concentrations of L-dopa than of dopamine are needed to achieve any degree of mydriasis (fig. 1).

L-dopa, unlike dopamine, can pass the blood-brain barrier and so may affect pupillary size through central mechanisms. To examine this possibility we injected L-dopa in mice pretreated with peripheral dopa-decarboxylase inhibitors. The use of these agents is expected to increase any central effect of L-dopa. The mydriatic effect of L-dopa was blocked completely by 300 mg/kg carbidopa or benserazide (fig. 2). Thus, the mydriasis produced by L-dopa in mice involves peripheral rather than central mechanisms. The abolition of L-dopa mydriasis by carbidopa precludes the possibility that L-dopa itself directly stimulates postsynaptic receptors. The mydriasis induced following L-dopa injection must therefore occur indirectly, for example after conversion to either dopamine or noradrenaline. Pretreatment with fusaric acid, an inhibitor of the enzyme dopamine-beta-hydroxylase, antagonized the pupillary dilation caused by L-dopa. Although we could not obtain a complete blockade of the mydriasis by fusaric acid (because higher doses of this drug proved to be toxic), the observed inhibition is significant (fig. 2).

Thus, our results indicate that the pupillary dilation produced in mice following the injection of L-dopa is caused by its peripheral conversion to noradrenaline, which in turn stimulates the alpha-adrenergic receptors in the dilator iridis, thus producing mydriasis. The complete blockade by phentolamine of L-dopa induced mydriasis (fig. 2) supports this conclusion. These results complement our recent report on the effects of dopamine in this system[8], which are consistent with the present findings.

Treatment with L-dopa in humans produces several autonomic side-effects[4]. The exact mechanism responsible for these is not clear but it is possible that some (mainly those which are prevented through the concurrent administration of decarboxylase inhibitors) may be underlain by mechanisms similar to those described here. Other effects, such as action on sympathetic ganglia[9], or on presynaptic dopamine receptors in noradrenergic terminals[10,11], may also contribute to autonomic dysfunction following treatment with L-dopa.

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Studies on the in vitro binding of D-penicillamine to cholestyramine

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Summary. Adsorption of D-penicillamine to cholestyramine depends on the amount of the resin, the pH and the presence of other compounds such as bile salts. In the usual drug to resin ratio (150 mg D-penicillamine and 4–8 g cholestyramine per single dose) the percentage of D-penicillamine adsorbed to cholestyramine was about 10% of the applied dose; Bile salts (10 mmoles/l) inhibited this small adsorption by 87%.

The anionic exchange resin cholestyramine[1,2] is used in the treatment of a variety of conditions including the pruritus of cholestatic syndromes[3,4]. It is believed to relieve itching by interruption of the enterohepatic circulation of bile acids[2,5], reducing their concentration in the serum[6]. In addition to bile acids, cholestyramine has been shown to bind a large number of other substances. Interactions of the resin with drugs may have clinical implications, since they may decrease serum levels of these compounds and hence reduce their therapeutic effect. Binding to cholestyramine has been demonstrated for digitalis[7,8] anticoagulants[9,10], antiinflammatory and analgesic drugs[11], antibiotics[12] and a number of other ionic[12] and non-ionic compounds[13]. The anionic drug D-penicillamine is used in patients with primary biliary cirrhosis[14,15] who often need cholestyramine therapy for itching. The potential interaction of cholestyramine with D-penicillamine must, therefore, be regarded as a problem of clinical interest. Although it has been recommended that simultaneous administration of these drugs should be avoided[16], exact information about the binding of D-penicillamine to cholestyramine is missing. Therefore, the binding of D-penicillamine to the resin was studied and the influence of bile salts on this interaction investigated.

Materials and methods. Cholestyramine in anhydrous form and of pharmaceutical grade (Lappe AG, Bergisch-Gladbach, FRG) was dried in a desiccator before use. D-penicillamine (β,β' dimethylcysteine) was generously supplied by Knoll AG (Ludwigshafen, FRG). The bile salts were of analytical grade and obtained from Sigma Chemicals (St. Louis, Mo. USA). The other chemicals were of analytical grade and purchased from commercial sources.

Experimental procedere: Anion-exchange studies were performed according to the method of Gallo et al.[12], slightly modified, by incubation of 5 µmoles D-penicillamine with various amounts (25, 50, 100 mg) of cholestyramine suspended in 5 ml 0.15 M saline (pH 7.7), corresponding to a drug to resin ratio of 1:33 to 1:133. pH dependence was studied from pH 1 to pH 10 with 400 mg cholestyramine and 5 µmoles D-penicillamine. To determine maximal

adsorption, a constant amount of the resin (400 mg) was incubated with increasing amounts of D-penicillamine (2,5, 5, 10, 20, 50 µmoles) for 2, 6, 18 and 24 h. To investigate the influence of bile salts, a mixture of the major bile salts of human duodenal bile[17] containing 21% glycocholate, 19% glycochenodeoxycholate, 25% glycodeoxycholate, 10% taurocholate, 6% taurodeoxycholate, and 16% taurochenodeoxycholate was added to the reaction medium to make a final bile acid concentration of 10 mmoles/l. After shaking with a Vortex mixer a homogenous suspension was obtained. The tubes were then incubated in a water bath (37 °C) and shaken horizontally (80/min) for 2 h if not stated otherwise. After centrifugation (4500 × g, 10 min), the supernatant was assayed for D-penicillamine.

Analytical procedure: D-penicillamine was determined by the colorimetric method of Pal[18].

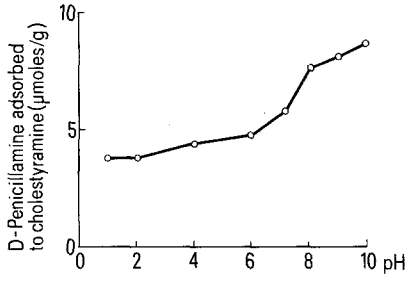
A 1-ml aliquot of the supernatant was incubated with $FeCl_3$ (15 mM) and KCN (0.2 M) at 65 °C for 5 min. After dilution to 5 ml with distilled water and centrifugation (10 min), absorption was measured at 623 nm. Calibration curves were linear from 0.1 to 1 mM. The amount of D-penicillamine bound by the resin was calculated from the difference between the D-penicillamine concentration in the supernatant and in a control containing all constituents except cholestyramine. All experiments were performed 6 times. The results were expressed as means ± SEM. The statistical significance of the differences between means was tested using the Mann-Whitney test for unpaired samples. $p < 0.05$ was considered statistically significant.

Results. The equivalents of D-penicillamine adsorbed to cholestyramine increased from 0.46 ± 0.04 to 0.96 ± 0.05 µmoles when the amount of the resin was increased from 25 mg to 100 mg in the incubation medium, corresponding to a drug to resin ratio of 1:33 to 1:133 (table). Maximum adsorption was obtained with 25 µmoles D-penicillamine and 400 mg cholestyramine after an incubation time of 18 h. It was 59.4 ± 1.2 µmoles of D-penicillamine per g resin (13.6 µmoles D-penicillamine per mmole equivalent resin). Adsorption of D-penicillamine to the resin decreased from 8.75 µmoles/g at pH 10 to 3.8 µmoles/g at pH 1 (fig.). Simultaneous incubation of D-penicillamine with bile salts (10 mmoles/l) resulted in a large decrease in adsorption of D-penicillamine to the resin (table). With 25 mg cholestyramine (drug to resin ratio 1:33) 0.06 ± 0.04 µmoles were adsorbed in the presence of bile acids as compared to 0.46 ± 0.04 µmoles in their absence ($p < 0.01$). This corresponds to 87.1% inhibition of adsorption. This effect of bile salts decreased with an increasing drug-to-resin ratio. At a drug-to-resin ratio of 1:133 it was as low as 10.5% (table).

Discussion. In this study we could demonstrate an in vitro adsorption of D-penicillamine to cholestyramine which depends on various conditions such as the amount of resin, the concentration of D-penicillamine, the pH and the presence of bile salts. The drug to resin ratio appears to be of considerable importance. The range of this ratio in patients with primary biliary cirrhosis receiving both D-penicillamine and cholestyramine may be between 1:26 and 1:52 (150 mg D-penicillamine and 4 to 8 g cholestyramine per dose)[16]. Adsorption of D-penicillamine to cholestyramine in this dose range amounted in our in vitro studies to about 10%. This small adsorption was further reduced by bile salts which exchange for D-penicillamine on the resin (table).

Such an effect of bile salts on adsorptive binding has been demonstrated for fatty acids, flufenamic and mefenamic acid and other compounds[11,12,19,20]. This may be due to the higher affinity and binding capacity for bile acids[1]. Despite the nearly complete dissociation of the carboxylic groups in both compounds as expected from their pK_a (pK_a of the carboxylic group of D-penicillamine: 1.8[22]; pK_a of the carboxylic group of taurocholate: 1.56–3.33, glycocholate: 2.78–4.35[1]) binding of the bile salts is greater because of additional attracting forces which have been demonstrated for bile salts[1]. On the other hand, repulsion of the partially positive charge of the amino group of D-penicillamine (pK_b 7.9[22]) and the quarternary ammonium residue of cholestyramine may weaken the adsorptive forces. This is consistent with the pH dependence of adsorption (fig.).

Predictions of in vivo interaction from in vitro studies are hazardous[10], but, as a rule, adsorption seems to be greater in vitro than in vivo[12]. From the findings of this in vitro study it appears unlikely that clinically relevant binding of D-penicillamine to cholestyramine in the gastrointestinal tract is to be expected when the 2 drugs are administered together in the usual dose range. However, bioavailability of D-penicillamine may be influenced by other factors than adsorption to the resin[21]. Therefore, only studies of D-penicillamine bioavailability with and without co-administration of cholestyramine will give the final answer.



pH dependence of D-penicillamine adsorption to cholestyramine (µmole/g resin). Each point represents the mean of 3 experiments.

Adsorption of D-penicillamine (5 µmoles) to cholestyramine in the presence and absence of bile salts

Cholestyramine (mg per 5 ml incubate)	Drug to resin ratio	D-penicillamine adsorbed (µmole) in NaCl (0.15 moles/l) medium	in bile salt (10 mmoles/l) medium	Percent inhibition of adsorption by bile salts
25	1:33	0.46 ± 0.04	0.06 ± 0.04	87.1 $p < 0.01$
50	1:66	0.56 ± 0.05	0.19 ± 0.07	66.1 $p < 0.01$
100	1:133	0.96 ± 0.05	0.86 ± 0.10	10.5 $p < 0.05$

The values are the means of 6 experiments ± SEM. Statistical calculations were performed comparing the incubations with and without bile salts.

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Structure elucidation of mammalian TCDD-metabolites

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Summary. Thin layer and gas chromatographic examination of the bile of dogs which were given tritium-labelled TCDD revealed the presence of several polar biotransformation products. The structure of 5 phenolic metabolites was elucidated by combined gas chromatography-mass spectrometry. A metabolic breakdown scheme for TCDD in the dog is proposed.

Quite high rates of conversion of 2,3,7,8-tetrachlorodibenzo-p-dioxin have recently been found in mammals,[2-4] whereas the metabolism of this compound by microorganisms appears to be very slow[5]. The differences among species in susceptibility towards TCDD[6,7] could be explained, at least partially, by different rates of metabolism, because earlier studies have shown that the dog, which is less sensitive to TCDD[6] than the rat, also converts the compound at a higher rate[8]. In addition, this animal species was chosen in our investigation because metabolites are excreted into the bile and, in the dog, bile duct cannulation is an ethically acceptable technique.

Tritium labelled TCDD (sp. act. 40 Ci/mmole; source: A. Kende, Rochester, NY) was purified by preparative gas chromatography (2 m×2 mm ID, DC 560, 220 °C) and mixed with unlabelled TCDD (source: Dow Chemical, Midland, MI), which was previously recrystallized several times from hot anisole, to yield a product with a sp. act. of 26.3 mCi/mmole. GC-MS analysis revealed only the presence of a penta-CDD (1%) but of no other isomers of TCDD. The radiochemical purity was higher than 98.5% (GC).

For the metabolism experiment a Thomas cannula was implanted in a 1-year-old beagle dog, weighing about 14 kg; the animal was then left to recover for a few weeks. A total amount of 5.4 mg of TCDD was administered enterally by direct introduction into the duodenal lumen in 4 portions of 1-2 mg, leaving time intervals of 2-7 days between applications. Treatment with TCDD was accompanied by very severe toxic symptoms, such as vomiting, anorexia and cachexy which ultimately caused the death of the animal (17 days after the 1st dose).

Since the principal aim of the experiment was the isolation of TCDD metabolites for structure elucidation, only a few parameters concerning the pharmacokinetics of the compound were investigated. Excretion of radioactivity in the bile reached a maximum on day 1 or 2 following administration, then decreased quite rapidly. This is an evident contrast to the elimination characteristics in the rat, in which the concentration of radioactivity in the bile remained constant over several days. The amount of radioactivity in the bile (expressed as a percentage of the amount administered) was 11 and 8% within 3 days after application of doses 3 and 4 on days 7 and 14, respectively.

GC-MS data of TCDD and methylated metabolites

Compound	Fraction	Elution temperature (°C)	MS-data M^+	No. of Cl atoms	Fragmentation
I (TCDD)		217.3	320	4	M^+-63; M^+-126
II	C1, C2	231	350	4	M^+-15; M^+-43
III	C1	223.9	316	3	M^+-15; M^+-43
IVa	C2	227	346	3	M^+-15; M^+-43
IVb	C2	228.8	346	3	M^+-15; M^+-43
V	B2	231.8	366	4	M^+-50
VI	B1	134.5	206	2	M^+-15; M^+-43

Finnigan 4000 quadrupole GC-MS instrument, EI 70 eV 240 °C; 24 m SP 2100 fused silica capillary; column conditions: 50 °C (2 min isothermal), 20 °C/min to 160 °C, 5 °C/min to 240 °C. Mass-spectra (m/z 100-450, 1.7 sec/scan) recorded using Finnigan 6115 data system.