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.

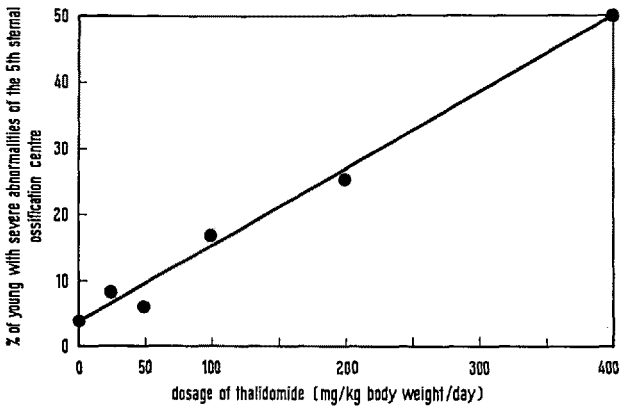

Fig. 2. Relationship between severe abnormalities of the 5th sternal centre and the dosage of thalidomide.

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.

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

level in each case. The increase in excretion from these mice when compared with female mice maintained always at +21°C was found to be significant at the 1% level ($P < 0.01$). It was also found that the i.p. injection of 1 ml of distilled water 1 h before dosing with 2-naphthylamine did not result in a significant increase in the excretion of 2-amino-1-naphthol and its conjugates. When the 2-naphthylamine was given by stomach tube the subsequent excretion of 2-amino-1-naphthol and its conjugates was found to be significantly raised ($0.05 > P > 0.02$).

These results are of interest in connection with industrial bladder carcinogenesis. Evidence has been presented that *ortho*-hydroxylated metabolites of aromatic amines (such as 2-amino-1-naphthol) are involved in the induction of bladder tumours by the carcinogenic amines (CLAYSON[3]). The present work suggests that cold and unpleasant working conditions add to the risks of handling the carcinogenic aromatic amines.

2-Naphthylamine appears to be metabolized by two major pathways; N acetylation followed by hydroxylation in the 6 position or hydroxylation in the 1 position without prior acetylation (WILLIAMS[4]). The hydroxylation of 2-naphthylamine to give 2-amino-1-naphthol is carried out by a N.A.D.P.H. dependent enzyme system associated with the microsomal fraction of the liver. The microsomal oxidative enzymes are involved in the metabolism of a wide range of drugs and foreign compounds (BRODIE[5]). The increase in excretion of 2-amino-1-naphthol and its conjugates when 2-naphthylamine is given by stomach tube implies that misleading results could be obtained from studies in drug metabolism in which the drug is administered to experimental animals in this way. The results suggest that the increases in excretion following fright and exposure to cold may be produced by different mechanisms. The increase in excretion during the building work was about 120%, during this period the animals were subjected to both cold and stress whilst the increase in excretion from animals subjected to cold alone or fright (dosing by stomach tube) was of the order of 60%. It may be, however, that the animals were exposed to a greater degree of stress during the building work than during the other experiments.

AXELROD and INSCOE[6] found that the power to hydroxylate acetanilide increased by 70%/g of wet liver and 87%/mg of microsomal protein in rats when they were subjected to cold. They also found that the power to form glucuronides was not affected and N demethylation activity was depressed. There have been few reports of the effects of cold and stress upon drug metabolism by the microsomal oxidative enzymes but the present work appears to be consistent with that of AXELROD and INSCOE.

The mechanism of the process may be associated with a decline in anabolism reducing the rate of oxidation of N.A.D.P.H. (This appears to be an essential co-factor for fat synthesis etc.) There is also evidence that the N.A.D.P.H./N.A.D.P.+ ratio is partly controlled by the rate of glucose 6 phosphate dehydrogenase activity which controls the rate of formation of N.A.D.P.H. by the pentose phosphate pathway. Thus the effects of increased catabolism and decreased anabolism in animals exposed to cold and stress would be to favour N.A.D.P.H. dependent enzyme systems such as the microsomal oxidative enzymes.

Zusammenfassung. An Mäusen wurde festgestellt, dass nach Exponieren in Kälte und Lärm bei dosierter Verabreichung von 2 Naphtylamin mittels Magenschlauch, eine Vermehrung des 2-Amino-1-naphtolderivats in der Exkretion stattfand.

F. DEWHURST

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The Effect of γ-Irradiation on the Amount of 5-Hydroxytryptamine in the Gut and Spleen in the Early Phase after Irradiation

It is known that 5-hydroxytryptamine (5-HT) is a normal constituent of various tissues in the body[1,2], and this substance is also known to be a radioprotective agent[3–6]. It was found previously that the barbiturate sleeping time was prolonged in X-irradiated rats[7] and that this effect of X-irradiation could be partly depressed by methysergide[8], a highly potent and specific 5-HT antagonist. It was therefore decided to study the effect of γ-irradiation and of cysteamine protection on the amount of two typical stores of 5-HT, the gut and the spleen.

Male rats weighing from 190 to 215 g were used in these experiments. γ-Irradiation was administered to rats by a cobalt-60 source. The animals received 900 r with an average of 44 r/min. The animals were sacrificed 24 and 48 h after irradiation. A piece of gut weighing 2 g and the whole spleen were taken out and extracted according to the method of CORREALE[9]. The extracts were assayed biologically on the isolated rat fundus[10] in the presence of atropine (0.1 μg/ml) and antazoline (5 μg/ml). A four point assay was used. In another series of animals, 12 rats in each group, irradiated in the same way and with the same dose, the number of platelets was counted in the peripheral blood in various periods of time after irradiation (24 h, 2, 4, 6, 8 and 14 days). The results are presented in Table I and Table II.

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