BIOCHEMISTRY OF INFLAMMATION

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Presented September 19, 1962, Seminar, New York City

INFLAMMATION ranks next to pain as the greatest symptom of body difficulty or pathology. Menkin (1) defines inflammation as the "complex vascular, lymphatic and local tissue reaction elicited in higher animals by the presence of viable or of nonviable irritants." More simply, it is the local response of small blood vessels to injury. Inflammation is characterized to the naked eye by swelling, increased heat, redness, pain and disturbance of function. The swelling is due to the edema and congestion in the area. The inflamed area feels hot in comparison with the surrounding areas because the dilated vessels bring a large amount of warm blood to the area. The redness results from the dilatation and congestion of the arterioles and capillaries. Pain is due to the swelling and tension on tissues with pressure on sensory nerves. The disturbance of body function is linked to the pain and destruction of the affected cells and tissue.

Inflammation is not a static single event but a sequence of constantly changing interdependent reactions, each triggered by a previous alteration, a dynamic process by means of which cells and exudate infiltrate, accumulate and finally destroy the integrity of connective tissue. Intimately and inseparably related to the inflammation is the repair process, whereby the tissues are protected from further injury. The agents causing the injury and hence leading to inflammation, may be of bacteriologic, physical, chemical or traumatic nature. Following a local acute injury there is disturbance in the flow through small blood vessels. A momentary constriction of the capillaries is rapidly replaced by dilatation with an increase in blood flow. This dilation is also transitory and the blood flow slows down to almost stagnation. These local changes result chiefly in an increase in the permeability of the capillaries to the plasma proteins. Fluid plasma and white blood cells escape through the capillary walls into the surrounding tissues. This accumulation of fluid and cells is called an "exudate." Exudation is the primary and pivotal response on which all subsequent inflammatory responses depend (2). The fluid, or serous part of the exudate is, as noted, largely plasma and, when abundant, may be referred to as inflammatory edema.

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The fluid plasma in the accumulated exudate coagulates in the area of injury with the precipitation of an abundant network of fibrin. This "walls off" the inflamed area, localizing the injurious agent and shielding adjacent tissues. The early fixation of, e.g., a bacterial irritant, allows time in which leukocytes can assemble to attempt to destroy the invader by phagocytosis. The first white cells to migrate through the vessel walls are the polymorphonuclear leukocytes, aided in some cases by a chemical stimulus, a process referred to as chemotaxis. Bacteria and products of injured tissue act as chemotactic agents, intensifying the emigration of the leukocytes from the blood vessels and directing them toward the injurious agent, leading them to actual contact with the foreign particle which makes phagocytosis possible.

Acidosis develops in the area of inflammation (3), injuring the cells. These local changes in the hydrogen ion concentration of the exudate appear to govern the cellular sequence in inflammation, consisting of polymorphonuclear leukocytes followed by mononuclear phagocytes (4–6). When the pH falls below 6, all types of white cells are injured, and pus results. Pus formation in acute inflammation is virtually a function of the hydrogen ion concentration (7). The mechanism of acidosis appears primarily referrable to a developing glycolysis (1), with the cellular sequence at the site of inflammation conditioned by the local pH, which in turn is determined by disturbance in the intermediary carbohydrate metabolism (7).

Connective Tissue

Inflammation can be considered to begin as a change in small blood vessels, or rather, as a change in the state of connective tissue components which determine the physiological properties of the small blood vessels. Whatever the organ affected, it is alteration in the connective tissue of the blood vessels which is fundamental to the development of inflammation. Connective and skeletal tissues are concerned throughout the body with the formation and maintenance of structure. They have as a common origin the embryonic mesenchymal cell, which in the course of differentiation forms the connective tissue proper, cartilage and bone (8). The texture of these tissues depends upon the orientation of the cells, their physical and chemical properties, the spatial organization of the various constituents with respect to each other and the relative amount of each substance present. The connective tissue cells are required to produce a wide variety of extracellular materials which determine to a large extent the processes of growth, regeneration and repair.

The chief connective tissue cells are the fibroblasts and mast cells. The mast cells synthesize and release several substances that are important in the metabolism of connective tissue (9). Fluctuations in the rate of forma-

tion, infiltration and lysis of these cells provide cellular mechanisms for several systems of balances and counterbalances that control activities of interstitial fluids and connective tissues (10). Mast cells synthesize, store and release histamine which increases capillary permeability, which in turn increases the plasma proteins in the interstitial fluid (11). Increased interstitial proteins stimulate formation of the stem cells of mast cells, and further synthesis of histamine. Mast cells are also able to synthesize and release mucopolysaccharides which appear to be necessary for the deposition of different collagen fibrils (12, 13) and play a role in the maintenance of normal cell permeability. Mast cells are the only cells in the connective tissues that contain acid mucopolysaccharides and they are able to release these substances to the ground substance. The concentration of tissue water is a stimulation to mucopolysaccharide release. Mast cells may be able to release histamine independently of simultaneous mucopolysaccharide release. Histamine may induce an increase in capillary permeability and produce edema; the edema provokes release of mucopolysaccharides that bind the water, changing it into a hydrated gel. The presence of acid mucopolysaccharides stimulates the deposition of collagen fibrils and thus connective tissue growth. It appears that fibroblasts as well as mast cells are actively and indispensably involved (14). Mast cells are present in increased numbers in some chronic skin inflammations, such as urticaria pigmentosa (15).

The fibroblasts are the precursor cells, or origin, of collagen. The collagen molecule is synthesized on the surface of the fibroblast (16) and released to the extracellular compartment, where the molecules become polymerized and oriented to collagenous fibers (17). The fibroblasts probably also secrete the ground substance (18).

The ground substance is a gelatinous material permeating loose connective tissue, acting as a substrate through which salts, water and a variety of proteins, as well as neutral sugars and mucopolysaccharides are transported to various cells of the body. The capacity to accumulate and bind water is one of the most important functions of the ground substance. Hyaluronic acid, one of the main mucopolysaccharides, lends to the ground substance its viscosity, gelatinous character and water-binding capacity (17).

The mucopolysaccharides of connective tissue ground substance are similar, but nevertheless, chemically quite distinct and probably are synthesized by different enzymes. It seems likely that these polysaccharides have different functions because they are not evenly distributed in connective tissue (19) and their proportions change with age, although they are apparently all linear unbranched polymers containing hexosamine and another sugar, usually uronic acid, arranged alternately. Apart from hyaluronic acid and chondroitin of cornea the connective tissue polysac-

charides are all sulfate esters and are therefore highly charged polyanions, which show considerable interaction with themselves and with proteins and which also bind salts and water to a marked degree. In the native state the polysaccharides of connective tissue are probably all combined with noncollagenous protein (20), forming very large complex molecules with molecular weights of several millions (21).

Efforts to delineate the mechanism of action of acid mucopolysaccharides (AMP) have been severely hampered because procedures for extraction are not quantitative, methods of separating different AMP from one another are insufficiently refined, and the knowledge of the mechanisms of their biosynthesis is still sketchy (22).

Chemical Mediators

The similarity of the inflammatory cycle in many different species in response to many diverse types of injury has led to the now generally accepted view that the vascular events of inflammation are due at least in part to the release of local hormones or mediators (23, 24). At some time or other, almost every active material extracted from blood or tissue has been incriminated as a causative factor in inflammation. These include potassium ions, acetylcholine, serotonin, catecholamines, adenyl derivatives (adenosine, adenylic acid, ATP), histamine, proteins, varied peptides, including particularly bradykinin, etc. (25). These substances display the most varied physiological actions. Only histamine, the kinin peptides, globulin proteins and catecholamines satisfy, in large part, the criteria for true mediators.

The beginning of modern mediation theory can be considered to begin with Lewis' (26) studies on wheal formation or hives. Possibly the outstanding dermatological example of the leakage of blood plasma from dilated small blood vessels into extracellular spaces is represented by wheal formation. Whealing represents circumscribed, superficial edema of the skin as it develops in response to various chemical, mechanical, thermal and actinic stimuli. There are three distinct steps in the development of wheals: local vasodilatation of the capillaries, causing local reddening; local increased capillary permeability causing local wheal formation; and a vasodilator axon-reflex complex with arteriolar dilatation, causing the red flare. This is known as Lewis' "triple response" of the skin to injury (26). It was Lewis who argued that these reactions, which appear quite uniformly in response to diverse physical and chemical inflammatory stimuli, are so uniform because all the stimuli eliciting the response do not act directly on vascular and nervous elements, but by liberating substances from the injured cells (24). This has led to the now generally accepted view that the release of local hormones or mediators by the injured cells reasonably explains some of the biological manifestations of inflammation. This

hypothesis has gained support from the discovery of naturally occurring substances with effects on small blood vessels similar to those seen in inflammation. In recent years the theory has been strengthened further by the demonstration of active forms of such compounds at the site of injury at the time when they should be exerting their effect (23).

A. Histamine. Lewis drew attention to the similarity of the action of histamine and the vascular events of early inflammation and postulated the release, by injury, of histamine or a histamine-like substance. Histamine is a diamine derived from the amino acid histidine. It is very widely distributed in the tissues of all mammals. It is formed by the enzyme histidine decarboxylase and destroyed by the diamine oxidase, histaminase (27). Mast cells synthesize, store and release histamine. Endogenous and exogenous histamine are potent inductors of hyperemia and of increased capillary and tissue permeability (10, 28, 29, 30, 31). Pathological tissues rich in mast cells contain very high concentrations of histamine. Analysis of tissue homogenates indicates that histamine is loosely held in the mast cells within definite granules in the mitochondrial fraction of the cell. It is evidently held in a readily diffusible form. Histamine is readily released from suspensions of these mitochondrial particles by freezing, hypotonic media, surface-active agents and a large variety of organic bases (24). The release of histamine from mast cells speedily induces dilatation of capillaries and increased permeability and reduces the viscosity of hyaluronic acid in interstitial fluid. Usually these changes result in increased passage of plasma proteins with the formation of protein rich edema (10, 32). This edema has been considered a factor in initiating and continuing collagen degeneration. Swelling of collagen fibers is one of the early changes in collagen diseases (33, 34). The release of histamine from the granules of mast cells primes the mast cell-histamine chain and possibly indicates a contributing factor in the predilection of the following tissues or organs to lesions in the collagen diseases (35): (a) abundance of mast cells in the pleura in pleurisy of rheumatoid arthritis (36); (b) "cuffing" of mast cells around arterioles in periarteritis nodosa (37); (c) foreign protein release of mast cell-histamine in serum sickness. Edema appears in skeletal muscle also in dermatomyositis (38) and in degeneration of muscle fibers.

The discovery of a group of drugs, the antihistamines, that antagonized more or less specifically the effects of histamine led to further advances. Treatment with these compounds greatly reduced the inflammation caused by antigen-antibody reactions in certain diseases such as allergic rhinitis and urticaria. However, the majority of inflammatory lesions were not affected by the antihistamine drugs (39). As a result it seemed that histamine played a minor part in the total inflammatory reaction. Another weakness of the "generalized" histamine theory is the case of the sunburn reaction (15), which has a latent period of one to several hours. Sub-

sequently, reddening develops which is sharply limited to the area of irradiation. This limitation cannot be compared to the widespread, suddenly appearing flare of Lewis' triple response (26). In burn reactions, eczematous types of dermatitis and tuberculin-type hypersensitivities, the role of histamine in the inflammation is also negligible. There are hardly ever any transitions between the urticarial and eczematous type of inflammations. Such transitions should be expected if the reactions depended only on the rate of histamine liberation and on its concentration in the tissue. The same lack of histamine liberation is found in the case of erythematous-edematous blistering reactions and other diseases with prevailing blister formation, such as smallpox, chicken pox, herpes simplex, herpes zoster and persistent papules (15).

The consensus of results (24, 40, 41) appears to indicate that the role of histamine in inflammation is to initiate the vascular changes, especially increased capillary permeability, and the subsequent sustenance of these changes is due to other mechanisms independent of histamine release (23). The rapidity with which the effects of histamine occur after injury may be explained by assuming that the histamine is rapidly released from the mast cells, which disrupt and liberate their granules in response to injury. The precise mechanism of the release is a complicated process not yet completely clarified, but appears to involve formation of ATP, dependent on the glycolytic cycle of carbohydrate metabolism. There is a possible final activation of lytic enzymes capable of lysing the structure of the mast cells (25, 42), and injury activates the enzymes. It has been suggested that the release of histamine follows from the rupture of a peptide or polar bond linking histamine to a protein, or an ion exchange reaction, releasing histamine from loose combination with an acidic body compound (43). There is also evidence that injury may cause increased activity of histidine decarboxylase and this leads to increased synthesis of histamine (44).

B. Serotonin. Serotonin, 5-hydroxy tryptamine, is a monoamine derived from the amino acid tryptophane. The primary role of serotonin is unknown but it is believed to have a part in the transmission of nerve impulses (45). In very low concentrations serotonin increases capillary permeability (46) and will cause local progressive collagenous and fibrous proliferation within the dermis on long-term injection, in the rat (47). However, it does not produce these effects in most other species and even in the rat the evidence of an important role in inflammation is meagre (48). Compounds exist which are more or less specific antagonists to serotonin, such as 1-methyl-d-lysergic acid butanolamide and cyproheptadine. Dosage with such substances considerably reduces the inflammatory reaction caused by serotonin in rats. These studies (48) however, did not prove that the observed effects were due to the inhibition of serotonin or related compounds. It is possible that some unknown pharmacologic action was responsible.

C. Peptides. The possible role of peptides in inflammation has been suspected for over a hundred years, but the study of the effect of peptides on capillaries really dates from the observations of Menkin in the middle 1930's (49, 50). His early studies indicated that injured cells at the site of inflammation release a permeability and chemotactic factor which was called "leukotaxine," and which appears to be a polypeptide. Leukotaxine is capable of essentially reproducing the effect of the whole exudate as far as increasing local capillary permeability and inducing the emigration of polymorphonuclear leukocytes (51). Unfortunately, leukotaxine has never been purified, its detailed structure is unknown and its significance not adequately defined. It might also be noted that leukotaxine appears after the inflammation is established.

Later work demonstrated that large numbers of different peptides from various sources were able to increase capillary permeability and that the most likely requisite for this peptide property was a molecule containing eight to twelve amino acids (52). These observations culminated in the discovery (53), purification (54), and synthesis (55) of one of the most important members of this group, bradykinin, a peptide derived by proteolysis from plasma globulin. It is released by proteolytic and coagulating venoms and by trypsin from the globulin fraction of normal plasma. The name "bradykinin" was given to indicate the slow movement of the guinea pig ileum produced by it, differing from the more rapid contractions of the ileum by histamine or acetyl choline. It is now customary to refer to all inflammatory peptides by the term "kinins."

In addition to stimulating smooth muscle, bradykinin causes all the inflammatory reactions of vasodilatation, increased capillary permeability, accumulation and migration of leukocytes, and especially pain (56, 57). Bradykinin is also known to have some effect on the nervous system, but precisely what it does is still not known. It will cause pain when injected under the skin and at the same time there will be a flushing of the skin in the region, but if the area is denervated the flushing will not take place. This appears to indicate that the redness is due to a specific effect of the bradykinin on the nerves.

When tissues are damaged by severe burns, or animal skins are experimentally heated, there is a marked increase in bradykinin concentration in the interstitial fluid and urine (58). Unfortunately, there is, as yet, little quantitative data to correlate the amount of cell damage. These findings, however, provide substantial evidence that a plasma kinin, possibly bradykinin, takes part in the inflammatory response.

Fortunately, there exists an efficient bodily system for the destruction of excess bradykinin and other kinins (58). If there were no such system, the kinins would keep building up in the blood stream and continue to enlarge the entire system of blood vessels, continually increasing their permeability.

This would result in general vascular collapse. A bradykinin-inactivating enzyme, kininase, is present in blood and extracts of renal tissue. In addition, an inactivator of kallikrein, mentioned as the bradykinin-forming enzyme, is present in high concentration in lymph nodes. This destruction by peptidases may in fact be a homeostatic mechanism for inactivating these powerful kinin substances once they escape from the site of inflammatory reactions.

Natural bradykinin was isolated in pure form (59, 60) after the release by trypsin from bovine plasma globulins. It was actually synthesized (61, 62) before its structure was known. The structure is now known to be a linear nonapeptide of the following amino acid sequence (58, 63, 64):

(H—Arg—Pro—Pro—Gly—Phe—Ser—Pro—Phe—Arg—OH)

However, bradykinin is not the only active peptide formed by the action of enzymes on the plasma globulins. A decapeptide, called kallidin, differing from bradykinin only by the addition of one amino acid residue, lysine, at one end, has been isolated and characterized from blood (65). The structure of kallidin is:

(H—Lys—Arg—Pro—Pro—Gly—Phe—Ser—Pro—Phe—Arg—OH)

Kallidin is less active than bradykinin on most tissues but has a similar spectrum of activity. Another potentially important kinin is the recently isolated peptide known so far only as "substance P" (66). The purified material has many of the properties of bradykinin, but its structure contains at least six amino acids not found in bradykinin. Since different proteolytic enzymes not only give rise to bradykinin, but to other peptides with similar properties, it has greatly complicated the problems of relating the various "kinins" to body function and particularly to inflammatory function.

D. Proteins. Kallikrein, the bradykinin-forming enzyme and certain globulin fractions of plasma have been shown to be capable of increasing capillary permeability, in very low concentration (41, 67). The plasma globulin is present normally as an inert precursor that can be activated by a variety of procedures, including dilution with saline, contact with organic solvents and incubation with minced tissues (23). It is thus an obvious candidate for the role of mediator of the vascular changes of acute inflammation. The protein could act directly on vessels (possibly enzymically) or could be either a protease, or less likely, a substrate in formation of kinin peptides. Kallikrein obviously exerts its effects on capillaries and smooth muscle by catalyzing the formation of kinins from globulin substrates (24).

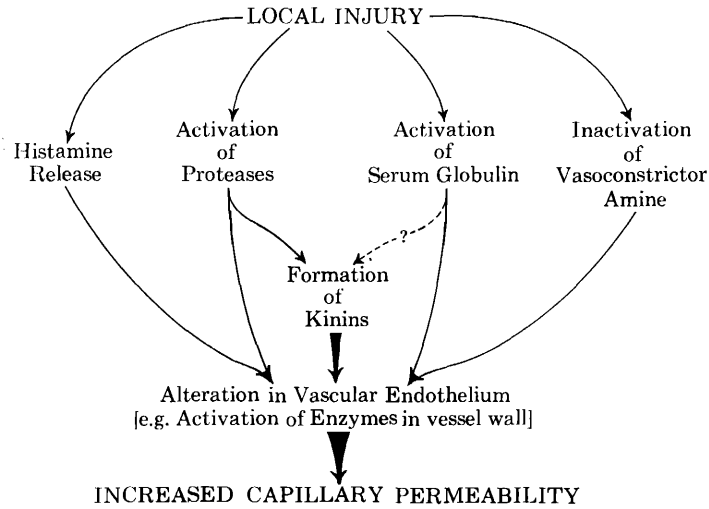
E. Vasoconstrictor Amines. The previously discussed mediators of

inflammation have all been active dilatators of blood vessels, increasing capillary permeability. Vasoconstrictor forces have been ignored. Yet the first vascular event following an acute injury is a momentary and transitory contraction of local blood vessels. A newer concept has recently arisen explaining initial vasoconstrictor forces, following the observation that the manifestations of rheumatoid arthritis and related diseases could be altered by the administration of small doses of iproniazid, an amine oxidase inhibitor (68, 69). This appeared to indicate that the vascular changes of acute inflammation are partly due to the destruction of an amine that would otherwise constrict and reduce the permeability of capillaries and oppose the action of compounds such as histamine and kinins. The most important endogenous compounds with these "antipermeability" actions on blood vessels are the catechol monoamines: adrenalin and noradrenalin. They are present in platelets, leukocytes and vessel walls, and at least two enzymes destroy them in the body, monoamine oxidase and catechol-O-methyl transferase. Administration of specific monoamine oxidase inhibitors greatly reduces the increased capillary permeability consequent to thermal or chemical injury. This result is explicable on the basis that inflammatory phenomena are partly due to inactivation of vasoconstrictor amines by monoamine oxidase. Competitive inhibitors of catechol-O-methyl transferase failed to modify the inflammatory reaction.

Recent evidence (70) dealing with the genesis of tissue destruction following locally administered bacterial extracts suggests that epinephrine is an important factor in mediating the subsequent inflammation. The mechanism by which catechol amines accelerate tissue damage is not well understood. Epinephrine does not seem to be involved in the local inflammatory response *per se* (71), but rather in the subsequent development of endothelial damage. While the evidence is not yet well substantiated, Cameron and Spector (23) postulated that, following injury, an adrenalin-like substance and an amine oxidase may be brought into contact. As a result the vasoconstrictor amine may be destroyed, and inflammation allowed to proceed. It seems possible that in the wall of the normal small blood vessel, adrenalin-like and histamine-like compounds compete for receptor sites, the interplay of their actions making for normal vascular reactions. In inflammation, not only are the vasodilator forces greatly augmented but the vasoconstrictor forces may be inactivated. The enzymic inactivation of the adrenalin-like substance could be precipitated either by local activation of monoamine oxidase or release of the amine from a site inaccessible to the enzyme (23).

Mechanism of Increased Capillary Permeability

Cameron and Spector (23) attempt to illustrate schematically the sequence of events of increased capillary permeability as follows:



It is suggested that the initial event appears to be a release of histamine from mast cells by a mechanism not yet fully understood but possibly involving activation of lytic enzymes which break down the structure of the mast cells. Histamine appears to exert its effects within a minute or two of injury, but continues to dilate capillaries and increase their permeability for some time afterward, for at least one to two hours.

At the same time as histamine is released, it is postulated that an adrenalin-like substance is brought into contact with the enzyme monamine oxidase which destroys it. The hypothetical destruction of the adrenalin-like substance leads to dilatation and increased permeability of small vessels that begins rapidly and may last for twenty-four hours or even longer. Soon after these initial events there may be an activation of globulins and peptides that increase capillary permeability.

Little to nothing is known of the intimate mechanism whereby histamine, globulins, and peptides increase capillary permeability. There is some evidence that all endogenous mediators of increased capillary permeability may exert their effects by activating an enzyme of the esterase-protease group in or near the vessel wall (72). The substrate of this enzyme could be a protein or phospholipid in the capillary wall of the precursor of yet another mediator substance which then acts on the blood vessel (73).

It is of interest that high concentrations of some antihistamine drugs not only exert a general antagonism to increased capillary permeability but also cause a general inhibition of electrolyte movements in damaged cells and mitochondria (23). This may possibly mean that increased capillary permeability to protein is in some way secondary to, or at least associated with, electrolyte disturbance in the vascular epithelium and that capillary

permeability factors such as histamine may act by altering the electrolyte and water balance of these cells. These speculations have gained some support by electron microscopy which has indicated that protein appears to leave histamine-treated vessels by transport through the endothelial cytoplasm rather than by passage through channels of molecular dimensions between the cells (74).

A new point of view has related the inflammatory processes and blood coagulation (75). There is no experimental proof that all the aforementioned mediators exert their effects by a simple direct action on the vascular wall. It is suggested that the intervention of a coagulating mechanism or fibrinogen production is a prerequisite to typical inflammatory vascular reaction. The assumed coagulation process in the vascular wall may be initiated by the inflammatory agent itself or by endogenous thromboplastic factors produced under the influence of these agents.

Anti-Inflammatory Drugs

A. Corticosteroids. Clinical anti-inflammatory agents, in order of their importance, are the corticosteroids, salicylates, phenylbutazone-antipyrine type, gold salts, antimalarial aminoquinoline compounds and enzymes. This review shall only discuss the corticosteroids since mechanistic data is sadly lacking in all the others. The corticosteroids represent the most successful anti-inflammatory drugs yet found. There are few physiologic processes which are not influenced directly or indirectly by the corticosteroids. Yet they do not appear to start any physiologic activity, but merely influence rates. Apparently, corticosteroids are not consumed in the process of exerting their physiologic effects, but rather they act as catalysts.

Corticoids tend to suppress the whole process of inflammation, inhibiting to various degrees the maximum development of any of the stages of inflammation (76). Unlike other inhibitors of inflammation they can check the inflammatory response at any stage from the initial swelling and increased capillary permeability to final dissolution of connective tissue (77). Hydrocortisone acts in a dose-response fashion, inhibiting to various degrees the maximum development of any of the stages of inflammation, depending on the nature and amount of the inflammatory stimulus (78–80).

The processes of regeneration and growth depend on well regulated processes in connective tissues. The mast cells and fibroblasts are the major active components. These processes of regeneration and growth are inhibited by the glucocorticoids. Mast cells diminish, become vacuolated and acquire irregular outlines. The number of demonstrable cells is diminished (81). The most consistent effect of the steroids appears to be an inhibition of mucopolysaccharide synthesis by the connective tissue. At a metabolic level, several investigators have demonstrated an inhibition of the incorporation of radioactive sulfate into the mucopolysaccharides of

connective tissue under the influence of cortisone or hydrocortisone (82–85). Inhibition of mucopolysaccharide synthesis by steroids was also demonstrated, following depletion of cartilage matrix by papain (85). When animals are intravenously injected with crystalline papain protease, all of the basophilic and metachromatic components of cartilage disappear within a few hours. This is associated with a loss of chondroitin sulfate from the cartilage and its appearance in the circulating blood. Reconstitution of cartilage matrix begins two days after the injection of papain, and chondromucoprotein is completely restored within the next three or four days. When cortisone is administered after papain, reconstitution of matrix is completely prevented. This inhibition seems to involve a direct action of cortisone on the cartilage. This inhibition of chondroitin sulfuric acid synthesis has been demonstrated in delaying the healing of wounds (86).

Cortisone inhibits the acccumulation of liberated histamine in the connective tissue (87). The steroid is a very effective inhibitor of the mast cell-histamine chain. Cortisone appears to maintain the tonus of small arterioles which are injured by histamine (10) and decreases permeability of existing capillaries and interstitial substances, possibly by inhibiting the action of hyaluronidases (10, 33), of histidine decarboxylase (88), and of histaminase (89).

Early in the process of inflammation, degenerative changes occur in the fibroblasts. The administration of hydrocortisone also produces morphological changes in many of the fibroblasts of loose connective tissue (90). Fibroblasts are among the first cells showing degeneration when connective tissue becomes inflamed. It appears that one essential action of the corticosteroids is the inhibition of the progressive destruction of fibroblasts in an area of potential inflammation (77, 91–93). The fibroblast appears to be the most common and dominating cell in connective tissue which sequesters and metabolizes hydrocortisone (94). It has been suggested (77, 95) that the degree of inflammation is enhanced by the autocatalytic destruction of fibroblasts, in which the destruction of one cell adds to the amount of inflammatory substances such as histamine or a kinin and leads to the destruction of other inflammatory substances. This chain reaction of cell breakage is interrupted by the action of hydrocortisone, which increases the resistance of some fibroblasts. While different research groups may differ upon the importance of steroidal effects on fibroblasts, it is clear that hydrocortisone is metabolized by fibroblasts. Even if a sufficient amount of hydrocortisone is produced by the adrenal cortex in individuals having inflamed tissue, if the hormone is inactivated at the fibroblastic site of action more rapidly than under usual normal conditions, anti-inflammatory influence could be insufficient to inhibit the inflammation (96). According to this theory, the peripheral cells may influence the disease process by their own capacities to metabolize the

steroid. Thus, the fundamental defects in chronic inflammatory diseases may not be at the hormone supply level but may be due to altered hormone metabolism at the fibroblastic level. Perhaps it is through the preservation of cellular integrity that hydrocortisone tends to minimize the subsequent phases of the inflammatory reaction.

The adrenal steroid actions contrast markedly with the effect of the estrogenic sex hormones. The sex hormones have been shown to increase the amount of intracellular water in the skin of mice, probably by increasing the amount of ground substance, while the corticoids appear to have an opposite effect (97). Hydrocortisone ointment causes progressive atrophy of collagen fibers, disappearance of interfibrillar mucopolysaccharides, dissociation of elastic fibers and atrophy of fibroblasts (98).

A most interesting observation has been made that corticosteroids can chelate potassium ions and are capable of binding copper (99). Cortisone causes a redistribution of copper in the body, with an increased renal and urinary concentration and a decreased concentration in other tissues and in serum. It was suggested that the anti-inflammatory effects of the steroids may occur because of the chelation of an essential metal activator of an undefined enzyme (99). A recent study (100) suggests that the ability to form complexes or chelates in or across a lipid phase is of anti-inflammatory importance. The potency of a number of anti-inflammatory drugs could be correlated with at least two physical properties: ability to form complexes with metal ions and the lipophilic character, favoring partition into the lipid rather than aqueous phase. As an index of potency, the investigator used the ability of the compounds to inhibit incorporation of inorganic sulfate into cartilage and corneal polysaccharides.

It has been well demonstrated that administration of cortisone will be followed by a diminished amount of circulating antibody (101). Cortisone inhibits the incorporation of labeled amino acids into tissue protein (102). Under most circumstances, it appears that cortisone has a distinct antianabolic effect on proteins, generally inhibiting the synthesis of protein. On a cellular level, corticosteroids inhibit the inflammation, which can readily be observed during the course of immunization with adjuvants such as alum, killed tubercle bacilli, and various vehicles and irritants (77). This may also be regarded as a kind of anti-inflammatory action of cortisone which results in inhibition of antibody synthesis.

While the corticosteroids are used principally for their anti-inflammatory effects, the mass of diverse effects cause additional actions which have come to be regarded as "side effects." While this paper is concerned chiefly with mechanisms, it will be concluded with a brief review of the broad side effects resulting from excess levels of corticosteroids.

Hydrocortisone indirectly controls adrenocortical secretion by restraining the secretion of adrenocorticotropic hormone (ACTH) by the pituitary.

It is becoming increasingly evident that the pituitary secretes ACTH in response to influences reaching it from the central nervous system. Hydrocortisone may suppress ACTH secretion by altering the rate at which corticotropin-releasing factors are elaborated by the central nervous system or by diminishing the responsiveness of the adenohypophysis to corticotropin-releasing factors. Whatever the mechanism, the higher the level of hydrocortisone, the greater is the restraint on ACTH secretion. Thus, when supraphysiologic doses of hydrocortisone are used in the treatment of inflammatory conditions, ACTH secretion is suppressed, and this leads to cessation of adrenocortical secretory activity, diminished responsiveness to exogenous ACTH, and progressive atrophy of the adrenal cortex. These changes in adrenocortical function are generally reversible if exogenous ACTH is administered or if hydrocortisone administration is discontinued, permitting recovery of endogenous ACTH secretion (103).

There are so many other diverse manifestations of supraphysiologic levels of hydrocortisone that they cannot all be treated here. It should be mentioned that the corticoids affect protein metabolism in a variety of ways. Which of the effects will ultimately come to be regarded as primary and which secondary cannot be judged with certainty. There is both an anabolic effect by uptake of amino acids by the liver (104) and catabolic interference with cellular uptake of amino acids (105). As a consequence of these actions, hydrocortisone causes clinical manifestations of protein wasting.

Corticosteroids, in excess dosage, promote the deposition of adipose tissue in the facial, abdominal and shoulder areas, as well as promoting sodium retention and potassium excretion by stimulating cation exchange by the renal tubule. Opposing this effect is the tendency of hydrocortisone to increase glomerular filtration rate which promotes sodium excretion.

(Received September 9, 1962)

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