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A note on the absorption and excretion of ^{14}C-labelled thalidomide in pregnant mice

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After oral administration of ^{14}C-labelled thalidomide to pregnant mice, similar concentrations of the drug are found in several maternal tissues and in the placenta and foetus. Thalidomide and one of its hydrolysis products, α-(*o*-carboxybenzamido)-glutarimide, have been identified in the foetus.

THE teratogenic action of thalidomide in man and several animal species is well known (Giroud, Tuchman-Duplessis & Mercier-Parot, 1962; Somers, 1962; Bignami, Bovet-Nitti & Rosnati, 1963; Felisati, 1964). Recently, Di Paolo, Gatzek & Pickren (1964) have demonstrated that the drug produces foetal malformations in the mouse and it was of interest to determine whether thalidomide or its hydrolysis products pass from the mother to the foetus in this animal.

Methods

Thalidomide labelled with ^{14}C in both carbonyl groups of the phthalimido moiety of the molecule (specific activity 4·7 μc/g) was dissolved in dioxane (15·2 mg/ml). Pregnant albino mice (A. Tuck & Son, Essex), 8 days before expected parturition, were given water (0·2 ml) by stomach tube immediately followed by the ^{14}C-thalidomide (38 mg/kg). The animals (6) were placed in small glass metabolism cages from which expired CO_2 could be collected into sodium hydroxide (40%). Two mice each were killed after 1·5, 4 and 8 hr and the organs and tissues were removed and weighed. All of the specimens were freeze-dried. Each tissue was then ground to a powder and extracted with dioxane (3 × 10 ml). The separate tissue extracts were combined and evaporated to dryness under reduced pressure at 40°. The residues were dissolved in known volumes of dioxane and the radioactivity of the solutions was determined by scintillation counting (Graham & Nicholls, 1959). All samples were counted before and after addition of a standard ^{14}C solution to correct for quenching. ^{14}C-Thalidomide added to tissues and extracted by this procedure gave a 92% recovery.

Chromatograms of the extracts of foetal tissue were run on Whatman No. 1 paper in isopropanol:water (4:1) and in the solvent systems suggested by Schumacher, Smith, Stagg & Williams (1964). The hydrolysis products of thalidomide containing a phthalic acid moiety (Faigle, Keberle, Riess & Schmid, 1962) were also run in these solvents, alone and in mixture with the extracts. The radioactive compounds were located by autoradiography and the non-radioactive marker substances by ultraviolet light and treatment with hydrazine (Schumacher & others, 1964).

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Results and discussion

Although this was a small scale experiment, it can be seen that in the mouse the ^{14}C is so distributed that similar concentrations occur in several tissues including the foetus after administration of ^{14}C-thalidomide (Table 1). This is in agreement with similar experiments with rats (Beckmann, 1962; Faigle & others, 1962; MacKenzie & McGrath, 1962). However, in the present work, maximum amounts of ^{14}C in the tissues appear to have been achieved earlier and the levels are 5 to 10 times less than would be expected from the results of these workers. A probable explanation is that a portion of the compound administered would remain in solution and the remainder would be precipitated as fine particles in the stomach. Both these factors would promote a more rapid absorption.

TABLE 1. DISTRIBUTION OF ^{14}C IN THE TISSUES OF PREGNANT MICE GIVEN ^{14}C THALIDOMIDE[1] (38 MG/KG, ORALLY)

Time after administration of drug (hr)	Concentration of ^{14}C in tissue[2] as μg ^{14}C thalidomide g of tissue								
	Brain	Liver	Kidney	Spleen	Lung	Muscle	Placenta	Foetus	Amniotic fluid[3]
1·5	1·4	4·2	1·1	0·3	0·4	0·37	0·74	0·42	0·005
4	0·5	0·5	0·5	—	—	0·08	0·18	0·8	0·003
8	0·3	0·8	0·85	0·2	0·3	0·10	0·03	0·08	0·003

[1] Specific activity, 4·7 μc/mg. [2] Determined by scintillation counting of extract of the freeze-dried tissues in dioxane. Each value is the mean result of 2 mice. [3] Expressed as % of dose administered.

The radioactive content of the smooth muscle of the gastrointestinal tract was considerably higher than that of the other tissues studied (Table 2). Using an autoradiographic technique, Koransky & Ullberg (1964) have demonstrated a high concentration of radioactivity in the stomach wall of pregnant mice receiving ^{14}C-thalidomide. This may point to excretion at these sites. There is also a relatively large amount of ^{14}C

TABLE 2. DISTRIBUTION OF ^{14}C IN THE MUSCLE OF THE GASTROINTESTINAL TRACT OF PREGNANT MICE GIVEN ^{14}C THALIDOMIDE[1] (38 MG/KG, ORALLY)

Time after administration of drug (hr)	Concentration of ^{14}C in tissue[2] as μg ^{14}C thalidomide/g of tissue			
	Stomach	Duodenum	Small intestine	Large intestine
1·5	16·0	4·0	2·2	1·6
4	12·1	0·6	0·6	0·36
8	6·4	1·4	0·4	0·74

[1] Specific activity, 4·7 μc/mg. [2] Determined by scintillation counting of extract of the freeze-dried tissues in dioxane. Each value is the mean result of 2 mice.

in the bile. After 8 hr about 70% of the dose administered is accounted for in the urine, faeces and contents of the gastrointestinal tract (Table 3). A small amount of radioactivity was detected in the expired CO_2. A similar finding has been made with rats (Faigle & others, 1962).

Chromatography of the extracts of the foetal tissue showed that 1·5 hr after administration only thalidomide could be detected. Four and 8 hr

after the drug, thalidomide and one of its hydrolysis products α-(*o*-carboxybenzamido)glutarimide were present. The radioactive spots from the chromatogram of the 4 hr extract were eluted with dioxane and counted. This showed that the concentration of thalidomide relative to that of its hydrolysis product was 1:8.

TABLE 3. EXCRETION OF ^{14}C BY PREGNANT MICE GIVEN ^{14}C-THALIDOMIDE[1] (38 MG/KG, ORALLY)

Time after administration of drug (hr)	Concentration of ^{14}C[2] as % of dose administered				
	Blood	Bile	Urine	Faeces and gastrointestinal contents	Expired[3] CO_2
1·5	2·1	0·02	6·3	*	*
4	1·5	0·3	14·4	*	*
8	0·7	0·01	31·0	42·1	0·09

[1] Specific activity, 4·7 μc/mg. [2] Determined by liquid scintillation counting of dioxan extracts of the freeze-dried materials. Each value is the mean result of 2 mice. [3] Expired CO_2 was collected in sodium hydroxide (40%) before counting. * Not determined.

Fabro, Schumacher, Smith & Williams (1964a) have demonstrated the presence of thalidomide and this hydrolysis product in rabbit blastocysts. Their investigations of the teratogenic activity in the rabbit of several glutarimide and phthalimide compounds suggest that thalidomide itself may be the active teratogenic agent (Fabro & others, 1964b). This could also be true in the mouse, although it should be noted that the animals used in the present experiments were not in the sensitive teratogenic period for this species.

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