infusion of calcium chloride.

Although in the present study, direct measurements of the diffusible calcium were not obtained, the previous investigation(1) did not demonstrate a significant difference between the per cent diffusible calcium in individuals receiving infusions of calcium gluconate and calcium chloride. However, the accuracy of ultrafiltration technics are such that small, but possibly physiologically significant, changes in per cent diffusible calcium could not be detected. If the diffusible component of serum calcium were slightly greater during the calcium gluconate infusion this would contribute to the differences observed between the two salts in the present study.

Following infusions of calcium gluconate in man there is a rise in urinary organic acid, primarily gluconate(7). It has been suggested that the gluconate ion may restrict the reabsorption of calcium from the tubular fluid (3), and this is the most likely explanation for the greater clearance of calcium during the gluconate infusion.

Summary. A calcium infusion of either the chloride or gluconate salt raised the filtered load of calcium an equivalent amount. At comparable filtered loads the clearance of calcium during the infusion of calcium gluconate was higher than during the infusion of calcium chloride. The interpretation of results of tests utilizing calcium infusions should take into consideration the type of calcium salt infused.

The authors wish to acknowledge the technical assistance of Mrs. C. Brewer, Mrs. A. Bishop and Miss R. Twiss.

1. Kleeman, C. R., Bernstein, D., Rockney, R., Dowling, J. T., Maxwell, M. H., *Yale J. Biol. and Med.*, 1961, v34, 1.

2. Lassiter, W. E., Gottschalk, C. W., Mylle, M., *Fed. Proc.*, 1962, v21, 435.

3. Howard, P. J., Wilde, W. S., Malvin, R. L., *Am. J. Physiol.* 1959, v197, 337.

4. Chen, P. S., Neuman, W. F., *ibid.*, 1955, v180, 623.

5. Walser, M., Browder, A. A., *J. Clin. Invest.*, 1959, v38, 1404.

6. Freeman, F., Chang, T. S., *Am. J. Physiol.*, 1950, v160, 335.

7. Crawford, M. A., Loughridge, L., Milne, M. D., Scribner, B. H., *J. Clin. Path.*, 1959, v12, 524.

Received June 4, 1962. P.S.E.B.M., 1962, v110.

Prevention by Calciphylaxis of the Progeria-Like Syndrome Induced by Chronic Dihydrotachysterol Overdosage. (27613)

HANS SELYE AND RALPH STREBEL*
Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

Calciphylaxis enables the organism to deposit calcium selectively in certain areas. Thus, following pretreatment with parathyroid hormone or dihydrotachysterol (DHT), various calciphylactic challengers (egg white, metals, serotonin, etc.) can induce selective calcification—often with inflammation, sclerosis, necrosis or degeneration—in the skin, muscles, cardiovascular system, pancreas, salivary glands, or uterus. The phenomenon represents an induced hypersensitivity reaction to certain compounds not known to be antigenic in the usual sense(1-5).

* Fellow of U. S. Nat. Inst. Health.

However, under given circumstances, calciphylaxis can also exert a considerable prophylactic effect against certain toxic compounds. Thus, the fatal generalized soft-tissue calcinosis, normally induced by acute overdosage with DHT, fails to occur if a calciphylactic challenger is brought into contact with a large connective-tissue surface. For example, if, after sensitization with DHT, albumen or iron compounds are injected into a subcutaneous air sac (granuloma pouch technic), enormous amounts of calcium precipitate in the directly challenged area and are thereby deviated from the more vital or-

gans (heart, kidney), in which they would otherwise produce fatal damage(6).

Chronic DHT-intoxication causes no generalized soft-tissue calcinosis, but a syndrome reminiscent of progeria. There is marked catabolism, loss of appetite, muscular weakness, wrinkling of the skin with loss of hair, cataracts, dental decay, collapse with calcification of intervertebral discs and kyphosis, but gross calcinosis is limited to the arteries and some cartilages (ribs and larynx). The question arose whether calciphylaxis could also protect against this progeria-like syndrome and, if so, whether such a prophylactic effect could be accomplished without any massive calcium deposition in a directly challenged area.

Materials and methods. Twenty female Holtzman rats with a mean initial body weight of 215 g (range 200-228 g) were subdivided into 2 equal groups, both of which received dihydrotachysterol, or "DHT" (Calcamin®, Wander) at the dose of 50 μg in 0.5 ml corn oil *per os* daily. Group 1 received no other treatment. Group 2 was given, in addition to DHT, a preparation of ferric hydroxide with dextran, or "Fe-Dex" (Imposil®, Benger) in amounts equivalent to 50 mg of metallic iron in 1 ml water i.p. every 5 days, beginning on the fifth day after initiation of DHT treatment. The rats were kept on ground "Purina Laboratory Chow" and tap water. The food had to be ground because the animals receiving no Fe-Dex soon developed dental anomalies, which prevented them from chewing the hard Purina cubes. All survivors were killed on the 50th day. Representative specimens from various organs were fixed in alcohol-formol (4 parts of absolute alcohol and one part of 10% formalin) for subsequent embedding in paraffin and staining with hematoxylin-phloxine (general structure), PAS (hyaline deposits), methylene blue (metachromatic material), Prussian blue (iron deposits) and celestin blue as well as the von Kóssa technics (calcified foci).

Results. During the first 10 days of the experiment, the animals of both groups lost approximately 30 g of body weight. Group 2 soon regained and in many instances even exceeded their original weight. Group 1 continued to lose weight during the entire observation period and, under the influence of DHT alone, the skin became wrinkled, the hair ruffled and sparse, the incisor teeth developed malocclusion and became separated along the midline, while the gingivae showed erosions, often exposing the proliferating edge of the alveolar processes. In addition, every animal of this group developed a pronounced xiphosis in the lower thoracic region and the deformed spinal column could not be reduced even by strong pressure (Fig. 1). Six animals of Group 1 died and the survivors were moribund by the end of the experiment; one rat developed a cataract. Conversely, the rats of Group 2 remained in perfect health and showed no visible anomaly apart from the brownish discoloration of the skin characteristic of iron deposition and occasional abscesses in cervical lymph nodes.

At autopsy widespread calcification with dilatation and hardening was observed not only in the aorta but in the entire arterial system (bamboo stick vessels) of every rat in Group 1. The coronary arteries were also markedly affected, while the cardiac muscle itself as well as the skeletal musculature and the renal parenchyme—all of which show extensive metastatic calcification as a result of acute DHT-overdosage—remained free of macroscopically visible calcium deposits. There was also intense calcification in the costal cartilages, xiphoid cartilage, trachea, vocal cords and intervertebral discs. The latter became very narrow and rigid, especially

TABLE I. Prevention by Calciphylaxis of Chronic Dihydrotachysterol Overdosage.

Group	Treatment	Body wt (g) Initial	Final	Gross calcification (arbitrary scale: 0 to 3) Heart	Vessels	Kidney	G.I. tract	Mortality (%)
1	DHT	214.6 ± 2.78	108.8 ± 1.44	3	3	0	1	60
2	DHT + Fe-Dex	215.4 ± 2.19	227.6 ± 3.20	0	0	0	0	0

in the region of the kyphosis. Throughout the skeleton there was marked osteosclerosis with both endosteal and periosteal proliferation but, apart from Mönckeberg sclerosis of the arteries, soft-tissue calcification was virtually limited to the smooth musculature and the mucosal stroma of the stomach. None of these lesions could be detected in any of the animals that received Fe-Dex in addition to DHT.

Histologic study confirmed the predominantly arterial localization of the calcinosis in Group 1, while in Group 2 the arteries were either free of calcium or contained only dust-like, minute granules of von Kóssa-positive material, usually in close asscciation with Prussian-blue-positive iron deposits, mastocytes and free metachromatic mastocyte granules (Fig. 1).

Discussion. Most of the early work on calciphylaxis was concerned with the production of soft-tissue calcification. Only when, using

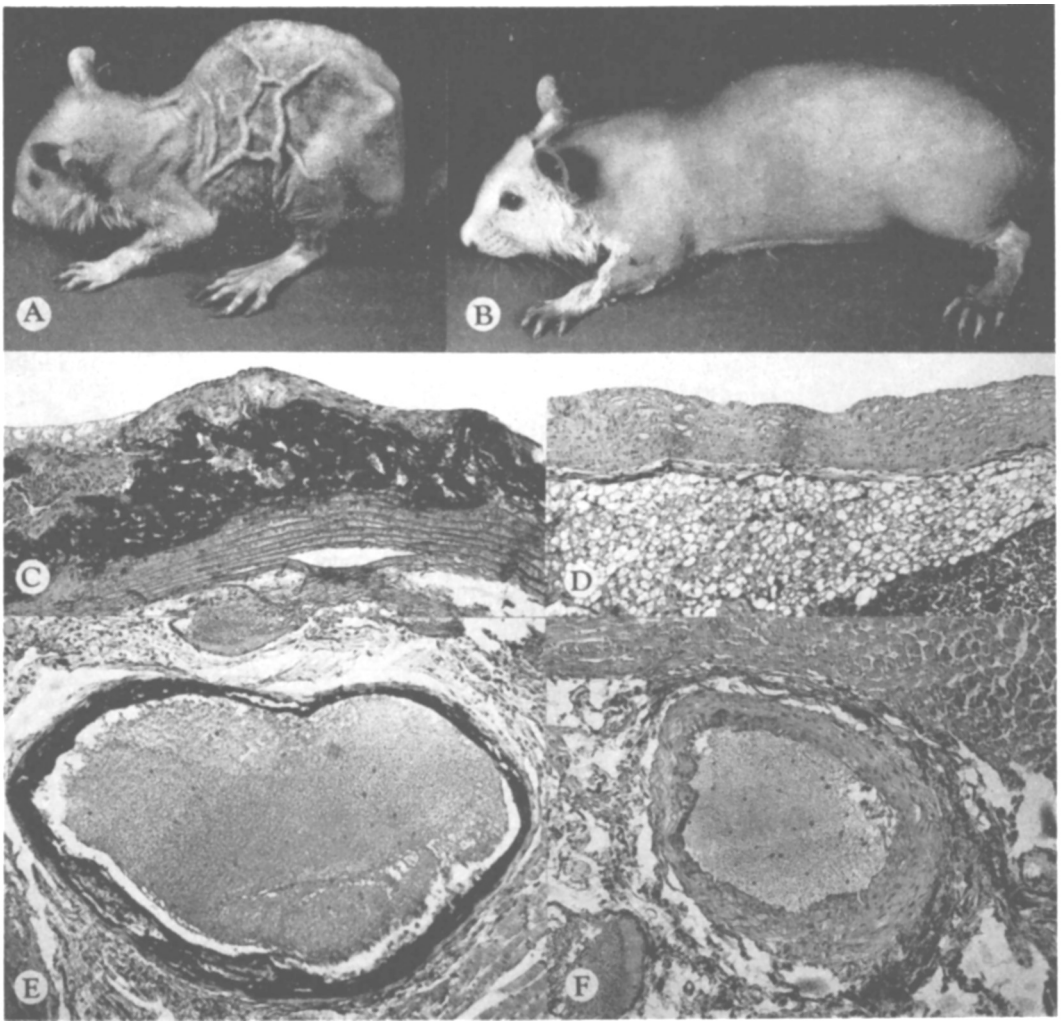

FIG. 1. Prevention of DHT intoxication by Fe-Dex. A: General appearance of a rat from Group 1 (DHT alone). Marked kyphosis. Pelvic bones and ribs visible through wrinkled, inelastic skin. B: Rat from Group 2 (DHT + Fe-Dex) of essentially normal appearance. Both these rats were shaved for better visualization 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 + Fe-Dex are essentially normal, but adventitia contains many iron-storing (here dark) phagocytes and mast cells. (All sections von Kóssa × 84.)

the granuloma pouch technic, extensive areas of connective tissue were exposed to challengers, was it possible to show that the reaction can also protect against the generalized calcinosis of acute DHT-overdosage. However, there, the prophylactic value of the response was attributed merely to a shift of the available calcium to the challenged granuloma-pouch area, leaving no calcium salts available for deposition elsewhere. By contrast, in the present experimental series a prophylactic effect was obtained without producing a calcium-fixation focus. Here, the intraperitoneally injected iron, administered in the readily diffusible and absorbable form of Fe-Dex, did not cause any selective massive challenge and calcium fixation at the injection site; it formed innumerable minute iron deposits fairly evenly distributed throughout the body, but none of these was apparently so much larger than the others as to compete with them successfully by attracting unabsorbable massive deposits of calcium salts. It should be mentioned, incidentally, that it is quite possible that challengers other than Fe-Dex may possess a similar effect, but up to now neither dextran alone nor any of the iron preparations tested (*e.g.*, ferric oxide saccharate, $FeCl_2$, $FeCl_3$) equalled the highly diffusible Fe-Dex in this respect.

Histochemical observations revealed that the iron granules are phagocytosed by countless reticulo-endothelial cells and fibroblasts in all parts of the organism, especially in and around metachromatically staining mastocyte granules. It has been shown that phagocytosis of other particulate substances (*e.g.*, India ink or even calcium granules) also tends to occur in the vicinity of disrupted mastocytes(1). The protection offered by Fe-Dex against DHT-intoxication might be due to formation of innumerable "calcium turnover points" at sites where minute iron granules are deposited under the influence of mastocytes. This hypothesis is still far from being proven, but if it were correct, it would explain the prevention of massive organ calcification by the widespread distribution of calcium to numerous dust-like granules, from which it can be easily mobilized for subsequent excretion, without formation of massive unabsorbable deposits at any one point.

The many similarities between chronic DHT-intoxication and the clinical syndrome of progeria in children (Hutchinson-Gilford syndrome) and adults (Werner's disease) has often been emphasized. Both in the child and in the adult progeria is frequently accompanied by wrinkling of the skin, cataracts and Mönckeberg's sclerosis of the arteries, as shown by the many case reports which we have recently surveyed(1). These similarities in appearance do not prove any fundamental relationship in pathogenesis, but it is noteworthy that experimentally produced manifestations of this type can be consistently prevented by a calciphylactic technic.

Summary. Experiments on rats indicate that the syndrome of chronic intoxication with dihydrotachysterol (DHT), including mortality, the Mönckeberg-type of arteriosclerosis, the loss of weight, and the loss of skin elasticity, can be prevented by repeated intraperitoneal administration of ferric hydroxide with dextran (Fe-Dex).

The experimental work on which this article is based was supported by grants from National Institutes of Health, U. S. Public Health Services and from Nat. Heart Foundation of Canada.

1. Selye, H., *Calciphylaxis,* Univ. of Chicago Press, 1962.

2. Selye, H., Grasso, S., Gentile, G., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 600.

3. Selye, H., Dieudonné, J.-M., Veilleux, R., *ibid.,* 1962, v109, 232.

4. Selye, H., Gentile, G., Prioreschi, P., *Science,* 1961, v134, 1876.

5. Selye, H., Jean, P., Veilleux, R., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 409.

6. Vasku, J., Padmanabhan, N., Selye, H., *Vnitrni lékar,* in press.

Received April 23, 1962. P.S.E.B.M., 1962, v110.