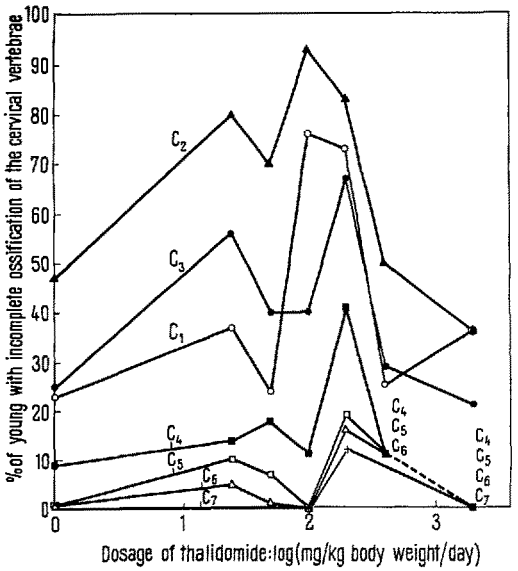
The width of the fontanels shifted from intermediate to narrow, but this seemed more related to the more favourable conditions for development in the smaller litters than to thalidomide doses.

For the peculiar pattern demonstrated by the cervical vertebrae another explanation should be taken into consideration. Since the number of mature young decreased definitely with increasing doses of thalidomide it is conceivable that with certain doses minor anomalies occur that do not cause death in early foetal life. Larger doses inducing additional damage might cause more animals to die that would have been susceptible already to smaller doses and a number of fairly normal animals to survive in the highest dosage groups. It might be important that the vertebral anomalies are in the region where a disturbance of the nerve supply of the upper extremities might be possible as well, but this cannot be investigated at the same time as the skeleton staining.

Conclusion. It has to be concluded that not only anomalies are induced by the drug given but that also fairly wide variations around the normal or average occur that are not related to the drug given. From the data of the control animals it appears that those bones that show already many variations under normal circumstances are the most susceptible to this drug. For future investigations it is important to know that more abnormalities might be found when giving small doses than giving large doses, since in the latter too many young will not survive until birth[2].



Relation between anomalies of the cervical vertebrae in new-born rats and the dosage of thalidomide in the mother-animals during pregnancy.

Zusammenfassung. Nach Thalidomidverabreichung an trächtigen Ratten fanden wir Fruchtresorption und Anomalien am fünften Brustbeinkern der Föten. Weitere ergänzende Skelettuntersuchungen ergaben, besonders bei mittelgrossen Thalidomidgaben, Verknöcherungsstörungen an den Halswirbelkörpern. Andere Skeletteile zeigten keine Thalidomid-abhängigen Entwicklungsvariationen.

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Laboratory of Physiological Chemistry, University of Amsterdam and the Netherlands Institute of Nutrition, Wageningen (The Netherlands), December 28, 1963.

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Effect of Histamine on the Diameter of Skeletal Muscle Fibers in Mice[1]

The occurrence of abnormally large numbers of tissue mast cells in skin of mice with hereditary muscular dystrophy[2] and the association of these cells with tissue content of histamine[3] and 5-hydroxytryptamine[4] suggests a possible interrelationship between these two substances and the muscle abnormality. Reports in the literature do not indicate that either compound affects skeletal muscle of any species. This study was undertaken to determine if repeated injections of different concentrations of histamine would modify the skeletal muscle fibers of mice. Selection of specific doses of the drug has been based on biological assays of histamine in normal mouse skin and in skin from mice with hereditary muscular dystrophy[5]. In addition, the effect of injecting an extremely high concentration of histamine has been included.

Materials and Methods. Experimental groups of mice were given intraperitoneal injections of histamine dihydrochloride (Calbiochem.) in 0.25 ml physiological saline twice daily for 21 days. Control animals were injected with equal volumes of saline on the same schedule. All mice were weighed daily. At 28 days after the initial injection the mice were anesthetized with ether and fixed *in toto* in Helly's or Bouin's solution for 24 h. Fixation by this method controlled the degree of muscle contraction in each animal. After fixation the tissues were washed, and the gastrocnemius and gracilis major muscles were removed, embedded, and sectioned at 7 μ. Sections were

[1] Supported by grant HD-00221, National Institutes of Health, U.S. Public Health Service. I wish to gratefully acknowledge the technical assistance of Miss F. VAUGHAN.

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[3] J. F. RILEY and G. B. WEST, J. Physiol. (Lond.) *120*, 528 (1953).

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[5] W. K. O'STEEN and J. J. HRACHOVY, unpublished results (1963).

stained with either Harris' hematoxylin and eosin or Mallory's phosphotungstic acid hematoxylin.

The mice were divided into three groups: (A) 22 normal mice from three litters of inbred strain 129 mice (Roscoe B. Jackson Laboratories) (9 controls, 13 experimental mice). Ages ranged from 63 to 95 days at the end of the experiment (Table I). Experimental animals were injected with 200 mg histamine per kg body weight at 9.00 a.m. and at 4.00 p.m. daily; (B) 10 normal Swiss Yale albino mice. Five experimental mice were injected with 25 mg per kg histamine twice daily; (C) 10 normal Swiss Yale albino mice. Five experimental mice received 50 mg per kg histamine twice daily. Mice in groups (B) and (C) were 70 days old at autopsy.

Measurements of muscle fiber diameter were made with an ocular micrometer at 400× magnification on cross-sections of the muscle. Counts were begun on the periphery of the section and continued toward the interior of the muscle until at least 25 fibers were measured on each section. At least four sections from different levels of each muscle were examined in this manner to ascertain if a statistical difference in fiber diameter occurred in the same muscle.

The statistical significance of differences between the means was evaluated by use of the Student t test.

Results. The externally visible effects of the highest concentration of histamine (400 mg/kg daily), such as deeper breathing, general inactivity, and dilated blood vessels in the ears, disappeared approximately 1 to 2 h after each injection. These symptoms were observed after each injection throughout the 21-day treatment period, and the animals did not appear to develop a tachyphylaxis. During the injection period the control group had a 14.4% average weight gain as compared with 5.8% for the experimental group (calculated at 21 days). However, during the week following cessation of histamine injections, or the 'recovery period', the experimental group had weight gains which resulted in statistically comparable body weights in both groups by the time of autopsy (28 days). Three mice in the experimental group died (at 8, 13, and 18 days after the initial injection). Systemic effects and the body weight change were not observed in the other two experimental groups (50 mg/kg and 100 mg/kg histamine daily).

A study of sections of muscle from these mice indicated that in each histamine-treated animal the diameter of muscle fibers was significantly smaller ($P < 0.001$) than in control mice (Tables I and II). Diameters of muscle fibers in all groups, both experimental and control, ranged from 10–50 μ, but experimental mice had a greater number of smaller fibers. The smallest fibers (10–15 μ diameter) were composed of nuclei with very little surrounding sarcoplasm. Large muscle fibers sometimes appeared to be splitting along their length, thereby forming 2 or 3 smaller fibers. The smaller fibers thus formed were morphologically normal, but their sarcoplasm seemed to be continuous with that of the larger 'parent fiber'. The effect of histamine on muscle fiber diameter apparently was not influenced by sex or by this particular range in age and was observed in both strains of mice (Tables I and II).

Although other histological changes were observed in the muscles of histamine-treated mice, they were not as dramatic as the diameter variance, and their occurrence was rare. In muscle of treated animals the nuclei within the fibers were frequently enlarged and spherical or oval with one to two masses of chromatin and a single enlarged nucleolus as compared with typical normal, elongate muscle nuclei with 2 to 4 chromatin masses. Rows of centrally arranged nuclei were found in some muscle fibers which were cut longitudinally. In other fibers, groups of as many as 8 to 10 nuclei were clustered into a localized area of the fiber and were surrounded with basophilic sarcoplasma (Figure). Small blister-like areas containing several nuclei surrounded by darkly basophilic sarcoplasm appeared to be evaginating from the surface of some skeletal muscle fibers (Figure).

Discussion. The pharmacologic effects of histamine on smooth muscle of the intestine and uterus has received much attention for several years and has led to the development of a biological assay method for tissue histamine[6]. However, a search of the literature did not indicate that this drug affected skeletal muscle. The observation of increased numbers of mast cells in mice with muscular dystrophy[2] and the direct correlation of mast cells with

Table I. Effect of intraperitoneal injections of histamine (400 mg per kg) on the diameter of skeletal muscle fibers (gastrocnemius m.) of Strain 129 mice. Each average fiber diameter (μ) is based on measurements of 25 fibers

Saline Controls			Histamine-treated		
Sex	Age (days)	Fiber diameter average μ	Sex	Age (days)	Fiber diameter average μ
F	63	29.68 ± 1.85	F	63	20.30 ± 0.88
F	63	30.88 ± 1.93	F	63	22.78 ± 0.99
M	63	28.72 ± 1.55	M	63	20.60 ± 1.02
Group Mean ± S.E.		29.76 ± 1.02			21.23 ± 0.57
P Value		< 0.001			
F	75	23.70 ± 1.08	F	75	21.50 ± 1.06
M	75	30.60 ± 1.97	F	75	22.92 ± 1.33
M	75	28.42 ± 1.74	M	75	18.38 ± 0.81
Group Mean ± S.E.		27.57 ± 0.99			20.94 ± 0.66
P Value		< 0.001			
M	95	34.30 ± 1.60	F	95	24.50 ± 1.00
M	95	30.63 ± 2.01	F	95	21.80 ± 1.19
M	95	28.48 ± 1.68	M	95	23.20 ± 1.18
			F	95	22.90 ± 1.02
Group Mean ± S.E.		31.14 ± 1.04			23.10 ± 0.55
P Value		< 0.001			

Table II. Effect of histamine dihydrochloride (i.p.) on the diameter of skeletal muscle fibers of male Swiss Yale albino mice. Each average fiber diameter is based on measurements of 25 muscle fibers from each animal or a total of 125 fibers in each group of 5 mice.

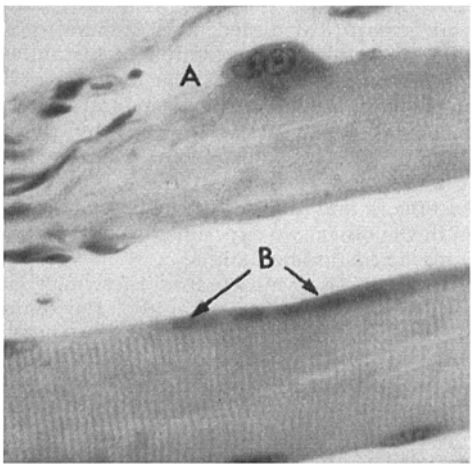
Treatment	No. of mice	Fiber diameter ± S.E. (μ)	P value
Saline control	5	26.74 ± 5.77	
Histamine (50 mg/kg)	5	23.58 ± 5.19	< 0.001
Saline control	5	28.49 ± 7.54	
Histamine (100 mg/kg)	5	23.81 ± 5.68	< 0.001

[6] G. E. W. WOLSTENHOLME and C. M. O'CONNOR, *Histamine* (Little, Brown and Co., Boston 1956).

tissue histamine[3] led to this investigation of the effects of the drug on skeletal muscle.

These experiments have demonstrated that skeletal muscle fibers from mice treated with histamine are consistently smaller in diameter than those from control animals (Tables I and II). Basophilic sarcoplasm in fibers and blister-like evaginations containing groups of nuclei which were present in treated mice were never seen in control animals. Some of the histological changes which were seen in histamine-treated mice have been described in mice of the same strain (strain 129) with hereditary muscular dystrophy. Mice with this genetic affectation have skeletal muscle defects such as reduced fiber size, degenerating muscle fibers, and central rowing of nuclei in the fiber[7]. Regenerating muscle fibers, which are also present and are characterized by basophilic sarcoplasm, have been described in radioautographs[8] and in diffusion chamber implants of dystrophic muscle[9]. It should be emphasized that the mice used in these experiments were from litters of homozygous normal mice and that they did not carry the gene for muscular dystrophy.

Therefore, these drug-induced changes cannot be attributed to genetic factors. The occurrence of muscle fibers with smaller mean diameters in histamine-treated Swiss Yale albino mice, which belong to a different strain, unrelated to strain 129, further accentuates that these variations from the control findings are the result of histamine administration.



Blister-like evagination of basophilic sarcoplasm containing several nuclei (A). Basophilic sarcoplasm (B) in lower fiber contains several nuclei not in focus at this level. × 1000.

Résumé. Des souris reçurent des injections intrapéritonéales de bichlorure d'histamine. Le diamètre moyen des fibres musculaires s'en trouva nettement réduit et des modifications histologiques furent notées chez les souris traitées.

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Department of Anatomy, University of Texas Medical Branch, Galveston (USA), December 16, 1963.

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Esterases in Avian Sera: Species Specific Pattern and Individual Variations

This report summarizes preliminary results concerning esterase activities in bird sera. The purpose of this investigation was to determine whether species differences could be observed in zymograms of birds, as they have been described for mammals[1].

Individual samples of serum were collected from adult specimens of both sexes. After agar gel electrophoresis, the esterases were revealed by incubation in one of the media described by URIEL[2]: (1) indoxyl acetate, barbital buffer pH 8.2; with Cu acetate as activator, (2) 1-(α) or 2-(β)-naphthyl acetate or β-carbonaphthoxycholine as substrates in phosphate buffer pH 7 and Diazo Blue B as dye-coupler, (3) butyrylthiocholine as substrate with tetrazolium salt Nitro BT, (4) inhibitors: prostigmin (eserin) M 10^{-4} and DFP M 10^{-6}.

The results with indoxyl as well as with α- or β-naphthyl acetates were the same, unlike what was seen in mammals[2–5]. The first reaction was hence most often used; its advantage is a good colour contrast between the indigo blue and the red of Ponceau S, the stain used for revealing proteins[6]. When both reactions are applied on the same plate, a direct and easy localization of esterases with respect to proteins is realized.

The sera studied were:

I. Gallinaceae:		
(1) Hens	(a) Rhode Island Red, strain M44	16
	(b) White Leghorn, without pedigree	12
	(c) without specification of race	12
(2) Guinea-fowls (blue)		16
(3) Pheasants, *Ph. colchicus*		12
Pheasants, *Ph. colchicus* var. *obscurus*		4
(4) Turkeys, black		8
Turkeys, white		2
(5) Quails of different origins but mainly from families with pedigree		80
II. Anatidae:		
(1) Ducks	(a) Pekin, mainly from an inbred strain	200
	(b) Khaki without specification	200
	(c) hybrids	200
(2) Barbary ducks		6
(3) Goose		1
III. Columbidae:		
Pigeons	(a) White Peacock	8
	(b) grey (Paris)	4
	(c) Cauchois (hybridized)	12