carbon tetrachloride[5]. After centrifugation at 2000 *g*, glycoside concentrations were determined in the organic and in the aqueous phases. A quotient Q = organic phase: aqueous phase was calculated.

Results and discussion. The substances tested show a range of Q from 0.004 to 70, indicating great differences in the polarity (Figure). The charcoal concentration needed to obtain half maximal adsorption of the glycosides and derivatives varies from 1.15 to 2.49 mg/ml. It is evident that a correlation exists between polarity and charcoal binding. The higher the polarity, the more charcoal is needed to obtain the same adsorption. With Q increasing > 10 no further increase of the charcoal-glycoside affinity is observed.

The results raise the question of in vivo adsorption of glycosides to charcoal. In the case of glycoside intoxication, charcoal would be a cheep and non-dangerous antidote. On the other hand, a reduction of bioavailability of the cardiac glycosides may occur when a patient on digitalis therapy receives charcoal from other indications.

The stoechiometric relations between glycoside binding and charcoal concentration should not be transferred without criticism to in vivo conditions, as an interference of the glycosides with other substances absorbed to charcoal is most probable.

Zusammenfassung. Die Adsorption von 10 Herzglykosiden und Geninen an Aktivkohle wurde untersucht. Es zeigte sich, dass mit zunehmender Polarität der Substanzen die zur Adsorption benötigten Kohlemengen anwuchsen.

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[5] H. F. Benthe, in *Probleme der klinischen Prüfung herzwirksamer Glykoside* (D. Steinkopff, Darmstadt 1968), p. 29.

Prevention by Cystamine of the Rat Liver Polysomal Disaggregation Induced by Carbon Tetrachloride (CCl_4)

Cystamine prevents some biochemical and morphological alterations induced by CCl_4 in the rat liver[1,2]. As suggested by Castro et al.[2], the protective effect of cystamine against CCl_4 intoxication can be explained in 3 ways: 1. cystamine inhibits CCl_4-activation leading to free radicals; 2. cystamine acts as free radical 'trapping agent'; 3. cystamine interacts with the target structures (the membranes of endoplasmic reticulum), in such a way that it shields the unsaturated lipids against the action of free radical.

Since the formation of free radicals from the homolytic scission of CCl_4 is considered the first step in the chain of reactions leading to liver damage[3], it seemed important to study whether cystamine prevents the polysomal damage (disaggregation) produced by CCl_4.

Methods. Male rats of Wistar strain weighing 180–230 g were fasted 12 h before treatment, water was given ad libitum. Cystamine dihydrochloride (Fluka) dissolved in saline, was given per os. Pure CCl_4 was given i.p. in the amount of 250 µl/100 g body wt. Controls were given equal volumes of saline, cystamine and CCl_4 respectively. Rats were sacrificed 30 min after CCl_4 administration. Polyribosomes sedimentation patterns were studied as described by Borghetti et al.[4]; the amount of post-mitochondrial supernatant stratified on a gradient was exactly determined, as suggested by Fleck and Munro[5], by the RNA content.

[1] J. A. Castro, E. V. Cignoli, C. R. de Castro and O. M. de Fenos, Biochem. Pharmac. *21*, 49 (1972).
[2] J. A. Castro, E. C. de Ferreyra, C. R. de Castro, M. I. Diaz Gomez, N. D'Acosta and O. M. de Fenos, Toxic. appl. Pharmac. *24*, 1 (1973).
[3] R. A. Recknagel, Pharmac. Rev. *19*, 145 (1967).
[4] A. F. Borghetti, B. Franchi. P. Comi, A. M. Gianni, B. Giglioni, S. Ottolenghi and G. G. Guidotti, Ital. J. Biochem. *19*, 397 (1970).
[5] A. Fleck and H. N. Munro, Biochim. biophys. Acta *55*, 571 (1962).

Table I. Effect of different doses of cystamine on the polysomal disaggregation caused by CCl_4

Treatment		Polysomes[c]
		Total ribosomes
Control[a]	(5)	0.30 ± 0.02
Cystamine[b] (mg/100 g body wt.)		
5	(5)	0.35 ± 0.01
10	(5)	0.47 ± 0.01
20	(5)	0.57 ± 0.008
40	(5)	0.57 ± 0.03
100	(5)	0.60 ± 0.008

[a]-Control receiving i.p. 250 µl/100 g b.w. of CCl_4 alone. [b]-Cystamine was given po 120 min before CCl_4. Animals were sacrificed 30 min after CCl_4. [c]-Calculated by areas of the polysomal patterns. Mean ± SE. In parentheses number of experiments.

Table II. Protection of cystamine administered at different times prior CCl_4, on CCl_4-polysomal disaggregation.

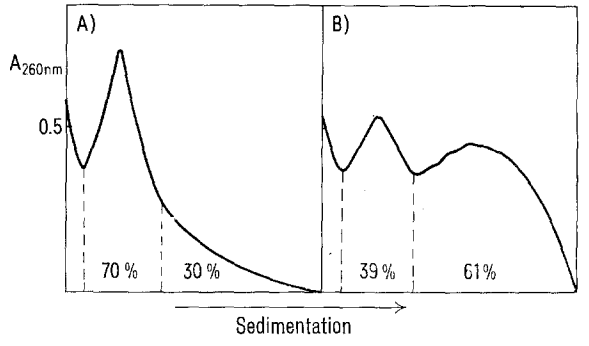
Cystamine pretreatment[a]		Polysomes[b]
time (h)		Total ribosomes
2	(5)	0.60 ± 0.008
12	(5)	0.57 ± 0.01
16	(5)	0.48 ± 0.007
24	(5)	0.40 ± 0.04
Control	(5)[c]	0.30 ± 0.02

[a]-Cystamine was given po at the dose of 60 mg/100 g body wt. Pretreated animals were sacrificed 30 min after CCl_4 administration. [b]-Calculated by areas of the polysomal patterns. Mean ± SE. [c]-Controls receiving i.p. 250 µl/100 g body wt. of CCl_4. In parentheses number of experiments.

Results and discussion. As shown in Table I, cystamine prevents the polysomal disaggregation caused by the i.p. administration of CCl_4. As can be observed, cystamine largely prevents the polysomal disaggregation when administered at the doses of 20, 40 and 100 mg/100 g body wt. ($P < 0.001$ in respect to controls). At the doses of 5 and 10 mg/100 g body wt. cystamine did not protect significantly.

The preventive effect of cystamine is shown also in the Figure, where we have reported the polysomal patterns of a single experiment.

As shown in Table II when cystamine is given 2 and 12 h before CCl_4, at the dose of 60 mg/100 g body wt. it clearly prevents CCl_4-induced polysomal disaggregation ($P < 0.001$).



Sedimentation patterns of liver polysomes from a rat treated with CCl_4 (A) and from a rat pretreated with cystamine (100 mg/100 g body wt.) and poisoned by CCl_4 (B).

At 16 and 24 h cystamine pretreatment did not significantly prevent CCl_4-induced polysomal disaggregation.

Our findings that cystamine inhibits CCl_4-induced polysomal disaggregation are in accordance with results of CASTRO et al.[1,2], however our results do not show how cystamine could act. Since the damage of polyribosomes caused by CCl_4 has been correlated to free radicals arising during the homolytic scission of CCl_4[6], the protective effect of cystamine may be attributed to the inhibition of CCl_4-activation to free radicals, as also suggested by CASTRO et al.[1,2]. This mechanism could be mediated by inhibition of the drug-metabolizing enzyme system that metabolizes CCl_4 and/or by an inhibition of microsomal lipid peroxidation, that could be responsible for the polyribosomes damage, although this last mechanism is unlikely, since it has been shown that cystamine does not prevent the increase in microsomal lipid peroxidation caused by CCl_4[2]. Further studies are therefore necessary to obtain a better understanding of this problem.

Riassunto. Il pretrattamento di ratti con cistamina impedisce la disaggregazione dei polisomi di fegato indotta dal tetracloruro di carbonio.

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[6] E. FARBER, H. LIANG and H. SHINOZUKA, Am. J. Path. *64*, 601 (1971).

Effect of PGE_2 on the Turnover of Calcium in Rat Uterus

It has been demonstrated on several muscle preparations that prostaglandins (PGs) affect the turnover of cellular calcium[1-8]. However, it has not yet been clarified whether PGs act primarily on the calcium transmembrane flux or on the release of calcium from intracellular storage sites; the first process seems to be important in the slow-contracting smooth muscle, the second one in the rapid-contracting cardiac and skeletal muscles[9-14].

The present investigations have been undertaken on rat uterus in order to clarify the site of action of PGs on the turnover of calcium in smooth muscles; for this purpose the extent of the intracellular exchangeable calcium and the wash-out curves of the ion have been investigated. PGE_2 has been used because it does not induce tachyphylaxis[15,16].

Methods. The mechanical stillstand of rat uterus has been obtained in estrogen-treated ovariectomized animals; the organs were incubated in Krebs solution containing 0,69 mM Ca^{++} at 32 °C and pH 7.4; the bathing fluid was aerated with 5% CO_2 in O_2. PGE_2 (kindly supplied by Upjohn Co. Kalamazoo) was used at the concentration of 0.1 μg/ml, $^{45}CaCl_2$ at the concentration of 0.1 μC/ml. Extracellular spaces have been determined with an inuline method[17]: no differences were found in controls and in PGE_2 contracted uteri (43.43 ± 2.38% and 42.46 ± 1.76% respectively).

Table I. Effect of PGE_2 (90 min contact) on the cellular calcium content of rat uterus

Treatment	No. of determinations	Ca(μEq/g fresh tissue)	*P*
—	11	2.89 ± 0.38	—
PGE_2	12	2.78 ± 0.33	>0.80

Evaluation of exchangeable calcium. Uteri incubated in labelled Krebs solution were withdrawn after different times of incubation (5, 15, 30, 45, 60, 90 min), dipped in cold bathing fluid, blotted on filter paper and heated at 200 °C with HNO_3-$HClO_4$ 1:1; the residue was dissolved in 0.1 *N* HCl and used for the determination of total calcium by atomic absorption spectometry, and for the radioassay by liquid scintillation counting. The percent of intracellular exchangeable calcium was calculated as the ratio between the specific radioactivity (cpm/μEq Ca) of the organ and of the bathing fluid, taking into account the amount of calcium and of radioactivity present in the extracellular spaces.

Wash-out curves. After 60 min incubation in labelled Krebs solution, the wash-out curves were performed by changing the bathing fluid (containing or not the usual dose of PGE_2) after 2, 4, 6, 8, 10, 15, 30, 45, 75, 90 min. A sample was taken at each time fo radioassay. Differences between control and PGE_2-treated organs have been evaluated by the Duncan's test.

Results and discussion. The influence of PGE_2 on the calcium exchangeability in rat uterus is shwon in Figure