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CULTURAL STUDIES ON RHEUMATOID ARTHRITIS AND RHEUMATIC FEVER

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THE literature on cultural studies in rheumatoid arthritis and rheumatic fever is so familiar to those interested in these diseases that no detailed description will be given; however, a few studies will be mentioned.

Cecil, Nicholls, and Stainsby¹ recovered streptococci from the blood and joints of the majority of their patients with rheumatoid arthritis, and Gray and Gowen² confirmed their observations. Dawson, Olmstead and Boots³ were unable to do so. McEwen, Bunim and Alexander⁴ recovered streptococci from the blood of 21 per cent. of their patients with atrophic arthritis and from 6 per cent. of the controls. Margolis and Dorsey⁵ recovered streptococci from the blood and tissues from some of their cases and attached significance to the presence of these bacteria. Collins⁶ obtained negative cultures from the synovial fluid of a large number of cases of atrophic arthritis. Blair and Hallman⁷ were unable to isolate any significant organism from the synovial fluid or tissue of similar cases. Sabin⁸, Findlay, Mackenzie and

MacCollum⁹, and Preston¹⁰ have all recorded negative results in their search for pleuropneumonia-like organisms in this disease.

The bacteriologic studies of rheumatic fever are just about as confusing and unsatisfactory. Cecil, Nicholls and Stainsby¹¹ and Clawson¹² have recovered streptococci in a high percentage of cases. Callow¹³ has recovered streptococci of different kinds from a large number of cases; however, similar results were obtained on controls. McEwen, Alexander and Bunim⁴ found streptococci in 17 per cent. of their cases, but the incidence was no more frequent than in controls. Green¹⁴ also reported on the recovery of hæmolytic streptococci from the heart valves of patients dead with rheumatic fever.

It is evident that most workers have placed more emphasis on one of these diseases and have concentrated their efforts on either the blood or synovial fluid.

In 1936 we started to culture all available clinical and post-mortem material from suitable cases of rheumatoid arthritis and rheumatic fever. This study was continued over a period of four years. The studies were made on blood, synovial fluid, tissue and subcutaneous nodules from patients with rheumatoid arthritis, and on pleural or pericardial fluid, pericardial tissue or heart valves from cases of rheumatic fever.

In addition to cultural studies, most of the material was inoculated into mice by various routes and on to the chorio-allantoic membrane of chick embryos. The chick membrane and cultural methods for the detection of pleuropneumonia-like organisms are entirely adequate for the growth of most micro-organisms so that observations made by these special techniques considerably enhance the value of these studies. Tissues and fluids from several cases were also concentrated in an air-driven high-speed centrifuge for one hour at 29,000 r.p.m., and cultures of the sediment made.

RHEUMATOID ARTHRITIS

The patients selected for this study included only those in the active stage of the disease; that is, with pain and swelling of the joints and usually an increase in the sedimentation rate. No case was included if there was any doubt concerning the diagnosis.

The blood cultures were taken whenever possible in a dust-free, air-filtered room. Masks were worn by the operators during all procedures, and the transfers of cultures were made with rubber

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bulbs on the pipettes. The aerobic clot method was used for most cultures. The principal medium used was dextrose phosphate broth. Repeated subcultures were made at intervals of five days for one month.

Forty-nine of 61 (80 per cent.) blood cultures made from 58 patients yielded no growth (Table I). The following organisms were obtained from 12 of the cultures: 5 yielded a large Gram-positive diplococcus (unclassified), 4 a *Staphylococcus albus*, 1 an alpha prime streptococcus, 1 a *Staphylococcus aureus*, and 1 a *B. subtilis*.

TABLE I.—BLOOD CULTURES ON PATIENTS WITH RHEUMATOID ARTHRITIS

No. of Patients.	No. of Cultures.	Sterile.	
		No.	Per cent.
58	61	49	80
Organisms Isolated			
Gram-positive diplococcus (unclassified)		..	5
<i>Staphylococcus albus</i> ..		..	4
<i>Staphylococcus aureus</i> ..		..	1
<i>Alpha prime streptococcus</i> ..		..	1
<i>Bacillus subtilis</i> ..		..	1
Total	..	..	12

Additional cultures were made of synovial fluid and tissue, as well as subcutaneous nodules on similar media enriched with horse blood (Table II). Twenty-four cultures of synovial fluid from 20 patients were sterile. Seven of 9 cultures of synovial tissue were sterile; a *Micrococcus tetragenus* was grown in 1 instance and a *Staphylococcus albus* in 1. Five of 9 subcutaneous nodules were sterile, 3 yielded a *Staphylococcus albus* and 1 a Gram-positive diplococcus.

TABLE II.—CULTURES OF JOINTS AND SUBCUTANEOUS NODULES FROM PATIENTS WITH RHEUMATOID ARTHRITIS

Material.	No. of Specimens.	Results.	
		No Growth.	Growth.
Synovial fluid	24	24	0
Synovial tissue	9	7	2
Subcutaneous nodules ..	9	5	4

Organisms Isolated			
TISSUE:	<i>Staphylococcus albus</i> ..	..	1
	<i>Micrococcus tetragenus</i> ..	..	1
NODULES:	<i>Staphylococcus albus</i> ..	..	3
	<i>Gram-positive diplococcus</i> ..	..	1

THE RHEUMATIC DISEASES

Special cultural studies were made according to the method of Klieneberger for the presence of a pleuropneumonia-like organism in the synovial fluid, synovial tissue or subcutaneous nodules from fifteen patients with rheumatoid arthritis (Table III). In no instance was such an organism recovered.

TABLE III.—CULTURAL STUDIES FOR PLEUROPNEUMONIA-LIKE ORGANISMS

Material Cultured.		No. of Specimens.	Pleuropneumonia-like Organisms.
RHEUMATOID ARTHRITIS:			
Synovial fluid and tissue	..	14	0
Subcutaneous nodules	..	5	0
RHEUMATIC FEVER:			
Synovial fluid		6	0
Pleural fluid		5	0
Pharyngeal washings		4	0
Heart		6	0
Lung		2	0

The synovial fluid, tissue or subcutaneous nodules from sixteen patients were inoculated on to the chorio-allantoic membrane of the chick embryo (Table IV); 141 eggs were inoculated and thirty subpassages made. No significant transmissible lesion was produced. The following organisms were isolated, one from each of three different cases: a *gamma streptococcus*, *Staphylococcus aureus* and *Staphylococcus albus*.

TABLE IV.—STUDIES ON CHORIO-ALLANTOIC MEMBRANE

	<i>No. of Speci- mens.</i>	<i>No. of Eggs.</i>	<i>No. of Pass- ages.</i>	<i>Results.</i>	
				<i>No Growth.</i>	<i>Growth.</i>
RHEUMATOID ARTHRITIS:					
Synovial fluid and tissue ..	11	98	23	11	0
Subcutaneous nodules ..	5	43	7	2	3
RHEUMATIC FEVER:					
Synovial fluid	4	93	21	3	1
Pleural fluid	1	18	5	0	1
Heart	3	28	6	3	0

Organisms Isolated

RHEUMATOID ARTHRITIS:					
<i>Gamma streptococcus</i>	..	..	..	..	1
<i>Staphylococcus aureus</i>	..	..	..	..	1
<i>Staphylococcus albus</i>	..	..	..	..	1
RHEUMATIC FEVER:					
<i>Alpha prime streptococcus</i>	..	..	..	..	1
<i>Staphylococcus albus</i>	..	..	..	..	1

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Similar material from seventeen cases of rheumatoid arthritis (Table V) was also instilled into the nares of 12- to 21-day-old white mice (Strain CF1) under light ether anaesthesia. Occasionally small areas of pneumonia developed after the first instillation. Thirteen of 127 mice injected in this manner developed small, patchy areas of pneumonia following the first instillation. Several passages (usually six) of normal and pneumonic lungs were made at intervals of from four to six days. In a few instances, a transmissible pneumonia developed; however, this was so irregular and inconsistent we feel that it was probably caused by a mouse virus or pleuropneumonia-like organism. Material from seven cases of rheumatoid arthritis was injected intravenously, intracerebrally, intraperitoneally, or subcutaneously into mice. No significant lesions were produced, nor was any transmissible agent recovered. Irradiated mice were used in many instances; however, no difference was found between these and the non-irradiated mice.

TABLE V.—RESULTS OF INTRAPULMONARY INOCULATIONS OF MICE.

	No. of Cases.	No. of Mice.	No. of Passages.	Pneumonia after First Instillation.
Rheumatoid arthritis ..	17	279	37	127/13
Rheumatic fever ..	16	486	85	145/14
Other diseases ..	32	183	19	116/16

RHEUMATIC FEVER

Tissues or exudates from 11 clinical cases and 10 autopsies with rheumatic fever were studied.

The following were cultured for pleuropneumonia-like organisms: synovial fluid (4), pleural exudate (3), pharyngeal washings (4), and heart valves with verrucous vegetations, pericardium or myocardium from five hearts obtained at autopsy (Table IV). Similar material from 11 of the cases which had been preserved by freezing was also cultured. In no instance were pleuropneumonia-like organisms recovered.

The pleural fluid from one and the synovial fluid from four clinical cases, as well as tissue from three hearts obtained at autopsy (Table IV), were inoculated on to the chorio-allantoic membrane and passed at intervals. No significant transmissible lesion was produced. An alpha prime streptococcus was obtained from one case and *Staphylococcus albus* from another.

Material from nine of the clinical cases and seven of the autopsies was instilled into the lungs of 145 mice and passages made as previously described (Table V). In all, 486 mice were used. Sixty-seven mice were inoculated by other routes with material from three of the clinical cases and six of the autopsies. The results of these studies were similar to those described above for rheumatoid arthritis.

SUMMARY

Extensive cultural studies on special media, including the chorio-allantoic membrane, as well as the inoculation of mice, have been made on 85 cases of rheumatoid arthritis and 21 cases of rheumatic fever. No organism of significance has been consistently isolated.

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