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Biology of Inflammation

Chemical Mediators and Cellular Injury

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Inflammation is a manifestation in vertebrates of severe cellular injury, and as such it represents the basis of all infectious processes. It can readily be defined as the complex vascular, lymphatic, and tissue response on the part of vertebrate tissue to the presence of an irritant. An irritant may be considered to be any agent that interrupts normal cellular metabolism. It may be physical in nature, or chemical, or viable, as in the case of microorganisms.

Inflammation consists of a number of interdependent sequences that lead to the localization and ultimate disposal of the irritant. These phases are often grouped under the somewhat loose term *inflammatory reaction*. Initially, there is a disturbance in fluid exchange, which is manifested by an alteration in the normal equilibrium of capillary filtration. There is at first, as shown by Landis, a transitory increase in capillary pressure (*1*), and this is followed by more permanent changes in the structure of the endothelial wall structure—namely, augmentation in its permeability (*2*). For instance, the enhancement in permeability can be readily demonstrated by introducing trypan blue into the circulating blood of a rabbit. The area of injury is rapidly stained, owing to the outward passage of the dye (*3*). The magnitude of increase in permeability is such as to allow particulate material to pass into the extracapillary spaces (*4*). The extent of increased capillary permeability is subject to measurement. In 1930 it was shown that, when a dye is introduced into the ventricle of a pithed frog with its mesenteric capillaries exposed on the stage of a microscope, one can evaluate with an appropriate scale the rate of change of concentration of the dye as it passes out into the extracapillary spaces. It was then found that with inflammation caused by certain irritants, the permeability of capillaries is enhanced about twofold (*3*).

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Leukotaxine and the Mechanism of Increased Capillary Permeability

The mechanism of increased capillary permeability in inflammation is an important factor, for it is the pivotal reaction on which all subsequent sequences depend. In 1924, Lewis, stimulated by the work of Ebbecke, postulated that the phenomenon is referable to the liberation of histamine or at least to a substance so closely allied to it that he termed it the "H-substance" (*5*). The argument utilized by Lewis is fraught with difficulty, for it is largely one of analogy. For this reason, I reinvestigated the whole problem in 1936 (*6*). It was readily shown that the exudative fluid recovered from the site of inflammation contains a factor that is capable of eliciting an increase in capillary permeability. Subsequently, chemical extraction was undertaken and a crystallinelike material was isolated. This substance is capable of increasing the permeability of capillaries with inflammation. It has been called leukotaxine (*7*).

Cullumbine and Rydon, Pasquali, and Morimoto have confirmed the isolation of this chemical substance (*8–10*). It is evidently not a protein. It has an α-amino group and an indole nucleus. There is evidence that it is a polypeptide to which some as yet unknown prosthetic group may be attached. The substance today, even though it is not chemically pure, appears definitely to be a chemical entity (*11*). It has marked biological specificity (*11*). Chromatographic studies, undertaken by my associate W. Kalnins, indicate the presence of at least five distinct amino acids (leucine, valine, α-alanine, glycine, and glutathione). Aspartic acid and glutamic acid appear as a fused unit in the chromatogram (*11*).

It has none of the properties of histamine, thereby throwing considerable doubt on the view of Lewis that the H-substance is primarily concerned in the mechanism of increased capillary permeability in inflammation (*5*).

The increase in capillary permeability induced by leukotaxine has been found to be counteracted by only one substance—namely, the extract of the adrenal cortex—and to be counteracted to a certain extent by some of the compounds derived from the adrenal cortex —namely, cortisone and hydrocortisone. This phase will be discussed subsequently.

Leukotaxine is concerned with another fundamental manifestation in inflammation—namely, the migration of polymorphonuclear leukocytes to the site of injury. This is referred to as "chemotaxis *in vivo*" (*11, 12*). Leukotaxine does not as yet explain the exact intrinsic cellular mechanism concerned in the motion of leukocytes, but it at least stands out as a concrete substance, liberated in exudates, that is definitely chemotactic. This fact in itself offers a distinct possibility for attacking the problem further in an endeavor to determine how leukotaxine specifically affects the mobility of white cells so as to induce their migration to the site of inflammation. Whether electric charges have anything to do with the phenomenon remains to be proved. The end product of blood serum extracted for leukotaxine yields no activity as far as cell migration is concerned. On the other hand, leukotaxine derived from exudates is definitely chemotactic, for its injection into tissues induces (within 13 to 14 minutes with some fractions) not only a marked increase in capillary permeability but also a distinct migration of polymorphonuclear cells outward into the area of injury (Figs. 1 and 2). The

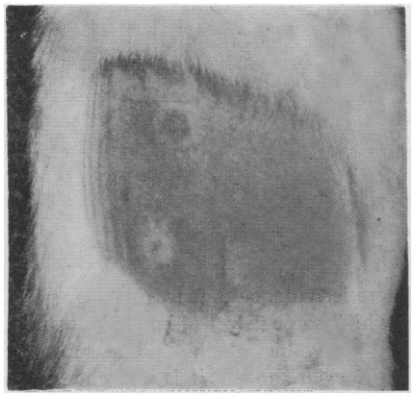

Fig. 1. Effect of leukotaxine extracted from an exudate (with the utilization of 1*N* acetic acid in the final analysis) on capillary permeability in the dermis of the abdomen of a rabbit. Six milliliters of 1-percent trypan blue in saline was injected intravenously. Note the accumulation of the dye within 25 minutes in the upper area owing to the increase in capillary permeability caused by leukotaxine. No such effect is elicited by the mere injection of saline (lower area).

chemotactic property of leukotaxine can be easily demonstrated by *in vitro* observations. The placing of leukotaxine on a slide containing a drop of exudate is soon followed by orientation, migration, and clustering of cells at the periphery of particles of leukotaxine. No such effect is observed with either carbon particles or reduced iron powder (*4, 11, 12*).

In conclusion, leukotaxine extracted from inflammatory exudates offers a reasonable explanation for two of the basic sequences in inflammation—namely, the primary mechanism of increased capillary permeability and the local migration of polymorphonuclear leukocytes. Besides these two functions, leukotaxine appears to be relatively innocuous as far as the induction of any appreciable degree of tissue injury is concerned.

Role of Inflammation in Immunity

Concomitantly with the increase in capillary permeability, induced primarily by leukotaxine, which is released in turn by injured cells at the site of an acute inflammation, there follows the rapid passage from the circulation of the plasma proteins into the area of injury. These are in order of magnitude: the albumin, globulins, and fibrinogen. In the presence of thrombokinase, the latter is precipitated out as a fibrinous network in tissues, now distended with edema. The lymphatic channels, which drain the particular area, are apparently more delicate in structure than capillaries and are therefore more readily damaged. They become filled with fibrinous plugs or thrombi. A true lymphatic blockade ensues. Material injected into such a "walled off" or circumscribed area is incapable of escaping through the lymphatics. Such material is mechanically fixed at the site of the acute inflammation owing to a blockage of lymphatics and the presence of a fibrinous network (*4*).

These observations have been extended to the problem of bacterial invasiveness. The rapidity and intensity through which lymphatic blockade is established in an inflamed area serve as a gage of the invasiveness of a microorganism—that is, if such a viable vector happens to be the irritant. Microorganisms disperse from their point of inoculation via lymphatics. In this way it was shown that staphylococci are localizing microorganisms largely because of their marked injurious effect on tissue at their point of entry. There then results the rapid establishment of a lymphatic blockade. On the contrary, the hemolytic streptococci produce a relatively mild local reaction. The lymphatics are maintained patent for as long as 2 days, thus allowing the microorganisms to disseminate freely.

One is dealing with a paradox. The streptococci are feared microbes, whereas the reverse is usually held for the ubiquitous staphylococci. This is primarily due to the potent lethal effect and the prompt establishment of a lymphatic blockade by the staphylococci at their point of entry. The streptococci, on the other hand, are harmful to the organism as a whole owing to their unhindered dispersive capacity through patent lymphatics (*13*). In this way it is seen that, from the standpoint of immunity, an acute inflammation is the regulator of bacterial invasiveness (*14*). One can formulate this concept as follows: $D = Kt/I$, where D refers to dissemination from the point of entry, I is the degree of induced local injury and t refers to time; K may be considered a constant that refers to the type of irritant and the anatomical location of the lesion. In other words, the invasiveness of a microorganism is to a large extent inversely related to the intensity of the local injury it induces (*4, 14*).

The fixation or localization described, which is referable to lymphatic blockade, is also an important principle. With some irritants, the "walled off" process occurs within 30 minutes after the injection of an irritant. This rapidity allows a definite interval for the irritant to be circumscribed and for the sluggish leukocytes to assemble for phagocytosis (*4, 15*).

Finally, the capillaries eventually likewise become occluded by thrombi. The inflamed area then becomes, so to speak, isolated from the rest of the organisms. It develops its own *p*H, metabolism, and local circulation. Prior to this type of local insulation, in which one may envisage that the organism is protected at the expense of local injury, various inert materials or bacteria that have been intravascularly injected concentrate in the area of acute inflammation because of the initial increased local capillary permeability and the early simultaneous establishment (with some types of irritants) of a lymphatic blockade (*4*).

The foregoing immunological view is of the utmost importance in our understanding of inflammation in its role in immunity. This, in the last analysis, is the most important aspect of inflammation in body organization.

Mechanism of Leukocytosis with Inflammation

The leukocytosis-promoting factor is another potent biologic substance that was isolated in 1940 from the site of inflammation (*16*). Leukotaxine introduced into the circulating blood fails to alter the number of white cells. Yet it is well known that in numerous inflammatory processes the level of circulating leukocytes is considerably elevated. The leukocytosis has been shown to be referable to chemical units. The injection of whole exudative material derived from a

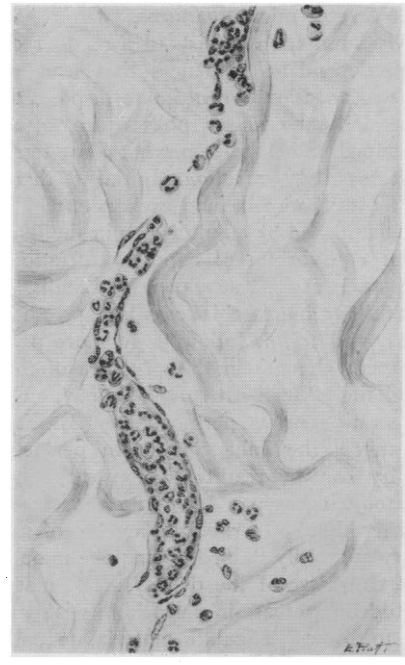

Fig. 2. Forty-four minutes after the introduction of leukotaxine into the cutaneous tissue of a rabbit. The material injected was crystallinelike in nature. It also induced a markedly increased capillary permeability. Note that the capillary lumen is crowded with polymorphonuclears actively migrating into the extracapillary spaces. (About × 350.)

dog with a concomitant leukocytosis into the blood stream of a recipient dog induces a prompt leukocytic response. Neither leukotaxine nor blood serum is capable of eliciting such a response. The evidence indicates that there is a leukocytosis-promoting factor present in inflammatory exudates. This factor, in contrast to leukotaxine, is nondiffusible through a cellophane membrane and it is destroyed by heat at 60°C. Its injection is accompanied by a discharge of immature leukocytes into the circulation. Chemical extraction of exudates yields activity in the pseudoglobulin fraction (*17*). Cataphoretic studies with a Tiselius apparatus indicate that the leukocytosis-promoting factor (LPF)is distributed between the $alpha_1$ and $alpha_2$ globulins of exudates (*18*). Other protein fractions, such as the albumin and the euglobulin, are ineffective in inducing a state of leukocytosis. The material is absent in normal blood serum, but it can be recovered from the serum of an animal with a concomitant inflammation (*19*). This fact suggests that it reaches the bone marrow by way of the blood stream. The material can be obtained from the exudates of dogs, of rabbits, and of human beings (*20*).

The leukocytosis-promoting factor not only discharges immature granulocytes into the circulation but also induces a marked and specific growth in the bone marrow (*21*). The result is a conspicuous hyperplasia of some of the hematopoietic cells, notably the polymorphonuclear leukocytes and the megakaryocytes (Fig. 3).

In brief, the leukocytosis-promoting factor of exudates offers a reasonable explanation for the mechanism of leukocytosis that accompanies numerous inflammatory processes. Its marked growth effect on some of the elements of the bone marrow may prove to have clinical implications. In fact, it has been shown that the canine material can be injected innocuously into human beings, in whom it likewise increases the white counts (*22*). The active group in the pseudoglobulin molecule has been shown to be due to a polypeptide (*23*). The factor also enhances the ordinary leukocytosis caused by an inflammation. This suggests the possible use of this substance on patients with various inflammatory disorders (*24*). There is another thermostable leukocytosis-promoting factor liberated in acid exudates that will be discussed briefly in a subsequent paragraph.

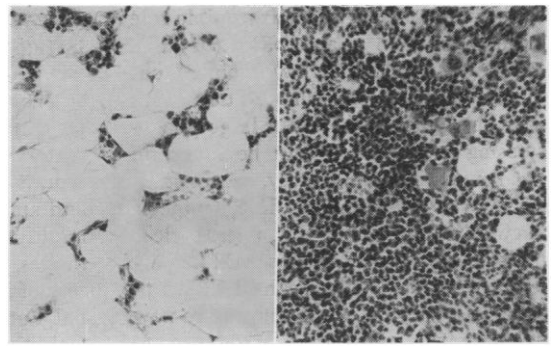

Fig. 3. (Left) Femoral bone marrow of a dog 2 days following an injection of 14.5 milligrams of pseudoglobulin derived from normal blood serum. There is clearly no evidence of any hyperactivity. (Right) Femoral bone marrow of a dog 2 days following a single injection of 13.5 milligrams of the leukocytosis-promoting factor. The hyperplasia is striking. (× 165) [From V. Menkin, *Am. J. Pathol.* 19, 1021 (1943)]

Cytologic Sequence in Inflammation

Another fundamental sequence in the development of the inflammatory reaction is the initial infiltration of polymorphonuclear leukocytes, which are subsequently replaced by macrophages. This is an almost invariable cytologic sequence. With some irritants, the polymorphonuclear phase may be lengthened and the macrophage phase correspondingly shortened—for example, a staphylococcal abscess, or, vice versa, a tubercle. Nevertheless, the same basic principle is maintained. In 1934 it was observed that this phenomenon is referable to a developing local acidosis at the site of an acute inflammation (*25*). It was demonstrated in 1931 that the local circulation in an inflamed area is markedly impaired. The lymphatic outlets become occluded (*26*), and in time thrombi are also found in the smaller vascular channels.

Thus, as stated in a preceding section, the area of inflammation becomes more or less isolated from the rest of the organism. It has already been pointed out that it develops its own hydrogen-ion concentration, its own circulation, and its own metabolism. The polymorphonuclear leukocytes brought to the site of injury primarily through the action of leukotaxine, as described, are unable to survive below a *p*H of 7.0, whereas the mononuclear phagocytes appear fairly normal at *p*H 6.9 to 6.8. At a somewhat lower *p*H all types of leukocytes tend to be injured. The result is a state of suppuration. Pus in acute inflammation is virtually a function of the hydrogen-ion concentration (*4*). For instance, a 24-hour exudate from the pleural cavity of a dog that has previously been injected with turpentine is primarily composed of polymorphonuclear leukocytes, whereas an exudate of several days' duration at *p*H 6.5 reveals an absence of normal polymorphonuclear cells. The rise in hydrogen-ion concentration tends to precede the change in the differential leukocyte picture (*25*).

On the other hand, if the reaction remains alkaline during the development of the inflammatory reaction, the cellular picture is characterized throughout by a predominance of polymorphonuclear leukocytes. That the general effect is an inability on the part of polymorphonuclear leukocytes to survive at an acid reaction has been demonstrated by subjecting leukocytes *in vitro* to buffers at various hydrogen-ion concentrations (*27*). In this way, it was readily shown that these cells tend to cluster and become swollen with coarse granulation at about *p*H 6.5. When they are supravitally stained, the nuclei take up the dye, indicating severe injury. These changes are absent in cells exposed to *p*H 7.4. It should be mentioned that this relationship does not apply in rabbits (*28, 29*). This may be referable to the absence or reduction of proteolytic enzymes in the leukocytes of this animal (*28, 30*).

It is important to determine the mechanisms of local acidosis in inflammation if one is to comprehend the forces at work in forming the cytologic picture at the site of an acute inflammation. The alkali reserve of the exudate tends to decrease with the developing local acidosis (*28*). This in itself might be expected to occur, for it is another means of expressing the rise in hydrogen-ion concentration. Incidentally, it is well to emphasize again that all the biochemical changes are local: the reaction of the blood remains essentially unaltered.

The local acidosis seemed to be primarily referable to a glycolytic process (*28*). The result was a true lactic acid acidosis. Several chemical variables had been studied in the exudate during the progress of the inflammatory reaction. These included the concentration of sugar and of lactic acid and the measurement of the *p*H, correlated in turn with the cellular picture. The conversion of glucose to lactic acid induced a local acidosis, with the resulting effect on the leukocytes that has been described.

In conclusion, the cellular picture or the survival of leukocytes in inflammation is primarily conditioned by the hydrogen-ion concentration, which in turn seems to be referable to a disturbance in the intermediary carbohydrate metabolism. The lymphocytes appear to be unaffected, at least directly, by the changes in hydrogen-ion concentration. The function of the latter cells is still somewhat obscure. The studies of McMaster and Hudack and the more recent

work of Harris suggest their possible role in the formation of antibodies (*31, 32*). Maximow and Bloom have pointed out that in inflammatory exudates transitions from the lymphocytes to the large phagocytosing macrophages can be found in great number (*33*). Finally, recent studies *in vitro* indicate that leukotaxine is likewise chemotactic to mononuclear phagocytes (*11*). This phase will be particularly significant if it can be shown to occur also *in vivo*. Such studies are now in progress.

Diabetes and Inflammation

The biochemical studies on the relationship of the hydrogen-ion concentration to the cellular picture at the site of an acute inflammation have led to further extensive investigation in an attempt to unravel the mechanism concerned in the intensifying of the diabetic state when there is a superimposed inflammation in diabetic dogs (*34*). An inflamed area is a focus of proteolysis. At the site of inflammation in a diabetic animal, the breakdown of proteins is considerably exaggerated.

Local sugar formation from the split part of the protein molecule by deamination readily explains the heightening in blood sugar following the diffusion of sugar previously formed at the site of injury. Close analysis indicates that, in general, even in nondiabetic animals, sugar can be formed by injured cells. Such cells are therefore potentially foci of gluconeogenesis (*35*). The process is merely exaggerated in the diabetic animal, and furthermore, there is an absence of insulin. There is also some suggestive evidence that urea, besides originating in the liver, is possibly formed at the site of an acute inflammation (*35*).

Studies performed in 1946 indicate that there is another toxic substance liberated at the site of injury, termed "necrosin," which by itself is also capable of heightening somewhat the level of blood glucose, presumably by releasing the latter from the liver (*36*). Thus, the state of diabetes complicated by inflammation appears to be reinforced by the formation of glucose locally from the injured cells and also perhaps by the release of sugar from the liver through the effect of necrosin (*36*). The aspect of carbohydrate metabolism and its possible relationship to inflammation has been treated critically and at greater length elsewhere (*12*). The possible additional participation in the mechanism concerned of various endocrine structures, such as the adrenal cortex, the pituitary, or the thyroid, has been considered and, therefore, requires further study (*12, 34*).

Chemical Basis of Injury in Inflammation

The fundamental stereopattern of injury in the development of the inflammatory reaction, irrespective of the irritant, is of great importance. The irritant and anatomic location of the lesion may modify the ultimate appearance. Nevertheless, close scrutiny reveals a basic pattern of injury, as manifested by the cardinal signs of inflammation described by Celsus at the beginning of our era. In addition, damaged cells may show granular elements strewn through their cytoplasm; the cells may be vacuolated; the collagenous bundles may appear to be swollen; and the nuclei may reveal various degrees of shrinkage. Another evidence of tissue injury found in a variety of acute inflammatory lesions is displayed by the fibrinous occlusion of lymphatics and also by the presence of small thrombi in the blood vessels of an inflamed area.

It may be asked whether the mechanism that accounts for the basic pattern of injury in inflammation is referable to a chemical unit. Leukotaxine scarcely induces any degree of injury to cells except for an augmentation of cellular permeability. The leukocytosis-promoting factor elicits essentially no injury to tissues. Studies in 1943, however, have indicated that the euglobulin fraction of exudates is highly injurious to the cutaneous structures of the rabbit and to some extent to those of the dog (*37*). There is thus an accumulated body of evidence that, either in the euglobulin fraction of particularly acid exudates or else associated with it, there is present an injury factor that is capable per se of reproducing the nocuous effects seen in inflammation. This substance or factor has been termed "necrosin" (*37*). Its injection into the skin of rabbits is accompanied by intense redness, edema, and usually a central area of necrosis (Fig. 4). No other fractions of exudate appear to be capable of inducing any such severe effect. Furthermore, the euglobulin fraction of normal blood serum elicits absolutely no appreciable lesion in the skin of rabbits. On the other hand, the same fraction from the serum of a dog with a concomitant acute pleural inflammation may induce a severe inflammation. This observation suggests that the toxic substance is absorbed from the site of inflammation into the circulation, from which it can be recovered. This fact is perhaps of significance in reevaluating the concept of a focus of infection and its far-reaching repercussions on other organs.

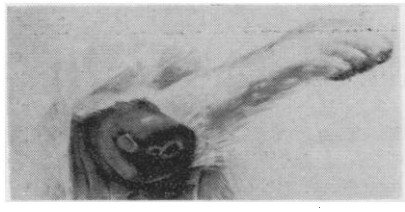

Fig. 4. Effect of necrosin injected into the skin of the foreleg of a rabbit. The acute inflammatory reaction with areas of central necrosis is about 1 day old. The necrosin was obtained from a dog's exudate. [From V. Menkin, *Arch. Pathol.* **36**, 269 (1943)]

Necrosin induces an acute inflammation with blockade of the lymphatics. A section through such an area reveals zones of dense leukocytic infiltration in which the lymphatic channels may be occluded by thrombi. Small vascular channels may also show evidence of clot formation. On the other hand, the euglobulin fraction of normal serum leaves the lymphatic channels essentially patent. About 10 minutes after the injection of necrosin into the skin of rabbits, the connective tissue bundles occasionally appear to be swollen, with a tendency to fuse together. The latter seems to be one of the first morphologic evidences of the injurious effect of necrosin on supporting structures (*37*).

As stated previously, necrosin can at times be recovered from the blood serum of an animal with an acute inflammation. This interesting observation, which may reasonably explain the toxic effect of a lesion on organs at a distance, has suggested the necessity for examining the generalized effect on organs following an intravascular injection of necrosin. Although several organs, such as those in the gastrointestinal tract, may show evidence of injury, or various lymphoid structures may reveal signs of enlargement and congestion, it is nevertheless only the liver and to some extent the kidneys that seem most frequently to show signs of damage. Many of the cells of the liver appear swollen and are laden with fat. The liver on gross examination is streaked with what seems to be yellowish or whitish foci. In some cases, the cell outlines have disappeared and the liver cords are stippled with coarse, black-stained granules that do not take the iron stain. The epithelial lining of the kidney tubules may show irregularities and vacuolation, and foci of leukocytic infiltration, separating some of the tubular structures, may also be present (*22, 37*). Perhaps it should be pointed out that the various injurious biological effects obtained by ionizing radiation may well prove to be referable to the liberation of necrosin, as well as to some of the other factors, by cells that are injured, in this case, by a physical irritant.

There are two additional properties of the euglobulin fraction of acid exudates that indicate its significance to the

student of pathology. The intravascular injection of this fraction into a dog may be accompanied by severe toxic manifestations, including vomiting and diarrhea. For a while the animal lies on its side and appears somewhat prostrated. Of greater significance is the abrupt fall in the number of circulating leukocytes, which is usually extremely marked. The white cell count may fall in an hour or two from about 10,000 to 1000 or 2000 per cubic millimeter. After several hours the dog appears to be clinically improved, and the white cell count rises. Subsequently, or on the following day, a leukocytosis may ensue. This leukocytosis, associated with the euglobulin of particularly acid exudates, has been shown to be referable to a thermostable leukocytosis-promoting factor. The mechanism of leukocytosis with inflammation is thus ascribed to at least two factors that are liberated by injured cells: (i) the thermolabile leukocytosis-promoting factor, (LPF), and (ii) a thermostable leukocytosis factor (*38, 39*).

On the other hand, as just pointed out, the depression in the number of circulating leukocytes or the state of leukopenia is also referable to two factors: (i) a leukopenic factor associated with the euglobulin of acid exudates (*40*) and (ii) leukopenin recovered in the pseudoglobulin fraction (*41*). Leukopenin is recovered in abundance from aged LPF (*41*) and it appears to be present in alkaline exudates (*41*).

It is difficult to predict accurately the number of circulating leukocytes when there is a concomitant acute inflammation. This indeterminate status is due to the fact, as just pointed out, that there are four factors involved. Two factors cause an elevation in the level of circulating leukocytes (LPF and the thermostable leukocytosis factor), and two factors induce a decrease in the level of white cells (leukopenin and the leukopenic factor). The ultimate level of leukocytes in the blood stream is a resultant of the interrelationship of these four factors (*12, 42*).

Of perhaps even greater interest is the fact that the intravascular injection of the euglobulin fraction of acid exudates in dogs is often accompanied by a marked rise in temperature simultaneously with the developing leukopenia, possibly of 3° to 5°F. The studies of Grafe in 1910 suggested that in infectious processes the fever is referable to the absorption of products of cellular disintegration. Other protein fractions of exudate—for example, the leukocytosis-promoting factor or the euglobulin fraction of normal serum —are incapable of inducing any fever when they are injected into the circulating blood of a dog. The fact that the euglobulin fraction of acid exudates seems to be the only fraction that has this capacity suggests strongly its possible role in the production of fever with inflammation (*37*).

In 1945 I succeeded in dissociating from the euglobulin fraction of exudates the fever-causing factor (*43*). I called this fever-inducing substance "pyrexin." Pyrexin seems to give a reasonable explanation for the development of fever with inflammation. It seems to be a protein split product, a polypeptide, to which there seems to be attached a carbohydrate group. It is quite possible that the latter group is associated with the thermostable leukocytosis factor; but this requires further study (*38, 39, 42*). Pyrexin was brought to the crystalline state in 1952 (*44*). There is no factual or experimental evidence that pyrexin is a contaminant by bacterial pyrogens. On the contrary, all available evidence indicates that pyrexin is a direct product of cellular injury, which is recovered particularly in acid exudates (*12, 45, 46*).

Anti-inflammatory Problem

In 1940 a report was made on the suppressing effect of whole adrenal cortical extract on the increased capillary permeability caused either by whole exudate or by leukotaxine (*47*). In 1942 the same repressing effect was found with cortisone (compound E) (*48*). These were the first observations on the anti-inflammatory capacity of cortisone.

Following the studies of Kendall, Hench, and their collaborators on the effect of cortisone on arthritis, these studies were resumed in 1951 (*49–51*). The earlier observations were confirmed. However, an interesting difference was also found. Whereas an alkaline exudate or its contained leukotaxine was repressed by whole adrenal cortical extract or cortisone, an acid exudate was incapable of being repressed by either adrenal cortical extract or by cortisone. As a consequence, it was found that at the stage of development when the inflammatory reaction is at an acid level (but independently of the *p*H of the exudate) another chemical factor appears to be liberated by the injured cells. This substance, like leukotaxine, is also capable of increasing capillary permeability, but only in the later stages of inflammation. This factor has been termed "exudin" (*50*). In contrast to leukotaxine, which increases capillary permeability in the earlier stages and induces the emigration of polymorphonuclear leukocytes to the site of inflammation, exudin does not cause any appreciable migration of the cells through the endothelial wall of the capillaries. Exudin contains about 10 percent nitrogen. It can be obtained after first discarding the euglobulin fraction of acid exudates and then following with repeated reprecipitation of the pseudoglobulin-albumin fraction at one-third saturation of $(NH_4)_2SO_4$. Leukotaxine is suppressed by either adrenal cortical extract, cortisone, or hydrocortisone (compound F). On the other hand, none of these corticosteroids inhibit exudin. But ACTH or corticotropin suppresses exudin (*51*). There are thus two components throughout the duration of an acute inflammation which increase capillary permeability, namely leukotaxine and exudin. These two components can be distinguished from each other by their differing reactions with some anti-inflammatory agents. These facts can be briefly summarized as shown in Table 1.

Table 1. Reactions of leukotaxine and exudin and some anti-inflammatory agents: 0 indicates suppression of capillary permeability to trypan blue; + indicates no effect on the increased capillary permeability to trypan blue.

Component	Adrenal cortical extract	Cortisone or Cpd. F	ACTH
Leukotaxine	0	0	+
Exudin	+	+	0

The effect of ACTH or corticotropin in repressing exudin is a direct one, for the same response can be elicited in adrenalectomized rats (*52*). This observation does not controvert the classical physiological effect of ACTH on the adrenal cortex in releasing hydrocortisone or compound F, but the local inflammatory reaction can be envisaged as a sort of sieve with a very extensive increase in local capillary permeability. As a consequence, the injection of ACTH into the circulation of the animal induces a certain amount of seepage of this substance into the inflamed focus, with subsequent direct action on the exudin that is liberated in turn by the injured cells. Recent and as yet unpublished observations with pure ACTH in the form of α-corticotropin, obtained through the kindness of Choh Hao Li, corroborate further the direct effect of ACTH with now this single chemical entity. α-Corticotropin represses in an inflamed area the increased capillary permeability referable to either an acid exudate or exudin. This study tends to obviate the possibility of an impurity in the commercial ACTH to explain the earlier results.

The mechanism involved has also been studied. The mechanism of an anti-inflammatory steroid, namely hydrocortisone or compound F, seems to be at a cellular level. Cell activity is suppressed. In the sea urchin ova, as an isolated sys-

tem, cell division, following fertilization, is utilized as a criterion of cellular activity (*53*). It is shown that cortisone, compound F, and ACTH reduce the incidence of cell division, whereas neither testosterone propionate as a control for the steroids nor somatrofin (STH) as a control for another anterior pituitary hormone has any effect in altering the pattern of cell division (*53*). More recent studies (in 1954) reinforce further the view of suppressed cell activity, for it has been shown that cortisone and ACTH significantly retard the rate of cleavage in sea urchin ova (*Arbacia punctulata*) (*54*). It has also been demonstrated that the permeability to water of these isolated marine eggs is definitely reduced by an average of 34 percent after preliminary exposure to cortisone (*55*).

These findings of suppression of normal cell activity have been transferred to a study of the mechanism of an anti-inflammatory steroid in the dog (*56*). The activity of the injured cell at the site of an acute inflammation is likewise suppressed. The formation of the chemical factors responsible for the inflammatory reaction appears to be inhibited. Repeated injections of compound F (hydrocortisone) into an inflamed area suppresses the activity of leukotaxine and the leukocytosis-promoting factor. In the case of the latter substance, at least, it is shown by *in vitro* studies that the corticosteroid does not interact directly with the LPF, but that it evidently suppresses its formation by the injured cell. Therefore, here likewise the mechanism seems to be at a cellular level. Compound F (hydrocortisone) is an anti-inflammatory substance because it represses the injured cell from forming the very chemical substances concerned in the development of inflammation (*11, 56*).

The anti-inflammatory corticosteroids are actually harmful and therefore contraindicated in numerous infectious lesions—for example, tuberculosis and poliomyelitis. It seems that cortisone, by doing away with the chemical factors liberated in an inflamed area, allows free rein for microorganisms to proliferate and thus accentuates the pathological lesions caused by the microbes. It is probable that the anti-inflammatory agents should primarily be employed in noninfectious forms of inflammation. They are also of value in various eye infections, which, if not interfered with, may be complicated eventually by corneal opacities (*57*).

Homeostasis in Inflammation

Homeostasis, as is well known, is a term coined by Walter B. Cannon in 1929 that indicates that there are in the physiological organism factors that tend to maintain the constancy of the internal environment (*58*). For instance, temperature, *p*H, or blood volume tend under physiological circumstances to maintain definite constancy. This view is essentially an extension of the concept evolved by Claude Bernard, namely, that there tends to exist a "milieu interne" in the body organization.

In a dynamic pathological process, such as that encountered in a local inflammation with the progress from an initial alkalinity to a frank acidity, there is the production by injured cells of a number of chemical factors. The common denominators liberated in the exudate seem responsible for the diversified biological manifestations of inflammation. Despite the changes in *p*H level at the site of an acute inflammation, there are produced various chemical factors having, nevertheless, the same biological properties—that is, regardless of whether a state of local alkalinity or local acidosis develops. In this way, a systemic homeostatic state is maintained throughout the duration of the inflammatory reaction (*50, 59*). The concept can be clarified further by listing the following examples.

1) Leukocytosis. In the initial alkaline stage of inflammation, the elevation in the number of circulating leukocytes is referable to the thermolabile leukocytosis-promoting factor (LPF). In the subsequent acid stage, the state of leukocytosis is ascribed to the liberation of the thermostable leukocytosis factor.

2) Leukopenia. In the alkaline phase of the inflammatory reaction, the liberation by injured cells of leukopenin seems to be the primary factor necessary to explain reasonably the occasional state of initial leukopenia, whereas in the acid stage the fall in circulating leukocytes is apparently due to the production of the leukopenic factor of acid exudates.

3) Permeability. The increased capillary permeability at the beginning of the inflammatory reaction is induced primarily by leukotaxine, whereas the increased seepage of fluid through the capillary wall in the acid stage seems to be referable to exudin.

4) Fever. Fever is primarily referable to the liberation of pyrexin. This substance is frequently although not invariably liberated in acid exudates. There is the possibility, however, that there is also a pyrogenic factor in polymorphonuclear leukocytes. This would possibly place this factor primarily in the initial or alkaline phase of the inflammatory reaction (*43–46, 60*).

In brief, it is by these various substances liberated at either an alkaline *p*H or at an acid *p*H, depending on the state of the inflammatory reaction at a given time, that the systemic manifestations are maintained in a constant state. In this manner, with a pathological process, physiological homeostasis of the organism as a whole is maintained despite the progressive local chemical changes in the area of acute inflammation.

Repair

An inflammatory lesion heals. When damage is merely to the epithelial lining, normal regeneration occurs by simple mitosis. When a clean-cut wound is made at operation, relatively aseptically, and when the edges are brought together by suture, healing occurs rapidly by "primary union," or, as it is sometimes termed, "healing by first intention." There is hardly any fibrin or exudate. Fibroblasts move to the line of incision. At the line of cut a few polymorphonuclear leukocytes may appear because of the injury caused by the knife. Within a few days, firm union occurs. More and more fibroblasts and their collagenous bundles are deposited. The result is a scar or a cicatrice that becomes bloodless and usually persists indefinitely.

When the wound is open or if it suppurates, healing is somewhat slower and it proceeds somewhat as follows. Fibroblasts grow in abundance once the irritant has been localized and disposed by the various forces and by some of the factors discussed in this article. Accompanying their growth, there is active proliferation of the capillary endothelium. New capillaries sprout, growing from the base of the wound upward. When the scab of a wound, for instance, is removed there is a raw, reddish-appearing surface, rather granular. This is often referred to as "granulation tissue." The combination of the reparative factors consisting of fibroblasts and capillary sprouts with numerous new lymphatic channels is termed "vascular organization." After a longer period, more collagen is deposited. There is a tendency for contraction to occur, and we have, as a result, a scar or cicatrice.

This is a brief account of the well-known essentials in the repair processes of an inflamed area. Now, what is the stimulus that favors the growth of fibroblasts and capillary endothelium? A number of years ago it was found that exudative material recovered from an area of inflammation appears to contain one or perhaps even several growth-promoting factors (*61*). The experimental procedure consisted in repeatedly injecting into the tragal region of the rabbit's ear 0.5 milliliter of inflammatory exudate. The course of injections ranged from 1 to 7½ months. Following the cessation of injections, an interval of time ranging from 1 day to 7½ months was allowed to elapse. At that time, it was found that varying degrees of growth re-

actions appeared. These involved the cartilage and epithelium. The epithelial layer manifested pronounced hyperplastic response, with islets of keratinization. The dipping down of epithelial tonguelike projections into the corium was somewhat reminescent of the picture seen in some of the tar-induced cancer in rabbits described by the earlier writers. However, there was no definite evidence of any neoplastic formation. The architecture of the original cartilage plate was in many instances obliterated. Heterogenous, irregularly branching, and newly deposited cartilage cells were in evidence. In a few instances, metaplastic bone transformation appeared at the site of the original cartilage plate.

Recently it has been shown that the growth factor or factors present in exudates can be recovered as a diffusible component following vigorous dialysis, and subsequent concentration of the diffusate in a vacuum at reduced temperature of about 40° to 50°C. Such diffusible material induces proliferation without any significant inflammatory reaction (*52, 62, 63*). This can also be shown to some extent after repeated injections in the tragal region of the ears of rabbits (*12, 62*). A more convenient region is the vicinity of the nipples of nonpregnant rabbits. Hyperplasia of breast tissue and cystic dilatation of the ducts develop following the repeated local injections (*63*). Control injections with the diffusate of blood serum or saline injections fail generally to induce proliferative lesions to the same extent as found in the experimental areas. The nondiffusible material remaining in the cellophane tube can be extracted for necrosin and shown to be markedly injurious to cells. In brief, an injury factor can be dissociated from a a proliferative factor or factors liberated into the exudate at the site of inflammation (Fig. 5). These findings may be of significance in our further understanding of the reaction of repair following an acute inflammatory process. A picture may result which resembles that encountered in chronic cystic mastitis. Some workers have considered this type lesion to be precancerous in character (*64*). Furthermore, the process appears to be accelerated with the superimposition of a carcinogenic hydrocarbon—for example, methylcholanthrene (*12, 63*).

In a recent study on a homogenized aqueous extract of the ovaries of the sea urchin, *Arbacia punctulata,* an accelerator mitotic factor was observed that per se accelerates the cleavage of fertilized sea urchin ova (*12, 65*). In the same extract, a different fraction was also obtained that tends to be antimitotic in character and that actually retards cleavage (*12, 66*). The accelerator cleavage factor in the extract of the now-injured ovaries is diffusible. It is thermostable, and absorption spectrum measurements indicate that it probably is a nucleotide (*12, 65*). The diffusible proliferative factor of exudate described here appears, however, not to be a nucleic acid derivative. Preliminary chromatographic studies suggest the possibility that a peptide may be involved (*12*).

Finally, the presence of a growth-promoting factor liberated by injured cells may also prove to be of importance in our understanding of carcinogenesis or in the development of neoplastic processes in which cellular disequilibration seems to be brought about by a variety of means (for example, chronic irritation, virus infection, and hormone imbalance). When such cellular disturbance occurs and is superimposed on a genetically susceptible organism or in the presence of a chemical carcinogen, neoplastic development may be favored (*12, 42*).

Conclusions

The presence of leukotaxine, exudin, the leukocytosis-promoting factors, necrosin, pyrexin, and the leukopenic factors in inflammatory exudates offers a reasonable explanation for the development of a fundamental pattern in inflammation. Observations suggest that an irritant, irrespective of its nature, severely injures cells, which in turn, as a result of their deranged metabolism, liberate these various common denominators. These chemical substances, which are to be regarded as constant products of marked cellular injury, are responsible for the recognized pattern of the inflammatory reaction.

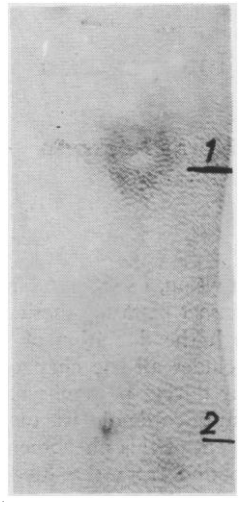

Fig. 5. The dissociation of the necrotizing factor in exudates (necrosin) from a present proliferative growth-promoting factor. (Area 1) The effect of necrosin extracted from the residual fraction of canine exudate after dialysis. Note the typical necrotizing inflammation. (Area 2) Note the absence of any pronounced visible effect when concentrated canine diffusate of an exudate was injected into the skin of a rabbit. [From V. Menkin, *Intern. Arch. Allergy and Appl. Immunol.* 4, 131 (1953)]

The interplay of the sequences described in this article (*67*) favors the localization and the ultimate disposal of the irritant. This leads to the final reparative phase of the injured area. Numerous observations indicate that repair may also be due to the liberation of a growth-promoting factor or factors by mildly injured cells. The growth-promoting factor or factors appear to be a diffusible component in exudative material. Necrosin seems to be released by the cell that is severely injured by an irritant, and thus the appearance of necrosin is significant in the development of the inflammatory process. Its chemical separation from the rest of the exudative fluid is a necessity in any endeavor to extract proliferative or reparative factors derived from probably less severely injured cells. Studies on invertebrate tissue (extract of ovaries of sea urchins) indicate the presence of a diffusible accelerator mitotic factor in the extract. This latter appears to be a nucleotide.

Sufficient material has been presented in the foregoing discussion to point out some of the important sequels of inflammation. For instance, it has been pointed out how the formation of glucose by injured cells complicates the all-important disease of diabetes. It has also been shown that the release of a toxic material, necrosin, from the area of inflammation may injure internal organs such as the liver and the kidneys. Pyrexin, which is liberated from injured cells at the site of an acute inflammation, is responsible at least in large part for the manifestation of systemic fever. It is thus clear that inflammation is not an exclusively localized reaction but that it may have far reaching effects on organs situated at a distance. Furthermore and conversely, as is pointed out here and elsewhere, various endocrine glands, such as the pituitary and the adrenal cortex, may exert definite influences on the course of the inflammatory reaction (*12*). The possible role of nerves is likewise not to be overlooked (*12*).

Finally, in the foregoing discussion, a biochemical theory of inflammation has been presented. There are, however, other biochemical aspects that can be described so that, necessarily, only a partial spectrum of the problem has been outlined here. A number of extremely important points have not been discussed for the reason that these aspects have either been very adequately treated previously and frequently by other investigators, or else they are not exactly relevant to the biochemical and immunological point of view of inflammation as I

have described it. Some of these have been treated elsewhere (*12*). For instance, the all-important subject of phagocytosis has intentionally been omitted here. Metchnikoff, the founder of the phagocytic theory, as well as his followers, have treated very adequately this paramount and well-known subject, now considered to be a cornerstone of inflammation.

References and Notes

1. E. M. Landis, *Am. J. Physiol.* 75, 548 (1926).
2. J. Cohnheim, *Lectures on General Pathology* (New Sydenham Society, London, 1889).
3. V. Menkin, *Arch. Pathol.* 12, 802 (1931).
4. ———, *Dynamics of Inflammation* (Macmillan, New York, 1940, reprinted 1950).
5. T. Lewis, *The Blood Vessels of the Human Skin and their Responses* (Shaw, London, 1927).
6. V. Menkin, *J. Exptl. Med.* 64, 485 (1936).
7. ———, *ibid.* 67, 129, 145 (1938).
8. H. Cullumbine and H. N. Rydon, *Brit. J. Exptl. Pathol.* 27, 23 (1946).
9. L. Pasquali, *Ricerche e studi med. sper.* 18, 209 (1947).
10. M. Morimoto, *Naika No Ryoiki.* 1, 401 (1953).
11. V. Menkin, *Ann. N.Y. Acad. Sci.* 59, 956 (1955).
12. ———, *Biochemical Mechanisms in Inflammation* (Thomas, Springfield, Ill., 1956).
13. ———, *J. Exptl. Med.* 57, 977 (1933).
14. ———, *Physiol. Revs.* 18, 366 (1938).
15. ———, *J. Exptl. Med.* 50, 171 (1929).
16. ———, *Am. J. Pathol.* 16, 13 (1940).
17. ———, *Arch. Pathol.* 30, 363 (1940).
18. M. L. Dillon, G. R. Cooper, V. Menkin, *Proc. Soc. Exptl. Biol. Med.* 65, 187 (1947).
19. V. Menkin and M. A. Kadish, *Arch. Pathol.* 33, 193 (1942).
20. V. Menkin, M. A. Kadish, S. C. Sommers, *ibid.* 33, 188 (1942).
21. V. Menkin, *Am. J. Pathol.* 19, 1021 (1943).
22. ———, *Arch. Pathol.* 41, 376 (1946).
23. ———, *Blood* 3, 939 (1948).
24. ———, *Arch. Pathol.* 43, 566 (1947).
25. ———, *Am. J. Pathol.* 10, 193 (1934).
26. ———, *J. Exptl. Med.* 53, 171 (1931).
27. ———, *Arch. Pathol.* 27, 115 (1939).
28. V. Menkin and C. R. Warner, *Am. J. Pathol.* 13, 25 (1937).
29. M. B. Lurie, *ibid.* 13, 612 (1937).
30. E. L. Opie, *Physiol. Revs.* 2, 552 (1922).
31. P. D. McMaster and S. S. Hudack, *J. Exptl. Med.* 61, 783 (1935).
32. S. H. Harris, T. N. Harris, M. B. Farber, *J. Immunol.* 72, 148, 161 (1954).
33. A. A. Maximow and W. A. Bloom, *A Text-Book of Histology* (Saunders, Philadelphia, 1930).
34. V. Menkin, *Am. J. Physiol.* 134, 517 (1941).
35. ———, *ibid.* 138, 396 (1943).
36. ———, *ibid.* 147, 379 (1946).
37. ———, *Arch. Pathol.* 36, 269 (1943).
38. ———, *Blood* 4, 1323 (1949).
39. ———, *Proc. Soc. Exptl. Biol. Med.* 75, 378 (1950).
40. ———, *Arch. Pathol.* 41, 50 (1946).
41. ———, *ibid.* 46, 145 (1948).
42. ———, *Newer Concepts of Inflammation* (Thomas, Springfield, Ill., 1950).
43. ———, *Arch. Pathol.* 39, 28 (1945).
44. ———, *Arch. intern. pharmacodynamie* 89, 229 (1952).
45. ———, *Federation Proc.* 13, 439 (1954); *J. Lab. Clin. Med.* 46, 423 (1955).
46. I. L. Bennett, Jr., and P. B. Beeson, *J. Exptl. Med.* 98, 477 (1953).
47. V. Menkin, *Am. J. Physiol.* 129, 691 (1940).
48. ———, *Proc. Soc. Exptl. Biol. Med.* 51, 39 (1942).
49. P. S. Hench *et al.*, *Proc. Staff Meetings Mayo Clinic* 24, 181 (1949).
50. V. Menkin, *Am. J. Physiol.* 166, 509 (1951).
51. ———, *ibid.* 166, 518 (1951).
52. ———, *Intern. Arch. Allergy and Appl. Immunol.* 4, 131 (1953).
53. ———, *Proc. Soc. Exptl. Biol. Med.* 82, 189 (1953).
54. V. Menkin and M. Pepper, *Biol. Bull.* 107, 316 (1954).
55. ———, *ibid.* 107, 316 (1954).
56. V. Menkin, *Science* 120, 1026 (1954).
57. ———, *Brit. J. Exptl. Pathol.* 34, 412 (1953).
58. W. B. Cannon, *Physiol. Revs.* 9, 399 (1929).
59. V. Menkin, *J. suisse med.* 82, 1184 (1952).
60. I. L. Bennett, Jr., and P. B. Beeson, *J. Exptl. Med.* 98, 493 (1953).
61. V. Menkin, *Cancer Research* 1, 548 (1941).
62. ———, extracted from *The Mechanism of Inflammation*, G. Jasmin and A. Robert, Eds. (Acta, Montreal, Quebec, 1953), p. 137.
63. ———, *Proc. Am. Assoc. Cancer Research* 2, 34 (1955).
64. J. Ewing, *Neoplastic Diseases* (Saunders, Philadelphia, ed. 2, 1922).
65. V. Menkin, *Biol. Bull.* 109, 364 (1955).
66. ———, *ibid.* 109, 365 (1955).

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Metamorphosis

A Physiological Interpretation

Morris Rockstein

During the past decade there has been evident an increasing number of cooperative efforts by scientists who represent research disciplines considered widely separated. However, a few areas of the biological sciences remain in which the techniques of the physical scientist and experimental biologist are generally overlooked. Thus, taxonomists continue to depend on gross morphological or anatomical criteria for their deductions (*1*). In the few cases in which experimental or analytic data have been so employed (*2*), this use has been through the efforts of a few biochemists and physiologists who have applied their findings to phylogenetic or taxonomic deductions. I should like to show here how recent findings by other physiologists and myself may serve as a basis for the critical examination, if not the revision, of the established concepts of insect metamorphosis.

Although the definition of metamorphosis includes all the changes in form that occur during postembryonic development, most entomologists tend to limit their concept of this phenomenon to the transformation from the juvenile form (the larva) to the adult, or imago. One result of this limited concept has been artificial grouping of various orders of insects into groups such as the Ametabola, Paurometabola, or Holometabola according to the extent to which the juvenile stages differ in outward appearance from their corresponding imagoes. A number of competent entomologists admit that such a classification is artificial, especially from the phylogenetic standpoint. In his excellent monograph, Snodgrass (*3*) writes that "insects cannot be classified taxonomically according to the type of metamorphosis they undergo" and that "true metamorphic characters are adaptive structures . . . that have no phylogenetic counterpart in the adult evolution." This point is best illustrated by insects with complete metamorphosis, for they can hardly represent a monophyletic group. A more likely hypothesis is that holometaboly arose independently on several occasions during the evolution of the different orders of insects (*4*). In the course of his discussion, Snodgrass (*3*) also cites postemergence metamorphic changes, of which several cases are known, and anamorphosis or body segmentation after hatching. Since entomologists consider metamorphosis to be complete, except for sexual maturation, when the juvenile characters are discarded, the usual interpretation of these changes is that they are exceptions to the rule.

My own investigations and those of other physiologists interested in metabolic pathways in insects at different stages of development indicate a different interpretation. These findings suggest that visible transformation to the adult should not be considered as a terminal event but as one in a series of changes

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