β_1-lipoprotein, considerable β_1, a_2 and a_1 globulins and albumin. Fraction 5 was similar to 4 except for markedly diminished a_1 and a marked increase in the other globulins and albumin.

Discussion. The behavior of frozen-dried β_1-lipoprotein in chloroform: methanol and in benzene as here described (Table I) renders less useful the notion that *all* protein-bound lipides can be estimated by the solvent partition method(6,12). In this case carotene and cholesterol, for example, seem to be loosely bound, *i.e.*, extractable by benzene. On the other hand it is apparent that the other blood lipoproteins (excepting chylomicrons) are more resistant to benzene extraction.

Summary. Normal, postabsorptive, human serum was fractionated by means of the ultracentrifuge and the lipoproteins studied with the aid of paper chromatography, ionophoresis and by chemical procedures. The characteristic features of β_1-lipoprotein and methods for its isolation are described.

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Development of Arthritis, Periarthritis and Periostitis in Rats Given Adjuvants.* (22179)

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In this investigation skeletal muscle was incorporated into a Freund-type water-in-oil emulsion and the mixture administered by various routes to rats. Within a short time, ranging from 14 to 45 days, a high percentage of the animals which received intracutaneous inoculations began to develop tender swellings of the ankles, the paws, the tail and the digits. Single or multiple joints were involved. The experiment was repeated on two further occasions. In the third trial a different strain of rats was used. The original results were readily reproducible.

Materials and methods. Originally both male and female rats of the Wistar strain were used. They ranged in weight from 230 to 280 g. The final series contained female rats of the Long-Evans strain, weighing 180 to 220 g. All animals were fed a standard pellet diet of Purina chow and water *ad libitum*. They were weighed frequently and inspected almost daily throughout the investigation period. The inoculum was prepared as follows: a healthy rat of the same species to be subsequently used in the study was killed

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TABLE I. Incidence of Clinical Alteration of Extremities and Tail in Relation to the Site of Inoculation—Series 55A1.

Site of inoculation	No. of rats (Wistar)	Severity of change				% animals affected
		None	Slight	Moderate	Severe	
Intracutaneous (posterior cervical)	17	4	6	2	5	77
Subcutaneous	8	6	1	1	0	25
Intraperitoneal*	4	0	0	0	0	0
Controls (no inoculations)	8	8	0	0	0	0

* Produced fibroplastic peritonitis but no peripheral alteration.
Mean time of alteration after initial injection—23 days (15-32 days). Avg No. of injections before alterations—2.9 (1-5).

by a sharp blow. Skeletal muscles from the thigh and the iliopsoas groups were freed from as much surrounding fat and connective tissue as possible and weighed in a sterile beaker. Ten grams of this muscle was homogenized in a Waring micro-blendor with 30 ml of sterile isotonic saline solution using intermittent action of the rotor blades to avoid overheating of the mixture. The resultant homogenate was then compressed through fine-mesh sterile gauze and to the filtrate was added 32 ml of refined thin mineral oil, 8 ml of the emulsifier Falba,* and 100 mg of heat-killed *Myco. phlei.* The mixture was emulsified by the syringe method in a sealed vial and stored at 4°C. It was warmed to 50°C for a few minutes each time inoculations were given. In the final series aqueous Merthiolate, to a final dilution of 1:10000, was added without significant effect on the final result. In one portion of the final experiment muscle tissue was excluded from the inoculum and was replaced by an approximately equal amount of sterile isotonic saline solution. In another portion of this experiment only saline and muscle were combined so that the final concentration of muscle homogenate was approximately the same as in the mixture with adjuvants added. All animals were given 0.1 ml of the emulsion at each inoculation. Injections were given in a series of 3 at 2-day intervals, then a 14-day rest period was followed by a second and in some animals a third and fourth series of injections. In the first experiment inoculations were given intracutaneously into the skin of the posterior cervical region after first carefully clipping the hair. Subcutaneous injections were also given into this region. A small group of animals were given intraperitoneal injections. In the second and third experiments all inoculations were given intracutaneously. A 21 gauge needle and tuberculin syringe were used for all injections.

Results. A total of 97 animals were used in the study which was divided into 3 experiments. The results of each will be presented separately but the discussion of the clinical and pathological changes will be combined since they were essentially similar in all groups. A detailed autopsy was performed on every animal that has been sacrificed. Some animals showing clinical change have been allowed to recover and are under continued observation.

Controls. Unrelated Deaths. Uninjected control animals from the same litter groups as the treated animals have been followed in each experiment. Clinically and at autopsy these have consistently failed to demonstrate any lesions of the joints. Two animals in the treated and one in the control group died from acute pneumonitis.

First Exp. Wistar rats were given inoculations by various routes. The results are shown in Table I and demonstrate the superiority of the intracutaneous mode of injection. The incidence and degree of response was similar in both sexes. *Second Exp.* The initial experiment was repeated, again with Wistar rats and a freshly prepared inoculum. All injections were given intracutaneously and the initial results were essentially reproducible as shown in Table II. *Third Exp.* Long-Evans female rats were used and the content of the inoculum was varied to determine the

† Obtained from the McNerney Chemical Corp., Los Angeles, Calif.

TABLE II. Incidence of Clinical Alteration of Extremities and Tail—Intracutaneous Inoculations.

Series	No. of rats (Wistar)	Severity of change				% animals affected
		None	Slight	Moderate	Severe	
55A 13-15	16	6	4	4	2	62
Controls	8	8	0	0	0	0

Mean time of alteration after initial injection—28 days (20-45 days). Avg No. of injections before alteration—4.1 (3-6).

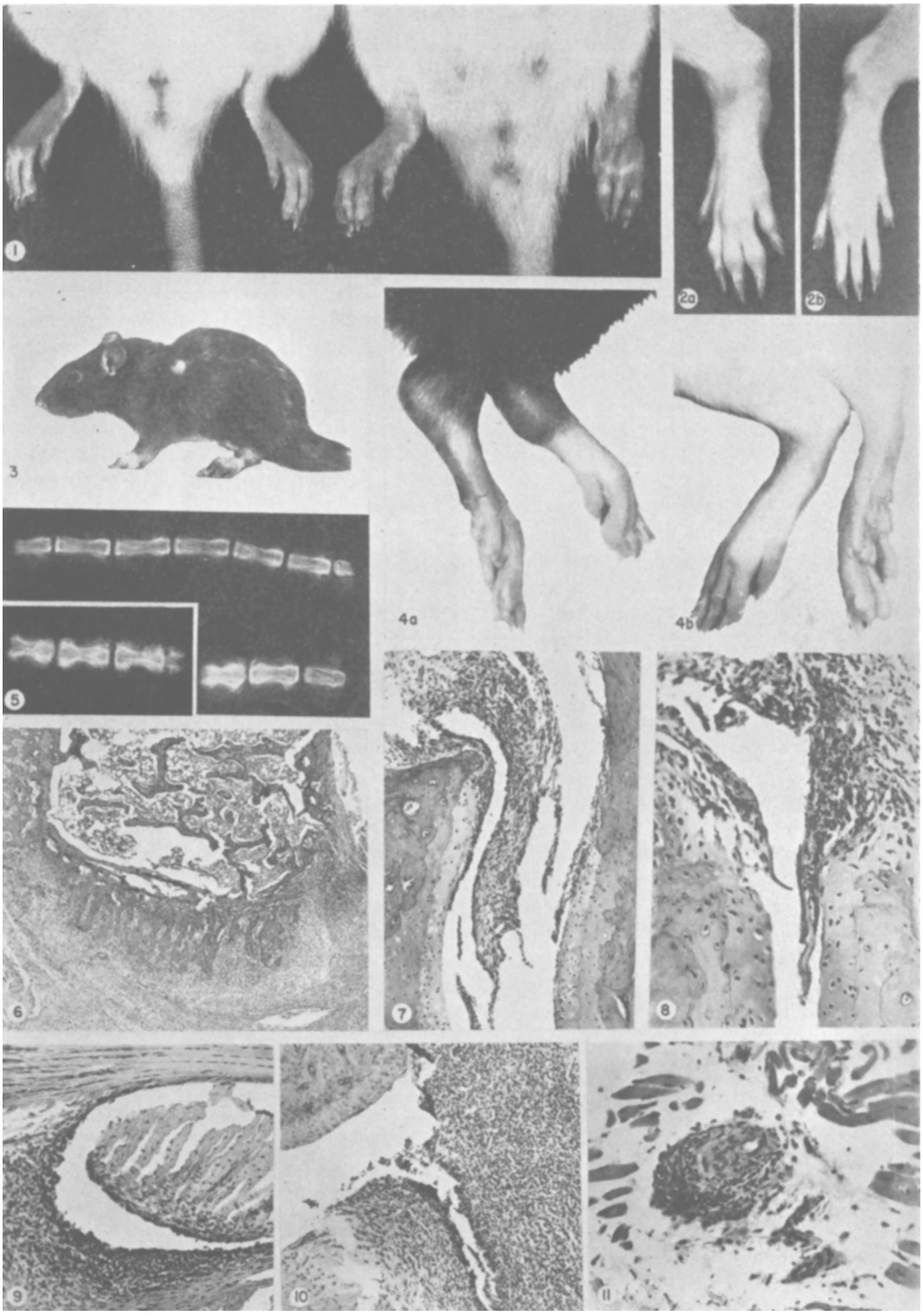
relative value of various components in inciting the pathologic changes as noted. The results show that although muscle tissue does not appear to be necessary for the development of these changes it may have a potentiating effect. Acid fast bacilli and probably the mineral oil are essential. The incidence of clinical alteration in this experiment is shown in Table III.

Time of Onset and Clinical Appearance of Limb and Tail Alterations. The earliest alterations occurred on the fourteenth day after the original inoculation. Initial alterations in other animals were delayed up to 45 days. The average time of onset was 22 days. The first changes usually were seen in the ankles, hind paws or proximal or middle phalangeal joints of the feet. In general the earlier the onset the more severe the clinical involvement but this was not always the case. In a small series, not included above, only one intracutaneous inoculation was given and 3 out of 8 animals have shown joint alterations. The ankles and tarsal regions were most frequently affected severely; then the forepaws, digits and tail. When markedly affected, serum exuded from the swollen red paws which were obviously painful and tender to touch. Frequently one or several digits would be completely spared from swelling although adjacent ones were 2 or 3 times their normal size (Fig. 1). The tail was more frequently involved in the Wistar strain and showed swelling, nodulation and desquamation, more marked in the proximal segments. Many severely affected animals lost weight. Lesser degrees of swelling affected many other animals and again the ankles were most frequently involved. Fusiform red swelling of one, several or all of the proximal interphalangeal joints was not uncommon (Fig. 2) especially in the Wistar strain. Diffuse digital swelling was more commonly seen in the Long-Evans strain. In animals that were observed for several weeks or months there was a gradual return of lost weight as evidence of acute inflammation subsided. Functional recovery did not continue beyond 3 months at which time there were still many residuals in the form of chronic firm enlargements especially about the ankle joints (Fig. 4), the metatarso- and metacarpophalangeal joints which showed typical flexion deformities (Fig. 3) resembling a "flipper hand," and large rigid tails. Firm ankyloses often partially or almost completely restricted motion especially at the ankles and in the tail joints. Histological study has not as yet been made on these chronic alterations. *Roentgenologic Observations.* After one month, early bone

TABLE III. Incidence of Clinical Alteration of Extremities and Tail with Varying Composition of Inoculation Material—Series 55A16-21.

Inoculum content	No. of rats (Long-Evans)	Severity of change				% animals affected
		None	Slight	Moderate	Severe	
Adjuvant + muscle + merthiolate (1:10,000)	8	3	2	1	2	62
Adjuvant alone*	8	5	2	0	1	37
Adjuvant (no acid fast bacilli) + muscle†	8	8	0	0	0	0
Saline + muscle	6	6	0	0	0	0
Controls (no inoculations)	6	6	0	0	0	0

* Small granulomas at injection site. † No granulomas at injection site.


1
2a
2b
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4a
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FIG. 1. Diffuse swelling of both tarsal regions and digits compared with control animal. Note absence of involvement of middle toes.

FIG. 2. (a) Fusiform swelling of proximal interphalangeal joints. (b) Control.

FIG. 3. Flexion deformities at metacarpo- and metatarsophalangeal joints. Duration of disease, 4 mo. Retouched photograph.

FIG. 4. (a) Chronic firm swelling of ankles with marked restriction of ankle motion. (b) Control.

FIG. 5. X-rays of tail vertebrae. The upper picture is a control. Lower X-ray demonstrates increased periosteal density, exostoses and narrowing of intervertebral disc spaces.

FIG. 6. Section through os calcis showing active periosteal osteogenesis and inflammatory reaction on the right with invasion by granulation tissue through the bony cortex and into the marrow cavities in this area. × 45 (before reduction).

FIG. 7. An ankle joint showing a broad, edematous, inflamed synovial villus projecting into the joint space. Note extension of granulation tissue over the surfaces of the articular cartilage. × 180 (before reduction).

FIG. 8. Fibrinoid necrosis of synovial margin of a tarsal joint. × 220 (before reduction).

FIG. 9. Peritendonitis and tendonitis. × 130 (before reduction).

FIG. 10. Intense inflammatory reaction, granulation tissue growth and synovial reduplication at the margin of a phalangeal joint. Note neutrophiles in joint space. × 180 (before reduction).

FIG. 11. Perivascular lymphocytic collection about a small intramuscular artery in one of the small muscles of the foot. × 220 (before reduction).

and joint changes were visualized. These were progressive during the "healing" phase and were maximal at 3 months. They were most marked about the os calcis and the ankle joint but were often intense on the lateral wings of the caudal vertebrae (Fig. 5). The change consisted essentially of a periostitis followed by the appearance of nodular exostoses along the entire surface or an isolated segment of bone. The joint spaces of the ankle were narrowed or completely obliterated and the intervertebral spaces of the tail were narrowed. Oblique views of formalin-fixed spine showed some bony reaction with partial or complete obliteration of the apophyseal joint spaces in the lower dorsal and lumbar spine. No other joints were altered radiographically.

Histopathologic Observations. Animals were sacrificed at various stages during the development and subsidence of the clinically observed bone and joint lesions. The process was essentially one of intense stimulation of the connective tissue elements with focal and diffuse inflammatory infiltrations. This response was limited to the regions about the affected bones, joints and tendons and rarely extended into the subcutaneous tissue. The overlying dermis was normal. Edema, fibroblastic proliferation and sparse mononuclear or polymorphonuclear infiltrations were noted in the earliest stages. After 10 or more days there was exuberant proliferation of granulation tissue about some bones, joints, tendons, synovial sheaths and synovia of the joints. This tissue was responsible for the focal swellings and hyperemia seen clinically. It was infiltrated to a variable degree, often intensely, with neutrophiles, plasma cells, lymphocytes and mononuclear histiocytes. Eosinophiles were rarely seen. The next most striking observation was the profound osteoblastic activity which arose mainly from the periosteum of cortical bone and rapidly formed broad spicules of new bone (Fig. 6). This bone was frequently surrounded at its outer border by a zone of immature cartilage which appeared to arise *in situ* in the adjacent hyperplastic connective tissue. Granulation tissue frequently invaded the cortical bone and spread out into the marrow cavity (Fig. 6). The synovia about tendon sheaths and joint spaces was hyperplastic and portions of tendon were often invaded (Fig. 9) and adherent to the side of the sheath. The synovial villi were enlarged, edematous and inflamed and frequently projected deeply into the joint spaces (Fig. 7). Occasionally fibrillar material surrounded the joint margin and resembled fibrinoid necrosis (Fig. 8). Small numbers of neutrophiles were present in some joint spaces (Fig. 10) along with an apparent slight increase in joint fluid. There was no primary involvement of the articular cartilages but granulation tissue from the margins of the synovial membrane was frequently observed growing over the surfaces of the articular cartilages (Fig. 7). The

above changes were seen in the ankle joints or the joints of the paws or fingers when clinical involvement was apparent. Lesser degrees of connective tissue reaction were occasionally noted about bones and joints not grossly affected.

Sections of the tail showed an essentially similar process plus early invasion of the margins of the intervertebral discs and the subchondral spaces by inflamed granulation tissue. Other findings of interest were focal lymphocytic and mononuclear cellular infiltrates in skeletal muscles near involved bones and joints. This was a true focal polymyositis with destruction of one or several striated muscle fibers. An arteritis or vasculitis was infrequently noted in the regions of bone and joint involvement (Fig. 11). It consisted of lymphocytic and mononuclear cellular infiltrates about a portion or all of a small intramuscular artery. There was no necrosis of the vessel walls. Focal areas of edema and necrosis, without surrounding palisading, were occasionally seen in the hyperplastic connective tissue. Occasional focal subcutaneous lymphocytic collections were noted.

Similar histologic changes were not noted in muscle and other tissues remote from the areas of clinical involvement. All other organs were histologically negative except for an occasional small granulomatous focus in the liver and varying degrees of a granulomatous pneumonitis in some animals. Similar pulmonary lesions have been frequently noted in guinea pigs following repeated injections of a Freund-type adjuvant(1). In summary the pathological changes consisted of: connective tissue proliferation and granulation tissue formation; acute and chronic inflammatory infiltrates; periostitis with periosteal and enchondral new bone formation; arthritis and periarthritis; synovitis; tendonitis and peritendonitis; focal myositis (near affected bones and joints only); perivasculitis, non-necrotising (rare).

Discussion. There have been many previous attempts to produce an arthritis in animals. Some of these have been successful by such varied methods as injecting large amounts of desoxycorticosterone acetate into rats previously nephrectomized and adrenalectomized(2), inoculating rabbits repeatedly with large amounts of horse serum(3) or killed beta hemolytic streptococci plus chondroitin sulfate(4), and by injection of viable pleuropneumonia-like micro-organisms into mice and rats(5).

The pathogenesis of the bone, joint and connective tissue alterations reported herein must at present remain a matter of speculation. There is suggestive evidence that hypersensitivity is an important factor in these reactions when it is considered that a known allergenic emulsion was used as the inciting agent. Studies are underway to determine if activation of pleuropneumonia-like microorganisms are responsible for these reactions. Periosteal osteogenesis has not been recorded as a feature of this infection, however it is a prominent factor in experimental lathyrism (6).

Pathologically the basic lesion appears to be an intensive focalized mesenchymal tissue response. There are several gross and microscopic similarities between these lesions and those observed in human rheumatoid arthritis but since it is likely that such tissues can only react in a limited number of ways to many stimuli the identification of such changes with any human counterpart is unwarranted.

Summary and Conclusions. A reproducible generalized or focal arthritis, synovitis, periostitis and tendonitis has been induced in rats by intracutaneous injections of a Freund-type adjuvant which was most potent when macerated striated muscle tissue was added to the inoculum. The pathogenetic mechanisms underlying the production of these changes is at present unclear.

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Plasma Thromboplastin Component: Influence of Coumarin Compounds and Vitamin K on Its Activity in Serum. (22180)

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A new blood clotting factor, termed plasma thromboplastin component (PTC), was recently described by Aggeler, *et al.*(1) and subsequently called Christmas factor by Biggs, *et al.*(2). Little is known about the formation of PTC or about its disappearance from the blood stream. Early workers demonstrated that the level of prothrombin in the blood stream is influenced by vit. K and the coumarin compounds. Since these compounds have been shown to influence "stable factor" as well(3,4), it is of interest to determine whether PTC is similarly affected. The present study demonstrates that patients deficient in vit. K develop a depression of PTC, and confirms the observation of Sise, *et al.*(5) that following the administration of the coumarin compounds there is also a depression of PTC. In both groups we find that PTC activity is restored, in part at least, by the administration of vit. K analogues.

Methods. One-Stage Prothrombin Time was determined by the Link-Shapiro(6,7) modification of Quick's method(8), using commercial rabbit brain thromboplastin* (normal: 14 seconds). *One-Stage Prothrombin Activity* ("per cent" of normal). Normal oxalated plasma was serially diluted with saline (90%, 80%, etc.), and the one-stage prothrombin times of various dilutions determined. Observed times were then plotted against dilution, to provide a standardization curve for comparison of unknown plasmas with normal ones. *Assay of Hemophilioid Factors; Principles Involved*: Thromboplastin is essential for normal conversion of prothrombin into thrombin. Thromboplastin obtained from rabbit brain will react with normal recalcified plasma to form a clot in 14 seconds. This is the basis of the one-stage prothrombin time test. Washed human platelets can form an equally potent thromboplastin, but unlike rabbit brain, must first be incubated with the hemophilioid factors and calcium(9). Hemophilioid factors include: plasma thromboplastin component (PTC), plasma thromboplastin antecedent (PTA) (10) and anti-hemophilic factor (AHF). The ability of these factors, in combination, to transform platelets into a potent thromboplastin, the equivalent of that obtained from rabbit brain, is the basis for the thromboplastin generation test, Biggs, *et al.*(9,11). In the present experiments the thromboplastin generation test is modified so that it becomes a quantitative rather than merely a qualitative assay. Thus the relative levels of any one of the hemophilioid factors can be determined. In the quantitative procedure a reaction mixture containing platelets and calcium is supplied with two of the hemophilioid factors, each in high titer. The third factor—the one to be assayed—is added as an unknown or test preparation. A deficiency of this third factor will be demonstrated by faulty "activation" of thromboplastin. Of the three possible assay procedures, only one was used in the present study—the assay for PTC. *Details of the PTC Assay*: A standard platelet suspension is obtained by the differential centrifugation of citrated normal

*Prepared by Warner-Chilcott Laboratories.