SPECIAL ARTICLES

PATHOGENIC PLEUROPNEUMONIA-LIKE MICROORGANISMS FROM ACUTE RHEUMATIC EXUDATES AND TISSUES

THE inoculation of chorioallantoic membranes of chicken eggs with exudates obtained from a number of patients with acute rheumatic fever has resulted in the development of characteristic lesions which have not appeared when similar membranes were inoculated with non-rheumatic exudates. These lesions have had the same general appearance in a number of different series where the inocula have been derived from arthritic exudates, pleural exudates or an excised erythema nodosum nodule. The characteristic lesions have usually not become definite until the third to fifth serial passage; but once having appeared they have been easily induced in as many as twenty-four passages at two- to four-day intervals. The use of a 10 per cent. human serum-saline mixture as a medium in which the ground membranes are suspended has proven an important part of the technique. Macroscopically the characteristic lesions consist of granules or pearl-like bodies, often best seen when viewed from the entodermal surface. Microscopically these areas eventually appear like globular structures surrounded with flattened epithelium, but containing in their center condensed eosinophilic material, apparently derived from the mesoderm, and varying amounts of mesodermal inflammatory reaction. The bodies appear to originate in the ectoderm; as they enlarge, they press downward into the mesoderm, and often push the entoderm ahead of themselves, so that they are most easily seen from the under surface of the membrane.

Occasionally membranes have become contaminated with ordinary bacteria. Under these circumstances they have presented quite a different appearance from that described above. When bacterial contamination has occurred, the ground membrane suspension has been subjected to Berkefeld N or V filtration, which has allowed the infectious agent to pass through.

Etherized mice inoculated intranasally with rheumatic arthritic or pleural exudates and with suspensions of chorioallantoic membranes, showing the characteristic lesions described above, have sickened and developed pneumonia in which the inciting agent has been transmissible from series to series by using as inocula, ground pneumonic lungs suspended in broth. Filtrates of these suspensions, passed through Berkefeld V candles, have induced the same type of pneumonia; and from these lungs ordinary bacteria have been absent, both from films and cultures. The equivocal nature of the evidence obtained from mouse pneumonia was recognized, because of the findings of Dochez[1] and Gordon,[2] respectively, and their collaborators, and of similar results in the Laboratories of the International Health Division.[3] In all those experiments, however, the induced pneumonia did not appear until after two or more mouse lung passages, while in our experience the characteristic pneumonia appeared after the primary intranasal inoculation. Suspensions of these pneumonic lungs which had been ground have induced the same characteristic lesions on the chorioallantoic membranes as those previously mentioned.

As Dr. Albert Sabin has consulted with us frequently during his work in recovering pleuropneumonia-like microorganisms from normal mice[4] and inducing with these cultures chronic arthritis in mice,[5] we applied the cultural techniques he was using and also some of the methods employed by Kleineberger.[6] After a few sub-cultures in beef-serum-dextrose-broth or on solid media rich in serum, it was possible to grow pleuropneumonia-like microorganisms from the pneumonic mouse lungs and also from the abnormal chorioallantoic membranes. This was highly suggestive evidence that this agent had arisen from a common source—*viz.*, the exudates or lesions of patients with rheumatic fever—although the possibility was recognized that these pleuropneumonia-like microorganisms might have come from carriers among the sick mice, even though that possibility seemed improbable.

It, therefore, became important to cultivate, if possible, these microorganisms directly from rheumatic exudates; and by using the same culture media and applying the same repeated passage techniques that were used in culturing the chorioallantoic membranes and pneumonic mouse lungs, similar appearing cultures and microorganisms have been obtained from the arthritic exudate of a child early in the course of her second attack of rheumatic fever, and also from an erythema nodosum nodule excised from a patient with typical rheumatic polyarthritis. This furnished evidence that the pleuropneumonia-like microorganisms obtained from both the chorioallantoic membranes and from the mouse pneumonic lungs were probably derived originally from the rheumatic exudates.

The pathogenicity of the cultures from the three different sources is being investigated. A culture, free from ordinary bacteria, was obtained from the nineteenth chorioallantoic membrane passage where the original inoculum was a rheumatic pleural exudate. One tenth of a cubic centimeter of this culture, after four days' incubation, was injected into the vitreous

[1] A. R. Dochez, K. C. Mills and B. Mulliken, *Proc. Soc. Exp. Biol. and Med.*, 36: 683, 1937.

[2] F. B. Gordon, G. Freeman and J. M. Clampit, *Proc. Soc. Exp. Biol. and Med.*, 39: 450–453, 1938.

[3] F. L. Horsfall, personal communication.

[4] A. B. Sabin, SCIENCE, 88: 575–576, 1938.

[5] A. B. Sabin, SCIENCE, 89: 228–229, 1939.

[6] E. Kleineberger, *Jour. Hygiene*, 38: 458–475, 1938.

of the eyes of three rabbits. Two of them developed marked iritis and also a systemic reaction indicated by diarrhea of several days' duration; the third had a panophthalmitis with some form of cocci as contaminating agents. Another set of three rabbits was inoculated with the seventh and eighth subcultures from an arthritic exudate (this culture had never undergone animal passage); one rabbit developed marked iritis and diarrhea, the second mild iritis; while the eye of the third has so far remained free from macroscopic lesions. Two out of three other rabbits inoculated with a 24-hour-old culture of the same strain developed definite iritis after 9 or 10 days; while the iritis in the first two groups appeared between the second and fifth days after inoculation and persisted from the seventh to tenth.

Four series of Swiss mice, of a stock known to be free from mouse typhoid infection, were inoculated intranasally with the same cultures that had been injected into rabbits' eyes. During the following six days, animals in each set were obviously sick and had dyspnea. When autopsied on the sixth or seventh days, 3 out of 5 mice inoculated with the 4-day-old culture showed only macroscopically equivocal pulmonary lesions. On the other hand, marked pneumonia was present in 2 out of 5 mice in each of the three sets inoculated with either 1- or 2-day-old cultures. Another macroscopically normal appearing mouse lung was found upon microscopic examination to have foci of interstitial pneumonia, perivascular hyperplasia and bronchi distended with polymorphonuclear cells, a picture that has been peculiar to all the pneumonic lungs examined.

It thus appears that pleuropneumonia-like microorganisms cultured directly from rheumatic exudates can induce the same type of pneumonia in mice that is obtained by inoculating these animals with rheumatic exudates, or with suspensions of chorioallantoic membranes in which characteristic lesions have been induced by these exudates. These pulmonic lesions have appeared in the first mice inoculated with these various materials, as well as in those where serial transfers have been carried out; hence we feel that the organotropism of these microorganisms is different from those of the pleuropneumonia-like microorganisms recovered from mice by Dr. Sabin, for he has been unable to induce pneumonia in mice with his cultures.[7] A few mice inoculated either intracerebrally, intravenously or intraperitoneally with cultures have, so far, shown no characteristic lesions, even though some of them have been obviously sick. The series has been too small, however, and the time since inoculation too short for final judgment concerning the pathogenicity of these cultures.

SUMMARY

In suitable cell-free media it has been possible to cultivate pleuropneumonia-like microorganisms from the following materials, first, from chorioallantoic membranes in which lesions were apparently induced by exudates from patients with rheumatic fever; second, from pneumonic lungs of mice inoculated with similar exudates or with suspensions of the above-mentioned abnormal membranes; and third, directly from the arthritic exudate of a patient with rheumatic fever, and also from an erythema nodosum nodule excised from a patient with this same disease. With three different subcultures from joint fluid, iritis has been induced in rabbits; and following intranasal inoculation with the same cultures there has developed in mice a pneumonia similar to that found in mice inoculated with rheumatic exudates and with suspensions of chorioallantoic membranes infected with rheumatic exudates. Therefore it seems probable that in all instances the pathogenic agent was derived from similar sources, *viz.*, patients with rheumatic fever. Further work will be required to demonstrate the etiologic significance of these pathogenic agents in rheumatic fever.

HOMER F. SWIFT
THOMAS MCPHERSON BROWN
HOSPITAL OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK, N. Y.

THE PROCESS OF CONTINUOUS DEAMINATION AND REAMINATION OF AMINO ACIDS IN THE PROTEINS OF NORMAL ANIMALS

WE have shown[1] that the feeding of *dl*-tyrosine with an increased concentration of the nitrogen isotope N^{15} to normal rats kept in nitrogen equilibrium leads not only to the incorporation of isotopic tyrosine into the tissue proteins, but also to the formation of other isotopic amino acids. This transfer of the nitrogen from one protein constituent to others could only have been due to chemical reactions, one of which must have involved the opening of peptide linkages. It was suspected that the mechanism responsible was that of deamination of tyrosine (to the corresponding α-keto acid?) coupled with the amination of another substance (α-keto acid?) to form the new amino acid. A process of this type was first proposed by Braunstein and Kritzman[2] and demonstrated in minced muscle. In our experiments the transfer of N^{15} from tyrosine into the α-amino group, but not into the ring,[3] of histidine offered strong evidence for the hypothesis that the shift of nitrogen from one amino acid to another is a normal event, which occurs at a rapid rate.

However, the possibility was not excluded that the

[7] A. B. Sabin, personal communication.

[1] R. Schoenheimer, S. Ratner and D. Rittenberg, *Jour. Biol. Chem.*, 127: 333, 1939.

[2] A. F. Braunstein and M. G. Kritzman, *Enzymologia*, 2: 129, 1937.

[3] R. Schoenheimer, D. Rittenberg and A. S. Keston, *Jour. Biol. Chem.*, 127: 385, 1939.