taken, piperazine (0.1 mM) slightly potentiated the carbachol responses whereas the higher concentration (0.5 mM) was ineffective (figure 1). Carbachol depolarizations were antagonized by tubocurarine which had a dissociation constant (average value = 0.41 μM, obtained by the dose ratio method) virtually identical to that calculated from the data of Jenkinson[11].

In the frog cord piperazine (0.1 mM) depolarized spinal roots (≤ 0.5 mV) and depressed (by 30–40%) the amplitude of the orthodromic ventral and antidromic dorsal root potentials. In unstimulated preparations piperazine produced a slowly-developing reduction (reversible on washing) of spontaneous ACh release, the peak effect being found 20 min after application of the drug (figure 2, A). 1 mM piperazine produced a similar depression with peak effect occurring in the 1st 10 min sample while a 50 μM concentration gave an insignificant decrease (10%) in ACh release. In order to check for a direct action of piperazine on functionally-identifiable cholinergic nerve terminals, ventral roots were stimulated antidromically for 10 min, commencing 30 min after application of the drug. Other cords were similarly stimulated, either in control Ringer or in the presence of the excitatory amino acid DL-homocysteate[13] (0.1 mM). Figure 2, B shows that piperazine reduced ACh release induced by antidromic stimulation whereas homocysteate produced an additive effect. This amino acid depolarized (about 0.5 mV) spinal roots and increased spontaneous ACh release by 75%.

Discussion. Piperazine had no antagonistic effect on nicotinic receptors of frog muscle although an inhibitory effect has been observed in other studies[5,6]. Since these authors measured a decrease in muscle contractions rather than changes in membrane potential, it is possible that piperazine antagonizes peripheral cholinergic transmission by acting not on postjunctional receptors but on contractile mechanisms. The slight potentiation of carbachol seen after 0.1 mM piperazine needs not to be interpreted as a change in cholinergic receptor sensitivity and is perhaps an unspecific action (e.g. due to an increase in membrane resistance). A presynaptic action of piperazine seems likely in view of its depressant effects on ACh release from the spinal cord. This effect is probably direct because it is present following antidromic ventral root stimulation which activates cholinergic[8,9,12] motor axon collaterals. It is not unlikely that the presynaptic action of piperazine contributes to the observed reduction in spinal cord potentials[4].

Axonal block cannot explain the reduced ACh release since the depression was less than that produced by the local anaesthetic tetrodotoxin[9] and large doses did not abolish evoked root potentials. Piperazine-induced reduction of electrically evoked release was larger than that calculated by simple subtraction of the spontaneous release in the presence of piperazine from the evoked release of untreated control cords. This implies that the release-depressing activity was greater during continuous electrical stimulation than at rest. Sustained nerve terminal depolarization reduces transmitter release due to nerve stimulation at the neuromuscular junction[14]. As piperazine and DL-homocysteate produced comparable depolarizations but had different actions on ACh release, it follows that ventral root depolarization did not reach the presynaptic terminals or was insufficient to depress release. In any case the action of piperazine on ACh release must have a different explanation. Since Ca^{2+} influx is a critical factor for ACh release[15], we might suppose that piperazine depressed release by interfering with presynaptic Ca^{2+} movements coupled with the excitation-secretion process.

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Interaction of chlorpromazine with organic solvents and fatty acids as studied by UV-spectrophotometry[1]

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Summary. The magnitude of the UV-spectral change of chlorpromazine increases in the presence of increasing concentrations of alcohols or fatty acids and with increasing chain length. A maximum is reached with 14.0- or 16.0-fatty acids. The difference spectrum is still larger with unsaturated fatty acids, a maximum effect being obtained with one cis-double bond. The spectral change is abolished by chaotropic and enhanced by antichaotropic agents.

Tissue-binding studies with basic lipophilic drugs[2] have revealed that the drugs used are mainly bound to microsomes and mitochondria of hepatic and extra-hepatic tissues. With chlorpromazine (CPZ) and related drugs, the binding could be further traced to membrane phospholipids, in particular to their fatty acid moieties[3]. Binding observed by equilibrium dialysis was confirmed by a spectroscopic technique, which is based on a red shift in the UV-spectrum of CPZ in the presence of both protein and lipid binders. Further investigations demonstrated that the magnitude of spectral change is dependent on the concentrations of both ligand and binder, that it is enhanced by a pH increase from 6 to 10, decreased by urea (8 M), and not changed by variation of the ionic strength from 0 to 0.5[4].

Table 1. Magnitude of spectral change (ΔA) of chlorpromazine in solutions of saturated fatty acid (FA) anions

FA	10^{-1} M	10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M
6.0	0.068 ± 0.005				
8.0	0.518 ± 0.019	0.030 ± 0.004			
10.0		0.199 ± 0.010	0.029 ± 0.005*		
12.0			0.190 ± 0.009	0.018 ± 0.013*	
14.0				0.241 ± 0.015	0.041 ± 0.004
16.0				0.076 ± 0.003	0.064 ± 0.008
18.0				0.044 ± 0.002	0.028 ± 0.006*
22.0				0.035 ± 0.005	0.019 ± 0.006*

CPZ 50 µM. Mean values ± SE of 4 to 14 experiments. * Statistically not significantly different from blank without FA (0.015 ± 0.006).

Table 2. Magnitude of spectral change (ΔA) of chlorpromazine in solutions of unsaturated fatty acid (FA) anions

FA	10^{-4} M	10^{-5} M	10^{-6} M
18.0	0.044 ± 0.002	0.028 ± 0.006*	
18.1 trans	0.460 ± 0.016	0.108 ± 0.010	
18.1 cis	1.065 ± 0.014	0.108 ± 0.003	0.011 ± 0.006*
18.2 cis, cis	0.907 ± 0.020	0.054 ± 0.003	0.010 ± 0.007*
18.3 cis, cis, cis	0.741 ± 0.022	0.067 ± 0.003	0.006 ± 0.002*
22.0	0.035 ± 0.005	0.019 ± 0.006*	
22.1 cis	0.621 ± 0.019	0.097 ± 0.002	0.006 ± 0.003*

See explanations table 1.

Based on the likely predominance of hydrophobic interactions in the binding of CPZ to membrane lipids, this study is aimed at quantifying the difference spectrum under various experimental conditions. Thus, the magnitudes of spectral change were measured upon transfer of CPZ from an aqueous into less polar environments. Agents known to disrupt or enhance hydrophobic interactions were also used.

Materials and methods. Solution of CPZ and of alcohol (< 50% v/v) or fatty acid anions, each in phosphate buffer (pH 7.4, 0.05 M), were placed separately in the compartments of sample and reference split-compartment mixing cuvettes. The baseline was recorded, after which the contents of the sample cuvette were mixed and the difference spectrum recorded.

When CPZ had to be measured against CPZ in alcohol solutions > 50% v/v or in nonpolar solvents, a modified technique was required: after recording of the baseline, the aqueous CPZ in the sample cuvette was replaced by buffer, and the pure solvent by CPZ in solvent. If the solvent was nonpolar (nonmiscible with water), 1 ml aqueous CPZ (100 µM) was mixed with 0.1 ml KOH (5 M) and the CPZ-base extracted into 5 ml of the solvent by shaking for 20 min.

The CPZ interaction spectrum with peaks at 260 and 325 and troughs at 245 and 280 nm has been discussed previously[3,4]. Spectra were recorded with a Unicam SP 800 spectrophotometer. The magnitudes of spectral change $\Delta A = \Delta A_{260} - \Delta A_{245}$ is the difference of the absorbances of the larger positive and negative peaks. The fluctuation in the baseline was accounted for by measuring blank ΔA-values without a solvent or binder. Only if test and blank ΔA-values were significantly different ($p < 0.05$) was the existence of a difference spectrum postulated.

Results. The red shift of the UV-spectrum of CPZ, which produces the typical difference spectrum in the presence of binders like serum albumin or lipids, is also obtained when CPZ is transferred from an aqueous into a nonaqueous environment. This is shown in figure 1 for a number of alcohols and nonpolar organic solvents. A continuous in-

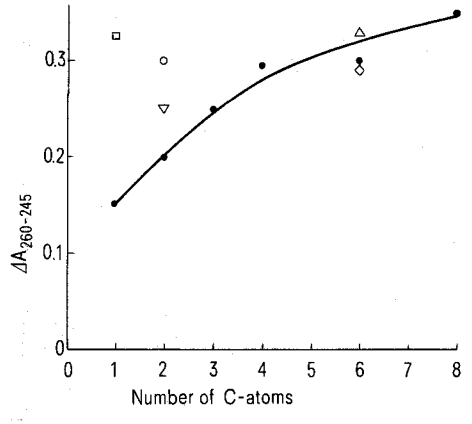

Fig. 1. Magnitude of spectral change (ΔA) of chlorpromazine in n-alcohols (●) of various chain lengths and in other solvents: □ chloroform, ▽ dichloroethane, ○ acetonitril, ◇ hexane, △ cyclohexane. Value for dibutylether was 0.460±0.003. CPZ 20 µM. Mean values of 4 to 6 experiments.

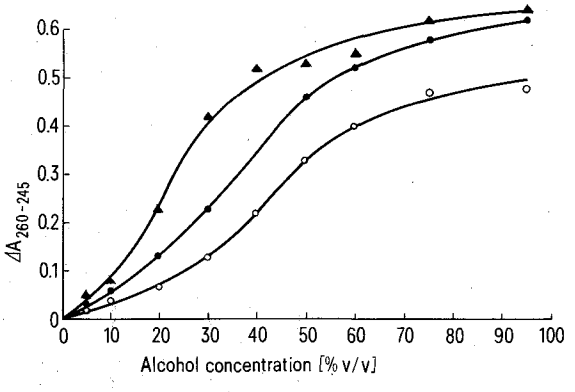

Fig. 2. Magnitude of spectral change (ΔA) of chlorpromazine in aqueous alcohol solutions at various concentrations. ○ methanol, ● ethanol, ▲ propanol. CPZ 50 µM. Mean values of 4 to 7 experiments.

crease in the ΔA-values was also observed in aqueous solutions with increasing concentrations of alcohols (figure 2).

The difference spectra elicited upon interaction of CPZ with lipids and fatty acids have been measured using individual fatty acids as aqueous anionic solutions. The results obtained with saturated fatty acids are summarized in table 1, the ones with unsaturated fatty acids in table 2.

In the presence of urea (8 M), a chaotropic agent, the difference spectrum elicited by CPZ (50 µM) in the presence of 18.1-cis- or 18.1-trans fatty acid (10^{-4} M) disappeared. This was in contrast to the antichaotropic ion, fluoride, which produced a 1.6fold increase in ΔA of CPZ in the presence of 18.1-trans fatty acid (10^{-4} M). Finally, the ΔA-value of CPZ in hexane (see figure 1) was not increased by the addition to the hexane solution of palmitic acid (16.0) even at a concentration of 10^{-2} M.

Discussion. The difference spectrum of CPZ was first observed with serum albumin[5] and was believed to express hydrophobic interactions. This was also concluded from analogous studies with lipids which are biologically important binders of the drug[2,3] and which elicit the difference spectrum at much lower concentrations[4]. CPZ can undergo self-association[6,7] which also results in the appearance of the difference spectrum[4].

The appearance of the difference spectrum of CPZ with alcohols, nonpolar solvents, and fatty acids, as well as the increase of ΔA with increasing hydrophobicity of the environment adds more evidence to this difference spectrum representing hydrophobic interactions. In the case of nonpolar solvents, self-association of CPZ can be excluded for thermodynamic reasons[8].

Additional problems are exposed by the experiments with fatty acids (tables 1 and 2). The critical micellar concentrations of the fatty acids[9] are likely to be about one order of magnitude higher than the concentrations at which the difference spectrum appeared, indicating that interaction occurs with both micellar and monomeric fatty acids. One or more double bonds introduced into a fatty acid produce an upsurge in ΔA without appreciable changes in hydrophobicity. Since the ΔA of the cis- and trans-isomers of a fatty acid may vary considerably, steric rather than electronic factors must be assumed to influence the interaction.

The difference spectrum was abolished by urea (8 M) which is known to dissociate hydrogen bonds and thus hydrophobic interactions[8,10]. The difference spectrum of CPZ with serum albumin or microsomal fractions was also abolished by urea, and this was paralleled by a decrease in their binding as determined with equilibrium dialysis[3,4]. In contrast, fluoride ions, which enhance hydrophobic interactions, increased ΔA.

In conclusion, the binding of CPZ to fatty acids is mainly due to hydrophobic interactions; however, steric factors of the fatty acids play an additional role in the magnitude of the interaction spectrum and thus possibly in the actual binding process.

1 Supported by a grant of the Swiss National Science Foundation.

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Erythrocytes within pancreatic B-cells of corticosteroid-treated mice

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Summary. An ultrastructural study of the endocrine pancreas of female ICR mice that received 21 daily injections of the synthetic glucocorticoid, Triamcinolone diacetate (8 mg/kg b.wt) revealed some examples of microhaemorrhage within islets of Langerhans, extravasation of erythrocytes, and the presence of erythrocytes within B-cells, where they undergo degradation to form myelin-like configurations.

Glucocorticoids are generally regarded as potential diabetogenic agents in both experimental animals and man[1-6]. During the course of prolonged administration to mice of a synthetic glucocorticoid, Triamcinolone diacetate, an analogue of cortisol, followed by an ultrastructural examination of their Islets of Langerhans, we have encountered some instances of erythrocytes apparently within B-cells. So far as we are aware, this is the first recorded report of such erythrocytic extravasation and we would like to record briefly the details.

Material and methods. Female ICR mice, 6 weeks old, received 21 daily s.c. injections of 8 mg/kg b.wt Triamcinolone diacetate ('Ledercort', Lederle Labs, American Cyanamid Co., New York). Untreated littermates maintained under identical conditions served as controls. All animals were maintained on mouse Purina chow (Ambar Purina Co. No. 19-210, Israel) and tapwater ad libitum. During the final 20 h before sacrifice, only water was made available. Mice were killed by neck dislocation and their pancreases carefully removed and immersed in ice-cold (4 °C) 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Pancreases in the fixative were cut into small blocks with ultrafine dissection scissors under a binocular microscope, by means of which the islets are readily visible, and fixed for 2 h. Blocks were stored overnight in the cacodylate buffer containing 7.5% w/v sucrose, postfixed in 1% osmium tetroxide, dehydrated in graded concentrations of ethanol and embedded in Epon 812. Epon sections, 0.5 µm thick, were stained with 0.1% toluidine blue in 1% borax for light microscopy. Sections, 60–90 nm thick, of selected blocks containing islets, were cut with glass knives on a Cambridge-Huxley Mk 1 ultramicrotome, collected on un-