## Effects of Thalidomide on the Skeleton of the Rat Foetus

Using rats as test animals for studying teratogenic effects of thalidomide, distinct effects on the development of the fifth sternal bone centre have already been reported. Even more striking effects of the thalidomide were seen on the frequency of foetal resorptions and litter size, without a disturbance of the number of implantations. Other parts of the skeleton have now been studied in detail, e.g. fontanels, cranial sutures, vertebrae, ribs, calcaneus, talus and other distal bones of the extremities.

Methods for staining the skeleton were described previously. Thalidomide was administered in doses from 0 up to 2000 mg/kg body weight per day and given from the sixth until the last day of pregnancy.

The results are given in the Table and the Figure. The large bones of the extremities as well as the shoulder and pelvis and the ribs showed no anomalies. The smaller bones of the extremities varied widely in state of development, which is quite a normal picture. With increasing doses of thalidomide the size of both the fontanels showed a tendency to decrease. In the width of the cranial sutures

no definite pattern could be observed. In the cervical vertebrae a peculiar preference for anomalies in ossification was seen in animals born in the 100 and 200 mg/kg thalidomide groups, especially in  $\mathrm{C_1}$  and  $\mathrm{C_2}$ ;  $\mathrm{C_3}$  and  $\mathrm{C_4}$  showed most abnormalities in the group with 200 mg/kg. In the other cervical vertebrae the effect is less pronounced but still present. The differences between the number of normals in the groups mentioned and that of all the other groups together proved to be highly significant for these vertebrae. In the thoracal, lumbal, sacral and caudal vertebrae no anomalies were found.

The observations on the fifth sternal bone centre complete the whole scale of skeletal anomalies.

Discussion. The explanation of the various effects seen cannot be identical. The changes in the fifth sternal bone centre were definitely pathological: bell shapes, wedge shapes, pin-point sizes, etc. and a complete absence were found in increasing frequency with increasing doses. With 2000 mg thalidomide per kg body weight there was not a single normal-shaped fifth sternal bone centre.

<sup>1</sup> H. J. Klein Obbink and L. M. Dalderup, Exper. 19, 645 (1963).

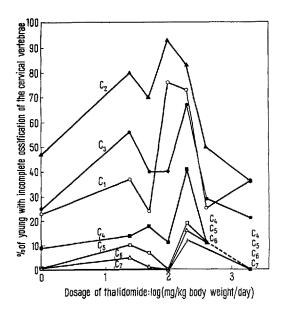
Results of the examination of the skeleton of new-born rats after administering thalidomide to the mother-animals during pregnancy

rkg y		te of	Anterior fontanel				Posterior fontanel				Sutures of the skull											
mide per ıt per day	young d			open			open		1	-	Sutura frontalis		Suturae coronales		Sutura sagittalis		Suturae lambdoideae					
mg thalidomide body weight per	Number of y investigated	Average size the litters	wide	intermediate	narrow	closed	wide	intermediate	narrow	closed	wide	narrow	closed	wide	narrow	closed	wide	пагтом	closed	wide	narrow	closed
_					%		-		%			%		l	%			%			%	
0	68	10,3	31	60	9		13	78	6	3	97	_	3	1	_	99	81	9	10	44	16	40
25	102	7,9	20	73	7		12	73	14	_	100	-		2	-	98	65	11	24	61	8	31
50	83	6,5	12	81	7	****	8	76	6	10	94	2	4	-	-	100	29	25	46	44	29	27
100	45	6,6	51	49	~		29	69	2	-	89	_	11	-	-	100	75	7	18	56	13	31
200	52	5,2	30	54	16		23	56	19	2	87	7	6	16	5	79	71	6	23	73	5	22
400	36	4,9	46	33	27		13	54	23	10	87		13	70	3	27	67	3	30	40		60
2000	15	2,1	31	15	54		-	8	92	-	100	_	North,	-	-	100	31	23	46	15	15	70

Ossification	of the bodies o	5th sternal bone centre	Ossification of the					
C-1	C-2	C-3	C-4	C-5	C-6	C-7	mal rmal	Calcaneus Talus
complete % slight none	complete slight none	complete slight none	complete slight	complete slight none	complete	complete slight none	normal severely abnormal severely abnormal	advanced slight % absent present absent
77 1 22 63 — 37 76 11 13 24 38 38 27 50 23 75 19 6 64 29 7	53 - 47 20 - 80 30 1 69 7 4 89 17 4 79 50 5 45 64 7 29	75 - 25 44 1 55 60 - 40 60 - 40 33 6 61 72 - 28 79 - 21	91 - 9 86 - 14 82 1 17 89 4 7 59 4 37 89 3 8 100	99 - I 90 - 10 93 - 7 100 81 4 15 89 3 8 100	99 - 1 95 - 5 99 1 - 100 84 10 6 89 8 3 100	100 100 100 100 88 6 6 100 100	96 - 4 78 14 8 82 12 6 54 29 17 47 28 25 22 28 50 - 7 93	12     41     47     -     100       20     50     30     -     100       38     49     13     6     94       11     62     27     -     100       44     31     25     2     98       67     19     14     8     92       24     38     38     8     92

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



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

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<sup>2</sup>.

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<sup>1</sup>

The occurrence of abnormally large numbers of tissue mast cells in skin of mice with hereditary muscular dystrophy2 and the association of these cells with tissue content of histamine<sup>3</sup> and 5-hydroxytryptamine<sup>4</sup> 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<sup>5</sup>. 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 dihy-

drochloride (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  $\mu$ . Sections were

<sup>&</sup>lt;sup>1</sup> 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.

W. K. O'STEEN and J. J. HRACHOVY, Texas Rept. Biol. Med. 20, 70 (1962).

<sup>&</sup>lt;sup>3</sup> J. F. RILEY and G. B. WEST, J. Physiol. (Lond.) 120, 528 (1953).

<sup>4</sup> E. P. BENDITT, R. L. WONG, M. ARASE, and E. ROEPER, Proc. Soc. exp. Biol. Med. 99, 303 (1955).

<sup>&</sup>lt;sup>5</sup> W. K. O'Steen and J. J. Hrachovy, unpublished results (1963).