ATTEMPTS TO REPRODUCE RHEUMATIC FEVER IN ANIMALS

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PLATES 1 TO 3

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INTRODUCTION

With the assumption that rheumatic fever* is due to some infectious agent, the isolation and identification of the agent has been attempted by bacteriological and immunological methods as well as by animal experimentation. Of the various organisms which have been described in connection with this disease, the streptococcus has held foremost attention and is at present the only one which deserves serious discussion with respect to etiology. An analysis of the present status of our knowledge on the cause of rheumatic disease, with few and practically negligible exceptions, amounts to an evaluation of the possible rôle played by the streptococcus.

The association of the streptococcus with rheumatic disease was first brought into prominence by Poynton and Paine.[1] Whilst many articles have been published on the subject, up to recent years those investigators who had conducted the greater number of animal experiments with streptococci paid little attention to the biochemical characteristics of the organisms with which they were dealing. It is particularly for this reason that it is difficult to analyze and evaluate much of the past literature on this subject.

The first obstacle which presents itself in the acceptance of the streptococcus as the etiologic agent of this disease is that, whereas many investigators claim an extraordinarily high percentage of positive blood cultures (Herry,[2] Freund and

*We feel that the terms "Acute rheumatic fever, Acute articular rheumatism, Rheumatic pancarditis," etc., are each in themselves too limited to cover descriptively all the possible manifestations of this disease. It would seem that the term "Rheumatic Disease" would be preferable inasmuch as this condition undoubtedly exists during afebrile periods.

Berger,[3] Clawson,[4] Suranyi and Forro[36]), many equally careful investigators have obtained a strikingly small percentage of positive blood cultures (McCrae,[5] Philipp,[6] Cole,[7] Harrison,[8] Swift and Kinsella[9]). These discrepancies, however, may be explained possibly on the basis of different technic employed.

The next objection lies in the variety of streptococci which have been found. Thus, while many investigators have obtained from various sources (though principally blood cultures) the *Streptococcus viridans* (Swift and Kinsella,[9] Freund and Berger,[3] Clawson[4]), some have obtained a non-methemoglobin producing organism (Small,[10] Birkhaug[11]) and some have obtained an occasional hemolytic streptococcus (Clawson,[4] Rosenow[12]). The difficulty is further enhanced by the fact that in the hands of most observers the organisms do not appear to fall into one serological group. The outstanding exceptions to this are: Clawson,[4] who found 7 of his 10 *Streptococcus viridans* strains to fall into one homogeneous serological group, Small,[10] who found that 31 strains of "*Streptococcus cardioarthritidis*" obtained from blood cultures, tonsils, feces, etc., fall into one serological group, and Kreidler,[13] who found that 107 strains of "*Streptococcus cardioarthritidis*" obtained from similar sources also fall into one serological group. It is noteworthy that none of these investigators mention absorption tests to prove that they were not dealing with group agglutination.

The only explanations which would still be consistent with such diametrically opposed findings as regards the streptococcus would be to assume either with Rosenow[12] that the streptococcus need not be of a specific type but with specific affinities, or that in this disease the human tissues respond by a specific reaction to non-type-specific agents, particularly under certain hypersensitive states. The latter hypothesis has been voiced by a number of workers and has recently been stressed by Swift[9] and his coworkers.

Another more outstanding discrepancy lies in the fact that, whereas many have claimed the reproduction by means of streptococci, etc., of one or more of the following lesions: endocarditis, arthritis, chorea, and, more significantly, Aschoff-like bodies (Wassermann, Westphal and Malkoff,[14] Meyer,[15] Poynton and Paine,[1] Shaw,[16] Beattie,[17] Rosenow,[12] Coombs, Miller and Kettle,[18] Jackson,[19] Harzell and Henrici,[20] Henrici,[21] Small,[10] Belk and Jodzis,[22] etc.), others have been able to reproduce similar lesions with a variety of organisms not from rheumatic sources (Cole,[7] Horder,[23] Davis,[24] Thalhimer and Rothschild,[25] Cecil,[26] Topley and Weir[27]).

In connection with the alleged production of Aschoff bodies, it must be pointed out that not only are the published photomicrographs of these lesions in animals entirely unconvincing, as has been pointed

out by some of the last named observers, but, also, that Miller[28] called particular attention to the spontaneous occurrence of inflammatory foci in the myocardium of the rabbit and of the guinea pig and suggested that these lesions may have been mistaken for Aschoff bodies in the past.

The interesting work by Birkhaug[11] which has recently been reported may be mentioned. Birkhaug has obtained a toxin from a non-methemoglobin forming streptococcus which he claims produces a positive skin reaction in a higher percentage of rheumatic patients than in non-rheumatic subjects. Injected into his own wrist joint (after previously sensitizing himself with subcutaneous injections of the toxin), it produced a wandering arthritis resembling rheumatic polyarthritis. No definite conclusions can, however, as yet be drawn from this work. Birkhaug's work on skin reactions has been confirmed by Kaiser,[29] Swift,[9] and his coworkers, and by Edith Irvine-Jones.[30]

Swift and his coworkers, however, showed that rheumatic patients are equally sensitive to filtrates from *Streptococcus viridans*, as well as to vaccines and nucleoproteins. In fact, they hold that rheumatic patients show an intensified reactivity (hyperergy) to these products and suggest the possibility that rheumatic disease may be the expression of some such "hyperergy" produced by a variety of streptococci.

Edith Irvine-Jones[30] found that filtrates made from streptococci of the alpha, beta and gamma variety,[37] whether obtained from throats of normal subjects or from those suffering of rheumatic disease, produced in the latter a higher percentage of positive skin reactions than in normal controls.

It is not our intention to review the literature on rheumatic disease in detail. It is obvious, however, from this brief survey that the subject is still in a chaotic state. For this reason, we thought it advisable to repeat some of the work reported in the past, particularly with the object of reproducing the disease in animals. Moreover, there was to be used a large number of animals of different species and a variety of materials obtained from rheumatic patients (see "experimental" below), including "pedigreed streptococci," *i.e.*, organisms obtained from patients in whom autopsy, biopsy (subcutaneous nodules), or clinical follow-up established the fact that they had suffered of rheumatic disease at the time that the cultures were made. In addition, there was to be employed a variety of procedures calculated to sensitize the animals or render them susceptible to the disease.

EXPERIMENTAL

The material and technique employed in these studies were as follows:

Inocula.—I. Twenty strains of streptococci whose source and cultural characteristics are shown in Table I. In order to indicate that these organisms were obtained from patients suffering of rheumatic fever, they will be referred to briefly as "Rheumatic streptococci." Many of the organisms were passed through animals in order to raise their virulence (see appendix). The procedure adopted was to place the organisms in pure culture with broth in collodion sacs. The sacs were transplanted into the peritoneal cavities in animals of the same species into which the organisms were ultimately to be injected. In the case of rabbits, the strains were passed through the knee joints of successive animals.

II. Apart from the streptococci, there was used other material from rheumatic cases, such as, whole blood, plasma and buffy layer, serum, pericardial and hydrocele fluid, and filtrates from tonsils, subcutaneous nodules, lymph nodes and nasopharyngeal washings. These were obtained from sources indicated in Table I. The lymph nodes, subcutaneous nodules, and tonsils were each ground with sterile sand, extracted with saline and filtered through Berkefeld candles. The filtrates were injected in the manner indicated in the protocols.

Animals.—Seven species of animals were employed, *viz.*, rabbits, guinea pigs, dogs, cats, swine, sheep and calves. Most of the rabbits employed and all of the dogs, cats, and guinea pigs were full grown animals. The sheep were approximately 8 months old. The swine and calves were used shortly after being weaned.

In the several groups of animals employed a variety of procedures were instituted in different combinations.

Portal of Inoculation.—Injections were made intravenously, intratracheally, intracardially, into the tonsils, joints, pericardium, peritoneal cavity and nasopharyngeal mucous membrane.

Physical State of Inoculum.—Washings of 18 hour agar slants, 18 hour cultures in various fluid mediums,* organisms agglutinated in homologous rabbit sera, organisms incubated (sensitized?) in sera from patients suffering of rheumatic disease, organisms mixed with finely divided agar, organisms suspended in 5% gum tragacanth.

Other Procedures.—Infected agar masses were injected subcutaneously into some animals. Large doses of Digitan were injected intravenously into some animals with the object of poisoning the heart muscle and thus lowering its resistance to infection.† In many animals an external jugular or a femoral vein was either

* Noguchi, Rosenow, dextrose veal infusion broth, dextrose mutton infusion broth, human hormone medium.

† These injections were carried out in rabbits only. Each rabbit received one intravenous injection of 0.5 cc. of Digitan per kilo twice a week to begin with. The dose and number of injections were increased gradually so that after three weeks the animals were receiving 1 cc. per kilo daily.

crushed or painted with 20% silver nitrate. The object of this was to produce a thrombus which might become the seat of infection with the organism subsequently injected intravenously. It was hoped in this way to produce a constant blood stream infection. Many animals were injected intraperitoneally with large doses of saturated Trypan blue in saline (20 cc. per kilo of rabbit injected weekly). The object of this procedure was to attempt a blockade of the reticulo-endothelial system in order to lower resistance to infection. Daily temperature readings were taken but only abnormal temperatures are indicated in the protocols.

For convenience of analysis the experiments will be summarized in three groups, *viz.*, Group A, Group B, Group C. In each group, animals of different species were used with the object of finding a species susceptible to the organism or virus and because it was hoped that the existence of blood vessels in the valves of some of these species (calves, sheep, swine) in fairly high per cent might furnish a means of localizing an infective agent in the valves.

In some of the groups the experimental procedures were relatively simple, hence an abbreviated summary of the technic will be given for these groups. Where the procedures were more complicated, detailed protocols will be given in the appendix.

Group A

Dietrich[31] has claimed that after sensitizing rabbits by means of successive intraperitoneal injections of first, dead organisms (staphylococci) then live organisms, followed after a suitable interval of time by a small intravenous injection of live organisms, he was able to obtain a high per cent of verrucous endocarditis in rabbits. We attempted to repeat this method of sensitization in a variety of species, using streptococci obtained from patients suffering of rheumatic disease, in the hope of producing verrucous endocarditis with, possibly, other rheumatic clinical and pathological manifestations.

Twenty-four rabbits, 12 guinea pigs, 5 dogs, 9 cats, 2 swine, 1 sheep and 2 calves were employed in this experiment. These were divided in three equal subgroups. The first subgroup was injected with Strains 7A-MB and 26A-IS. The second subgroup was injected with Strains 5A-MK, 9A-IS, 3A-EB, 6A-MT, 10A-IS, 4A-RD, and 11A-RG. The third group received Strain 8A-NG. Since, however, there were no conspicuous differences in the results found in these subgroups the procedures detailed in the following pages will refer only to "rheumatic streptococci" without indicating the identity of the individual strains.

TABLE I

No.	Nature of material	Age	Joints	Pericarditis	Nodules	E.C.G.	X-ray	Attacks	Clinical diagnosis
1A-RG	Pos. bld. clt. *Strep. gamma**	10			4+	Neg.	Pos.††	1	Rheum. heart dis.
2A-JW	Pos. bld. clt. *Strep. mitis*†	7				Neg.	Pos.	2	Rheum. heart dis. Chorea
3A-EB	Pos. bld. clt. *Strep. saliv.*	6		+			Pos.	1	Acute rheum. dis. Pericard.
4A-RD	Pos. bld. clt. *Strep. fecal.*	9				?	Pos.	1	Acute rheum. dis.
5A-MK	Pos. bld. clt. *Strep. fecal.*	13	R. elbow, foot				Pos.	2	Acute rheum. heart dis.
6A-MT	Pos. bld. clt. *Strep. gamma*	5				?	Neg.	1	Acute rheum. dis.
7A-MB	Pos. bld. clt. *Strep. fecal.*	7	L. hip	+	+			1	Acute rheum. dis.‡°
8A-NG	Pos. bld. clt. *Strep. fecal.*	9	R. knee, foot	+		Pos.**	Pos.	2	Acute rheum. dis. Pericard.
9A-IS	Pos. bld. clt. *Strep. fecal.*	11		+		Neg.	Pos.	4	Acute rheum. dis. Chorea
10A-IS	Pos. bld. clt. *Strep. fecal.*	28	Hips, knees and ankles			?	Pos.	1	Acute rheum. dis.
10B-IS	Buffy layer of cit. bld.								
11A-RG	Pos. bld. clt. *Strep. fecal.*	28	Mult. arthrit.			Neg.	Pos.	2	Acute rheum. dis.
12A-LH	Pos. bld. clt. *Strep. gamma*	45				Pos.		6	Acute rheum. dis. Aur. Fib.
13A-R to 17A-R, Pos. bld. clts., Swift, *Strep. virid.* = his B-39, 38-D, B-38, A-141, A-135, respectively.									
18A-VF	Sterile peric. fld. P. M.	27		+		Pos.		2	Acute rheum. dis. Pericard.
18B-VF	Cit. and defib. bld.								
19A-RB	Cit. and defib. bld.	12		+		Pos.		3	Acute rheum. dis. Aur. Fib.
20A-SY	Tonsil filtrate	33				Pos.	Pos.	3	Rheum. dis.
21A-VS	Sterile bld.	9	Mult. arthrit.			Neg.	Neg.	1	Polyarthritis
21B-VS	Tonsil filtrate								
22A-YL	Sterile bld. clt.	19				L.V.P.	Pos.	3	Chr. cardio-valv. dis.
23A-MB	Sterile bld. clt.	32	Mult. arthrit.			L.V.P.		1	Polyarthritis
24A-AW	Sterile bld. clt.	12	Knees, feet			Neg.	Neg.	1	Acute rheum. dis.
25A-RG	Sterile bld. clt.	17	Ankles, wrists	+		?	Pos.	1	Acute rheum. Pericard.

26A-IS	Valve clt. *Strep. fecal.*	41				Pos.		2	Chr. cardio-valv. dis.‡
27A-SD	Valve clt. *Strep. ignav.*	16		+	+	Pos.	Pos.	2	Acute rheum. dis. Pericard.‡°
28A-FB	Tonsil clt. *Strep. mitis*	10		+	4+	Neg.	Pos.	3	Acute rheum. dis. Pericard.
29A-MM	Sterile peric. fld.	10		+	3+	?	Pos.	1	Acute rheum. dis. Pericard.‡°
29B-MM	Filt. of lymph node								
30A-MG	Hydrocele fld.	15		+		Pos.	Pos.	2	Acute rheum. dis. Aur. Fib.
31A-AW	Buffy layer of cit. bld.	5		+	+		Pos.	1	Chr. valv. dis. Ac. Pericard.
31B-AW	Tonsil filtrate								
31C-AW	Serum								
32A-AK	Cit. and defib. bld.	20				Pos.		1	Acute rheum. dis. Pericard.
32B-AK	Serum								
33A-HH	Sterile defib. bld.	35	Shoulders			Pos.	Pos.	6	Chr. cardio-valv. dis. Aur. Fib.
34A-JR	Tonsil filtrate	8						4	Chr. cardio-valv. dis.
35A-MS	Tonsil filtrate	11				Pos.	Neg.	4	Chr. cardio-valv. dis.
36A-HM	Serum	10			+	Pos.	Pos.	3	Ac. rheum. dis. Chr. valv. dis.
37A-JR	Filt. nasophar. wash.	37	Wrists and			L.V.P.	Neg.	1	Subacute rheum. arthritis
37B-JR	Cit. and defib. bld.		L. shoulder						
38A-MP	Tonsil filtrate	35	Shoulder				Neg.	1	Subacute rheum. arthritis
39A-EZ	Buffy layer of cit. bld.	15		+		Pos.	Neg.	3	Chr. cardio-valv. dis.
40A-AL	Filt. of nodule	12		+	+	?		2	Acute rheum. dis.‡°
40B-AL	Filt. of lymph node								

*All organisms in this table except 13A-R to 17A-R belong in the gamma group of Brown.[37] **Pos. E.C.G. = finding of either prolonged conduction time, A-V block, S-A block, or auricular fib. †Holman classification.[38] ††Pos. X-ray = mitral or aortic configuration, pericardial effusion, singly or in combination. ‡Confirmed by autopsy. °Aschoff bodies present.

Rabbits

Twenty-four rabbits received from 1 to 6 injections of "rheumatic streptococci." The first intraperitoneal injection consisted of an amount of saline suspension of killed* organisms equivalent to 3 sq. cm. of an 18 hour growth on glucose agar. In the following three intraperitoneal injections, given at 4 day intervals, the number of organisms was doubled each time. The fifth intraperitoneal injection was similar to the first except that living organisms were employed. The sixth injection was given intravenously after a 12 day interval and consisted of one loopful of an 18 hour agar slant of the organisms.

Only six of these rabbits lived long enough to receive an intravenous loopful. These rabbits were exsanguinated under ether anesthesia in from 2 days to 1 month after the last injection. One of the rabbits showed, microscopically, several small scattered areas of accumulations of lymphocytes and mononuclear cells in the heart muscle. Otherwise the gross and microscopic findings in all six rabbits were negative. The remaining 18 rabbits died in from 6 days to 4 weeks after the initial injection. Eleven of the rabbits gave completely negative results. Of the remaining seven, five died with purulent pleurisy and lobular or lobar pneumonia due to organisms other than the streptococci injected. Four showed, microscopically, small scattered areas of scarring in the heart muscle, two showed a low grade pericarditis with mononuclear cells. One showed small focal interstitial collections of lymphocytes in the myocardium. One showed an acute focal interstitial myocarditis. One showed a large, irregular, thrombotic mass on the posterior cusp of the mitral valve. This thrombotic mass contained no demonstrable bacteria. There were no evidences grossly or microscopically of embolic phenomena in any of the organs. It is rather interesting to note that this rabbit received only two intraperitoneal injections of dead, pooled organisms.

Guinea Pigs

Twelve guinea pigs received from 8 to 10 injections of "rheumatic streptococci." The first intraperitoneal injection consisted of an amount of a saline suspension of killed organisms equivalent to 1.5 sq. cm. of an 18 hour growth on glucose agar. In the following three intraperitoneal injections, given at 4 day intervals, the number of organisms was doubled each time. The next three intraperitoneal injections were similar to the first three injections, respectively, except that live organisms were employed. After a lapse of 1 week, the equivalent of one-half a loopful of an agar slant of live organisms was injected intravenously. Six of the guinea pigs received two more intravenous injections of live cultures each equivalent to 500 loopfuls of agar slant, at weekly intervals.

Two guinea pigs died before receiving the intravenous injection. The rest were bled out under anesthesia at intervals varying from 1 day to 4 months after

* Killed by weak phenol solution.

receiving the last injection. None of the animals presented clinical or gross pathological abnormalities. Microscopically, six showed focal interstitial accumulations of lymphocytes and large mononuclear cells in the heart muscle. There was a tendency toward perivascular localization of these inflammatory foci. They were also localized, apparently with predilection, in the auricular muscle at the base of the mitral valve.

Dogs

Five dogs received from 6 to 9 injections of "rheumatic streptococci." The first intraperitoneal injection consisted of an amount of a saline suspension of killed organisms, equivalent to 9 sq. cm. of an 18 hour growth on glucose agar. In the following three intraperitoneal injections given at 4 day intervals, the number of organisms was doubled each time. The next four intraperitoneal injections were similar to the first four injections, respectively except that live organisms were employed. After a lapse of 2 weeks the equivalent of three loopfuls (in 2 dogs, 300 loopfuls) of an agar slant of live organisms was injected intravenously.

The dogs were bled out under anesthesia in intervals of from 2 weeks to 4 months. One dog died of lobar pneumonia before receiving the intravenous injection of live organisms. The other dogs showed no gross or microscopic pathological lesions. Clinically, the dogs had been normal.

Cats

Nine cats received each from 8 to 9 injections of "rheumatic streptococci." The first intraperitoneal injection consisted of an amount of a saline suspension of killed organisms equivalent to 6 sq. cm. of an 18 hour growth on glucose agar. In the following three intraperitoneal injections, given at 4 day intervals the number of organisms was doubled each time. The next four intraperitoneal injections were similar to the first four injections respectively, except that live organisms were employed. After a lapse of 2 weeks the equivalent of two loopfuls (in 1 cat, 1000 loopfuls) of an agar slant of live organisms was injected intravenously. The cats were bled out under anesthesia in intervals of from 1 week to 4 months.

None of the cats showed clinical or gross pathological evidence of disease. One cat showed several interstitial foci of necrosis with lymphocytic infiltration, large mononuclear cells and occasional binucleated cells in the heart muscle (Fig. 1). Four cats showed focal interstitial (in one case, perivascular, Fig. 2) accumulations of lymphocytes and mononuclear cells in the heart muscle. The organs were otherwise normal.

Swine

Two swine received each 9 injections of "rheumatic streptococci." The first intraperitoneal injections consisted of an amount of a saline suspension of killed

organisms equivalent of 12 sq. cm. of an 18 hour growth on glucose agar. In the following three intraperitoneal injections, given at 4 day intervals, the number of organisms was doubled each time. The next four intraperitoneal injections were similar to the first four except that live organisms were employed. After a lapse of 1 week, the equivalent of four loopfuls of an agar slant of live organisms was injected intravenously.

One swine developed slightly swollen knee joints which were neither red nor fluctuating, and a temperature of 104.6° 1 day after receiving the intravenous injection. No cultures were taken from these joints. In several days the joints subsided. 2 weeks after receiving the intravenous injection of organisms, the animal was bled out under anesthesia. All the organs including the joints were negative, macroscopically and microscopically.

The other swine also showed somewhat swollen knee joints the day following the intravenous injection of live organisms. There was no elevation of temperature. These joints were neither red nor fluctuating and subsided in a week. 1 week later the equivalent of 600 loopfuls of an agar slant of live organisms was injected intravenously. 1 month later the animal was bled out under anesthesia. The gross and microscopic findings were negative.

Sheep

One sheep received an intraperitoneal injection of an amount of saline suspension of killed organisms equivalent to 18 sq. cm. of an 18 hour growth on glucose agar. In the following three injections, given at 4 day intervals, the number of organisms was doubled each time. The next four intraperitoneal injections were similar to the first four injections, respectively except that live organisms were employed. Following a lapse of 2 weeks the equivalent of six loopfuls of live organisms was injected intravenously followed in 2 weeks by a similar injection of the equivalent of 600 loopfuls of live organisms. 1 month later the animal was bled out under anesthesia.

Following the third intraperitoneal injection of dead organisms the temperature rose to 107.4°F. 2 days later it fell to normal and continued so until the animal was bled out under anesthesia.

The only positive pathological finding was a few interstitial foci of lymphocytes and mononuclear cells in the heart muscle of the left ventricle.

Calves

Two calves received each 9 injections of "rheumatic streptococci." The procedure and doses were exactly the same as for the sheep in the previous experiment except that 3 weeks after the last intraperitoneal injection of live organisms the calves received each the equivalent of 600 loopfuls of live organisms injected intravenously. 4 months later both calves were bled out under anesthesia. The temperature remained normal throughout the experiment. The findings were the same as those of the sheep in the previous experiment.

Summary of Clinical and Pathological Findings in Group A

Out of the 24 rabbits, 12 guinea pigs, 5 dogs, 9 cats, 2 swine, 1 sheep and 2 calves used in this experiment, marked temperature elevation was noted only in the sheep and in one of the swine. Non-fluctuating arthritis which persisted for a short time only was observed in the swine. Most of the pathological lesions were found in the rabbits as follows: low grade, non-specific inflammatory, myocardial foci in 2, myocardial scarring in 4, purulent pleurisy and lobar or lobular pneumonia in 5, low grade pericarditis in 2, acute focal myocarditis in 1, and a non-bacterial thrombus in the mitral valve of 1. Six guinea pigs and five cats showed low grade, non-specific, inflammatory, myocardial foci. One dog showed a lobar pneumonia.

Group B

In this group 32 rabbits were employed. The object here was to produce in varying combinations thrombosis of a large vein with streptococcal invasion of the thrombus, blockage of the reticulo-endothelial system, poisoning of the heart muscle with Digitan and a continuous toxemia (if this were possible) by the injection subcutaneously of 15 cc. agar mass subsequently infected with "rheumatic streptococci."

The technic employed is described under "methods" (see above). The subgrouping of this experiment was as follows:

(a) 4 rabbits received Trypan blue, infected agar mass, thrombosis.
(b) 4 rabbits received Trypan blue, infected agar mass, thrombosis, Digitan.
(c) 4 rabbits received Trypan blue, thrombosis.
(d) 4 rabbits received Trypan blue, thrombosis, Digitan.
(e) 4 rabbits received thrombosis.
(f) 4 rabbits received thrombosis, Digitan.
(g) 4 rabbits received infected agar mass.
(h) 4 rabbits received infected agar mass, Digitan.

Each rabbit received in addition daily intravenous injections of 10 cc. broth culture of live pooled "rheumatic streptococci" consisting of Strains 1A-RG, 2A-JW, 3A-EB, 12A-LH, 26A-IS, 27A-SD, 28A-FB, 13A-R, 14A-R, 15A-R, 16A-R and 17A-R.

It may be said at the outset that there were no special differences in the findings of the various groups. The animals died or were killed in from 1 week to 5 months after the beginning of the experiment. Only seven rabbits survived over 2 months, at about which time they all showed swollen suppurating knee joints from which streptococci were cultured.

All but one of the rabbits had negative blood cultures. In most of the cases,

however, insufficient amounts of blood were obtained for culture because we did not wish to bleed directly from the heart. The positive culture was obtained from a rabbit who had suppurating knee joints and was bled out under anesthesia 3 months after the beginning of the experiments.

Another rabbit with suppurating knee joints was bled out and showed a bacterial (streptococcus) endocarditis involving the entire mitral valve, with acute focal suppurative myocarditis. The myocardium showed numerous streptococcus emboli in the blood vessels. The kidneys showed streptococcus emboli in the arterioles and glomerular loops. There was also a focal embolic glomerulonephritis not quite typical of the Loehlein lesion (Fig. 3). The spleen showed a large amount of brown pigment and an increase in large mononuclear cells.

Apart from this, three rabbits showed focal suppurative nephritis, two showed chronic interstitial nephritis, four showed scattered lymphocytic collections in the myocardium, one showed focal necrosis with calcification in the myocardium, four showed small scattered areas of scarring in the myocardium; one showed a chronic (lymphocytic) interstitial valvulitis of the mitral valve, one showed a suppurative pericarditis.

Group C

For these experiments there were employed 11 swine, 3 calves, and 2 sheep. The procedures in this group were so varied and extensive that it is desirable to record them in abstracted protocols (see appendix). Briefly stated, the animals in this group received numerous injections of agglutinated and unagglutinated "rheumatic streptococci;" of blood clot infected with "rheumatic streptococci;" of possible "virus" material represented by whole blood; plasma; serum; pericardial, pleural, and hydrocele fluid; and filtrates from tonsils, subcutaneous nodules, lymph nodes, and nasopharyngeal washings. This "virus" material was obtained from patients suffering of rheumatic disease (Table I). These materials were introduced through one or more of the following routes: intravenously, intraperitoneally, intracardially, and into the tonsils, trachea, or joints. As will be noted, the procedure was varied somewhat for the different animals.

Summary of Clinical and Pathological Findings in Group C

Swine

The experiments lasted from 1 week to 1 month. The temperature of some of the animals rose at times to 105.7°F., but this could not be correlated with any

of the other findings. None of these animals developed swollen joints or other discernible clinical manifestations. They were all bled out under anesthesia.

One swine (Swine 1) which had received an intracardiac injection, showed at autopsy an adherent pericarditis with acute and subacute inflammatory foci in the pericardium (Fig. 4) as well as in the superficial layers of heart muscle. No bacteria were found in the microscopic sections. Four showed occasional interstitial foci of lymphocytic and mononuclear cells in the heart muscle. Two showed a low grade chronic inflammation of the pleura associated with a fibrinous pericarditis. Two showed a small amount of scarring in the media of the aorta. One showed a mild interstitial valvulitis.

Calves

The three calves used in this experiment died 2 weeks, 5 weeks, and 3 months, respectively, after the beginning of the experiment. The temperature showed no unusual fluctuations. All the injections in these animals were done intravenously.

Two of the calves developed a suppurative arthritis of the knee joints from which streptococci were cultured. One of the calves developed the arthritis 3 weeks after the beginning of the experiment. At autopsy this calf showed an organizing synovitis. The findings were otherwise negative. The other calf (Calf 4) developed arthritis 10 weeks after the beginning of the experiment. At autopsy there was found a small warty vegetation in the left ventricle at about the middle of the interventricular septum (Fig. 5). The vegetation measured 2 x 3 mm., was raised 2 mm. above the endothelial lining, was smooth, with a broad base, and could be scraped off with the finger. Microscopic preparations with hematoxylin and eosin stains showed the vegetation to be covered by endothelium. The bulk of the vegetation showed purplish-pink staining, more or less hyaline material with a scattering of lymphocytes at its base. A few streptococci and Gram positive bacilli were found in the superficial layers of this thrombotic mass. The impression gained was that these organisms were post-mortem invaders. On the other hand, the heart muscle, spleen, and kidney showed many streptococcus emboli in the smaller vessels. A fibrinous pericarditis, a suppurative arthritis and a lobar pneumonia were the other findings in this animal.

The third calf (Calf 5) disclosed at autopsy a lobar pneumonia and a small warty nodule situated on the middle of the right posterior cusp of the aortic valve within the sinus pocket, 2 mm. below the free edge. The thrombotic mass measured 2 x 2 mm., was round and fairly smooth. On section it proved to be a typical streptococcus vegetation. The other organs were negative grossly. Unfortunately, however, the spleen and kidneys were lost before microscopical material was taken.

Sheep

The two sheep employed in this experiment died 9 and 10 weeks, respectively, after receiving the first injection. The temperature remained within normal limits.

One sheep received intravenous injections only. At autopsy there were disclosed lobar pneumonia and, microscopically, a few small interstitial foci of mononuclear cells in the myocardium. The other sheep (Sheep 3) received only intracardiac injections. After the third injection, 9 days after beginning of the experiments, positive blood cultures (streptococci) were obtained. This bacteremia remained until the death of the animal. 2 days before death, small petechiae which appeared to be white-centered were found in the conjunctivae of the animal. At autopsy there was found an organizing non-bacterial, fibrinous pericarditis, massive vegetative endocarditis of the aortic and mitral valves, fresh splenic infarction and bacterial emboli in the spleen, kidneys and heart muscle.

DISCUSSION

We have described a rather extensive attempt to induce rheumatic fever in animals by the use of streptococci isolated from patients suffering of rheumatic disease, as well as by the use of other material which might reasonably be supposed to contain the "virus" of the disease.

By consulting Table I it will be seen that many of the strains of streptococci were obtained from blood cultures in uncontaminated form. An analysis of the older up to the most recent literature on the subject brings out a striking similarity between the cultural characteristics of our organisms and many of those previously reported.

Some of the strains as well as "virus" material, it will be noted, were obtained from patients where a subsequent autopsy confirmed the diagnosis of rheumatic disease. The "virus" material was obtained in all instances from patients in whom the past history, present illness, and subsequent history left little doubt as to the correctness of the diagnosis. We desire to lay especial emphasis on our use of material from cases in which histological examination of biopsy material (subcutaneous nodules) as well as autopsy material made the diagnosis absolutely certain.

In contrast to this, much of the experimental work reported in the literature was carried on with streptococci isolated from contaminated sources, *e.g.*, tonsils, feces, post-mortem material, etc., or blood cultures from patients in whom the diagnosis of rheumatic disease was not confirmed by autopsy. It may, therefore, be argued that the equivocal results obtained by these workers were at least in many instances due to the fact that the etiological agent responsible for the disease (*i.e.*, the specific strain of streptococcus) was not employed.

Scattered references in the literature deal with observations on streptococci as secondary invaders in various conditions. Libman[33] has stressed this point for a number of years. In a recent article on general infections by bacteria, he states:

> As I have repeatedly emphasized, we now realize the fact that the anhemolytic streptococci constitute the most important secondary aerobic invaders with which the clinician has to reckon. They can be regarded as almost ubiquitous organisms, having been found in the blood in a great variety of conditions, in lymph nodes of various kinds removed during life, and quite regularly in post-mortem examination in organs studied in connection with etiologic investigations of several epidemic diseases.

The questionable value of the employment of streptococci obtained from post-mortem material has been further emphasized by the observations of Epstein and Kugel[32] in this laboratory. These workers, using routine autopsy material from non-rheumatic cases, have obtained streptococci of various types from perfectly normal valves in 40% of their cases, from heart muscle in 47%, from bone marrow in 67%, and from blood cultures in 79% of their cases.

It is obvious, therefore, that the finding of small numbers of such organisms in a vegetation, joint, pericardial cavity, subcutaneous nodule, etc., is by itself slim evidence as to their relation to the disease studied. In the absence of immunological reactions in the patients' blood against these organisms we must rely, at the present time, entirely upon the experimental production of the pathognomonic lesion of rheumatic disease, the Aschoff body, before concluding that the organism is responsible for the disease.

In the evaluation of our work we set for ourselves certain definite criteria which had to be fulfilled in order to establish the production of this disease. These criteria are the reproduction of (1) the Aschoff body, (2) non-bacterial pericarditis, (3) non-bacterial verrucous endocarditis. Of these, we held the Aschoff body to be, par excellence, the desideratum.

The production of migratory arthritis even of a non-bacterial type held for us but passing interest, first because it is not an essential concomitant of the disease in the human, secondly, because such joint conditions can be produced by a variety of agents. It is in the recognition of the experimentally produced Aschoff body that most

of the work in the past has been uncritical. For, whilst it is well known that the Aschoff body in the human heart is somewhat pleomorphic, one is reassured of his diagnosis of this lesion in the human heart even when it occurs in its less characteristic form, by the almost invariable coexistence of verrucous endocarditis, the less frequent occurrence of fibrinous non-bacterial pericarditis, the pathognomonic subcutaneous nodules and the long studied and fairly characteristic clinical course, together with the electro-cardiographic signs. One is further aided by the extensive knowledge of the pathology of the human heart where the tremendous fund of data gathered has shown that there are very few lesions with which the Aschoff body in its most characteristic form can be confused to the critical eye.

This is obviously not the case with experimental animals. Miller[23] has shown that in rabbits and guinea pigs, focal perivascular accumulations of large polyhedral cells with single or multiple nuclei are not infrequently found as spontaneous lesions. This we have been able to confirm. The spontaneous lesions which may occur in other experimental animals are not well known and perhaps it is not superfluous to state that one must guard against confusing such lesions with those intended to be produced experimentally. Further, the rabbit, and possibly other animals, are peculiarly prone to the formation of multinucleated cells in collections somewhat resembling Aschoff bodies following the injection of a variety of toxic substances. Fig. 6 shows such a lesion frequently produced in the rabbit's myocardium by the injection of an organism from the hemorrhagic septicemia group. This illustration can almost be superimposed upon one appearing in a recent publication from Small's laboratory by Belk and Jodzis.[22] These investigators produced this lesion by injections of "*Streptococcus cardio-arthritidis*" in rabbits and make the unequivocal statement that it is an Aschoff body in spite of the fact that they describe the finding of calcium deposition with giant cells in the heart.

Fig. 4 shows foreign body giant cells and polyhedral cells, with red staining protoplasmic granules as shown with the Unna Pappenheim stain, produced in the pericardium of Swine 1 in Group C. No bacteria were found in the preparation. Such a lesion can be easily confused with an Aschoff body to one who is not thoroughly familiar with the protean appearances of the latter.

With our associate, Dr. Benjamin Sacks, we submit the following structure as constituting an Aschoff body. For further details as to localization, Sacks'[34] excellent review may be consulted:

The Aschoff bodies consist essentially of focal collections of characteristic large cells belonging to the family of histiocytes, forming nodules of variable size and shape, those present in the myocardium being located in the interstitial connective tissue, in close relation as a rule to the coronary arterioles, and in the subendocardial tissue not in close proximity to blood vessels. The nodules are rounded, oval, globular, fusiform, spindle-shaped or entirely irregular in outline and save in very exceptional instances are not large enough to be seen with the naked eye. The interstitial nodules develop in the adventitia or peri-adventitial tissue of the coronary arterioles, or at some distance away from the vessel. The nodule may approach the vessel at only one point in its circumference or may spread out in both directions until it surrounds half or even the whole of the circumference. The close proximity of the nodule to the vessel may lead to compression of its lumen, especially when there are two nodules at opposite poles of the circumference. The interstitial connective tissue about the nodules is often edematous and some of the surrounding muscle fibers may undergo degeneration.

The predominating and essential cell of the Aschoff body is a large, polygonal element of variable size and shape, containing one or more nuclei. The cytoplasm, which has an indefinite, often ragged, limiting membrane, is rather homogeneous, non-vacuolated, not distinctly granular and in hematoxylin-eosin sections is distinctly, though not deeply *basophilic*. The nucleus may be ovoid or polymorphous. The former is somewhat vesicular and exhibits a *sharply defined nuclear membrane* with one or more nucleolus-like structures. The polymorphous type of nucleus is generally dark staining, large and occupies a relatively large part of the cell. The cytoplasm when stained by the Unna Pappenheim method with methyl green-pyronin assumes a distinctive brilliant red color, but this tinctorial property is shared by other cells (young cells) to such an extent that it is but rarely, if ever, useful for purposes of identification. The cells do not contain detectable phagocytosed material and no microorganisms or inclusion bodies have thus far been discovered. The multinucleated cells contain two to seven nuclei and in rare instances even more, but the staining properties of these cells are identical with those having a single nucleus. The multinucleated cells differ from the Langhans cells of tuberculosis in the central arrangement of their nuclei and resemble to a certain extent the Dorothy Reed cells of Hodgkin's disease. Their number in the Aschoff nodules is variable; at times they are few and at other times they may comprise as many as half of the characteristic cells. Mitoses have not been encountered. Intermingled with the most peripheral of the large cells and especially at the margins of the nodules there may be a variable number of other types of cells, including polymorphonuclear leucocytes, lymphocytes and plasma cells. At times these cells may be quite numerous.

In the fresher nodules it is common to find the characteristic cells arranged about a central zone of necrotic substance containing little or no fibrin. As the Aschoff bodies grow older, the cells become elongated and lose their characteristic appearance. Fibroblasts grow in among the cells and finally replace them. *Ultimately, the arteriole about which nodules have developed becomes surrounded by an oval or circular band of connective tissue which gives the interstitial tissue in rheumatic infection of the myocardium a very characteristic appearance.* In recurrent or chronic cases, new nodules may develop in such periarterial scars.

No description of the Aschoff bodies is complete without emphasing the extraordinary variability in the number, size, localization and appearance of these lesions, some of these peculiarities already having been referred to. At times the myocardium may be fairly riddled with large nodules accompanied by severe disorganization of the adjacent muscle fibers and what appears to be edema of the entire myocardium. In some cases, sheets of the characteristic cells may be found in localized areas of myocardium or pericardium or invading the valves from the rings. In the endocardium of the left auricle which is often the seat of a gross lesion, the Aschoff bodies are often located in rows about a necrotic collagen or elastic tissue fiber, the nuclei being perpendicular to the fibers or they may be located more deeply in the endocardium or subendocardium in the neighborhood of an irregular area of necrotic tissue, in both cases at some distance away from arterioles.

For the identification of Aschoff bodies, chief emphasis should be laid upon the presence of these nodules in the periarterial tissue of the myocardium with the subsequent development of circular perivascular fibrosis, the tendency for the component cells to arrange themselves about necrotic material (collagen?) and the morphology and tinctorial properties of the characteristic mononuclear and multinucleated cells, especially the basophilic cytoplasm and the dark nucleus with its sharply defined nuclear membrane and nucleoli.

It is precisely because there is here room for individual interpretation that we have insisted on the other two criteria as well, namely, the non-bacterial verrucous endocarditis and the non-bacterial pericarditis.

A number of reports on the experimental reproduction of rheumatic endocarditis describe what is obviously vegetative endocarditis (so-called malignant endocarditis). Apart from the fact that a great variety of organisms can produce this condition (Menzer,[35] Cole,[7] Horder,[23] Thalhimer and Rothschild[25]), we can under no circumstances accept such a lesion as of rheumatic origin.

It might be contended that we are too stringent in our postulates, that possibly species other than human may present an entirely different pathological reaction to the same agent or, indeed, may not

be susceptible to the disease. While such a state of affairs would be highly regrettable, it does not justify the assumption that vegetative endocarditis is verrucous endocarditis or that focal inflammatory accumulations in an animal's myocardium are the equivalent of Aschoff bodies in the human. And yet just such assumptions have been made.

Following this preliminary discussion there is little further we need say in the appraisal of our work. We have found in 24 of our animals focal accumulations of inflammatory cells—at times closely resembling Aschoff bodies and yet so distinct from the latter that we can unconditionally state that we have *not* reproduced the classical Aschoff body. We have also found pericarditis in 8 animals, arthritis in 12 and vegetative endocarditis in 4. Furthermore, we feel reasonably certain that these lesions were not spontaneous occurrences. Indeed, in one case (Fig. 5), a lesion was produced in the outflow tract of the left ventricle showing a remarkable resemblance to a rheumatic vegetation. This animal, however, showed embolic bacterial lesions in the kidney and spleen. In short, whilst many lesions have been produced, we cannot state that we have induced rheumatic disease in animals by the use of streptococci nor by other materials obtained from human cases of this disease; this, despite the fact that we have employed a large number of animals, a variety of species and a variety of procedures far greater than that hitherto reported in the literature. To this we must add the statement that we have also employed the streptococcus in conjunction with possible "virus" material in the same animals with equally unsuccessful results.

Before drawing final conclusions, we must emphasize that this work does not represent an attempt to disprove the streptococcal theory of the etiology of rheumatic fever. It is certainly possible that our negative results may have been due to the fact that despite the complexity of our experiments and our deliberate attempts to sensitize our animals to the streptococcus, we failed to induce the necessary receptivity of the tissues, (Swift *et al.*,[9] Semsroth and Koch[39]) if indeed, such a state of receptivity is an essential prerequisite.

As we have indicated, however, quite positive conclusions have been reached by previous workers. Inasmuch as we have repeated much of the work reported in the past, and have amplified these experiments

in many ways and on an extensive scale, it seems justifiable to point out that rheumatic fever, recognizable as such, has not been reproduced, and to conclude on the basis of such work that either the streptococcus is not the etiological agent of rheumatic fever, or that the species of animal employed are not susceptible to this disease—at least under the conditions of these experiments—or finally that rheumatic manifestations in the species employed differ markedly from those found in the human. Until it is proved that the last of these assumptions is correct, one is not justified in making it.

CONCLUSIONS

Experiments have been described in which we attempted to reproduce in animals the lesions characteristic of rheumatic fever in the human. A large number of animals representing 7 species was employed. Among other materials, streptococci isolated in pure culture from the blood of rheumatic patients (proved to be so by biopsy or by autopsy) as well as whole blood, plasma, serum, pericardial, pleural and hydrocele fluid, filtrates from tonsils, subcutaneous nodules, lymph nodes, and nasopharyngeal washings obtained from such patients were used in a variety of combinations and with a number of procedures calculated to predispose the animal to the disease.

A discussion is given of the criteria whose fulfillment is essential for the establishment of the experimental production of rheumatic disease in animals.

Judged by these criteria, we have failed to reproduce the disease. This conclusion, we believe, holds true for all the work thus far reported in the literature.

APPENDIX

Protocols of Group C

(The following abbreviations are employed: pld = pooled; IV = intravenously; IP = intraperitoneally; IT = intratracheally; cit. & def. = citrated and defibrinated; NP = nasopharyngeal mucous membrane; agg = agglutinated.)

Swine 1.—(10/30/25) 10 cc. pld Mass 1,* IV. (11/2/25) femoral vein crushed,

* Mass 1 consists of pooled 1A-RG, 2A-JW, 26A-IS, 27A-SD, 28A-FB.

10 cc. pld Mass 1, IP. (11/5/25) 10 cc. pld Mass 1, into tonsils. (11/6/25) 10 cc. pld Mass 1 into tonsils, and 10 cc. IV. (11/9/25) 10 cc. pld 2A-JW, 21A-VS, 35A-MS, IV. (11/10/25) blood culture taken (streptococci), 10 cc. pld 2A-JW, 27A-SD, 21A-VS, 35A-MS, IV. (11/11/25) 10 cc. pld 24A-AW, 28A-FB, 27A-SD, 2A-JW, IT. (11/17/25) 10 cc. pld 1A-RG, 28A-FB, 24A-AW, 26A-IS, 35A-MS, into heart. (11/18/25) Swine very sick, can scarcely stand. 10 cc. pld 27A-SD, 2A-JW, 22A-YL, into joints. (11/30/25) Bled out under anesthesia. (For findings see page 53.)

Swine 4.—(12/9/25) 15 cc. pld 2A-JW, 26A-IS, 3A-EB, 1A-RG, IP. (12/12/25) 15 cc. culture of streptococci recovered from Swine 1, IP. (12/16/25) 3 cc. pld 3A-EB, 32A-AK, 23A-MB, 33A-HH, IV. Animal died. Organs grossly negative. Auricular muscle lymphocytic interstitial myocarditis.

Swine 5.—(12/8/25) blood culture taken (negative). 5 cc. cit. & def. blood (32A-AK), IV, and 25 cc., IP. (12/9/25) 10 cc. serum (32B-AK), IV. (12/16/25) 1 cc. cit. & def. blood (19A-RB), IV, 60 cc., IP. (12/28/25) 20 cc. cit. & def. blood (18B-VF), IP. (12/31/25) Blood culture taken (negative.) Bled out under anesthesia. Findings negative.

Swine 6.—(12/20/25) Blood culture (negative). Superficial vein crushed. 10 cc. pld 1A-RG, 2A-JW, 12A-LH, 3A-EB, 26A-IS, IV, 5 cc. into each tonsil, 5 cc. into NP, 1 cc. into each ankle joint, and 25 cc. 23A-MB, IP. (12/21/25) 10 cc. agar mass infected with pld Mass 1, IP. (12/22/25) 5 cc. pld Mass 1, IT. (12/23/25) 10 cc. pld Mass 1, IV. (12/24/25) Bled out under anesthesia. Auricle shows occasional perivascular mononuclear accumulations. Otherwise findings negative.

Swine 7.—(12/20/25) Blood culture (negative). Large superficial vein crushed. 15 cc. pld Mass 1, IV. 5 cc. into each tonsil and NP, 1 cc. into each of 4 joints. 25 cc. 23A-MB, IP. (12/21/25) 10 cc. agar mass infected with pld Mass 1, IP. (12/22/25) 5 cc. pld Mass 1, IT. (12/23/25) 7 cc. pld Mass 1, IV. (12/28/25) 20 cc. cit. & def. blood (18B-VF), IP. (12/29/25) 20 cc. pld 12A-LH, 3A-EB, 2A-JW, IV, and 30 cc. IP. 15 cc. agar mass infected with these cultures, IP. 4 cc. post-mortem pericardial fluid (18A-VF) ground up with blood clot of Swine 7, IV. Swine developed edema of lung. (12/31/25) 17 cc. cit. & def. blood (37B-JR), IP. (1/2/26) Bled out under anesthesia. Temperature of this swine rose to 106°F. on 12/26/25. Only findings are organizing peritonitis.

Swine 8.—(12/20/25) Blood culture (negative). Large superficial vein crushed. 10 cc. pld Mass 1, IV, 1 cc. into each of 2 joints, 5 cc. into tonsil and NP, 25 cc. 23A-MB, IP. (12/21/25) 10 cc. agar mass infected with pld Mass 1, IP. (12/22/25) 5 cc. pld Mass 1, IT. (12/23/25) 10 cc. pld Mass 1, IV. (12/28/25) 20 cc. cit. & def. blood (18B-VF), IP. (12/29/25) Swine died. Organizing fibrinous pericarditis and peritonitis. Scattered interstitial collections of lymphocytes and monocytes in pericardium.

Swine 10.—Treatment similar to Swine 8. (1/7/26) Bled out under anesthesia. Blood culture (negative). Fibrinous and mononuclear pericarditis.

Swine 12.—Treatment the same as Swine 8. (1/14/26) Bled out under anesthesia. A few scattered mononuclear interstitial foci. Lymphocytic interstitial valvulitis of mitral valve.

Swine 13.—Treatment the same as Swine 8. (12/29/25) Bled out under anesthesia. Blood culture (negative). Negative findings.

Swine 15.—Treatment the same as Swine 8. (1/18/26) Bled out under anesthesia. Blood culture (negative). Fibrinous pericarditis and pleuritis. Focal lymphocytic and monocytic interstitial collections in myocardium.

Calf 1.—(6/17/26) 50 cc. pld "R" cultures,* IV. (6/18/26) Left femoral vein painted with 20% silver nitrate. 15 cc. ground up blood clot from Calf 2 previously incubated for 24 hours with 1 cc. pld "R" cultures, IV. 40 cc. pld "R" culture, IV. (6/23/26) 50 cc. pld Mass 1 and 2,† IV. (6/24/26) 50 cc. pld Mass 1 and 2, IV. (6/25/26) 10 cc. nasal washings (37A-JR) incubated with 2 cc. pld "R" cultures for 24 hours, IV. 40 cc. Mass 1 and 2 agg with homologous rabbit serum and washed, IV. (6/26/26) 2.5 cc. "R" cultures, 22.5 cc. Mass 1 and 2, and 25 cc. agg Mass 1 and 2, IV. (6/28/26) 30 cc. pld "R" cultures, IV. Repeated for following 2 days. (7/2/26) 20 cc. nasal filtrate (37A-JR) and 40 cc. pld "R" cultures, IV. (7/7/26) 30 cc. hydrocele fluid (30A-MB) and 20 cc. agg "R" cultures, IV. (7/9/26) Blood culture (negative). 20 cc. ground up blood clot from Calf 1 which had been incubated over night with 20 cc. pld Mass 1 and 2, IV. (7/10/26) 20 cc. infected blood clot (as above), 20 cc. hydrocele fluid (30A-MB), and 5 cc. tonsillar filtrates (31B-AW), intravenously. (7/15/26) 24 cc. serum (31C-AW) and 5 cc. "R" cultures, IV. Calf died. Findings completely negative.

Calf 4.—(6/30/26) Collodion sacs containing respectively, 3A-EB, 13A-R, 14A-R, 26A-IS, and pld "R" cultures in broth implanted IP. Left femoral vein painted with 20% silver nitrate, 50 cc. pld "R" cultures, IV. (7/7/26) 50 cc. agg "R" culture, IV. Blood culture taken (negative). (7/10/26) 25 cc. infected blood clot (similar to Calf 1), and 5 cc. tonsil filtrate (31B-AW), IV. (7/15/26) Treatment same as Calf 1. (7/24/26) 10 cc. plasma and buffy layer (31A-AW), and 10 cc. 25A-RG, IV. (7/28/26) 10 cc. filtrates of subcutaneous nodules (40A-AL), 10 cc. filtrates of lymph nodes (40B-AL), 10 cc. pld Mass 1 and 2, and 10 cc. agg Mass 1 and 2, IV. (7/30/26) 10 cc. agg pld "R" cultures and 5 cc. plasma and buffy layer (39A-EZ), IV. (7/31/26) 5 cc. pericardial fluid (29A-MM), 5 cc. tonsil filtrate (34A-JR), and 5 cc. pld "R" cultures, IV. (8/3/26) 10 cc. pericardial fluid (29A-MM), 5 cc. serum (36A-HM), and 5 cc. pld "R" strains, IV. (8/7/26) Right knee joint markedly swollen. (8/19/26) 15 cc. pld "R" cultures, 5 cc. pericardial fluid (29A-MM), 5 cc. lymph node filtrates (29B-MM), IV. (8/26/26) 30 cc. agg Mass 1 and 2, and 5 cc. pericardial fluid

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* "R" cultures consist of 13A-R, 14A-R, 15A-R, 16A-R, 17A-R.

† Mass 2 cultures consist of pooled 2A-EB, 12A-LH, pooled "R" cultures, 2A-JW, and strains recovered from Swine 1.

(29A-MM), IV. (9/4/26) 40 cc. pld "R" cultures and 10 cc. tonsil filtrate (38A-MP), IV. Swelling gone from knee joint. (9/11/26) 30 cc. pld "R" cultures and 10 cc. tonsil filtrate (20A-SY), IV. (9/17/26) Similar to Sheep 1 on 9/16/26, IV. (9/22/26) Capsules removed from peritoneal cavity and cultured on blood plates and veal broth. (9/24/26) 50 cc. pld rheumatic capsule strains from Calf 4, IV. (9/25/26) Calf very sick. (9/27/26) Abdominal wound broke open, dressed. (9/26/26) Calf died. (For findings see page 53).

Calf 5.—Treatment the same as Calf 4, except that no capsules were implanted IP. No swollen joints developed. (8/7/26) Calf died. (For findings see page 53).

Sheep 1.—(7/29/26) Collodion capsules, containing respectively individual "R" strains, 3A-EB, 26A-IS, organisms obtained from blood of Swine 1, and 2A-JW, implanted IP. 30 cc. pld Mass 1 and 2, IV. (8/18/26) 20 cc. pld Mass 1, 5 cc. each of pericardial fluid (29A-MM) and lymph nodes filtrate (29B-MM), IV. (8/26/26) 20 cc. agg Mass 1 and 2, and 5 cc. pericardial fluid (29A-MM), IV. (9/4/26) 25 cc. agg Mass 1 and 2, and 5 cc. tonsil filtrate (38A-MP), IV. (9/11/26) 25 cc. agg Mass 1, and 5 cc. tonsil filtrate (20A-SY), IV. (9/15/26) IP capsules removed and contents cultured on blood slants and mutton broth. (9/17/26) Capsule strains emulsified with 2% agar, 20 cc. injected IV. (10/2/26) 50 cc. pld capsule strains from Sheep 1, IV. (10/8/26) Stitches of wound infected. Capsule cultures emulsified with gum Tragacanth (5% emulsion), 25 cc. injected IV. 1 hour later sheep died. A few interstitial mononuclear foci, otherwise *findings negative.*

Sheep 3.—(10/15/26) 50 cc. agg pld capsule strains from Sheep 1, intracardially. This was repeated on 10/19/26, and on 10/23/26. (10/25/26) Blood cultures show Gram positive cocci. (10/26/26) 80 cc. agg pld capsules strains of Sheep 1, and 10 cc. tonsil filtrate (20A-SY), intracardially. (10/29/26) 80 cc. agg pld capsule strains from Sheep 1, intracardially. Blood culture (streptococci). (11/4/26) 30 cc. agg pld capsule strains from Sheep 1, intracardially. (11/9/26) Similar to 11/4/26, but 50 cc. injected. (11/12/26) Similar to 11/9/26. Blood culture (Gram positive cocci). (11/19/29) Blood culture (streptococci). (12/7/26) 5 cc. plasma and buffy layer (10B-IS), 40 cc. agg pld capsule strains from Sheep 1, and 5 cc. 7A-MB, intracardially. (12/29/26) Sheep died. (For findings see page 54).

BIBLIOGRAPHY

1. Poynton and Paine, Researches on Rheumatism, London, 1913.
2. Herry, *Bull. Acad. roy. méd. Belg.*, 1914, **27**, ser. 4, 76.
3. Freund and Berger, *Deutsch. Med. Wochenschr.*, 1924, **50**, 625.
4. Clawson, *Jour. Infect. Dis.*, 1925, **36**, 444; *Arch. of Path. and Lab. Med.*, 1926, **2**, 799; *Amer. Jour. Path.*, 1928, **4**, 565.
5. McCrae, *Jour. Amer. Med. Assoc.*, 1903, **40**, 211.
6. Philipp, *Deutch. Archiv. f. klin. Med.*, 1903, **76**, 150.
7. Cole, *Jour. Infect. Dis.*, 1904, **1**, 714.

8. Harrison, *Jour. Roy. Army Med. Corps*, 1913, **20**, 1.
9. Swift and Kinsella, *Arch. Int. Med.*, 1917, **19**, 381; Andrewes, Derick and Swift, *Jour. Exper. Med.*, 1926, **44**, 35; Derick and Swift, *Proc. Soc. Exper. Biol. and Med.*, 1927, **25**, 222 and 224; Swift, Hitchcock and Derick, *Proc. Soc. Exper. Biol. and Med.*, 1928, **45**, 312; Swift, Derick and Hitchcock, Rheumatic Diseases, The Bath Conference, Bath, 1928.
10. Small, *Amer. Jour. Med. Sci.*, 1927, **173**, 101; *Amer. Jour. Med. Sci.*, 1928, **175**, 638 and 650.
11. Birkhaug, *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 541; *Jour. Infect. Dis.*, 1927, **40**, 549; *Jour. Infect. Dis.*, 1928, **43**, 280.
12. Rosenow, *Jour. Infect. Dis.*, 1912, **11**, 210; *Jour. Infect. Dis.*, 1914, **14**, 61; *Jour. Amer. Med. Assoc.*, 1915, **65**, 1687.
13. Kreidler, *Jour. Infect Dis.*, 1926, **39**, 186; *Jour. Infect. Dis.*, 1928, **43**, 415.
14. Wassermann, Westphal, and Malkoff, *Berl. Klin. Wochenschr.*, 1899, **36**, 638.
15. Meyer, *Deutsch. Med. Wochenschr.*, 1901, **27**, 81.
16. Shaw, *Jour. Path. and Bact.*, 1904, **9**, 158.
17. Beattie, *Jour. Path. and Bact.*, 1904, **9**, 272; *Jour. Exper. Med.*, 1907, **9**, 186; *Jour. Path. and Bact.*, 1910, **14**, 432; Beattie and Yates, *Jour. Path. and Bact.*, 1911–1912, **14**, 247; *Jour. Path. and Bact.*, 1912–1913, **17**, 538.
18. Coombs, Miller and Kettle, *Lancet*, 1912, **2**, 1209.
19. Jackson, *Jour. Infect. Dis.*, 1912, **11**, 243; *Jour. Infect. Dis.*, 1913, **12**, 364.
20. Harzell and Henrici, *Jour. Amer. Med. Assoc.*, 1915, **64**, 1055.
21. Henrici, *Jour. Infect. Dis.*, 1916, **19**, 572.
22. Belk and Jodzis, *Arch. Path.*, 1928, **6**, 812.
23. Horder, Thirty-sixth Ann. Rep. of the Local Gov. Report of the Med. Officer, 1906–7, 279; *Quart. Jour. Med.*, 1908–9, **2**, 289.
24. Davis, *Jour. Amer. Med. Assoc.*, 1912, **58**, 1283.
25. Thalhimer and Rothschild, *Jour. Exper. Med.*, 1914, **19**, 417 and 444.
26. Cecil, *Jour. Exper. Med.*, 1916, **24**, 739.
27. Topley and Weir, *Jour. Path. and Bact.*, 1921, **24**, 333.
28. Miller, *Jour. Exper. Med.*, 1924, **40**, 525 and 543.
29. Kaiser, *Jour. Infect. Dis.*, 1928, **42**, 25.
30. Irvine-Jones, *Arch. Int. Med.*, 1928, **42**, 784.
31. Dietrich, Zeitschr. f. d. ges. exp. Med., 1926, **50**, 85.
32. Epstein and Kugel, *Trans. New York Path. Soc.*, 1928; *Jour. Inf. Dis.*, 1929, **44**, 327.
33. Libman, *Jour. Amer. Med. Assoc.*, 1923, **80**, 813; New York Acad. of Med. Lectures on Med. and Surg., (Hoeber), 1928.
34. Sacks, *Amer. Heart Jour.*, 1926, **1**, 750.
35. Menzer, Die Aetiologie des Gelenkrheumatismus, Berlin, 1902.
36. Suranyi and Forro, *Klin. Wochenschr.*, 1928, **7**, 453.
37. Brown, Monographs of The Rockefeller Inst. for Med. Res., No. IX, 1919.
38. Holman, *Jour. Med. Res.*, 1916, **29**, 377.
39. Semsroth and Koch, *Proc. Soc. Exper. Biol. and Med.*, 1929, **26**, 516.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. Area of focal necrosis with lymphocytes and large mononuclear cells in cat's myocardium (Group A).

FIG. 2. Perivascular foci of lymphocytes and large mononuclear cells in cat's myocardium (Group A).

PLATE 2

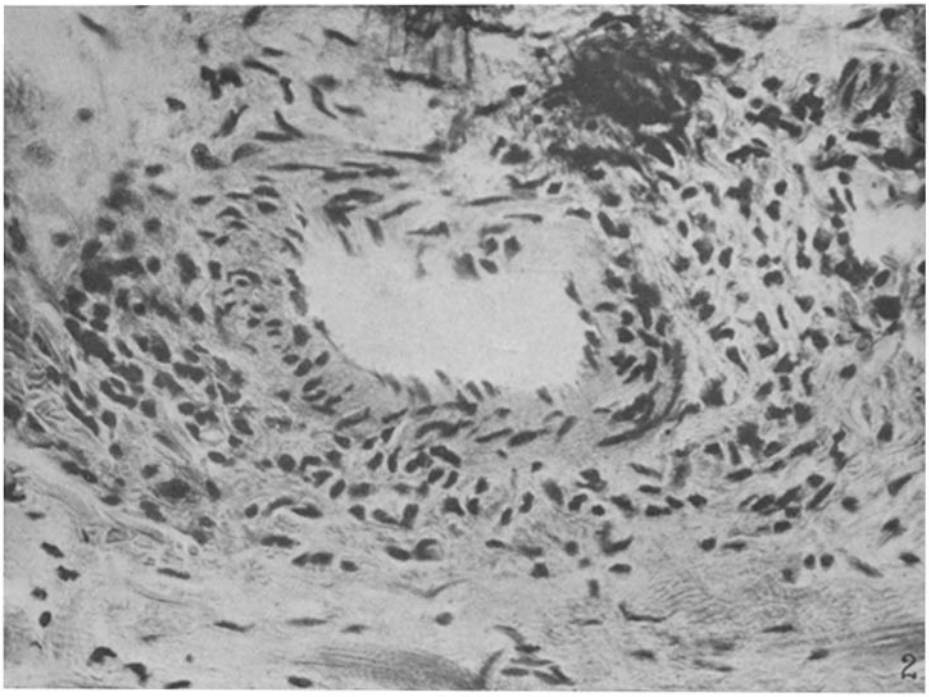
FIG. 3. Focal embolic glomerulo-nephritis in kidney of rabbit (Group B).

FIG. 4. Nodule in pericardium of Swine 1 (Group C), showing collection of large mononuclear cells and giant cells.

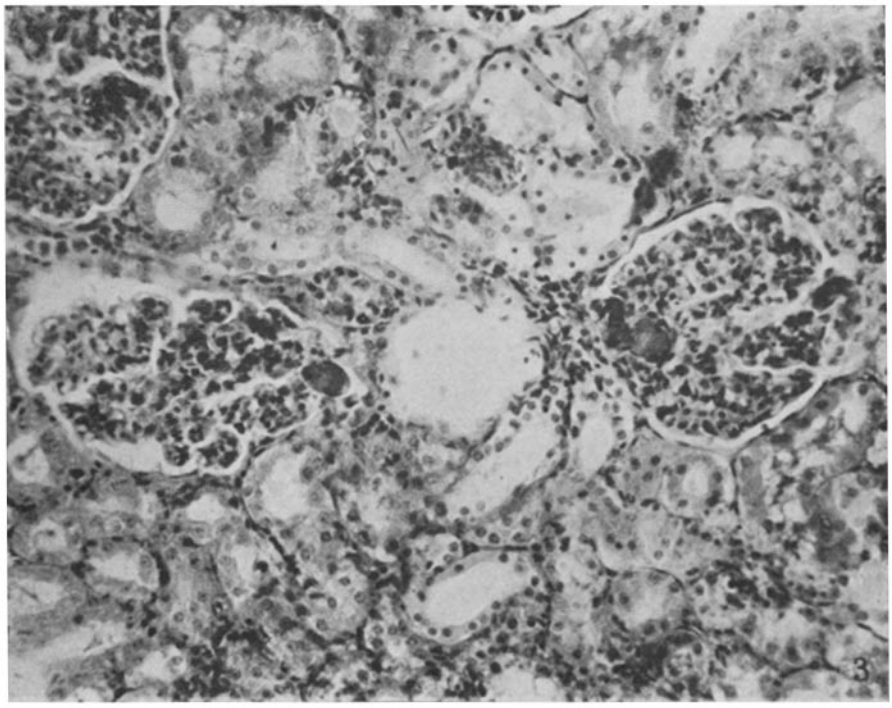
PLATE 3

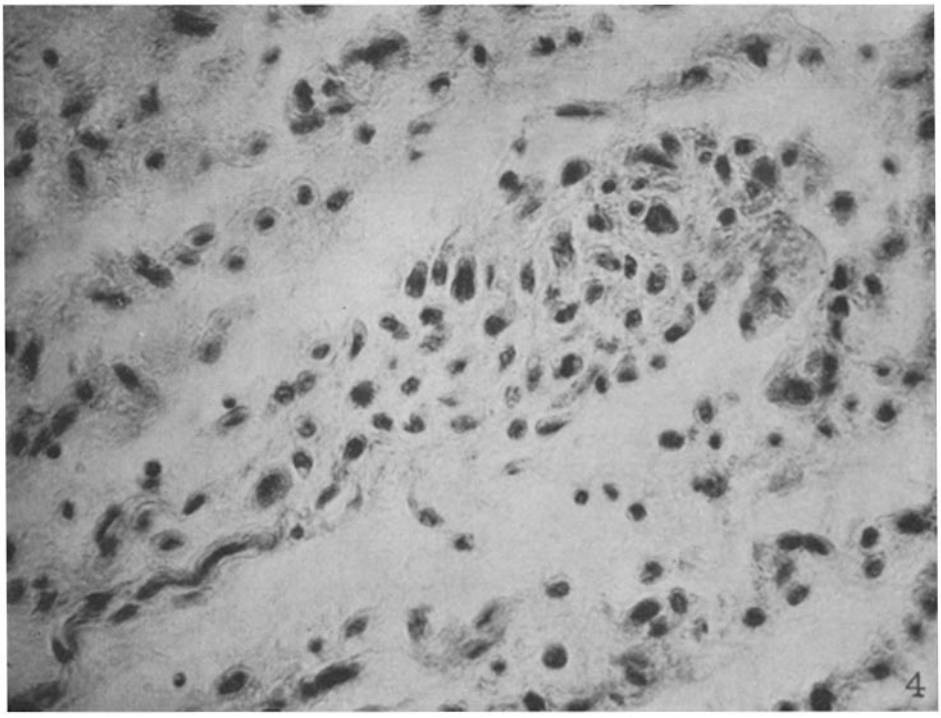
FIG. 5. Verrucous nodule in left outflow tract (interventricular septum) of Calf 4 (Group C).

FIG. 6. Foreign body giant cells and calcium deposit in left auricle of rabbit.

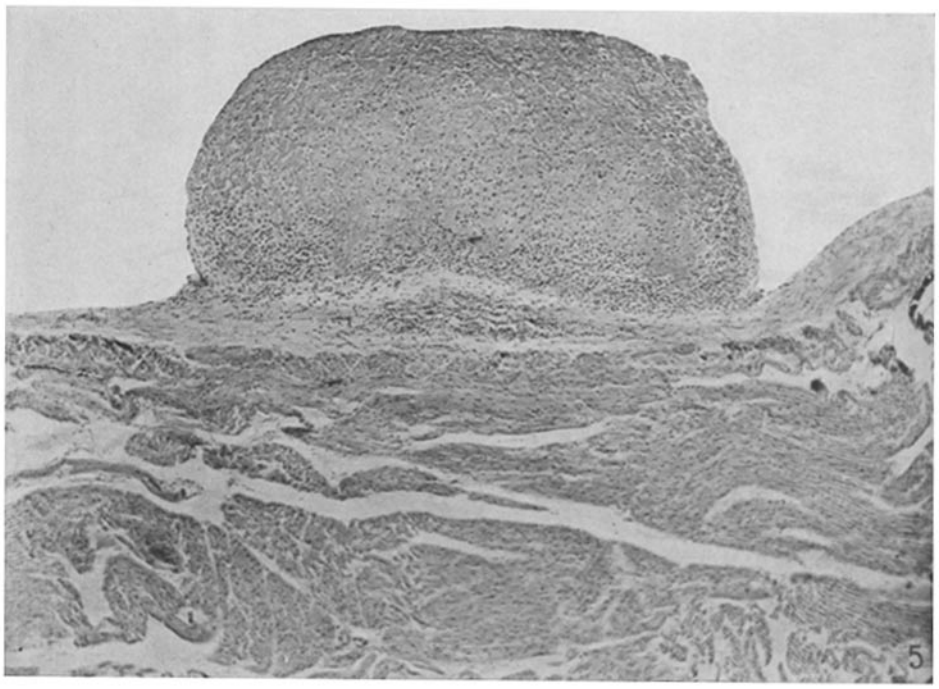


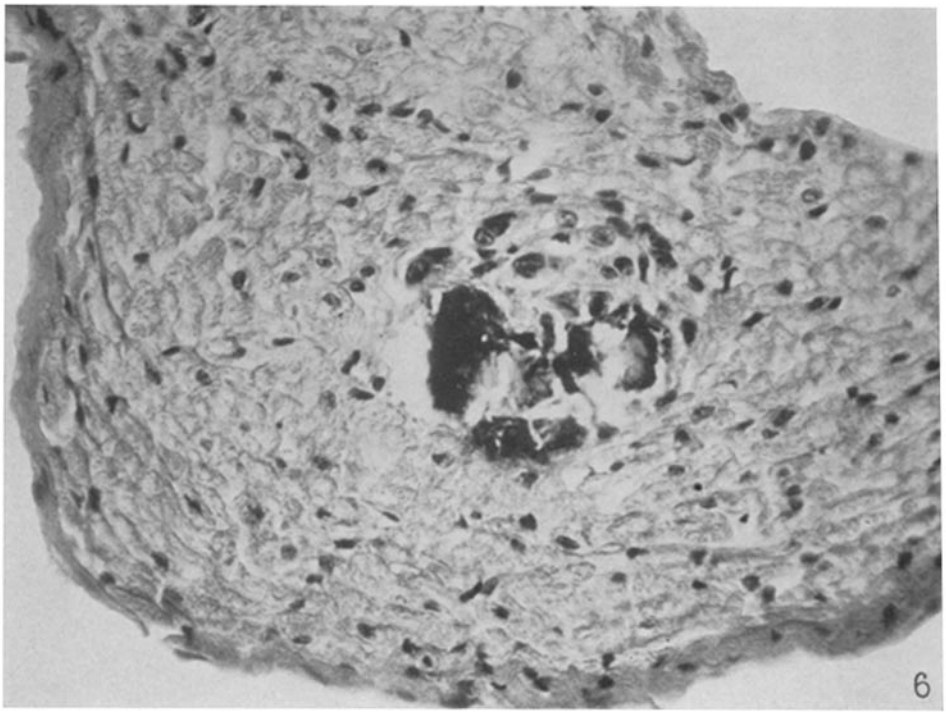
(Gross *et al.*: Rheumatic fever in animals)





(Gross *et al.*: Rheumatic fever in animals)





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