The Pulmonary Excretion of $^{14}CO_2$ in Patients with Ileal Resection, Crohn's Disease and Control Subjects Following Administration of Glycine-1-^{14}C

The technique described by FROMM and HOFMANN[1] to test for bile salt deconjugation in patients with intestinal disease depends on the conversion of glycine-1-^{14}C, released from an oral test dose of cholyl glycine-1-^{14}C, to ^{14}C-carbon dioxide which is detected in the breath. Several of the enzymes mediating glycine metabolism (Figure 1) are vitamin dependent and in patients with ileal disease and malabsorption, pulmonary excretion of $^{14}CO_2$ may be altered compared with normal subjects. To examine the possibility of such an alteration in the pulmonary excretion of $^{14}CO_2$, glycine-1-^{14}C was administered to patients with bile salt deconjugation and to a control group with normal ileal function, and the excretion of $^{14}CO_2$ in the breath measured.

Materials and methods. Patients were divided into 2 groups. 1. Controls: 4 patients were recovering from myocardial infarction, 1 from cerebrovascular thrombosis, and 2 were being treated for protrusion of an intervertebral disc. 2. Ileal resection and Crohn's disease: positive evidence of bile salt deconjugation was obtained by the detection of abnormally high amounts of $^{14}CO_2$ in their breath following ingestion of cholyl glycine-1-^{14}C. 6 patients had Crohn's disease with ileal resection, 1 patient had an ileal resection following ischaemia of the small intestine, and the remaining patient had radiological evidence of Crohn's disease.

Procedure. 1 ml of glycine-1-^{14}C solution (2.5 μCi per ml 90% ethanol, specific activity 55 mCi/mmole) was added to a little water and given to the patient to drink immediately before breakfast. Normal meals were allowed throughout the day. Carbon dioxide was collected[1] at $^1/_2$, 1, $1^1/_2$, 2, 3, 4, 6 and 8 h after ingestion of the test dose and the specific activity of the exhaled carbon dioxide measured. The rate of elimination of radioactivity in the breath (R_{Ci}) was estimated by multiplying the specific activity of the exhaled carbon dioxide by the rate of respiration of carbon dioxide[1,2]. The area under the curve relating R_{CI} with time elapsed after administration of the dose, gave the amount of test dose eliminated in the breath during the chosen time interval.

Results. The fraction of the original test dose excreted in the breath of the control subjects in the period 0–8 h ranged from 0.153 to 0.262; mean 0.210, SD 0.041. For patients with ileal resection the range was 0.116–0.213; mean 0.157, SD 0.031 (Figure 2). The reduction in the pulmonary excretion of $^{14}CO_2$, in the 8 h following ingestion of glycine-1-^{14}C, observed in patients with ileal resection was statistically significant ($p < 0.02$).

In both groups of subjects the pulmonary excretion of $^{14}CO_2$ obeyed bi-exponential kinetics (Figure 3) and an attempt was made to calculate the theoretical initial sizes (C_1 and C_2) of the two pools of $^{14}CO_2$ and the rate constants (k_1 and k_2) for the processes of excretion of $^{14}CO_2$, by fitting the data directly to the equation

$$R_{CI} = k_1C_1e^{-k_1t} + k_2C_2e^{-k_2t}$$

using Seidel's method of successive approximations[3]. In these calculations the rate at $^1/_2$ h was not used because the pulmonary excretion of $^{14}CO_2$ had not reached a maximum at this time in the ileal resection group. The results are listed in the Table.

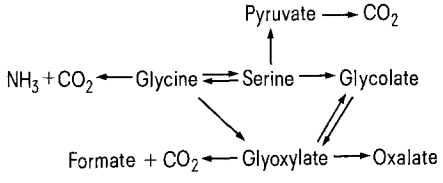

Fig. 1. Metabolic pathways linking glycine, oxalate and carbon dioxide[6–11].

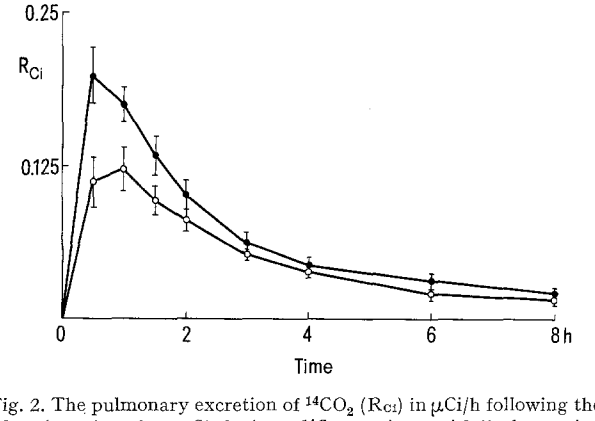

Fig. 2. The pulmonary excretion of $^{14}CO_2$ (R_{Ci}) in μCi/h following the administration of 2.5 μCi glycine-1-^{14}C to patients with ileal resection and Crohn's disease (○, 8 subjects) and to a control group (●, 7 subjects). Each point is the mean of observations in that group. The bar represents the standard error of the mean.

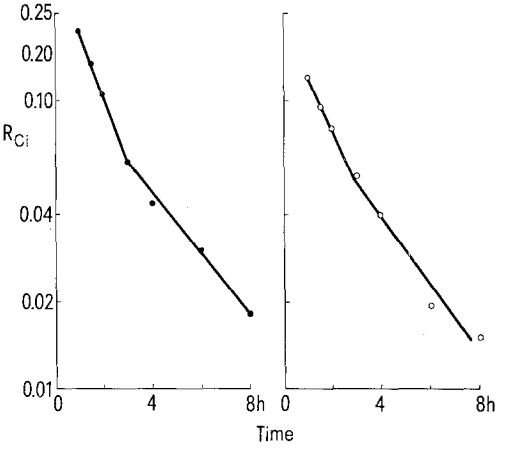

Fig. 3. The mean pulmonary excretion of $^{14}CO_2$ (R_{Ci}) in μCi/h following glycine-1-^{14}C in patients with ileal resection and Crohn's disease (○) and a control group (●). R_{CI} is plotted on a logarithmic scale.

[1] H. FROMM and A. F. HOFMANN, Lancet *2*, 621 (1971).
[2] H. S. WINCHELL, H. STABELIN, N. KUSUBOV, B. SLANGER, M. FISH, M. POLLYCOVE and J. H. LAWRENCE, J. nucl. Med. *11*, 711 (1970).
[3] J. F. KENNEY and E. S. KEEPING, *Mathematics of Statistics*, 2nd edn. (D. Van Nostrand Company Inc., Princeton, N.J. 1951), part 2, p. 289 and 331.

Discussion. Figure 4 shows that the bi-exponential model is probably correct for both groups of patients. Measurements beyond 8 h may indicate more complex behaviour. The pulmonary excretion of $^{14}CO_2$ following glycine-2-^{14}C has been shown to have 4 components, the slowest having a half life of 71.5 days[4]. The reduction in k_1 and C_1 in patients with ileal disease has contributed most towards the reduction in pulmonary excretion of $^{14}CO_2$, the difference in cumulative excretion being greatest in the period 0–3 h following the test dose.

Three of the patients with ileal resection had low serum pyridoxal phosphate concentrations and 2 subnormal serum folates. Partial metabolic blocks due to deficiency of dietary co-factors may be the cause of the reduction in the amount of glycine metabolized to carbon dioxide. In our 2 groups of subjects normal absorption of glycine may be expected because the proximal small intestine is the site of absorption of amino acids[5], and in Crohn's disease, the terminal ileum is the part of the small intestine affected. Further experiments using i.v. glycine-1-^{14}C would be necessary to confirm this point.

Although a reduction in the pulmonary excretion of $^{14}CO_2$ in patients with Crohn's disease and ileal resection has been demonstrated, it is not of sufficient magnitude to invalidate the use of the bile salt deconjugation test of FROMM and HOFMANN[1]. The measurement of $^{14}CO_2$ excretion following oral glycine-1-^{14}C is a worthwhile preliminary to the bile salt deconjugation test. An erroneous impression of the extent of intestinal bile salt deconjugation may be obtained if there is no prior knowledge of the patient's ability to convert glycine-1-^{14}C to $^{14}CO_2$.

Mean values for the constants describing the bi-exponential pulmonary excretion of $^{14}CO_2$ from 2 groups of subjects following the administration of glycine-1-^{14}C

Constant	Control (mean ± SD)	Ileal resection (mean ± SD)	Significance of difference
k_1 (h^{-1})	1.738 ± 0.235	1.092 ± 0.356	$p < 0.01$
k_2 (h^{-1})	0.240 ± 0.023	0.267 ± 0.025	
C_1 (μCi)	0.287 ± 0.038	0.097 ± 0.030	
C_2 (μCi)	0.507 ± 0.028	0.415 ± 0.018	

The significance of the differences between the 2 groups was tested by the *t*-test using 6 degrees of freedom.

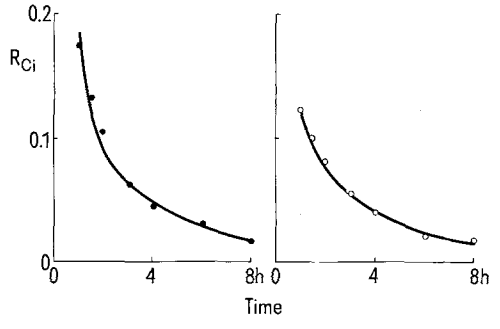

Fig. 4. The rates of pulmonary excretion of $^{14}CO_2$ *calculated* for 2 groups of patients from the parameters in the Table. Circles represent the mean *observed* rates for patients with ileal resection and Crohn's disease (○) and the control group (●).

Zusammenfassung. Mittels Cholinglycin-^{14}C-Atemtest für eine bakterielle Konjugation im Darm wird gezeigt, dass der Glycinmetabolismus zwischen Normalpersonen und solchen mit Ileumresektion und Morbus Crohn unterschiedlich ist.

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Interaction of Chinoform with Electron Transfer System of Rat Liver Microsomes

Since massive dosage of chinoform, 5-chloro 7-iodo 8-quinolinol, has been believed in Japan to cause a neuropathy, called SMON (*s*ubacute *m*yelo-*o*ptico *n*europathy), the toxicity of this drug should be studied from the viewpoint of its metabolism in the animal body. Although the detoxication of this drug through its esterification, such as glucuronization and sulfation, has been reported[1,2], the metabolism of this drug in microsomes has to be investigated. The present paper, therefore, deals with interaction of this drug with liver microsomal electron transfer system.

As experimental animals, male Wistar rats, weighing about 100 g, were used. Liver microsomes were prepared as usual. The perfused liver was homogenized in 5 volumes of 1.15% KCl and the nuclear-mitochondrial fraction was removed by centrifugation at 10,000 *g* for 10 min. The microsomes were collected by centrifugation

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