THE DERMATOLOGIC IMPLICATIONS OF STRESS AND CALCIPHYLAXIS

HANS SELYE, M.D.

Institut de Médecine et de Chirurgie expérimentales Université de Montréal, Montreal, Canada

It is indeed an honor to have been invited to give the Annual Herman Beerman Lecture this year on stress and calciphylaxis in their relationship to dermatology. Throughout my research career I have been chiefly interested in the varying degrees of biologic specificity. This approach to physiology led me to formulate the concept of stress as the most nonspecific biologic reaction-form to any kind of stimulation or damage and to recognize the general adaptation syndrome (G.A.S.) and the local adaptation syndrome (L.A.S.) as the prototypes of systemic and topical nonspecific reactions, respectively.

Now, my associates and I are chiefly engaged in the clarification of a new biologic reaction-form which we named "calciphylaxis." Calciphylaxis is more specific than the stress responses both as regards the agents that elicit it and the target regions that respond; yet, calciphylactic reactions share certain cardinal features with the stress responses: they are elicited by numerous agents and they primarily affect the almost ubiquitous connective tissue and stromal elements. These, unlike the so-called "noble elements" of parenchymal tissues, are diffusely distributed throughout the body and, hence, well placed to regulate those general reactions of the body that govern physiologic and pathologic responses affecting the organism as a whole.

Perhaps the most characteristic feature of stress reactions is inflammation with its corollary, the "collagen diseases," whereas calciphylaxis is primarily characterized by calcification. The fact that inflammation is a connective-tissue reaction requires no comment, but it is well to point out that the same is essentially true of calcification: the principal physiologic site of calcification in the body is the collagen of bones, and even pathologic soft-tissue calcification—especially as it occurs in calciphylaxis—affects primarily the connective-tissue elements. Since the skin consists mainly of connective tissue, it is not unexpected that both stress and calciphylaxis play a particularly important role in dermatology.

The concept of stress and the stress-induced "diseases of adaptation" has been described at length in several monographs (5–7), and even its particular dermatologic implications have been the subject of several reviews (1–4); hence, we need not deal with these topics exhaustively here. Recently, there also appeared a systematic treatise on calciphylaxis (8) but, since this subject is still comparatively new, we shall have to outline its essential features before turning our attention to the many interesting relationships between nonspecific stress, calciphylactic reactivity, and the physiopathology of the skin.

BRIEF CHARACTERIZATION OF CALCIPHYLAXIS

Definition.—Calciphylaxis is a condition of induced systemic hypersensitivity in which tissues respond to appropriate challenging agents with local calcification.

The term was coined in analogy with such designations as anaphylaxis, tachyphylaxis, or skeptophylaxis that likewise refer to induced systemic alterations in the body's responsiveness. Apparently, calciphylaxis is a fundamentally defensive (phylactic) response which, depending upon circumstances, can either produce calcification by concentrating calcium salts in more or less circumscribed foci, or prevent calcinosis by "deviation" or dispersion of the metal throughout the body. The concentrating form of calciphylaxis often provokes inflammation and sclerosis through the selective deposition of irritating calcium salts in the challenged area; it can thereby help sequestrate a pathogen with granuloma tissue, thus increasing resistance to topical injury. However, this focal form of calciphylaxis can also become the cause of morbid lesions if an excessive amount of mineral is deposited in the tissues. Deviating calciphylaxis, on the other hand, can interfere with the most varied forms of focal calcification by dispersing

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calcium to innumerable minute turnover points throughout the body; here, no major unabsorbable mineral deposit is formed and any excess of calcium is readily metabolized.

Among the *concentrating forms* we distinguish:

1) *topical calciphylaxis* induced by the direct application of the challenger to the responsive tissue, from

2) *systemic calciphylaxis* in which the challenger is distributed throughout the organism (e.g., after intravenous or intraperitoneal administration), but produces a response only in tissues for which it has a selective affinity.

Conversely, *deviating calciphylaxis* is induced by gradually saturating the organism with a readily diffusible challenger, for example, with ferric dextran, or "Fe-Dex." When given in this manner, the challenging iron atoms are dispersed throughout the body into the connective-tissue elements, particularly the phagocytes and, since all the minute iron foci attract calcium, they compete for it. As a consequence of this competition, calcium is fairly evenly distributed throughout the organism and circumscribed, massive soft-tissue calcifications cannot occur.

CONCENTRATING CALCIPHYLAXIS

Examples.—If a rat (weighing about 100 g.) is given a single oral dose of dihydrotachysterol, or "DHT" (e.g., 1 mg. in 0.5 ml. of corn oil), the subcutaneous injection of as little as 25 μg. of $FeCl_3$ (in 0.2 ml. of water) on the following day elicits a precipitous local deposition of calcium salts within the next two to three days. Macroscopically, this *topical calciphylactic response* is characterized by the appearance of a hard white patch at the site of injection. Histologically, we note calcareous incrustation of dermal and subcutaneous connective-tissue fibers, followed by reactive inflammatory infiltration (in which eosinophils and pseudoesosinophils predominate) and eventually sclerosis.

If under similar circumstances, instead of the subcutaneous application of $FeCl_3$, 1 ml. of a ferric oxide saccharate, or "Fe-OS" (containing 20 mg. of metallic iron), is injected intravenously on the day of sensitization with DHT, a *systemic calciphylactic syndrome* results. Here, calcification occurs predominantly in the left auricular appendage of the heart, the subepicardial layers of the ventricular myocardium, the bile ducts, the duodenum and the renal cortex. Presumably, this distribution is due to the fact that iron, when given in this form, tends to accumulate selectively in these regions and attracts calcium to them.

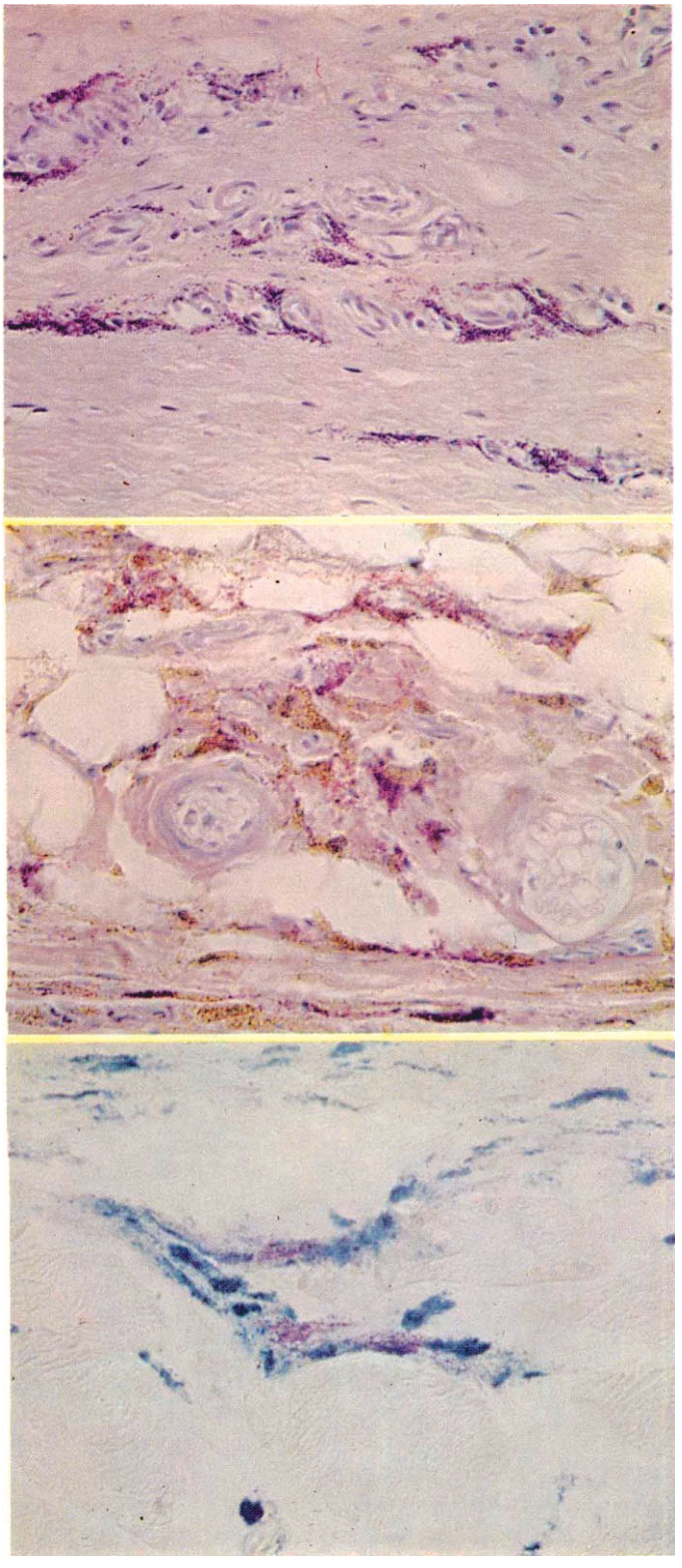
As we shall see, there are many other sensitizers, or "systemic calcifiers," that can replace DHT, and numerous challengers can substitute for $FeCl_3$ or Fe-OS in the production of such calciphylactic responses; yet, almost invariably the interval elapsing between the application of the systemic sensitizer and the local challenger is of decisive importance. In the examples of topical and systemic calciphylaxis just mentioned, the *"critical period"* for the most efficacious application of the challenger happens to be respectively, 24 hours and 0 hour after sensitization; however, the length of this interval varies, depending upon the sensitizers and the challengers used. Indeed, some types of calciphylaxis can only be obtained by applying the challenger before the sensitizer, and even the quality of the response (e.g., the distribution of the lesions in systemic calciphylaxis) may depend upon the timing of the two types of pathogens.

Both topical and systemic calciphylaxis are truly pluricausal morbid lesions, since in themselves neither the sensitizing DHT nor the challenging iron preparations can evoke them.

THE CALCIFIER (SYSTEMIC SENSITIZING AGENT)

Most of the original work on calciphylaxis was performed on animals sensitized with *DHT*, a readily available and extremely active, calcification-promoting agent whose actions are essentially those of parathyroid hormone.

Degranulation of mastocytes induced by DHT.—Intense degranulation of mastocytes is seen following chronic treatment with DHT, both in the otherwise untreated (top) and in the Fe-Dex-protected (middle and bottom) rats. Following Fe-Dex treatment, however, the iron granules are closely intermixed with the discharged metachromatic mastocyte granules. *Top:* Virtually fat-free skin of rat treated with Fe-Dex alone. There are numerous mastocytes around the cutaneous vessels, but all of them have discharged their metachromatic (purple) granules (cresyl violet, × 420). *Middle:* Corresponding skin lesion of a Fe-Dex-protected rat shows well developed fat tissue, but the mastocytes around vessels and nerves nevertheless discharged their granules (purple). The latter are intermixed with the iron granules, which are yellowish-green because unstained (cresyl violet, × 420). *Bottom:* Small blood vessel in the cutaneous muscle of a DHT + Fe-Dex-treated rat. Here, the iron granules are stained blue, while the mastocyte granules are again metachromatically purple. Note close topographic relationships between the two types of granules (Prussian blue plus cresyl violet, × 420).



TOP

MIDDLE

BOTTOM

We still do not know how calciphylactic sensitization acts, but all sensitizers tested share with DHT the property of mobilizing calcium and predisposing for calcification in general; that is why these agents may also be referred to simply as calcifiers. However, the nonspecific "metastatic" calcification caused by a mere excess of such calcifiers (without the need for additional treatment with an exogenous challenger) differs essentially from calciphylaxis in that it occurs only in certain naturally predisposed sites (e.g., gastric mucosa, cardiovascular system, kidney). Hence calcification thus produced—e.g., by excessive amounts of parathyroid hormone or DHT, alone—offers no possibility of altering the distribution pattern of the resulting lesions by directing calcium at will to predetermined sites.

Among the calciphylactic sensitizers examined to date, *vitamin D_2*, *vitamin D_3*, *parathyroid hormone and sodium acetylsulfathiazole* (*NaAST*) have also proven to be very effective. Under appropriate conditions of dosage and timing pretreatment with any of these compounds "conditions" for the induction of a precipitous local calcification of the connective-tissue fibers (followed by sclerosis or even necrosis) at sites subsequently treated with challengers (e.g., $FeCl_3$). Of course, when given in very high doses, any of these calcifiers can produce nonspecific calcinosis at the previously mentioned sites of predisposition, but even at much lower doses they sensitize other tissues for calcification under the influence of challenge.

The vitamin-D compounds are close chemical analogues of DHT; like the latter, they appear to act directly and not through stimulation of parathyroid-hormone secretion, since they remain effective even after parathyroidectomy. On the other hand, NaAST acts as a calcifier only in the presence of the parathyroids.

The possibility of producing calciphylaxis with NaAST shows that this response can be elicited by autologous parathyroid hormone in the amounts secreted as a consequence of renal damage.

Even various *surgical interventions* (e.g., bilateral nephrectomy, ligature of both ureters, obstruction of the pylorus combined with the establishment of a gastric fistula) can produce metastatic calcification at the usual sites of predisposition. Presumably the effect of these operations is also mediated through parathyroid stimulation, since it is abolished by parathyroidectomy. Yet, all these interventions are only moderately effective sensitizers, perhaps because they produce intense stress and an alarm reaction tends to prevent calciphylaxis.

Thus we have learned to distinguish between *direct systemic calcifiers* that sensitize for calciphylaxis in themselves and *indirect systemic calcifiers* whose action depends upon a secondary reaction, the increased elaboration of an endogenous sensitizer (e.g., parathyroid hormone).

Many drugs can cause "*dystrophic calcification*" without any special sensitization by virtue of their destructive action upon certain organs that are naturally predisposed to take up calcium; yet, these drugs do not necessarily sensitize for calciphylaxis. For example, intoxication with $HgCl_2$ causes severe calcification of the renal tubules damaged by mercury, but fails to sensitize other tissues to the calciphylactic action of potent challengers. Other compounds, the "*direct calcifiers*" (e.g., $PbCl_2$, $CeCl_3$, $ZnCl_2$, $KMnO_4$) can produce local calcification anywhere in connective tissue without the need for sensitization, although they are much less damaging than certain irritants and corrosives (e.g., croton oil, HCl, NaOH) that cause no topical calcinosis. Apparently there exist essential differences between dystrophic calcification, direct calcification and calciphylaxis.

We have already mentioned that some sensitizers act directly, others indirectly, (e.g., through parathyroid stimulation). A similar distinction may be made as regards the challengers. Most of these act at the site of application; for example, they produce cutaneous calcinosis wherever they are subcutaneously injected in a DHT-sensitized rat. Among these *direct challengers* are salts of iron, chromium, aluminum, manganese, thorium, cerium, zirconium, titanium and lead; but certain organic compounds (e.g., egg white, egg yolk) and even the mild mechanical trauma of plucking the hair or pinching the skin are also very effective in this respect.

Here again—as with the direct calcifiers—there appears to be no proportionality between the damaging effect of an agent (as judged by its ability to cause necrosis or inflammation) and its calcifying action: many strong inflammatory irritants and corrosives are quite ineffective, while typical challengers produce calciphylactic

responses even at dose levels at which by themselves they elicit no demonstrable tissue-damage.

Most of the direct challengers that cause topical calciphylaxis upon subcutaneous injection also proved to elicit a systemic calciphylactic syndrome when administered intravenously or intraperitoneally. However as we shall see, the distribution and structure of the resulting lesions differ greatly, depending upon the particular organ affinities of the various challengers and upon the reactivity of the experimental animal. For example, in rats sensitized with DHT, the intravenous administration of egg white produces an almost selective calcification of the skin and pancreas, whereas egg yolk causes calcification in the spleen and the Kupffer cells of the liver (presumably because the yolk globules tend to be phagocytosed by the cells of the RES). Both these systemic calciphylactic reactions are, in turn, quite unlike that produced under similar circumstances by Fe-OS, which we have already discussed. Factors affecting the reactivity of the organism (e.g., age, genetic background, hypophysectomy, drugs, stress) can likewise markedly alter the intensity and quality of calciphylactic responses.

Indirect challengers are agents that cause little or no topical calciphylaxis when directly applied to an otherwise receptive site, such as the skin of a suitably sensitized animal, but elicit systemic calciphylactic syndromes when introduced into the general circulation. For example, in the DHT-sensitized rat histamine liberators (e.g., 48/80, dextran, polymyxin, glucocorticoids) cause virtually no local cutaneous calcinosis upon subcutaneous administration, but they can elicit calciphylactic responses in various distant organs if injected intravenously or intraperitoneally. Indeed, if sufficiently large amounts of such compounds are injected subcutaneously, systemic calciphylaxis may result even though the site of administration fails to undergo calcification. As a working hypothesis, we assume that compounds of this type act only indirectly through the liberation or activation of some endogenous challenger (e.g., mastocyte granules, iron).

THE ADJUVANT (TOPICAL ACTIVATOR OF CHALLENGER)

Certain substances that have little or no challenging action can enormously increase the activity of threshold doses of topical challengers. For example, in the DHT-sensitized rat, dextran does not cause calcification but if otherwise ineffective amounts of $FeCl_3$ are injected subcutaneously in dextran solution, severe topical cutaneous calcinosis results. Unlike dextran, egg white possesses considerable direct challenging potency, but if high dilutions of albumen (in themselves almost ineffective) are subcutaneously injected with subthreshold traces of $FeCl_3$, the result is again a greatly increased topical calciphylactic response. Here, the dextran and egg white apparently potentiate the action of iron, somewhat as adjuvants can increase the efficiency of antigens.

THE "CRITICAL PERIOD"

It is impossible to reproduce certain calciphylactic phenomena consistently without strictly observing the critical period that must elapse between treatment with sensitizer and challenger. The length of this period is not the same for all forms of calciphylactic responses; indeed, we may obtain qualitatively different reactions by merely altering the time interval between treatment with sensitizer and challenger. For example, if female rats weighing about 200 g. are first given 1.5 mg. of DHT p.o. and 24 hours later 1 ml. of ferric dextran, or "Fe-Dex" (~50 mg. Fe) i.p., they develop intense calcification in the pancreas and retroperitoneal fat, but not in the uterus. On the other hand, if the experiment is repeated under otherwise identical conditions except that now the Fe-Dex is given 24 hours before the DHT, the pancreas and adipose tissue fail to react, while the uterus undergoes intense calcification. Numerous other examples illustrating the decisive importance of the critical period have been listed elsewhere (9).

It is not yet clear how minor differences in the timing of the treatment with sensitizer and challenger can so radically change the form of a calciphylactic response. In anaphylaxis, a rest period after sensitization is necessary to allow time for the formation of antibodies before the challenging antigen is applied, but in calciphylaxis we have no evidence of any antigen-antibody reaction. It may be argued that here, time is required for the absorption of the sensitizer and for the mobilization of calcium from the bones. However, as we have said, some calciphylactic reactions are best elicited by simul-

taneous treatment with sensitizer plus challenger (e.g., the cardiovascular, renal and biliary-tract lesions induced by DHT + Fe-CS, i.v.), whereas others (e.g., uterine calcification after Fe-Dex, i.p. + DHT) are most readily obtained after pretreatment with the challenger. Of course, the formation of a calcifiable matrix is also important. The tissues that undergo calcification become PAS-positive and the accumulation of PAS-tingible material may have to be adjusted to the available calcium and phosphate before certain forms of mineralization can develop.

In view of these facts, it is unlikely that the time-lapse required for the development of any one metabolic change could account for the length of the critical period in all types of calciphylaxis.

DEVIATING CALCIPHYLAXIS

Example.—If a rat (weighing 100–200 g.) is given 50 mg. of iron intraperitoneally every five days in the form of a readily diffusible iron complex, such as Fe-Dex (ferric dextran*), it develops a generalized hemosiderosis owing to the formation of diffusely distributed, minute iron deposits. In animals thus pretreated, the most diverse forms of soft-tissue calcification are inhibited. Topical treatment with direct calcifiers no longer produces local calcification; heavy overdosage with DHT or parathyroid hormone fails to elicit the customary calcification in the normally predisposed cardiovascular system, kidney or lung; and calciphylactic responsiveness to challengers is greatly diminished or totally suppressed. Apparently, here, the diffuse impregnation of the organism with a challenger results in protection against the induction of large focal calcium deposits.

DERMATOLOGIC IMPLICATIONS

It would be redundant to describe here all of the cutaneous lesions that can be elicited in animals by the calciphylactic technics, that have been described in detail elsewhere (9). Suffice it to mention a few experimentally induced calciphylactic changes of the skin, that are reminiscent of certain diseases of man. It must be strongly emphasized, however, that the names given to these experimental syndromes (e.g., calciphylactic scleroderma, dermatomyositis or psoriasis) are meant to serve only for identification; they should not be considered to imply any proven relationship to clinical entities that bear similar designations.

Cutaneous calcinosis induced by topical challenge.—Following calciphylactic sensitization (e.g., by DHT or parathyroid hormone), subcutaneous injection of minute doses of challengers (e.g., a few micrograms of $FeCl_2$, $FeCl_3$, $CeCl_3$, $CrCl_2$, egg white or egg yolk) or even mere plucking of the hair produces massive topical calcium precipitation in the treated skin area. As soon as 24 hours after challenge it is possible to demonstrate histochemically (e.g., with the $AgNO_3$ technic) that the calcium deposits begin to precipitate around collagen and elastic fibers in the subcutis and cutis, often also effecting the hair follicles. X-ray diffraction studies (kindly performed by Dr. A. S. Posner of the National Institutes of Health, Bethesda, Maryland) revealed that in these deposits—as in the bones and in pathologic soft-tissue calcifications of man—the calcium occurs in the form of hydroxyapatite crystals. Mild deposits of this type may subsequently be absorbed, but heavy calcium precipitates usually induce secondary inflammation with sclerosis and often even necrosis of the skin surface through which the calcified tissue debris is eliminated. In all these respects the experimentally induced lesions resemble cutaneous calcinosis as it occurs in man.

Soft-tissue calcification is generally regarded as a result of "decreased tissue vitality" secondary to chronic inflammation and necrosis. It should be emphasized, therefore, that the calciphylactic challengers produce no demonstrable tissue damage (as judged by the absence of inflammation or necrosis) in the nonsensitized organism and that even after sensitization calcification precedes any histologically detectable sign of tissue damage. Numerous observations suggest that at least some types of injury may predispose tissues to calcification under certain conditions; yet, in view of the experimental observations just mentioned, we must also admit that the precipitation of endogenous calcium can be the cause rather than the result of inflammation and necrosis.

The fact that nonspecific tissue damage is not the decisive factor in the induction of calciphylactic skin lesions is further substantiated by

* Available for example in the form of the trade preparations Imferon®, Imposil®, of the Benger Laboratories, London, England.

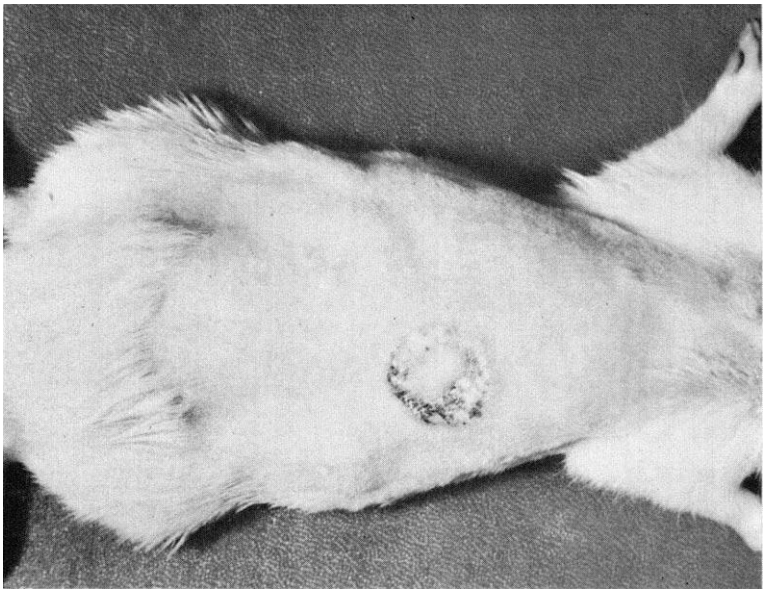


FIG. 1. *Calciphylactic wheal with central "overchallenge."* Circinate cutaneous calcinosis on margin of skin patch infiltrated with 10% yolk. Central area remains unaffected.

other findings. If the skin of a sensitized animal is treated with very heavy doses of chemical challengers or intense mechanical trauma, calcification occurs only in the form of circinate lesions around the directly affected area and not in the most severely damaged central tissue region itself. (Fig. 1).

In their histologic structure, the calciphylactically induced skin lesions are virtually indistinguishable from cutaneous calcinosis and scleroderma seen in man, but of course, this does not prove an essential similarity in their evocative pathogenetic mechanisms (Fig. 2 and 3).

It may be mentioned incidentally that, if most of the body surface of a sensitized rat is infiltrated with a challenger (e.g., egg white), the entire challenged area undergoes calcification with subsequent detachment of the resulting hard "carapace". This phenomenon is reminiscent of the molting or exuviation that normally occurs in lower animals (e.g., crustaceans and snakes). Normal molting is usually accompanied by a considerable increase in the calcium content of the affected skin, but here again, we wish to point out that we have no proof of any participation of calciphylactic reactions in the mechanism of physiologic molting. (Fig. 4).

Calciphylactic scleroderma with esophageal and joint lesions.—In calciphylactically sensitized (e.g., DHT-pretreated) rats the intravenous injection of certain challengers, such as ferric dextrin (Fe-Din) or Thorotrast®, elicits a syndrome reminiscent of calcifying scleroderma. It is usually accompanied by severe esophageal lesions and sometimes by calcareous bursitis (Figs. 5–7).

Although it is generally estimated that approximately 40% of the patients who suffer from calcinosis universalis also exhibit manifestations of scleroderma, macroscopically visible calcification is by no means a constant accompaniment of the latter disease in man. Nevertheless, some relationship between scleroderma and calcium metabolism is suggested by the therapeutic effect of calcium chelators in this as well as in many other collagen diseases. Furthermore, both calcifying scleroderma and esophageal lesions due to systemic interventions are extremely rare and, hence, the association of such changes both in clinical scleroderma and in certain calciphylactic syndromes is noteworthy. Therefore, it may be well to give serious attention to the possibility that derangements in calcium metabolism similar to those induced by calciphylaxis may play a role in scleroderma and perhaps also in other related collagen diseases.

Psoriasiform calciphylaxis.—If following the usual sensitization rats are challenged by the

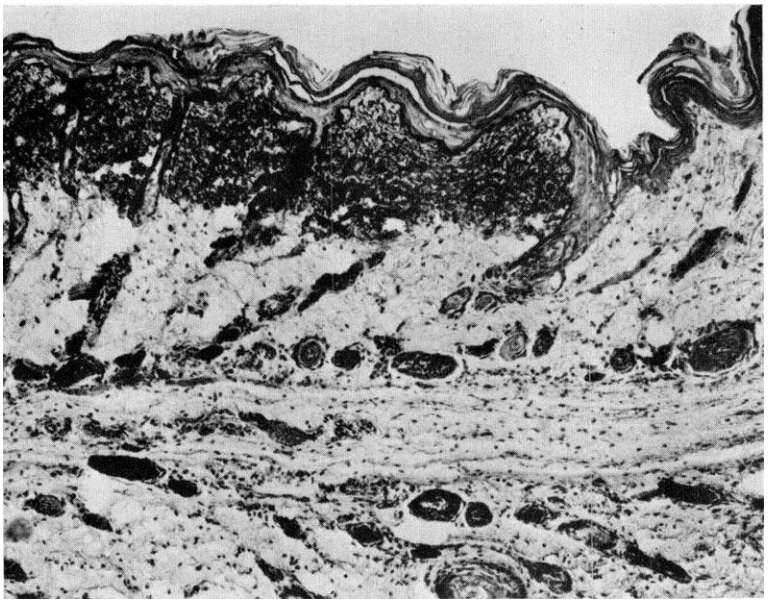


FIG. 2. *Topical cutaneous calcinosis produced by DHT + plucking of scalp hair.* Rat sensitized with DHT and challenged by plucking hair over scalp and back. Histologic appearance of typical calcified skin wheal in epilated area. Calcium deposition is limited to subepithelial connective-tissue fibers of sharply circumscribed region. (von Kóssa, × 120.)

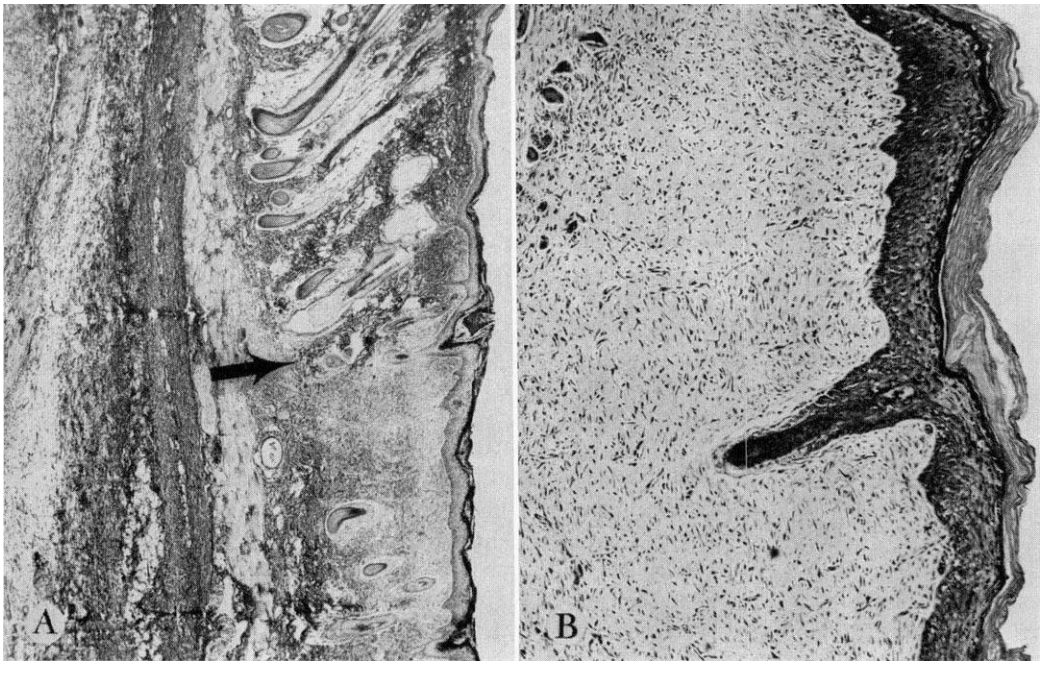


FIG. 3. *Various chronic forms of cutaneous calcinosis produced by DHT + epilation (hair plucking).* *A:* Borderline between healthy and sclerosed skin during period of healing. In the affected region (below arrow) there is dense connective tissue, but sebaceous glands are absent, hair follicles rare, and the epidermis is thickened. (Fuchsin, × 23.) *B:* Calcium is no longer visible in dense connective tissue of this healing patch. Only few calcified granules remaining in foreign-body giant cells near upper left corner. (von Kóssa, × 65.)

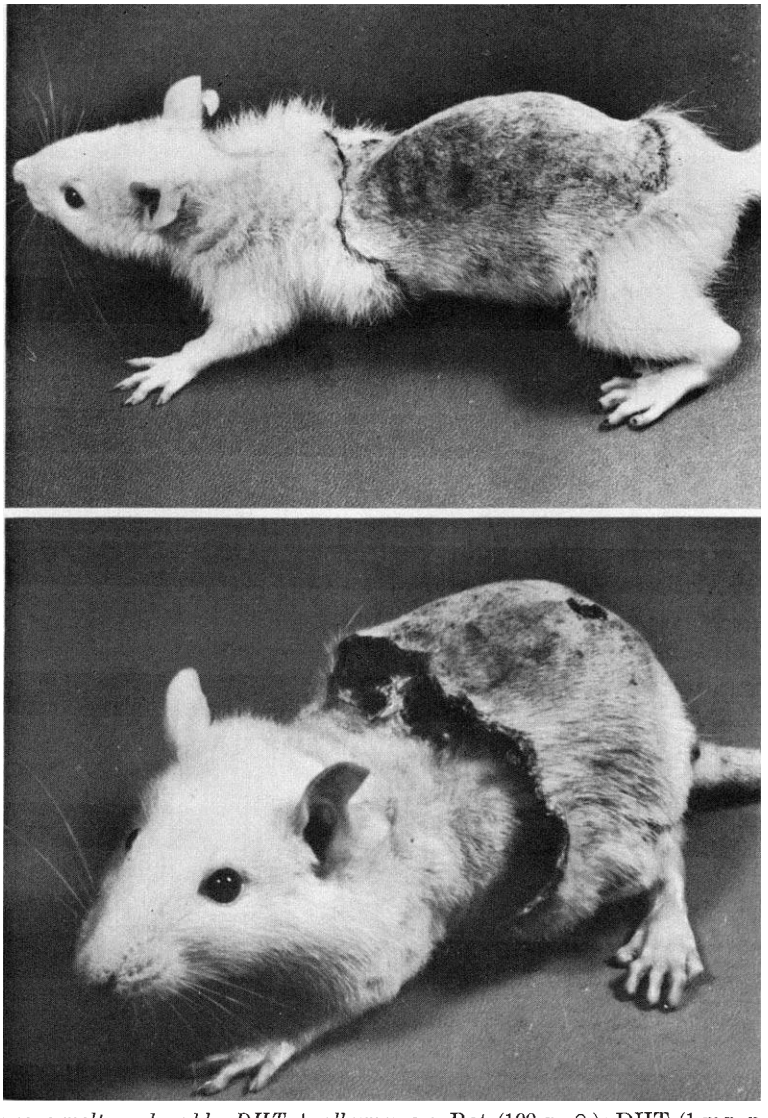


FIG. 4. *Cutaneous molt produced by DHT + albumen s.c.* Rat (100 g. ♀): DHT (1 mg. p.o.) 1st day + albumen (50%, 10 ml. s.c. infiltration of entire body surface, except head, ano-genital region and extremities) 2nd day; killed 25th day. *Top:* Complete circular skin carapace formed by cutaneous calcinosis of infiltrated region (photographed 18th day). *Bottom:* Rat exuviates its old integument and emerges with new skin on 24th day.

intraperitoneal injection of a single large dose of Fe-Dex, there develops a chronic dermatosis characterized by multiple reddish-brown, more or less sharply demarcated, dry papules and plaques covered by a thin layer of silvery scales. The lesions are usually symmetrically distributed on the back and thighs, often also affecting the chest and scalp. During the initial stages there may be widespread erythroderma with exfoliative dermatitis. Even gentle removal of the scales produces typical, slightly bleeding surface patches, not unlike those of Auspitz' sign in clinical psoriasis (Fig. 8). However, these experimental lesions differ fundamentally from true psoriasis in that they are invariably accompanied by more or less pronounced calcium precipitation in the affected regions. Hence, in this case it is particularly doubtful whether any relationship exists between the experimental condition and the clinical disease, which it resembles only superficially.

Calciphylactic dermatomyositis.—Widespread

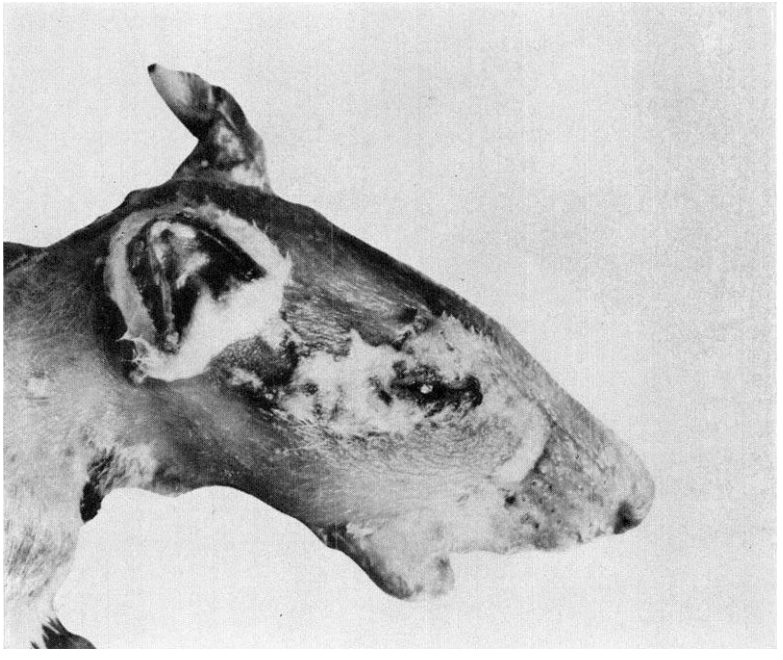


FIG. 5. *Cutaneous calcinosis of the face produced by DHT + Fe-Din i.v.* Rat (110 g. ♀): DHT (1 mg. p.o.) 1st day + Fe-Din (1 ml. = 20 mg. Fe, i.v.) 2nd day; died 5th day. After shaving head and cutting off right external ear at root, white calcium deposits become clearly visible in skin of snout, lips, eyelids and their surroundings, as well as at root of external ear.

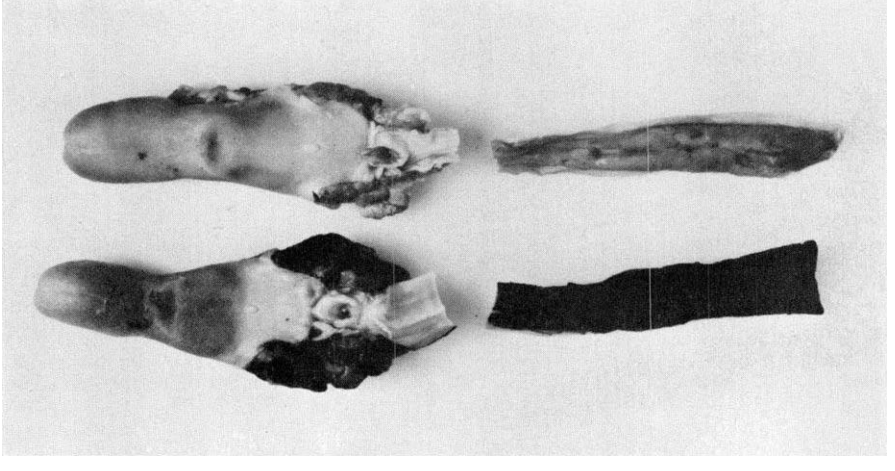


FIG. 6. *Calcification of the tongue, pharynx and esophagus produced by DHT + Thorotrast® i.v.* Rat (100 g. ♀): DHT (1 mg. p.o.) 1st day + (bottom) Thorotrast® (1 ml. i.v.) 2nd day; killed 6th day. Pronounced calcium deposition at root of tongue on both sides of glottis as well as along entire length of esophagus except its most cranial portion. *Top:* Fresh. *Bottom:* $AgNO_3$-stained specimen.

inflammation of the skin and muscles can be elicited in DHT-sensitized rats if they are subsequently given an intravenous injection of Fe-Dex simultaneously with a subcutaneous injection of a mastocyte discharger, such as polymyxin or 48/80. Under these conditions, there develops a symmetrical inflammatory edema, particularly in the neck, affecting the skin, the fascial layers of the nuchal muscles, and the forelimb musculature. This inflammation is

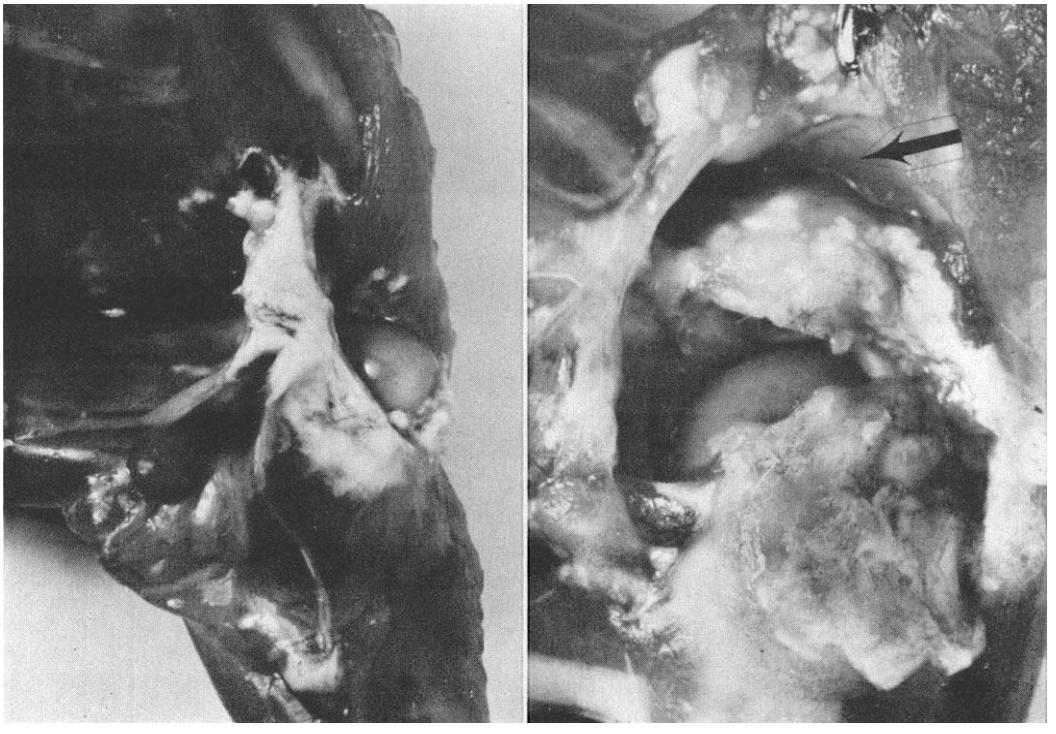


FIG. 7. *Articular lesions produced by DHT + Thorotrast® i.v.* Rat (210 g. ♀): DHT (2 mg. p.o.) 1st day + Thorotrast® (1 ml. i.v.) 2nd day; killed 10th day. *Left:* Ring-shaped calcium deposition in and around shoulder joint. Exposed head of humerus shows intact cartilage. *Right:* Opened shoulder joint exposes subacromial bursa (arrow); white calcium deposits throughout joint region.

associated with calcium precipitation in varying degrees as well as edema, hyalinization and fragmentation of muscle fibers. These changes are reminiscent of the lesions that characterize dermatomyositis in man, a disease that is likewise frequently associated with calcium precipitation in the involved areas.

Histologic study of the skin and muscle regions affected by calciphylactic dermatomyositis reveals that in this case the mastocytes play a prominent pathogenetic role. Under the influence of the histamine discharger, the mastocytes release their metachromatic granules, which subsequently become impregnated with iron and calcium salts. It is highly probable that here the role of the metachromatic mastocyte material is merely to attract the irritating calcium salts, which then induce edema and inflammation. This concept is further supported by the observation that pretreatment with mastocyte dischargers prior to sensitization protects against the subsequent induction of calciphylactic dermatomyositis by the usual procedure (Figs. 9 and 10).

Influence of deviating calciphylaxis upon the skin.—We have already mentioned the fact that generalized impregnation of the connective tissue, particularly the histiocyte system, with granules of a challenger (e.g., Fe-Dex) can protect the organism against various forms of soft-tissue calcification. For example, if a normal (nonsensitized) rat receives repeated intraperitoneal injections of Fe-Dex, the iron compound is rapidly absorbed from the peritoneum and produces a general hemosiderosis, owing to the deposition of iron particles not only in the reticulo-endothelial system but also in the histiocytes, fibroblasts and to a lesser extent the collagen fibers of the connective tissue throughout the body. Rats thus pretreated become singularly resistant not only to the various forms of calciphylaxis but also to the calcium precipitation normally induced by the subcutaneous injection of direct calcifiers or the generalized, predomi-

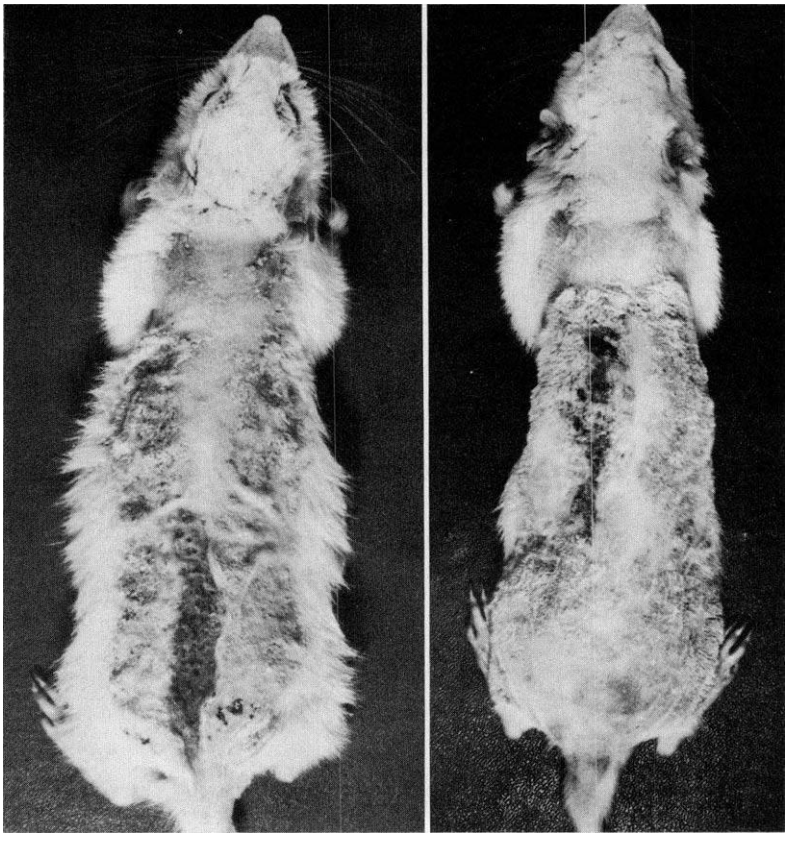


FIG. 8. *Calciphylactic psoriasis produced by DHT + Fe-Dex i.p.* Rat (200 g. ♀): Fe-Dex (1 ml. = 50 mg. Fe, i.p.) 1st day + DHT (2 mg. p.o.) 4th day; photographed 15th day. *Left:* Extensive nodular calcium infiltration with marked scaling of skin. Scale removal (by gentle scratching along midline over lumbar and sacral regions) caused diffuse surface bleeding corresponding to Auspitz' sign in psoriasis. *Right:* Auspitz' sign in another similarly treated rat with less advanced lesions.

nantly vascular, cardiac and renal calcinosis that is otherwise induced by acute overdosage with vitamin-D derivatives or parathyroid hormone.

More recently my associate, Dr. Ralph Strebel, and I observed that if rats are chronically treated with very small doses of DHT, they do not tend to show calcification in the cardiac muscle and renal parenchyma, such as is elicited by heavy, acute intoxication with the same compound; instead, they develop a syndrome reminiscent of progeria. Calcification occurs almost exclusively in the arterial system, the cartilages of the ribs, larynx and vocal cords. There is also kyphosis with calcification of the intervertebral discs and deformation of the vertebrae, intense catabolism, loss of cutaneous elasticity with wrinkling of the skin, loss of hair and discharge of cutaneous mastocytes. The teeth tend to spread apart, thereby causing malocclusion with erosion of the enamel. Occasionally there is cataract formation. All these lesions are strikingly similar to those characteristic of advanced senility, but unlike in the latter condition, they are not accompanied by osteoporosis—on the contrary, the skeleton becomes unusually sclerotic. Despite their bulk, the affected bones are not particularly strong and tend to be deformed by the stress of weight bearing. In the course of bone reconstruction the original Haversian lamellar pattern is destroyed and replaced by random foci of newly formed osseous tissue. Furthermore, narrow cement lines can be seen between the original and newly formed lamellar systems; these create a characteristic "mosaic pattern," such as is typical of osteitis deformans (Paget's disease).

It remains to be seen whether there is any

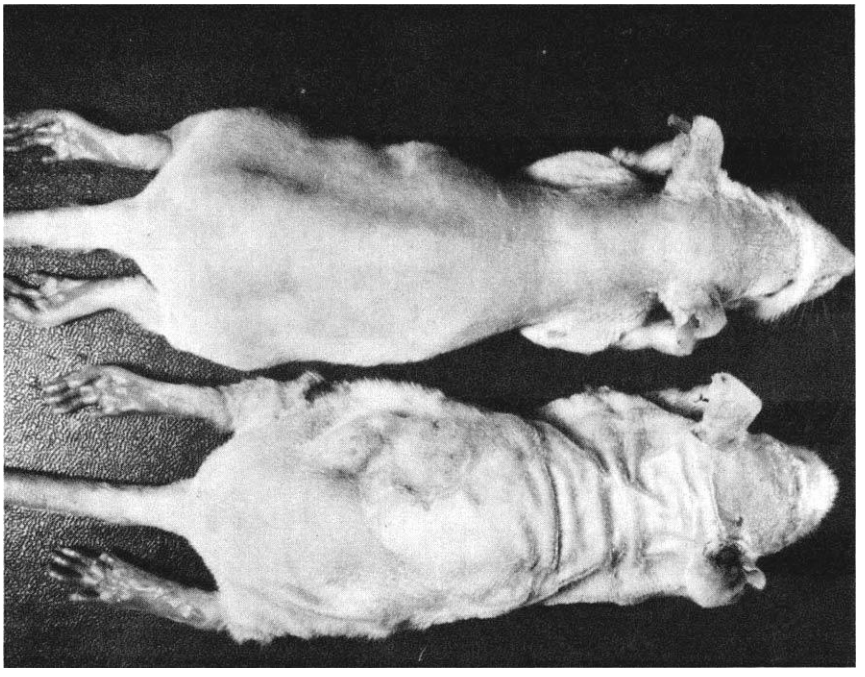


FIG. 9. *Calciphylactic dermatomyositis produced by DHT + Fe-Dex i.v. + PMX s.c.* Two rats (100 g. ♀): DHT (1 mg. p.o.) 1st day + Fe-Dex (1 ml. = 1 mg. Fe, i.v.) 2nd day + (bottom) PMX (2 mg. in 0.2 ml. water, s.c. in left flank region) 2nd day; killed 6th day. *Top:* Combined treatment with DHT + small dose of Fe-Dex produced no obvious macroscopically visible change. *Bottom:* Additional administration of PMX elicited particularly severe calciphylactic dermatomyositis, characterized by thick infiltration of skin and by swelling of musculature, especially in head and neck regions.

relationship between this experimental disease and progeria, true senility or osteitis deformans. However, it is interesting, in any case, that all the changes induced by this form of chronic DHT intoxication can be completely prevented by Fe-Dex. While all animals treated with DHT alone eventually succumb, those that in addition receive Fe-Dex survive in apparently perfect health (Fig. 11).

Histologically, the skin of the rats treated with DHT alone exhibits many changes characteristic of senility. The surface epithelium, sebaceous glands, and the cutaneous muscles are atrophic and the adipose tissue has virtually disappeared. The collagenous connective tissue appears to be shrunken and dense owing to the practically total absorption of the fluid matrix between the fibers. Particularly thick sheaths of dense connective tissue are found around the cutaneous nerves and, to a lesser extent, around the arteries that are severely affected by Mönckeberg sclerosis.

An especially striking feature of this lesion is the nearly complete degranulation of all mastocytes throughout the cutis and subcutis. However, unlike following mastocyte depletion by histamine-dischargers (e.g., polymyxin, 48/80), the extruded metachromatic granules do not disappear but remain in the connective tissue between the fibers especially in the vicinity of blood vessels. Here they form dense, perivascular cuffs in and around the adventitia sometimes apparently penetrating into the vascular wall.

By contrast in the animals protected by Fe-Dex, virtually all the cutaneous structures remain normal, though heavily infiltrated by iron containing phagocytes. Only one result of DHT-overdosage is uninfluenced by Fe-Dex: the discharge and perivascular accumulation of mastocyte granules. Here the metachromatic granules are frequently seen in close association with iron particles around the blood vessels; indeed sometimes both types of granules can be identified within the body of the same cell.

The functional significance of this extraordinarily pronounced accumulation of mastocyte debris around the blood vessels remains to be elucidated. However, since calcifiable tissue is

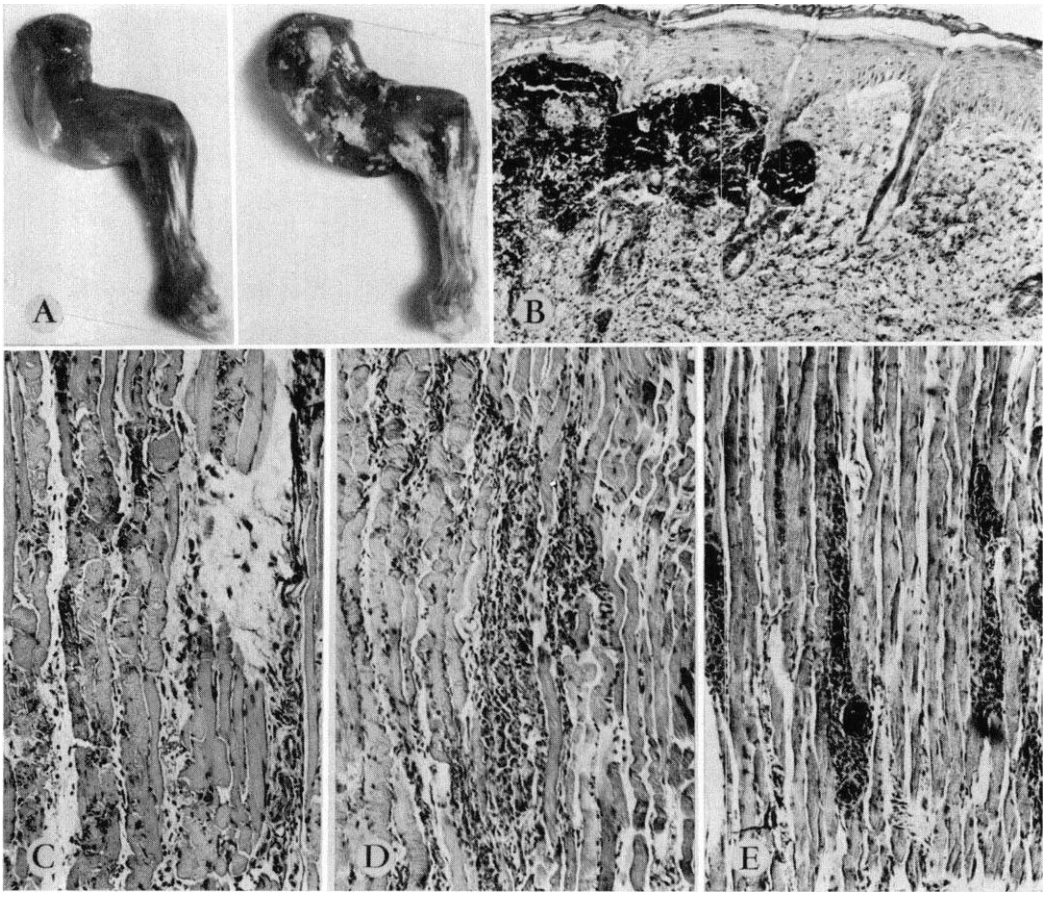


FIG. 10. *General appearance of the musculocutaneous lesions produced by DHT + Fe-Dex i.v. + PMX s.c.* Two rats: DHT + Fe-Dex + (All but A, left) PMX. *A:* Only animal on right shows lesions in and around muscles of left front paw. *B:* Histologic appearance of severely affected skin after DHT + PMX + Fe-Dex. Scaling with inflammation and calcification of derma. Liquefaction necrosis along epithelial attachment and (left upper corner) within epithelium itself. (von Kóssa, × 95.) *C:* Edema, hyalinization and fragmentation of muscle fibers in acute stage. (von Kóssa, × 95.) *D and E:* Nuclear proliferation and inflammation in more advanced stage. Dark spots correspond to calcium deposits. (von Kóssa, × 95.)

Figs. 1–10 after Selye (9). Courtesy of the University of Chicago Press

frequently metachromatic, it may well be asked whether a perivascular cuff of mastocyte granules could not deviate calcium from the media where it would otherwise form unabsorbable thick plaques. If this interpretation were correct, the accumulation of free mastocyte debris might be looked upon as physiologic defence reaction, which offers essentially the same kind of protection as Fe-Dex. Both the mast cell and the iron particles could provide innumerable minute foci of material which by virtue of its own calcium affinity, could protect the adjacent vessels against mineralization by successfully competing with them for calcium. It is not incompatible with this view that, of all the lesions induced by DHT, only the accumulation of discharged mastocyte granules fails to be prevented by Fe-Dex prophylaxis since this particular change does not appear to be a manifestation of damage but of defence.

In this connection, it is noteworthy that intravenously injected India ink particles are normally deposited around free mastocyte granules —for example, when mastocyte discharge is produced by topical trauma or by treatment with histamine discharging agents—while after DHT-

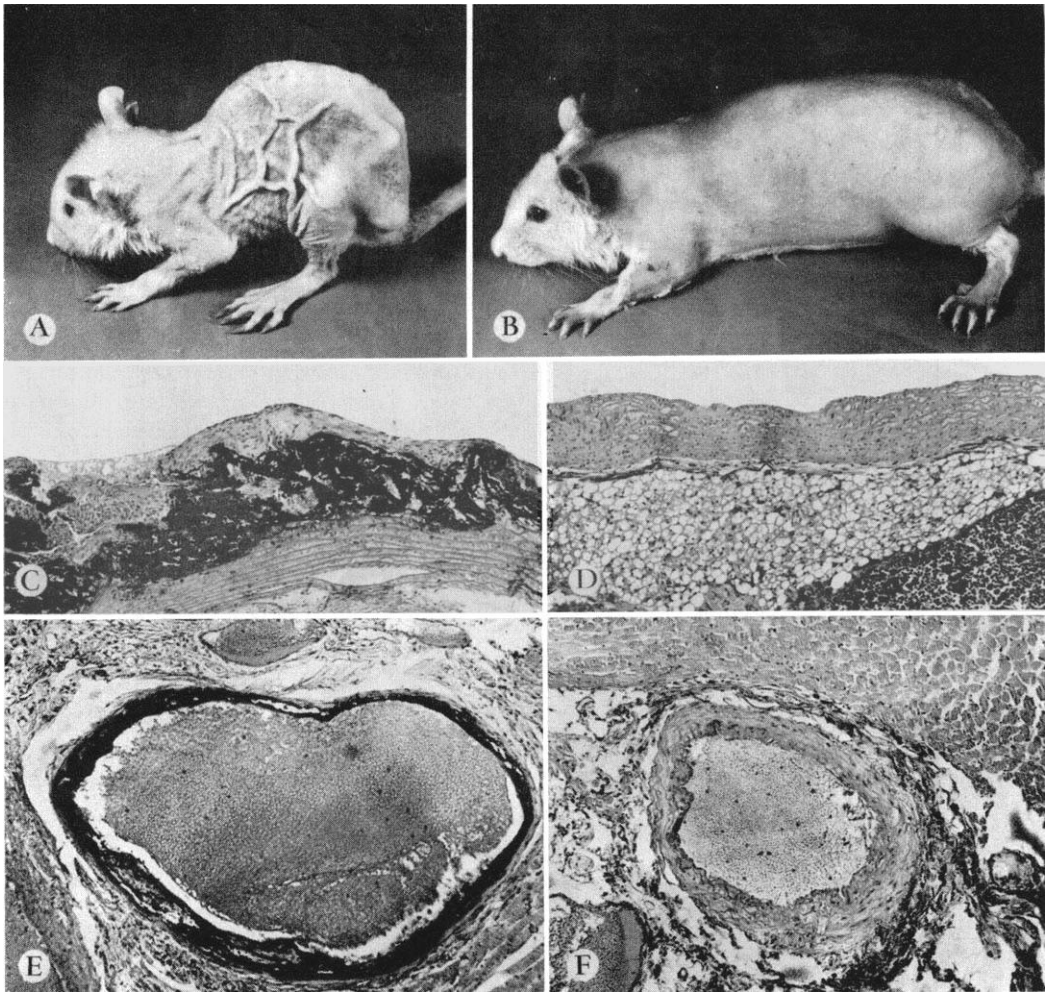


FIG. 11. *Prevention of DHT intoxication by Fe-Dex. A:* General appearance of a rat that received DHT only. Marked kyphosis. Pelvic bones and ribs visible through wrinkled, inelastic skin. *B:* Rat treated with DHT + Fe-Dex; essentially normal appearance. Both these rats were shaved for better visualization of body. *C:* Aorta near arch, and *E:* left circumflex coronary artery of rat treated with DHT alone shows intense calcinosis and distortion. *D* and *F:* Corresponding vessels of rat treated with DHT and Fe-Dex are essentially normal, but adventitia contains many iron-storing (here dark) phagocytes and mast cells. (All sections von Kóssa, × 77.) [After Selye and Strebel (10), courtesy Proc. Soc. Exper. Biol. & Med.]

treatment the mastocyte debris does not attract carbon particles from the blood. It is possible—though quite unproven—that in the latter case the mastocyte granules fail to attract carbon because they are saturated with calcium. In any event, if calcium is attracted by the granules, its turnover must be very efficient, since no von-Kóssa-positive material is demonstrable on them. It is equally possible, however, that the perivascular dispersion of calcifiable metachromatic material acts merely by preventing the latter from being accumulated in the soft tissues and bones where it could induce massive calcium precipitation.

Some relationship between mastocyte granules and calcification is also suggested by the previously cited observations on calciphylactic dermatomyositis. Here, von-Kóssa-positive calcified material is histologically demonstrable around mastocyte granules (9). Furthermore, the

intense aggravation of DHT-induced arterial calcification that can be induced by mastocyte dischargers (8) might be ascribed to the destruction of mast cell granules and a consequent inactivation of an important calcium deviating mechanism. Let me reemphasize, that none of these concepts has as yet been proven; they are mentioned here only because they suggest promising new experimental approaches to our problem.

SPECULATIONS CONCERNING POSSIBLE INTERRELATIONS BETWEEN STRESS AND CALCIPHYLAXIS

We still know very little about the basic biochemical reactions regulating the L.A.S. and G.A.S. (that is, the local and general adaptation syndromes to stress), on the one hand, and the local and systemic calciphylactic reactions, on the other. Hence, it is possible to discuss the interrelations between these two types of mechanisms in only the vaguest terms. Yet, it may be opportune to compare these phenomena now, since interesting connections appear to exist between them.

Both the stress reactions and calciphylaxis evolve primarily in the mesenchyma, especially the *connective tissue* and the *vascular system.*

The principal changes elicited by the simplest form of local stress are inflammation, hyalinization and sclerosis. The "adaptive hormones," particularly the pro- and anti-inflammatory corticoids, regulate the systemic response to stress by increasing or decreasing the inflammatory potential. The "collagen diseases," which are some of the most typical "diseases of adaptation" are characterized by hyalinization of connective tissue and vascular elements owing to the deposition of PAS-positive material presumed to consist of mucopolysaccharides. Quite similar changes can be produced in animals by overdosage with the pro-inflammatory mineralocorticoids (e.g., desoxycorticosterone, aldosterone).

The most striking feature of calciphylaxis is the precipitation of calcium. However, this is possible only after the formation of a calcifiable matrix that is likewise PAS-positive and presumably rich in mucopolysaccharides. Here again, the process gives rise to inflammation with subsequent sclerosis.

Thus both the hyalinoses and calciphylaxis are predominantly diseases of the mesenchyma.

Both the response to stress and calciphylaxis are regulated by *hormones and electrolytes.*

The corticoids play a prominent role in stress, especially through their influence upon extracellular sodium and chloride. Their manifold actions appear to depend largely upon the regulation of the equilibrium between intracellular potassium and extracellular sodium.

In calciphylaxis the principal endogenous stimulus is undoubtedly parathyroid hormone, which regulates the metabolism of calcium and phosphates. (Although vitamin-D derivatives may also be synthesized in the body and thereby contribute to the action of parathyroid hormone through their essentially similar metabolic actions.)

Other hormones, especially ACTH and STH, likewise exert an important influence upon stress reactions and calciphylaxis, for example, hypophysectomy interferes with both types of reactivity. However, it would be premature to analyze the participation of the entire endocrine system in these reactions because the facts available are still too few.

The primary *purpose* (if the use of such a teleologic term may be forgiven) of the L.A.S. and G.A.S. is the induction of resistance against local and systemic stress, respectively; stress being defined as "the rate of wear and tear in a biologic system during a given period of time" (7).

On the other hand, calcification appears to be intimately related to biologic (as opposed to chronologic) aging: "the sum of all the wear and tear suffered during life." It is not yet clear whether derangements in calcium metabolism are merely the results of aging or whether the tissue changes characteristic of senility (degeneration of connective tissue, atrophy of bones and muscles, kyphosis, otosclerosis, senile cataracts, arteriosclerosis, calcification of cartilages, delayed wound healing, diminished resistance to stress, etc.) are, at least in part, consequences of a primary disturbance in calcium metabolism. The latter interpretation receives some support from the experiments on deviating calciphylaxis, in which interference with pathologic calcification resulted in the prevention of all the changes characteristic of aging, even those that are not associated with manifest calcinosis.

Finally, some relationship between stress and calciphylaxis is suggested by the observation

that most *calciphylactic syndromes can be prevented by previous exposure to stress* (9).

The skin and the skeleton are the two principal accumulations of connective tissue in the body, the former being also the main reservoir of sodium and chloride, the latter of calcium and phosphate. It is not wholly unexpected, therefore, that both the "diseases of adaptation" to stress and the calciphylactic syndromes frequently affect the skin, though skeletal participation is more common in the latter.

These concluding remarks are based on very incomplete evidence; that is why we entitled them "speculations." However, we feel that a sufficient number of objective observations has now been made regarding the responses of the skin to stress and calciphylaxis to justify an interim report of this kind before your Society. I am firmly convinced that further progress along these lines will depend largely upon the clinical evaluation of these first tentative efforts toward the creation of an Experimental Dermatology as a systematized branch of your science.

SUMMARY

Following a brief outline of the concepts of stress and calciphylaxis, a number of cutaneous calciphylactic syndromes were described.

Calciphylaxis is a condition of induced hypersensitivity in which tissues respond to appropriate challenging agents with local calcification. We distinguish a focal form, in which calcium precipitation is concentrated in a circumscribed area, from a deviating form of calciphylaxis, which, by contrast, prevents focal tissue calcification, presumably by distributing the metal evenly in the organism and thereby preparing it for excretion.

Finally, special attention has been given to the relationship between calcification and aging. Through the induction of diffuse calciphylaxis it became possible to prevent the development of many experimental organ lesions characteristic of senility.

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