Symposium

The Mast Cell

Recent Problems

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Identical cutaneous lesions can be elicited by several combinations of the following pathogens that in themselves are inactive and essentially different: (1) "sensitizers," which induce a latent predisposition for a specific reaction form (eg, inflammation, calcification, thrombosis and hemorrhage, necrosis); and (2) "challengers," which unmask this predisposition by making the disease manifest and determining its location.

It may be significant that in all the disease models mentioned in this review, mast-cell dischargers and mast-cell products act as potent elicitors for diverse skin reactions; it therefore appears justified to conclude that close relationships exist between mast cells and tissue resistance to numerous pathogens.

THE ROLE OF the mast cell as a source of heparin and histamine is now well established. At least in certain species, mast cells also produce serotonin and possibly a number of additional substances including catecholamines and slow-reacting substance (SRS). In addition the mast cells are extraordinarily rich in a great variety of enzymes. Under the influence of various traumatic injuries, and particularly during anaphylaxis and anaphylactoid inflammation, the mast cells discharge their secretory products. A recent review of the literature on mast cells lists more than 30 theories concerning their functions,[1] but their final participation in physiological and pathological processes is still poorly understood.

This paper discusses more recent problems concerning the physiopathology of the mast cells, which are of special interest in that they help to clarify the concept of the "pluricausal diseases."

To analyze this concept, I have selected several types of pluricausal disease models, all of which are dependent on mast-cell action. The principal reaction forms that we have studied in animals—anaphylactoid inflammation, necrosis, calcification, thrombosis and hemorrhage—represent important constituents of many spontaneous diseases of man.

A pluricausal disease is defined as a derangement produced by combined treatment with two or more agents, none of which is effective in this respect by itself. Most of the pluricausal diseases examined up to now were elicited by combined treatment with two agents; one of them induces a particular type of disease proneness and is therefore called the "conditioner" or "sensitizer," while the other agent makes this predisposition manifest and determines the location of the resulting changes and thus acts as the "challenger."[2]

By such a dual treatment it has been possible to produce calcification,[3] thrombosis with hemorrhage,[4] and the so-called "acute conditioned necrosis,"[5-7] all predictably localized at certain sites.

The basis of our work on these phenomena was laid as far back as 1937, when we

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first observed the *anaphylactoid edema* in rats following intraperitoneal injection of egg white.[8] This edema is characterized by an acute serous inflammation with degranulation of the mast cells (which are particularly plentiful in the anaphylactoid shock organs), as well as by hyperemia and edema in the lips, ears, tongue, paws, and the anogenital region. Subsequent investigations showed that essentially similar responses can be produced by a variety of mast-cell dischargers (eg, dextran, dextrin, compound 48/80, polymyxin); indeed, the anaphylactoid reaction presumably depends upon the liberation of histamine and serotonin from mast cells, since inhibitors of these substances can prevent its development.[9] This basic reaction to mast-cell discharge is certainly not a recent problem, because by now a literature of well over 1,000 publications deals with the various aspects of the anaphylactoid reaction.

In 1963, we reported on experiments which showed that through "*mastopexis*," discharged mast-cell material can capture blood-borne particulate substances and thereby participate in the localization of disease.[10]

A subcutaneous injection of the mast-cell discharger polymyxin, given simultaneously with an intravenous injection of India ink, causes localization of carbon particles, particularly in a halo around the injection site of polymyxin. Here, the India ink and the discharged mast-cell granules are found in close association within the connective tissue and in the walls of small blood vessels, perhaps because mast-cell material can fix blood-borne particles by increasing the adhesiveness of tissues.

If a similar injection of polymyxin is given somewhat prior to the intravenous administration of India ink, there is no local fixation of carbon particles in the subcutis, but numerous pulmonary capillaries are occluded by thrombi that contain aggregates of India ink. Here, the discharged mast-cell granules may have already been carried from the injection site of polymyxin to the pulmonary circulation by the time the India ink is introduced into the circulation, and it is perhaps for this reason that the carbon particles are fixed in the lung.

Essentially similar observations were made when ferric oxide saccharate (Fe-OS) was administered intravenously instead of India ink. However, since iron is more toxic to tissues than is India ink, the local precipitation of Fe-OS, especially in the center of the polymyxin injection site, may cause a necrosis similar to that seen in the local *Shwartzman-Sanarelli phenomenon.*

Repeated intravenous administration of Fe-OS to older rats produced heavy selective iron precipitation in the choledochus and duodenum; this resulted in extreme dilatation of the bile ducts and development of duodenal ulcers with necrosis. Here again, the iron particles precipitate predominantly in the immediate vicinity of the mast cells which are particularly numerous around the extrahepatic bile ducts and the duodenum of the rat. In order to test the hypothesis of a causal relationship between mast-cell granules and iron precipitation, a mast-cell discharge was produced prior to the administration of Fe-OS; it was found that, after depletion of the mast cells, the bile duct and choledochus lesions normally elicited by the iron preparations were prevented.

While continuing our studies on the participation of mast cells in the localization of blood-borne metals, we noted that massive calcification can be induced in the anaphylactoid shock organs by the newly discovered techniques of calciphylaxis and calcergy.[3,11]

Calciphylaxis is a phenomenon that induces selective calcification in various organs. It is brought about by pretreatment with a systemic calcifying sensitizer (eg, parathyroid hormone or vitamin-D derivatives), followed after a time interval (the "critical period") by an eliciting agent that acts as the challenger.

Calcergy is an essentially different reaction; it is produced without previous sensitization by parenteral administration of so-called direct calcifiers or "calcergens"[12,13] such as salts of lead, lanthanum, indium, or cerium.

Most calciphylactic challengers and calcergens are metallic compounds but, while all calcergens so far tested are also active as challengers, the reverse is not necessarily true.

Through their mast-cell-discharging effect, histamine liberators play an important role in the mechanism of certain calciphylactic

syndromes,[14] and the same applies to some forms of calcergy.[15] These mast-cell-dependent reactions types have been designated as *mastocalciphylaxis*[16] and *mastocalcergy*,[17] respectively. Here, a mast-cell discharge presumably participates in the distribution of the challenging metal and of calcium salts to different tissues. Through mastocalcergy, topical calcification of the skin can be obtained, for example, if a rat first receives lead acetate intravenously and immediately afterwards a subcutaneous injection of a mast-cell discharging histamine-liberator (eg, polymyxin, compound 48/80) or a mast-cell constituent (eg, histamine, serotonin). Calcification of the anaphylactoid shock organs is obtained if the rat pretreated for calciphylactic[3] or calcergic[11] reactivity is simultaneously given an intraperitoneal or intravenous injection of a histamine liberator that induces an anaphylactoid reaction.

In comparing the effects of different mast-cell dischargers we have noted that, depending upon the compound used and the route of its administration, the resulting anaphylactoid reaction can affect different parts of the body preferentially; the paws or the snout may react most intensely, or almost the entire skin surface may be more or less uniformly affected. Furthermore, when an anaphylactoid reaction is produced by different types of histamine dischargers, subsequent treatment with calciphylactic or calcergic sensitizers causes mast-cell discharge followed by calcification of the discharged granules and their surroundings. This occurs with great selectivity not only in the classical shock organs, but even in the palate, esophagus, bile duct, or the autonomic nervous system.[3,18,19]

From these experiments it became evident that, in animals in which calcergic reactivity was induced by a single intravenous injection of lead acetate, treatment with various mass-cell-discharging histamine liberators, histamine or serotonin causes calcification in different regions of the body. Depending upon the mast-cell discharger or mast-cell component used and upon the route of their administration, the distribution of the resulting lesions varies in a predictable manner. Histologic studies showed that, at least in the case of calcification induced by mast-cell dischargers, this variation appears to be due to their selective effect upon the mast cells of certain regions. Thus, we found that thorium dextrin, given intravenously, causes intense mast-cell degranulation selectively in the perivaginal connective tissue, which subsequently calcifies, but not in the adjacent adipose tissue of the pelvis, which remains uncalcified. Furthermore, it could be shown that five hours after the injection of thorium dextrin, the mast cells in the affected region have already discharged their granules. But at this time there is no sign of mineralization while after 24 hours the released mast-cell granules become impregnated with calcium salts that are demonstrable by the von Kóssa technique. The fact that even mast-cell constituents can cause calcification in the rat pretreated with lead acetate shows that this effect is not dependent upon any direct action of mast-cell dischargers.[3,20-23]

Presumably, depending upon their chemical structure and the route of their administration, various mast-cell dischargers can act selectively on mast cells of certain regions without affecting those in other areas. We can, therefore, no longer speak of "the anaphylactoid reaction," but must recognize the existence of various forms of this phenomenon. Even before using the technique of mastocalcergy, we had noticed that different histamine liberators can cause more pronounced edema in one or the other region. However, mild anaphylactoid swellings are not always very obvious, especially in internal organs. Now, with the aid of mastocalcergy, the affected sites can be permanently marked by massive mineral deposits and it is clear that qualitatively different forms of anaphylactoid reactions become demonstrable through this technique, permitting the predictable selective induction of calcification at different sites. This is accomplished by permanently marking otherwise inconspicuous reaction sites with calcium deposits by means of the mast cell dependent type of calcergy.

In connection with the possible role of mast cells in calcification, it is also noteworthy that, in weanling rats placed on a low calcium diet, numerous mast cells accumulate on the surface of, within, or under the endosteum when growth becomes arrested. There is no corresponding increase in the

mast-cell population elsewhere in the body. Treatment with therapeutic doses of vitamin D restores the normal structure of the bones and leads to the disappearance of excess mast cells, whereas injections of parathyroid hormone merely produce osteitis fibrosa without diminishing the mast-cell population in the bones. The question arises whether mast cells might not produce precursors of ground substance, alkaline phosphatase, or other materials necessary for osteogenesis.[24] It might even be asked whether some of the spontaneous calcinoses of man could not depend upon hypersensitivity reactions since, in exceptional instances, the cutaneous lesions of urticaria may undergo rapid and massive calcification.[25] It would be interesting to explore whether here, mast-cell degranulation is causally related to the precipitation of calcium.

While attempting to produce systemic calcergy by intravenous injection of various metals plus compounds suspected of being appropriate challengers, we hit upon many combinations that proved ineffective in eliciting calcification but induced thrombosis with hemorrhage in the challenged area. Thus we stumbled upon what has subsequently been called the *thrombohemorrhagic phenomenon (THP)*. Here, sensitization is obtained by metallic compounds (eg, scandium chloride, indium chloride) or sulfated polysaccharides (carrageenan, agar) and, here again, among the most effective challengers are mast-cell dischargers and mast-cell products.[4]

Similar considerations apply to the so-called *acute conditioned necrosis* (ACN).[5-7,26] We first observed this phenomenon in the course of certain experiments on the THP in which we used serotonin to influence the thrombohemorrhagic changes that occur in the brain of animals receiving large amounts of hypertonic saline, urea, or glucose. It is known that subcutaneous injections of such solutions produce an acute shrinkage of the brain when given in doses quite well tolerated by the tissues at the site of injection. However, when animals so treated were concurrently given an intravenous or intraperitoneal injection of serotonin, the skin underwent acute necrosis over the whole subcutaneous region infiltrated with the hypertonic solution. As always in such instances, our immediate reaction was that the experiment had failed. Evidently the action of serotonin on these brain lesions could not be examined because the animals had lost a large portion of their skin surface and developed enormous, gaping ulcers. It was only as an afterthought, presumably triggered by a flashback to earlier experience with other pluricausal lesions, that we realized we may have developed an excellent model for the induction of a necrotizing diathesis.[27]

Our hope proved to be well founded. Subsequent systematic studies showed that subcutaneous injections of normally well-tolerated amounts of hypertonic sodium chloride or urea solutions produce extensive topical necroses in rats systemically treated with various mast-cell dischargers (compound 48/80, polymyxin, egg white), and mast-cell products (histamine, serotonin). Moreover, we found that not only hypertonic solutions but various other inflammatory irritants can act as challengers for this type of response at dose levels not normally conducive to necrosis. This response is considered to be closely related to mast-cell function, for it cannot be duplicated by systemic treatment with a variety of other agents.[26]

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