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.

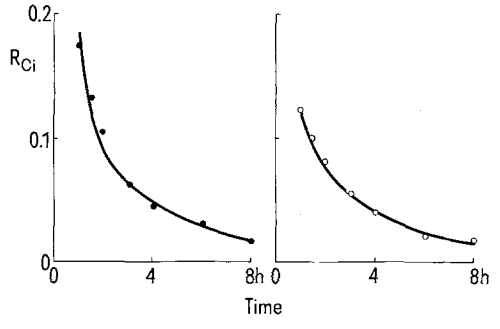

Fig. 4. The rates of pulmonary excretion of $^{14}CO_2$ *calculated* for 2 groups of patients from the parameters in the Table. Circles represent the mean *observed* rates for patients with ileal resection and Crohn's disease (○) and the control group (●).

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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Table I. Effect of incubation with chinoform on microsomal electron transfer system

Incubation time (min)	Addition	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)	NADPH-cytochrome c reductase activity (μmoles/min/mg protein)	NADH-ferricyanide reductase activity (μmoles/min/mg protein)
0	—	0.623	0.460	0.029	1.46
30	—	0.531	0.405	0.022	1.30
60	—	0.400	0.388	0.019	1.30
30	+	0.340	0.342	0.016	1.09
60	+	0.327	0.282	0.014	1.09

at 105,000 *g* for 60 min, washed with the same KCl solution, and suspended in 0.1 *M* phosphate buffer, pH 7.5. Microsomal protein was determined by the biuret method[3].

To examine the metabolism of chinoform in microsomes, the reaction mixture was made to contain 3 mg protein of microsomes, 4.2×10^{-3} *M* isocitrate and 0.5 unit of isocitrate dehydrogenase as NADPH generating system and 2.7×10^{-4} *M* ^{14}C-chinoform (specific radioactivity: 1.63 mCi/mmole) in 3.0 ml of 0.1 *M* phosphate buffer, pH 7.5. After incubation at 30 °C for 100 min, the reaction was stopped by heating in boiling water for 5 min, and the mixture was shaken with 2 ml of benzene-pyridine mixture (9:1, v/v). The radioactivity was quantitatively transferred to benzene layer, which was then subjected to thin layer chromatography using benzene-acetic acid (10:1, v/v) as solvent. The thin layer chromatogram was scanned with an ALOKA radioautoscanner model JTC-201. The radioactivity was exclusively found in the fraction of free chinoform. When other solvents, ethanol-acetic acid-water (7:1:2, v/v) and chloroform-acetic acid (10:1, v/v) were used, the same results were obtained. This indicates that chinoform is hardly hydroxylated or dehalogenated through electron transfer system in liver microsomes.

On the other hand, effect of this drug on the components of electron transfer system in rat liver microsomes was studied. After rat liver microsomes were incubated in the buffer solution suspended with chinoform (1.0 m*M*) at 30 °C for a definite time, the amounts of cytochrome P-450 and cytochrome b_5 were assayed according to the method of OMURA and SATO[4,5], and the activity of NADPH-cytochrome c reductase and that of NADH-ferricyanide reductase by the methods of OMURA and TAKESUE[6] and MIHARA and SATO[7], respectively. Although independent series of experiments revealed some experimental deviation, all the experimental data accord with each other in that chinoform decreases all the components of the electron transfer system. Typical data are shown in Table I. Even though the components in the control decrease with time of incubation, the decrease is enhanced by the coexistence of chinoform. The enhancement occurred markedly before 30 min of the incubation, indicating that the effect could be provoked in the initial period of the incubation.

The effect was also examined in in vivo experiment. Chinoform was dissolved in 0.1 *N* NaOH to 1.0 mg/0.1 ml and injected to rats i.p. After the injection of 2 mg of chinoform daily for 5 days, the animal was killed by decapitation and liver microsomes were prepared. Control animals were injected with 0.2 ml of 0.1 *N* NaOH in the same manner. The amounts of the components of electron transfer system were measured according to the methods described above. The results which are summarized in Table II, obviously indicate that chinoform cannot increase the amount of cytochrome P-450, but apparently decreases it. This is also the case for NADPH-cytochrome c reductase. Both the amounts of cytochrome b_5 and the activity of NADH-ferricyanide reductase were also reduced in the microsomes of rat administered chinoform. The data shown in Table II accord in principle with the data reported with mice by YANO et al.[8].

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Table II. Effect of administration of chinoform on microsomal electron transfer system

Administration	Cytochrome P-450 (nmoles/mg protein)	Cytochrome b_5 (nmoles/mg protein)	NADPH-cytochrome c reductase activity (μmoles/min/mg protein)	NADH-ferricyanide reductase activity (μmoles/min/mg protein)
—	0.653 ± 0.071 ($n = 3$)	0.349 ± 0.018 ($n = 4$)	0.027 ± 0.001 ($n = 4$)	1.14 ± 0.08 ($n = 4$)
+	0.367 ± 0.115 ($n = 3$)	0.228 ± 0.055 ($n = 3$)	0.019 ± 0.004 ($n = 3$)	0.81 ± 0.25 ($n = 3$)

All these results indicate that chinoform cannot be metabolized by microsomal electron transfer system, but decreases the components of the electron transfer system. Accordingly, main mechanism of detoxication of chinoform might be ascribed to its esterification in the liver, and chinoform, if it is not esterified, could be toxic or harmful to biological systems such as microsomal electron transfer system.

Zusammenfassung. Nachweis, dass Chinoform, 5-chloro 7-iodo 8-quinolinol, mit Mikrosomen der Rattenleber weder hydroxyliert noch dehalogeniert werden kann. Inkubation mit Chinoform vermindert die Komponenten des elektronischen Transfersystems, was auch mit in vivo-Versuchen festgestellt wurde.

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Die Wirkung des Glyzins auf chronischen Morphinismus

Vor kurzem konnten wir zeigen, dass die synthetische «Substanz P» (SSP) Glyzin (G) im ZNS erhöht[1]. Wir konnten ebenso zeigen, dass SSP die Abstinenzsymptome chronisch morphinisierter Mäuse (CHMM) zurückdrängt[2]. Dieser Befund kompliziert sich durch die Tatsache, dass Nalorphin, welches die Abstinenzsymptome hervorruft, ebenso G im ZNS erhöht[3]. Wir haben in einer früheren Arbeit erwähnt, dass G, für welches wir zeigen konnten, dass es die hemoenzephale Barriere durchdrängt[4], die Abstinenzsymptome der CHMM nicht potenziert[3]. Aus diesen Gründen glauben wir, dass es interessant wäre, die Wirkung des G auf die Abstinenzsymptome CHMM im eventuellen entgegengesetzten Effekt, d.h. im eventuellen Zurückdrängen der Abstinenzsymptome, zu prüfen. Im Versuch waren weisse Mäuse von 20 ± 2 g (eigene Zucht); chronischen Morphinismus haben wir nach der Methode HUIDOBRO und MIRANDA[5] durch Tablettenimplantation (s.c. 70 mg Morphiumbase) hervorgerufen. Die Tabletten wurden nach der Beschreibung von WAY et al.[6] hergestellt. Am 4. Tag bekamen die Mäuse 200 und 400 mg/kg Glyzin i.p. und 30 min nach Glyzin Nalorphin-Bromid 100 mg/kg i.p. Die Abstinenzsymptome, die sich als Springen und Unruhe manifestierten, wurden in den folgenden 10 min gezählt.

Wie aus der Tabelle ersichtlich, werden bei CHMM die Abstinenzsymptome durch G unterdrückt. Darnach wirken SSP und G in diesem Experiment synergistisch. Sie unterdrücken Abstinenzsymptome; Nalorphin, welches ebenso G im ZNS erhöht, wirkt umgekehrt. Wir konnten schon früher zeigen, dass G auch auf andere Erregungszustände beruhigend wirkt, z.B. psychomotorische Unruhe, hervorgerufen durch β-β-iminodipropionitril, oder auch Aggressivität der männlichen isolierten Mäuse[7]. Es ist interessant, zu erwähnen, dass Nalorphin die durch β-β-iminodipropionitril erzeugte psychomotorische Unruhe inhibiert, während dieselbe Substanz ohne Wirkung auf die Aggressivität der männlichen isolierten Mäuse ist. Mephenesin das ebenso G im ZNS erhöht, unterdrückt die Abstinenzsymptome CHMM und die Aggressivität der isolierten aggressiven Mäuse[7]. SSP beruhigt diese Art von Aggressivität[2]. Diese Versuche zeigen, dass SSP und G in einigen Fällen dieselbe Wirkung auf Erregungszustände haben. Es stellt sich die wichtige Frage, ob G auch bei chronischem Morphinismus bei Menschen die Abstinenzsymptome unterdrückt, was von grossem Nutzen sein könnte. Entsprechende Versuche sind im Gange.

Gruppe	Anzahl Tiere	Dosis (mg/kg)	Sprungzahl innerhalb von 10 min	p
Kontrollen	6	—	38,6 ± 2,9	
Versuchstiere	10	400	21,3 ± 2,5	< 0,01
Kontrollen	6	—	66,5 ± 6,2	
Versuchstiere	6	200	42,5 ± 3,5	0,05

Summary. Glycine inhibits withdrawal induced by nalorphine-like synthetic 'substance P' on mice chronically treated with morphine.

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The Effects of a Novel Insecticide on Insect Cuticle

A new insecticide under development by Philips-Duphar, PH 60–40, interferes with the development of insect cuticle such that the insect has difficulty in moulting, and dies because the new cuticle ruptures before hardening. Some evidence[1,2] suggests that this is due to the inhibition of chitin incorporation, thus weakening the cuticle. There is no published information on what happens to the protein fraction in the cuticle of