ORGAN LESIONS PRODUCED IN RABBITS BY GROUP A STREPTOCOCCI AND SOME OF THEIR EXTRACELLULAR PRODUCTS

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Many studies have been made of tissue alterations due to infections with Group A streptococci in laboratory animals. Cardiac lesions characterized by muscle necrosis, myocarditis, and giant-cell formation have been reported in a variety of laboratory animals following injections of living Group A streptococci and some of their products.[1-5] Some of these lesions were described as quite similar to the Aschoff bodies of rheumatic carditis.[4] The mechanism of formation of these cardiac lesions has been attributed to toxic effects of streptococcal products,[6,7] to immune response to streptococcal components (hypersensitivity or autoimmunity),[8-10] or to combinations of these processes.

Among these studies, the pharyngeal cavity of animals was used as the portal of entry of streptococci only in that of Glaser *et al.*,[11] who found cardiac lesions within 72 hr. of a single intratonsillar injection of virulent Group A streptococci. These lesions were focal, and showed muscle necrosis, infiltrations of mononuclear cells, and occasional giant cells. No streptococci could be isolated from such lesions, and it was concluded that the lesions were probably not caused by an immunologic process.[11]

The present report describes the induction of lesions in cardiac and other tissues of rabbits following injection, by intratonsillar and other routes, of streptococcal extracellular products (SEP) obtained from Group A streptococci grown in steady-state culture. These lesions occured soon after single injections of these materials and probably reflect direct toxic effect on the cells of these tissues.

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MATERIAL AND METHODS

Animals

Rabbits weighing 2–3 kg., male or female, were obtained from a local breeder and kept on a Purina pellet diet and water ad libitum.

Bacterial Strains

Three strains of Group A streptococci, Type 6, 11, and 12, were employed. The strains were kindly supplied by Dr. S. Rabinowitz, of the Streptococcus Reference Laboratory, Central Laboratories of the Ministry of Health, Jerusalem, and were passed several times in mice to increase their virulence. All strains killed mice weighing 20 gm. on intraperitoneal injection of approximately 10^3 organisms.

Streptococcus viridans isolated from human saliva was identified as *Streptococcus salivarius* by growing the bacteria on Salivarius-Mitis broth (Difco).

Staphylococcus, a coagulase-positive, hemolytic strain, was isolated from a human leg ulcer.

All bacterial strains were cultivated in Todd-Hewitt broth (Difco). The bacterial cells were harvested from the logarithmic phase of growth, washed twice with 100 ml. of saline buffered with 0.025 M phosphate, pH 7.4, resuspended in buffer, and used within 1 hr.

Streptococcal Products

Streptolysin S (SLS). RNA-hemolysin was prepared from Type 3 streptococcus (Strain S84) as described by Ginsburg, Bentwich, and Harris.[12] The hemolysin was sterilized by passing through a Millipore filter (0.22 μ). Preparations containing 1000–5000 hemolytic units per milligram were employed.

Streptococcal Extracellular Products (SEP). SEP were obtained by growing Type 4 streptococci (Strain H44) in steady-state culture (chemostat), using a complete synthetic medium, as described elsewhere,[13] with separate pH control [14] to free the culture from constraints of pH limitation which occurs in steady-state cultures of this organism. The supernatant fluid was concentrated tenfold by pervaporation, and the proteins were precipitated by saturation with ammonium sulfate, dialyzed against distilled water, and lyophilized as described previously.[15]

Under standard conditions 1 L. of chemostat culture yielded 20–40 mg. of extracellular streptococcal proteins. Two pools of such protein, SEP-1 and SEP-2, were used for the injection of rabbits. In either case, SEP was dissolved in buffered saline at 10 mg./ml., and portions of this solution were mixed with equal volumes of complete Freund's adjuvant (Difco). Two milligrams of SEP-1 or SEP-2 were injected intramuscularly to each of 5 rabbits. The injections were repeated 3 times at 1-week intervals, and the animals were bled 10 days following the last injection.

When serum samples obtained prior to the injections of microorganisms or their products and at intervals thereafter were analyzed by immunoelectrophoresis, antiserums to SEP-1 showed 6–8 precipitin lines, while antiserums to SEP-2 showed 10–12 lines. Both SEP-1 and SEP-2 were found to contain desoxyribonuclease (DNAse), ribonuclease (RNAse), diphosphopyridinenucleotidase (DPNase), hyalyronidase, and streptokinase (SK). SEP-2 also contained streptolysin O (SLO) and traces of proteinase (Table I). Since early experiments with the two preparations revealed no difference between their pathologic effects, SEP-1 or 2 was used for these experiments according to availability of material, most of the experiments reported here being performed with SEP-1. When SEP-2 was used, the antigen pool was incubated with 100 μg./ml. of cholesterol for 10 min. to neutralize the activity of SLO.

Injection of Microorganisms, SLS, or SEP

Rabbits were anesthesized with sodium pentothal (20–30 mg./kg.). The jaws were opened with a retractor and 0.25 ml. of a suspension of washed streptococci was

TABLE I

ENZYMATIC ACTIVITIES OF THE PREPARATION OF STREPTOCOCCAL EXTRACELLULAR PRODUCTS

Product	*SEP-1* * *(U./mg.)*	*SEP-2* *(U./mg.)*
SLO	0	120
SLS	0	0
Proteinase	0	4
Proteinase precursor	0	4
DNAse	2500	10,000
RNAse	16	4
DPNase	5000	20,000
Streptokinase	2	10
Hyaluronidase	5000	40,000

* See Reference 25.

injected into each tonsil with 25-gauge needles, as described by Glaser *et al.*[11] Streptolysin S (SLS) or SEP was dissolved in buffered saline solution at appropriate concentrations, sterilized by passing through a Millipore filter (0.22 μ), and injected in a similar manner. SLS and SEP were also injected intramyocardially as described previously.[2] At intervals thereafter, 2 ml. of blood was withdrawn from the ear vein for inoculation into Todd-Hewitt broth and for determination of enzymes and lipids as indicated below.

Injections of Antigen-Antibody Complexes

Serums of animals given injections of SEP-1 or SEP-2 were incubated at 37° C. with various amounts of SEP or with a lyophilized commercial preparation of streptococcal kinase and DNAse (SK-SD) (Lederle Laboratories). The concentrations of SEP or SK-SD which barely precipitated all the corresponding antibodies were used to prepare complexes at the zone of equivalence. Soluble complexes were prepared by dissolving such complexes with an excess of the antigens. Usually 1 ml. of rabbit anti-SEP, containing approximately 4 mg. of these antibodies, was mixed with 35 mg. of SEP or with 50 mg. of SK-SD. Anti-SEP gave 2–3 precipitin lines when tested against SK-SD by immunoelectrophoresis. Both soluble and insoluble complexes were injected either intravenously or intraperitoneally.

Determinations of Total Lipids and Enzymes

Prior to the experiment and 1–7 days following injection of SEP, the serums of the animals were analyzed for total lipids by the method of Kunkel,[16] for their content of glutamic-oxalacetic transaminase (GOT) by the method of Reitman and Frankel,[17] and for sorbitol dehydrogenase (SOD) as described by King.[18] Unesterified fatty acids were determined by the method of Dole.[19]

Histologic Techniques

The animals were sacrificed by means of an intravenous injection of sodium pentothal. Sections from the base to the apex in all parts of the heart, as well as samples from liver, kidney, spleen, diaphragm, and tonsils were fixed in 10% neutral formalin. The tissues were embedded in paraffin and 6-μ sections were stained with hematoxylin and eosin. Sections of heart, diaphragm, and liver showing lesions were also stained with van Gieson, azan, alizarin PAS, colloidal iron, alcian blue, Prussian blue, toluidine blue, Feulgen, and elastic tissue stains. In some cases paraffin sections were treated with trypsin, hyaluronidase, and amylase prior to staining with the periodic acid-Schiff (PAS) technique.

Evaluation of the Results

The lesions induced in the rabbits following the injections of microorganisms and SEP varied considerably in size, severity, and distribution in different organs and in different groups of animals. Cardiac lesions were classified into four categories:

Grade 0: Absence of lesions in all sections studied, or presence of a few very small focal accumulations of round cells in the myocardial interstitial tissue, with no necrosis

Grade 1: Mild focal interstitial inflammatory infiltrations accompanied by mild regressive alterations of the myocardial fibers

Grade 2: Degenerative and/or necrotic alteration of the myocardial fibers, with pronounced interstitial inflammatory infiltration

Grade 3: Widespread necrosis of myofibers, usually associated with granulomatous reaction and calcification

No attempt was made to quantitate the lesions in other organs.

RESULTS

Following Intratonsillar Injection of Streptococci

Injection of 10^8 Streptococci. Eight rabbits received injections of Type 11 streptococci, and 10, Type 6. Of these 18 rabbits, 3 died within 1 day and the remaining 15 were sacrificed after 3 days. The histologic findings did not differ between the 2 groups. Of the 15 rabbits, 9 showed Grade 2 pathologic changes in the heart. The lesions, characterized by necrosis, were focal and showed no relationship to blood vessels. The necrotic areas were infiltrated with inflammatory cells, predominantly with lymphocytes and histiocytes (Fig. 1), and, occasionally, with smaller numbers of plasma cells and cells with nuclei resembling those of Anitschkow cells. In 4 animals milder changes, Grade 1–2, were observed. Of the 3 animals which died within 24 hr. of the injection, 1 showed interstitial edema with patchy granulocytic infiltration, many of the inflammatory cells showing karyorrhexis (Fig. 2); the second had patches of necrosis of the myofibers and diffuse granulocytic infiltration; and the third showed no lesions. In all rabbits given intratonsillar injections of streptococci, blood cultures showed positive results up to the forty-eighth hour following injection. Cultures at time of sacrifice did not yield streptococci. No pathologic changes were found in the liver, spleen, lung, kidney, or diaphragm. The tonsillar area was markedly inflamed, with a yellowish white exudate. The lesions described in the hearts of the rabbits are similar to those described by Glaser *et al.*[11]

Injection of Streptococci in a Larger Dose. Eight rabbits received 2.5×10^{10} Type 12 streptococci in each tonsil. One animal died within 24 hr., and the remaining 7 were sacrificed 4 days following the injection. In 6 animals a sharp rise in both SOD and GOT was detected 24 hr. after the injection. Two animals showed a marked rise in lactic dehydrogenase levels and 4 had elevated serum lipids 24 hr. following

injection. A rise in leukocyte count (6000–4000) was also found in 5 of the 7 animals in this group. This increased leukocyte count persisted up to the time of sacrifice. Cardiac lesions were found in 2 rabbits. In one (Grade 3) the many myocardial lesions were of varied size and partly confluent. They involved loss of myocardial tissue, and in some cases ghost-like remnants of myofibers were seen. The lost muscle was replaced by reparative tissue composed chiefly of histiocytes, a few of them multinucleated, and varying numbers of lymphocytes and granulocytes (Fig. 3). In the other animal cardiac lesions (Grade 2) similar to those described in the previous section were found. Both animals had liver damage characterized by a marked focal necrosis of the parenchymal cells. The lesions appeared to be at different stages, some necrotic foci showing only acute reactive changes, while others were surrounded by granulation tissue containing many foreign-body giant cells, frequently enclosing delicate central basophilic rods (Fig. 4).

Effects of Intratonsillar Injections of Streptococci and SLS

The appearance of cardiac lesions in rabbits shortly after a single intratonsillar injection of living streptococci suggested that they were caused by a toxic component(s) produced by the streptococci. Among the many extracellular products of this organism, streptolysin S (SLS) has been shown to be highly cytotoxic to mammalian cells in vitro and in vivo.[20,21] The possibility that SLS participated in the induction of the lesions was investigated.

Seven rabbits received an intratonsillar injection of SLS (2000 H.U.), and were sacrificed 3 days later. No pathologic alterations were found in any of the tissues of these animals. The possibility that SLS would enhance the pathologic effect of living streptococci was then examined. Ten rabbits were given intratonsillar injections of living streptococci mixed with 5000 HU of SLS, and were sacrificed 3 days later. Five animals had cardiac lesions of Grade 2, which were essentially similar to those observed in animals receiving streptococci alone. Three animals had focal lesions graded 1–2, while 3 animals showed no lesions in the heart (Table II).

Effects of Combined Intratonsillar Injection of Streptococci and Streptococcal Products

The results of the previous experiment gave no indication that SLS in the amounts injected participated in the induction of cardiac lesions. Since Group A streptococci are known to produce many extracellular products,[22] the role of a pool of such products in the induction or exacerbation of cardiac lesions was examined by the simultaneous intra-

TABLE II

DATA ON RABBITS GIVEN INJECTIONS OF STREPTOCOCCI AND SOME OF THEIR PRODUCTS AND DATA ON CONTROL GROUPS

Group No.	*Injection Material*	*Rt.*	*Time of sacrifice (days)*	*No. of rabbits*	*No. of animals with lesions*						
					*Heart **						
					Grade				*Incidence*	*Diaphragm*	*Liver*	*Kidney*
					0	*1*	*2*	*3*				
	EXPERIMENTAL RABBITS											
	Strep. T-6 (10^8)	I.T.	3	8	2	2	4	—	9/18	—	—	—
	Strep. T-11 (10^8)	I.T.	3	10	3	2	5	—		—	—	—
	Strep. T-12 (5×10^{10})	I.T.	4	8	6	—	1	1	2/8	—	2	—
	SLS (2000 U.)	I.T.	3	7	7	—	—	—	0/7	—	—	—
	Strep. T-6 + SLS (5000 U.)	I.T.	3	10	4	2	4	—	4/10	—	—	—
	Strep. T-6 + SLS (5000 U. + SEP, 2 mg.)	I.T.	4	7	—	—	—	4	4/7	—	2	—
	SEP (2 mg.)	I.T.	3	18	5	—	—	9	11/25	2	4	—
	SEP (2 mg.)	I.T.	7	7	3	—	—	2		—	—	—
	SEP (4 mg.)	I.T.	1	6	—	—	—	—	3/11	—	—	—
	SEP (4 mg.)	I.T.	7	5	2	—	3	—		1	3	—
	SEP (6 mg.)	I.T.	1	2	2	—	—	—	6/10	1	1	—
	SEP (6 mg.)	I.T.	3	3	—	—	—	3		3	3	3†
	SEP (6 mg.)	I.T.	7	5	1	1	2	1		2	—	—
	SEP (2 mg.)	I.V.	7	11	5	2	2	2	7/25	3	5	—
	SEP (2 mg.)	I.P.	7	14	6	5	2	1		7	3	—
	CONTROL RABBITS											
1	None	—	3	10	9	1	—	—		—	—	—
2	Strep. T-6 (10^8), heated at 100° C.	I.T.	3	5	5	—	—	—		—	—	—
3	SEP (2 mg.) heated at 100° C.	I.T.	3	5	5	—	—	—		—	—	—
4	*Strep. salivarius* (10^8)	I.T.	3	5	4	—	1‡	—		—	—	—
4	*Staph. aureus* (10^8)	I.T.	3	8	6	—	—	2§		—	3§	5§
5	SLS (10,000 U.)	I.V.	5	6	5	—	1	—		—	—	—
5	SLS (50,000 U.)	I.P.	20	7	7	—	—	—		—	4¶	—
5	SLS (10,000 U.) heated to 100° C.	I.V.	5	4	4	—	—	—		—	—	—
6	SEP-anti-SEP complexes (soluble)	I.V.	3	4	2	2	—	—		—	—	2**
6	SEP-anti-SEP complexes (soluble)	I.P.	6	6	5	1	—	—		—	—	3**
6	SK-SD-anti-SK-SD complexes (soluble)	I.V.	3	5	—	1	—	—		—	—	—
7	Human serum albumin	I.T.	3	4	4	—	—	—		—	—	—

Route of injection: intratonsillar (I.T.); intravenous (I.V.); or intraperitoneal (I.P.).

* Lesions graded as indicated in text; animals listed under Grade 3 lesions also showed Grade 2 lesions; only animals showing Grades 2 and 3 lesions are included in *Incidence*.

† Animal showed masses of amorphous basophilic material in the tubules.

‡ Bacterial thromboendocarditis.

§ Pyemic lesion.

¶ Acute liver necrosis.

** Proliferative glomerulitis.

tonsillar injection of living streptococci, SLS (5000 HU) and SEP (2 mg. per animal). The amount of SEP used in the experiment was approximately equivalent to 50–100 ml. of streptococcal culture supernate. Of 7 animals receiving injections of such mixtures and sacrificed on the fourth day, 4 revealed cardiac lesions, which fell into two categories. The first category consisted of Grade 2 lesions similar to those shown in Fig. 1. The second type of lesion was more severe, Grade 3, consisting of relatively large circumscribed areas of myocardial fiber necrosis with prominent inflammatory infiltration including neutrophils, lymphocytes, and histiocytes. Within the necrotic areas, remnants of myofibrils were discernible. These were fragmented, deeply stained basophilic muscle fibers devoid of longitudinal and cross striation and nuclei. These lesions were similar to those induced by large numbers of streptococci, but with the addition of large, sharply demarcated masses of basophilic material usually at the periphery of these foci (Fig. 5). Five of the animals showed a marked elevation of GOT 24 hr. following the intratonsillar injection. In 2 animals hepatic lesions were also present: areas of necrosis of various shapes and sizes, merging or separated from each other by loose edematous granulation tissue. Within the necrotic debris were seen amorphic basophilic masses and ghost-like remnants of the cords of liver cells. The surrounding granulation tissue was rich in histiocytes and contained unevenly distributed multinuclear giant cells.

Effects of Intratonsillar Injection of SEP Alone

The preceding experiments indicated that the presence of SEP in the injected material elicited a severe myocardial lesion not present in animals given injections of living streptococci alone. The possibility that such severe granulomatous lesions could be induced by SEP alone was therefore examined, intratonsillar injections being given in the following doses: 2 mg. into 25 rabbits, 4 mg. into 10, and 6 mg. into 11. Six of the animals given 4 mg. of SEP and 2 animals receiving 6 mg. died within 24 hr. following the injection. Of the remaining animals, approximately half of each group was sacrificed after 3 days, and half after 7 days.

Cardiac Lesions. The lesions in the animals receiving the 3 different doses of SEP (2, 4, and 6 mg.) were similar in nature. Of the 21 animals given SEP and sacrificed 3 days following injection, 12 revealed Grade 3 lesions which were similar to those shown in Fig. 5, as well as some Grade 2 lesions. Of 17 animals sacrificed 7 days following injection, 2 showed a multitude of small, partly merging foci of myocarditis. In such areas the myocardial fibers had largely disappeared. They were replaced by a chronic inflammatory exudate with some fibroblastic proliferation and syncytial giant cells that often still had the structure

of myofibrils (Fig. 6). In 2 animals, there were, in addition, areas in which groups of myocardial fibers had been replaced by interstitial edema and connective tissue (Fig. 7), as determined by connective-tissue stains. There was a mild round-cell infiltration and some fibroblastic proliferation, and some remnants of distorted and altered myocardial fibers were seen. In 3 animals peculiar granulomatous lesions were found. These were of various sizes and shapes and sharply demarcated from the surrounding myocardium, which was apparently normal. These areas were completely devoid of recognizable myocardial fibers and contained basophilic masses, usually in groups (Fig. 8). A large proportion of these masses were stained with the von Kossa and alizarin stains, indicating calcification. The masses were present within newly formed, highly cellular granulation tissue (Fig. 8). There was a marked fibroblastic reaction and only a moderate formation of collagen fibers. The inflammatory cells consisted of lymphocytes, histiocytes, and a few neutrophils. Surrounding the masses were many multinucleated foreign-body giant cells, some containing the masses (Fig. 9). Occasionally some of the amorphous material was seen within the giant cells.

Hepatic Lesions. Of the 46 animals, 11 had lesions in the liver which consisted of round foci of necrotic liver tissue, filled with histiocytes, fibroblasts, granulation tissue, and amorphous material (Fig. 10). Within the lesions large numbers of multinucleated giant cells were present (Fig. 11). The lesions were quite similar to some of the lesions described in the cardiac tissue (Fig. 8 and 9).

Diaphragmatic Lesions. Nine of the rabbits injected with SEP had diaphragmatic lesions. In 2 animals which died 24 hr. following the injection there was patchy disappearance of the muscle fibers, leaving empty sarcolemmal sheaths. Other muscle fibers showed fragmentation, homogenization, and waxy degeneration. There was a sparse inflammatory response, with evidence of phagocytosis in some of the macrophages. Three animals surviving for 7 days had a number of sharply demarcated foci of muscle destruction in which fibers were replaced by granulation tissue containing many giant cells and amorphous material essentially similar to that described in the heart (Fig. 12).

Kidney Lesions. Three of the animals given intratonsillar injections of 6 mg. of SEP showed accumulations of an amorphous basophilic material in the renal tubules. This material was similar to the amorphous material present in the heart, liver, and diaphragm.

Effects of Intraperitoneal and Intravenous Injection of SEP

In order to test whether the induction of these lesions was specific for the tonsillar route of injection, 11 rabbits were given intravenous and

14 intraperitoneal injections of 4 mg. of SEP. All the animals were sacrificed 7 days following the injections. Of the 11 animals receiving intravenous injections, 2 showed Grade 1 cardiac lesions, 2 had Grade 2 lesions, and 2 showed granulomatous lesions of Grade 3. Five animals had lesions in the liver and 3 showed granulomas in the diaphragm. The lesions in the liver and diaphragm were essentially similar to those described above. Of the 14 animals given SEP by the intraperitoneal route, 3 had heart lesions graded 2–3, 3 had granulomas in the liver, and 7 had granulomas in the diaphragm (Table II). Thus, although the cardiac lesions described above did not occur solely after intratonsillar injection, the incidence of cardiac lesions following the tonsillar injection was higher than that following intravenous or intraperitoneal injection. On the other hand, the incidence of liver damage was higher following the intravenous route, and of the diaphragm, following the intraperitoneal route. These results are summarized in Table II.

Effects of the Intramyocardial Injection of SEP

The nature and the mode of induction of the cardiac lesions in rabbits given SEP injections are not as yet known. In order to see whether the lesions involve some localization of some streptococcal products in the myocardium, 2 mg. of SEP was injected intramyocardially[2] into 5 rabbits, which were sacrificed 5 days later. Myocardial lesions similar to those shown in Fig. 8 were found, but these were confined to the area of injection.

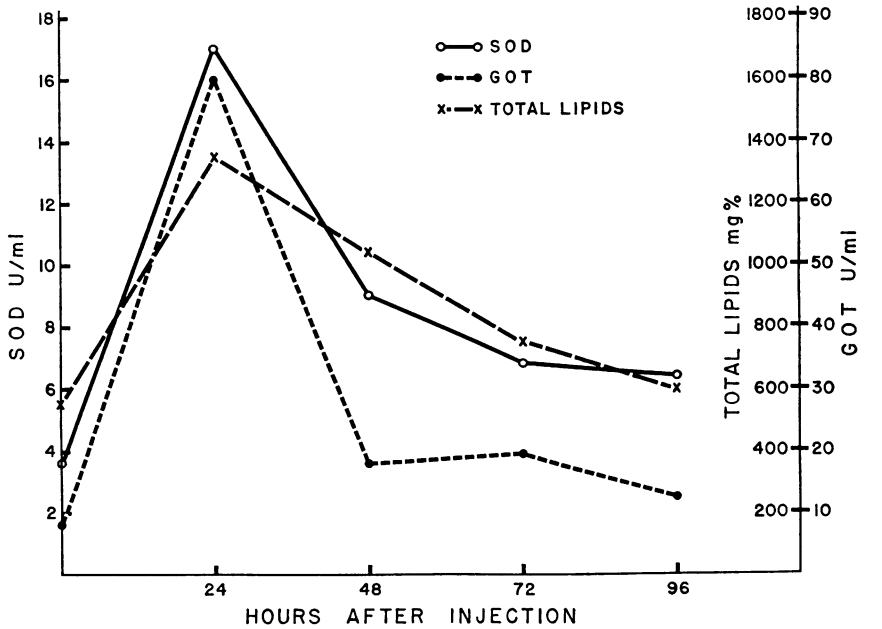
GOT, SOD and Total Lipids in Animals Given Intratonsillar Injections of SEP

Approximately 50% of rabbits given intratonsillar injections of 2–6 mg. of SEP showed a marked elevation in GOT, SOD, and serum lipid levels as early as 16 hr. following injection. The levels remained high for 48 hr. and returned to starting levels on the seventh day following injection. The increase in total lipids was due to elevation of free fatty acids. The averages of results obtained in 5 animals are shown in Text-fig. 1. The rise in levels of the 2 enzymes was observed at the time of appearance of cardiac and liver lesions.

Staining Properties of Myocardial Lesions Appearing After Intratonsillar and Intramyocardial Injection of SEP

The similarity in the cardiac lesions induced in the rabbits by the intratonsillar and intracardiac injections of SEP led to an examination of some staining properties of such myocardial lesions. The results were the same in 2 rabbits given injections by different routes: Rabbit 734, which

died 96 hr. after receiving an intratonsillar injection of 6 mg. SEP-2; and Rabbit 745, which was sacrificed 4 days after an intramyocardial injection of 2 mg. SEP-2. The amorphous basophilic material within the granulomatous lesions in the heart was stained positively by the von Kossa and alizarin techniques, indicating the presence of carbonate and



TEXT-FIG. 1. Sorbitol dehydrogenase, glutamic-oxalacetic transaminase, and serum lipid levels in rabbits following intratonsillar injection of SEP-1 (average data of 5 animals).

calcium. The lesions proved to be hematoxylinophilic when stained with hematoxylin and eosin. The masses in both rabbits were stained with the PAS technique. However, digestion by hyaluronidase, amylase, or trypsin did not alter the staining properties of the material. All the other staining techniques (colloidal iron, alcian blue, toluidine blue, elastica, Feulgen, and Prussian blue) gave negative results. Studies on the nature of the amorphous material present in the liver and diaphragm are under way.

Control Groups

A total of 69 rabbits divided into 7 groups served as controls. Results are given in Table II.

Group 1 consisted of 10 untreated rabbits. One rabbit had a few foci of interstitial myocarditis of Grade 1. None of the animals had any lesions in any of the other organs studied.

Group 2 consisted of 5 rabbits given intratonsillar injections with 10^8 streptococci (Type 6) which had been heated to 100° C. for 10 min. No lesions were found in any of the organs tested.

Group 3 consisted of 5 rabbits given intratonsillar injections of 2 mg. of SEP-2 previously heated to 100° C. for 5 min. No pathologic lesions were found.

Group 4 consisted of 5 rabbits which were given intratonsillar injections of *Streptococcus salivarius* (10^9 organisms) and of 8 animals injected with 10^8 coagulase-positive staphylococci. One of the former rabbits had bacterial thrombendocarditis in the endocardium of the left ventricle, but no myocardial, hepatic, or diaphragmatic lesions were found. Of the 8 rabbits given staphylococci injections, 2 had multiple myocardial micro-abscesses, 3 had pyemic lesions in the liver, and 5 had acute interstitial nephritis.

Group 5 consisted of 13 animals which received 10,000 to 50,000 HU of SLS intravenously or intraperitoneally, and of 4 animals given injections of 10,000 HU of SLS which had been heated to 100° C. for 5 min. One animal given an intravenous injection had cardiac lesions of Grade 1. Intraperitoneal injection induced acute focal necrosis of liver parenchyma in 4 rabbits. No cardiac or diaphragmatic lesions were found in the other animals.

Group 6 consisted of 10 rabbits which received soluble SEP-anti-SEP complexes and of 4 rabbits which received soluble complexes of SK-SD-anti-SEP. Of the 14 animals, 4 had cardiac lesions of Grade 1, and 5 had mild to severe proliferative glomerulitis (to be reported).

Group 7 consisted of 4 rabbits given intratonsillar injections of 5 mg. of bovine serum albumin. No pathologic lesions were found.

Discussion

The data presented show that cardiac lesions characterized by muscle necrosis associated with an inflammatory reaction were obtained in approximately 50% of rabbits given intratonsillar injections of living Group A streptococci. These results are similar to those described earlier by Glaser *et al.*[11] Injection of the other bacteria tested—e.g., *Streptococcus salivarius*—did not result in any cardiac lesions. (In the study of Glaser *et al.* pneumococci also failed to produce such lesions.) Intratonsillar injections of virulent staphylococci caused multiple abscesses in the heart, liver, and kidneys, but these lesions were quite distinct from those induced by Group A streptococci.

The appearance of cardiac lesions relatively shortly (1–3 days) after a single injection into the tonsils suggests that toxic factor(s) produced by the streptococci in vivo are responsible for the initiation of the cardiac

lesion described here. Streptolysin S, previously shown to be cytotoxic to heart cells grown in tissue culture,[19] failed to cause any cardiac damage. Also, SLS did not appear to enhance the pathologic effects caused by streptococci alone. On the other hand, the intratonsillar injection of a pool of streptococcal extracellular products (SEP), which did not contain either SLS or SLO, induced similar but more severe cardiac lesions following a single injection. In animals dying 24 hr. following the injection of SEP, there was already damage of the heart, liver, and diaphragm. The cardiac damage induced by SEP was characterized by necrosis associated with severe mesenchymal response, including multinucleated giant cells engulfing calcified amorphous material (Fig. 5, 8, and 9). Since the amount of SEP which was effective in our experiments was equivalent to approximately only 25–50 ml. of streptococcal culture supernate, it is possible that evanescent changes of the kind shown here may occur in severe cases of tonsillitis, in which large amounts of toxic products of Group A streptococci may be released in vivo.

In addition to cardiac tissue, both the diaphragm and liver were affected by SEP. The lesions in both these organs were essentially similar to those seen in the heart, with parenchymatous necrosis, infiltration by inflammatory cells, and multinucleated giant cells surrounding a basophilic amorphous material.

The relation between the tonsillar route of injection and cardiac tissue was not specific. Animals receiving intraperitoneal and intravenous injections also showed lesions in the heart, in addition to those of the liver and diaphragm. However, the incidence of cardiac lesions was much higher after intratonsillar injection than after injection by other routes, and hepatic and diaphragmatic lesions were more frequent following intravenous and intraperitoneal injection. These findings, if corroborated with larger series, will point out the importance of the tonsils as a route of entry of streptococcal toxic factors into the body under conditions of natural infection.

The lesions observed in the heart, diaphragm, and liver were granulomatous in nature. The presence within the granulomatous tissue of giant cells abutting on and often engulfing amorphous material makes it certain that we are dealing here with a foreign-body reaction. The nature of the material eliciting the granulomatous reaction is still uncertain. The SEP employed contains at least 12 molecular species, as indicated by the antigenic analysis. It is possible that some native or altered streptococcal products became localized in the affected tissues, with the formation of granuloma, or that some of the streptococcal products were localized within parenchymal cells, causing cellular death, with alteration of cellular components and the resulting formation of foreign mate-

rial. Injection of SEP directly into the myocardium produced lesions indistinguishable from those induced in the hearts of rabbits given intratonsillar injections of SEP. Since, in the above-mentioned experiment, there was similarity both in morphology and in tinctorial properties of the tissues affected, it is highly probable that the tissue changes reflect cell destruction, such as would be caused by a toxic product. This inference is supported by the findings that the majority of the animals which showed lesions after SEP injections had high levels of GOT and SOD as soon as 16 hr. following injection. In some cases there was a rise in both enzymes. On the other hand, in a few rabbits only the GOT was elevated, indicating that only the heart and/or diaphragm was severely affected. In addition, most of the rabbits given SEP injections showed a steep rise in total serum lipids (Text-fig. 1), which was due to an increase in free fatty acids. The reason for such a rise is still obscure. It is possible that this is a secondary reaction to changes caused by streptococcal components. (It has recently been shown that rabbits receiving intravenous injections of papain also show a rapid rise in total lipids.[23]) Although the cause of the rise in lipids is not understood, it always accompanied the rise in GOT or SOD, and even preceded them, thus giving early evidence of the tissue damage. A few of the lipemic serums showed very high nonspecific antistreptolysin O activity that returned to normal levels with the decrease in the inflammatory reaction.

The nature of the streptococcal factor(s) in SEP responsible for the initiation of organ lesions is not known. SLO, SLS, or proteinase, previously shown to be highly cytotoxic to heart tissue both in vitro and in vivo,[6,7,18,19] were not present in detectable amounts in the SEP employed. The role of SK, DNAse, hyaluronidase, or DPNase, and the other additional 6–8 antigens present in the SEP, is currently being tested. It is of interest that we have not found any cytopathogenic effects of SEP in rabbit or rat heart cells grown in tissue culture. This suggests that the toxic effect may be mediated through some still unknown in-vivo mechanism.

The fact that the toxic factor(s) responsible for the tissue damage is thermolabile and destroyed by trypsin suggests that protein is involved.

The possibility that the lesions induced by SEP are immunologic in nature must also be considered, but the facts that no antibodies to any of the streptococcal antigens used in such tests could be demonstrated either prior to the injection or at time of sacrifice (3–7 days), and that heart and liver damage was apparent as soon as 12 hr. following injection, tend to rule out immunologic mechanisms as responsible for these lesions (see also Glaser *et al.*[11]). That the toxic component(s) in SEP are antigenic is suggested by the experiments in which both insoluble

and soluble complexes of SEP-anti-SEP were injected intraperitoneally into rabbits. None of the rabbits thus treated showed any lesions in the heart, liver, or diaphragm, but some of the animals given injections of soluble complexes developed mild to severe proliferative glomerulitis 3–7 days following injections of complexes. These results are in accord with the findings that soluble antigen-antibody complexes may initiate kidney damage.[24]

The appearance of pathologic changes with substantially greater frequency in cardiac tissue after the intratonsillar injections suggests that this would be a useful route of injection for studies of other pathologic effects of streptococcal products on cardiac tissue. For instance, studies of hypersensitivity to streptococcal proteins in cardiac tissue in experimental animals might serve to elucidate the role of Group A streptococci and their extracellular products in the initiation of poststreptococcal complications in man.

Summary

Rabbits were given injections via the tonsils with Group A hemolytic streptococci, streptolysin S (a known primary toxin of this organism), and with protein concentrates from steady-state cultures of the hemolytic streptococcus. In most of the rabbits that received killed streptococci, cardiac lesions were found 3 days later. These were largely moderately severe areas of focal necrosis with infiltration of inflammatory cells, not in direct relation to blood vessels. The addition of streptolysin S to the streptococcal cells caused no aggravation of these lesions. When the concentrate of streptococcal extracellular proteins (SEP) was injected along with killed streptococci and streptolysin S, lesions similar to those described were found within 3 days, as well as an additional lesion consisting of large areas of necrosis of myocardial fibers with infiltration by inflammatory cells and, within the necrotic areas, deeply stained basophilic muscle fibers and clumps of amorphous basophilic material. Such lesions were also found when the SEP was injected alone into the tonsils, without the accompanying streptococci and streptolysin S. Here, in addition, some foreign-body giant cells were found surrounding some of the clumps of amorphous basophilic material in the cardiac muscle, and there was some evidence of early calcification. Approximately 50% of the rabbits also showed marked increase in serum concentration of two enzymes generally associated with cell destruction, serum glutamic-oxalacetic transaminase and sorbitol dehydrogenase, as well as an increase in total serum lipids.

Lesions generally similar to the cardiac lesions described here were found in the liver and the diaphragm of about one-quarter of these rabbits, and in all 3 organs following intravenous and intraperitoneal in-

jection of SEP. There was, however, a higher frequency of association of cardiac lesions with intratonsillar injection.

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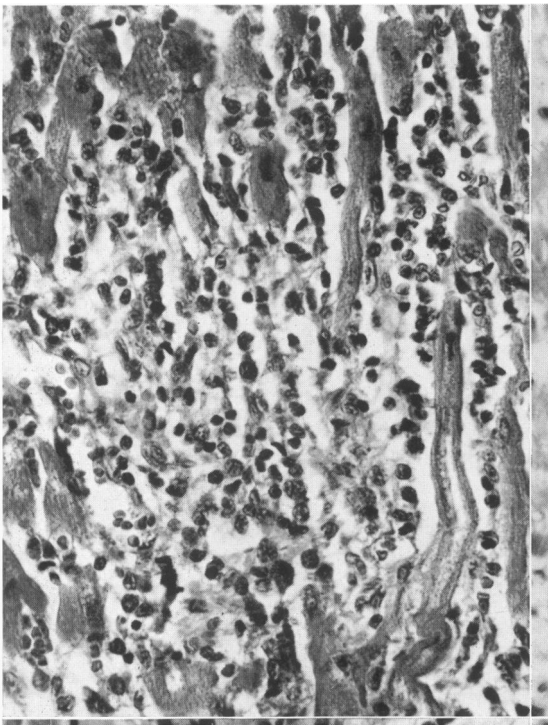
LEGENDS FOR FIGURES

FIG. 1. Early myocardial lesion in rabbit 24 hr. following intratonsillar injection of living streptococci. Note muscle degeneration and mild granulocytic reaction. × 450.

FIG. 2. Myocardial lesion in rabbit sacrificed 3 days following injection of living streptococci. There is sharply outlined necrosis, and infiltration by mononuclear inflammatory cells. × 450.

FIG. 3. Myocardial lesions in rabbit 4 days following intratonsillar injection of 10^{11} streptococci. Lesions consist of areas of necrosis in which muscle is replaced by reparative tissue composed chiefly of histiocytes. × 450.

FIG. 4. Liver of rabbit 4 days following intratonsillar injection of 10^{11} streptococci showing granulomatous reaction and many foreign-body giant cells. × 450.



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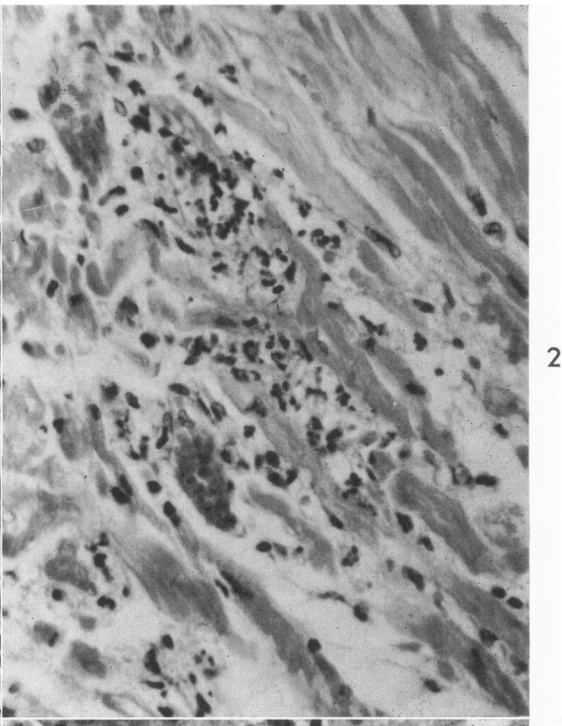

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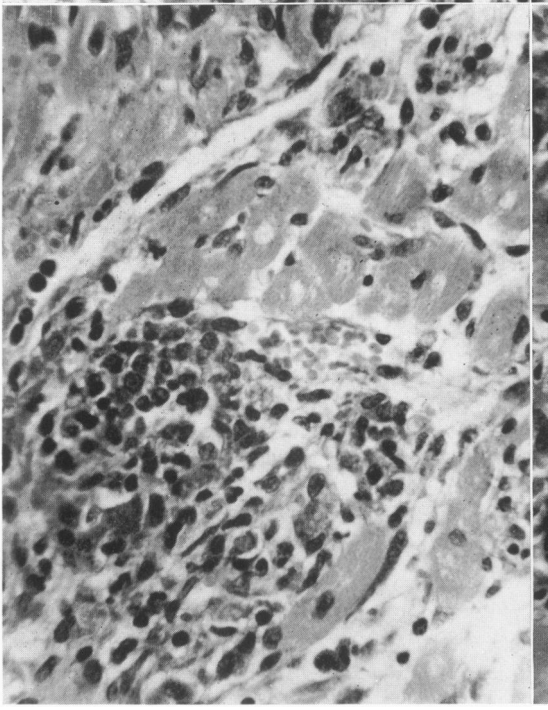

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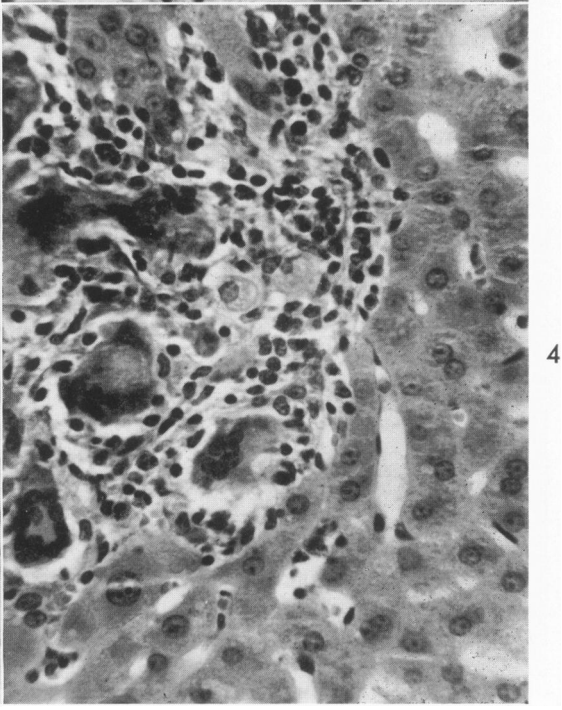

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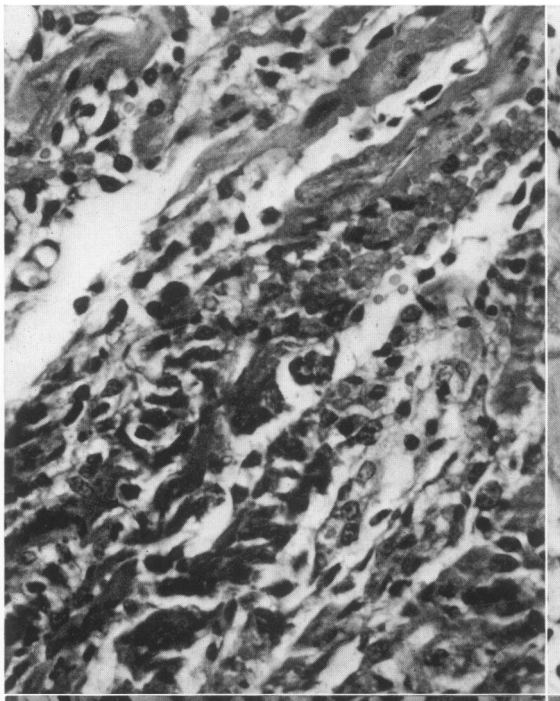
FIG. 5. Myocardium of rabbit 7 days following intratonsillar injection of SEP-1 and 5000 HU of SLS. Muscle tissue has been replaced by granulation tissue containing oval areas comprised of partly merging, roundish, calcified amorphous clumps. × 150.

FIG. 6. Myocardium of rabbit 7 days following intratonsillar injection of 2 mg. of SEP-1, showing loss of muscle fibers and mild round-cell infiltration. There are a few syncytial giant cells suggesting remnants of myofibrils. × 450.

FIG. 7. Myocardium of rabbit 7 days following intratonsillar injection of 2 mg. of SEP-1. Subtotal loss of muscle fibers and interstitial edema. × 450.

FIG. 8. Myocardium of rabbit 7 days following intratonsillar injection of SEP-1. Muscle tissue has been replaced by granulation tissue containing oval areas made up of partly merging, roundish, calcified amorphous masses. × 150.



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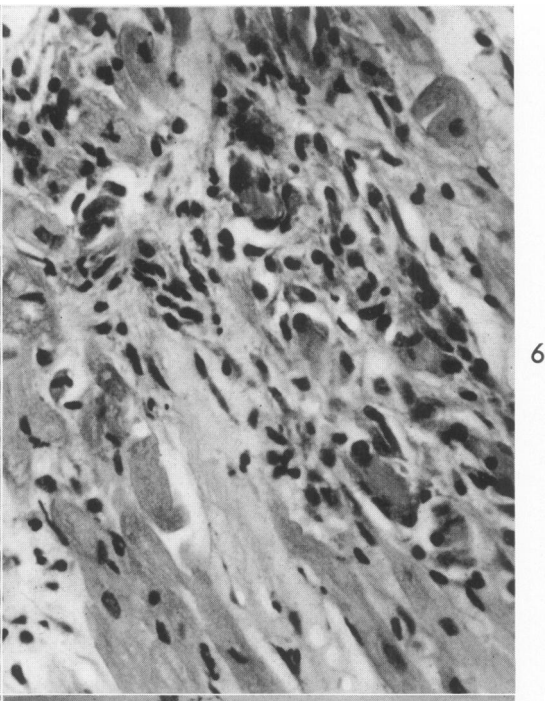

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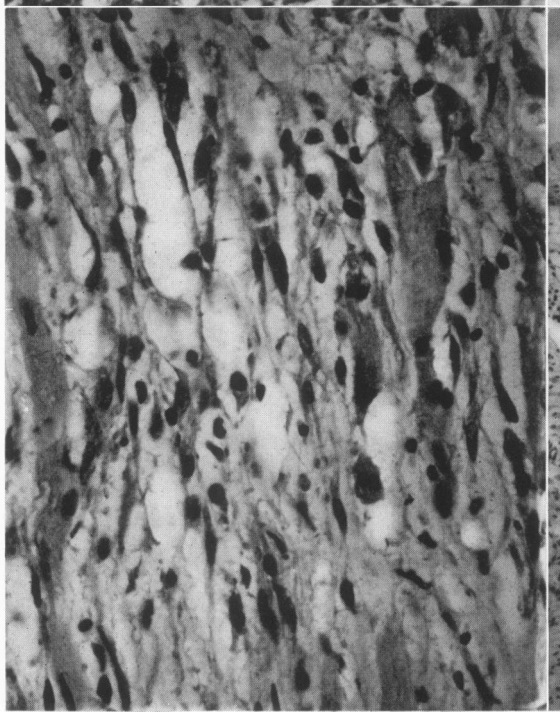

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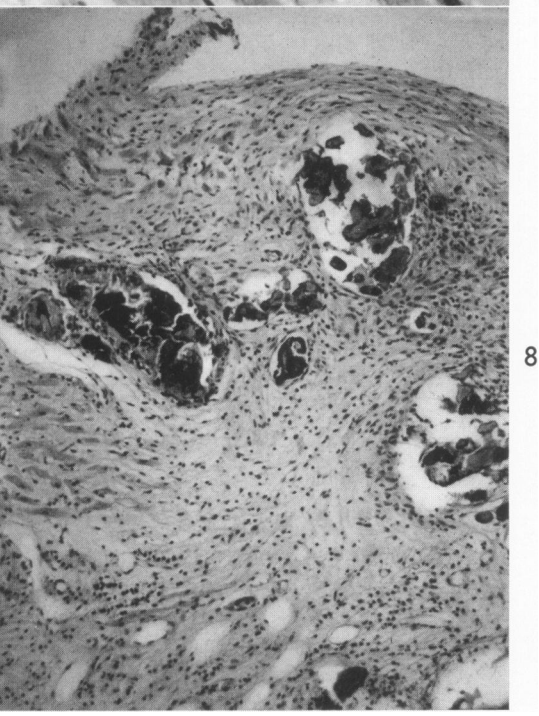

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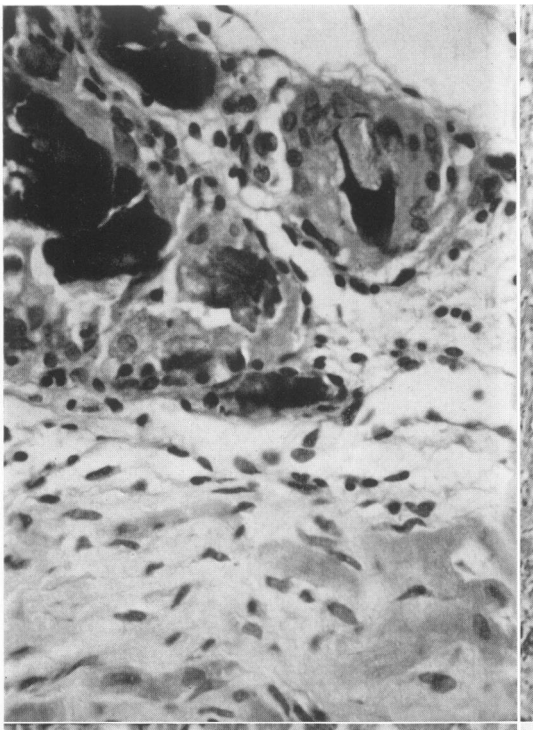
FIG. 9. Higher magnification of Fig. 8, showing multinucleated foreign-body giant cells engulfing amorphous masses. × 450.

FIG. 10. Liver of rabbit 7 days following intratonsillar injection of SEP-1. Numerous sharply outlined areas of parenchymatous loss contain granulation tissue and calcified amorphous masses. × 110.

FIG. 11. Higher magnification of Fig. 10 showing granulation tissue and giant cells engulfing amorphous material. × 270.

FIG. 12. Diaphragm of rabbit 7 days following intratonsillar injection of SEP-1. Sharply outlined area of granulation tissue contains many calcified amorphous masses. × 450.



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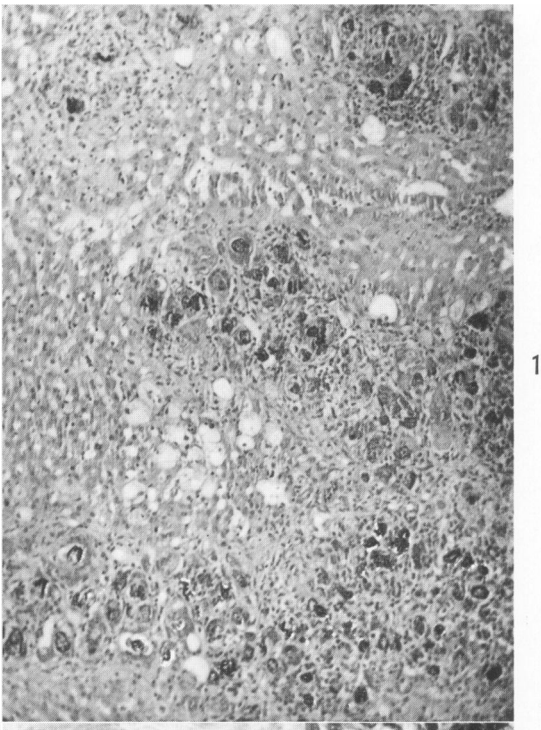

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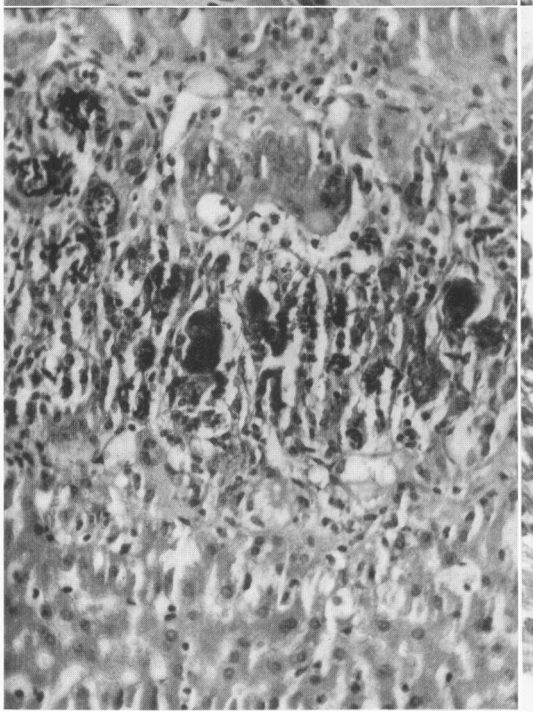

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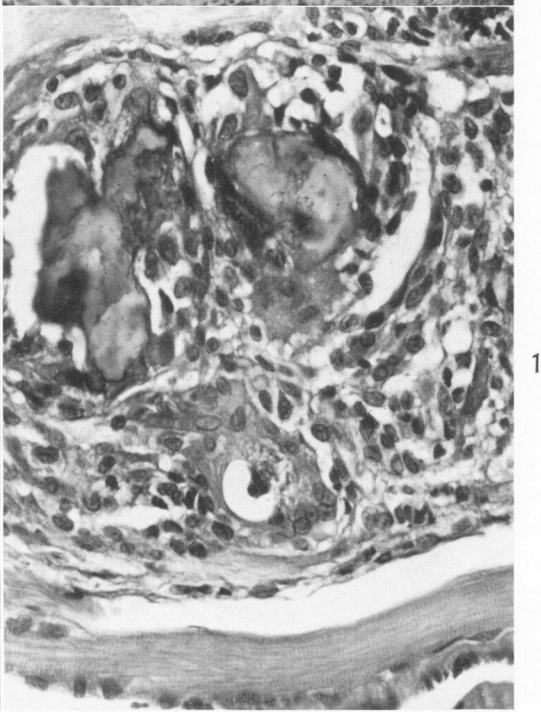

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