LACTOGENIC HORMONE INDUCED POLYARTHRITIS IN THE WHITE RAT: A SUGGESTED METHOD FOR THE LABORATORY EVALUATION OF ANTI-INFLAMMATORY AGENTS

by

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PREFACE

The study and development of new modes of therapy for man's afflictions is, perhaps, one of his oldest undertakings. In this quest two main avenues of endeavor are open. The first stems from the dawn of civilization. It continues even today with doctors or laymen making empirical observations that administration of certain substances alleviates disease in man or animals. The second, the scientific approach, is of more recent development. With the latter method a deliberate attempt is made to examine the effect of various agents in altering the course of artificially produced disease states in vitro or in vivo. In this rational approach to the discovery of effective therapeutic agents, it is often necessary that the disease syndrome be duplicated as closely as possible to that existing in man by setting up experimental "models" in animals (83). In the case of infectious diseases this is easily accomplished. More difficult is the replication in animals of chronic organic diseases such as congestive myocardial failure, nephritis, cirrhosis, or chronic rheumatoid arthritis. When disease "models" in small animals are devised which can be easily reproduced and provide measurable means for evaluating the course of the disease process, it will then be possible to accurately assess the effectiveness of drugs used for treatment of these diseases.

Progress in developing suitable and effective medications for the therapy of rheumatoid arthritis has been notably slow. Without adequate experimental methods for evaluating the many remedies suggested, resort

to the empirical method of trial and error has resulted. No specific therapy is available today after years of research both at the clinical and laboratory level.

Several factors hinder a more complete knowledge of the physio-pathologic processes and the biochemical and metabolic changes taking place in rheumatoid arthritis. To start with, information as to the exact etiology of this disease would provide an attack point in devising more specific therapy. Fortunately, as more is learned about the mechanisms of action of the various drugs now employed in treating rheumatoid arthritis, more facts are revealed as to the underlying pathology and basic etiology of the disease. Another difficulty has been the inability to foretell and follow the natural fluctuations occurring during the course of the disease. In rheumatoid arthritis periods of exacerbations of the acute inflammatory process alternate with remissions of variable length for no explainable physio-pathologic reason so far known. This makes the evaluation of drug therapy doubly hazardous since it is uncertain whether improvement represents the result of therapy or is simply a remission which would have occurred anyway. Similarly, extraneous subjective factors such as emotional upsets, climatic changes, occupational activities and capabilities as well as endogenous physio-pathologic alterations influence the course of the disease process in rheumatoid arthritis. Not only do these several factors render difficult an accurate assessment of the value of drug therapy in this disease but they point out some of the problems that must be faced in reproducing a typical pattern or "model" of the disease in animals.

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INTRODUCTION

The high incidence of chronic rheumatoid arthritis in man and the crippling disability this disease causes bear out the need for finding better methods for its treatment and alleviation of symptoms. The following points out important facts about the tremendous significance of arthritis as a disease(35). If arthritis and related diseases are considered under the broad category of "rheumatism", at least one person out of twenty in the United States has some form of rheumatic disease. Among the chronic diseases, rheumatism cripples the largest number but kills the fewest leaving the afflicted person crippled or unable to work gainfully and to suffer pain more or less constantly. From the standpoint of days lost from work it is surprising to find that arthritis surpasses injury from automobile and other accidents. Moreover, rheumatism ranks second to nervous and mental diseases as a cause of days lost from work due to chronic disease. Certainly the social, as well as the economic significance of rheumatism and arthritis call attention to the need for further knowledge of the etiology, pathogenesis and, especially, the treatment of these disabling and painful diseases.

The research work reported in this thesis deals with three phases of endeavor. The first concerned the problem of producing experimental arthritis in small animals which would accurately duplicate in its physiopathologic features and anatomic findings the changes found in rheumatoid arthritis in man. The second problem was to develop a simple, reproducible, objective, evaluation technique for measuring and grading the stage and

severity of the inflammatory process. With these first two phases satisfactorily produced and allowing quantitation, it would then be possible to continue with the next step in this research. The third endeavor concerned the practical application of the "model" arthritic disease, together with the severity grading system, in a comparative study of the effectiveness of the various anti-inflammatory agents available today as anti-arthritic agents.

The present research required a survey and laboratory trial of several different procedures used to reproduce the pathologic changes resembling arthritis in small animals. During this preliminary study it became evident that inflammatory arthritis in animals is a progressive, worsening condition so that landmarks would have to be set up and calibrated periodically during the active process in order to establish a time-disease relationship. This partitioning of the progressive pathologic process would then permit evaluation of the effects of drug therapy at any particular stage of the disease in the "model". It was with these requirements in mind that the experimental procedure, later described, was selected for producing and studying the development of experimental arthritis in the white rat.

In selecting suitable methods for reproducing inflammatory disease in small animals, it seems logical that attention should be given to the etiology of rheumatoid arthritis. Unfortunately, there are a number of theories as to the etiology of rheumatoid arthritis and, consequently, a variety of experimental procedures have been tried. Over the years one sees a striking relationship between the experimental models devised

and the theory of the etiology of rheumatoid disease in vogue at that time. A discussion of the possible etiology of rheumatoid arthritis, which Short (86) has grouped into seven broad classifications, follows.

THEORIES OF THE ETIOLOGY OF RHEUMATOID ARTHRITIS

1. Neurogenic - At the turn of the century one of the more popular theories was the neurogenic theory of etiology. Evidence which supported this proposition was the clinical-syndrome of muscle weakness, tremor, paresthesias, muscle and skin atrophy, and certain changes in the deep reflexes.

2. Vascular - Many clinical signs and symptoms in the arthritic patient indicate instability of the vasomotor function. Some patients give a history of Raynaud's Phenomenon while others demonstrate a very labile vascular system. At autopsy many arthritic patients show the pathologic picture of a vasculitis or an arteritis. However, a pertinent question which arises is whether the vascular lesion is a primary or secondary change, in the disease. Perhaps it is secondary to a neurogenic etiology which may be on an autonomic nervous system basis rather than a central nervous system origin.

3. Infection - For years attempts have been made to isolate an organism or organisms which might be responsible for the production of rheumatoid arthritis. The few reports suggesting this possibility (3) have not been confirmed and the concensus today is that infection is not the primary cause of the disease although it may play a role in the pathogenesis.

4. Psychogenic - Until recent years, the theory that stress could serve as an etiologic background for rheumatic diseases was hardly considered (31), (33). Proponents of the psychogenic theory of etiology point to the numerous clincial cases where emotional stresses seem to be directly related to the onset of rheumatoid disease or to exacerbations of the disease (73). Perhaps the emotional stimulus results in certain vasomotor changes or muscular "tension" which then gives rise to the clinical signs and symptoms (28). Some authorities believe that a consistent personality pattern has been demonstrated in psychiatric studies of groups of rheumatoid patients (42). Whether the psychological makeup is the result of the disease rather than the cause is not known. This theory is under investigation currently, especially regarding the state of "contracture" and resultant arthritic symptoms.

The last three classifications of etiology pointed out by Short (86), those of hypersensitivity, metabolic and endocrinological abnormalities, seem to be related. Certainly they do reflect the influence of the times and the various techniques of study which have become available relatively recently.

5. Hypersensitivity - The theory that rheumatoid arthritis is a manifestation of hypersensitivity has been present for at least twenty-five years. According to this theory the patient develops antibodies to some component of his own connective tissue or articular tissues so that an antigen-antibody reaction results. This, in turn, gives rise to the chronic inflammatory lesions which can be seen. Why the patient develops these antibodies is unknown. It is postulated that this may be

due to ingestion or injection of foreign proteins. At one time foci of infection were thought to be possible sources of the antigen. With the discovery of the pleuro-pneumonia-like-organisms (PPLO) (89) further studies were conducted without success to try to determine if they might be the responsible antigenic agent.* The discovery of the rheumatoid factor and the development of multiple serological tests to determine its presence have provided some evidence in favor of the hypersensitivity theory (98). Other evidence consists of the profound effect of the adrenal cortical steriods which are known to alter antibody production.

6. Metabolic Abnormalities - This theory is based upon the clinical analogy of gouty arthritis in which there is a known inherited disturbance in metabolism of uric acid. Also, certain electrolyte abnormalities have been postulated in patients with rheumatoid disease. The presence of marked tissue wasting, negative nitrogen balance, hypoalbuminemia, hypergammaglobulinema and mild impairment of hepatic glycogenolysis has suggested some hepatic dysfunction with varied metabolic abnormalities postulated as etiologic agents. Although some twenty to thirty-three per cent of rheumatoid patients have evidence of some minor hepatic dysfunction at some time, there is no evidence for any primary dysfunction as the

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*In spite of negative results to date, Bauer states that persistence of an altered bacterial form is compatible with the current concept that immune mechanisms play an important role in the pathogenesis of the disease (2).

etiology in rheumatoid disease (19).

7. Endocrine Abnormalities - The final theory is the proposal that endocrine imbalance serves as an etiologic agent. At an early date, it was noted clinically that a certain per cent of rheumatoid patients demonstrated a low basal metabolic rate and also there appeared to be an increase in incidence of the disease at the time of menopause. Selye's work, which resulted in his theory of the "Diseases of Adaptation", played a large part in the development of this theory (81). In his work with the stress concept Selye observed that the production of endocrine abnormalities resulted in widespread systemic disease in his experimental animals among which was a form of arthritis. He believed that the disease was the result of an excessive output of the electrolyte regulating hormone (desoxycortisone) of the adrenal cortex plus a relative decrease in the production of the gluco-corticoids (cortisone). These animal experiments have met considerable criticism and are somewhat controversial, but have done much to arouse interest in this possible etiology of rheumatoid arthritis (33) (74).

8. Hereditary Predisposition - An eighth broad classification might be added, hereditary predisposition, perhaps based upon the lack of some as yet unknown enzyme needed for normal connective tissue metabolism. Various studies have demonstrated the tendency for arthritic symptoms to occur in families as well as for serologic abnormalities to be present in the asymptomatic relative of rheumatoid patients (54), (95), (111). The production of a strain of rats which is particularly susceptible to arthritic disease is an interesting possibility for further study.

Further factors which may be related etiologically include sex, race and climate.

METHODS USED TO PRODUCE EXPERIMENTAL ARTHRITIS

In order to provide a basis for the selection of the technique used in this study, a review of the various methods used to produce experimental arthritis follows.

1. Physical Methods - Physical methods for producing arthritis were used as early as 1850 when changes in cartilage cells of damaged joints were described (27). Methods of direct local trauma have included compression and distortion of knee joints in guinea pigs; rearrangement of tendons to produce local trauma; epiphyseal displacement and femoral subluxation; local resection of bone, cartilage or synovia; and injection of irritant fluids (such as blood). Other substances used have been kaolin suspension, turpentine oil or talc in water. Other techniques for causing local trauma include direct cauterization with hot needles; local electrolysis; ultrasonic vibration; deprivation of nerve or blood supply; and local cooling.

Experimental traumatic arthritis more closely resembles the pathology of degenerative joint disease seen in the human rather than true rheumatoid arthritis. Although these methods for producing local trauma result in osteoarthritic like changes, they are still used today for inducing pathologic changes in animals for evaluating anti-arthralgic, anti-rheumatic, analgesics.

2. Infective Methods - Many forms of infective arthritis have been studied experimentally. However, none have shown similarity in all respects to human rheumatoid arthritis with the latter's symmetrical, yet migratory joint involvement, and its chronic course characterized by exacerbations and remissions and lack of bacteremia at time of onset, joint abscesses or exudate.

Most widely known is the work dealing with streptococci. This is of especial interest because of the postulated streptococcus infection preceding rheumatic fever. In 1884 septic arthritis was produced in rabbits following intravenous injection of virulent hemolytic streptococci (25). In 1913 Jackson (37) found that experimental arthritis could be produced in rabbits by intravenous injection of beta-hemolytic streptococci obtained from a milk borne epidemic of sore throat.

Later, in 1939, Cecil (12) reported that chronic arthritis in rabbits could be produced by streptococci, pneumococci, staphylococci, and paratyphoid A. He also confirmed that the type of disease from which the streptococcus was isolated was unrelated to its capacity to produce arthritis. The arthritis appeared within a few days after intravenous injection during the stage of bacteremia and was never migratory. Although these organisms could cause lesions microscopically similar to rheumatoid arthritis, Cecil questioned if such lesions were non-specific. It would appear that such is the case, especially in the light of Selye's work with regard to non-specific responses to stress.

Brinch (6) emphasized the resemblance of streptococcus-induced arthritis to degenerative joint disease rather than to rheumatoid

arthritis. In spite of this disparity such models are still under investigation for use in drug evaluation as illustrated by the report of Pershin in 1959 (64). Pershin found that intravenous injections of streptococci and staphylococci in rats lead to the formation of multiple osteomyelitic foci and polyarthritis in seventy to ninety per cent of animals. It is evident that the pathological picture of rheumatoid arthritis is not simulated with this technique.

Following the discovery of naturally-occurring swine arthritis due to Erysipelothrix rhusipathiae, it was found that joint disease could be produced following intra-articular, subcutaneous, intradermal or intravenous inoculation of this organism into rabbits. This particular model yields many of the non-specific histological changes found in human rheumatoid arthritis but is not thought to be related since no organisms have been identified in the human disease. It is noteworthy that clinical recognition of this experimental arthritis was difficult and drew attention to the need for histological confirmation.

In 1930 a description of a polyarthritis of the vertebral column and subcutaneous nodules following spontaneous or experimental infection of mice with Streptobacillus moniliformis appeared. This disease was characteristically fatal and in the spontaneous form amputation of the infected limbs was seen. It was shown by work in the chick that blood stream invasion was followed by localization of the organisms in the synovial membrane of the joints where they functioned as facultative intracellular parasites. Because of the localization of organisms in synovial membrane and subsequent histological changes Gardner (27)

states that ". . . Streptobacillus moniliformis may be thought to provide as satisfactory a replica of the human disease as any other known organism."

Jones (43) found that crude polysaccharides from Klebsiella pneumoniae and Shigella paradysenteriae given intravenously to guinea pigs produced acute arthritic lesions of one to two weeks duration. Changes included increased mucoid material in the joint cavity, vacuolization and increase in the number and size of synovial cells. In subsequent studies polysaccharide complexes were labelled with C^{14}. The tagged material entered the joint space immediately from the plasma and histologic changes closely followed its appearance in the joint. The C^{14} was found to concentrate in the synovium. The author hypothesized a direct local effect on the synovium leading to production of non-suppurative exudate which in turn caused proliferation of synovial cells with further extrusion of plasma or protein into the joint spaces (44). Although chronic, remitting arthritis was not reported with this method further studies seem warranted.

Finally, there has been extensive work with pleuro-pneumonia-like organisms (PPLO). Sabin (76) reported development of a progressive, proliferative polyarthritis in mice with a filtrable, PPLO isolated from a mouse brain. Following intraperitoneal injection, migratory joint swelling with fusiform swelling of isolated digits appeared in four to five days. Sabin found proliferation of the joint capsule, synovium and changes in articular cartilage.

In 1942, Preston (69) found that the lesions were primarily periarticular. Later workers agreed that the animal PPLO infection

provided a useful experimental model for evaluation of therapeutic agents (gold, aureomycin, sulphonamides, cortisone, ACTH) and effects of cold and ultra-violet light etc., but that this method did not produce a replica of any particular human disease. Parkes (63) in 1951 concluded that the method could not be used as a valid screening test because of the purulent nature of the peri-articular changes and spontaneous resolution of the lesions which did not resemble any rheumatic disease. Despite this fact studies still appear with this method. In 1956, a polyarthritis in the white rat following injection of exudate from a Murphy rat lymphosarcoma pouch was described. Later this was found to be due to PPLO contamination. This exemplifies the need for consideration of infection in the role of experimental arthritis regardless of how induced. This point is also emphasized by Ward (106) who described pathological changes following PPLO administration as indistinguishable from other forms of experimental suppurative arthritis except by isolation of the pathogenic agent.

3. Chemical - Many inorganic and organic chemicals have been used over the past one-hundred years to induce experimental arthritis but none has been found to duplicate the pathogenesis of rheumatoid arthritis. Jordon (45) produced microscopic changes in rabbit knee synovium by injection of xylene or turpentine. Key (46) injected guinea pig knee joints with carbolic acid, iodine, or alcohol and concluded that the nature of the injected substance was not important provided that damage was caused. This damage, if continued, would then result in deforming arthritis.

Perhaps the best known example is the "formalin" arthritis or "topical" arthritis technique of Selye (82) (83). By injection of dilute formalin into the joint or adjacent area Selye produced initial hyperemia and edema which progressed to a chronic arthritis in white rats. Bourne (5) confirmed the observations of various investigators that the arthritis so produced was more accurately described as a peri-arthritis. Bourne claimed that the joints were not directly involved and neither cartilaginous erosion nor granulation tissue could be found. Part of this discrepancy may be due to the difficulty of making true intra-articular injections of the protein-denaturing agent into small rodents. This technique has been and continues to be used as a screening process for evaluating new anti-inflammatory compounds, cortisone, salicylates, phenylbutazone, etc.

Other substances which have been used range from distilled water or normal saline to caustic injections of acid or alkali. The organic chemicals which have been used unsuccessfully include indole, tryptamine, various toxoids, tyramine, trypsin, hyaluronic acid, histamine, proprionitriles, papain, caragheenin and mustard.

Of particular interest is the recent use of hydralazine (Apresoline^{R}) following reports of a "rheumatoid-arthritis-like" state in patients treated with large doses for hypertension over long period of time (49), (78), (92). If hydralazine therapy was continued this syndrome progressed into a condition simulating systemic lupus erythematosus. Unfortunately this syndrome has not been duplicated in animals and no

joint changes analogous to those of the human disease have been produced (14) (57) (60).

4. Endocrine - The concept of producing arthritic changes resembling those of rheumatoid arthritis secondary to endocrine imbalance is largely the result of Selye's work (80). He postulated that stress resulted in the release of some factor from the anterior pituitary which, in turn, caused the release of certain hormones responsible for the adaptation to stress. In his work Selye used adrenalectomized animals and the various hormones and extracts known. He found that high doses of desoxycortisone acetate produced nephrosclerosis, changes in electrolytes and increased blood pressure. Then he discovered that unilateral nephrectomy (later shown by Harrison (32) to be due to damage to the arterial supply of the adjacent adrenal) and one per cent sodium chloride fluid intake enhanced these toxic effects of the hormone so that rare cases developed arthritis. Next, he found that adrenalectomy or thyroidectomy plus a large dose of desoxycortisone acetate produced arthritis evidenced grossly by swelling, hyperemia, increased temperature of the affected paw; and, microscopically by pronounced edema of the peri-articular connective tissue, hyalinization and necrosis of the synovial membrane and granulation tissue. Selye concluded that the adrenal cortex may play a role in the pathogenesis of rheumatoid conditions in man, which he categorized as "Diseases of Adaptation".

This work has given rise to considerable controversy since many animals died from intercurrent infection and the possible role of

infection in causing the arthritis was not adequately studied. Various workers have either been unable to confirm his results or feel that the inflammatory reaction produced is not analgous to the human disease (75). Subsequent investigators have studied experimental arthritis and such factors as the effects of desoxycortisone acetate in normal, adrenalectomized, thyroidectomized, and thyroparathyroidectomized rats. More recent investigations used anterior pituitary hormones (27).

Reinhardt and Li (71) recorded sluggishness, decreased muscle tone, irritability, and evidence of knee and ankle joint tenderness, along with transient episodes of joint swelling, in growth hormone-treated, adrenalectomized, ovariectomized rats. They observed radiological abnormalities at the end of six months, especially of the knee, with this technique. Although interference with the pituitary-adrenal axis or endocrine imbalance was considered as a possible etiology, the authors noted that sensitization to growth hormone or production of hypersensitivity to other allergenic factors had not been ruled out.

Jasmin (39) (41) reported the production of an acute polyarthritis in Sprague-Dawley rats using Prolactin^{R} with or without growth hormone in 1959. The latter method will be described in detail under the methods section.

5. Immunological - Although there is no direct evidence that rheumatoid arthritis is the result of altered immunological mechanisms, or hypersensitivity, serological changes are associated with the condition. The separation of experimental methods for inducing immunological abnormalities from bacteriological or endocrinological procedures is

somewhat arbitrary as various agents and organisms may be used in all three techniques.

Studies based on immunological concepts consider that the organisms or the proteins function indirectly as antigens leading to a hypersensitivity reaction rather than as direct, local invaders of the joint. This work is based on the theory that arthritis may be a manifestation of allergy (altered reactivity) to joint tissue. That is, the host becomes allergic to antigens present in his own joint tissues which leads to a reaction resulting in the manifestations of arthritis and release of additional antigen.

Work in this field dates back to 1913 when sterile homologous serum was injected into the knee joints of sensitized rabbits (27). It continues with injection of foreign proteins other than serum; local, followed by systemic injection of antigenic material and, finally, the injection of homologous tissue with or without adjuvant (20). The latter method is perhaps more widely known at the present time.

In 1959 Pearson (65), (66), (68), (105) reported the development of arthritis in the rat following injection with an adjuvant. His adjuvant contained mineral oil, normal saline, emulsifier, and acid fast bacilli (Mycobacterium phlei). Ninety percent of the rats developed evidence of arthritis in eleven to sixteen days. No rats developed the disease prior to ten and one-half days. This is significant if an antigen-antibody reaction is the mechanism causing the arthritis. The arthritis was recurrent and fluctuant for several weeks, which is reminiscent of rheumatoid arthritis. However, Silverstein(87) has

presented evidence that the inflammatory swelling of the rat's paw produced by this technique is not a true arthritis but rather a periostitis and periarthritis secondary to migration of the adjuvant materials from the depot site. The explanation of this localization is not known.

Surprisingly, the most direct approach to a model of this type of disease has produced negative results. Favour (24) conducted studies based on the model of nephrotoxic nephritis wherein anti-rat kidney serum from rabbits is injected into rats with resultant nephritis. In his studies rabbits were immunized with guinea pig synovia and, secondly, guinea pigs were immunized with guinea pig synovia both with and without adjuvant. The anti-joint serum was tagged with I^{131} and given intravenously or intra-articularly. No evidence of localization of the material in the joint or synovial tissue could be obtained. (It has been suggested that the lack of response to homologous transplants of the vascular tree in humans may represent a similar lack of antigen-antibody response).

In speaking of immunological mechanisms of inducing arthritis Gardner (27) states, "Like the varieties of arthritis induced by chemical and physical agencies, they retain some value in the testing of analgesic drugs, but it cannot be accepted that they have as yet thrown light on the pathogenesis of rheumatoid arthritis."

The present investigation does not propose to investigate the pathogenesis of the disease process as already stated.

METHODS OF EVALUATION IN CLINICAL ARTHRITIS

Problems and considerations encountered in clinical evaluation will be briefly discussed before reviewing experimental methods of evaluation. Methods must be sensitive enough to indicate changes for individuals as well as groups. Manifestations of the disease must be selected which are readily observed and representative of total disease activity in terms of systemic and articular involvement. Many of the apparently obvious changes of inflammation are very difficult to measure objectively, especially when more than one observer is concerned.

JOINT CLASSIFICATION

An early attempt to consider total amount or spread of disease activity consisted of inscribing "ciphers" (symbolizing signs and symptoms such as tenderness, swelling, etc.) over the appropriate joint on an outline of the human form (38). It provided no means of numerical scoring or summation. Lansbury (51) (52) devised a numerical scoring system (Articular Index) based upon the number and size of involved joints. His technique provided a quantitative measure, permitting summation, correlating with total amount of joint activity, with insignificant intra-observer variation. Other clinicians believe such a technique gives undue emphasis to large joints where accurate clinical evaluation is impossible. Smyth (93) claims that expressing total inflammation on a simple one to four plus scale permits summation and gives equally reliable results.

FUNCTIONAL CLASSIFICATION

Early schemes of classification consisted of vague descriptive categories such as "severe, moderate or slight" (101). Following the separation of functional class from anatomical stage and therapeutic response (97) a number of set tasks were devised to provide quantitative measures of performance and muscle weakness (90). To date no tests have been developed for evaluation of overall function in experimental arthritis.

INDEXES

Steinbrocker and Blazer (96) were among the first to assign numerical values. In his review Lansbury (53) cites weaknesses of most clinical index systems to date. These weaknesses are equally true of experimental methods and include: use of arbitrary numbers, lack of adequate quantitative definitions of the severity of component items, and the preponderance of joint findings. In spite of these criticisms such methods have produced encouraging results in clinical comparison of therapeutic responses to anti-inflammatory compounds. An example is Smyth's (91) (93) comparison of response between phenybutazone, aspirin, cortisone, and others. Smyth used an Inflammatory Index based upon a qualitative four plus scale and a Functional Index based upon only three criteria.

Perhaps the most widely known method of evaluation is the combination

of articular and systemic indexes devised by Lansbury (50). He was the first to quantitate the functional criteria of stiffness and fatigue which subsequent investigators have shown to be valuable (104). The chief contribution of Lansbury's work is the method of summation and the de-emphasis of a single variable.

METHODS OF EVALUATION IN EXPERIMENTAL ARTHRITIS

One of the chief problems in experimental work is the lack of quantitative methods. This difficulty is encountered in studies regarding pathogenesis of a particular experimental arthritis or evaluation of the therapeutic response of the disease process. The most frequent procedure is to report inflammatory changes on the basis of one to four plus scales.

Various attempts have been made to quantitate the degree of joint swelling which include: 1) simple weight change in the swollen joint compared to a control (requiring amputation) (18), 2) volumetric determination by the use of a plethysmograph technique (requiring anesthetization) (17), and 3) direct measurement of the involved joint with calipers (subject to considerable inaccuracy as suitable landmarks in the small laboratory animal are difficult to define).

Possible methods of objectively measuring pain threshold of swollen joints have been suggested by the work of Brodie (7) and Randall (70). Quantitative techniques have not been applied to study of experimental arthritis to date.

Signs and symptoms of joint inflammation have been the chief

manifestations observed in experimental arthritis. Sabin (77) was the first to devise a system which indicated extent or "spread" of the arthritic process. He observed the joints of all extremities in mice given PPLO arthritis and reported use of a diagrammatic record of the afflicted joints which he called an "arthrogram". This consisted of a square for each mouse containing symbols for each small joint involved. The thickness of the symbol indicated the severity of the arthritis. No criteria were given as to how severity was graded nor were methods of summation proposed.

Tripi and co-workers (102) modified this arthrogram system to signify the extent of arthritis in terms of numerical values. They assigned values from zero to four for each anterior extremity and from zero to five for each posterior extremity. Thus, the arthrogram score for the individual rat ranged from zero, indicating no demonstrable arthritis, to eighteen indicating all four extremities involved to a maximum degree. Single composite arthrogram scores were calculated for each animal and group of animals to represent the maximum group involvement during the six month period of observation. Illustrations included the larger joints (elbows, shoulders, knees and hips) plus the factor of paralysis but the criteria on which the symbols were based were not defined. Again, no method of summation was proposed and constitutional factors were not considered.

In an attempt to increase sensitivity of this technique Pearson (65), in his investigation of adjuvant induced rat arthritis, applied a scale of zero to four for each joint of the four paws and tail, in which grade

four represented the most severe involvement. The maximum score possible for any animal on any given day was one hundred and eighty points, even though the larger joints were not considered. Again, observations are limited to inflammatory findings in the joints which are never clearly defined. Pearson has attempted numerical summation of the extent of joint involvement which may be graphed against time. This approaches the "Articular Index" useful in human disease but does not account for any of the factors considered in the "Functional Index".

Other evaluation techniques include radiological, pathological and serological studies. Most x-ray changes require months to appear and have chiefly been referred to in a descriptive manner with no attempt at quantitation. Pathological changes might be assumed to be amenable to some ranking system so that a numerical index might be applied. Few attempts to do this or form a basis for summation of results have been reported.

The discovery of serological changes in rheumatoid disease at first seemed to provide a model for experimental evaluation (4). However, studies have been disappointing in this regard. Lerner et. al. (55) (56) reported discovery of positive bentonite flocculation tests and sheep cell agglutination tests in rats developing infectious arthritis following injection of broth culture of Streptococcus moniliformis. There was no correlation between the level of the bentonite flocculation test response and the severity of joint disease. It was later found that the presence of human protein in the culture medium used for the microbe was essential for the production of the elevated bentonite flocculation

test titers in the sera of infected or immunized rats. The factor responsible for the bentonite flocculation test reaction was distinct from human rheumatoid factor and appeared to be an antibody to human protein rather than an auto-immune response on the part of the experimental animal. In 1952 Svartz (99) found that certain enterococci and pneumococci were capable of inducing antisheep cell agglutinens in rabbits, but the significance of this finding is uncertain. More recently, Svartz (100) reported formation of a protein similar to rheumatoid factor, in white rats with experimental arthritis, induced by injections of beta streptococci (Streptococcus agalactiae). This work needs confirmation but suggests that further attempts in this direction may provide objective means of studying experimental disease processes.

Factors of decreased range of motion, muscle weakness, pain and fatigue are significant in rheumatoid arthritis. Technical factors have obviated measurement of range of motion in the small laboratory animal. Tests of muscle weakness or fatigue would have to be based on maximum performance (such as stress tests using swimming time in cold water, ability to perform on a forced runway, ability to grip a rotating rod, etc.) which would be very difficult to standardize and interpret. (In addition, adrenalectomized animals would not be expected to perform such tasks without considerable mortality).

Various techniques have been reported in the literature for measurement of gross motor activity in the small animal. It was hypothesized in this study that gross motor activity might provide a measure of

"functional capacity" whether it relates specifically to muscle weakness, fatigue, or simply decreased activity due to joint tenderness and pain. Such measurements have not been previously applied in the study of experimental arthritis.

METHODS OF MEASURING GROSS ACTIVITY

The two most widely used methods of measuring general activity are the revolving drum technique or "running wheel" and the tambour mounted cage technique (62) (88). The running wheel has been widely used in psychological and pharmacological research with many modifications (40). The usual activity wheel provides a measure of the total number of revolutions or the total distance traveled in a given time period. The tambour mounted cage provides a measure of the time of occurrence of the rat's movements and the duration of rhythms which appear throughout the day.

Schulte (79) recorded with a wire cage suspended from a spring which moved up and down in response to each movement or shift in balance of the animal. Although movement could be summated for several hours with this technique, it raises the question of how an unstable environment affects activity.

Each (22) used a technique of open field counting. The open field was marked with six inch squares, the measure of activity being the number of squares traversed in a stated period.

Winter and Flataker (109) were among the first to use the principle of recording interference of a light beam by the animals movement. Their apparatus consisted of a box lined on two sides with mirrors. A light

beam was admitted through an opening in one mirror, was reflected twice, and struck a photo-electric cell. This cell operated a solenoid counter which registered the number of times the beam was interrupted. Counts for a ten minute period were taken as an index of activity. Similar devices have been cited by Munn (62). Melander (59) reported use of four pairs of photocells placed centrally on the sides of the cage and in both diagonals. This author obtained a numerical value for activity using only a five minute counting period before and one hour after drug administration. His study is noteworthy because of the combination of the photocell technique and a photographic technique. Individual mice were painted with a dye which emitted visible light when exposed to ultraviolet light. Movement was recorded on panchromatic film for a five minute period immediately after the corresponsding photocell count was obtained.

LABORATORY TECHNIQUES FOR EVALUATION OF ANTI-INFLAMMATORY AGENTS

Laboratory evaluation of anti-inflammatory drugs has been chiefly based upon effects following peri-articular injections producing acute edema (48) (70), "topical arthritis" (83), cotton pellet implantation (10) (21), dermal application or injection of irritants (8) (23) (103), dermal exposure to ultraviolet light (107) (108), or the related model of the granuloma pouch (18) (72) (83).

PRELIMINARY METHODS

Preliminary studies were conducted using five methods to produce an arthritic model in the rat as follows: high dosage administration of desoxycortisone acetate in adrenalectomized white rats; formalin "topical arthritis" technique; granuloma pouch technique as modified by Robert (72); high dosage administration of hydralazine; and, finally, Prolactin^{R} injection in adrenalectomized, desoxycortisone acetate maintained white rats as described by Jasmin and Bois (39).

The first four methods were discarded either because no arthritic lesions could be observed (such as following hydralazine) or because of the fleeting and mild nature of lesions produced. The method of Jasmin and Bois (39) was selected since the severity of involvement ranged from mild to fatal.

METHOD OF PRODUCING ARTHRITIS

The original method of Jasmin and Bois consisted of treating 125 - 150 gm. female, Sprague-Dawley, adrenalectomized, white rats with subcutaneous injections twice daily of Prolactin^{R} (Lactogenic Hormone) in the following dosage:

15 International Units (in 0.2 cc. physiological saline)
for an eight day period followed by

30 International Units (in 0.2 cc. physiological saline)
for a seven day period.

In addition animals were given 1.0 mg. desoxycortisone acetate every other day (crystalline suspension in 0.2 cc. physiologic saline) to maintain salt and water balance.

The method was modified as follows:

First, Prolactin^{R} treatment was not initiated immediately following adrenalectomy because of the alteration in general activity level after the trauma of surgery. Since the activity level was one of the chief criteria of arthritic involvement in this study, it was necessary to provide an opportunity for the activity to return to pre-operative levels or at least to become stabilized. This modification plus the time necessary to obtain pre-operative activity levels accounts for slightly older age of our animals at the time of treatment.

Second, it was desirable to find ways to increase the total number of moderately and severely involved animals in order to be able to rank those anti-inflammatory drugs with varying degree of therapeutic effect. Although Hall (30) found no alteration in incidence or severity of arthritis in parabiotic female rats given one per cent saline to drink, other authors have reported the enhancement of severity and incidence of hormonally induced arthritic lesions by the substitution of one per cent saline ad lib for drinking water (39) (80). Since hormonally-induced arthritis was the object of the study, all animals received one per cent saline ad lib for their drinking water.

Third, in the initial phases of this study, desoxycortisone acetate was administered in sesame oil. In later experiments it was given as a micro-crystalline suspension in physiologic saline.

Fourth, the natural course of the induced disease was observed over a period of time rather than sacrificing the animals at fifteen days as was done by Jasmin and Bois. Upon death of the animal due to the disease process or other natural causes, autopsy was performed with especial attention given to the state of all joints, kidneys, heart, lungs, and to the perirenal area to be sure that no adrenal tissue remained. Appropriate tissues were taken from these organs and fixed in ten per cent formalin. Hematoxylin and eosin sections were then made of selected specimens.

GENERAL METHODS

Clinically, rheumatoid arthritis has a sex ratio of occurrence of about four female patients to one male. Female rats have been shown to be more susceptible to experimental arthritis than male rats by previous investigators (65). In this study only female Sprague-Dawley rats were used.

All animals were housed in standard 7x7x9½ inch wire cages and given Purina Rat Chow ad lib. The temperature of the animal quarters varied from summer to winter but during any given experiment was maintained as nearly constant as possible. During activity measurements, the difference in rat room temperature and activity room temperature was kept between 0 to 1½° C. with few exceptions.

Adrenalectomy was performed by lumbar approach (16). Various anesthetic agents used include: Trilene^{R} alone, ether alone, Nembutal^{R} (35 mg.kg.) alone, and with Trilene^{R} or ether supplement. The most satisfactory combination, from the standpoint of short but adequate duration of anesthesia plus low mortality rate, was intraperitoneal injection of 24 mg./kg. Nembutal^{R} in 10% alcohol.

METHODS OF EVALUATION OF ARTHRITIS

1. Arthrogram - Joint involvement was recorded and transcribed into a numerical system which permitted summation and comparison between different animals. In the scoring system devised values were assigned

to the degree of redness, swelling, tenderness, heat, flexion, flaccidity, and gait disturbance. It was soon evident that certain factors could be broken down into four gradations whereas others were limited in variation and only one or two gradations could be determined. As shown in Table I those factors with limited variation were scored on a 0 to 2+ scale and the remaining factors scored on a 4+ scale.

After each joint had been evaluated a method of correlation between the number and size of involved joints was required. This was done by using the multiplication system noted in Table I, Part B. The total score for each factor, such as redness, was multipled by 1/2, 1, or 2 dependent upon the number of joints involved in each extremity, with the exception of the variables with an asterisk (where a maximum of 2+ per extremity could be observed). When all joints of the metatarsal-phalangeal, all of the metatarsal-tarsal and the entire ankle joint were involved, these joints were considered as three groups and the maximum multiplication factor was six. This system was adopted because of the need to emphasize maximum or near maximum involvement in spite of the difficulty in determining exactly whether all of the joints of these three groups were involved.

The longest period of observation and scoring was performed for the initial study done on ten animals. Daily observations and arthrogram scores were computed for the first two months and subsequently observations were made every third day for the next two months. In all subsequent experiments data was collected daily for the first month after Prolactin^{R} was started. In some cases observations were then continued

TABLE I

ARTHROGRAM SCORE SYSTEM

Part A.

OBSERVATION	+ SCALE
Redness	0-4
Swelling	0-4
Tenderness	0-4
Increased temperature	0-2*
Flexion	0-2
Vasodilatation	0-2*
Flaccidity	0-2*
Altered gait	0-2*

BASIS OF NUMERICAL SCALE

0 - Normal
1+ - Slight change
2+ - Moderate, but definite, change
3+ - Marked change
4+ - Maximum change

*2+ maximum per extremity

Part B.

By gross observation it is impossible to determine if all five metatarsal-tarsal joints or only a fraction are involved with inflammatory changes. Thus, the definition of a "composite joint" or group of closely related joints which grossly undergo change as a unit was developed. The three "composite joints" used for application of the multiplication system are the metatarsal-phalangeal joints; the metatarsal-tarsal joints; and the ankle joint.

The score for involvement of each variable above in each extremity is multiplied by one of the following dependent upon the number of involved joints per extremity.

Factor for each joint	Degree of Spread	Total multiplication factor
1/2	Single joint or Single "composite joint"	1/2
1	Interphalangeal joint + one "composite joint" or Two "composite joints"	2
2	Entire extremity (all three "composite joints")	6

The TOTAL ARTHROGRAM SCORE = THE SUM OF SCORES FOR EACH OBSERVATION IN ALL EXTREMITIES

every other day for the next month. At this time, the arthritic process had stabilized with minimum inflammatory activity and observations were concluded unless animals were to be retreated. Body weight was recorded at the same time observations were being made for the presence of inflammatory changes.

2. Actophotometer - Gross activity of the animal was measured with the Acophotometer[R] activity recording chamber. This device consists of a metal cylinder thirteen inches in diameter with six photo-electric cells placed so that any movement within the chamber interferes with a light beam which activates a solenoid counter.

All measurements were made in a darkened room in order to insure identical light conditions for each run and also because the animal might be expected to run maximally in the dark.

Initial experiments with this apparatus revealed that a period of three to four sham runs was necessary before the animal became accustomed to the recording chamber. Following these "rehearsals" the animal no longer became unduly excited and reproducible results could be obtained. It was also found that activity measurements had to be done at least once every seven days or else the "strange" surroundings influenced the animal's activity.

In preliminary experiments activity was recorded for 3, 5, 10, 15, 20, 30, 45 and 60 minute periods after the animal was placed in the Actophotometer[R]. It was determined that the largest mean activity with the least deviation between animals and between different days was present when activity was measured for a period of twenty or thirty

minutes. A thirty minute period of observation in the activity chamber was used throughout this study.

3. Photography - Gross photographs were taken of selected animals prior to, during, and after Prolactin^{R} treatment in order to obtain a further objective record of the degree of swelling, flexion and site of involvement.

4. Pathology - At autopsy sections of heart, lungs, kidney, muscle and the involved joints were placed in ten per cent formalin. Hematoxylin and eosin stains of these tissues were then made following decalcification where necessary. It was suggested that special connective-tissue stains such as the Mallory stain would not aid greatly in interpretation.

5. Electromyography - Because of the recent reports of electromyographic changes present in rheumatoid arthritic patients (1) (29) (34) (61) attempts to record potentials over the experimental rat's lower extremity were made. Unfortunately these records were unsatisfactory for technical reasons. In addition to sixty cycle interference the necessity for anesthetizing animals prevented further pursuit of this technique. The use of a general anesthetic was felt to be contra-indicated because the animals were already subject to increased risk of pulmonary infection and the severely involved cases were quite debilitated.

6. Hematology and Serology - The mean blood volume in adult male Sprague-Dawley rats has been reported to be 4.95 cc./100 gm. of body weight (84). The average weight of the oldest animals included in this study did not exceed 290 grams at the completion of Prolactin^{R} injections.

It was felt that the removal of slightly over 1 cc. of blood in order to determine sedimentation rate, red cell count, serological reactivity, and serum protein electrophoretic pattern would produce severe anemia and would be inadvisable. The risk of secondary infection was also a contraindication to venipuncture. In addition, until the recent report of Svartz (100), results of serological studies in experimental arthritis have been negative.

7. Radiology - The purpose of this arthritic model was to provide a means of evaluating anti-inflammatory drugs over a relatively short period of time. Because radiological changes require months to appear such examinations were not performed.

METHOD OF DRUG EVALUATION WITH "MODEL" ARTHRITIS

Two types of experiments were performed to test the effects of administration of anti-inflammatory compounds upon the "model" disease.

In the first, the anti-inflammatory agent was withheld until the animal had developed maximum involvement with inflammation of all extremities and signs of impending death. A single subcutaneous injection of the drug was then given and changes in the arthrogram recorded. This technique was limited because of the low incidence of severely involved animals.

The second experiment was designed to test the effects of administration of the anti-inflammatory agent prophylactically. The anti-inflammatory agent was administered for a 15 day period simultaneously with the Prolactin^{R} treatment. All agents were given in a single subcutaneous injection in 0.2 cc. physiologic saline. Comparison was based

upon whether the agent altered the time of onset, number of involved animals, severity (mortality), or the course of the inflammatory changes.

To determine if this method was sensitive enough to distinguish between different types of anti-inflammatory compounds, test drugs were selected from three broad categories as follows:

Test drug number 1 — Sodium salicylate - representative of the classic "anti-rheumatic" activity of the salicylates.

Test drug number 2 — Phenylbutazone - representative of a new class of synthetic anti-inflammatory agents of the pyrazolone group of compounds.

Test drug number 3 — Hydrocortisone - representative of the potent anti-inflammatory steriods.

Selection of Drug Dosage - Dosage of salicylate was based upon comparison with the usual maintenance dosage in the human rheumatoid arthritic patient which varies between 2.6 and 5.8 gm./day. The usual dosage is approximately 50 mg./kg./day of sodium salicylate which was selected for use in this study.

The human maintenance dose of phenylbutazone is 100 - 200 mg./day, or approximately 2.1 mg./kg./day. Because of the more rapid rate of bio-transformation of this drug in the rat, as compared to the human (9), the equivalent dose is about 24.2 mg./kg./day. Most experimental studies report use of doses four to eight times this value. Studies have shown that following two subcutaneous doses of 150 mg./kg. of phenylbutazone, 50 out of 50 rats will develop gastric ulcers within 24 hours after the

first injection. To avoid this complication, and to provide a means of comparison with sodium salicylate on a weight for weight basis a lower dose of 50 mg./kg./day of phenylbutazone was selected (approximately twice the human maintenance dose).

In a study of the effect of hydrocortisone upon the mycobacterial adjuvant type of experimental arthritis, Pearson and Wood (66) used a dose of 2 mg./kg./day for a 16 day period. To enable comparison with their study as well as with the dose of phenylbutazone selected above, a dose of 2.5 mg./kg./day of hydrocortisone was selected (approximately twice the human maintenance dose).

The design of this second experiment is shown in the following protocol:

Number of Young Adult Rats	Procedure	Anti-inflammatory Drug
Controls 6	Adrenalectomy + DOCA and saline maintenance	NONE (saline only)
Controls 10	Above + Prolactin^{R} treatment	NONE (saline only)
Group A 9	Above	+ Sodium salicylate 50 mg./kg./day
Group B 8	Above	+ Phenylbutazone 50 mg./kg./day
Group C 10	Above	+ Hydrocortisone 2.5 mg./kg./day

Arthrogram scores were computed and body weights recorded daily for all rats over a 30 day period. Actophotometer counts were obtained prior to treatment and on days 2, 5, 9, 12, 16, 19, 23, 26, and 30 following

initiation of Prolactin[R] and drug administration. All activity measurements were obtained at approximately the same time of the day by the same observer for each animal.

R E S U L T S

ARTHROGRAM STUDIES

In the initial study ten young adult rats whose average weight was 146 gm. were started on Prolactin^{R} injections. No changes were evident in the appearance of the rats until the 12th day when signs of an inflammatory process appeared. These began with slight to moderate redness occurring usually in the metatarsal-tarsal joint and progressing to involve the tarsal bones. In the more severely involved animals, the process continued to spread and involved the interphalangeal joints, metatarsal-phalangeal, tarsal and tibio-tarsal joints. No changes were evident in the knee, shoulder, spinal or tail joints. The hind paws were affected most frequently but, with maximal involvement, the distal joints of the upper extremities were similarly affected.

Swelling was the next change noted and it usually appeared in the metatarsal or tarsal area first. In progressive cases swelling was observed in the interphalangeal, metatarsal-phalangeal, tarsal and tibio-tarsal joints (see Figure 1). It was associated with tenderness, warmth, vasodilatation and altered gait in severely involved animals. Some cases did not progress beyond redness and swelling. In most instances the arthritis was migratory and involved multiple joints. Unless swelling involved the entire foot it infrequently produced fusiform swelling of the inter-phalangeal joints.

The signs of inflammation usually remitted in the reverse order of their appearance but occasionally swelling and stiff gait persisted after all other signs had disappeared.

Figure 1

GROSS APPEARANCE
of
PROLACTIN^{R} TREATED RAT'S FOOT

A. Normal plantar view of rat's foot prior to Prolactin^{R} treatment.

B.-C.-D.
Lateral, plantar, and dorsal views of same animal on the 13th day after Prolactin^{R} treatment was started. At this time all extremities were 3+ red, 3+ swollen, both feet were tender, warm and some flexion deformity was present.

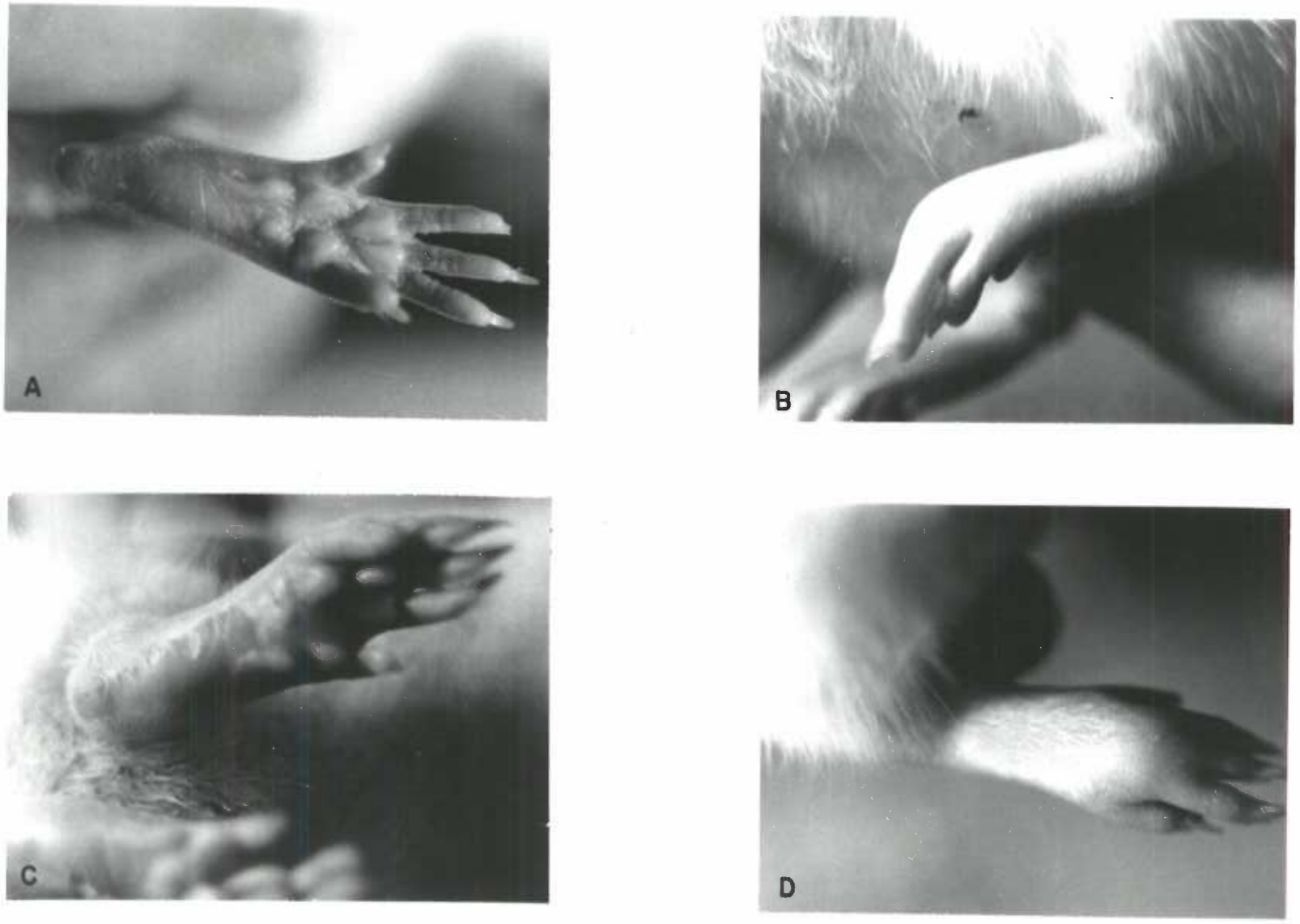


Figure 1

In one animal showing progressive inflammatory changes, death occurred on the 16th day. This animal had a generalized inflammatory reaction with all extremities red, swollen, painful, and warm. Even the ears, tail and mucous membranes appeared inflamed. This condition was associated with severe debility of the animal, diarrhea, conjunctivitis and mucoid to blood-tinged nasal secretions (see Figure 2). Inability to feed itself in the terminal stage was perhaps a chief cause of death as the animal appeared too weak to lift its head.

The incidence of involved animals reached a maximum during the 15th to 25th day following injection. During this same period involvement in individual animals reached a peak. The maximum group average arthrogram occurred on the 16th day. Reference to Table II shows that all ten animals in Experiment I developed some signs of arthritis. Nine had moderate involvement, and only one had maximum involvement (an arthrogram score over 20). The death of the severely involved animal partially accounts for the sharp drop in group average arthrogram score as depicted in Figure 3. It should be emphasized that Figure 3 represents the average degree of involvement for the ten rats and that a great range existed in individual involvement.

Following the acute polyarthritis, the animals developed a low grade migratory arthritis usually involving the metatarsal-phalangeal or metatarsal-tarsal joints. In some instances all signs of involvement disappeared completely only to exacerbate at a later date. At sixty days, because very few alterations in the signs of arthritis were apparent, observations were extended to every third day and continued for the next

Figure 2

GROSS GENERAL APPEARANCE
of
NORMAL AND PROLACTIN^{R} TREATED RAT

A. Normal rat:
Note stance, smooth fur and especially, the size and appearance of the hind paw.

B. Prolactin^{R} treated rat:
Severe, generalized inflammatory reaction was present when the picture was taken on the 13th day after Prolactin^{R} was started.

Note the listless, weak stance, the ruffled fur and especially the swollen, flexed hind paw.

Figure 2

TABLES II and III

A SUMMARY OF OBSERVATIONS WITH THE ARTHROGRAM

Correlation of Onset of Appearance, Severity and Peak Incidence of Arthritic Changes, and Mortality

TABLE II

Exp. No. I — Procedure: Prolactin^{R} Regimen

Number of rats started: 10

Average weight when Prolactin^{R} started: 146 gm. (range 140-160)

Onset of arthritis day: 12

Total Number of rats developing arthritis: 10

Total Number of rats dying in first month: 2

Peak incidence of arthritis in group: days 15 (8/10) to 22 (9/9)

Variation in severity:

Arthrogram score	Number of rats
0	0
0.5 to 5	0
5+ to 20	9
20+	1

Highest arthrogram score..... 73

Mortalities on days 16 and 26

TABLE III

Exp. No. I — Procedure: Prolactin^{R} Regimen retreatment

Number of rats started: 6

Average weight when Prolactin^{R} started: 272 gm. (range 240-290)

Onset of arthritis day: 8

Total number of rats developing increased arthritis: 5

Total number of rats dying in first month: 0

Peak incidence of arthritis in group: days 10 (6/6) to 18 (5/6)

Variation in severity:

Arthrogram score	Number of rats
0	0
0.5 to 5	2
5+ to 20	3
20+	1

Highest arthrogram score 24

Figure 3

VARIATION IN SEVERITY OF ARTHRITIS

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities following Prolactin^{R} treatment of 10 young adult rats.)

NOTE: A total of 2 fatalities occurred. The average arthrogram was computed for the remaining animals.

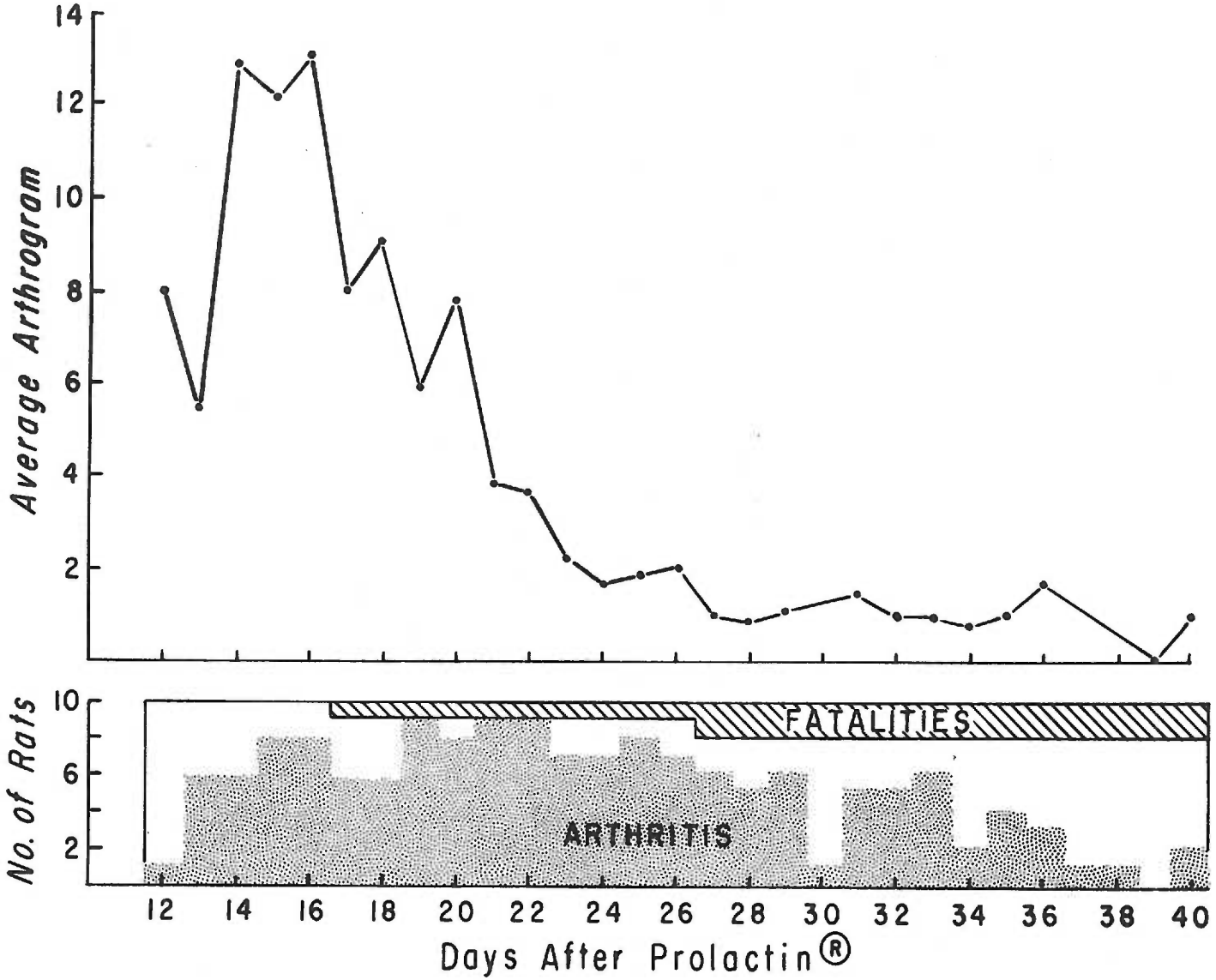

Average Arthrogram
14
12
10
8
6
4
2
No. of Rats
10
6
2
FATALITIES
ARTHRITIS
12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Days After Prolactin®

Figure 3

seven weeks. A slight increase in redness and swelling of the lower extremities occurred at three and one-half months. At this time observations were recorded every second day. During this interval minimal activity was present but it was so slight that drug evaluation would not have been possible. Therefore, at four months the six surviving animals were retreated with the same dosage schedule of Prolactin^{R}.

The retreated group showed a definite shortening between treatment and appearance of increased signs of arthritis (see Figure 4). That is, appearance of increased arthritis on the 8th day occurred in two out of six animals, compared to onset of arthritis on the 12th day in the initial experiment. On the 9th day a third animal showed increased inflammation. Only one animal failed to show some increased response. This data is suggestive of an "anamnestic response". Table III shows that five of the six animals developed increased peri-articular inflammation. Three animals had moderate involvement and one animal had maximum involvement. The peak group average arthrogram occurred on the 11th day of retreatment. No fatalities occurred during the retreatment period.

In the next study seven mature rats averaging 256 grams weight were started on Prolactin^{R} injections. Late on the 11th day of the experiment, one animal developed slight redness of both metatarsal-tarsal joints. On the 12th day six animals had minimal evidence of redness with no swelling. Minimal signs of inflammation were present in six animals with a peak incidence during the 12th to 22nd day as shown in Table IV. Only one animal developed moderate disease activity

Figure 4

VARIATION IN SEVERITY OF ARTHRITIS

(Showing correlation of the average arthrogram and the number of animals with increased arthritic involvement following re-treatment with Prolactin^{R} four months after the initial period of Prolactin^{R} treatment. No fatalities occurred.)

NOTE: The shorter period between the time of treatment and exacerbation of the arthritis and the slightly increased severity of the process when compared to results of initial treatment (see Figure 3) are suggestive of an "anamnestic" response.

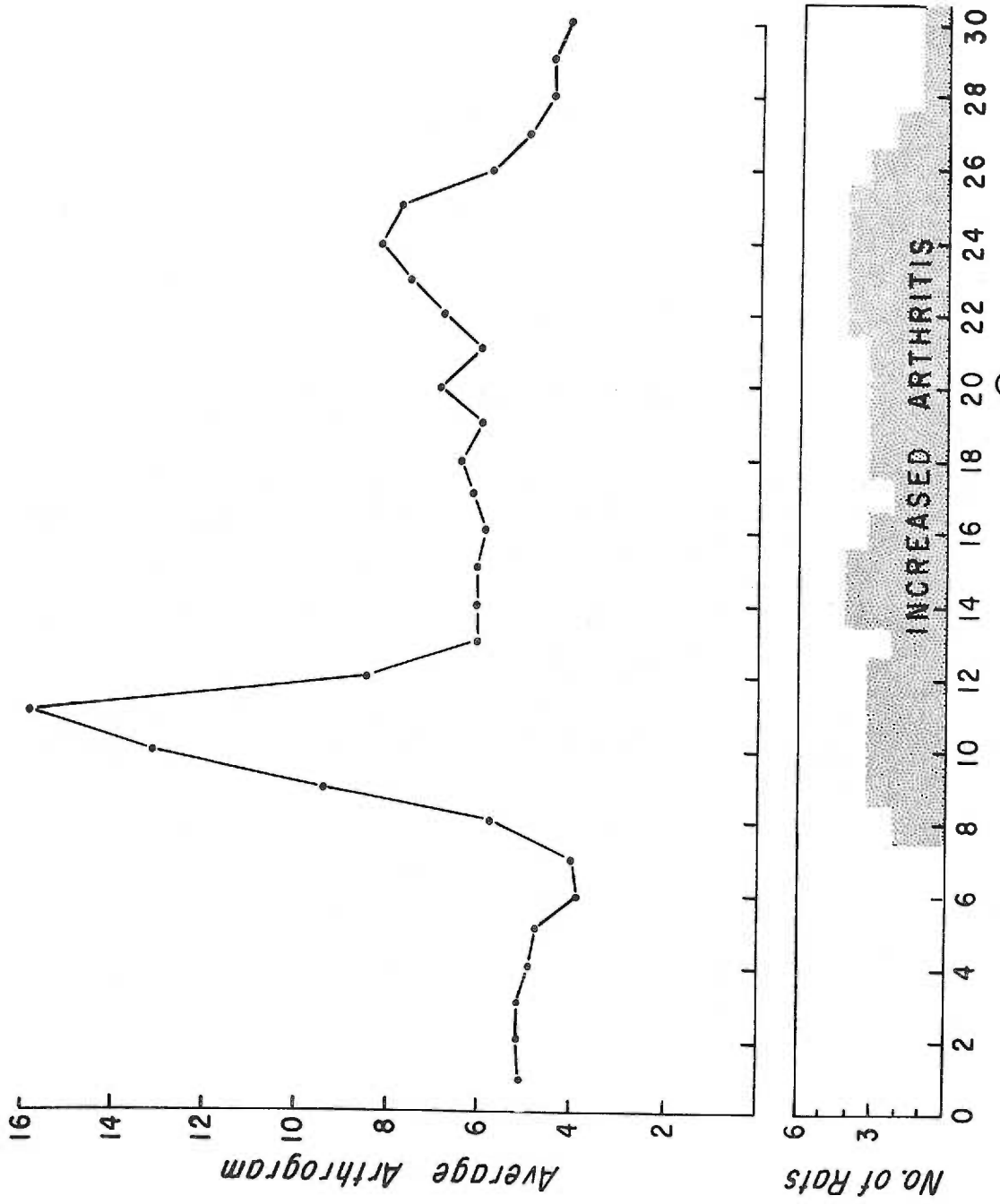

Average Arthrogram
16
14
12
10
8
6
4
2
No. of Rats
6
3
INCREASED ARTHRITIS
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Days After Prolactin ®

Figure 4

TABLES IV and V

A SUMMARY OF OBSERVATIONS WITH THE ARTHROGRAM

Correlation of Onset of Appearance, Severity and Peak Incidence of Arthritic Changes, and Mortality

TABLE IV

Exp. No. II — Procedure: Prolactin^{R} Regimen

Number of rats started: 7

Average weight when Prolactin^{R} started: 256 gm. (range 230-280)

Onset of arthritis on day: 11

Total number of rats developing arthritis: 7

Total number of rats dying in first month: 0

Peak incidence of arthritis in group: days 12 (6/7) to 22 (6/7)

Variation in severity:

Arthrogram score	Number of rats
0	0
0.5 to 5	6
5+ to 20	1
20+	0

Highest arthrogram score..... 11.5

Mortalities on days 72 and 82

TABLE V

Exp. No. III — Procedure: Prolactin^{R} Regimen

Number of rats started: 10

Average weight when Prolactin^{R} started: 230 gm. (range 210-260)

Onset of arthritis on day: 10

Total number of rats developing arthritis: 9

Total number of rats dying in first month: 3

Peak incidence of arthritis in group: days 15 (8/9) to 23 (5/7)

Variation in severity:

Arthrogram score	Number of rats
0	1
0.5 to 5	3
5+ to 20	3
20+	3

Highest arthrogram score..... 190

Mortalities on days 12, 20, 22

with a maximum arthrogram score of 11.5 on the 16th day. The peak group arthrogram of 3.4 occurred on the 16th day. There were no progressive fatal cases. There was no increase in signs of inflammation at three and one-half months.

A second group of ten mature rats with an average weight of 230 gm. was started on Prolactin^{R}. Onset of arthritis occurred on the 10th day in two rats. One animal developed progressive, generalized signs of severe inflammation and died on the 12th day, with an arthrogram score of 190. A second animal was treated with 2mg. of the corticosteroid Hydeltrasol^{R} because of similar progressive inflammation. A single dose was given subcutaneously on the 13th day. The subsequent course for this animal is depicted in Figure 5. In contrast to these two animals with severe involvement, one animal failed to develop arthritis, three developed minimal involvement and three developed transient moderate involvement (see Table V). The peak group arthrogram score of 22 occurred on the 12th day and the peak incidence occured between the 15th to 23rd day. Figure 6 represents the average group arthrogram for the seventeen mature rats. The apparent increase in activity on the 27th day shown in Figure 6 is due to the fact that one animal developed marked redness and swelling in one extremity following intramuscular injection of tetracycline for a respiratory infection. It is likely that this reaction was due primarily to the drug injection but the role of the respiratory infection or the local stress in precipitating latent inflammatory processes in this animal can not be disregarded. None of the four control animals given desoxycortisone maintenance and saline ad lib following adrenalectomy developed any signs of inflammation.

Figure 5

ALTERATION OF PROGRESSIVE INFLAMMATION

IN THREE ANIMALS

BY CORTICOSTEROIDS

(Each line represents the arthrogram score for a single animal. Corticosteroid therapy was withheld until the arthrogram score was greater than 100, representing a severe, progressive, inflammatory condition.

In two animals the single subcutaneous dose, as indicated, was sufficient to rapidly ameliorate the signs of inflammation. One animal received two doses of triamcinalone, as indicated, and then slowly returned to minimal arthritic activity.)

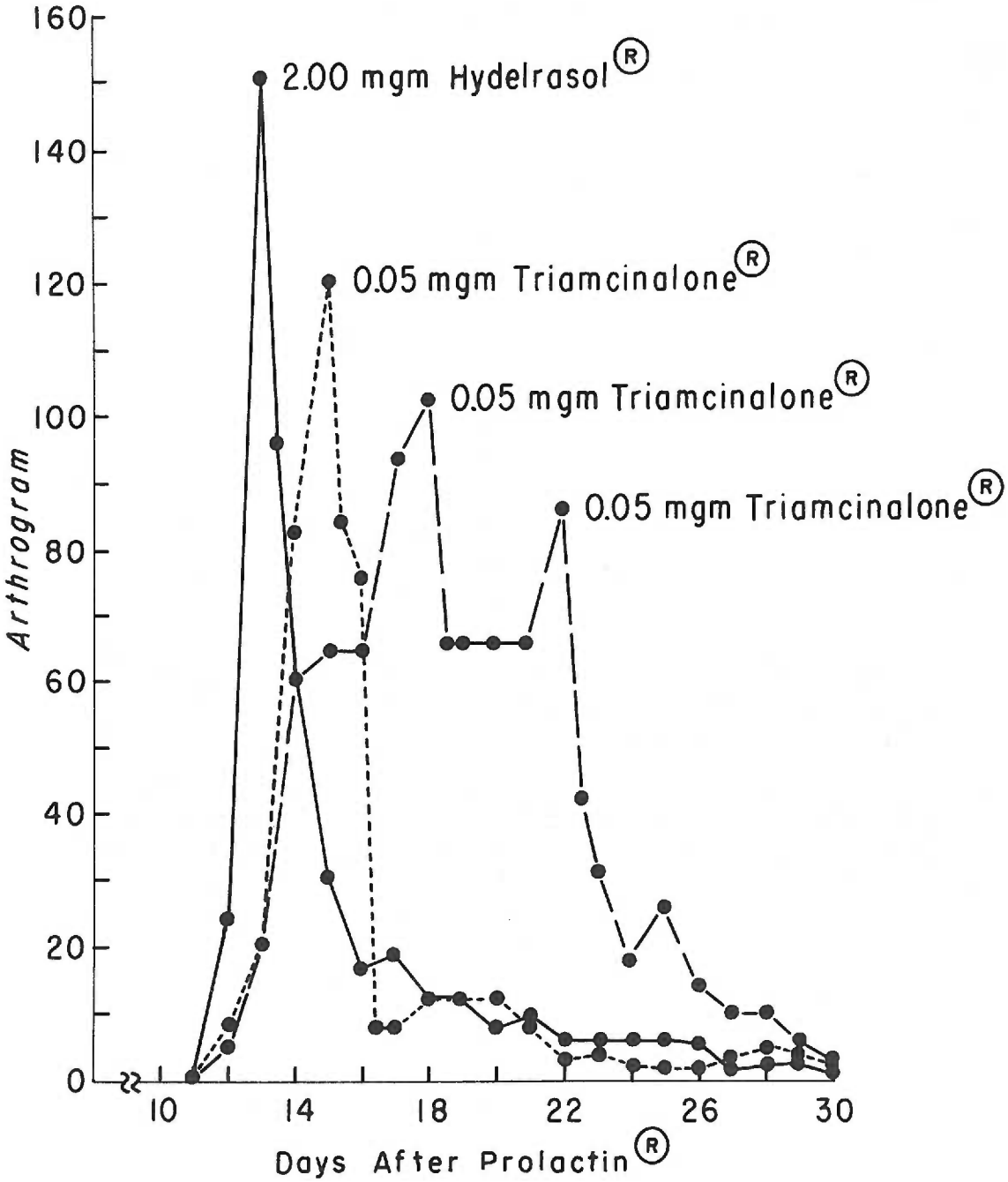

160
140
120
100
80
60
40
20
0
Arthrogram
2.00 mgm Hydelrasol®
0.05 mgm Triamcinalone®
0.05 mgm Triamcinalone®
0.05 mgm Triamcinalone®
10
14
18
22
26
30
Days After Prolactin®

Figure 5

Figure 6

VARIATION IN SEVERITY OF ARTHRITIS

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities following Prolactin^{R} treatment of 17 mature rats.)

NOTE: A total of 3 fatalities occurred and 1 animal received steroid therapy. The average arthrogram was computed for the remaining animals.

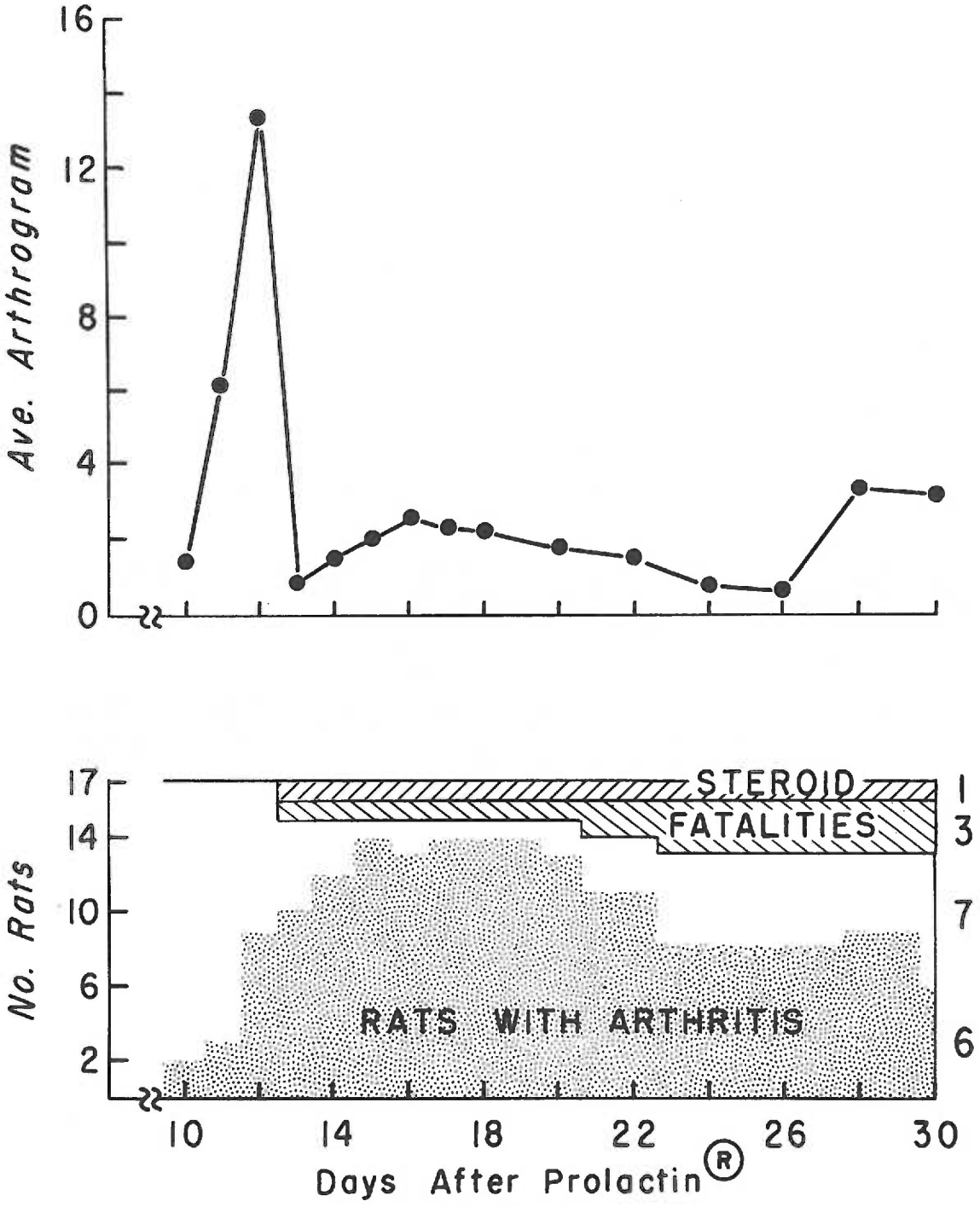

Ave. Arthrogram
16
12
8
4
0
No. Rats
17
14
10
6
2
STEROID
FATALITIES
1
3
7
RATS WITH ARTHRITIS
6
10
14
18
22
26
30
Days After Prolactin®

Figure 6

In contrast to the minimal involvement in the seventeen mature animals shown in Figure 6, Figure 7 shows the response of eighteen young adult rats (weight 160 gm. when Prolactin^{R} started). Three animals developed slight redness of the metatarsal-tarsal and heel joints on the eleventh day. This was rapidly progressive in one case with death on the twelfth day. Death was associated with redness, swelling, warmth and tenderness of both lower extremities resulting in an arthrogram score of 60. On the twelfth day ten animals had developed some signs of inflammation. The pattern of redness followed by swelling in the metatarsal-phalangeal, metatarsal-tarsal and heel joints appeared as described previously. It was progressive with warmth, tenderness, vasodilatation, and altered gait in five animals. Three mortalities occurred during the first month. Seventeen of the eighteen animals developed migratory inflammatory changes with a peak incidence for the group between the 13th and 21st days as shown in Table VI. Predilection for the hind paws was evident with upper extremities involved only in the severe cases. The maximum individual arthrogram of 190 appeared on the 14th day shortly before death. The maximum group arthrogram of 23.5 occurred on the 14th day. A second animal rapidly progressed to an arthrogram of 120 on the 15th day. This animal was given 0.05 mg. of the corticosteroid triamcinalone, subcutaneously, and the dramatic reversal of inflammatory signs is illustrated in Figure 5. Figure 5 also depicts the second steroid treated animal given the same dose of triamcinalone on the 18th day and repeated on the 22nd day. Following the acute phase signs of inflammation regressed in all

Figure 7

VARIATION IN SEVERITY OF ARTHRITIS

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities following Prolactin^{R} treatment of 18 young adult rats.)

NOTE: A total of 3 fatalities occurred and 2 animals received steroid therapy. The average arthrogram was computed for the remaining animals.

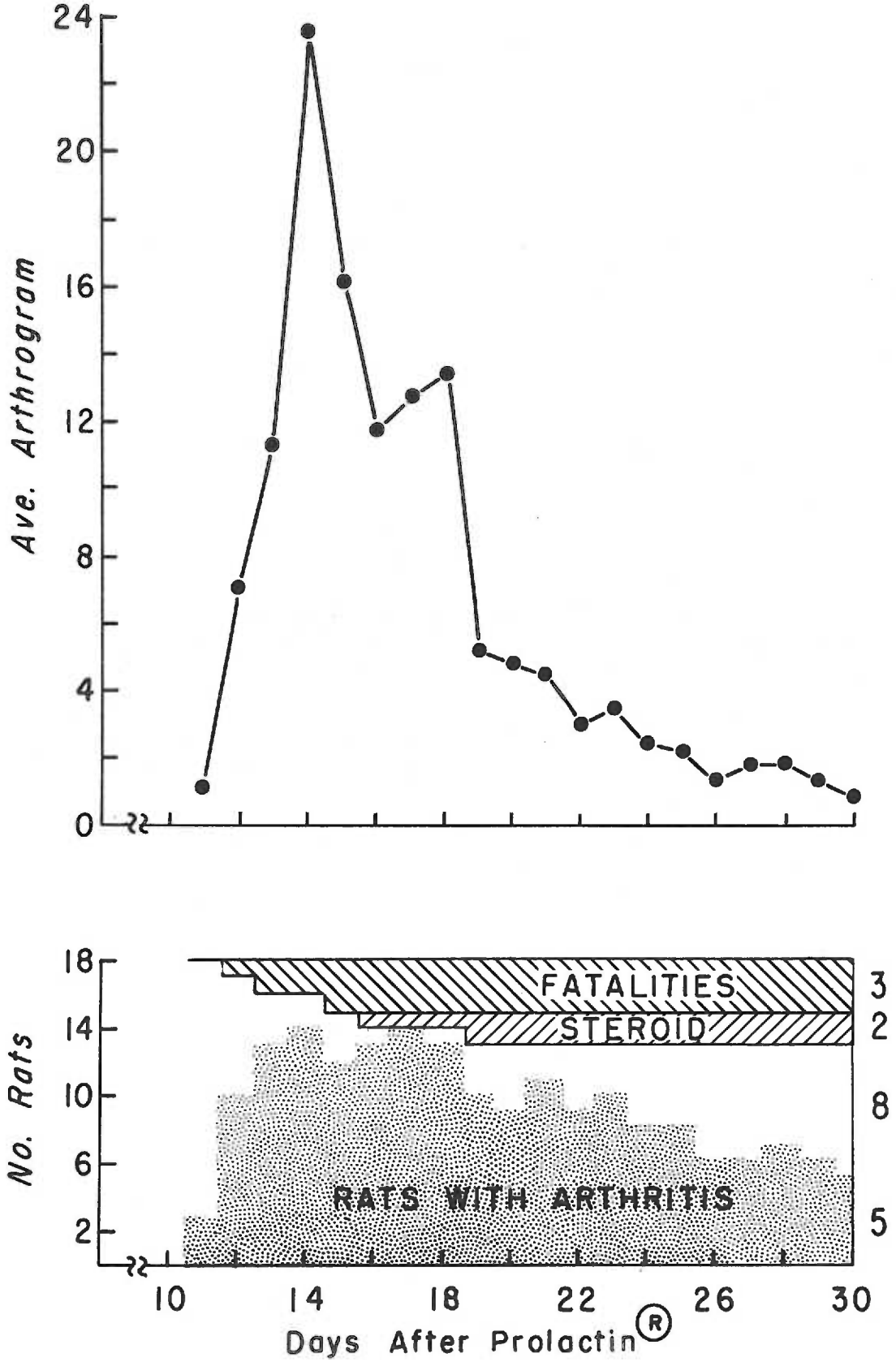

Ave. Arthrogram
24
20
16
12
8
4
0
No. Rats
18
14
10
6
2
FATALITIES
STEROID
RATS WITH ARTHRITIS
3
2
8
5
10
14
18
22
26
30
Days After Prolactin®

Figure 7

animals in reverse order of appearance with some persistence of swelling and altered gait and a minimal chronic arthritis.

None of the twelve desoxycortisone, saline maintained, adrenalectomized animals developed any signs of inflammation. No changes were apparent in five unoperated control animals.

The above experiments were designed to demonstrate the natural course of the disease in both young adult and mature rats and to observe the effects of steroid administration after development of arthritis.

The results described below refer to experiments designed to evaluate the prophylactic administration of anti-inflammatory compounds given simultaneously with the arthritogenic agent in young adult rats.

Ten control animals (weight average 211 gm. when Prolactin^{R} started) were not given any anti-inflammatory compound. As shown in Table VII, onset of arthritis occurred on the 12th day. All ten animals developed arthritis with a peak incidence between the 15th and 25th days as illustrated in Figure 8. Six animals developed moderate or marked involvement. The maximum group arthrogram of 24 occurred on the 20th day. The highest individual arthrogram score of 108 occurred on the 20th day. There were two mortalities during the first month, on the 18th and 20th days.

None of the six desoxycortisone, saline maintained, adrenalectomized control rats developed any signs of arthritis.

Eight rats (average weight 196 gm. when Prolactin^{R} started) were given phenylbutazone 50 mg./kg./day, subcutaneously, during the fifteen day Prolactin^{R} treatment period. Onset of redness of the metatarsal-tarsal joints occurred in one rat on the 12th day. As shown in Table VIII

TABLES VII and VIII

A SUMMARY OF OBSERVATIONS WITH THE ARTHROGRAM

Correlation of Onset of Appearance, Severity and Peak Incidence of Arthritic Changes, and Mortality

TABLE VII

Exp. No. V — Procedure: Prolactin^{R} Regimen

Number of rats started: 10

Average weight when Prolactin^{R} started: 211 gm. (range 190-220)

Onset of arthritis on day: 12

Total number of rats developing arthritis: 10

Total number of rats dying in first month: 2

Peak incidence of arthritis in group: days 15 (8/10) to 25 (5/8)

Variation in severity:

Arthrogram score	Number of rats
0	0
0.5 to 5	4
5+ to 20	3
20+	3

Highest arthrogram score 158

Mortalities on days 18, 20, 36

TABLE VIII

Exp. No. V — Procedure: Prolactin^{R} Regimen + Phenylbutazone

Number of rats started: 8

Average weight when Prolactin^{R} started: 196 gm. (range 170-220)

Onset of arthritis on day: 12

Total number of rats developing arthritis: 4

Total number of rats dying in first month: 0

Peak incidence of arthritis in group: days 15 (3/8) to 18 (4/8)

Variation in severity:

Arthrogram score	Number of rats
0	4
0.5 to 5	3
5+ to 20	1
20+	0

Highest arthrogram score 9.5

Figure 8

VARIATION IN SEVERITY OF ARTHRITIS
and
IN MOTOR ACTIVITY

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities, the average 30 minute Actophotometer count and the growth curve following Prolactin^{R} treatment of 10 young adult rats.)

NOTE: The mean counts per minute (c./min.) $\pm$ 1 S.D. for the 30 minute observation period is illustrated for each day that activity was recorded. The mean c./min. is designated by the mid-point of the enclosed vertical bar; the two extremes of the vertical bar represent the range of the mean c./min. $\pm$ 1 S.D.

This convention will be used in all subsequent graphs of results of activity measurement.

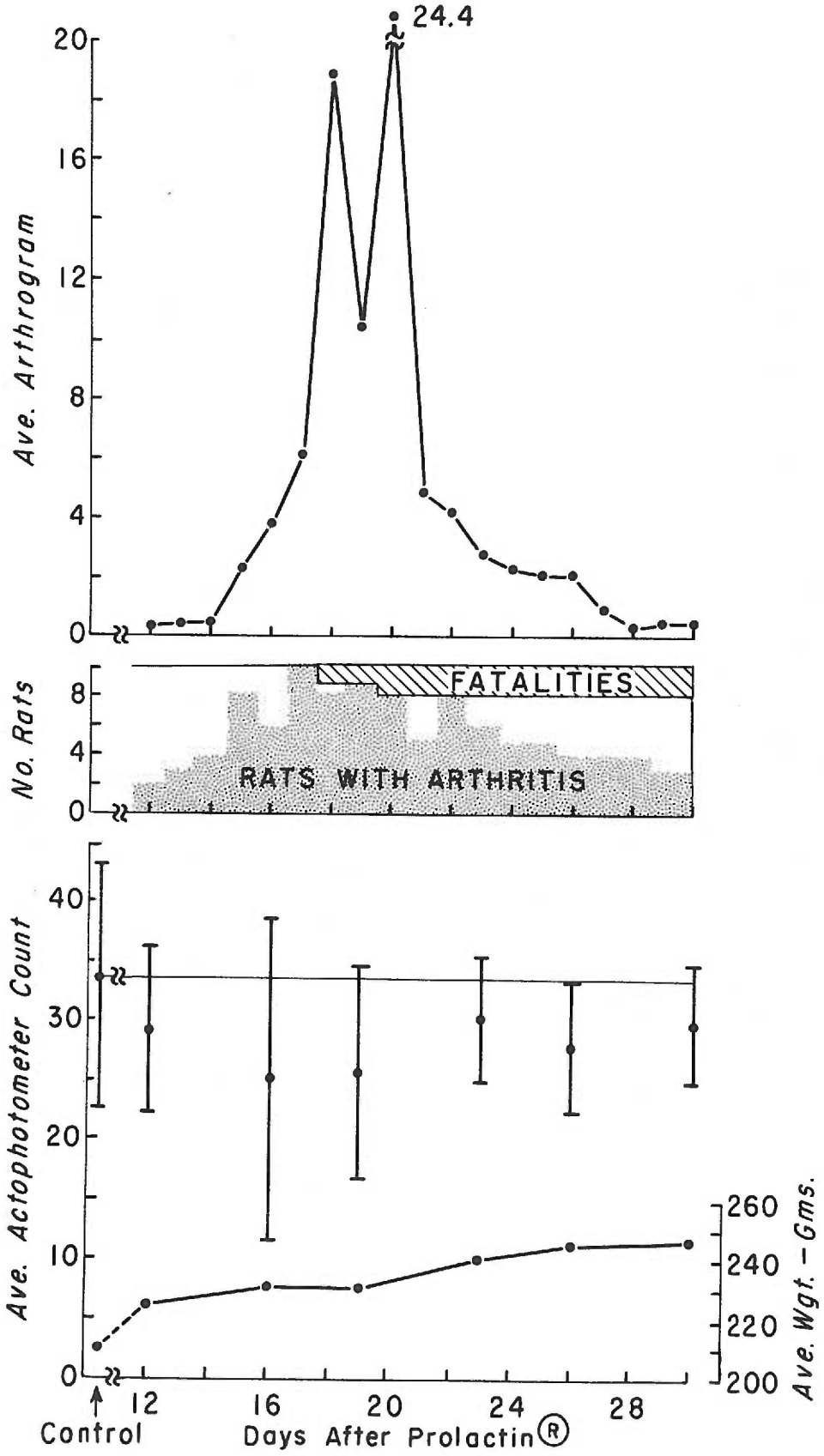

24.4
Ave. Arthrogram
20
16
12
8
4
0
No. Rats
FATALITIES
RATS WITH ARTHRITIS
8
4
0
Ave. Actophotometer Count
40
30
20
10
0
260
240
220
200
Ave. Wgt. - Gms.
12
16
20
24
28
Control
Days After Prolactin®

Figure 8

four animals developed minimal arthritis. The average group arthrogram is shown in Figure 9. The peak incidence occurred between the 15th and 18th days with a maximum group arthrogram of 1.3 on the 17th day. The maximum individual arthrogram of 9.5 occurred on the 17th day. There were no mortalities during the first month.

Ten rats (average weight 211 gm. when Prolactin^{R} started) were given subcutaneous injections of hydrocortisone 2.5 mg./kg./day for the fifteen day period. Onset of redness of the metatarsal-tarsal joints occurred in one rat on the 12th day. As shown in Table IX three animals developed minimal to moderate arthritis. The average group arthrogram is shown in Figure 10. The peak incidence occurred between the 19th and 23rd days with a maximum group arthrogram of 3.7 observed on the 17th day. The maximum individual arthrogram of 19 occurred on the 17th day. There were no mortalities during the first month.

Nine rats (average weight 127 gm. when Prolactin^{R} started) were given subcutaneous injections of sodium salicylate 50 mg./kg./day for the fifteen day period. Onset of slight redness of the metatarsal-tarsal joint occurred in one animal on the 19th day. As shown in Table X three animals developed minimal arthritis. The average group arthrogram is shown in Figure 11. The peak incidence occurred on day twenty-six when two animals showed minimal involvement. The maximum group arthrogram of 0.2 occurred on the same day as well as the maximum individual arthrogram of 1.5. There were no mortalities during the first month.

Comparison of the three different drug groups with the control

Figure 9

VARIATION IN SEVERITY OF ARTHRITIS
and
IN MOTOR ACTIVITY
with
PROPHYLACTIC PHENYLBUTAZONE TREATMENT

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities, the average 30 minute Actophotometer count and the growth curve following Prolactin^{R} and prophylactic phenylbutazone treatment of 8 young adult rats.)

NOTE: These animals received a single subcutaneous dose of phenylbutazone, 50 mg./kg./day, simultaneously with Prolactin^{R} injections for a 15 day period.

(The mean c./min. $\pm$ 1 S.D. for the 30 minute activity count is illustrated as in Figure 8.)

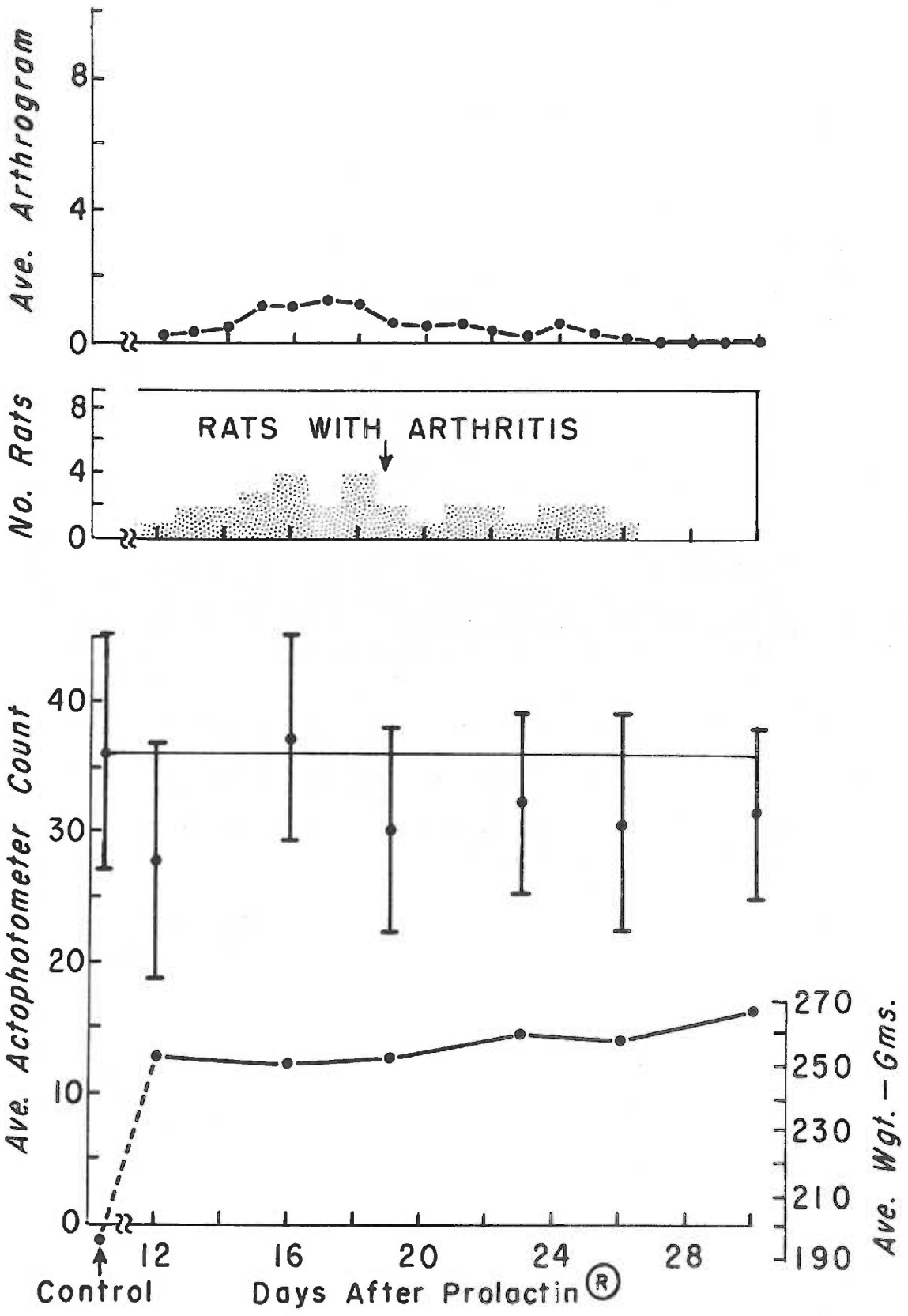

Ave. Arthrogram
8
4
0
No. Rats
8
4
0
RATS WITH ARTHRITIS
Ave. Actophotometer Count
40
30
20
10
0
270
250
230
210
190
Ave. Wgt. - Gms.
12
16
20
24
28
Control
Days After Prolactin ®

Figure 9

TABLES IX and X

A SUMMARY OF OBSERVATIONS WITH THE ARTHROGRAM

Correlation of Onset of Appearance, Severity and Peak Incidence of Arthritic Changes, and Mortalities

TABLE IX

Exp. No. V — Procedure: Prolactin^{R} Regimen + Hydrocortisone

Number of rats started: 10

Average weight when Prolactin^{R} started: 211 gm. (range 200-220)

Onset of arthritis day: 12

Total number of rats developing arthritis: 3

Total number of rats dying in first month: 0

Peak incidence of arthritis in group: days 19 (3/10) to 23 (3/10)

Variation in severity:

Arthrogram score	Number of rats
0	7
0.5 to 5	1
5+ to 20	2
20+	0
Highest arthrogram score	19

TABLE X

Exp. No. V — Procedure: Prolactin^{R} Regimen +Sodium salicylate

Number of rats started: 9

Average weight when Prolactin^{R} started: 177 gm. (range 150-200)

Onset of arthritis on day: 19

Total number of rats developing arthritis: 3

Total number of rats dying in first month: 0

Peak incidence of arthritis in group: days 26 (2/9)

Variation in severity:

Arthrogram score	Number of rats
0	6
0.5 to 5	3
5+ to 20	0
20+	0
Highest arthrogram score	1.5

Figure 10

VARIATION IN SEVERITY OF ARTHRITIS
and
IN MOTOR ACTIVITY
with
PROPHYLACTIC HYDROCORTISONE TREATMENT

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities, the average 30 minute Actophotometer count and the growth curve following Prolactin^{R} and prophylactic hydrocortisone treatment of 10 young adult rats.)

NOTE: These animals received a single subcutaneous dose of hydrocortisone, 2.5 mg./kg./day, simultaneously with Prolactin^{R} injections for a 15 day period.

(The mean c./min. $\pm$ 1 S.D. for the 30 minute activity count is illustrated as in Figure 8.)

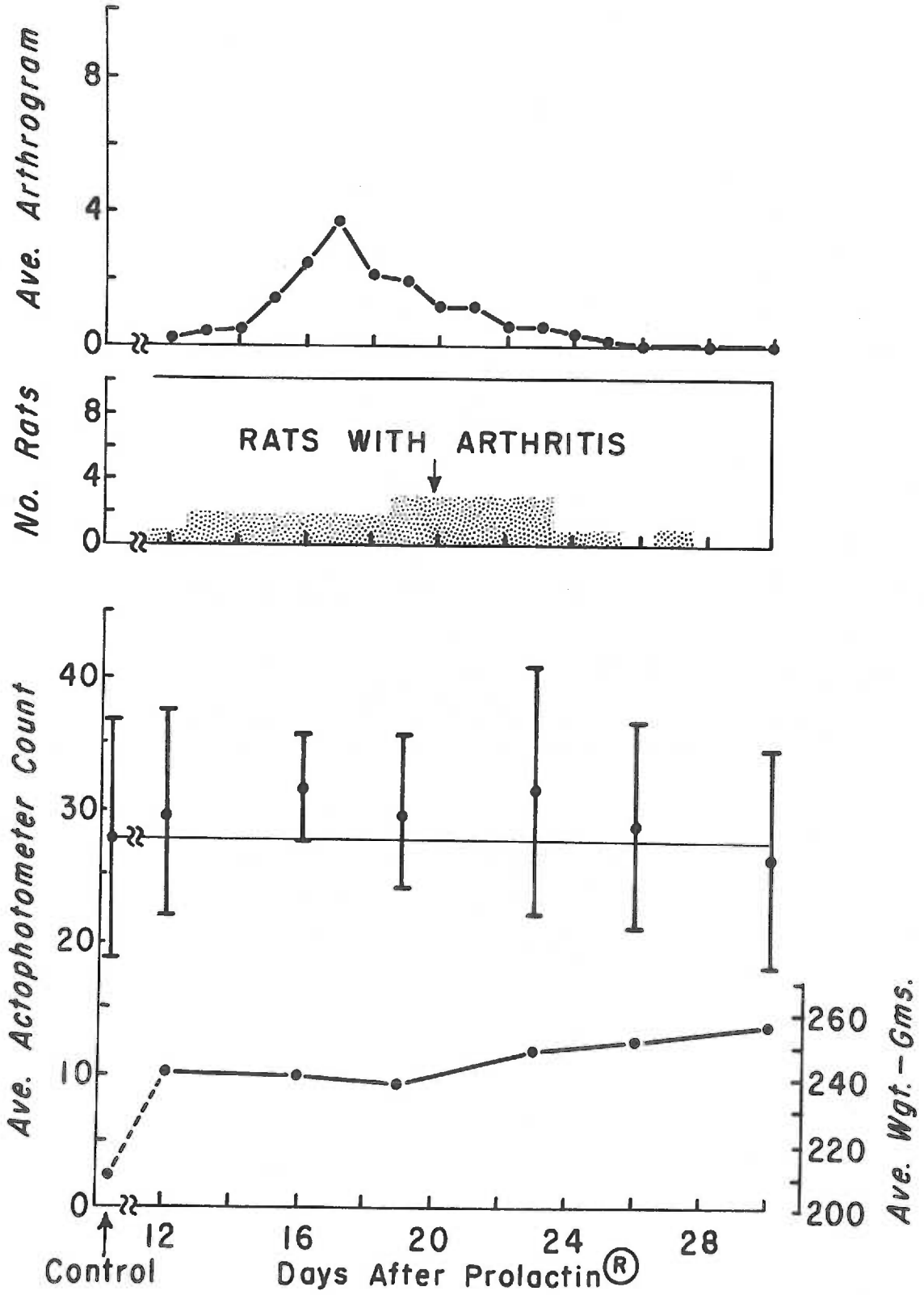

Ave. Arthrogram
8
4
0
No. Rats
8
4
0
RATS WITH ARTHRITIS
Ave. Actophotometer Count
40
30
20
10
0
260
240
220
200
Ave. Wgt. - Gms.
12
16
20
24
28
Control
Days After Prolactin®

Figure 10

Figure 11

VARIATION IN SEVERITY OF ARTHRITIS
and
IN MOTOR ACTIVITY
with
PROPHYLACTIC SODIUM SALICYLATE TREATMENT

(Showing correlation of the average arthrogram, the number of arthritic animals and fatalities, the average 30 minute Actophotometer count and the growth curve following Prolactin^{R} and prophylactic sodium salicylate treatment of 9 young adult rats.)

NOTE: These animals received a single subcutaneous dose of sodium salicylate, 50 mg./kg./day, simultaneously with Prolactin^{R} injections for a 15 day period.

(The mean c./min. $\pm$ 1 S.D. for the 30 minute activity count is illustrated as in Figure 8.)

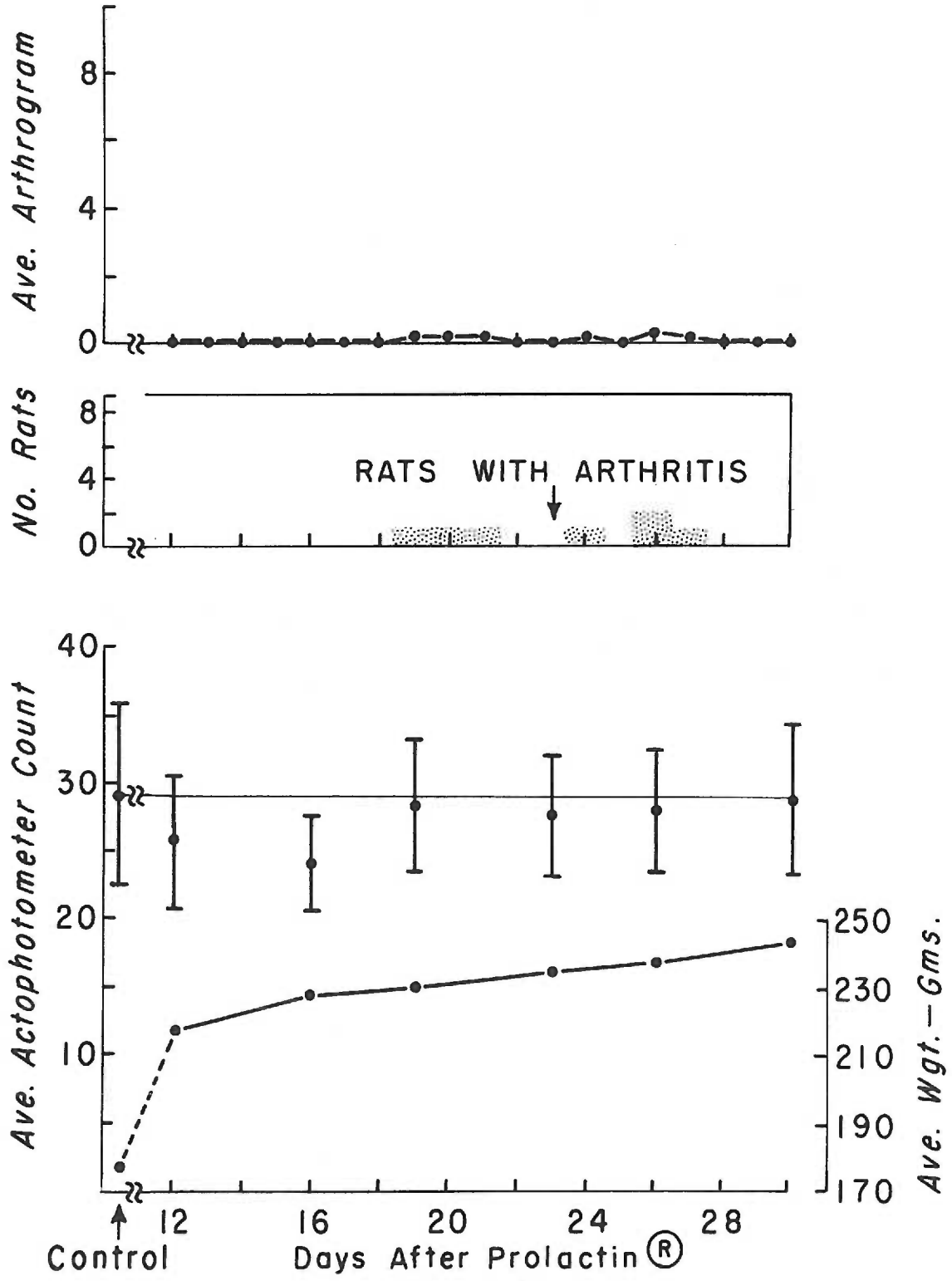

Ave. Arthrogram
8
4
0
No. Rats
8
4
0
RATS WITH ARTHRITIS
Ave. Actophotometer Count
40
30
20
10
250
230
210
190
170
Ave. Wgt. — Gms.
12
16
20
24
28
Control
Days After Prolactin ®

Figure 11

group was based upon the criterion of the number of animals with an arthrogram score equal to, or greater than, one-half. No animals developed arthritis prior to the 12th day. Therefore, the number of animals with arthritis during the period of nineteen days from the 12th to the 30th day was used in comparing drug effects (see Table XI). Using the Chi Square method a statistical analysis of these results revealed the following:

All three drugs used to ameliorate the arthritic condition produced a significant decrease in the severity of the disease and the number of animals involved. Sodium salicylate produced the most consistent decrease in incidence of arthritis with a probability level of 0.01 or greater on 11 of the 19 days. During the 15th to 22nd days, when the incidence and severity of arthritis reached a peak in the control group, the decreased number of animals showing arthritis in the salicylate treated group was significant at the 0.001 level on 6 of the 8 days. No significant difference was observed on 4 of the 19 days.

In contrast to salicylate, phenylbutazone failed to produce a significant difference in the number of involved animals on 12 of the 19 days. A significant difference (at the 0.01 level or greater) was present on 5 of the 7 days between the 17th and 23rd day of observation.

Comparison of the number of arthritic animals in the hydrocortisone and the control group revealed a significant difference

TABLE XI

COMPARISON OF THE INCIDENCE OF ARTHRITIS IN PROLACTIN^{R} TREATED CONTROL ANIMALS VERSUS ANIMALS RECEIVING PROLACTIN^{R} AND ANTI-INFLAMMATORY AGENT SIMULTANEOUSLY

P Values Obtained from Chi Square Analysis

Control 10 Rats		Phenylbutazone 8 Rats		Sodium Salicylate 9 Rats		Hydrocortisone 10 Rats	
Rats with Arthritis	Day	Rats with Arthritis	Chi Square P	Rats with Arthritis	Chi Square P	Rats with Arthritis	Chi Square P
2	12	1	N.S.	0	N.S.	1	N.S.
3	13	2	N.S.	0	N.S.	2	N.S.
4	14	2	N.S.	0	0.05	2	N.S.
8	15	3	N.S.	0	0.001	2	0.01
6	16	4	N.S.	0	0.01	2	N.S.
10	17	2	0.001	0	0.001	2	0.001
8	18	4	N.S.	0	0.001	2	0.01
9	19	2	0.01	1	0.001	3	0.01
8	20	1	0.01	1	0.001	3	0.01
5	21	2	N.S.	1	0.05	3	N.S.
8	22	2	0.01	0	0.001	3	0.01
6	23	1	0.01	0	0.01	3	N.S.
5	24	2	N.S.	1	0.05	1	0.05
5	25	2	N.S.	0	0.01	1	0.05
4	26	1	N.S.	2	N.S.	0	0.02
4	27	0	0.05	1	N.S.	1	N.S.
4	28	0	0.05	0	0.02	0	0.02
3	29	0	N.S.	0	0.01	0	0.05
3	30	0	N.S.	0	0.01	0	0.05

(at the 0.01 level or greater) for 6 of the 8 days between the 15th to 22nd day of observation. During this time the peak incidence of arthritic involvement occurred in the control group withheld from hydrocortisone. The difference in incidence was not significant on 7 of the 19 days.

On the basis of the above analysis the three drugs which were given in approximately equivalent dosages might be ranked in decreasing order of effectiveness as follows: sodium salicylate > hydrocortisone > phenylbutazone. To determine if this apparent ranking represented a significant difference between drugs, the incidence of involved animals was compared between paired drug-treated groups. Chi Square analysis was used to test the hypothesis that the difference in incidence between groups receiving different drugs was greater than expected by chance alone.

As shown in Table XII there was no significant difference between animals receiving prophylactic sodium salicylate or hydrocortisone. Similarly, Table XIII shows that there was no significant difference between the animals receiving prophylactic hydrocortisone or phenylbutazone.

In Table XIV the effect of sodium salicylate is compared to the effect of phenylbutazone. On three days the number of involved animals in the salicylate group was significantly lower at the 0.2 or 0.1 level of significance. This partially confirms the impression gained from comparison of the two drugs with untreated

TABLE XII

COMPARISON OF THE INCIDENCE OF PROLACTIN^{R} INDUCED ARTHRITIS IN ANIMALS RECEIVING PROPHYLACTIC SODIUM SALICYLATE OR HYDROCORTISONE

P Values Obtained from Chi Square Analysis

	Prolactin^{R} + Sodium Salicylate	Prolactin^{R} + Hydrocortisone	
	9 Rats	10 Rats	
Day	Rats With Arthritis	Rats With Arthritis	Chi Square P
12	0	1	N.S.
13	0	2	N.S.
14	0	2	N.S.
15	0	2	N.S.
16	0	2	N.S.
17	0	2	N.S.
18	0	2	N.S.
19	1	3	N.S.
20	1	3	N.S.
21	1	3	N.S.
22	0	3	N.S.
23	0	3	N.S.
24	1	1	N.S.
25	0	1	N.S.
26	2	0	N.S.
27	1	1	N.S.
28	0	0	N.S.
29	0	0	N.S.
30	0	0	N.S.

TABLE XIII

COMPARISON OF THE INCIDENCE OF PROLACTIN^{R} INDUCED ARTHRITIS IN ANIMALS RECEIVING PROPHYLACTIC HYDROCORTISONE OR PHENYLBUTAZONE

P Values Obtained from Chi Square Analysis

Day	Prolactin^{R} + Hydrocortisone 10 Rats: Rats With Arthritis	Prolactin^{R} + Phenylbutazone 8 Rats: Rats With Arthritis	Chi Square P
12	1	1	N.S.
13	2	2	N.S.
14	2	2	N.S.
15	2	3	N.S.
16	2	4	N.S.
17	2	2	N.S.
18	2	4	N.S.
19	3	2	N.S.
20	3	1	N.S.
21	3	2	N.S.
22	3	2	N.S.
23	3	1	N.S.
24	1	2	N.S.
25	1	2	N.S.
26	0	1	N.S.
27	1	0	N.S.
28	0	0	N.S.
29	0	0	N.S.
30	0	0	N.S.

TABLE XIV

COMPARISON OF THE INCIDENCE OF PROLACTIN^{R} INDUCED ARTHRITIS IN ANIMALS RECEIVING PROPHYLACTIC SODIUM SALICYLATE OR PHENYLBUTAZONE

P Values Obtained from Chi Square Analysis

Day	Prolactin^{R} + Sodium Salicylate — 9 Rats: Rats With Arthritis	Prolactin^{R} + Phenylbutazone — 8 Rats: Rats With Arthritis	Chi Square P
12	0	1	N.S.
13	0	2	N.S.
14	0	2	N.S.
15	0	3	0.01
16	0	4	0.02
17	0	2	N.S.
18	0	4	0.02
19	1	2	N.S.
20	1	1	N.S.
21	1	2	N.S.
22	0	2	N.S.
23	0	1	N.S.
24	1	2	N.S.
25	0	2	N.S.
26	2	1	N.S.
27	1	0	N.S.
28	0	0	N.S.
29	0	0	N.S.
30	0	0	N.S.

animals shown in Table XI.

A final ranking might be expressed as salicylate being equal to hydrocortisone, with both drugs slightly more effective than phenylbutazone. To provide conclusive data a larger series would be required.

ACTOPHOTOMETER STUDIES

Table XV illustrates the effect of measuring activity over different periods of time (time intervals) in seven untreated control rats. The mean and standard deviation of counts per minute (c./min.) is shown for each duration of activity measurement. The shorter periods of measurement have the largest means which might be desirable if a decrease in activity is to be readily demonstrated after treatment. However, the 5 and 10 minute intervals have large standard deviations which would make small changes in activity almost negligible. That is, only a marked decrease in activity for any one rat in the group could be shown to be statistically significant. At the opposite end of the scale the 60 minute measurement has a standard deviation of only 8.88 counts per minute (c./min.) but the mean for this period is so low (27.70 c./min.) that activity would have a very limited range over which a change could be demonstrated. The 20 or 30 minute duration of measurement avoids these factors with a relatively high mean activity and yet the standard deviation for these periods is small. Since the period of 30 minutes duration had the smallest deviation in this and subsequent experiments it was selected for all activity measurements reported in this paper.

Some rats seemed to require a "warm up" period in order to become accustomed to a new environment before attaining their usual activity. Others seemed to start immediately with maximum activity when placed in the counting chamber. The question thus arose as to whether or not the first few minutes should be excluded. Table XVI shows the results of tabulating the 30 minute mean and standard deviation of counts per

TABLES XV and XVI

TABLE XV MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT VI

(Seven Rats - Three Observations)

COUNTS PER MINUTE FOR VARIOUS TIME PERIODS

	5 min.	10 min.	20 min.	30 min.	45 min.	60 min.
MEAN	89.29	70.00	52.19	43.55	34.25	27.70
S.D.	17.15	16.08	11.79	10.02	9.09	8.88

TABLE XVI MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT VII

(Sixteen Rats - Three Observations)

EFFECT ON VARIANCE OF FIRST TEN MINUTES

Counts Per Minute		Counts Per Minute	
TOTAL THIRTY MINUTE PERIOD		THIRTY MINUTE PERIOD LESS FIRST TEN MINUTES	
MEAN	34.04	MEAN	23.48
S.D.	10.01	S.D.	9.49
VARIANCE	100.184	VARIANCE	90.150

$$F \text{ Ratio} = \frac{100.184}{90.150} = 1.111 \qquad \text{Not Sig.}$$

minute for sixteen rats on three different days in the left column. The right column shows the mean and standard deviation of counts per minute for only the final 20 minutes of the same 30 minute period. In this case the counts for the first 10 minutes after placing the rat in the counting chamber have been disregarded. To determine if the variance estimate might be less, or at least different, for these two methods of recording activity the F Ratio was computed for both determinations (58). As shown in Table XVI the F Ratio was not significant. Thus the elimination of the initial 10 minute counting period does not cause a significant difference in the standard deviation, even though the means differ. A similar result was found in a duplicate study performed later. On this basis the total 30 minute activity count was selected for use in all subsequent measurements.

Since it has been demonstrated that muscles of adrenalectomized rats fatigue more quickly than those of normal rats (36), and our animals had been subjected to this procedure, it seemed of interest to study the effect of adrenalectomy alone upon activity. In carrying out this experiment a second variable, the age of the animal entered into consideration. Table XVII indicates the mean and standard deviations for the activity of a group of six young adult rats, during three pre-operative observations and on post-operative days 12 and 19. Using the analysis of variance ("F Test") technique (58) no significant difference in mean activity between individual rats or between pre-operative observations and post-operative periods was noted. Table XVIII presents the mean and standard deviations for the activity of seven

TABLE XVII MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

BEFORE AND AFTER ADRENALECTOMY

EXPERIMENT XII
(Six Young Adult Rats)

	BEFORE SURGERY			AFTER SURGERY	
	COUNTS PER MINUTE			COUNTS PER MINUTE POST-OP DAY	
				DAY 12	DAY 19
MEAN	43.99	43.34	42.70	39.28	35.24
S.D.	9.71	5.24	6.12	7.25	6.82

F TEST
Between Runs - Not Sig.
Between Ind. - Not Sig.

TABLE XVIII MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT VII
(Seven Mature Rats)

	BEFORE SURGERY			AFTER SURGERY	
	COUNTS PER MINUTE			COUNTS PER MINUTE POST-OP DAY	
				DAY 16	DAY 23
MEAN	43.83	46.64	39.50	17.34	41.76
S.D.	9.64	8.22	8.40	3.43	4.75

F TEST
Between Runs - 0.001
Between Ind. - Not Sig.

	BEFORE SURGERY			AFTER SURGERY	
	COUNTS PER MINUTE			COUNTS PER MINUTE POST-OP DAY	
				DAY 23	DAY 29
MEAN	43.83	46.64	39.50	41.76	43.72
S.D.	9.64	8.22	8.40	4.75	7.58

F TEST
Between Runs - Not Sig.
Between Ind. - Not Sig.

mature rats during three pre-operative observations and on post-operative days 16 and 23. While there was no significant difference between the performance of individual animals, a definite decrease in activity post-operatively occurred which was significant at the 0.001 level. To see whether or not this difference remained permanently, further post-operative activity counts were recorded for this group. When the data for post-operative days 23 and 29 were compared to the pre-operative control data for this group, no significant difference between pre-operative and post-operative activity existed. Apparently, older mature animals with a less rapid growth rate recover less rapidly from the trauma of surgery than do young animals. Most young adult rats return to their normal activity about two weeks post surgery. Therefore, in young adult rats the effect of adrenalectomy upon activity may be disregarded. This enables the use of post-operative activity in comparison with activity after Prolactin^{R} treatment in the evaluation of the "model" disease. Since no pre-operative period of activity measurement is required younger animals may be selected for Prolactin^{R} treatment. Hereafter, all references to "control" activity are based upon post-operative measurements beginning approximately two weeks post-surgery.

Since rats are known to be nocturnal animals it might be expected that the time of day when activity was measured would correlate with changes in activity. Table XIX, Part I shows the grand mean and standard deviation of six rats whose activity was measured at the same hour, plus or minus 30 minutes, for each animal. In Table XIX, Part II the same animals were observed during different four hour periods of the day. Each animal contributed the same number of activity counts in the two parts of Table XIX. It is evident that the grand mean and standard

TABLE XIX MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT XXII
(Six Rats)

PART I: Variance Estimate With Activity Measurement Taken At Same Time Of Day

Animal No.	COUNTS PER MINUTE			
1	58.27	50.13	40.37	
2	42.70	43.20	41.00	
3	29.20	22.70	24.60	26.23
4	38.73	25.67	29.77	
5	34.70	40.30	27.40	31.03
6	40.67	35.43	29.60	

GRAND MEAN 35.58
S.D. 9.22
VARIANCE 85.0760

PART II: Variance Estimate With Activity Measurement Taken At Different Time of Day

Animal No.	11 am 3 pm	3 pm 7 pm	7 pm 11 pm	11 pm 3 am
	COUNTS PER MINUTE			
1	52.83	58.27	50.73	
2	47.80	37.87	41.80	
3	28.13	22.97	29.20	36.33
4		36.83	38.73	30.17
5	25.23	26.43	34.70	38.23
6		35.00	40.67	30.33

GRAND MEAN 37.11
S.D. 9.56
VARIANCE 91.4313

F Ratio = $\frac{91.43}{85.08}$ = 1.075 Not Sig.

deviation for the two activity measurements are very close numerically. The F Ratio test shows that there is no significant difference between the standard deviations of counts per minute for the two groups of data. It is probable that if all other variables could be controlled a significant difference could be demonstrated correlating with time of day. No correlation could be demonstrated in this investigation.

The influence upon activity of the temperature where the rats were housed and where their activity was counted was also considered. It has been shown that adrenalectomized rats exhibit a markedly increased rate of cooling when exposed to cold air (26). One theoretical explanation for this rapid cooling is that there is a decrease in muscular activity and shivering. With the method of measuring activity used in this investigation, no correlation between individual or group activity and room temperature could be demonstrated.

The possibility of variation in activity being caused by the fact that different observers handled the animals was also considered. In a separate comparison, the group means and standard deviations obtained by two different observers were computed. There was no statistically significant difference between the data of the two observers.

In the final group of experiments the measurement of rat activity was applied in the evaluation of three anti-inflammatory agents, as follows.

To show the reliability of the method and, also, to rule out an infectious etiology of arthritic changes, a control group of six young

adult rats was employed. The top half of Table XX shows the computation of the mean and standard deviation of counts per minute for six post-operative activity counts. No significant difference between counts was noted although there was a definite difference between animals which was significant at the 0.001 level. This merely represents the biological variation existing between animals. In the bottom half of Table XX computation of the mean and standard deviation of counts per minute for additional activity measurements for these rats is shown. These measurements were made during the month corresponding to the 15 day treatment period and subsequent observation period in drug treated groups. Again, there is no significant difference between mean counts per minute during the second month of observations. Figure 12 illustrates the activity (mean c./min. $\pm$ 1 S.D.) and body weight of these control animals. It may be compared with the activity and body weight of Prolactin^{R} treated animals shown in the lower portion of Figure 8. Table XXI shows the comparison of each animal's control mean $\pm$ 1 S.D. and subsequent activity counts for that animal. At the bottom of the table the data is summarized in terms of per cent of animals with activity above, below, or within the range of the control mean $\pm$ 1 S.D. On only one occasion do more than 33 per cent of animals show a decrease in activity below the - 1 S.D. range.

To serve as a basis for comparison with drug treated groups a group of ten young adult rats received Prolactin^{R} treatment only (see Figure 8). Table XXII shows the computation of post-operative control activity. In this instance the difference between means was significant

TABLE XX MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

DURING POST-OPERATIVE PERIOD
(EXPERIMENT XXII)

Animal Number	Counts Per Minute and Date of Measurement						
#	1/19	1/25	1/28	2/7	2/10	2/17	
1	58.27	52.83	50.13	50.73	36.80	40.37	
2	42.70	47.80	43.20	41.80	34.90	41.00	
3	29.20	28.13	22.70	16.87	32.70	24.60	
4	38.73	36.83	25.67	27.47	30.17	37.13	
5	34.70	27.53	40.30	38.23	32.23	27.40	
6	40.67	26.30	35.43	30.33	29.97	29.60	
MEAN	40.71	36.57	36.24	34.24	32.80	33.35	GRAND MEAN 35.65
S.D.	9.85	11.42	10.52	11.90	2.67	7.04	S.D. 9.45

	Level of Sig.
F Test Between Runs	Not Sig.
F Test Between Individuals	0.001

CONTINUED POST-OP CONTROL ACTIVITY

Animal Number	Counts Per Minute and Date of Activity Measurement					
#	2/24	3/3	3/10	3/17	3/20	
1	36.10	28.27	36.13	42.03	45.63	
2	37.87	35.73	32.70	35.97	40.27	
3	22.97	26.23	40.93	28.13	36.33	
4	28.83	29.77	33.63	33.27	27.23	
5	26.43	31.03	27.30	30.80	34.13	
6	29.93	36.73	30.83	35.57	31.40	
MEAN	30.35	31.29	33.59	34.30	35.83	GRAND MEAN 33.06
S.D.	5.69	4.16	4.65	4.81	6.52	S.D. 4.55

	Level of Sig.
F Test Between Runs	Not Sig.
F Test Between Individuals	0.001

F Test Between Two Control Periods Not Sig.

Figure 12

VARIATION IN MOTOR ACTIVITY

AFTER ADRENALECTOMY

(Showing normal variation of the average 30 minute Actophotometer count and the growth curve following adrenalectomy in 6 young adult rats.)

NOTE: These animals received no treatment other than maintenance desoxycortisone, 1 mg. every other day, and 1 per cent saline ad lib for drinking water. "Sham" injections of 0.2 cc. physiologic saline were given on days when other animals received injections.

(The mean c./min. $\pm$ 1 S.D. for the 30 minute activity count is illustrated as in Figure 8.)

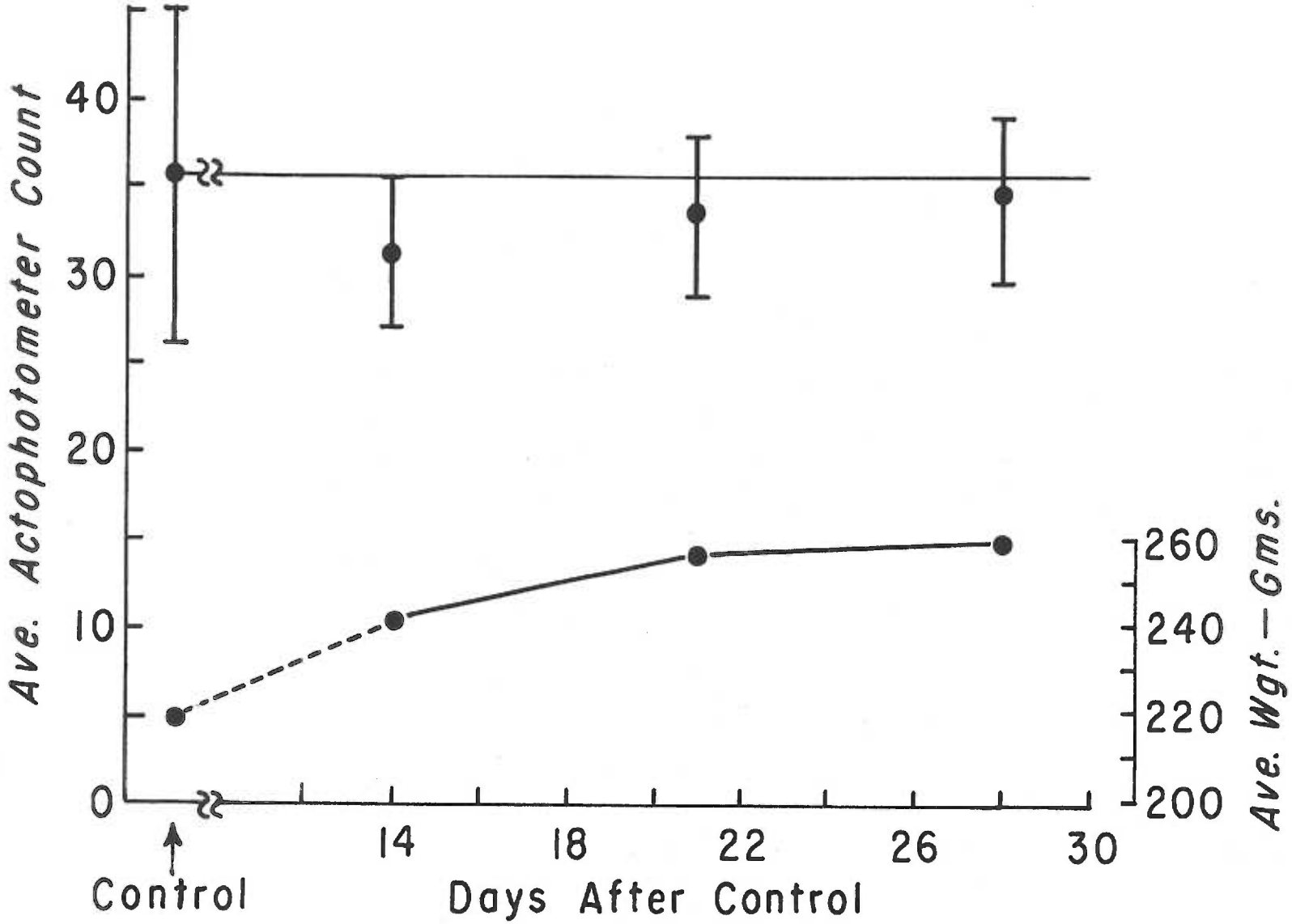

Ave. Actophotometer Count
40
30
20
10
0
260
240
220
200
Ave. Wgt. — Gms.
Control
14
18
22
26
30
Days After Control

FIGURE 12

TABLE XXI MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

VARIATION IN ACTIVITY WITHOUT TREATMENT**

EXPERIMENT XXII

(Six Rats)

Post-Op Control (Six control runs)				DAYS AFTER INITIAL CONTROL PERIOD				
Animal Number	MEAN	S.D.	Range ± 1 S.D.	7	14	21	28	31
1	48.19	8.05	40.14-56.24	B	B	B	W	W
2	41.90	4.17	37.73-46.07	W	B	B	B	W
3	25.70	5.57	20.13-31.27	W	W	A	W	A
4	32.67	5.59	27.08-38.26	W	W	W	W	W
5	33.40	5.38	28.02-38.78	B	W	B	W	W
6	32.05	5.14	26.91-37.19	W	W	W	W	W
% Within Range Control ± 1 S.D. = %(W)				66	66	33	83	83
% Below Control - 1 S.D. =%(B)				33	33	50	17	0
% Above Control + 1 S.D. =%(A)				0	0	17	0	17

** "A" Activity above Control MEAN + 1 S.D.
"W" Activity within Control MEAN ± 1 S.D.
"B" Activity below Control MEAN - 1 S.D.

at the 0.01 level. This is probably an artifact secondary to the fifth observation when several animals appeared depressed for no apparent reason. The individual difference between animals was significant at the 0.001 level. Table XXIII shows the computation of mean and standard deviation of counts per minute for nine additional studies during and after Prolactin^{R} treatment on days 2, 5, 9, 12, 16, 19, 23, 26 and 30. During the first six studies the difference between means was not significant and the difference between individuals decreased to the 0.05 level of significance. The difference between the grand mean (33.16 c./min.) of the control activity period and the grand mean (29.65 c./min.) of the treatment period is significant at the 0.001 level. Table XXIV shows the comparison of each animal's control mean $\pm$ 1 S.D. and activity counts for that animal on nine subsequent days. The data is summarized as in Table XXI in terms of per cent of animals with activity above, below, or within the range of the control mean $\pm$ 1 S.D. Variation tabulated in this manner shows little deviation from the untreated group with activity in 50 per cent of animals below - 1 S.D. on the 16th day only.

In the following three drug treated groups the computations from raw data will not be shown but results will be summarized using the same methods illustrated for the control groups. Figures 9, 10, and 11 show the activity and body weight of the three drug treated groups.

Table XXV shows the results in eight young adult rats receiving phenylbutazone simultaneously with Prolactin^{R}. The difference between means of the six control observations and of the nine observations during and post therapy was significant at the 0.05 level. The difference

TABLE XXII MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

DURING POST-OPERATIVE PERIOD

EXPERIMENT XX
(Ten Rats)

Animal Number	Counts Per Minute and Date of Measurement						
#	1/17	1/20	1/27	2/3	2/6	2/10	
1	29.97	27.80	31.53	30.43	33.50	23.83	
2	26.93	30.90	25.57	17.53	21.93	26.30	
3	43.77	41.33	37.67	38.57	23.13	41.67	
4	36.03	47.30	48.33	44.40	45.50	35.50	
5	28.70	35.97	28.47	28.90	10.83	22.60	
6	17.97	17.83	17.37	16.20	11.20	24.43	
7	34.63	45.17	36.47	41.97	29.87	33.33	
8	27.07	31.00	29.80	37.57	27.60	31.07	
9	40.27	48.93	40.60	43.53	40.67	42.60	
10	52.90	45.67	56.07	37.47	24.07	41.50	
MEAN	33.82	37.19	35.19	33.66	26.83	32.28	GRAND MEAN 33.16
S.D.	9.98	10.19	11.27	10.19	11.27	7.85	S.D. 10.19

	Level of Sig.
F Test Between Runs	0.01
F Test Between Individuals	0.001

TABLE XXIII MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

FOLLOWING PROLACTIN^{R} TREATMENT WITHOUT ANTI-INFLAMMATORY COMPOUND

EXPERIMENT XX

(Ten Rats)

Animal Number #	Counts Per Minute and Days After Treatment Started 2	5	9	12	16	19	23	26	30	
1	28.00	21.57	29.53	31.37	24.53	23.10	27.27	34.60	30.07	
2	28.30	28.90	28.20	24.33	27.93	26.00	29.60	23.40	22.93	
3	37.43	54.00	44.57	34.13	36.73	32.67	32.33	30.27	37.13	
4	40.40	36.57	44.90	20.90	11.63	DEAD				
5	32.20	31.47	25.77	33.77	17.17	19.20	29.57	32.43	34.97	
6	12.50	28.17	30.50	17.90	20.33	21.63	25.47	23.87	24.17	
7	30.47	23.83	35.00	26.63	19.93	23.30	24.83	18.47	32.40	
8	32.73	29.50	34.57	32.07	39.13	34.13	30.70	31.23	25.70	
9	52.90	41.37	14.50	30.90	5.07	11.60	DEAD			
10	38.30	33.67	33.00	40.70	50.53	41.17	41.43	28.90	31.27	
MEAN	33.32	32.90	32.05	29.27	25.30	25.87	30.15	27.90	29.83	GRAND MEAN 29.65
S.D.	10.39	9.39	8.85	6.83	13.67	8.87	5.23	5.46	5.14	S.D. 8.18

F Test Days 2 - 19 with N = 10

	Level of Sig.
Between Runs.....	Not Sig.
Between Individuals....	0.05

TABLE XXIV MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

VARIATION FOLLOWING PROLACTIN[R] TREATMENT WITHOUT ANTI-INFLAMMATORY COMPOUND **

EXPERIMENT XX
(Ten Rats)

Post-op Control No. = 10
(Six control runs)

DAYS AFTER TREATMENT STARTED

Animal Number	MEAN	S.D.	RANGE ± 1 S.D.	2	5	9	12	16	19	23	26	30	
1	29.51	3.35	26.16-32.86	W	B	W	W	B	B	W	A	W	W
2	24.86	4.60	20.26-29.46	W	W	W	W	W	W	A	W	W	W
3	37.69	7.47	30.22-45.16	W	A	W	W	W	W	W	W	W	W
4	42.84	5.65	37.19-48.49	W	B	W	B	B	DEAD				
5	25.91	8.52	17.39-34.43	W	W	W	W	B	W	W	W	A	A
6	17.50	4.24	13.26-21.74	B	A	A	W	W	W	A	A	A	W
7	36.91	5.68	31.23-42.59	B	B	W	B	B	B	B	B	W	B
8	30.68	3.76	26.92-34.44	W	W	A	W	A	W	W	W	B	W
9	42.77	3.28	39.49-46.05	A	W	B	B	B	B	DEAD			
10	42.95	11.59	31.36-54.54	W	W	W	W	W	W	W	B	B	W
% Within Range Control ± 1 S.D. = %(W)				70	50	70	70	40	66	63	50	50	75
% Below Control - 1 S.D. = %(B)				20	30	10	30	50	33	13	25	25	13
% Above Control + 1 S.D. =%(A)				10	20	20	0	10	0	25	25	25	13

** "A" Activity above Control MEAN + 1 S.D.
"W" Activity within Control MEAN ± 1 S.D.
"B" Activity below Control MEAN - 1 S.D.

TABLE XXV MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT XXI
(Eight Rats)

POST-OP CONTROL ACTIVITY

GRAND MEAN ± 1 S. D. (Six Runs)

36.05 ± 9.39 Counts Per Minute

	Level of Sig.
F Test Between Runs	0.05
F Test Between Individuals	0.001

ACTIVITY DURING AND AFTER PROLACTIN[R] AND PHENYLBUTAZONE TREATMENT

GRAND MEAN ± 1 S. D. (Nine Runs)

31.94 ± 7.12 Counts Per Minute

	Level of Sig.
F Test Between Runs	0.05
F Test Between Individuals	0.001

F Test Between Control and Treatment ... 0.01

	DAYS AFTER TREATMENT STARTED								
	2	5	9	12	16	19	23	26	30
% Within Range Control MEAN ± 1 S.D. =%(W)	62	38	50	38	50	25	38	50	62
% Below Control MEAN - 1 S.D. =%(B)	38	50	50	62	25	62	50	38	38
% Above Control MEAN + 1 S.D. =%(A)	0	12	0	0	25	12	12	12	0

between the grand mean of the control period (36.05 c./min.) and the grand mean (31.94 c./min.) of the treatment period was significant at the 0.01 level. The summary in terms of per cent of animals with activity below control mean - 1 S.D. shows that 50 per cent or greater were in this category on five out of the nine days.

Table XXVI shows the results in ten young adult rats receiving hydrocortisone simultaneously with Prolactin^{R}. The difference between means of the six control runs is significant at the 0.001 level. This variation is a reflection of intercurrent respiratory infection in the colony. The infection largely cleared prior to the treatment period and the difference between means of the treatment period is not significant. In both groups the difference between individual animals was significant at the 0.001 level. The difference between the grand mean of the control period (27.96 c./min.) and the grand mean (30.01 c./min.) of the treatment period is significant at the 0.001 level and probably can be explained on the basis of the respiratory infection since group activity during the treatment period did not vary significantly. The summary in terms of per cent of animals with activity below control mean - 1 S.D. shows that a maximum of 10 per cent fell into this category on five occasions, whereas, more animals showed increased activity.

Table XXVII shows the results in nine young adult rats receiving sodium salicylate simultaneously with Prolactin^{R}. The difference between means of the three control observations is not significant. The difference between means of the nine runs during and post therapy

TABLE XXVI MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT XXIII
(Ten Rats)

POST-OP CONTROL ACTIVITY

GRAND MEAN ± 1 S.D. (Six Runs)

27.96 ± 8.98 Counts Per Minute

	Level of Sig.
F Test Between Runs	0.001
F Test Between Individuals	0.001

ACTIVITY DURING AND AFTER PROLACTIN[R] AND HYDROCORTISONE TREATMENT

GRAND MEAN ± 1 S. D. (Nine Runs)

30.01 ± 7.27 Counts Per Minute

	Level of Sig.
F Test Between Runs	Not Sig.
F Test Between Individuals	0.001

F Test Between Control and Treatment ... 0.001

		DAYS AFTER TREATMENT STARTED								
		-2	5	9	12	16	19	23	26	30
% Within Range Control MEAN ± 1 S.D.	= %(W)	80	80	80	80	80	90	60	80	78
% Below Control MEAN - 1 S.D.	= %(B)	0	0	10	10	0	0	10	10	11
% Above Control MEAN + 1 S.D.	= %(A)	20	20	10	10	20	10	30	10	11

TABLE XXVII MEASUREMENT OF RAT ACTIVITY (ACTOPHOTOMETER COUNTS)

EXPERIMENT XXIV
(Nine Rats)

POST-OP CONTROL ACTIVITY

GRAND MEAN ± 1 S.D. (Three Runs)

29.24 ± 6.63 Counts Per Minute

	Level of Sig.
F Test Between Runs	Not Sig.
F Test Between Individuals	Not Sig.

ACTIVITY DURING AND AFTER PROLACTIN® AND SALICYLATE TREATMENT

GRAND MEAN ± 1 S.D. (Nine Runs)

27.33 ± 4.42 Counts Per Minute

	Level of Sig.
F Test Between Runs	0.05
F Test Between Individuals	0.001

F Test Between Control and Treatment ... 0.05

		DAYS AFTER TREATMENT STARTED								
		2	5	9	12	16	19	23	26	30
% Within Range Control MEAN ± 1 S.D.	= %(W)	89	67	56	67	67	78	67	89	67
% Below Control MEAN - 1 S.D.	= %(B)	0	22	33	33	22	11	22	0	11
% Above Control MEAN + 1 S.D.	= %(A)	11	11	11	11	11	11	11	11	22

is significant at the 0.05 level. The difference between the grand mean of the control period (29.24 c./min.) and the grand mean (27.33 c./min.) of the treatment period is significant at the 0.05 level only. The summary in terms of per cent of animals with activity below control mean - 1 S.D. shows that a maximum of 33 per cent fell into this category on only two occasions.

HISTOPATHOLOGICAL STUDIES

Microscopic changes were evident as early as 16 days after Prolactin^{R} injection (Figures 13-B, 15-A, B, C and 16-B) and were also present in a 335 day specimen. The earliest joint changes included edema, increased vascularity and proliferation of the synovial membrane. In some instances the synovial changes included infiltration of the synovium with mononuclear cells (plasma cells and lymphocytes). There was no evidence of acute infection and few polymorphonuclear cells invaded the inflamed area. In some instances the hypertrophied synovium appeared to form multiple papillae projecting into the joint cavity (Figure 14-B, C and Figure 15-A, B, C) similar to pannus formation in the human rheumatoid arthritic patient (47).

Synovial changes were seen in the tarsal bones most frequently but were also present in the metatarsal-phalangeal and interphalangeal joints, the tibio-calcaneal joint and the knee joint in more severely involved cases. Examples of involvement in these locations are illustrated in Figures 13, 14 and 15. No similar changes were present in the control animals illustrated for comparison with pathological cases.

Microscopic changes were present in most animals with clinical involvement as recorded by the arthrogram. A few animals did not appear to have microscopic changes. This does not rule out abnormality as only selected joints were examined in each animal and serial sections might have missed minimal pathology. The importance of serial sections is shown by Figure 15-E, F which demonstrates changes in a knee joint

Figure 13

PHOTOMICROGRAPHS:

HISTOPATHOLOGICAL CHANGES IN THE

INTERPHALANGEAL AND METATARSAL JOINTS

A. Normal proximal interphalangeal joint.

B. Proximal interphalangeal joint 16 days after Prolactin^{R} was started.

Note the periarticular edema and proliferation of the synovium with extention into the joint cavity.

C. Higher magnification of section B.

Note the increased vascularity and edema of the proliferating synovial membrane.

D. Metatarsal-phalangeal joint 251 days after Prolactin^{R} was started.

Changes shown are similar to those in sections B and C, plus the additional finding of resorption of the articular cartilage.

E. Higher magnification of section D.

Examination reveals hypertrophied synovium with increased vascularity and multiple thrombi, edema and mononuclear cell infiltration of the synovium.

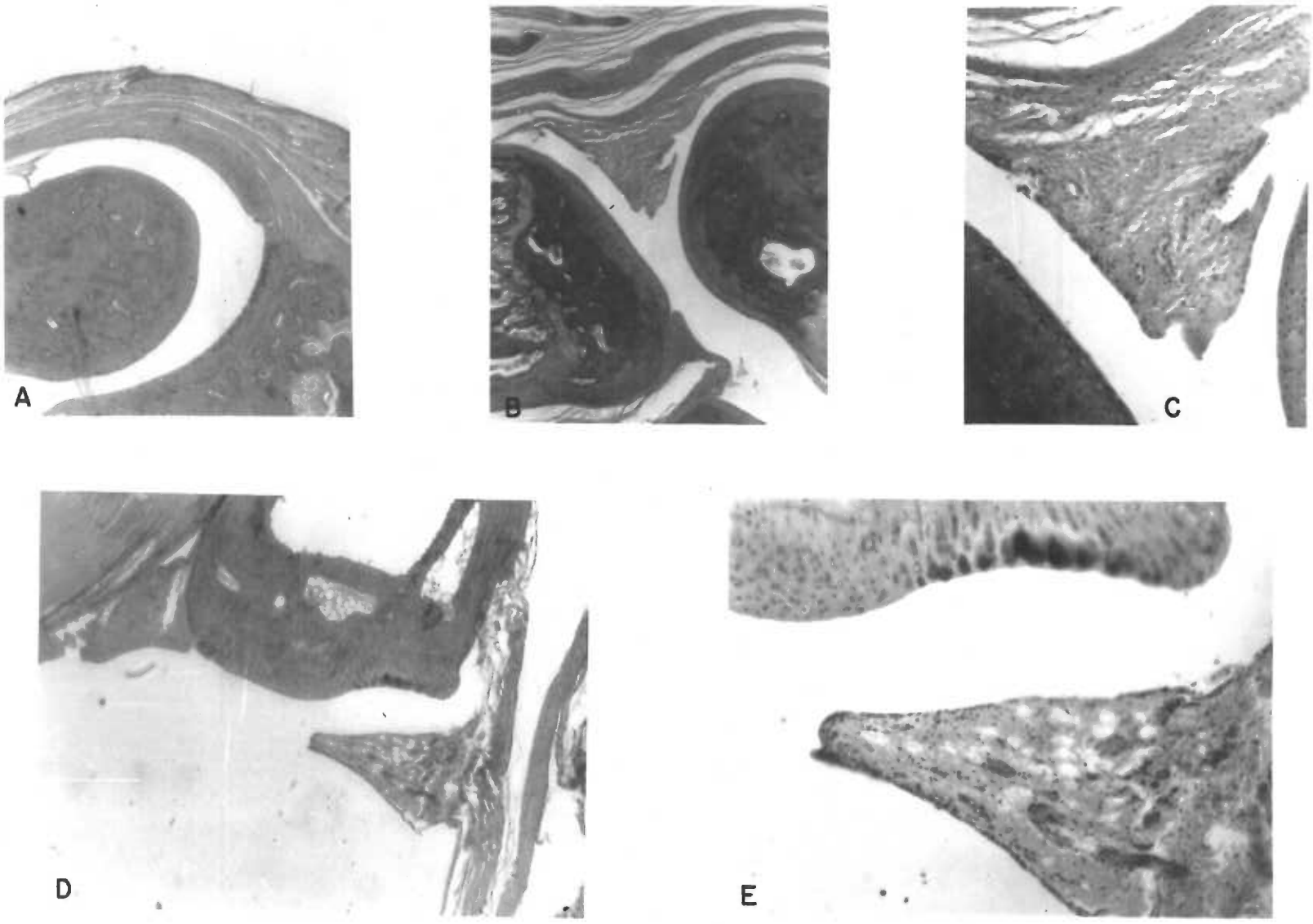


Figure 13

Figure 14

PHOTOMICROGRAPHS:

HISTOPATHOLOGICAL CHANGES IN THE

TARSAL JOINTS

A. Normal tarsal joint.

B. Metatarsal-phalangeal joint 251 days after Prolactin^{R} was started.

Note the proliferation of the synovium with invasion and destruction of the articular cartilage and periosteum.

C. Higher magnification of section B.

Note the increased vascularity of the synovium and the associated periosteal reaction.

D. High power view of hypertrophied synovium extending between two tarsal bones 55 days after Prolactin^{R} was started.

Increased vascularity, mononuclear cell infiltration and "fibrin" deposition on the surface of the invading synovium are apparent.

E. Tarsal joint 251 days after Prolactin^{R} was started.

Synovial changes are similar to changes described above.

F. Higher magnification of section E.

This section also shows increased vascularity of the synovium with edema of the synovial adipose tissue.

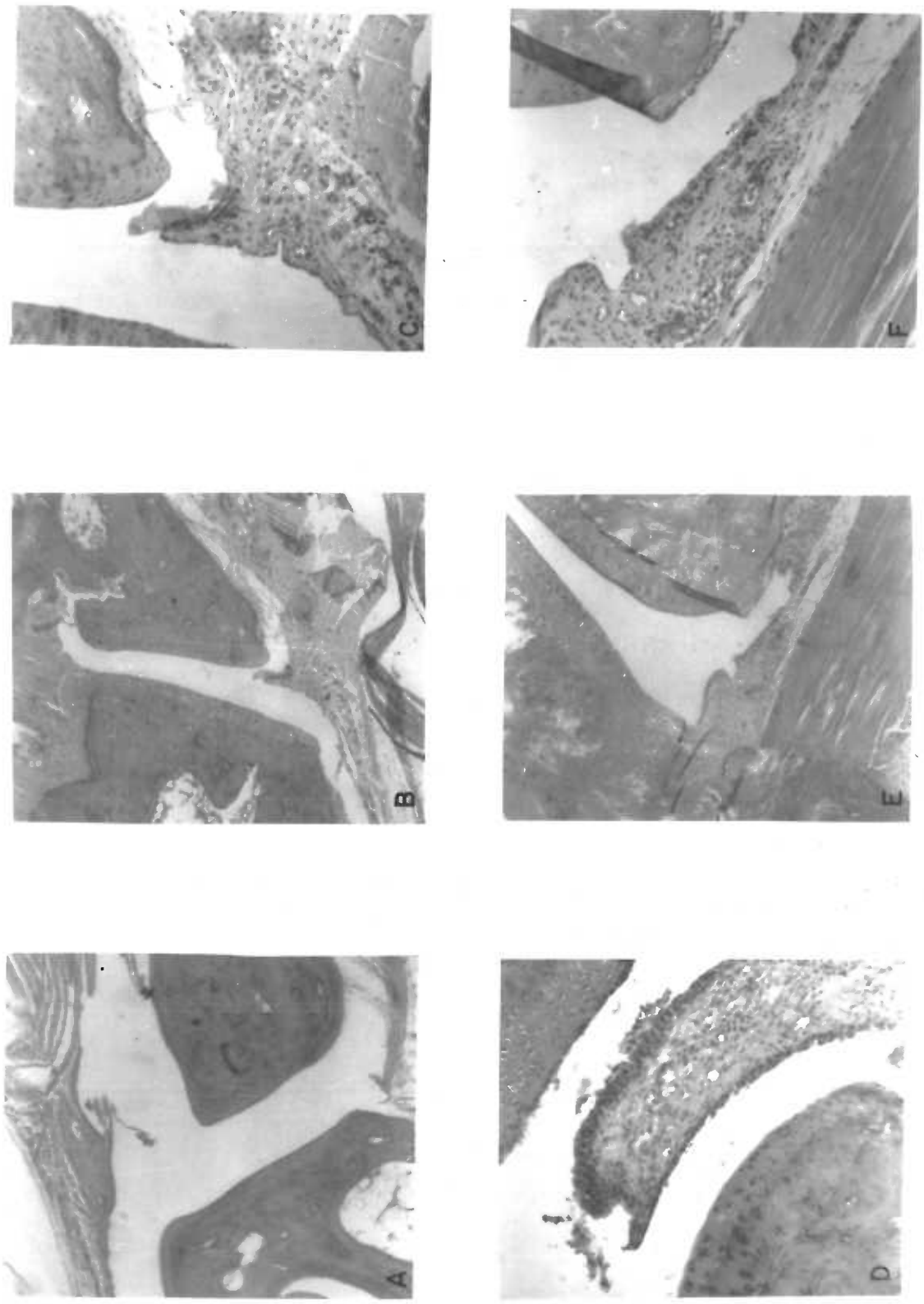


Figure 14

Figure 15

PHOTOMICROGRAPHS:

HISTOPATHOLOGICAL CHANGES IN THE

CARPAL AND KNEE JOINTS

A. Carpal joints 16 days after Prolactin^{R} started.

A severe, generalized inflammatory reaction was clinically evident in this animal. The synovium appears to project into the two articulations visualized.

B. Higher magnification of the upper articulation in section A.

Multiple synovial papillae (simulating pannus formation) are seen projecting into the joint cavity in association with edema, increased vascularity and mononuclear cellular infiltration of the synovial tissue.

C. Higher magnification of the lower articulation in section A.

Changes are present as described above.

D. Normal knee joint.

E. Higher magnification of knee joint 18 days after Prolactin^{R} was started.

Some synovial reaction and distortion of the articular cartilage are suggested.

F. Serial section adjacent to tissue shown in E.

The extent of destruction of the articular cartilage by the synovium is demonstrated.

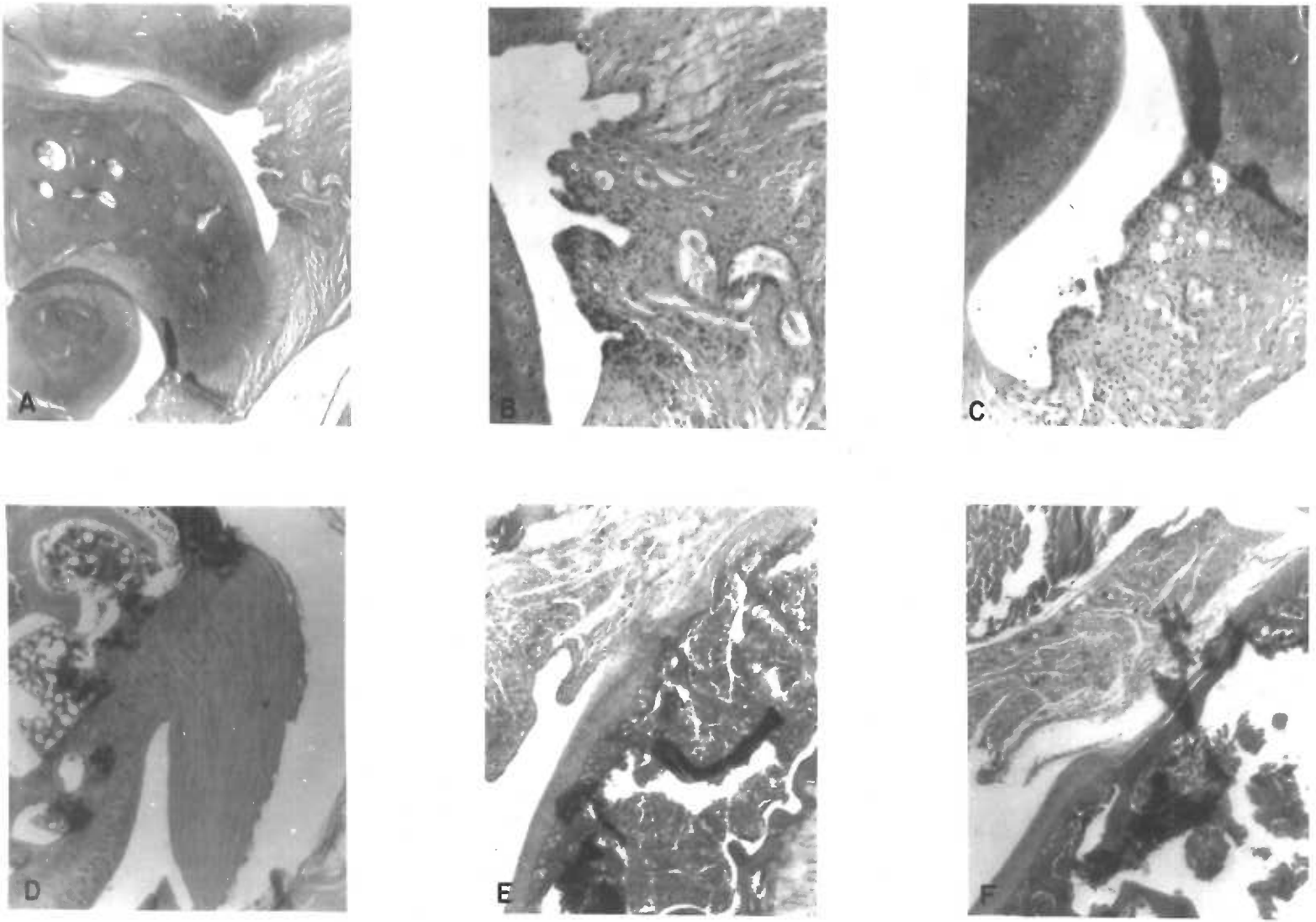


Figure 15

18 days after Prolactin^{R} injection. The extent of invasion of the articular cartilage of the femur is only fully apparent in the second serial section. Conversely microscopic examination revealed pathological changes in some joints where no clinical involvement had been observed.

In association with the synovial reaction there occurred remodeling of the bony trabeculae and in one case fibrous ankylosis of the tibial-calcaneal joint. The latter was clinically apparent as a persistent stiff gait. The change was present at the time of death on day 55 following Prolactin^{R} treatment. This animal also demonstrated a "fibrin deposit" upon the surface of the invading synovium (see Figure 14-D). Other changes observed include edema and mononuclear cell infiltration of the surrounding muscle and peri-articular tissues. No lymphoid nodules were observed in muscle sections.

Pathological changes were also present in the joints of the forepaws in animals with extensive clinical involvement (see Figure 15-A, B, C). No sections were obtained of spinal articulations. Pathological study of the joints correlated with clinical arthrogram scores especially in the severely involved animals.

Examination of the heart did not reveal any changes of the myocardium, aortic or mitral valves. In one case a perivascular infiltrate of mononuclear cells was seen in relation to a coronary vessel but there was no consistent evidence of vasculitis in the heart, muscles or kidneys examined.

Probably unrelated changes found in the kidney include: occasional collection of lymphocytes (lymphoid nodule?) near arterial vessels,

hyalinization of glomeruli and some increase in cellularity. There was no consistent evidence of glomerulonephritis in these animals.

In many cases the lungs grossly appeared to contain multiple abscesses which on cut section revealed yellowish to green caseous material. The gross appearance of the lungs in Prolactin^{R} treated animals was similar to the lungs of rats treated with Freund's adjuvant (87). A large proportion of the microscopic sections examined revealed some evidence of chronic granulomatous lung infection with increased vasculature, congestion, lymphocytic infiltration, abscess formation with necrosis, and in one case, "giant cells" (see Figure 16-A, B, C, D). The cellular infiltration sometimes appeared to have a perivascular location but in most cases assumed the form of an abscess in the lung parenchyma.

Figure 16

PHOTOMICROGRAPHS:

HISTOPATHOLOGICAL CHANGES IN THE

LUNG TISSUE

A. Normal rat lung.

B. Lung tissue obtained 16 days after Prolactin^{R} was started.

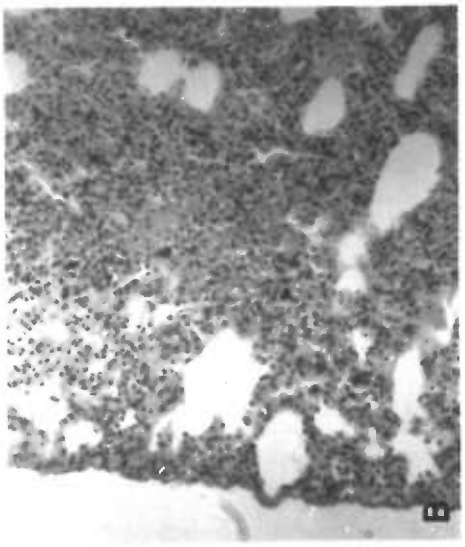
Consolidation, mononuclear cellular infiltration and phagocytosis are present.

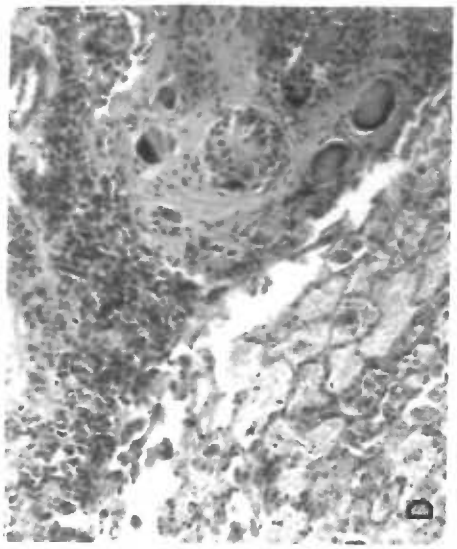
C. Lung tissue obtained 251 days after Prolactin^{R} was started.

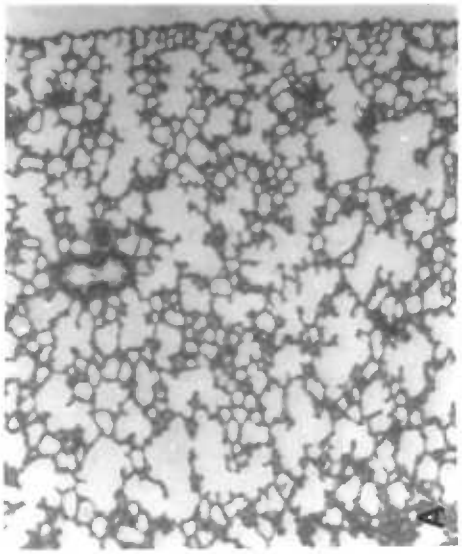
This section shows marked destruction and distortion of the lung parenchyma by chronic granulomatous inflammatory reaction. The border of a necrotic abscess is demonstrated in the upper portion of this field.

D. Higher magnification of the lower right portion of slide C.

This view shows further evidence of a chronic granulomatous reaction with numerous "giant" cells demonstrated.







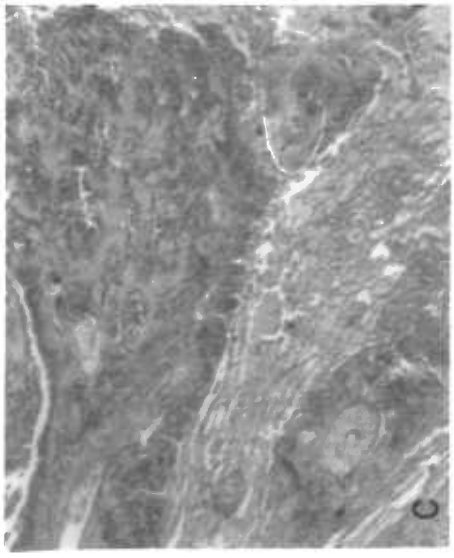


Figure 16

DISCUSSION

An acute polyarthritis has been experimentally induced in the adrenalectomized white rat maintained on desoxycortisone and saline and following treatment with Prolactin^{R}, as described. Young adult rats seem more susceptible to this procedure than mature animals as shown by the difference in the severity of lesions. The disease typically follows a pattern of onset of the arthritis in a few animals on the 12th day following treatment. The peak incidence of afflicted animals occurs between the 15th and 22nd days after starting Prolactin^{R} with from 80 to 100 per cent of the animals showing some signs of inflammation. Initial signs of arthritis consist of redness followed by swelling usually located in the metatarsal-tarsal or metatarsal-phalangeal joints of the hind limbs. This process may either regress or continue with spread of the inflammatory signs to all joints of the lower extremity distal to the knee. With severe involvement joints of the upper extremity are involved in a similar distribution. In severe cases the redness and swelling is associated with tenderness, heat, vasodilatation, and in some cases flexion deformity or flaccidity without true paralysis. In terminal stages severe debility with weight loss and weakness may be fatal unless anti-inflammatory drug therapy is administered. The process continues as a low grade migratory arthritis in 70 to 90 per cent of the animals after the first month of observation.

The arthrogram system provides a useful means of summation and comparison of the degree of the disease involvement in individual animals.

Although it is subject to the criticism of an arbitrary numerical system, results are relatively reproducible once the criteria are defined for scoring. Attempts at quantitation of the signs of inflammation by the use of a color scale (to match degree of redness) or calipers (to quantitate swelling) did not provide more reliable results than the arthrogram numerical scoring system based upon clinical observations. It is possible that use of a thermistor might be a useful modification of the evaluation procedure to provide an objective measure of increased temperature over involved joints. One disadvantage of the arthrogram method is the length of time required for transcription into the numerical score.

The results obtained using the Actophotometer activity measurement device show that the method is reproducible when activity is measured over a total 30 minute time period. There is a definite trend for motor activity to decline as signs of inflammation appear. However, because of the multiple factors which may alter activity, group mean activity before and after Prolactin^{R} does not always demonstrate a significant decrease. All individual animals with severe inflammation developed significantly decreased motor activity, whereas, the moderately involved or minimally involved animals sometimes showed increased motor activity. Further study with this technique under temperature controlled, humidity controlled, soundproof and light controlled conditions would be ideal and might be expected to show a better correlation between activity and inflammatory changes.

Treatment with Prolactin^{R} apparently caused a slowing down (plateau)

in the normal growth rate. However, decrease in body weight did not correspond to the degree of inflammatory involvement except in severe terminal stages where weight loss was a consistent finding.

Comparison of the results we obtained with the Prolactin^{R}-induced arthritis and Mycobacterial adjuvant-induced arthritis described by others (65), (66), (67), (68) reveals many similarities. In the latter, onset of arthritis occurs on the 10th day or later with a peak incidence between the 11th and 16th days. In both types of experimental arthritis the hind paws are affected more severely and consistently. In contrast to the Prolactin^{R}-induced arthritis, reported in this research, the "adjuvant" type of arthritis is described as producing fusiform swelling about the proximal interphalangeal region (66). In both experimental methods severely involved animals appear generally debilitated with weight loss and coarse, ruffled fur. No subcutaneous nodules or genital tract lesions were observed in this study in contrast to findings with "adjuvant" arthritis (66) (68). With both techniques a scaling skin lesion appears about the tail, head and face of occasional rats. In Prolactin^{R} disease we found no correlation between this dermatitis and the severity of the arthritis. In addition, some control animals in this study developed a similar dermatitis with no inflammatory joint findings. In "adjuvant" type arthritis, however, there is an association between the severity of the dermatitis and the arthritis (66).

Occasionally, an animal develops a conjunctivitis with or without purulent secretion when the "adjuvant" technique is used. In this study conjunctivitis was noted but it was a self limiting, transient process.

Also, conjunctivitis was observed in control animals without arthritis, and in the experimental animals without apparent relationship to arthritic lesions or altered motor activity. Probably, the conjunctivitis was secondary to an endemic infection or perhaps Vitamin A deficiency. In "adjuvant" type arthritis the conjunctivitis coincides temporally with the development of arthritis and has been shown to be associated with intraocular lesions by slit-lamp examination (66).

The results of the histopathological studies in the Prolactin^{R}-induced arthritis did not reveal evidence of cellulitis or a purulent arthritis. Synovial hypertrophy with extension into the joint cavity and round cell infiltration was observed, comparable to the findings of Pearson (66) in "adjuvant" arthritis. Pearson describes early lesions of synovitis, periarthritis, peritendonitis, joint exudation, fibrin deposition in the periarticular tissues and joint lumen, invasion of subchondral bone with pannus and late lesions of fibrous and bony anklyosis in adjuvant disease. He concluded that these findings represent an acute arthritis (67). Silverstein has suggested that these lesions of adjuvant arthritis should be classified as a periarthritis and periostitis (87).

Collins (13) has pointed out that there is no single pathonomonic histopathological change characteristic of rheumatoid arthritis. The principle lesion is a chronic synovitis with proliferation of the synovium and massive lymphoid or plasma cell infiltration forming foci frequently. Although lymphoid nodules were not identified in tissues from Prolactin^{R} treated animals the synovial reaction is compatible with

an arthritic process similar to rheumatoid disease. An example of the rheumatoid synovial reaction is shown in Copeman's text (15). Neither segmental vasculitis nor muscle and nerve lesions characterized by focal lymphocytic infiltration and focal degeneration of the involved tissues, comparable to the human rheumatoid disease process (47) (94) were seen in the experimental Prolactin^{R} disease.

In this study desoxycortisone was employed in an attempt to maintain normal electrolyte balance following adrenalectomy. It was not thought to contribute to the arthritic changes. The absence of microscopic evidence of an acute interstitial myocarditis, vasculitis, glomerulonephritis or other changes found in the hypertensive "hyalinosis syndrome" (75) (81) in our experimental animals tends to discredit the role of desoxycortisone in producing the arthritic changes. It would be of interest to know whether blood pressure was altered significantly following the treatment regimen used in this study.

Ward (106) has emphasized the need to consider infectious agents in experimental arthritis. The finding of granulomatous lesions in the lungs of Prolactin^{R} treated rats raises the possibility of Mycoplasmal, or PPLO organisms contributing to the inflammatory changes observed. Microscopic examination of the involved joints in Prolactin^{R} treated animals did not reveal evidence of a purulent arthritis typical of PPLO arthritis (63) (106). PPLO organisms are known to be susceptible to tetracycline. Treatment of our experimental animals with penicillin, tetracylcine or chloramphenicol did not alter the course of the arthritic disease. If an arthritogenic infectious agent were present in the

experimental animals it might be expected that some control animals would develop inflammatory joint changes. No such changes were observed in any control animals in this study. Finally, treatment with hydrocortisone was demonstrated to suppress inflammatory changes following Prolactin^{R} treatment. However, Silverstein (87) has noted that suppression of inflammatory joint disease by cortisone is not compelling evidence that the disease is due to hypersensitivity rather than to infection. If an infectious agent was responsible for the arthritis it is unlikely that it would have produced the "anamnestic" response observed in retreated animals. This response is suggestive of a hypersensitivity reaction. No passive transfer experiments were performed or cultures taken in this study.

Application of this experimental "model" for comparing the anti-inflammatory efficacy of several drugs shows the following:

A definite suppression of inflammatory activity, in terms of the number of animals affected and the severity of involvement, was observed following prophylactic drug treatment. All three compounds tested (sodium salicylate, phenylbutazone, and hydrocortisone) produce a statistically significant decrease in the incidence of arthritic animals when compared to the control group. Sodium salicylate and hydrocortisone appear to exert a slightly greater anti-inflammatory effect than phenylbutazone.

Interpretation of the motor activity measurements is less clear-cut. A decrease in overall group activity was partially suppressed in comparison to the control animals in the case of salicylate and phenylbutazone treated

animals. Hydrocortisone treated animals appeared to show an increase in activity level following treatment probably related to an inter-current respiratory infection during the pre-treatment period.

The anti-inflammatory drugs did not completely inhibit the decrease in rate of growth of animals during the Prolactin^{R} treatment period.

S U M M A R Y

An acute polyarthritis has been experimentally induced following Prolactin^{R} (Lactogenic Hormone) treatment, as described, in the adrenalectomized, desoxycorticosterone and saline maintained, white rat. The "model" disease first appears in a few animals on the 12th day following Prolactin^{R} treatment and reaches a peak incidence during the 15th to 22nd days after treatment is initiated. Low grade migratory arthritis is present in 70 to 90 per cent of animals after the first month. Signs of inflammation appear in 80 to 100 per cent of treated animals and range from very mild to severe, generalized, progressive, fatal disease. Histopathological studies reveal periarticular and synovial changes compatible with the non-specific pathological changes observed in rheumatoid arthritis.

An arthrogram scoring system was designed to permit summation and comparison of inflammatory involvement on a numerical basis. This system was found to provide a reproducible means of summation and comparison of the degree of inflammatory involvement in individual animals or groups of animals.

The Actophotometer recording device was used to measure changes in motor activity as a reflection of altered "functional capacity" in the "model" disease. A definite trend for motor activity to decline as signs of inflammation occurred was observed. Because of the multiple factors which may alter activity, group mean activ-

ity before and after development of the arthritic process did not always decrease significantly. All individual animals with severe inflammation developed significantly decreased activity as recorded by this method. Further study with this technique under temperature controlled, light controlled, humidity controlled, soundproof conditions might provide a more direct correlation between the degree of inflammatory changes and alteration in motor activity.

Application of this experimental "model" disease for comparison of anti-inflammatory efficacy of three compounds showed a definite suppression of inflammatory activity as measured by the arthrogram technique. Sodium salicylate and hydrocortisone appeared to exert a slightly greater anti-inflammatory effect than phenylbutazone in terms of decrease in the incidence of arthritis in Prolactin^{R} treated animals. Data is suggestive but not conclusive, that the anti-inflammatory compounds partially suppressed the decrease in overall group activity associated with Prolactin^{R} disease. Further studies are indicated to see whether or not this technique might be used to provide a laboratory method of ranking anti-inflammatory compounds with different degrees of effectiveness.

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A P P E N D I X

DEFINITIONS OF TERMS USED IN THESIS

In this thesis references have been made to the terms "arthritis", "polyarthritis", "true arthritis", "periarthritis", "model" disease, "pannus", "anamnestic response" and "arthrogram".

Arthritis or true arthritis may be defined as inflammation of the joint structures. The synovitis (or inflammation of the synovial membrane of the joint cavity) found in rheumatoid arthritis might be cited as an example of a true arthritis. The term arthritis is modified by the prefix "peri" to indicate inflammatory pathological changes present around the joint structures per se. The prefix "poly" is added to indicate inflammatory involvement of multiple joints.

The term "model" disease was used in this study to indicate a disease process deliberately produced in the experimental animal in an attempt to produce a replica of the human disease process. That is, some feature of the human disease was duplicated in the experimental "model" disease.

Pannus is defined as "a layer of connective tissue derived from the margin of synovial membrane where it joins with cartilage".[1] Pannus appears grossly to be a reddish, roughened, tongue-like protrusion of tissue extending into the joint cavity.

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[1]Rosenberg, Edward F. The pathology of rheumatoid arthritis. In J.L. Hollander (Ed.) Arthritis and allied conditions: a textbook of rheumatology. Philadelphia, Penn.: Lea & Febiger, 1960. p. 183.

The term anamnestic response refers to an immunological phenomenon. Following a second injection of antigen a much more rapid rise in antibody titer is obtained and the quantity of circulating antibodies usually is maintained at a high level for a longer period of time than occurs after a primary injection. The prompt and efficient production of antibodies following secondary injections is referred to as an anamnestic reaction.[2]

The term arthrogram was used in this investigation to refer to the numerical scoring system devised for description of the severity of joint inflammation.

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[2]Smith, D.T., & Conant, N.F., et. al. (Eds.) Bacteriology. New York: Appleton-Century-Crofts, 1957. p. 134-135.

CLASSIFICATION AND DESCRIPTION OF MAJOR FORMS OF ARTHRITIS

Hollander[3] has stated that most cases of arthritis fall into one of five major groups as follows:

1. cases that are possibly infectious but of unproved etiology (example: rheumatoid arthritis)
2. cases representing degenerative forms of joint disease (example: osteoarthritis)
3. cases in which the arthritis results from metabolic abnormalities (example: gouty arthritis)
4. cases in which the arthritis results from direct trauma to the joint
5. frankly infectious cases of arthritis caused by specific organisms (example: gonorrheal arthritis)

Because of the numerous references in this thesis to the differences between rheumatoid arthritis and osteoarthritis a description of their clinical and pathological characteristics is included in this appendix.

Short[4] has provided a useful although somewhat general definition of rheumatoid arthritis as follows: "Rheumatoid arthritis is a chronic inflammatory disease of unknown etiology and pathogenesis

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[3]Hollander, J.L. Introduction to arthritis and the rheumatic diseases. In J.L. Hollander (Ed.) Arthritis and allied conditions: a textbook of rheumatology. Philadelphia, Penn.: Lea & Febiger, 1960. p. 20-21.

[4]Short, C.L. Rheumatoid arthritis: historical aspects. J. Chron. Dis., 1959. 10, 375.

which is systemic in nature and characterized by the manner in which it involves joints." He also notes that it is a familial disease in that it occurs more frequently in the relatives of patients than of controls. Synonyms for rheumatoid arthritis include atrophic arthritis, proliferative arthritis, chronic infectious arthritis, and others.

Degenerative joint disease is defined as a chronic arthropathy (pathologic change of the joints) characterised pathologically by degeneration and hypertrophy of cartilage and bone. Synonyms for degenerative joint disease include osteoarthritis, hypertrophic arthritis, arthritis deformans, senescent arthritis and others.

The following table adapted from McEwen[5] permits easy comparison of the features of rheumatoid arthritis and osteoarthritis.

	Rheumatoid Arthritis	Osteoarthritis
Age of onset	Usually between 20-45 years	Most often after 40 years
Involvement of hands	Especially proximal interphalangeal and metacarpophalangeal joints; very rarely distal interphalangeal joints.	Especially terminal interphalangeal joints but proximal interphalangeal joints also.

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[5]McEwen, Currier, The diagnosis and differential diagnosis of rheumatoid arthritis. In J.L. Hollander (Ed.) Arthritis and allied conditions: a textbook of rheumatology. Philadelphia, Penn.: Lea & Febiger, 1960. p. 239-240.

	Rheumatoid Arthritis	Osteoarthritis
Number of joints involved	Usually many	Usually few
Joint involvement	Most often symmetrical	Often asymmetrical
Type of onset	Usually insidious; may be abrupt	Insidious
Constitutional symptoms	Fever, weight loss, anemia, tachycardia; enlarged lymph nodes and spleen	Usually none
Type of joint swelling	Usually fusiform or spindle shaped	Usually irregular or knobby
Muscular atrophy	Usually present and may be marked	Not frequent; not marked
Joint effusion	Usually present; may be marked	Usually none unless trauma superimposed
Subcutaneous nodules	Present in 15 per cent	Absent
Sedimentation rate	Usually rapid	Usually normal
X-ray findings	Early: fusiform periarticular soft tissue swelling; rarefaction of trabeculated ends of bones and systemic decalcification Later: decrease of joint space, lipping and osteophytes, punched out areas of bone at articular margins, and possibly ankylosis, subluxations, etc.	Early: narrowing of the joint space due to destruction of cartilage; formation of osteophytes and lipping Later: cysts at ends of bone, deformities from pressure and traction; no ankylosis
Primary pathological changes	Synovitis	Degeneration of hyaline articular cartilage; no inflammatory changes