THERAPEUTIC SUPPRESSION OF TISSUE REACTIVITY

I. Comparison of the Effects of Cortisone and Aminopterin

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The remarkable advances in chemotherapy in recent years have conditioned medicine of this era to consider disease in terms of specific cures, just as medicine of the era following Pasteur and Koch became conditioned to view disease in terms of specific causes. The period intervening was one of elucidation and therapeutic application of specific host immunologic defenses. In this age of specific causes, specific responses and specific cures, general pathology as a conceptual guide in clinical medicine, which flourished so productively in the last century, has become overshadowed and some of its generic lessons have become obscured.

The reaction to injury is, regardless of etiology, basically similar in its local manifestations, which constitute inflammation, both morphologically[11] and dynamically[30], and in the general bodily changes largely mediated via stimulation of the pituitary-adrenal axis to which Selye[41] has applied the terms alarm reaction and adaptation syndrome.

Although the process of inflammation is the first and most important line of defense of the body, its characteristics are such that defensive and reparative processes may themselves be inimical to the body's welfare. Phagocytosis of organisms which exist parasitically in macrophages as in tuberculosis, coccidioidomycosis, psittacosis and histoplasmosis, abet and aid in perpetuating and disseminating the pathogen. Reduction in vascularity, which characterizes inflammatory processes of a more chronic nature, sets up a barrier to the penetration of humoral immune bodies or pharmacological agents.

More important is the deleterious effect of the repair process itself, which like Aladdin's djinn, may wreak havoc. Mechanical obstruction may ensue following a lye burn of the esophagus, or as in cicatricial pyloric stenosis in peptic ulcer. Organ function may be impaired as in cirrhosis, uveitis, scarring of the heart valves in the wake of rheumatic fever, derangement of articular and periarticular structures in rheumatoid arthritis, pulmonary fibrosis secondary to silica or beryllium, to which may be added various granulomatoses, to cite only random illustrations. In such circumstances disease is itself the repair process of the body stimulated to respond in such manner that there ensues distortion of morphology and interference with function. The reparative process is most closely associated with mesenchymal derivatives, notably fibroblasts and reticuloendothelial cells, but other organs such as the skin in which repair is an important function may be similarly subject to overactivity of the repair process as illustrated by keloid formation and the excessive desquamation of psoriasis. Such disorders are distinguished from neoplasia, which similarly may be provoked by irritative noxious agents causing a proliferative reaction, in that they are responses of tissues rather than in-

dividual cellular elements. The distinction, however, is not absolute, as is perhaps best illustrated by the histopathology of Hodgkin's disease in which multiple cellular elements are combined in neoplastic formation.

It appears appropriate to group such disorders produced by the repair process as diseases of tissue reactivity. Such nosological grouping in disregard of etiology seems warranted, for the causative agents, be they microorganisms, or nonorganic irritants such as silica, share the common property of acting as incitant to tissue responses of proliferation, which comprise the disease entities. It is apparent that therapy in the large group of disorders of tissue reactivity must be directed at the host response and that specific measures aimed solely at the etiological agent may be inadequate and unavailing.

Until recently such a concept would have been of little practical moment. Newer knowledge of the functions of the adrenal cortex indicate that potent agents may be employed to combat the lesions of tissue reactivity by modifying host responses of the vulnerable shock tissue.

The adrenal cortex, vital though it be in mobilizing body reactions to stress is, as stated by Albright[1], "anti-anabolic". The lesions of Cushing's disease are characterized by marked generalized atrophy of tissues, notably striated and smooth muscle, bone and skin. As indicated by Albright, this is due not to excessive breakdown of tissue but rather to an inhibition of tissue protein synthesis by the glycogenic steroids of the adrenal. Numerous investigations have established the inhibitory effect of adrenocorticotropic hormone (ACTH) and Compound E of the adrenal cortex on body growth[23,25], and in suppressing development of mesenchymal tissues[2,3,37]. Baker[2], in a comprehensive study, has shown that in addition to general inhibition of growth ACTH causes reduction in cellularity of connective tissue with atrophy of collagenous fibers and retardation of wound healing, disappearance of osteoblasts with cessation of proliferation of epiphyseal cells, atrophy of marrow, lymphoid structures and the thymus, and a marked atrophy of the entire epithelial tissues including the epidermis, sebaceous glands and hair. Such suppressive effects on tissue proliferation provide an adequate explanation for the increasing number of disorders that are being benefited by ACTH and cortisone, all of which have as their common denominator excessive tissue reactivity.

Interest in the therapeutic applications of ACTH and cortisone has centered particularly on the rheumatic diseases, largely since it was in these conditions that the dramatic ameliorative effects of these hormones were first reported by Hench and his coworkers[20]. Rheumatic fever and rheumatoid arthritis are somewhat unique in that the mesenchyme is at once the offending agent and the victim in the disease process. Antibodies to hemolytic streptococcal infection, which are generated in the immune body-producing cells of the mesenchyme (reticuloendothelial, plasma, and lymphoid cells), are the noxious agents that produce the characteristic pathological lesions in connective tissue, another mesenchymal derivative[16]. It has been a logical line of investigation to attempt to prevent the antibody response to hemolytic streptococcal infection in treating rheumatic fever, and evidence has accumulated suggesting that the beneficial action of massive salicylate therapy may be due to suppression of the immune response[6,8,45]. The adrenal cortical hormone in large doses suppresses antibody formation experimentally[33], and it has been suggested that this may be a mechanism whereby cortisone alleviates rheumatoid arthritis

and allied conditions[10]. In man, however, neither ACTH nor cortisone inhibits antibody formation[32]. Numerous studies indicate furthermore that cortisone operates by suppressing the reactivity of connective tissue itself rather than by acting at the level of immune responses. Thus there may be mentioned suppression of the Schwartzman Phenomenon[42] and of connective tissue responses to such local irritants as beryllium dust[26] and formalin periarthritis[40] where immune mechanisms are not involved.

Although suppression of antibody formation may be produced experimentally by agents such as salicylate, adrenal cortical hormone, and aminopterin[34], this cannot be readily accomplished in man, and it is probable that the depression in blood globulins observed with cortisone and ACTH reflects the general inhibition of protein synthesis earlier reported by Albright[1], rather than any specific effect on antibody formation. Neither nitrogen mustard, spray radiation, urethane nor aminopterin produce any lowering of gamma globulin in rheumatic fever or rheumatoid arthritis[18].

Aminopterin does, however, despite the lack of any indication of effect on immune responses, produce amelioration of symptoms and decrease in articular and periarticular exudative changes. In a study carried out in collaboration with Dr. J. J. Oleson of the Lederle Laboratories it has been found that in rats pretreated with aminopterin there is suppression of exudative and proliferative changes produced by periarticular injection of formalin, similar to the findings reported by Selye with cortisone[40]. In these experiments male albino rats weighing 70 to 85 gm. received 10γ of aminopterin divided into 2 oral doses daily. On the second day 0.1 cc. 1% formaldehyde was injected into the right hind paw at the plantar aponeurosis 4th day, and on the 5th day an injection of 0.1 cc. of 2% formaldehyde was given at the plantar aponeurosis of the left hind paw. Severe inflammatory reaction and marked swelling of the hind feet occurred in control animals, which was largely although not entirely suppressed in the animals receiving aminopterin. The inhibitory effect of aminopterin on formalin arthritis occurred in adrenalectomized animals, so that the suppression of the inflammatory response was directly due to aminopterin and not mediated via adrenal stimulation. A somewhat greater suppression of inflammatory changes was observed in 2 rats given cortisone in dosage of 5 mg. divided into 4 daily doses. The steroid artisone in the same dosage was devoid of any effect.

Indication of the suppressive effect of aminopterin on tissue reactivity is seen clinically, particularly in psoriasis where the therapeutic response is more uniform and more dramatic[17]. Histologically psoriasis is characterized by a great overgrowth of the epithelium, with downward growth of the interpapillary processes[14]. It is of interest that improvement in psoriasis has been observed on administration of cortisone[12].

In 18 subjects treated with aminopterin in daily dosage of 1 to 2 mg. numerous indications of an inhibition of tissue reparative and proliferative responses have been observed. In 10 subjects with psoriasis, cessation of scaling has occurred regularly within 3 to 7 days, frequently accompanied by slight hemorrhagic crusting at the site of the lesions followed by rapid thinning of the skin in the areas involved. Similar changes in skin lesions have been observed in a case of lupus erythematosus and in a subject with chronic atopic eczematoid dermatitis. In the latter the observation was made by the patient that his associated asthma, which for several months had required

ephrine and 7 to 8 capsules of ephedrine daily, improved so markedly during the period of aminopterin administration that no therapy other than an occasional ephedrine capsule was required. Three weeks after discontinuing aminopterin his dermatitis and asthma both recurred and an identical response was obtained with a 2nd course of aminopterin given in dosage of 1.5 mg. daily for 11 days.

In 4 subjects temporary incomplete alopecia followed administration of aminopterin. Hair loss is produced likewise by ACTH[2]. Lack of healing was a noteworthy finding in 3 subjects with superficial ulceration of the scrotum, penis and neck respectively, and in 2 patients with pustular infections. In these subjects absence of granulation and epithelialization was conspicuous and bleeding occurred with any rubbing of the wound surface. Impairment of wound healing has likewise been observed with ACTH and cortisone[2,37]. Further indication of inhibition of connective tissue proliferation is seen also in the suppressive effect of aminopterin on experimental sarcoma [27,34,39], an effect that is also exhibited by cortisone[21].

A uniform finding among patients treated with aminopterin was the occurrence of patches of shallow ulceration of the buccal mucosa generally associated with abdominal cramps. These changes generally developed during the 2nd week of aminopterin administration after a total dose of 10 to 20 mg., although in a few instances as much as 40 mg. was given before changes in the oral mucosa or cramps developed. These findings are due, just as in the skin, to inhibition of proliferation of epithelium in the oral cavity and gastrointestinal tract, the mucosal thinning favoring the development of superficial infection and ulceration. Similar observations have been made in rats and dogs given aminopterin[35], and

kemia, in whom temporary remissions followed by refractoriness may be observed[4,31,38], similar to experience with ACTH and cortisone[43]. Weight loss and muscle wasting, which are produced experimentally by aminopterin[35], and are also seen with experimental administration of ACTH[24], and in the hyperadrenalcorticism of Cushing's disease, were observed in 1 subject given 40 mg. of aminopterin over a 3 week period.

It is not intimated from these analogous effects of aminopterin and cortisone that their actions are the same. Both are anti-anabolic, but cortisone is concerned with whole protein moieties[9]; whereas the locus of action of the folic acid antagonist aminopterin lies more particularly in inhibition of the effects of folic acid on the synthesis of purines, pyrimidines and desoxyribonucleic acid[22,36], and the utilization of tyrosine, histidine, and other amino acids in the regulation of protein synthesis[13,15,19,48].

Folic acid is now recognized as essential in nucleoprotein formation and cell growth, acting as a coenzyme in enzyme systems concerned with synthesis of thymine and purines[44]. The effect of aminopterin is to block the reduction of folic acid to the formation of the citrovorum factor, which is the biologically active derivative of folic acid[5,47].

Observation that the citrovorum factor or thymidine reverses the inhibitory effect of aminopterin[5,47] has provided a useful means to control some of the toxic effects of aminopterin. In the present study citrovorum factor in dosage of 40 to 100 million units was given intramuscularly 2 to 3 times weekly to 4 subjects receiving aminopterin. An increased tolerance to aminopterin was observed, as compared to previous courses of aminopterin administration several months earlier. There was partial but not complete

of aminopterin, that is, stomatitis and abdominal cramps, but it was also observed that the amelioration of arthritic symptoms and of psoriasis was less complete than when aminopterin alone had been given. Aureomycin given prophylactically in several subjects in dosage of 1 gm. daily was found effective in decreasing stomatitis and gastrointestinal symptoms and did not appear to modify sensibly the therapeutic effect of aminopterin.

Although the citrovorum factor and aureomycin are helpful in alleviating the toxic effects of aminopterin, the margin between the therapeutic and toxic dose of aminopterin is so narrow as to preclude its clinical use. The observations of an ameliorative effect on the exudative phenomena of rheumatoid arthritis reported separately in greater detail[17] are of interest principally to indicate that the mechanism of action of cortisone is not a specific hormonal effect but is due to suppression of mesenchymal reactivity, as is also accomplished by aminopterin. It is to be emphasized that the effects of aminopterin are not so marked as those produced by cortisone, although the action is more protracted, usually persisting for some weeks after discontinuing medication. Sustained pharmacological effect is a desideratum in controlling diseases of tissue reactivity and it is possible that other anti-folic compounds will be found more suitable for clinical employment, or that aminopterin in smaller dosage may be useful as a supplement to cortisone administration. The use of such measures may be envisaged as a potent therapeutic weapon to suppress the unfavorable host responses of tissue reactivity, in conjunction with specific measures aimed at the etiological agent. Thus, for example, ACTH promptly suppresses granulomatous formation and ulceration of laryngeal lesions, and systemic signs of illness, in agents that inhibit unfavorable host responses in tuberculosis over extended periods may prove of considerable value as an adjunct to specific chemotherapy with streptomycin and other bacteriostatic agents.

Both cortisone and aminopterin exert generalized effects on all tissues; this limits their therapeutic value. A study is currently in progress to establish whether the striking remissions in psoriatic lesions observed with oral administration of aminopterin can be achieved by topical applications so that the effects of the drug can be confined to the tissues involved. If, as appears probable, the beneficial effect of such measures as cortisone and aminopterin in the rheumatic state is due to suppression of mesenchymal reactivity, it would appear desirable to find an agent that specifically inhibits connective tissue. Such a substance has been found in extracts of malt, ungerminated grain and oranges, which totally inhibit the growth of fibroblasts and other mesenchyme cells in concentrations permitting the free growth of epithelial tissues[28]. Further study has revealed this differential growth-inhibiting action to be possessed by parasorbic acid and coumarin[7,29]. It is of interest that coumarin is structurally allied to salicylate. It would appear worth while to investigate the therapeutic potentialities of parasorbic acid and various of the coumarin series. Considerable investigation has been done in the screening of therapeutic agents in experimental sarcoma, and this line of investigation may prove fruitful in finding connective tissue inhibiting agents of value in diseases involving mesenchymal tissues such as respond to cortisone.

Summary. Attention is drawn to the similarity in action of cortisone and the folic acid antagonist aminopterin. Although the locus of their biochemical effects does not appear to be the same,

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