STUDIES OF ARTHRITIS AND OTHER LESIONS INDUCED IN RATS BY INJECTION OF MYCOBACTERIAL ADJUVANT

V. Changes Affecting the Skin and Mucous Membranes. Comparison of the Experimental Process with Human Disease*

By CARL M. PEARSON, M.D., BYRON H. WAKSMAN,‡ M.D., AND JOHN T. SHARP, M.D.

(From the Department of Medicine, University of California Medical Center, and Wadsworth Hospital, Veterans Administration Center, Los Angeles, and the Departments of Bacteriology and Immunology and Medicine, Harvard Medical School, and the Neurological and Medical Services, Massachusetts General Hospital, Boston)

Plates 55 to 65

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A migratory polyarthritis is produced experimentally in rats by the simple injection of Freund's adjuvant without "antigen" (1–3). This interesting process appears to be non-infectious, since it has not proved possible to culture pathogenic bacteria or *Mycoplasmataceae* (pleuropneumonia-like organisms) from the blood or affected joints, to transfer the disease to normal rats with material from diseased animals (4, 5), or to suppress it by treatment with large doses of a variety of antibiotics (2, 3). Evidence has been obtained to suggest, rather, that the disease is a generalized immunologic response to constituent(s) of the tubercle bacilli in the injected adjuvant (3, 6, 7). In summary, the evidence consists of the requirement for injection of adjuvant, a potent technique of immunization, the latent period of 10 days to 2 weeks, the shortening of the latent period following reinoculation with adjuvant, the failure of very young rats to develop the disease, and the suppression of the disease response in animals pretreated with tubercle bacilli at birth.

The present paper is one of a series of articles dealing with this disease process in which morphologic, bacteriologic, and immunologic data obtained in two widely separated laboratories are presented jointly (3, 5, 7, 8). The experimental findings have been closely similar in our two laboratories, although

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‡ National Neurological Research Foundation Scientist.

different strains of rats obtained from different breeders, different sources of mycobacteria, and indeed different techniques of preparing and injecting the adjuvant have been employed. We are concerned in the present report with certain additional features of the experimental disease, not hitherto described, which show that the process has a much broader specificity than that suggested by the name "arthritis" and suggest that it may possess considerable interest as a possible model for certain categories of human disease.

Methods

A large proportion of the animals used in the present investigation were Sprague-Dawley rats, weighing 150 to 200 gm. They were obtained from the Charles River Breeding Laboratories, Brookline, Massachusetts and were studied at the Massachusetts General Hospital, Boston. These were kept in groups of 5 or 6 in roomy cages, cleaned weekly, and were fed Purina lab chow without antibiotics and water ad libitum. These rats were injected in one foot-pad with 0.05 ml. of a suspension of heat-killed tubercle bacilli of the Jamaica 22 strain[1] suspended in light mineral oil (Bayol F) or *Nocardia asteroides*[1] in oil (each at a concentration of 3 mg./ml.), or of various control materials.

Other rats, studied at the University of California, Los Angeles, were of the Long-Evans or Wistar strains and most were females. They were obtained from the Curds Caviary, La Puente, California. These rats were likewise kept in roomy cages in groups of 8 to 10 and fed Purina lab chow and water *ad libitum*. They were injected with adjuvant composed of light mineral oil (4 parts), an emulsifier (Falba) (1 part), and saline (4 parts), to which was added the wax D fraction[2] of the Canetti or Brévannes strains of the tubercle bacillus (9) in a concentration of 1 mg. per ml. of final adjuvant. All inoculations were given intracutaneously into the posterior cervical region; usually 0.5 ml. was administered once at one time in several sites to each animal.

All rats were examined daily for approximately 3 weeks following injection of the adjuvant mixture and somewhat less frequently thereafter for the presence and severity of arthritis, diarrhea, or changes in the eyes, genitalia, skin, or other tissues. Histologic observations were made on hematoxylin and eosin or phosphotungstic acid—hematoxylin-stained paraffin sections prepared from formalin-fixed specimens of joints, skin, or other tissues obtained at different intervals following inoculation or following the onset of symptoms. All photographs are of sections stained with hematoxylin and eosin.

RESULTS

Nodular Lesions.—Indurated, erythematous, nodular lesions were observed on the ears, tail surface, prepuce, and the exposed surface of the feet of rats developing arthritis. The ear lesions were the most sharply defined and easy to observe (Figs. 1 and 2). These are therefore described in detail here. Similar lesions in the other areas mentioned evolved in an entirely parallel manner. Ear nodules showed a close correlation, both in the time at which they appeared and in their size, with the time course and severity of the arthritis (Table I). A few rats, however, showed quite marked nodule formation in the presence of relatively mild arthritis. The nodules appeared, reached

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[1] Obtained through the courtesy of Dr. Jules Freund of the National Institute of Allergy and Infectious Diseases.

[2] Graciously provided by Dr. E. Lederer, Institut de Biologie Physico-Chimique, Paris.

a maximum within 1 to 5 days, and slowly receded over a period of a few days to 2 or 3 weeks. In some ears, 2 or more could be seen, almost always in clear relation to small or medium sized vessels. Nodules tended to be greatly enhanced in number and size immediately adjacent to areas of trauma, produced either by injection of an irritant or by an ear punch (Fig. 2A), sometimes appearing when no nodules occurred

TABLE I

Correlation of Ear Nodules with Arthritis in Rats of a Single Experiment

Rat	Site of inoculation	Arthritis			Ear nodules		
		Onset	Peak	Degree	Onset	Peak	Degree
1*	Food-pad	12	18	+++	19	19	+
2	"	12	19	+++	13	13	+
3‡	"	13	?	+++	12	13	++
4	"	12	14	++	15	17	+
5	"	12	17	++	14	15	+
6	"	14	16	++	14	17	++
7	"	14	19	++	26	26	+
8	"	15	19	+	21	21	+
9	"	17	17	+	—	—	0
10	Food-pad§	19	24	+	20	22	+++
11	"	19	26	+	26	26	+
12	"	24	24	+	21	24	+
13	"	—	—	0	—	—	0
14	"	—	—	0	—	—	0
15	Intradermal, flank	12	20	++	19	22	++
16	" "	13	16	+	14	14	+
17	" "	—	—	0	—	—	0
18	" "	—	—	0	—	—	0
19	" "	—	—	0	—	—	0
20	" "	—	—	0	—	—	0

* Autopsied on 20th day.

‡ Autopsied on 13th day.

§ 600 r whole body irradiation, 8 days before inoculation.

in untraumatized parts of the ear. Typical nodules were 1 to 3 mm. in diameter, dark red, hard to the touch, and approximately round. Irregular nodular masses were produced by confluence of several of these. Some nodules showed clear cut central ischemia (Fig. 1A), or ulcerated after being present for some time, especially those at the edge of the ear or at the margin of a punch. In rats given an anamnestic stimulus with adjuvant at 48 days, the nodules reappeared in conjunction with the reappearance of arthritis, their intensity again reflecting the apparent severity of the joint disease. Male and female rats showed these lesions to an equal extent.

Histologically, small ear nodules of short duration were found to consist of peri-

venous foci of lymphocytes and histiocytes and a more diffuse infiltration of activated histiocytes, without obvious perivenous localization, in the deep dermal connective tissue, separated from both the cartilage and the overlying epidermis by muscle. Few polymorphonuclear leukocytes were present. In larger nodules, there was massive infiltration from epidermis to cartilage, often involving both surfaces of the ear (Figs. 2B and 2C). The cartilage was not invaded. There were isolated foci of necrosis with many polymorphonuclears and permeation of the involved tissue with eosinophilic, amorphous material resembling fibrin. Sometimes a zone of similar "fibrinoid" necrosis was seen along the surface of the perichondrium. Such necrotic foci appeared to be secondary to the basic mononuclear infiltrative lesion. The epidermis over large lesions was much thickened, showed some invasion by mononuclear cells, and presented spongiosis, the formation of small multilocular vesicles, and of a limited number of larger multilocular pustules (Fig. 2C). These changes, which often accompanied nodules of less than 48 hours' duration, could not be distinguished from the changes characterizing the "chronic" skin lesion described below.

Acute Skin Lesions.—A transient, papular rash was observed in approximately one-third of the rats of both sexes developing moderate or severe arthritis (Fig. 1B). This "exanthem" appeared within 1 or 2 days following the onset of arthritis, and lasted from 2 to 3 days to a week. It was not well correlated with the presence of nodules in the ears or of scaling nodular lesions on the tail. In some animals, the rash appeared to involve extensive areas of the body surface; in others it was limited to areas traumatized by shaving a day or 2 earlier. The lesions were light red or colorless, convex, irregular papules 0.5 to 3 mm. in diameter. Histologically, they were found to consist of a pleomorphic mononuclear cell infiltration in the subcutaneous fat and connective tissue septa and the deepest layers of the dermis (Fig. 3B). There was a moderate perivascular accentuation of the infiltrate, but this was not a conspicuous feature of the lesions. There was no necrosis or deposition of "fibrinoid."

In a single rat, 2 days after the onset of maximal arthritis, a rash was observed which differed from that described above. Scattered over the back and the dorsolateral surface of the thighs were large, perfectly round, red, maculopapular lesions (Fig. 1C). These were 4 to 12 mm. in diameter and slightly raised. The largest showed central clear zones, but there was no gross vesiculation. Similar lesions have not been seen in other rats of a number of experiments. The evolution of the lesion is not known. Histologically (Fig. 3A and 3C), there was minimal perivascular infiltration in the uppermost level of the dermis, predominantly histiocytic and lymphocytic in character but with appreciable numbers of neutrophils and occasional eosinophils. The epidermis showed acanthosis throughout the affected region and mild to marked spongiosis of the basal layers. There were areas of separation of the epidermis from the basement membrane and dermal collagen, *i.e.* subepidermal bulla formation, and the resultant spaces contained precipitated material. The deep fat and connective tissue presented an infiltrative lesion indistinguishable from that seen with the "exanthem" (Fig. 3C).

In a small number of rats, the aponeurosis beneath the paniculus carnosus was found to be the seat of an extensive inflammatory lesion, in which large areas of "fibrinoid" necrosis alternated with zones of intense and very pleomorphic mononuclear cell infiltration (with formation of occasional multinucleate giant cells) and other zones of tortuous, congested vessels, and fibroblastic proliferation. There was

no clearcut organization of these zones into nodules nor any relation to the more superficial lesions already described.

Chronic Skin Lesions.—In each experiment, many animals with arthritis showed a characteristic late skin disease, first recognizable in the gross at 35 to 40 days. This change was particularly prominent about the head, neck, and shoulders (Fig. 1D). The affected skin was puffy, cracked, scaling, and crusted. Discoloration and loss of hair were also observed. There was a clear parallel (Table II) between the occurrence and severity of the skin change and the severity of the antecedent arthritis. Male rats with intense arthritis developed more severe disease than female animals observed at the same intervals after inoculation. The lesion tended to progress gradually in rats with severe arthritis but remained constant or receded in rats whose arthritis had disappeared. After an anamnestic stimulus with adjuvant, the skin changes recurred in association with the arthritis. In some animals, the distribution of lesions appeared to be determined by the site of the second injection of adjuvant.

TABLE II

Relation of "Chronic" Skin Disease to Severity of Antecedent Arthritis

Arthritis	No. of rats with skin disease			
	0	+	++	+++
0	18	1	1	0
+	2	8	4	0
++	0	4	2	0
+++	0	0	3	1

The histologic evidence indicates that the lesion must have been recognized long after its actual onset; *i.e.*, that gross skin alterations were late consequences of the underlying skin lesion. Of 6 rats autopsied at 35 days, before the appearance of visible skin disease, 3 showed small focal collections of lymphocytes and histiocytes in the upper cutis, with limited areas of invasion of the epidermis by these cells. 1 of these 3 had similar infiltrates of mononuclear cells, accompanied by some neutrophil and eosinophil granulocytes in the deeper dermal connective tissue, and a 4th animal showed inflammation only in the deeper tissue. The remaining 2 of the 6 animals presented focal infiltrative lesions of much greater intensity, still limited largely to the subepidermal connective tissue, and consisting of lymphocytes and histiocytes with perhaps 10 per cent polymorphonuclears (Fig. 4). There were no plasma cells. The epidermis over the infiltrated zones was greatly thickened. There was a striking invasion of the overlying epidermis (and much less frequently the epidermis of hair follicles) by inflammatory cells with destruction of the basal layers of epidermis. In many areas, small numbers of mononuclear cells, perhaps 3 to 8, could be found inside adjacent spongiotic epidermal cells with pyknotic nuclei, the whole thus making up a small multilocular vesicle within the epidermis. At what appeared to be a more advanced stage of the same lesion there was polymorphonuclear infiltration of similar vesicles with the formation of small or medium sized spongiform pustules (see Fig.

2C). The intensity of the skin lesions found histologically in this group of rats, autopsied at 35 days, was correlated with the severity of their arthritis. In animals autopsied at 50 days, the lesions were similar, there being, however, a higher proportion of polymorphonuclears among the inflammatory cells invading the epidermis, and, in 1 animal, many eosinophils. Milder lesions, of essentially the same type, were found in animals autopsied as late as 99 days. In these, areas of parakeratosis and hyperkeratosis overlying an acanthotic epidermis could be found, often without obvious inflammation in the corium.

The nature of the lesion appeared to vary little with the site of its occurrence. Similar changes were observed in skin of the face, neck, ear, abdominal wall, tail, and prepuce as well as on the nipples of the mammary glands. Characteristic microscopic skin lesions were particularly common over regions of disease; *i.e.*, over nodular lesions in the ear (Fig. 2) or penis (Fig. 8A) and near severely involved joints.

TABLE III

Incidence of Genital Lesions in Male Rats with Arthritis*

Experiment	No. of male rats	No. with arthritis	No. with balan tis‡
1	10	6	1
2	1	1	1
3	7	6	2
4	4	3	3
5	6	3	2
6	7	7	2
7	9	9	8
Total.....	44	35	19

* Experiment 7 was conducted on rats of the Lobund strain, obtained from Dr. James Reyniers, Tampa, Florida. All other rats were of the Long-Evans strain.

‡ Only 1 animal with balanitis had arthritis less than grade ++ in severity.

Genitourinary Lesions.—Male rats with arthritis frequently showed penile lesions or urethritis (Tables III and IV). These were most frequent and most intense in animals with severe arthritis and nodular lesions, and tended to occur in association with diarrhea (Table V). Like the other lesions reported here, penile and urethral lesions usually appeared 1 or more days after the onset of arthritis and disappeared again within a few days. They rarely lasted longer than 1 week. The most characteristic findings were edema and hyperemia of the glans and penile shaft and a red, brawny induration of the prepuce, usually accompanied by seropurulent exudation from glands at the margin of the prepuce. Phimosis was a common complication sometimes resulting in actual necrosis of part of the penis. The skin adjacent to the penis and the skin of the scrotum were frequently boggy and erythematous and occasionally excoriated. Urethritis was infrequent, but when it occurred was manifested by a mucopurulent urethral discharge.

Histologic changes agreed closely with *ante-mortem* estimates of degree of genito-

urinary disease. Several types of lesion could be distinguished according to their morphology and anatomical location. The first was a reaction in the shaft of the penis. It consisted, in essence, of histiocytic and fibroblastic proliferation within the lumina of the corpora cavernosa (Figs. 5 and 6). Normally these structures are tortuous vascular channels with infolded septa of dense, relatively acellular connective tissue (Fig. 6, insert); their inner surfaces are lined by endothelium. The earliest lesion was

TABLE IV

Genitourinary Involvement in Male Rats with Arthritis*

Arthritis		No. of rats	No. with penile lesions		Average onset
Severity	Average onset		+	++	
0	—	11	0	0	—
+	16	15	5	2	19
++	13	18	5	4	16
+++	12	37	9	16	15

* Rats of the Sprague-Dawley strain. Results in 3 separate experiments are shown.

TABLE V

*Association of Diarrhea and Genitourinary Disease in 39 Male Rats with Arthritis**

Observed changes	Severity of arthritis			Average onset
	0	+ or ++	+++	
Diarrhea	0	4	4	17
Balanitis	0	1	2	17
Diarrhea and balanitis	0	1	4	13, 19
Diarrhea and urethritis	0	0	1	15, 15
Diarrhea, balanitis, and urethritis	0	0	3	15, 16, 15
None	8	9	2	—

* Rats of a single experiment in which adequate observations were recorded.

a mucinous, edematous swelling of this endothelium, and its infiltration by histiocytic or fibroblastic cells, plasma cells, and an occasional neutrophile (Figs. 5A and 5B). Thereafter, the vascular lumen was invaded by actively proliferating connective tissue which intertwined throughout the channels of the corpora and on occasion completely occluded large segments (Fig. 6). The most characteristic feature of the cell infiltrate was its extreme pleomorphism. Lymphocytes, histiocytes, plasma cells, and masses of epithelioid cells were all to be seen, the predominant form being the histiocyte. The collagenous septa became more cellular than normal, but the inflammatory reaction here was limited to a few small foci of lymphoid cells. There were also perivascular collections about small vessels adjacent to the corpora cavernosa (Fig. 5A).

The second type of reaction occurred in the prepuce and the scrotal skin. This consisted of a loose appearance (interpreted as edema) of the subepidermal connective tissue, vascular dilatation, perivenous infiltration of histiocytes and lymphoid cells (Fig. 7), and occasional focal dense collections of lymphocytes and histiocytes which vaguely resembled granulomas. These lesions were closely similar to the nodules seen in the ear and tail. There were large edematous areas containing many mononuclear cells in the base of the prepuce. Smaller, more compact nodules were seen more superficially (Fig. 8A), and particularly in relation to the preputial glands and their ducts (Fig. 8B). These were present even 8 weeks after inoculation, when the arthritic process had largely subsided.

The skin lesion seen in other body areas were also present on both inner and outer surfaces of the prepuce near nodular lesions (Fig. 8A). It consisted of infiltration of the epidermis by mononuclear leukocytes and formation of small, multilocular, intraepidermal vesicles and pustules. In a few specimens taken 2 to 3 days after the onset of urethritis, the same type of infiltrative lesion was observed in the urethral epithelium. At 6 days, in some animals, this epithelium was largely destroyed (Fig. 8C).

In the fat and connective tissue adjacent to the testes and seminal vesicles and in the dense periprostatic connective tissue, there was a distinctive perivasculitis, with occasional proliferation of cells within the walls of involved vessels. Concentric collections of lymphoid cells completely surrounded small vessels and extended into the adjacent connective tissue. There was an inflammatory reaction, concentrated chiefly along the connective tissue septa which separate the fat into lobules, but extending also into the adjacent fat and sometimes seen as isolated foci in the fat (Fig. 9). It consisted of intense fibroblastic and histiocytic proliferation and a lesser degree of infiltration with neutrophils and lymphocytes. Focal concentrations of these cells resembled granulomata, but fully developed epithelioid cells were rarely seen. In 1 animal, granuloma-like lesions were found; many of these contained 1 or more multinucleate giant cells.

In 12 animals which were studied in detail no other urogenital lesions were noted. The kidneys, ureters, testes, seminal vesicles, epididymis, spermatic ducts, prostate, bladder, and periurethral and other glands were all entirely normal.

In female rats with moderate to severe arthritis, leukorrhea and occasionally urethritis were observed (Table VI), appearing 1 or more days after the onset of the articular and nodular lesions. In most instances, leukorrhea was observed only for a day or 2; in some rats, it persisted for approximately a week. The histologic changes corresponding to this clinical finding were unimpressive. Of 6 rats autopsied at 11 to 18 days while leukorrhea was present, 1 presented in the cervical and vaginal epithelium the same lesion as has been described in the skin. Almost all showed minimal accumulations of lymphocytes and histiocytes in the uterine mucosa, a few small lymphocytic foci in the uterine muscle mass, and an appreciable number of eosinophiles about the cervix. One female rat with severe arthritis developed marked swelling, hyperemia, and mucosal denudation of the vulva and about the urethral orifice. Intense edema, perivasculitis, and focal cellular collections were found in sections of the vulva and periurethral tissues of this animal, lesions essentially identical with the reactions noted in the male prepuce and scrotum. There were also extensive deposits of crystalline material in the bladder and urethra without accompanying inflammatory response.

Gastrointestinal Tract.—Diarrhea was commonly observed in rats inoculated with adjuvant mixtures, even in encephalomyelitis experiments in which arthritis was relatively infrequent and mild (Table VII). It occurred almost exclusively in male rats, frequently in association with balanitis or urethritis (Table V). It affected as

TABLE VI

Genitourinary Involvement in Female Rats with Arthritis*

Arthritis		Leukorrhea and/or urethritis		
Severity	Average onset	No.	Average onset	Duration
				days
0	—	0/4	—	—
+	15	1/8	18	1
++	14	2/8	19	1
+++	11	6/10	13	1 to 6

* 30 female rats from 8 litters, inoculated simultaneously in a single experiment.

TABLE VII

Incidence of Diarrhea in Rats with Arthritis or Encephalomyelitis Following Injection of Adjuvant Mixtures*

Experiment	No. of rats	No. with encephalomyelitis‡	No. with arthritis§	No. with diarrhea	Average onset
1	38	19		4	14
2	33	23		0	—
3	23	17		9	15
4	88	62		16	12
5	14		10	2	28
6	60		53	9	13
7	70		45	17	16
8	29		29	7	17

* Rats of both sexes shown in this table. All rats with diarrhea were male except for 2 in Experiment 3.

‡ Inoculation intradermally in 2 flank sites with mixture of rat spinal cord and adjuvant. Few of these rats developed arthritis.

§ Inoculation in 1 hind foot-pad with adjuvant alone.

many as half the males in a given experiment, almost always animals with severe arthritis (or encephalomyelitis). Thus, about 20 per cent of the total number of rats given adjuvant showed diarrhea. It always appeared later than the articular and cutaneous lesions, having its onset in some cases while other lesions were receding. In most animals it was transient, but in a few persisted as long as 2 weeks.

The histologic alteration underlying the diarrhea in these animals proved difficult to evaluate. 15 control rats of both sexes, varying widely in age, some uninoculated

and others sacrificed before the onset of arthritis, were studied in detail. These animals presented small disseminated lymphoid nodules throughout the small and large bowel. These were usually pressed against the basement membrane of the lamina propria or less frequently, lay just beneath the epithelium. Larger collections, occasionally with distinct germinal center, *i.e.*, typical Peyer's patches, were also present, though much less frequent, at all levels of the gut. These filled the entire submucosa and frequently extended through the muscular coats of the intestinal wall. The epithelium was intact above and adjacent to these lymphoid masses. The only histologic abnormality which could be identified in animals with diarrhea was a moderately

TABLE VIII

Relation of Histologic Changes to Presence of Diarrhea in Rats with Arthritis

Day of autopsy	Sex	No. of rats	No. with diarrhea	No. with histologic abnormality	Segment of colon affected*
0 to 8	M	6	0	1	A
	F	6	0	0	
11 to 13	M	5	2	0	—
	F	4	2	0	—
19	M	5	4	2	AT, AD
	F	4	0	1	D
28	M	6	2	4	ATD, AT, AT, A
	F	6	0	1	AT
35	M	1	0	1	TD
	F	4	0	0	

* A, ascending; T, transverse; D, descending.

intense, non-specific cellular infiltration of the lamina propria (Fig. 10). There was a marked diversity of cell types. The principal cell in the infiltrating mass was the histiocyte; lymphocytes, plasma cells, and some polymorphonuclears were present as well. The loose connective tissue between the crypts was distended with cells, and a dense layer of cells separated the base of the crypts from the basement membrane. Mitoses were frequent. No micro-abscesses were observed and there was no ulceration, the epithelium remaining normal in appearance. This change was not recognizable at the time of onset of arthritis and diarrhea; it appeared to increase in intensity with the time from inoculation (Table VIII). It was found almost exclusively in male rats, in particular those with diarrhea, late in the experiment. In some animals, massive accumulations of histiocytes and a few granulocytes were found in the small bowel, again in the tunica propria. Here there was loss of normal architecture with replacement of the glandular structure by the infiltrating mass (Fig. 11). The arrangement of villi and the surface mucosa remained normal. This change was correlated with the severity of the arthritis, being absent in animals with little or no disease.

Other Tissues.—Of 30 Sprague-Dawley rats inoculated with adjuvant, of which 23 had moderate or severe arthritis, one showed massive histiocytic and lymphocytic meningitis, perivascular cuffing in both grey and white matter of the spinal cord, and neuronophagia of anterior horn cells. 4 others showed minimal meningitis. In a large series of Long-Evans rats with arthritis, no central nervous system abnormality was found. The sciatic nerve in the uninoculated extremity of rats with severe arthritis presented a diffuse increase in the number of histiocytes in the epineurial fat and connective tissue, small focal collections of the same cells in the perineurium, some increase in the number of mast cells, which formed small groups, and a diffuse (agonal?) overlay of polymorphonuclear leukocytes. In the parenchyma of the nerve, there were isolated fibers undergoing Wallerian degeneration. The degree of epineurial disease was correlated with the severity of arthritis affecting the limb from which the nerve was taken. In rats autopsied after the arthritis had subsided, no abnormality of the nerve was found. The sciatic nerve from the inoculated extremity showed varying degrees of diffuse and/or focal mononuclear infiltration in the epineurium and epineurial fat. The lesions were true granulomata in animals with severe diseases, and in some of these one could recognize oil droplets, presumably derived from the injected adjuvant.

In skeletal muscle taken from various sites in 40 rats with arthritis, no significant lesions were found other than necrosis of an occasional fiber or group of fibers and minimal cell infiltrates adjacent to lesions of tendon, periosteum, or periarticular tissue. In the hearts of 29 rats, of both sexes, with arthritis of varying intensity and duration, one or two insignificant foci of mononuclear cell infiltration, whether in the myocardium, in the papillary muscle, or under the epicardium or endocardium, were observed 9 times. Similar changes were found in other rats which failed to develop arthritis. These were therefore not regarded as significant.

The great majority of rats injected with adjuvant mixtures showed typical adjuvant granulomata in lymph nodes draining the injection site and in the lungs and liver, and an appreciable number presented some degree of periportal inflammation or of pneumonitis. Perivascular collections of mononuclear cells were observed in the kidneys and lungs of occasional animals, both with and without arthritis. These lesions were regarded as unrelated to the disease under investigation. Lesions were not found in other viscera.

DISCUSSION

The experimental induction of a generalized disease affecting the joints, eye, skin, genitourinary, and gastrointestinal tracts, in the absence of significant lesions of other organs or tissues, is unique in experimental pathology. While the present paper is principally concerned with a description of the nodular and mucocutaneous lesions, all the lesions will be considered in the following discussion in their proper place as part of the over-all disease process.

Nature of the Rat Disease

The *arthritis* has been described in some detail in previous publications (1–3) and will be the subject of a more complete histologic study. It consists of an

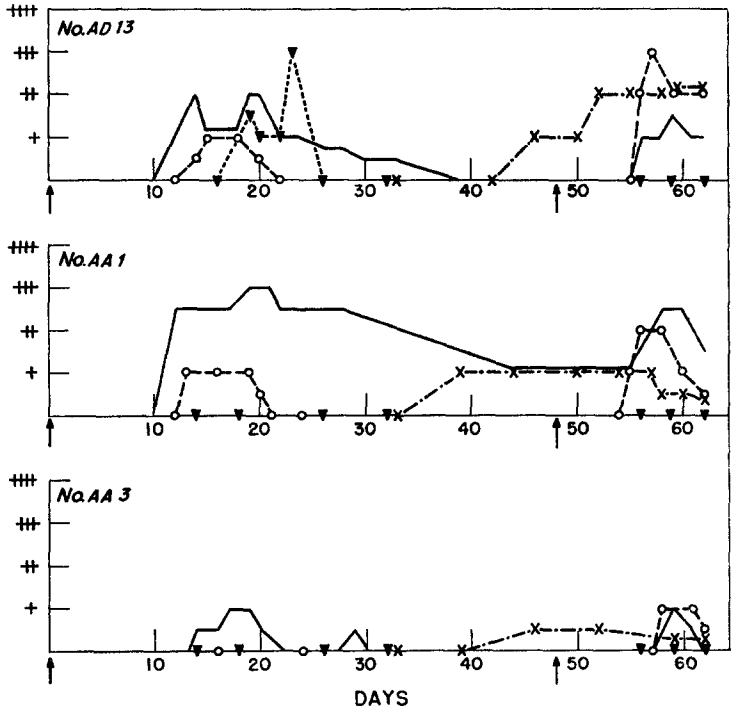
acute or subacute, migratory polyarthritis affecting the ankles, wrists, and smaller joints of the extremities, including not infrequently the terminal interphalangeal joints. It presents, histologically, as a synovitis followed by periarthritis, peritendinitis, periostitis, pannus formation, destruction of cartilage and bone, and finally fibrous or bony ankylosis. Joints with mild or moderate disease return completely to normal. At the same time there is *spondylitis*, affecting the spine as a whole and the tail in particular. The lesion of the intervertebral joints appears to start as periostitis with secondary involvement of the intervertebral discs. The arthritis is accompanied by *tendinitis* and *tenosynovitis*, most marked near involved joints and at the insertion of tendons into muscle or bone. Minimal non-specific inflammatory infiltrates are also found in adjacent muscles and nerves. The eye lesion (described in detail in reference 8), includes *conjunctivitis*, *keratitis*, and *iridocyclitis*, usually of the "non-granulomatous" type, occasionally "granulomatous" or combined. The remaining lesions, described in reference 3 and in the present report, include *nodular lesions* on exposed surfaces (ear, feet, tail, penis) and especially at sites of trauma, *transient rashes*, *a chronic skin disease* with formation of spongiform vesicles and pustules, and mucous membrane lesions, specifically *urethritis* and *diarrhea*. The cardiovascular apparatus remains entirely normal, and no other consistent lesion is found in a variety of viscera, aside from isolated granulomata in the lungs and liver (these are commonly found in animals of any species injected with adjuvant).

Histologically the earliest lesions are quite similar in all the affected tissues. They may be seen in their simplest form in the nodular lesions, especially in the external ears. They appear to start with congestion of small veins and arteries and perivascular accumulations of histiocytes, lymphocytes, and a few polymorphonuclears. Soon there is wider dissemination of these cell types into the surrounding tissues and the appearance of interstitial edema fluid in varying quantity. There may be focal areas of fibrin deposition and occasional foci of necrosis, apparently ischemic in origin (10). In the eye, skin, and nodules, the lesions persist for variable periods and continue to show this general histological pattern. In the extremities, and to a lesser degree in the genital lesions, there is superimposed during the early active stages a distinctive response of the cellular elements of the connective tissue. There may be intense fibroblastic proliferation, multiplication of synovial lining cells of the joints and tendon sheaths, and activation of the periosteal osteoblasts and osteoclasts, with rapid formation of periosteal new bone and remodeling of some of the original cortical and medullary bone. These reactions, especially the fibroblastic proliferation, produce activated granulation tissue which invades the tendons and bone as pannus chiefly in the subchondral regions, and this in turn may lead to fibrous ankyloses and other restrictive changes. In the external genitalia there is a remarkable proliferation of connective tissue cells

in the lumina of the corpora cavernosa of the penis, in addition to perivascular and diffuse cellular infiltrates. While the small, early infiltrates in the synovia may occasionally resemble Aschoff nodules and focal collections of lymphocytes and histiocytes in the subcutis sometimes resemble poorly defined granulomata, the principal lesions do not, in general, have a granulomatous character. Characteristic granulomata, containing 1 or 2 multinucleate giant cells, have been observed on rare occasions in the peritesticular fat, as in the lungs, liver, and some lymph nodes. Near the center of a few such cellular foci, fairly large, clear vacuoles resembling oil droplets could be seen. These were possibly embolized droplets of the injected adjuvant or its oil fraction.

The diverse lesions described are elements of a distinctive disease process which occurs as a subacute episode with its onset approximately 10 days to 2 weeks following a single inoculation with killed tubercle bacilli or its fractions in mineral oil. The disease is self-limited, rarely lasting more than a month, but recurs following subsequent inoculations. The mucocutaneous lesions, specifically the diarrhea, urethritis, and dermatitis, show a marked predilection for male rats. Virtually all animals with arthritis present nodular lesions. Almost all those with maximal arthritis also show diarrhea (in the case of males) and the late skin lesion; of those with milder arthritis, perhaps half show these changes. Genitourinary disease is present in half to two-thirds of rats, even with maximal disease. Finally, clinically evident eye lesions and the skin rashes are found in about 20 per cent of rats with arthritis. Rats failing to develop arthritis also fail to develop other lesions. During the first attack, arthritis affecting the extremities is almost always the first clinically recognizable alteration. It is sometimes accompanied by the appearance of a rash, nodules, or iridocyclitis. More commonly, however, these changes as well as the genital lesions and diarrhea appear 3 to 4 days later. The nodular, ocular, and mucosal changes reach a peak within a few days and usually are subsiding or have entirely disappeared before the 20th day. The arthritis increased more slowly to a peak of severity at 20 to 25 days and involutes slowly. The joint lesions may become chronic or occasionally spontaneously recurrent. Involvement of the spine appears at the same time as the peripheral arthritis or somewhat later and also tends to be long lasting. Chronic skin disease is not evident in the gross until the 35th or 40th day after adjuvant inoculation, when the other lesions have largely disappeared. It appears probable that a slowly developing dermal lesion is present for some time prior to its clinical recognition, however, since the typical skin change is often observed on the readily visualized skin of the tail during the peak of severity of the arthritis (this later subsides completely or occasionally becomes chronic and excoriated) and histologically, typical lesions are found early in relation to nodular lesions in the ears and in the skin of the genitalia. Following reinoculation, the arthritis and nodular lesions recur but usually in appreciably milder form than during the first at-

tack. The other changes, in general, fail to recur, except for the skin disease and diarrhea, which may progress during and after a second attack. The typical sequence of changes is illustrated in Text-Fig. I, in which the course of disease in 3 female rats inoculated twice with adjuvant is plotted. Diarrhea and genito-urinary lesions did not occur in these animals.



TEXT-FIG. 1. Clinical course of disease in 3 rats inoculated in one hind foot-pad and reinoculated at 48 days in a fore-foot. Arthritis———, nodular lesions O---O---, skin disease×—•—•—, iridocyclitis ▼----▼----.

Comparison with Human Disease

It is with considerable hesitation that one attempts a comparison of experimental lesions, like those described in the present study, with the lesions of specific human diseases of unknown etiology. It is a commonplace that lesions of similar morphology may be produced by quite different pathogenetic processes. Nevertheless, since one purpose of the study of experimental disease is to work out techniques for identifying pathogenetic mechanisms in the hope that these techniques may be applied to the study of human disease, it is clearly desirable to be aware of similarities when they exist. Resemblances either in clinical course or in histologic character need not be assumed to imply that processes are the same or even necessarily related in mechanism.

In the light of these comments, let us examine in turn various diseases and syndromes in which arthritis is associated with ocular and mucocutaneous lesions, considering, first, the histologic features which characterize each lesion and, second, the constellations of features commonly observed, and pointing out similarities as well as differences when they appear to be clear cut. The conditions which are of interest for such a comparison are: rheumatoid arthritis, ankylosing spondylitis, and arthritis with psoriasis; arthritis with ulcerative colitis, post-gonorrheal arthritis, Reiter's syndrome, and Behçet's syndrome (this merges into the Stevens-Johnson or oculo-mucocutaneous syndrome or erythema multiform exudativum); rheumatic fever, erythema nodosum, and the acute arthritis accompanying other infections (*e.g.*, coccidioidomycosis); and finally, arthritis with sarcoidosis. Other generalized "diseases of connective tissue," such as systemic lupus erythematosus, dermatomyositis, scleroderma, polyarteritis nodosa, and serum disease are too remote in their major characteristics from our experimental rat disease to require consideration. The reader is referred to references 11–18 for general reviews in which these various human conditions are described and, in particular, to the introductory Chapter of reference 13, in which the characteristics differentiating rheumatoid arthritis from other forms of arthritis are summarized.

Joint Lesions.—Kulka (19), after careful histologic analysis of the various lesions of rheumatoid arthritis, concludes that the primary change in this disease is a segmental vasculitis, largely venular, accompanied by formation of nodular or granulomatous cell collections, marked proliferation of connective tissue elements, deposition of fibrin, and focal areas of ischemic necrosis. The latter lead, in the case of the joint, to destruction, first of cartilage and finally of the joint as a whole. The full-blown joint lesion of rheumatoid arthritis (12–14, 20) includes exudation of fibrin, polymorphonuclear leukocytes, and some mononuclear cells into the joint space, proliferation of the synovial endothelium and stroma with hypertrophy of the villi, and intense synovitis, the inflammatory infiltrate being made up largely of lymphocytes and plasma cells, with smaller numbers of histiocytes, polymorphonuclears, eosinophils, and occasional giant cells. The cells first form perivascular foci, then increase in number and become diffusely distributed, and later give rise to the formation of typical lymphoid follicles without germinal centers. The congested and inflamed synovia extends as a pannus above and beneath the joint cartilage which is progressively destroyed. "Granulation tissue" and follicle-like accumulations of lymphocytes appear in the bone marrow near the joint. Bone destruction proceeds both from the marrow and from the inflamed periosteum and is accompanied by new bone formation. Finally fibrous or bony ankylosis occurs. The only feature of this picture said to be pathognomonic for rheumatoid arthritis is the formation of lymphoid follicles in the synovia (13, 20), but even these may be found in other types of arthritic process (21, 22). In ankylosing spondylitis, the axial diarthrodial joints (sacroiliac, apophyseal, costovertebral, costotransverse) show entirely similar changes (15, 23). However, the lesion of the cartilaginous intervertebral discs is described (23) as starting with periostitis and osteitis near the joint and about sites of tendon insertion in the bone. The discs themselves are affected only secondarily.

More acute forms of arthritis such as Reiter's disease and rheumatic fever are decribed (14, 24, 25) as showing congestion, edema, and even hemorrhage in the synovia, with a variable infiltration of polymorphonuclear leukocytes. Diffuse infiltration of lymphocytes and perivascular mononuclear cell foci appear early, together with hypertrophy and hyperplasia

of the synovial elements, the appearance of areas of fibrinoid degeneration, and proliferation of histiocytes and fibroblasts. Usually there are few chronic changes. However, destructive joint lesions do occur in Reiter's disease (17, 15, 25); there may be fibrin deposition, pannus formation, and lymphoid aggregates like those of rheumatoid arthritis. Rheumatic fever may lead to typical rheumatoid arthritis in rare instances (see reference 13).

Of the other forms of arthritis with which we are concerned, the arthritis occurring with psoriasis is pathologically indistinguishable from rheumatoid arthritis (13, 26). Post-gonorrheal arthritis and the arthritis of Behçet's disease are very similar to Reiter's arthritis. The arthritis associated with chronic ulcerative colitis (13, 27) may be indistinguishable from the arthritis of erythema nodosum or more like that of Reiter's syndrome. It also leads occasionally to chronic changes. In sarcoid, while some patients have bone lesions of the hands and feet leading to a clinical appearance like rheumatoid arthritis (17), an occasional polyarthritis of the rheumatic fever or erythema nodosum type is also seen (28, 29)

The peripheral joint lesions in our rats presented all the features said to characterize rheumatoid arthritis except for their relatively non-progressive character and the absence of lymphoid follicles in the synovia. On the other hand they differed from the arthritis of Reiter's disease and rheumatic fever by the considerable degree of joint destruction shown in severe cases. These findings are consistent with the possibility, on the one hand, that the experimental arthritis is similar to rheumatoid arthritis but less progressive in character, or on the other hand, that it is more comparable to an acute arthritis but with a greater intensity resulting from the efficient sensitization achieved with the use of adjuvants. The tail lesions were found to affect primarily the periosteum and connective tissue adjacent to the intervertebral joints. In severe cases the discs themselves were invaded and partially destroyed by inflammatory "granulation tissue." In other words, they presented the very features said to characterize ankylosing spondylitis except for their non-progressive character. Massive tendinitis and tenosynovitis accompanied all but the mildest lesions.

Nodules.—The most common nodular lesions associated with arthritis are erythema nodosum and the nodules of rheumatoid arthritis and rheumatic fever. Erythema nodosum lesions (30–32) occur on exposed skin surfaces, especially where there has been recent thermal or mechanical injury. The site of the nodules is the skin and the subcutaneous fat and connective tissue septa. Early lesions consist largely of edema and diffuse infiltration of polymorphonuclear leukocytes, lymphocytes, and histiocytes. Later there is a dense infiltrate, primarily of mononuclear cells, with a perivenous accentuation. Granulomatous foci are formed with epithelioid and even giant cells, and there may be foci of fibrinoid degeneration. There is a tendency to the formation of clefts in the involved tissue with some palisading of histiocytes. The lesions resolve completely in days to weeks.

A great deal has been written about the nodules of rheumatoid arthritis and of rheumatic fever (14, 20, 33–37). While some authors believe these to be quite distinct lesions (14, 36) others (15, 34, 35, 37) regard them as differing only in intensity and chronicity. Rheumatic fever nodules arise most frequently from tendons, tendon sheaths, periarticular ligaments periosteum, or superficial aponeuroses, especially on extensor surfaces and at sites of trauma. They are small, occur in crops, and involute spontaneously in a few days to a few weeks. Their principal histologic features are granulomata (frequently compared with Aschoff bodies)

consisting of small foci of fibrinoid degeneration surrounded or infiltrated by a zone of mononuclear cells and an ill-defined region of edema, increased vascularization, and infiltration with lymphocytes, histiocytes, and plasma cells. Rheumatoid nodules occur singly over bony prominences (elbows, knees, ankles, knuckles, occiput, sacrum), are larger, and persist for months or years. They do not appear to be induced by traumas of brief duration. The typical nodule shows one or more central foci of necrosis, each surrounded by a palisade of large mononuclear cells, variously interpreted as histiocytic or fibroblastic in origin, and by a zone of dense and relatively avascular fibrous tissue diffusely infiltrated with lymphocytes and plasma cells. Histologic evidence suggests that the necrotic center is derived from the fusion of areas of fibrinoid necrosis which in turn begin with a primary vasculitis, thrombosis, and infarct necrosis (14, 15). Aschoff bodies are found throughout the connective tissues of the body in rheumatic fever but are most common in the heart and subcutaneous tissues. Typical rheumatoid nodules occur in tendons and in the sclera, the heart, the pleura, and occasionally other viscera.

The nodular lesions in our rats did not show either the chronic progressive character of the rheumatoid nodule or the sharp division into three zones characteristic of the lesion. In duration, location, and histologic character, these nodules are consistent with the descriptions of erythema nodosum and of rheumatic fever nodules. Their localization by minor mechanical or other traumata is also consistent with either possibility. The papular "exanthem" appeared to be a lesion of the same type.

Mucocutaneous Lesions.—The chronic skin lesion observed in the rats of the present study merits comparison with psoriasis and with keratodermia blennorrhagica, a skin lesion which occasionally accompanies post-gonorrheal arthritis and Reiter's syndrome. These two types of disease are similar pathologically. In psoriasis (32, 38), the dermal papillae are elongated and edematous, the capillaries in their tips are dilated and tortuous, and there is an infiltration of lymphocytes and histiocytes in the upper corium and about the rete ridges. Polymorphonuclear leukocytes invade the epidermis from the tips of the papillae to form micro-abscesses (Munro) in the outer prickle cell layer or in parakeratotic lamellae. The epidermis shows acanthosis, elongation of the rete ridges, thinning over the papillae, and patches of parakeratosis mixed with hyperkeratosis. The typical lesions can be produced at sites of mechanical or other trauma (Köbner phenomenon). In keratodermia blennorrhagica (32, 18), there is an inflammatory infiltrate (lymphocytes, plasma cells, polymorphonuclears) in the upper corium and papillae, the formation of spongiform vesicles or pustules in the upper epidermis, and, at the same time or later, acanthosis, elongation of the rete ridges, and parakeratosis. The balanitis and the mucosal lesions of Reiter's syndrome resemble the skin change of keratodermia blennorrhagica both in the gross and histologically (17, 18, 25, 32). Those of Behçet's disease are described as ulcerating papular lesions with no distinguishing histologic features.

In our rats, a mononuclear cell infiltrate of the upper corium and invasion of the epidermis by these cells and by polymorphonuclear leukocytes, with the formation of both spongiform vesicles and pustules in the upper epidermis, were the most conspicuous findings. Acanthosis was prominent and patches of parakeratosis and hyperkeratosis were occasionally seen. However, a fully developed lesion comparable to that of psoriasis was not observed. The genitourinary lesions in our rats showed the characteristics of both the nodular lesions

and the skin lesion already described. However, the fibrosing process in the channels of the corpora cavernosa has no known human counterpart.

There is no general agreement at present as to the nature of the earliest lesion in ulcerative colitis (39, 40). Both vasculitis and formation of micro-abscesses in the crypts have been incriminated. The fully developed lesion (41) presents shallow and deep ulcerations, sometimes confluent, with edema and fibrosis of the submucosa and varying degrees of infiltration with chronic inflammatory cells.

The lesions in our rats consisted largely of an infiltration of the submucosa with lymphocytes, histiocytes, and plasma cells. Since this type of change is quite non-specific and since ulceration was also absent, there is no basis at present for a comparison of the rat lesion with the lesion of ulcerative colitis. It must simply be regarded as a non-specific diarrhea.

Eye Lesions.—"Endogenous" uveitis is frequently found in association with arthritis in man (42–46), specifically in ankylosing spondylitis, Reiter's syndrome, post-gonorrheal arthritis, Behçet's syndrome, sarcoid, and rheumatoid arthritis. While in rheumatoid arthritis the eye may present a "non-granulomatous" uveitis, it is more common to find the characteristic rheumatoid lesion in the form of either a brawny scleritis or scleromalacia perforans. In sarcoid, uveitis is usually "non-granulomatous" in character. However, it is not uncommon to find a nodular iritis, sometimes with mutton fat keratitic precipitates and other signs of acute inflammation, but often with little or no accompanying inflammation. In the other diseases mentioned, the usual eye lesion is an acute "non-granulomatous" uveitis lasting a few days or weeks, resolving spontaneously, and leaving few sequelae. In Reiter's and Behçet's syndromes and post-gonorrheal arthritis, it is frequently accompanied by conjunctivitis and keratitis.

The lesion in our rats, because of its non-progressive character and the association of uveitis, keratitis, and conjunctivitis, was more comparable to the eye disease in the latter group of diseases. However, the presence of nodular iris lesions and mutton fat keratitic precipitates in some animals would suggest a possible similarity to sarcoid as well. An eye lesion resembling the rheumatoid nodule was not observed.

Association of Lesions in Different Disease Syndromes.—Let us turn now to the constellations of features which characterize individual diseases or syndromes.

Rheumatoid arthritis (12–14, 19, 20) occurs more frequently in females. It presents as a migratory, frequently symmetrical polyarthritis affecting large and small peripheral joints. Characteristic nodular lesions occur in about 20 per cent of patients, similar lesions being found on occasion in the sclera, pericardium, heart valves, and pleura. Tendinitis, tenosynovitis, and bursitis also are found, as are inflammatory foci in muscle, nerve, spleen, lymph nodes, lacrimal and salivary glands, heart lesions like those of rheumatic fever, enlargement of spleen and lymph nodes in about 20 per cent of cases, and constitutional symptoms. *Ankylosing spondylitis* (12–15, 19, 23) occurs predominantly in males. It affects the joints of the axial skeleton, but peripheral joints are involved also in about half the patients. Aortitis and iridocyclitis are very common features. If subcutaneous nodules occur in spondylitis, they do

so only in patients also presenting peripheral joint involvement. The *arthritis with psoriasis* (12, 13, 26, 47, 48) is closely similar to rheumatoid arthritis except for the frequent involvement of terminal interphalangeal joints. In most patients with this syndrome the skin lesion precedes the arthritis. The two lesions may wax and wane in parallel but do not necessarily do so. The outstanding quality of rheumatoid arthritis, spondylitis, and the arthritis with psoriasis is their relentlessly progressive character in many patients.

Arthritis occurs *with ulcerative colitis and regional enteritis* (12, 13, 27, 49–53) in an incidence of 5 to 10 per cent. Some cases closely resemble cases of spondylitis, and a smaller number rheumatoid arthritis. Others are episodic, occurring in parallel with the episodes of colitis, and are "cured" by colectomy. The disease, in these last cases, is subacute in course, tends to affect only a few of the larger joints, often asymmetrically, and leads to little or no residual damage. The eye is rarely involved. Erythema nodosum is present in 2.5 to 20 per cent of ulcerative colitis patients (54) and is closely associated with "colitic arthritis" (13, 27, 50–52). In *Reiter's syndrome* (13–15, 25, 47, 55–57), arthritis occurs in association with eye lesions (conjunctivitis in most cases, iridocyclitis and keratitis in 5 to 10 per cent) and with urethritis, and less frequently other genitourinary lesions (cystitis, seminal vesiculitis, balanitis, inflammatory lesions of testis and epididymis). Lesions of the buccal mucosa and diarrhea are not uncommon and a late skin change (keratosis blennorrhagica) occurs in a small percentage of cases. The arthritis is polyarticular, acute in character, and resolves without sequelae in most cases. The spine is also involved, and there may be extensive tendinitis and tenosynovitis. This syndrome occurs following attacks of non-gonococcal urethritis and following various types of bacterial dysentery (14, 58, 59). About 90 per cent of the cases occur in males. There are recurrences in over half the cases (25, 56, 60). The arthritis which follows gonorrhea (17, 61) may be indistinguishable from Reiter's syndrome and is believed by many to be the same disease (14, 59). The frequent involvement of the axial skeleton in Reiter's may suggest a relation to ankylosing spondylitis (13, 62, 63). Similarly, a relationship to *Behçet's syndrome* is suggested by involvement of the central nervous system in some cases (58, 64) and by the mucocutaneous lesions (65). In Behçet's and in the closely related Stevens-Johnson syndrome (erythema multiforme exudativum), there are recurrent attacks of arthritis, eye lesions (iridocyclitis, retinitis, keratitis, conjunctivitis), central nervous system disease (meningitis and disease of grey matter in brain stem and spinal cord), and nodular and ulcerative lesions of the skin, genitalia, and mucous membranes (66–69). Erythema nodosum is a common accompanying lesion and there is frequent involvement of the respiratory and gastrointestinal tracts.

In *rheumatic fever* (13–15, 33, 35, 70) recurrent acute or subacute attacks of systemic connective tissue disease occur, frequently if not always in relation to hemolytic streptococcal respiratory infections. There are myocarditis, valvulitis, pericarditis, pneumonitis, and inflammation of small vessels in a number of regions; and Aschoff nodules or similar inflammatory foci are found in joints, tendons, tendon sheaths, aponeuroses, fasciae, and subcutaneous connective tissue, and less frequently in skeletal muscle, liver, kidney, spleen peritoneum, eye (as episcleral nodules), and the central nervous system. The clinical picture may be dominated by the heart lesions, by the symmetrical, migratory polyarthritis, or by chorea, which in turn are accompanied by fever, subcutaneous nodules, and erythema nodosum, erythema multiforme, or erythema marginatum. The arthritis generally heals completely. The nodules usually appear after the onset of the arthritis, even at a time when the joint involvement is subsiding. *Erythema nodosum* (30–32, 54, 71) occurs as a disseminated, nodular eruption in many of the types of arthritis we have discussed. It occurs as an independent event in relation to a large variety of infections, among them tuberculosis, hemolytic streptococcal infection, coccidioidomycosis, histoplasmosis, trichophytosis, lymphogranuloma venereum, and leprosy. In each instance, its occurrence appears to be correlated with a recent or current infection and with the change from a non-allergic state to a state of sensitivity; to *e.g.*, tuberculin, cocci-

dioidin, or histoplasmin. The use of drugs, such as sulfonamides which cause the release of bacterial contents, the use of vaccines or toxoids, even skin tests with such products as tuberculin may occasion a flare-up of the disease. Its localization at sites of thermal or mechanical injury is well known. Erythema nodosum is very frequently accompanied by fever and arthralgia and more than one-fourth of cases have an arthritis similar to that observed in rheumatic fever. A few cases present erythema multiforme. Short *et al.* (12, 13) list over 30 specific infections which may give rise to arthritis, with or without erythema nodosum. Several are said to give rise to chronic, progressive, symmetrical involvement of small joints. Coccidioidomycosis (17, 72) is an example of an infection which produces (in about one-third of patients during the acute phase) an arthritis similar to rheumatic fever, accompanied by conjunctivitis, and erythema nodosum, lasting about 1 month and leaving no sequelae.

It is clear that *sarcoid* must be related to the other conditions under discussion, since some cases have a migratory polyarthritis at some time during their disease (73), erythema nodosum is also present in about one-fourth of cases, and a "non-granulomatous" type of uveitis is very common, these lesions often being associated in the same patient (see references 28 and 29).

The experimental disease in the rats we have studied presented individual features suggestive of each of these human diseases, but differed from each of them in the constellation of findings. While the joint lesions were quite destructive and frequently led to deformity as is the case in rheumatoid arthritis, spondylitis, and arthritis with psoriasis, they were non-progressive in character, unlike these three conditions in man. Nor were the pathognomonic features of the rheumatoid joint lesion, eye lesion, and nodule, or the skin lesion of psoriasis observed, although it was possible to say in each case that similar histologic elements were present in the rat lesions studied. On the other hand, the acute character of the arthritis and its relation to an antecedent injection of microbial constituents, its subacute course and recurrence with reinoculation, are features suggesting a similarity to erythema nodosum, to rheumatic fever, and to Reiter's syndrome. The histologic nature of the rat lesions would support a comparison with any of these conditions. Certainly the simultaneous involvement of peripheral joints and spine, of eye, genitourinary tract, and skin makes up a picture very suggestive of Reiter's syndrome (and post-gonorrheal arthritis, and the relatively rare Behçet's syndrome). The combination of transient nodular lesions with short lived arthritis might be regarded as resembling rheumatic fever or erythema nodosum with arthritis. Nevertheless, the degree of destruction in the most severely affected joints greatly exceeded the degree of joint damage generally seen in any of these human diseases. In some rats, the combination of arthritis affecting both the peripheral and axial skeleton with transient nodular lesions and severe episodic diarrhea closely mimicked the peculiar combination of arthritis and erythema nodosum which occurs in conjunction with ulcerative colitis. Again, the destructiveness of the joint lesions seems inappropriate to this comparison. Finally, the association of sarcoid with arthritis, uveitis, and erythema nodosum is suggestively similar to the combination of nodular lesions, arthritis, and uveitis in many of our rats. However the joint, skin, and eye lesions did not

have the character of sarcoid granulomas. It may be appropriate to remark that in the guinea pig, injection of adjuvant produces a disease somewhat like the rat disease we have described (74). Here, however, nodular and infiltrative skin lesions are more prominent and are said histologically to be made up of granulomata much like those of sarcoid.

It appears wisest to conclude that the experimental disease we have studied has characteristics suggesting a relationship to each of several well defined syndromes in man, but that more precise information about the mechanisms of lesion formation will be required before we are justified in identifying it with any of them.

SUMMARY

A generalized disease is induced experimentally in the rat by administration of Freund's adjuvant. The primary clinical and pathologic lesions are arthritis, periarthritis, peritendinitis, and periostitis in the joints of the extremities and tail. Accompanying the arthritis in some cases, and never observed in its absence, are other specific tissue lesions including iridocyclitis, nodular lesions in the glabrous skin (ear, genitalia, feet, tail), transient rashes, a chronic skin disease, genitourinary lesions, and diarrhea. These make up a striking and characteristic picture. The arthritis usually precedes the other lesions and, together with the skin disease may show a prolonged and fluctuating course. Visceral lesions do not occur.

Histologically, the basic lesion is a lymphocytic and histiocytic infiltration, initially perivascular and subsequently more disseminated. In addition, in the region of the joints and in the corpora cavernosa of the penis, there is extensive proliferation of mesenchymal cells, especially fibroblasts. Foci of fibrinoid necrosis are seen in the articular and nodular lesions, and destructive lesions of the joints are common.

Such a combination of tissue lesions has not previously been described in experimental pathology. The experimental disease is shown to have both similarities to and differences from Reiter's syndrome, rheumatoid arthritis, and certain other disorders which occur in man.

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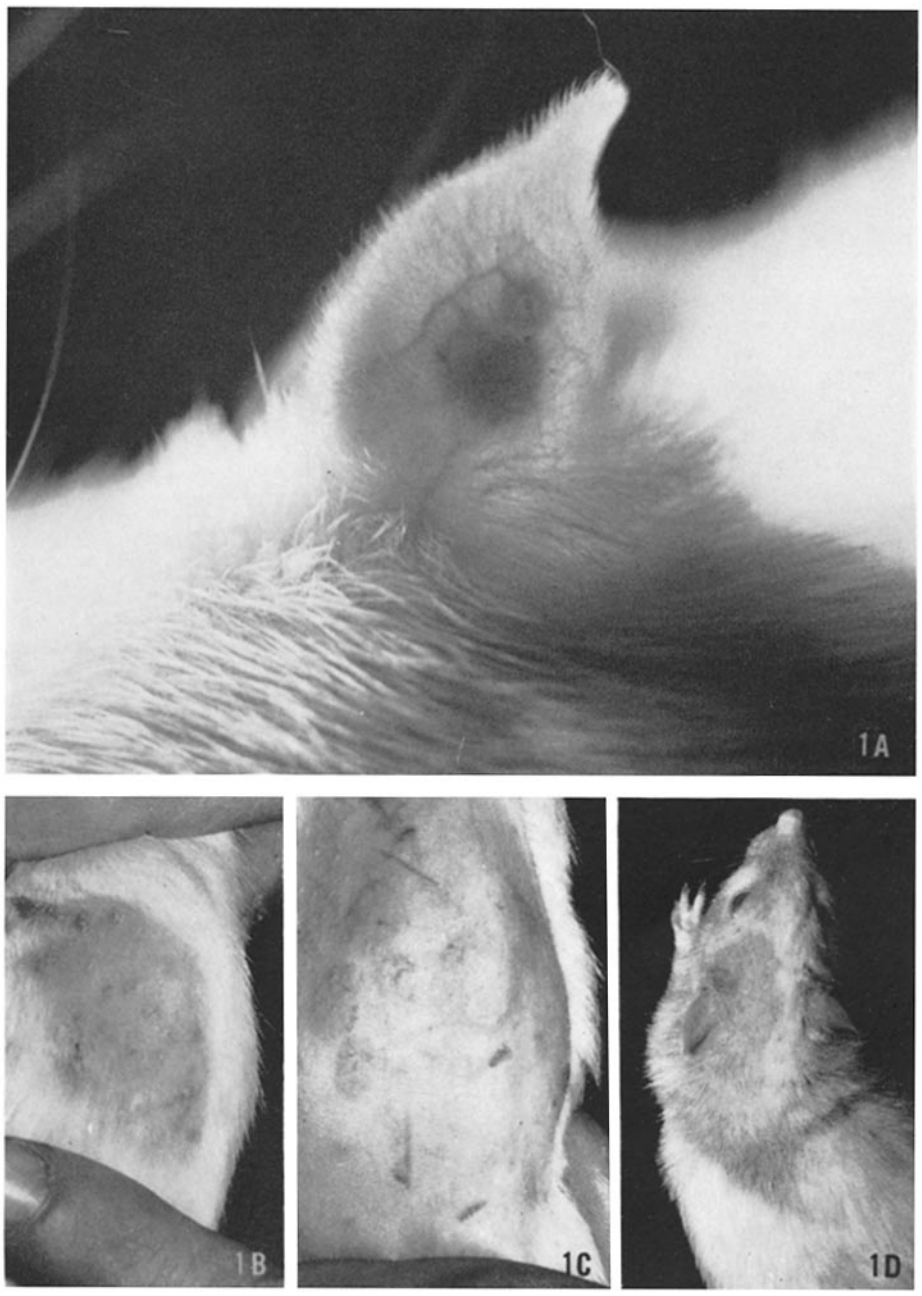
EXPLANATION OF PLATES

PLATE 55

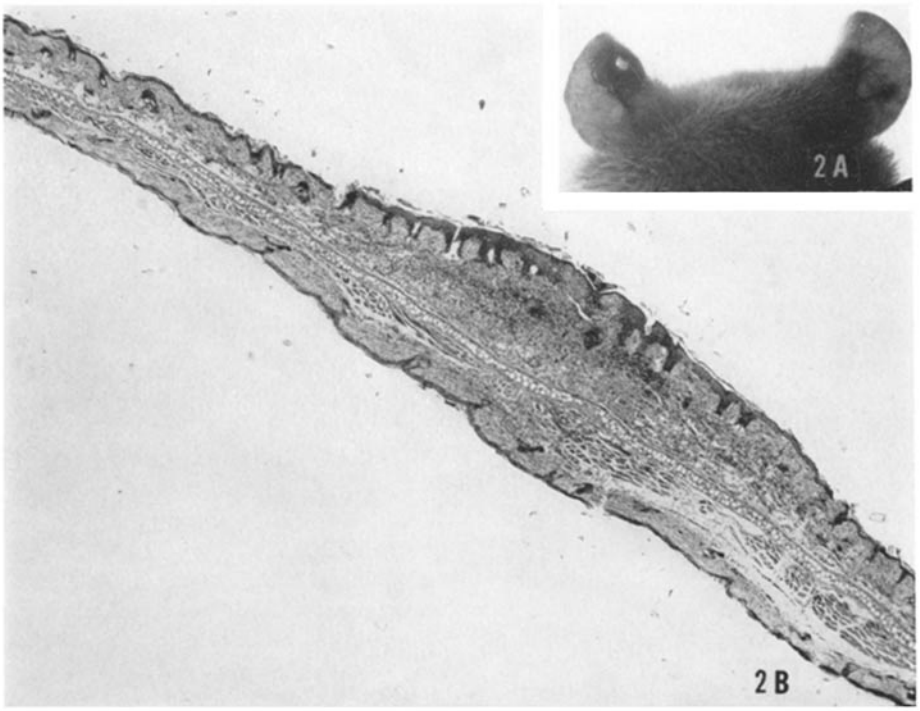
FIG. 1. A. Ear nodules of 2 days' duration in Wistar rat, photographed 14 days after inoculation with adjuvant. One nodule shows central ischemia. B. Papular rash in Sprague-Dawley rat at onset of arthritis, 15 days after inoculation with *Nocardia asteroides* in oil. Area shaved 4 days earlier. C. Maculopapular eruption in rat 13 days after inoculation with wax D-Brévannes in water-in-oil emulsion and 2 days after onset of arthritis. Largest lesion shows central clearing. D. Chronic skin lesion in rat 9 weeks after primary inoculation (left hind foot) and 4 weeks after second inoculation (left fore foot) with tubercle bacilli in oil. Note limitation of lesion to area related to site of reinjection.



(Pearson *et al.*: Arthritis and other lesions induced in rats)

PLATE 56

FIG. 2. Ear nodules in Sprague-Dawley rat No. 3 (see Table I). A. Photograph 12 days after inoculation with adjuvant, 48 hours after left ear punch, on day of onset of both nodules and arthritis. Note intense nodular reaction about site of earpunch. Animal sacrificed 24 hours later. B and C. Larger nodule in right ear, × 37 and × 250. Note thickening of epidermis over lesion and vesicles and pustules within epidermis.



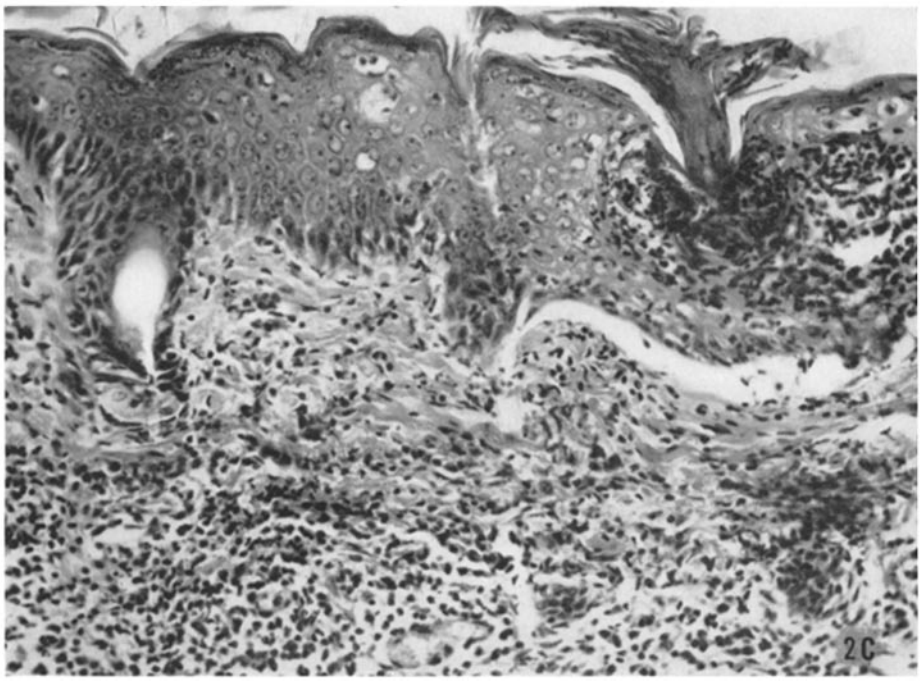


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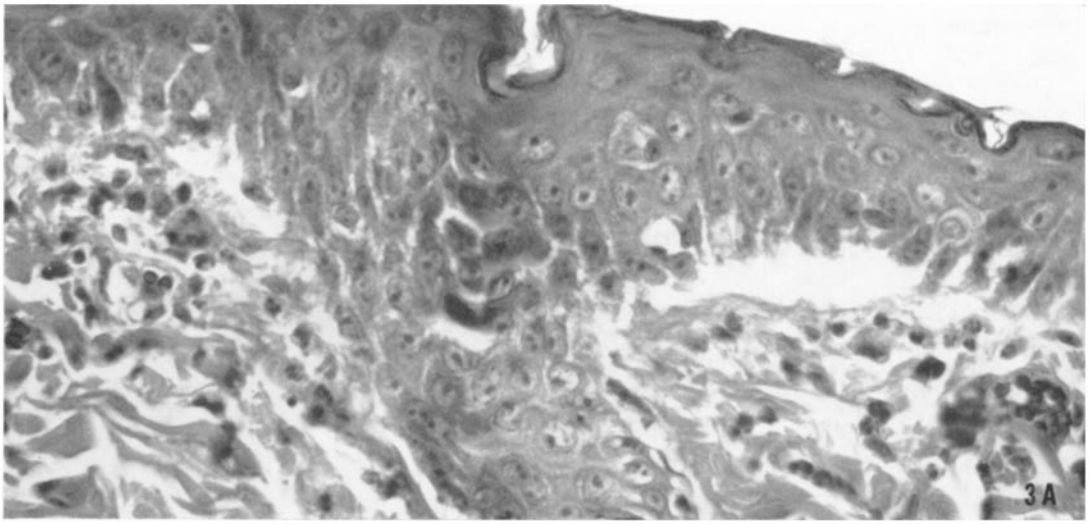
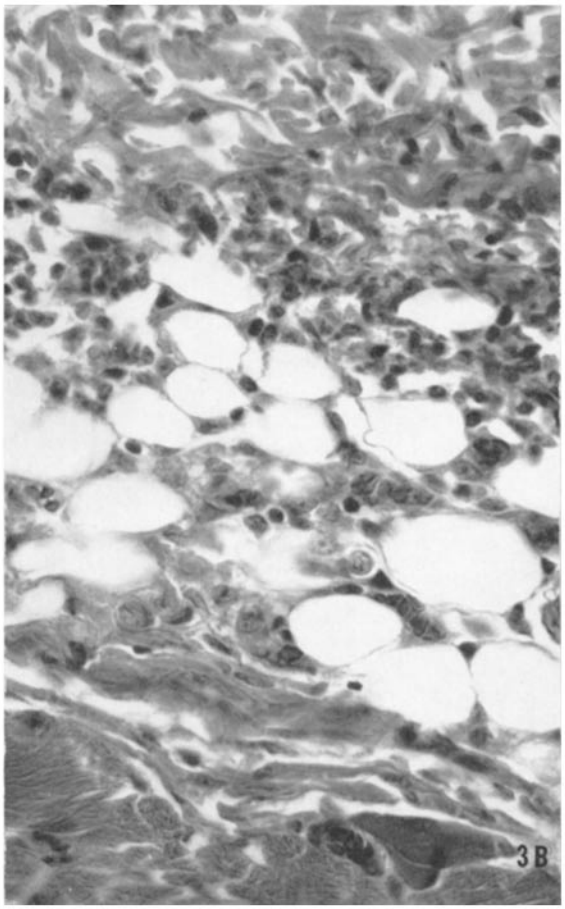
PLATE 57

FIG. 3. A. Superficial inflammatory lesion and detachment of epidermis from basement membrane in maculopapular lesion shown in Fig. 1C. B. Papular lesion like that illustrated in Fig. 1B. Mononuclear infiltrative lesion in subdermal fat. C. Simiar deep infiltration from same lesion shown in Figs. 1C and 3A. All, × 420.





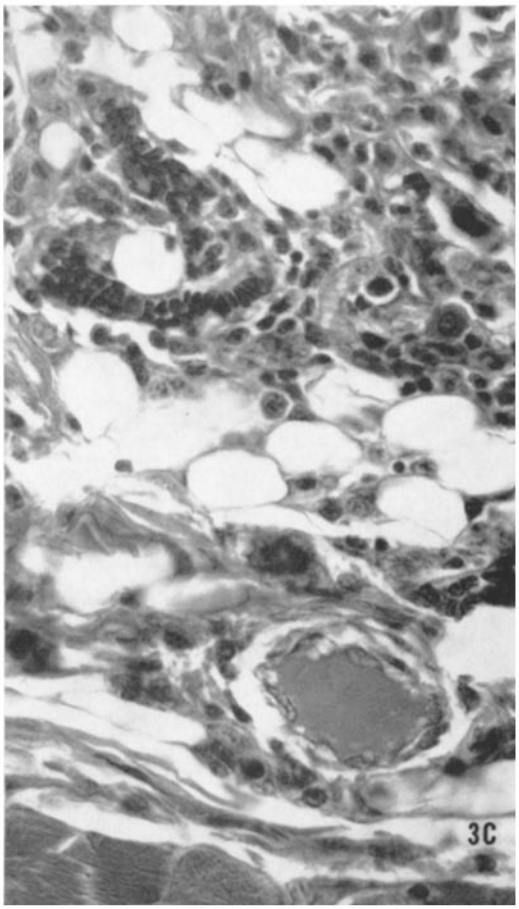


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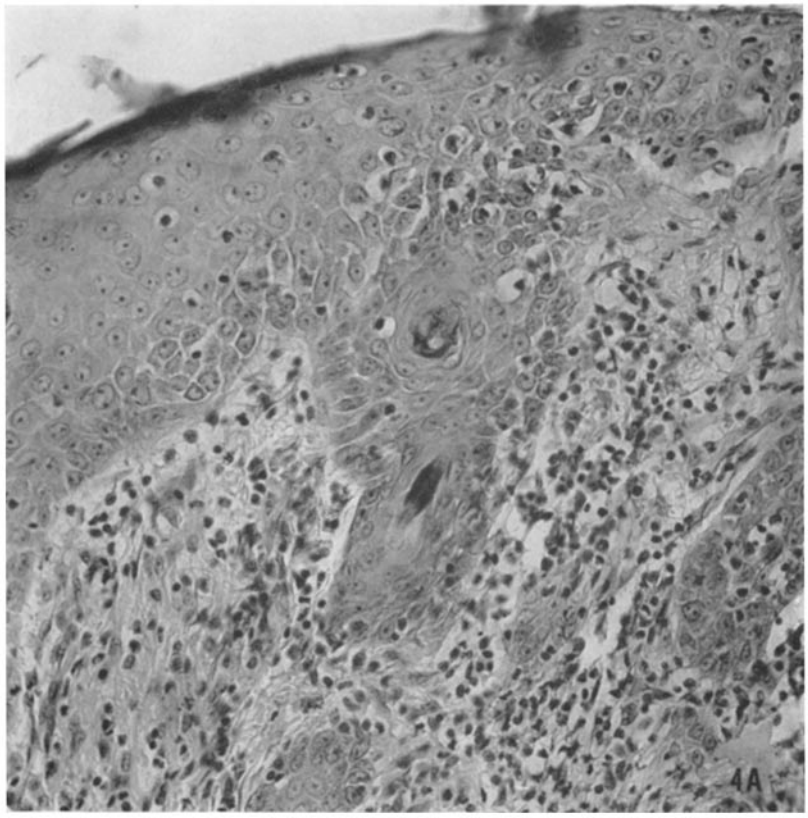
PLATE 58

FIG. 4. A. Neck skin of rat autopsied at 35 days, × 250. Skin disease not clinically apparent. Note inflammatory infiltrate in upper corium, acanthosis, spongiosis of basal layers of epidermis, and invasion by mononuclear cells with beginning vesicle formation. B. Cheek skin of rat autopsied at 50 days, × 450. Onset ++ skin disease at 40 days. Formation of large multilocular vesicle and destruction of basal layer of epidermis by inflammatory infiltrate.



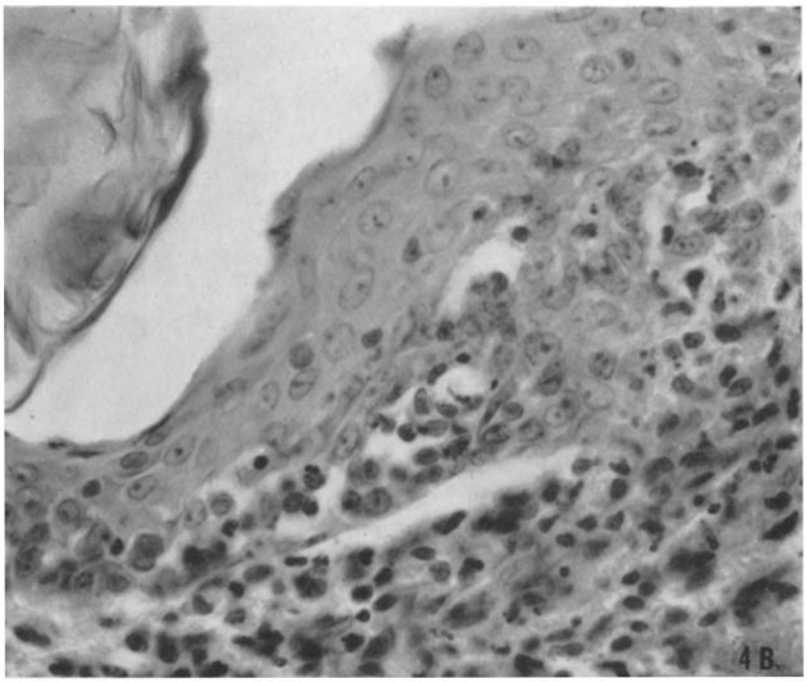


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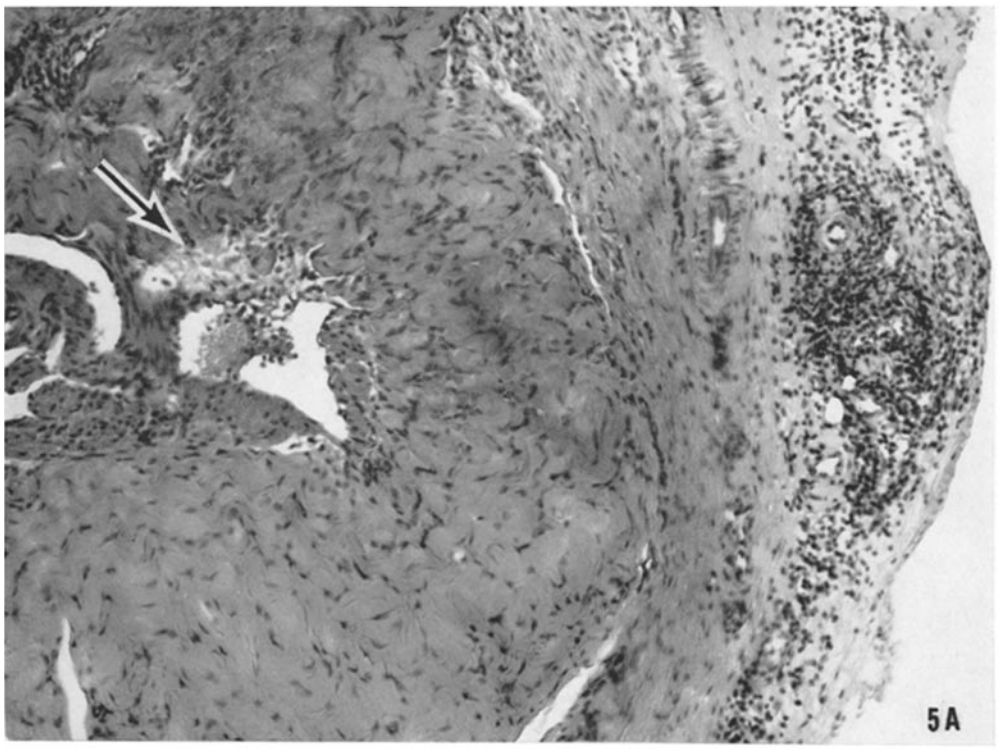
PLATE 59

FIG. 5. B. Penis of rat with arthritis and balanitis autopsied 16 days after inoculation with adjuvant. A. Mononuclear vasculitis and perivasculitis in the marginal tissues, and early edema of a portion of a vascular channel of the corpora cavernosa (at arrow). × 162. B. Edema of the endothelial lining of a channel of the corpora cavernosa; one of the earliest changes noted. There is infiltration with plasma cells and histiocytes, and proliferation of fibroblasts. × 650.



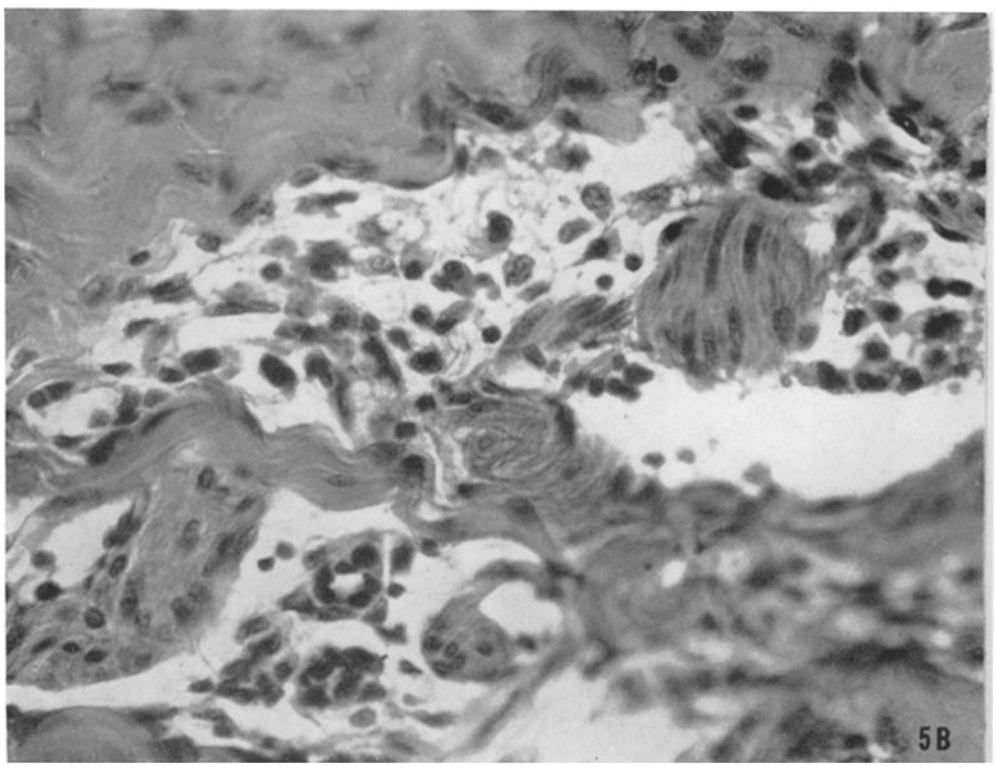


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PLATE 60

FIG. 6. Penis of rat autopsied 21 days after inoculation. Interlacing bands of fibrous connective tissue completely occlude the vascular channels of the corpora cavernosa. Insert shows normal corpora. × 162.

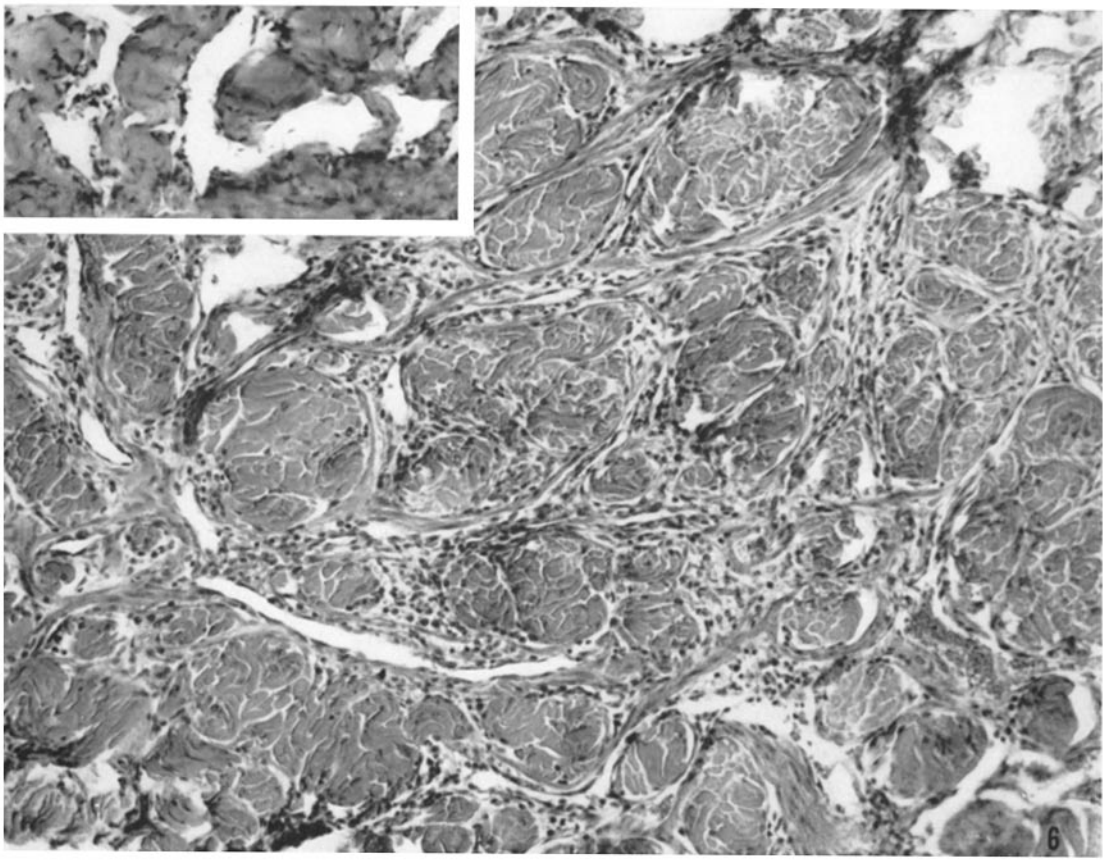

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PLATE 61

FIG. 7. Subepidermal tissue of the scrotum from rat with balanitis and severe arthritis, the latter of 5 days' duration. Vascular congestion is prominent and there is massive mononuclear infiltration. A, × 162; B, × 650.

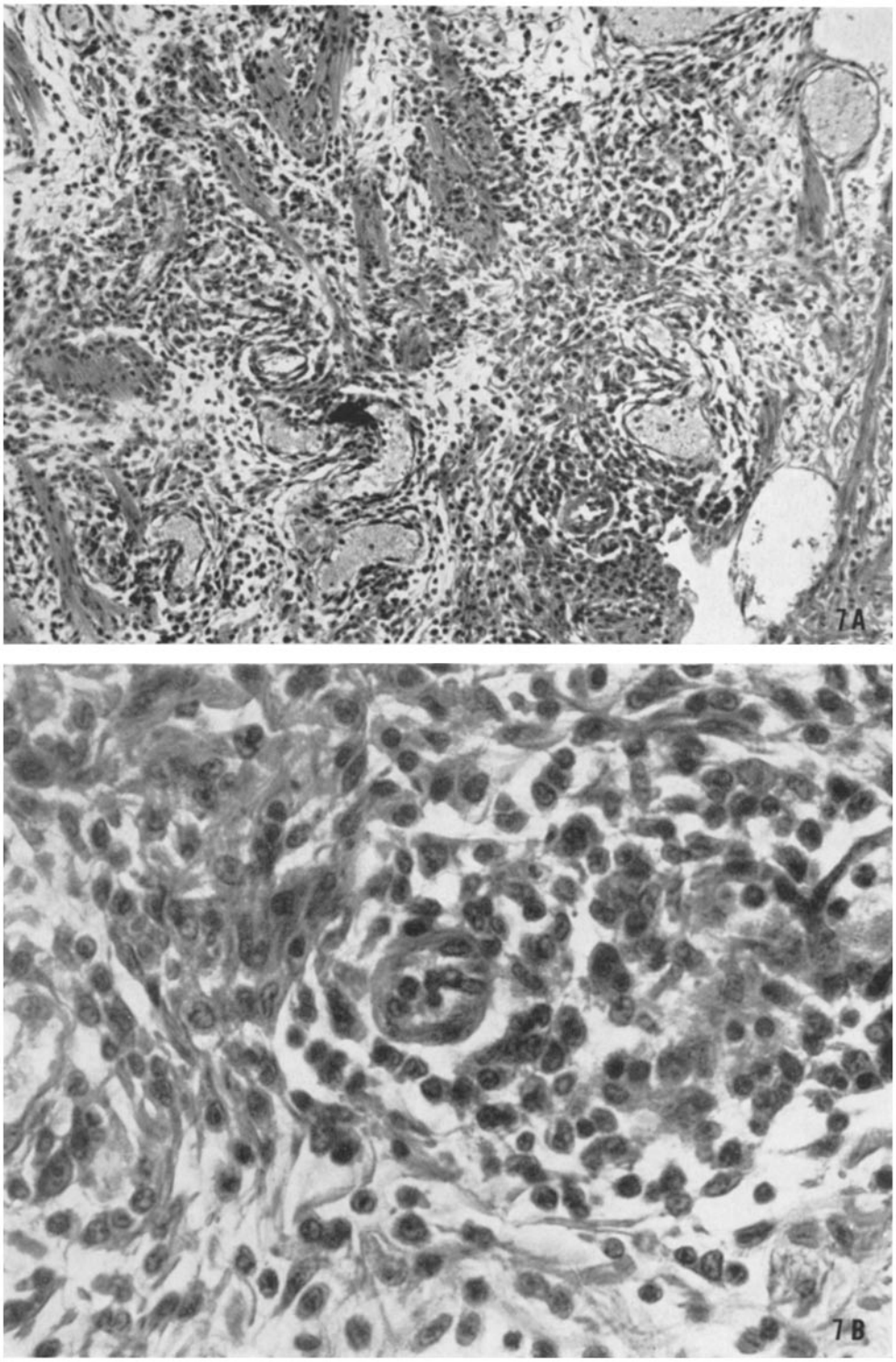

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PLATE 62

FIG. 8. A. Penis of rat autopsied at 22 days with mild balanitis and urethritis of 6 days' duration. Nodular lesion in prepuce and tiny infiltrative epidermal lesion above. × 80. B. Rat autopsied at 43 days. Animal showed balanitis and urethritis at the height of its arthritis, between 12 and 20 days. Nodular lesion below and to right of gland on shaft of penis. × 56. C. Urethral lesion in rat with severe arthritis autopsied at 19 days. There is complete destruction of upper half of mucosa shown in photograph. Urethritis and balanitis of 6 days' duration. × 120.

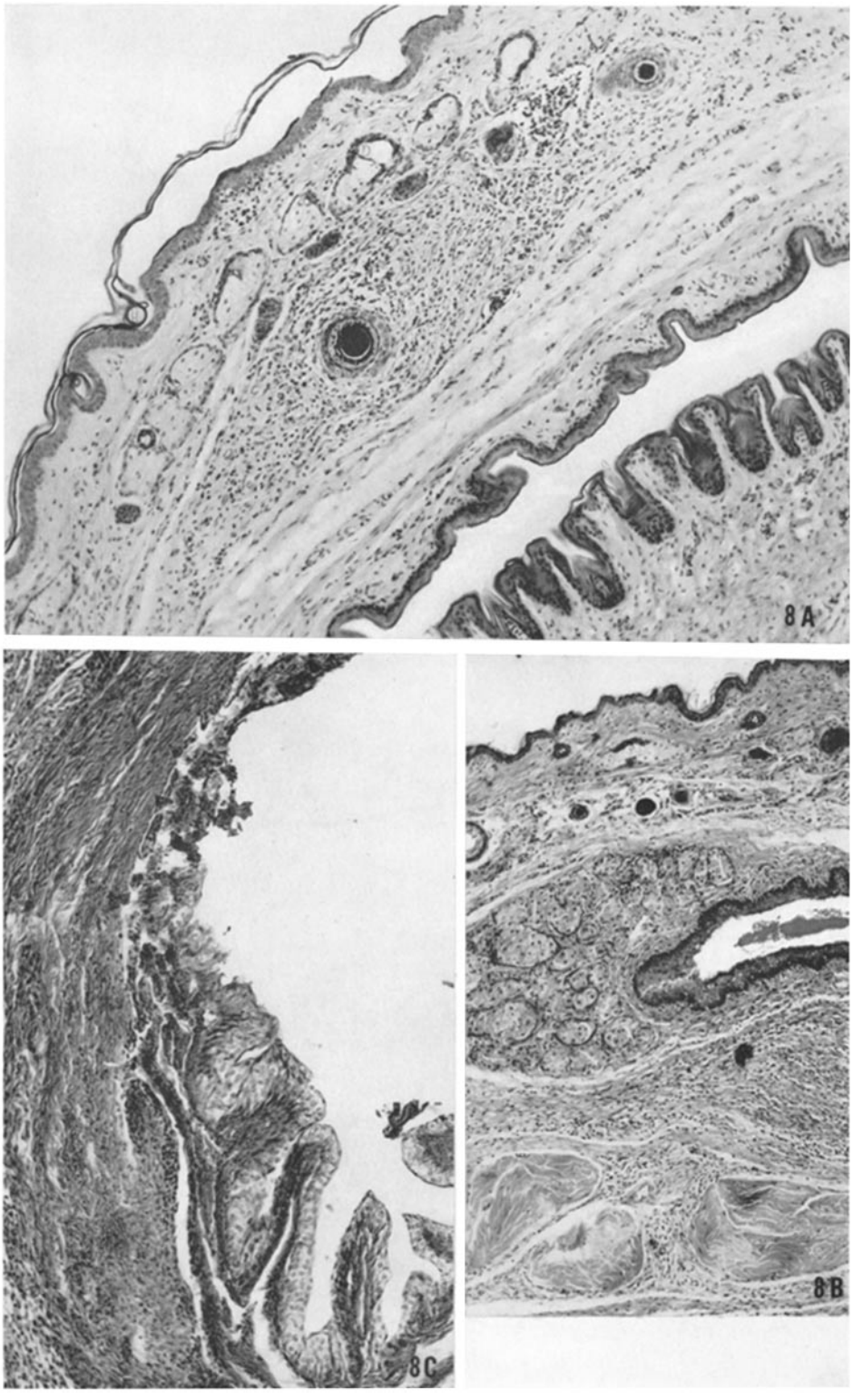

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PLATE 63

FIG. 9. Non-caseating granuloma in the peritesticular fat of rat autopsied 21 days after inoculation. × 162

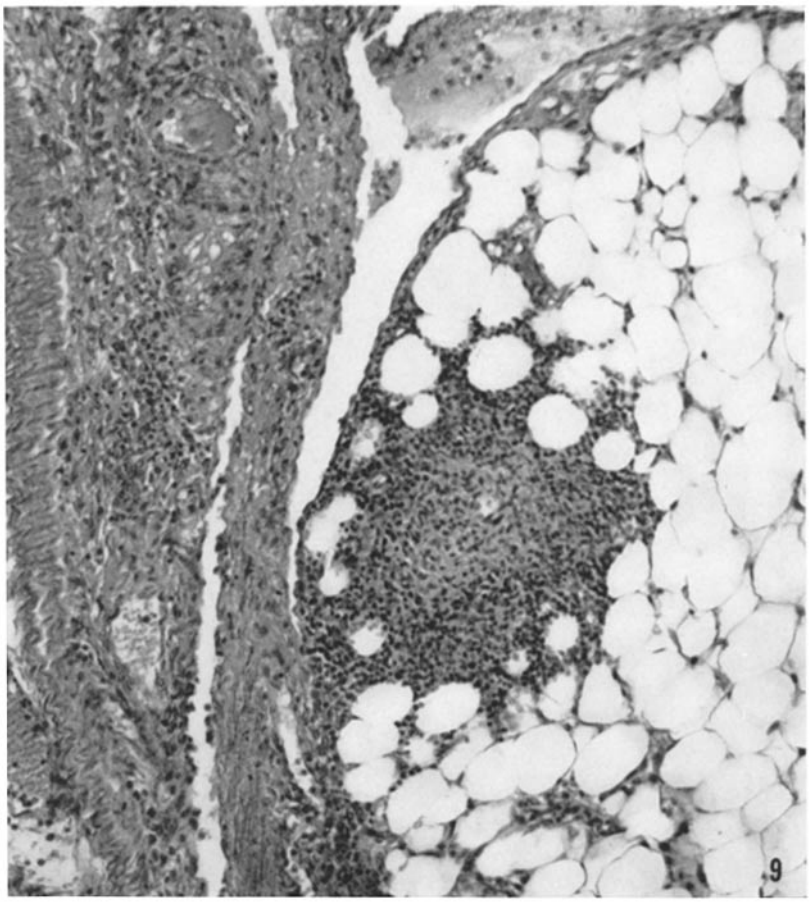

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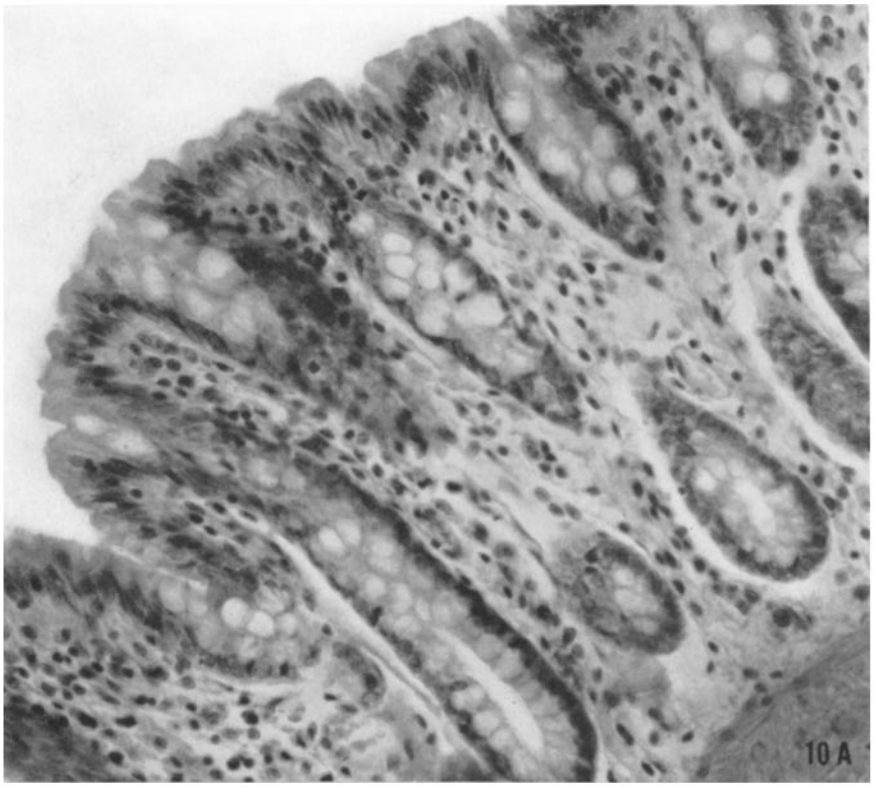
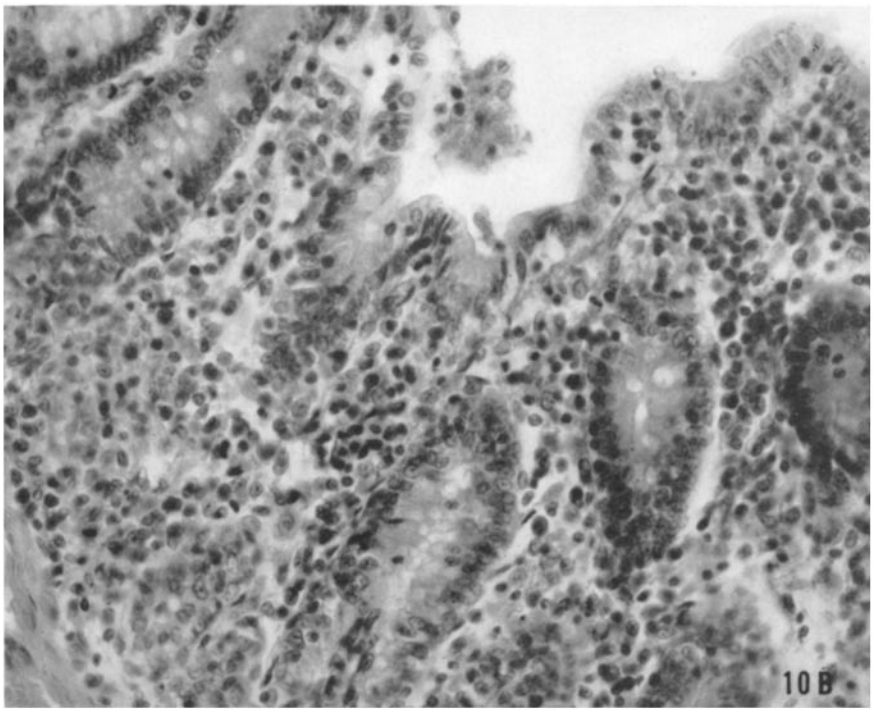
PLATE 64

FIG. 10. Descending colons of rats autopsied at 33 days (A) and 28 days (B) after inoculation and graded respectively as normal and as showing disease. × 250.

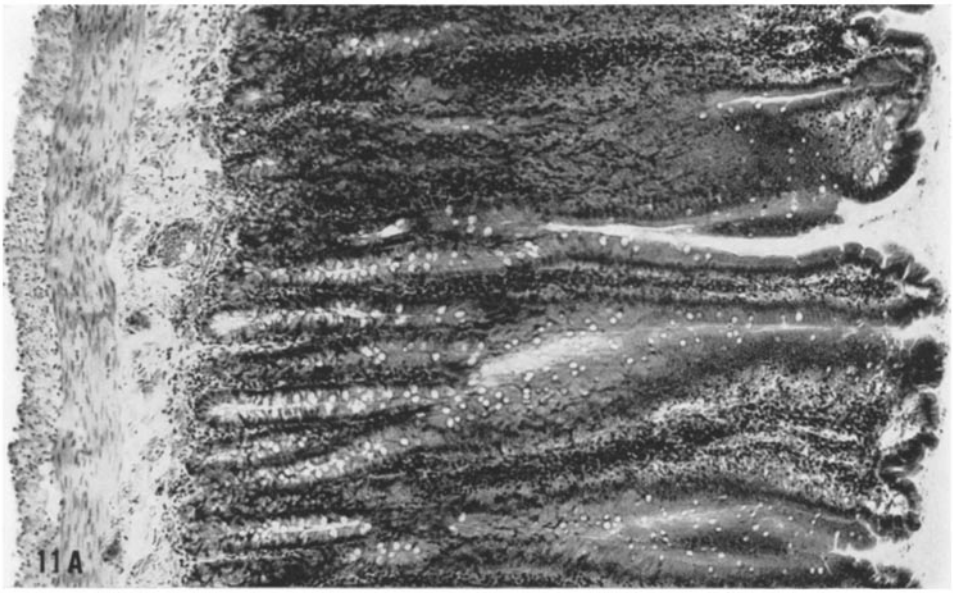


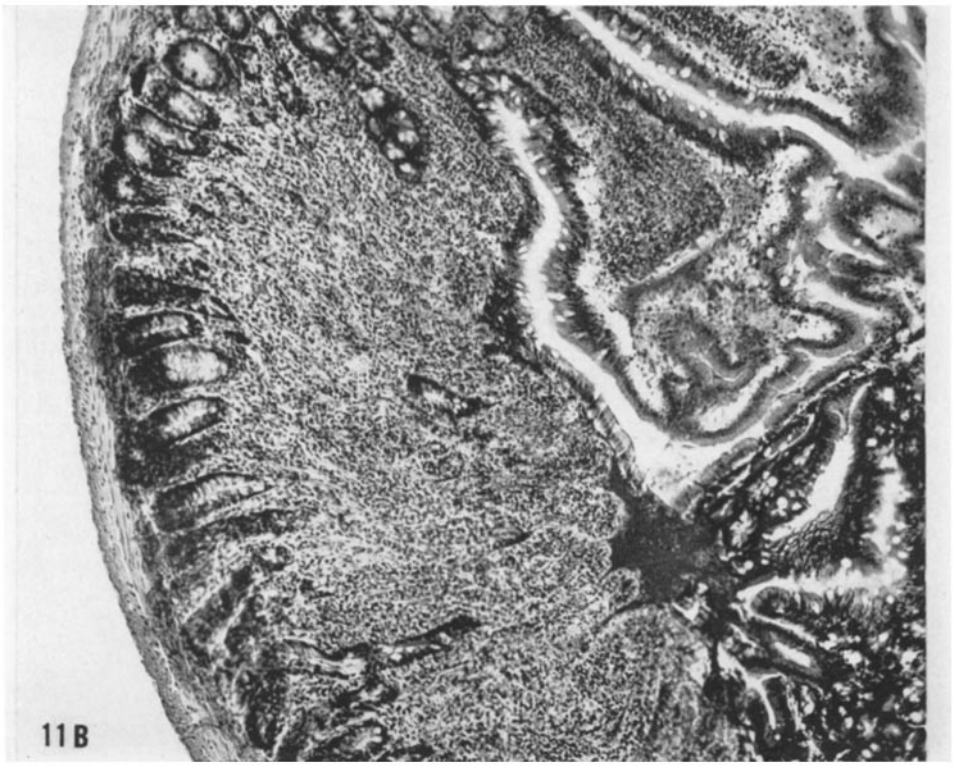


(Pearson *et al.*: Arthritis and other lesions induced in rats)

PLATE 65

FIG. 11. A. Jejunum of rat inoculated with adjuvant in flank and failing to develop arthritis. B. Jejunum of rat inoculated in foot-pad with adjuvant and developing severe arthritis. Both autopsied at 68 days, × 80.





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