Myotonia was induced in 20-day-old white, male and female Wistar rats with daily s.c. injections (0.2 mg/kg) of 25-azacholesterol. After 5 weeks of injections laboratory evidence of myotonia was clearly established in the anterior tibialis and gastrocnemius muscles of male rats. The soleus muscle of these animals remained completely normal. Electromyographic examination of the fast-twitch muscles showed persistent spontaneous activity and myotonic responses (trains of muscle action potentials oscillating in frequency and amplitude). At the end of this 5-week period clinical manifestations of the disease were not apparent in either the male or female rat.

Female rats treated equivalently showed either very little or no abnormal electrical activity in the fast-twitch muscles. Thus, after the 5-week period of administration of the cholesterol analog only the fast-twitch muscles of male rats develop electrical evidence of myotonia whereas female rats and the soleus in the male remain normal.

When daily injections were continued for an additional 2 weeks the effects were quite striking (see Figure). Clinical symptoms of myotonia became obvious. This was especially so in male rats, resembling those seen in humans. The animals were reluctant to move, ambulation became slow and awkward and limbs were spread far apart. The eyes were usually half closed. The fur, instead of being white, was yellow in color and very shaggy.

Coincident with these symptoms, 2 important factors were observed: (1) female rats which after 5 weeks of treatment showed no spontaneous activity, now were clearly myotonic; (2) the soleus muscles were consistently and strongly affected.

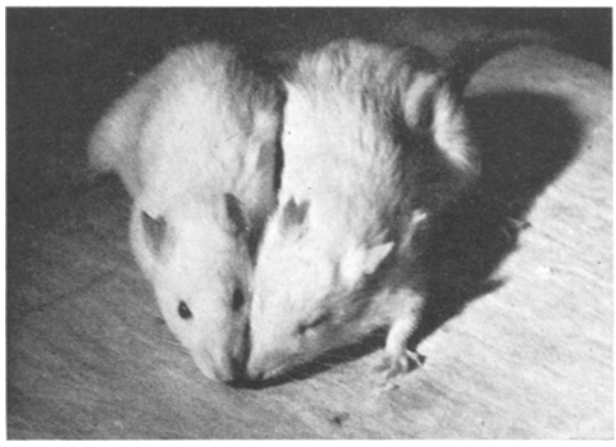
Myotonia was demonstrable electromyographically for about 10 weeks after cessation of drug administration. After 12 weeks the myotonia disappeared. This agrees with Burns et al.[3] who found that myotonia disappeared by the 81st day after termination of diazacholesterol administration in goats.

These results demonstrate conclusively (1) that the fast-twitch muscles develop myotonia earlier than the slow-twitch muscles, (2) that with prolonged drug administration the soleus muscles become as involved as the anterior tibialis, and (3) female rats follow the same delayed time course in developing myotonia as the soleus of male rats.

Although the mechanism by which 25-azacholesterol induces myotonia is not completely understood, an explanation for the difference in myotonic development between red and white muscles may be proposed. Since 25-azacholesterol is a steroid inhibitor of cholesterogenesis, it has been suggested by Winer et al.[4] that myotonia might result from 'the combined effect of desmosterol accumulation and agents with specific structural features, namely, a steroid nucleus with a side chain containing a nitrogen atom at or near a terminal dimethyl or diethyl group'. Winer et al.[4] reported a large accumulation of desmosterol and a reduction of cholesterol in the plasma in the myotonic rats.

A recent paper[5] has shown that rat red muscle contains about 50% more cholesterol and phospholipide than white muscle. Thus the delay in development of myotonia in the soleus may be due to the longer time necessary to change the cholesterol level and/or configuration in the muscle fibers. Similarly, the fact that female rats have higher plasma cholesterol than do male rats[6,7] may explain the longer period of drug administration necessary to induce myotonia[8].

Zusammenfassung. Nach chronischer Applikation von 25-Azacholesterol kann auch in den langsamen Muskelfasern Myotonie erzeugt werden.



Normal (left) and myotonic (right) rat of the same age.

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Mucosal Growth Effect of Vitamin D on the Duodenum[1]

The biochemical details of the mechanism of action of vitamin D on the small intestinal mucosa are being intensively investigated. A specific metabolite of vitamin D_3, 25-hydroxycholecalciferol has a direct stimulatory effect on small intestinal calcium transport[2]. Vitamin D_3 stimulates template activity of rat intestinal mucosa chromatin[3] as well as synthesis of RNA[4]. A chromosomal receptor for a vitamin D metabolite has been isolated from the chick small intestine[5]. The specific relationship of these findings to a calcium binding protein[6] localized to the luminal border of the intestinal mucosal cell[7] is as yet uncertain. Recently, vitamin D has also been shown

to increase intestinal absorption of the amino acid L-histidine[8]. We report a growth effect of vitamin D on the intestinal mucosa.

Male albino rats of the Simonsen strain weighing 50–70 g were randomly assigned to cages and depleted of vitamin D for 11–16 weeks. Details for housing, diet and method of vitamin D repletion have been fully described[9]. Animals selected at random were repleted per os with 12.5 μg vitamin D_2 (Calciferol, Kremers-Urban Co., Milwaukee, Wisconsin) 4 and 8 days before study and compared with unrepleted control rats. Under anesthesia (i.p. Dial with Urethane, CIBA Pharmaceutical Co., Summit, New Jersey), 10 cm segments of duodenum and 10–15 cm segments of terminal ileum were dissected out, weighed, laid flat and measured for length, opened lengthwise, spread on a glass plate and the mucosal surface scraped firmly with the edge of a microscope slide to separate villi (here called mucosa) from underlying tissue. The 2 tissue fractions were separately weighed, dried in a vacuum oven at 80 °C for 24 h and reweighed. The weight of dry mucosa was expressed as % of the combined dry weights of mucosa and underlying tissue. A separate but identically managed group of rats was given 2 mg of vitamin D_2 48 h before study. Half of these rats were compared with depleted controls from the same group using the methods described above. The other half of this repleted group and an equal number of depleted control rats were used to study in vivo intestinal calcium transport[9]. After perfusion, the intestinal segments were dissected out and analyzed in the same manner as the unperfused tissue.

Table I. Intestinal mucosa as percent of total dry segment weight[a]

	(Mean ± 1SE) Duodenum	$p <$[b]	Ileum	$p <$[b]
Depleted	46.3 ± 4.3	–	53.3 ± 3.4	–
Repleted 4 days	62.4 ± 0.9	0.05	61.7 ± 3.0	0.1
Repleted 8 days	62.4 ± 0.2	0.01	61.4 ± 2.8	0.1

[a] Six rats were studied 4 days and a further 6 rats were studied 8 days after repletion with 12.5 mg vitamin D. [b] p values compare corresponding depleted and repleted tissue in each instance.

The mucosal growth effect of vitamin D is shown in Table I. The proportion of total intestinal mass represented by the duodenal mucosa was increased significantly by 4 days, and there was no further increase by 8 days. The ileal mucosa showed a smaller increase with vitamin D which was not significant. The turnover time of intestinal mucosa of the rat is between 30–40 h[10]. Thus, if mucosal growth is primarily due to vitamin D, it should be possible to produce an acute pharmacological effect by high dosage over a short time. Table II shows this effect produced by 2 mg of the vitamin in 48 h. Again duodenal mucosa increased significantly and there was no effect on the ileum. Data for rats perfused with a luminal solution to study calcium transport show the same effect.

The data of Tables I and II show the same relationship when expressed on a wet weight basis. Hence, vitamin D had no effect on tissue water content. Thus, intestinal growth could be evaluated in terms of wet weight per unit segment length, the 2 experimental measurements made before scraping. Just as with similar data already published[9] segments from the vitamin D treated animals showed a small but consistent increase in wet weight per unit length of the full thickness segment but the increase was not significant ($p > 0.05$). Thus, growth takes place primarily in the mucosal portion of duodenal tissue rather than both mucosa and underlying tissue (Tables I and II). Localization of growth to the mucosa would account for our failure to find a significant increase in full thickness segment weight per unit length with vitamin D.

Therefore, in addition to molecular processes now being elucidated[2–7], there is a specific anatomical effect on one of the target organs of vitamin D action, the duodenal mucosa. Soft tissue growth stimulation by vitamin D has been suggested[11], but not previously documented.

Table II. Intestinal mucosa as percent of total dry segment weight

	(Mean ± 1SE) Duodenum Depleted	Repleted	$p <$	Ileum Depleted	Repleted	$p <$
Not perfused[a]	53.7 ± 2.9	61.5 ± 0.9	0.05	50.8 ± 5.2	50.6 ± 4.5	1.0
Perfused[a]	59.9 ± 1.7	69.2 ± 2.8	0.01	49.5 ± 4.9	50.4 ± 4.2	0.9

[a] Six rats were repleted with 2 mg vitamin D, 48 h before study, and compared with 6 depleted animals.

Zusammenfassung. Nach mehrmonatiger Vitamin-D-freier Diät erhielt eine Testgruppe von Ratten Vitamin D. Diese zeigte in Vergleich zur Kontrollgruppe eine Zunahme des Schleimhautgewichtes im Duodenum, nicht aber im Ileum.

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