Rheumatism Germ Found, Say Rockefeller Doctors

May Solve Problem of One of Most Serious Diseases of Mankind

Two doctors of the Hospital of the Rockefeller Institute for Medical Research today announced the discovery of a germ which may solve the problem of rheumatic fever and rheumatic heart disease.

One of the most serious diseases of mankind because it is so often followed by progressive heart impairment, particularly in children, rheumatic fever has baffled medical scientists seeking both its true cause and an effective cure.

Dr. Homer F. Swift, considered the world's leading research scientist on rheumatic fever, and Dr. Thomas McPherson Brown, announce their discovery in the current issue of Science.

Arthritis Germ Discovered, Too

There is a possibility that the rheumatic fever germ they have found is the same one announced two weeks ago by Dr. A. B. Sabin, also of the Rockefeller Institute, as a probable cause of arthritis.

The two diseases, rheumatic fever and arthritis, together constitute one of the major causes of chronic illness and disability.

For years medical scientists have pursued the belief that the two diseases have been caused by some type or types of the common streptococcus, the most frequent cause of infections.

Serums, vaccines and antitoxins have been produced with only slight success to fight both diseases.

The new germ or germs, both called "pleuropneumonia-like microorganisms" by the three doctors, may be the missing element in the cause of rheumatic fever and arthritis, with the streptococcus also playing a causative role.

The germ isolated by Drs. Swift and Brown was found after using highly ingenious techniques to cultivate a bacteriological response from secretions developed by a child with rheumatic fever.

The germ cultivation was done by implantation of the secretion in one of the special membranes of chicken eggs. It was only after taking the fluid from one planting and placing it into a new one for five successive times that a recognizable response was observed. This compares with ordinary bacteriological germ cultivation in which suspected material is planted in a medium and germ growth begins immediately.

Drs. Swift and Brown were able to produce a lung inflammation in mice with their germ, a response which Dr. Sabin was unable to do with his almost identical arthritis germ.

The two doctors believe that the difference may be only one of selectivity of site by a different type of the same germ. Dr. Sabin's germ produced lesions in the joints of rabbits but not pneumonia.

All three doctors are now concentrating on better methods to cultivate the germ and to further establish its connection with human arthritis and rheumatic fever.

Once this is done, research will be started to find ways to fight the germ, just as ways have been found to fight other diseases once the true cause was established.

THE

Rheumatic Fever Cause Clues Are Found By Science

Vicious Disease Of School Age Children In Northeast Frequently Kills

NEW YORK, March 23.—(AP)—Clues to the unknown cause of rheumatic fever, a disease that attacks 1 of every 100 Americans, were announced today in science.

Rheumatic fever is a particularly vicious disease of school age children in the northeastern United States. It frequently results in damaged hearts, and often in death a few years after the rheumatic attack.

An unidentified streptococcus has been suspected. But at the Rockefeller Institute a new microorganism, said to resemble the pneumonia germ, has been found in rheumatic fever sufferers.

The evidence on this new cause of disease is unusual, all being "backhanded." Two Rockefeller scientists, Homer F. Swift and Thomas McPherson Brown, announce that this new organism causes pneumonia in mice and even other ills in animals deliberately infected with it.

Two weeks ago A. B. Sabin of the institute reported discovery that this same new organism caused chronic arthritis in mice.

In all cases the new germ, or virus, was obtained from human beings with rheumatic fever. This cumulative evidence puts the new germ on the spot as the possible cause of the rheumatism.

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PATHOGENIC PLEUROPNEUMONIALIKE MICROORGANISMS FROM ACUTE RHEUMATIC EXUDATES AND TISSUES

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HOMER F. SWIFT

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The inoculation of chorioallantoic membranes of chicken egg 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, 80 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 result 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 pleuro-pneumonia-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.

1. A. H. 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.

After a few sub-cultures in beef-serum-dextrose-broth or on solid media rich in serum, it was possible to grow pleuro-pneumonia-like microorganisms from the pneumonia mouse lungs and also from the abnormal chorioallantoic membranes.

This was highly suggestive evidence that this agent had arisen from a common source–*vis.*, the exudates or lesions of patients with rheumatic fever although the possibility was recognized that these pleuro-pneumonia-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 polyarthritia.

This furnished evidence that the pleuro pneumonia 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-day incubation, was injected into the vitreous 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 day 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 lot were obviously sick and had dyspnoea.

When autopsied on the sixth or seventh day, 3 out of 5 mice inoculated with the 4-day-old culture showed only macroscopically equivocal pulmonary lesions.

0n 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 pleuro pneumonia-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 organ tropism of these microorganisms is different from those of the pleuro-pneumonia-like microorganisms recovered from mice by Dr. Sabin, for he has been unable to induce pneumonia in mice with his cultures.[7]

7. A. B. Sabin, Personal communication.

A few mice inoculated either intracerebrally, intravenously or intraperitoneally with cultures have, so far, [have] 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 pleuro-pneumonia-like microorganisms from the following materia1s:

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 abovementioned 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 the 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 the 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.

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