and a titer reduction of 6 logarithmic units predicted from Fig. 1. The mean titer reduction actually obtained was probably closer to 5 logarithmic units, which would indicate that the effective quantum density at a flow-rate of 150 ml/min was somewhat less than the calculated value. These experiments demonstrate, however, that differential inactivation of SV_{40} in poliovirus preparations is feasible at flow rates applicable to commercial production. Experiments on a full production scale using naturally infected tissue culture fluids would be necessary to determine whether or not this process, or some variation of it, can contribute appreciably to the safety of live attenuated poliovirus vaccine. Additional information would also be needed to verify that stability and immunogenic potency of the poliovirus are not appreciably reduced by the treatment.

Summary. The vacuolating virus, SV_{40}, is inactivated by visible light in the presence of toluidine blue (6 μg/ml) at quantum densities which have previously been shown to have almost no effect upon infectivity of poliovirus. The possible application of this process as a means of differential inactivation of SV_{40} in suspensions of live poliovirus was demonstrated by continuous-flow experiments.

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Calciphylactic Muscular Dystrophy Induced by DHT plus 5HT.* (27163)

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Calciphylaxis is a condition of induced systemic hypersensitivity in which, during a "critical period" after sensitization by a systemic calcifying factor (*e.g.*, vitamin-D compounds, parathyroid hormone), treatment with certain challengers (*e.g.*, metallic compounds, albumen, mastocyte depletors) causes acute local calcification with inflammation and sclerosis(1,2). Recently we found that in certain calciphylactic reactions, the precipitation of calcium within the target area may be minimal and evanescent, although even then it apparently plays an important pathogenetic role. Following sensitization with a single oral dose of dihydrotachysterol (DHT) a generalized and often fatal muscular dystrophy with minimal calcinosis can be produced in the rat by a single subcutaneous injection of 5-hydroxytryptamine (5HT).

Methods. 160 female Holtzman rats with a mean body weight of 196 g (180-212 g) were subdivided into 10 groups and treated as indicated in Table I.

Dihydrotachysterol, "DHT" (Calcamin®, Wander S.A.) was invariably administered at the dose of 2 mg in 0.5 ml corn oil by stomach tube on the fifth day. 5-Hydroxytryptamine, "5HT" (serotonin creatinine sulfate, Abbott), was given as a single dose of 5 mg in 0.5 ml water, subcutaneously but on different days, to determine the "critical period" for its calciphylactic challenging effect. Of necessity, the intensity of the muscle lesions had to be gauged semiquantitatively in terms of an arbitrary scale 0 to ++++. The means

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TABLE I. Calciphylactic Muscular Dystrophy Induced by DHT plus 5HT.

Group	No. of rats	Day of treatment with: DHT	5HT	Muscle lesions*	Mortality (%)
1	30	—	1	0	0
2	10	5	1	0	0
3	10	5	3	0	0
4	20	5	5	+	0
5	20	5	6	+	0
6	20	5	7	++++	60
7	20	5	8	++++	50
8	10	5	9	++++	80
9	10	5	12	++	0
10	10	5	15	+	0

* Scale 0 to ++++.

of these readings and the percentual mortality rate are indicated in Table I.

The experiment was terminated on the 20th day. The muscles were first examined with a dissecting loupe after removal of the skin. Some muscle specimens were fixed in alcohol-formol (4 parts absolute alcohol, 1 part 10% neutral formalin) and stained with the von Kóssa technic (for demonstration of calcium phosphate), others in Susa solution saturated with picric acid and stained with the PAS procedure.

Results. Immediately after the 5HT injection, all rats (whether or not pretreated with DHT) showed diminished muscular tonus and tended to lie flat on their abdomens or sides. In more instances, there developed a cutaneous hyperemia particularly of the acral regions, which is apparently a modified anaphylactoid response and has been seen before(3). In the animals treated with 5HT only, these derangements vanished rapidly; subsequent behavior of the rats and their appearance at autopsy was normal, except for occasional occurrence of minor degrees of the renal lesions that are characteristic of 5HT intoxication. Even when given prior to, on the day of, or on the day following DHT treatment, 5HT produced only minor and transitory motor impairment, especially in the hind legs. There was no mortality and at autopsy only insignificant muscle lesions were observed. The result was quite different when 5HT was given on the 7th, 8th or 9th day (*i.e.*, 2, 3 or 4 days after sensitization with DHT). Here, profound shock followed immediately upon administration of 5HT and many rats died within the subsequent 24 hours.

The survivors exhibited a lasting paralysis especially of the hind limbs (Fig. 1). The animals that died during the first 24 hours after 5HT treatment showed only indistinct reddish patches in the musculature. Conversely, the rats killed at termination of the experiment exhibited very obvious greyish-yellow and somewhat prominent (edematous) patches throughout the musculature, but particularly in the gluteal musculature and the extensor muscles of both fore and hind limbs (Fig. 2). 5HT treatment on the 12th and 15th day (*i.e.*, 8 or 11 days after DHT sensitization) caused similar muscle lesions but their intensity decreased in proportion to the lapse of time after DHT treatment, and there was no mortality.

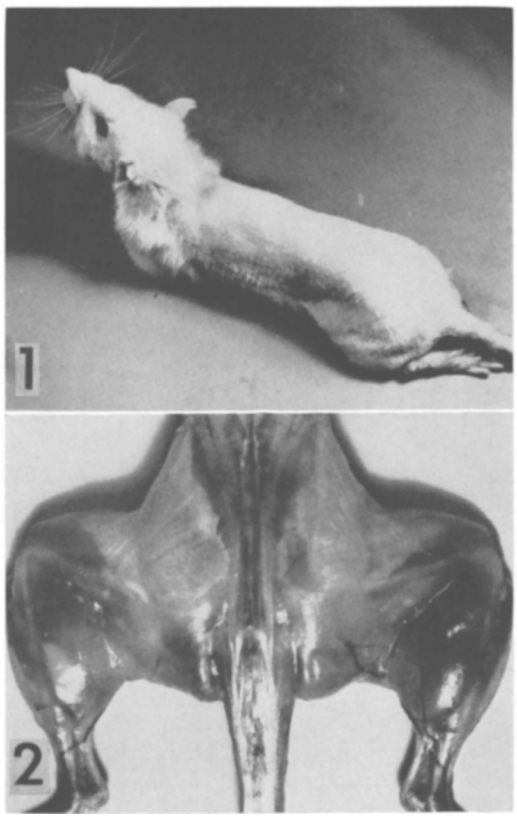

FIG. 1. The rat drags its completely paralyzed hind limbs, but motility of forelimbs is also handicapped.

FIG. 2. Large, light patches of dystrophic musculature in gluteus maximus and proximal part of quadriceps.

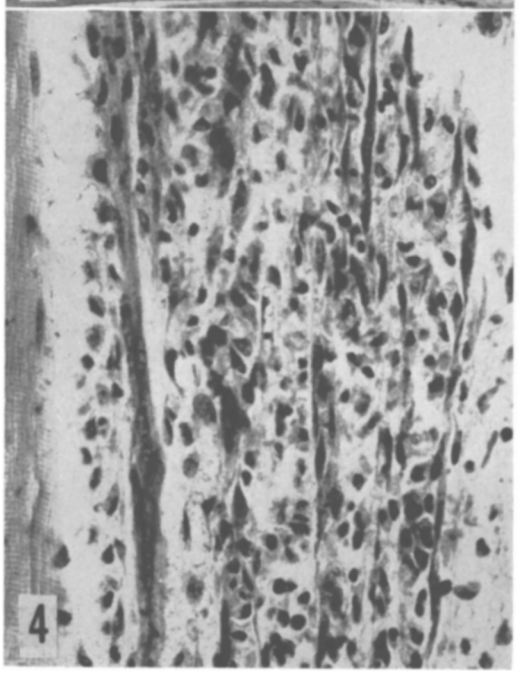

FIG. 3. Large focus of dystrophic musculature with coagulation necrosis, edema and predominantly histiocytic infiltration (v. Kóssa × 87).

FIG. 4. Higher magnification from lower portion of previous figure, showing one normal striated muscle fiber near left margin and parallel to it several rod-shaped and tubular regenerating myocytes with sarcolemma nuclei linearly arranged. Virtually no calcification is detectable in this field (v. Kóssa × 302).

Histologically, the affected regions were characterized by swelling, acidophilic waxy coagulation and disintegration of the muscle fibers with histiocytic infiltration and giant-cell formation. Edema was prominent only in animals that died early. Proliferation of sarcolemma nuclei led to formation of long chains of cells forming rod-like or tubular structures (Figs. 3 and 4). Calcification of the connective tissue or of the adjacent skin (especially characteristic of other calciphylactic responses) was not seen. Some of the necrotic muscle fibers underwent calcification, but this is quite typical of muscle tissue damaged in various ways (*e.g.*, by trauma or anoxemia).

Discussion. The muscular changes produced by 5HT under these circumstances, though dependent upon sensitization with a calcifying agent (DHT), do not exhibit a significant amount of calcification. We emphasize this fact because it suggests that the nature of similar calciphylactic reactions, should they occur in man, may easily escape attention owing to the paucity or absence of calcium granules in the affected foci. Yet, the structural resemblance of the calciphylactic muscular dystrophy to the progressive muscular dystrophy of man does not necessarily imply any fundamental relationship between them, since the histologic changes in both types of derangements are essentially nonspecific expressions of muscular injury.

Summary. In rats sensitized by a single oral dose of dihydrotachysterol (DHT), a single subcutaneous injection of 5-hydroxytryptamine (5HT), given during a "critical period" (2 to 4 days after DHT administration), produced a widespread and often fatal muscular dystrophy. However, a similar 5HT injection can cause some muscular lesions even as late as 11 days after a single dose of DHT.

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