cortisone. Since homograft immunity, like other delayed types of hypersensitivity, is clearly mediated by lymphoid cells, a substantial depression of such cells by corticosteroids would be expected to allow a prolongation of skin homograft survival. Nevertheless, the endocrine situation is complex. While the normal mouse secretes principally corticosterone and little if any cortisone and hydrocortisone (*7*), Medawar and Sparrow (*8*) have shown that injection of the latter compounds but not of corticosterone will prolong homograft survival time in mice. An analysis of the endogenous corticosteroid levels in stressed mice now under way in this laboratory should indicate whether such mice preferentially secrete the hormones known to prolong homograft survival. The possibility remains, of course, that the stress-induced inhibition of the homograft reaction is mediated mainly through channels other than the adrenal corticoids. In this connection, studies with rats (*9*) have revealed that adrenal corticoid output may actually decrease below normal levels during prolonged stress.

It should be noted that our control mice were unfortunately exposed to the periodic noise of building reconstruc-

Table 1. Summary of results.

Group	Donor-recipient combination	No. of mice	Median survival times (days) with 95% confidence limits
Control	A→C57B1	7	8.2 (7.2–9.3)
Stressed	A→C57B1	11	9.6 (9.3–9.9)
Control	C57B1→Swiss	23	8.5 (8.3–8.7)
Stressed	C57B1→Swiss	21	9.2 (8.6–9.9)

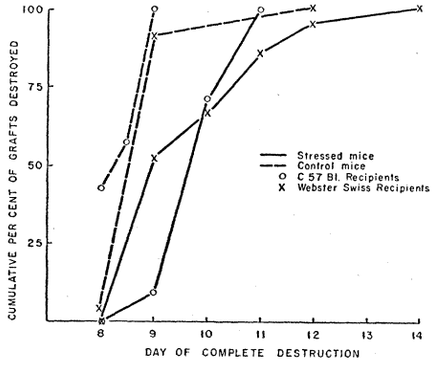

Fig. 1. Cumulative time-mortality curves for skin homografts in stressed and non-stressed mice.