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REFERENCES

AHLENIUS, S., BROWN, R., ENGEL, J. & LUNDBORG, P. (1973). *Naunyn-Schmiedeberg's Arch. Pharmac.*, **279**, 31–37.
ANDÉN, N.-E., ROOS, B.-E. & WERDINUS, B. (1964). *Life Sci.*, **3**, 149–158.
KELLOGG, C., LUNDBORG, P. & ROOS, B.-E. (1972). *Brain Res.*, **40**, 469–475.
KORFF, J., ROOS, B.-E. & WERDINUS, B. (1971). *Acta chem. scand.*, **25**, 333–335.
LUNDBORG, P. (1972). *Brain Res.*, **44**, 684–687.
ROOS, B.-E. (1965). *J. Pharm. Pharmac.*, **17**, 820–821.

The fate of [^{14}C] lactose administered into the lungs of rats and monkeys

Disodium cromoglycate (cromolyn sodium) used in the treatment of asthma is administered as an aerosol powder into the respiratory tract. To improve the flow properties of the powder it is combined with 50% by weight of lactose (Intal).* Lactose administered orally is hydrolysed by intestinal β-D-galactosidase (Weser, Sleisenger & others, 1967) but the lung apparently lacks significant amounts of this enzyme (Cohen, Tsou & others, 1952). We have therefore investigated the fate of lactose administered intratracheally to rats (as a solution) and to monkeys (as a powder aerosol).

Two rats (CSE Ash, Spraque-Dawley derived, female, 225g) were anaesthetized with sodium pentobarbitone. Lactose, 2·5 mg (5 μCi) (D-glucose-[1-^{14}C] the Radiochemical Centre, Amersham, England) in 0·1 ml of water, was intubated into the trachea and as far into the bronchial tree as possible. The animals were allowed to recover in Metabowl† cages where they were kept for 24 h. Food and water were provided and the air flow was kept steady. Exhaled CO_2 was absorbed with ethanolamine in ethylene glycol monomethyl ether (1:2 v/v) contained in two Dreschel bottles in series. The $^{14}CO_2$ produced was determined by liquid scintillation counting and accounted for less than 1% of the dose.

Peak $^{14}CO_2$ exhalation occurred between 1 and 2 h after administration. Since absorption from the lung occurred rapidly it seems likely that the $^{14}CO_2$ was a product of general metabolism of lactose throughout the body rather than metabolism of lactose in the lung. Finely chopped fresh rat lung tissue (250 mg) was incubated at 37° in air with [^{14}C] lactose in Warburg flasks using Krebs-Ringer phosphate buffer at pH 7·4. The CO_2 produced was collected in alkali and the ^{14}C content determined. The $^{14}CO_2$ produced in 70 min was less than 0·5% of the radioactivity added indicating that little lactose metabolism occurred *in vitro*. Tissue viability was confirmed by normal oxygen uptake.

Tracheal and carotid arterial cannulae were inserted into four rats (male, 340–370 g) anaesthetized with sodium pentobarbitone. Each animal received heparin (5 mg) intra-arterially followed by 0·5 mg [^{14}C] lactose directly into the trachea as

* 'Intal' is the registered trademark of Fisons Ltd. It contains disodium cromoglycate and lactose (1:1 w/w).

† Jencons Ltd., Hemel Hempstead, U.K.

Table 1. *Plasma levels of [^{14}C] lactose and total radioactivity in rats after intratracheal administration* (0·5 *mg*).

Time after dosing (min)	[^{14}C] plasma levels, μg ml^{-1}* ± s.e. (4 animals)	
	[^{14}C] lactose	Total radioactivity
5	19·1 ± 3·1	18·2 ± 3·2
10	23·4 ± 5·5	24·7 ± 3·7
20	24·9 ± 6·5	23·9 ± 5·4
30	20·3 ± 4·6	22·2 ± 5·3
60	21·0 ± 5·2	22·5 ± 5·3
120	17·2 ± 6·2	17·1 ± 4·6

* Expressed in terms of unchanged [^{14}C] lactose.

before. Arterial blood was collected into EDTA tubes at 5, 10, 20, 30, 60 and 120 min. Plasma aliquots were applied to polyamide coated thin-layer plates (Merck) which were then developed in ethyl acetate–methanol–acetic acid–water (60:15:15:10 v/v) and examined for radioactivity using a Radiochromatogram scanner. Radiochromatogram scans showed a single peak in every case which corresponded in R_F to standard lactose. The areas under the peaks were expressed as μg lactose by comparing with the peak size for standard [^{14}C] lactose (Table 1). No significant activity due to [^{14}C] glucose was found. Second plasma aliquots placed in small Cellophane bags were combusted to H_2O and CO_2. The $^{14}CO_2$ formed was determined by liquid scintillation counting.

The [^{14}C] lactose present in blood corresponded quite well considering the inherent errors involved in radiochromatogram scanning, with values for total radioactivity (Table 1) which showed that little metabolism had occurred. Thus intratracheally administered [^{14}C] lactose was rapidly absorbed into the general circulation of the rat and persisted in the blood for at least 2 h. Weser & others (1967) found a similar rate of elimination of lactose from the blood of intravenously dosed rats. After parenteral administration of lactose to rats, Dahlquist & Thomson (1964) and Weser & others (1967) also found little metabolism with most of it being excreted unchanged. The plasma concentration of lactose may account for much of the administered dose (Table 1). For example plasma at 20 min contained 250 μg lactose or 50% of the dose (assuming 10 ml plasma).

Since lactose is administered as a powder clinically we studied the fate of the dry powder in monkeys. Lactose (D-glucose [1-^{14}C]) and disodium cromoglycate were mixed in the ratio and in the particle size used clinically (British Pharmacopoeia, 1973).

The operative procedures, the method of intratracheal administration of the powder, and methods for determining total radioactivity levels in tissues have been published (Moss & Ritchie, 1970). Five monkeys (male, 5–9 kg, *Macaca arctoides*) were each dosed intratracheally with 20 mg of [^{14}C]lactose/disodium cromoglycate mixture (i.e. 10 mg lactose), killed after 0·5, 1·5, 2·5, 3·5 or 4·5 h and tissue levels of ^{14}C determined. Lactose remaining in the trachea, large bronchi, cannula and cartridge was considered not to have been absorbed. This value (3–6 mg) was deducted from the administered dose to give the absorbed dose. All results (Table 2) are relative to the absorbed dose. Lactose was readily cleared from the lungs and excreted in urine but not in bile.

When Intal is taken by patients most of the lactose is deposited in the mouth and throat. Some is left on the inhalation device (Spinhaler, Fisons) and the remainder reaches the lungs. Lactose deposited in the mouth and throat may be hydrolysed

Table 2. *Tissue retention values (% of dose remaining in organ) and excretion after inhalation of [^{14}C] lactose by monkeys (dose 4 to 7 mg).*

	Time after dose (h)				
	0·5	1·5	2·5	3·5	4·5
Organ or body fluid	% of absorbed dose (expressed as lactose)				
Lung	11·3	4·5	7·3	10·7	3·3
Liver	3·8	4·2	1·9	5·1	2·2
Kidney	4·0	1·2	1·6	1·5	1·2
Urine	3·8	11·7	44·7	16·7	64·6
Bile	not collected	n.s.	0·4	n.s.	n.s.

n.s. not significant.

there by galactosidases of bacterial origin present in normal saliva (Menguy, Masters & Desbaillets, 1970) or swallowed, to be subsequently metabolized by intestinal galactosidases. The lactose used consists of 30–60 μm diameter particles. Since particles larger than 10 μm have little chance of reaching the deeper parts of the lung (Hatch & Gross, 1964), it seems unlikely that much of the lactose inhaled will do so. However, these experiments show that any lactose reaching the lung is likely to be readily absorbed unchanged and excreted in urine. Enna & Schanker (1972a, b) concluded that certain disaccharides were rapidly absorbed from rat lung by a process of passive diffusion. However they did not study lactose. The maximum possible levels of lactose which could be achieved in the blood after inhaling Intal are only a fraction of those found in normal blood (Hubbard & Brock, 1935).

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REFERENCES

British Pharmacopoeia, (1973). p. 429.

COHEN, R. B., TSOU, K. C., RUTENBURG, S. H. & SELIGMAN, A. M. (1952). *J. biol. Chem.*, **195**, 239–249.

DAHLQUIST, A. & THOMSON, D. L. (1964). *Acta physiol. scand.*, **61**, 20–33.

ENNA, S. J. & SCHANKER, L. S. (1972a). *Am. J. Physiol.*, **222**, 409–414.

ENNA, S. J. & SCHANKER, L. S. (1972b). *Ibid.*, **223**, 1227–1231.

HATCH, T. F. & GROSS, P. (1964). *Pulmonary deposition and retention of inhaled aerosols*, p. 67. New York: Academic Press.

HUBBARD, R. S. & BROCK, H. J. (1935). *J. biol. Chem.*, **110**, 411–420.

MENGUY, R., MASTERS, Y. F. & DESBAILLETS, L. (1970). *Proc. Soc. exp. Biol. Med.*, *134*, 1020–1025.

MOSS, G. F. & RITCHIE, J. T. (1970). *Toxic. appl. Pharmac.*, **17**, 699–707.

WESER, E., SLEISENGER, M. H., DICKSTEIN, M. & BARKLEY, F. H. (1967). *J. clin. Invest.*, **46**, 499–505.