Technical Papers

Experimental Production of Arthritis[1] in Rats by Hypophyseal Growth Hormone[2]

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The temporary reversal of the course of rheumatoid arthritis and other related conditions following treatment with certain adrenal steroids or adrenocorticotropic hormone (ACTH) has suggested that the adrenal gland plays only a secondary etiological role, and that the effects of such treatment are of importance only in diminishing the activity of an as yet unknown factor or agent acting at the cellular or tissue level. The adrenal steroids seem, at least in part, to protect tissues and cells from the effects of such a factor.

Certain lines of evidence have repeatedly suggested that endocrine imbalances may result in the production of, or be associated with, various types of chronic arthritis (*1–3*). Exact experimental demonstration of such relationships has not been attained, principally for lack of definition of the experimental conditions required for the production of arthropathies after treatment with purified hypophyseal hormones. Silberberg (*4*) has, however, reported the production of an "acromegalic arthropathy" in the guinea pig, induced after short-term injection of extract of anterior pituitaries of cattle, and Selye (*3*) has likewise noted the production of an arthritis of rheumatoid type in otherwise intact rats after treatment with lyophilized anterior pituitaries. On the other hand, adrenalectomy has been shown to predispose to spontaneous (*5*) or desoxycorticosterone-induced arthritis (*2*) in rats.

The above and other clinical observations have suggested that pituitary hormones are of importance in the production of experimental arthritis. A working hypothesis that may be formulated from these observations holds that some hormonal factor(s) in the anterior pituitary can be antagonized directly or indirectly by certain adrenal steroid hormones, and that temporary alleviation of signs and symptoms of various connective tissue and joint lesions by such adrenal steroids may represent, at least in part, either a decrease in hormonal production by the pituitary and/or an antagonism of hormones at the peripheral tissue levels.

The experiments herein described present preliminary evidence that points to a significant role of pituitary growth hormone in the production of chronic arthritis in the absence of the adrenals and gonads. Of 38 plateaued female rats (6–8 months old), 18 were adrenalectomized and ovariectomized, and the others were retained as controls. Ten of the rats in each group were injected intraperitoneally with gradually increasing daily doses (Fig. 1) of pituitary growth hormone (*6*) administered over a 6-month period. All animals were maintained on 1% NaCl drinking water and stock diet in comparable environmental conditions.

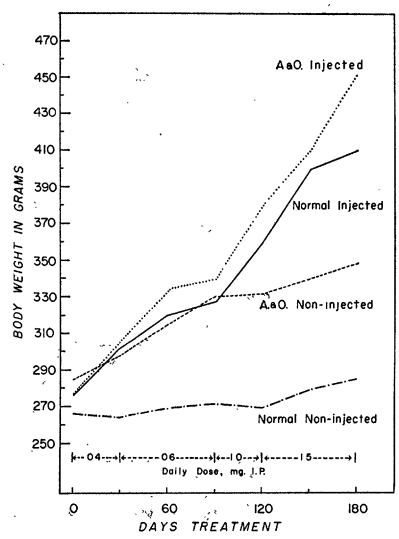

FIG. 1. Body weight changes produced by administration of hypophyseal growth hormone to normal and adrenalectomized-ovariectomized adult rats. Dose of growth hormone in mg given daily (6 times weekly) by intraperitoneal injection. Curves represent average body weight changes for adrenalectomized-ovariectomized (A. and O.) and normal rats injected with growth hormone. Other curves represent average body weight changes for untreated adrenalectomized-ovariectomized and normal rats.

During the course of regular observations, it was noted that both groups of growth hormone–treated animals grew at a comparable rate (Fig. 1), their weight being approximately 65% greater than that of the untreated normal controls,[3] with a commensurate increase in body dimensions, at the end of 6 months of treatment. It was further observed that the physical condition of the growth hormone–treated, adrenalectomized-ovariectomized animals progressively altered. General physical activity became sluggish,

[1] Since the arthritis described here is not necessarily rheumatoid and yet presents certain of its characteristics, the term "chronic arthritis" is employed with reservations.

[2] This investigation was supported in part by a grant from the Albert and Mary Lasker Foundation.

[3] It is of interest that adrenalectomy did not inhibit the increase in weight known to follow ovariectomy in the plateaued female rat (Fig. 1). The suggestion must be made in this connection that a correlation may exist between cessation of gonadal function, the increased incidence of arthritis at the menopause, and alterations in endogenous growth hormone secretion and/or responsiveness of the peripheral tissues to growth hormone. In addition, since all groups of animals were maintained continuously on 1% NaCl solution for drinking purposes, it is apparent that NaCl per se did not inhibit the normal responses to growth hormone, nor was it of immediate importance in the production of the changes described.

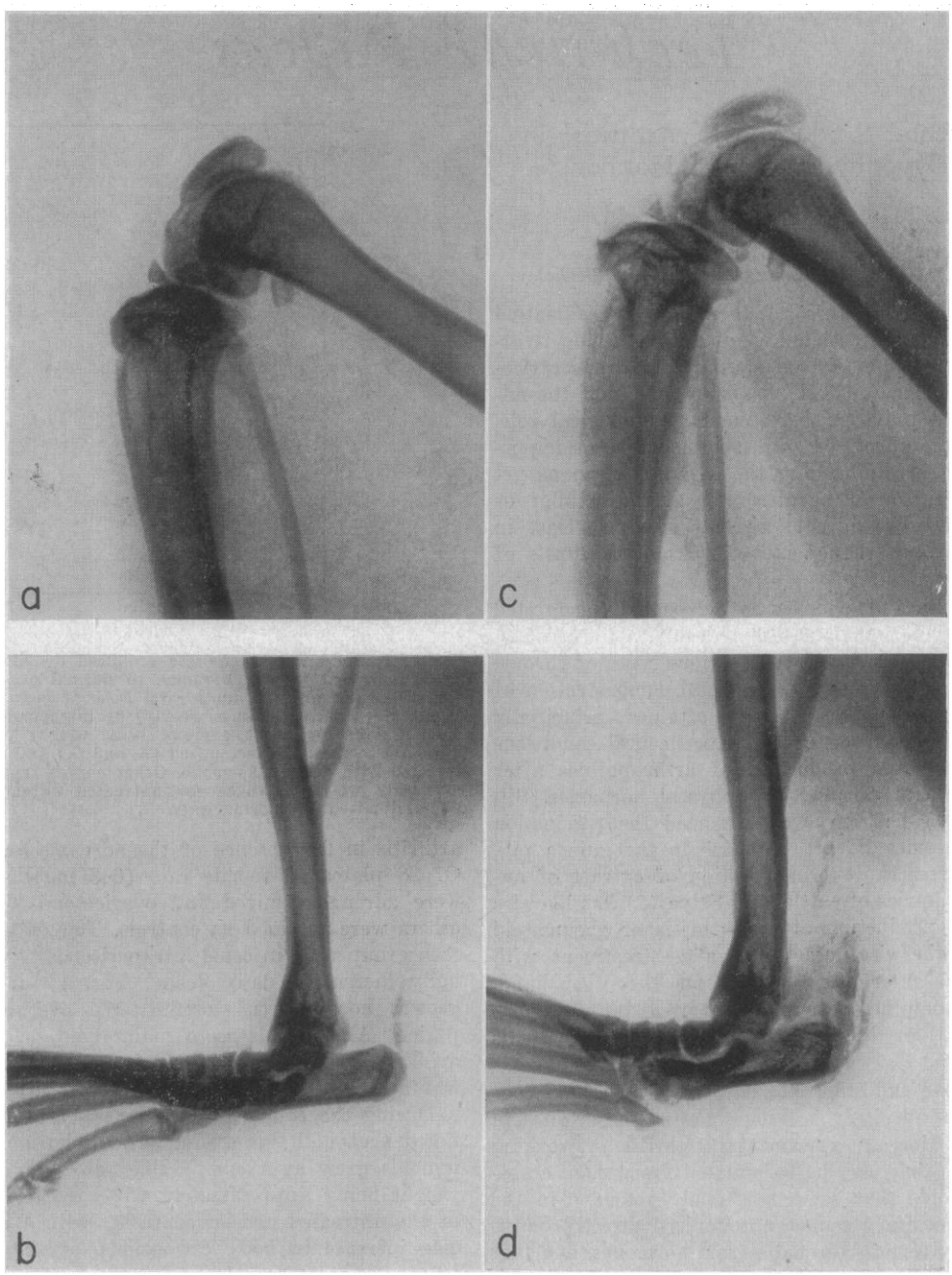

FIG. 2. Radiographic demonstration of articular and extra-articular changes in knee and ankle regions in growth hormone–treated, adrenalectomized-ovariectomized rats (*c* and *d*) as compared with normal control (*a* and *b*).

muscle tone was diminished, the animals appeared irritable, and evidence of knee and ankle joint tenderness, along with transient episodes of joint-swelling, became apparent. Five of the adrenalectomized ovariectomized animals treated with growth hormone and 3 of the untreated operated group succumbed to intercurrent infections or exhibited signs and symptoms resembling delayed terminal adrenal insufficiency. During the course of the experiment, two of the growth hormone–treated, adrenalectomized-ovariectomized rats, in poor condition, were treated with hydrocortisone for one week, with apparent symptomatic relief and diminution of joint-swelling and tenderness.

Radiographic study of all animals at the end of the treatment period disclosed evidences of joint disturbances, particularly at the knee, characterized by irregularities and erosions of condylar margins, localized osteoporotic areas in the condyles, with evidence of lipping and calcification at joint margins (Fig. 2).

These changes were present in varying degrees in all the growth hormone–treated, adrenalectomized-ovariectomized rats, in but one of the growth hormone–treated normal controls, and in none of the untreated controls. In addition, both groups of growth hormone-treated animals exhibited distinct extra-articular calcifications at the ankle joint and in the neighborhood of the Achilles tendon and in adjoining fascial planes.

The importance of these observations is provisionally thought to be related to the well-known antagonism existing between the pituitary growth hormone and certain adrenal steroids (as well as ACTH acting indirectly) on the various manifestations of growth. It should be noted that the demonstrated arthropathic effects of purified pituitary growth hormone are *not* mediated by the adrenal gland (or gonads).

If the above observations are confirmed, the groundwork can be laid for the verification of a hypothesis which holds (1) that the pituitary growth hormone may be of direct etiological importance in the chronic arthritides and in related conditions; and (2) that the ameliorative antiarthritic effects of ACTH, cortisone, and hydrocortisone may be considered to represent either suppression of pituitary growth hormone secretion, or antagonism to growth hormone (or to its local effects) at the tissue level, or both. It should be noted, however, that the experimental evidence described herein does not preclude the possible existence of sensitization to growth hormone (endogenous or exogenous) or of production of hypersensitivity to other allergenic factors or agents.

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The Relation of Bacteriophage to the Change of *Corynebacterium diphtheriae* from Avirulence to Virulence[1]

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Freeman (*1, 2*) has reported that exposure of an avirulent strain of *Corynebacterium diphtheriae* to a specific bacteriophage results in the production of virulent *C. diphtheriae*.[2] In addition he has observed that all virulent strains derived in this manner exhibit lysogenicity when tested against the parent avirulent strain. These results, confirmed in part by Parsons and Frobisher (*3*), have been fully confirmed in our own laboratory (*4*). Two possible explanations for the origin of these virulent organisms have been advanced (*1, 2*). They are (*a*) that virulent mutants develop in the avirulent culture and are subsequently selected for by bacteriophage action, and (*b*) that infection and the establishment of the lysogenic state alter the metabolism of the infected cells, with resultant production of toxin. The present report provides evidence compatible with the hypothesis linking lysogenicity and virulence and inconsistent with the hypothesis of mutation and selection.

Strain 444 of avirulent *C. diphtheriae*[3] as designated by Freeman (*1*) was used in the work to be described. This parent avirulent strain and the derived virulent strains will be referred to as 444A and 444V, respectively. The bacteriophage employed throughout has been designated 444V/A. It was isolated from strain 444V, produced by exposing 444A to bacteriophage B, described by Freeman (*1*), and was propagated on 444A. In all probability it is identical with bacteriophage B. Investigation of this phage-host system (*4*) indicates that, although it is strongly lytic, lysogenic cells are produced with extraordinary facility. It is similar in this respect to systems described by Burnet and Lush (*5*) for a *Staphylococcus* and Boyd (*6*) for *Salmonella typhimurium.*

The correlation between virulence and lysogenicity observed by Freeman (*1*) in the derived strain 444V, and repeatedly confirmed in the course of the present work, is highly suggestive of a causal relationship between the two changes in character. Nevertheless, it can be argued that a virulent mutant arising independently of bacteriophage action might simultaneously become receptive to a state of lysogenicity. If this occurred the establishment of the lysogenic state would be a result of the change to virulence rather than its cause. Thus, other evidence is required before any significance can be attached to this correlation.

Strong evidence against the mutation-selection hypothesis was obtained in the following manner. Samples were removed periodically from a mixture of *C. diphtheriae* 444A and bacteriophage 444V/A. Each sample was plated for total bacterial count and analyzed for the relative numbers of virulent and avirulent cells present. A differential medium exploiting the visibility of toxin-antitoxin precipitates in an *in vitro* system was used to distinguish between virulent and avirulent colonies. Plate differentiation was confirmed by guinea pig intracutaneous tests and *in vitro* virulence tests (*7*). Lysogenicity was demonstrated using parent strain 444A as the indicator strain. Because of the clumping exhibited in the normal growth of *C. diphtheriae* the counts obtained represent "clump" counts. The data are presented in Table 1.

[1] Supported in part by State of Washington funds for medical and biological research.

[2] The terms "avirulent" and "virulent" are used synonymously with "nontoxigenic" and "toxigenic."

[3] Kindly supplied by V. J. Freeman.