Effects of Thalidomide in the Rat Foetus

Although several workers have reported that the rat is not a suitable animal for testing the teratogenic action of thalidomide[1-3], BIGNAMI et al.[4] found a low frequency of malformations in rats. We have carried out experiments with Wistar albino rats on doses of thalidomide ranging up to 400 mg per kg body weight per day. The rats were mated and the male animals removed from the cages after 4 days. The females received the thalidomide-containing ration from that day until parturition. The diet consisted of 52% whole wheat flour, 30% skimmed milk powder, 9% margarine, 8.36% sugar-thalidomide mixture, 0.5% cod-liver oil and 0.14% salts.

After birth the litters were weighed, and the animals were examined for gross malformations and killed by ether inhalation. The skin and all internal organs were removed and the preparations fixed in 96% ethanol, made transparent in 1% KOH, stained with 0.01% alizarin red in 1% KOH for 1–2 weeks, placed in Mall's solution – 1 KOH, 20 glycerol, 79 water – for 1 week and stored in glycerol. With this procedure muscles and other soft tissues become transparent, but the preparations remain anatomically intact, whilst the skeleton stains bright red.

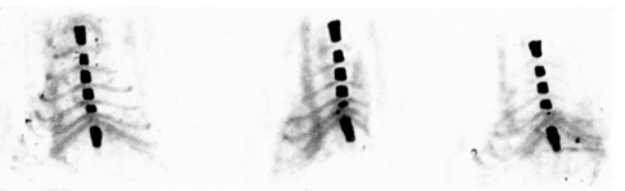
By external inspection no malformations were seen in the new-born rats. In all groups a few young were stillborn but there were more in the group fed 400 mg thalidomide per kg body weight. The average litter size decreased with increasing dose of the drug (Table I). The number of implantations was nearly the same in all groups.

The average weight of the young increased somewhat on ncreasing the thalidomide dose. This should be regarded, however, as a normal symptom of higher birth weights of young in small litters compared to large litters.

The stained skeletons revealed no gross malformations; the long limb bones were all present and of normal size.

Wide sutures in the bony skull were present in the thalidomide groups as well as in the control group and the phenomenon seemed to be related to the litter size. The young of large litters are somewhat behind in development in comparison to those of small litters.

The only noteworthy effect of thalidomide was an increasing frequency of abnormal 5th ossification centres of the sternum. Although this abnormality was not completely absent in the controls, increasing doses of thalidomide caused a markedly higher incidence and also more serious malformations of this bone centre. A complete range from normal size to smaller, flatter, wedge-shaped, dumb-bell-shaped, and various irregular shapes to very small irregular fragments (and even one or two pin-point size fragments) was seen (Figure 1). Table II gives the



A B C

Fig. 1. Sternum of new-born rats. Six ossification centres. A, normal (control); B and C, reduction in size of the 5th centre (400 mg thalidomide per kg body weight). Magnification, 2 ×.

[1] G. F. SOMERS, Lancet i, *1962*, 912.
[2] A. GIROUD, H. TUCHMANN-DUPLESSIS, and L. MERCIER-PAROT, Lancet ii, *1962*, 298.
[3] D. FELISATI, Lancet ii, *1962*, 724.
[4] G. BIGNAMI, D. BOVET, F. BOVET-NITTI, and V. ROSNATI, Lancet ii, *1962*, 1333.

Table I. Litter size in rats after administering various doses of thalidomide during pregnancy

mg Thalidomide per kg body weight per day	Number of animals	Average size of the litters	Average weight of the new-born rats (g)	Average number of implantations
none	7	10.3	5.0	11.0
25	15	7.9	5.2	12.2
50	13	6.5	5.4	9.2
100	7	6.6	5.3	10.2
200	10	5.2	5.3	10.0
400	9	4.9	5.5	10.4

Table II. Numbers of new-born rats with normal and abnormal 5th sternal bone centres

mg Thalidomide per kg body weight per day	Number of young	5th ossification centre of the sternum					
		Normal		Slightly abnormal		Severely abnormal	
		Number	%	Number	%	Number	%
none	54	52	96	0	0	2	4
25	102	80	78	14	14	8	8
50	84	69	82	10	12	5	6
100	41	22	54	12	29	7	17
200	53	25	47	15	28	13	25
400	32	7	22	9	28	16	50

distribution of this peculiarity in the various groups. Figure 2 shows that there is clear relationship between severe abnormalities of the sternal centre and the dosage of thalidomide. This effect is not limited to rats. Very similar abnormalities of the 5th sternal centre were also seen in mice.

The 2nd and 4th bone centres of the sternum were very rarely found to have an abnormal shape.

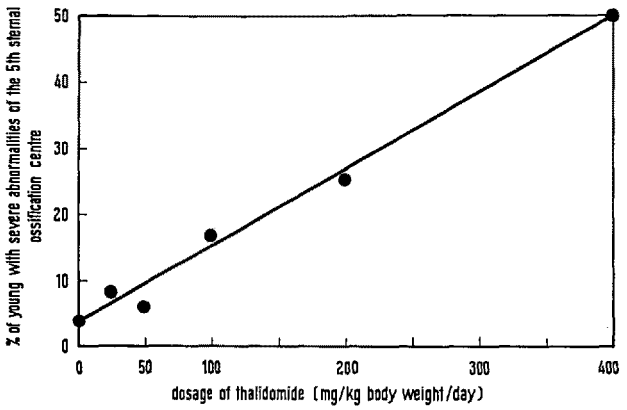


Fig. 2. Relationship between severe abnormalities of the 5th sternal centre and the dosage of thalidomide.

Further details of the skeletons of these animals will be studied. Experiments on the teratogenic action of thalidomide in other animals are in progress.

It must be concluded that thalidomide has some influence on the ossification process in the rat, although gross malformations were not seen. An increasing number of foetal resorptions was found with increasing thalidomide doses. This is in agreement with reports by other workers[5].

Zusammenfassung. Thalidomidverabreichung an schwangere Ratten führte zu keinen schweren Skelettmissbildungen bei den Neugeburten. Lediglich der fünfte Brustbeinkern blieb in der Entwicklung stark zurück und zeigte, besonders bei Tieren, welche Thalidomid in hohen Dosen erhielten, bizarre Formen. Die Zahl der Fruchtresorptionen war bei diesen Tieren erhöht.

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The Effect of Stress upon the Metabolism of 2-Naphthylamine in Mice

During an investigation of factors influencing the metabolism of 2-naphthylamine in rodents (DEWHURST[1]) certain structural alterations had to be carried out on the building housing the animals. This building work necessitated the exposure of the animals to the noise of pneumatic drills and the disconnection of the heating system during a spell of particularly cold weather. It was found that mice, both male and female, showed a significantly raised ($P < 0.01$) excretion of 2-amino-1-naphthol and its conjugates, after being dosed with 2-naphthylamine, during this period of stress (see Table).

The animals used in this investigation were Strong A strain mice aged between 12 and 16 weeks. The animals were given water and commercial rat cake *ad libitum* up to the time they were dosed with 2-naphthylamine (2 mg per mouse of the amine hydrochloride either by intraperitoneal (i.p.) injection as a 0.2% aqueous solution or by stomach tube as a 1% aqueous solution). After dosing, the animals were placed in groups of about five in metabolism cages and the urine excreted collected for 18 h. Previous experiments had shown that there was little or no excretion of 2-amino-1-naphthol and its conjugates in the faeces or in the urine beyond the first 18 h after dosing. The amount of 2-amino-1-naphthol and its conjugates in the urine was estimated by the method of CLAYSON[2], the analyses being performed in duplicate. Probabilities (P) were calculated by means of 'Students *t* function'.

To investigate these stress effects further female mice were kept caged, in two groups of about twenty-five, for seven days in a cold room which varied in temperature between −3 and +9°C as against the normal animal room temperature of +21°C. These two groups of mice were given 2-naphthylamine by i.p. injection and one group kept in the cold room after injection whilst the other group was transferred to a room at +21°C. It was found (see Table below) that the excretion of 2-amino-1-naphthol and its conjugates was raised to about the same

The urinary excretion of 2-amino-1-naphthol and its conjugates from mice dosed with 2 mg of 2-naphthylamine each[a]

Conditions under which the mice were kept	Number and sex of the mice	µg of 2-amino-1-naphthol hydrochloride excreted per mouse
Building work in progress	25 M	828 ± 154
Building work in progress	25 F	857 ± 268
Normal (+21°C)	63 M	379 ± 116
Normal (+21°C)	44 F	382 ± 93
Cold room (−3 to +9°C) before and after dosing	23 F	597 ± 138
Cold room (−3 to +9°C) then normal conditions (+21°C) after dosing	25 F	598 ± 125
Normal, 1 ml of water given i.p., 1 h before dosing	30 F	396 ± 59
Normal, 2-naphthylamine given by stomach tube	32 F	618 ± 193

[a] The 2-naphthylamine (2 mg of the hydrochloride per mouse) was given by intraperitoneal injection (i.p.) unless otherwise stated.

[1] F. DEWHURST, Naturwiss. *50*, 404 (1963); Brit. J. Cancer *17*, 365, 371 (1963).

[2] D. B. CLAYSON, Biochem. J. *47*, XLVI (1950).