curves which were parallel to that of adenosine (Figure 1). The cyclic nucleotides were much weaker inhibitors than their parent 5′-phosphates or nucleosides (Table II), and although platelet clumping was reduced in the presence of millimolar concentrations of the cyclic nucleotides, it was not abolished. c2-MeSAMP and c2-ClAMP were significantly more potent than cAMP ($p = 0.05$ and $p < 0.05$, respectively), which had minimal inhibitory activity. The samples of the two analogs used in platelet studies were free of their breakdown products, 2-methylthioadenosine 5′-phosphate and 2-chloroadenosine, which are potent inhibitors of platelet aggregation (Table II).

When the cyclic nucleotides were stirred in PRP for periods up to 20 min prior to the addition of ADP, a moderate increase in potency was observed in each case (Figure 2). Such potency increases might be anticipated if sheep PRP plasma converted the cyclic nucleotides to their more potent linear congeners. c2-MeSAMP and c2-ClAMP are substrates for cyclic 3′,5′-nucleotide phosphodiesterase prepared from rat heart, and have K_m values similar to that of cAMP, and V_{max} values which are 60% of that of cAMP (unpublished results of LUKAS and MAGUIRE). Paper chromatographic analysis of mixtures of PFP with cAMP or c2-ClAMP which were incubated for 2 h at 37 °C detected only the starting nucleotides, indicating that cyclic 3′,5′-nucleotide phosphodiesterase was absent from sheep plasma. Similarly only the cyclic nucleotides were found in mixtures of PRP incubated with cAMP and with c2-ClAMP. These observations are in accord with reports of the absence of cyclic 3′,5′-nucleotide phosphodiesterase from human plasma[3,12,13]; the enzyme has an intraplatelet location[13], and is released in both human and rat PRP on sonication[13,14]. When cAMP was incubated with sonicated sheep PRP, paper chromatography detected both cAMP and inosine, demonstrating that release of intraplatelet cyclic 3′,5′-nucleotide phosphodiesterase had occurred, with resultant hydrolysis of cAMP to AMP; sheep plasma metabolises AMP to inosine via adenosine (GOUGH and MAGUIRE, unpublished observations). These findings indicate that the cyclic nucleotides are not converted to their more potent inhibitory metabolites in intact citrated sheep PRP, and that they inhibit ADP-induced platelet aggregation per se. The mechanism of action of cAMP in inhibiting ADP-induced platelet aggregation is not understood. It is thought to involve the passage of cAMP into platelets thereby augmenting intraplatelet cAMP levels[3], however the incorporation of intact cAMP into platelets has not been demonstrated. The log-dose response curves obtained for cAMP and its two analogs in this study were parallel to that of adenosine, suggesting that the effects of the cyclic nucleotides might result from competitive interference with the action of ADP at the platelet membrane, as is indicated for adenosine and AMP[5,6,15]. Whatever the site of action of cAMP, c2-MeSAMP and c2-ClAMP, it is clear that 2-substitution of cAMP by methylthio and chlorogroups yields analogs having significantly greater inhibitory activity than cAMP itself. These analogs, in which the salient structural features of cAMP, i.e. the cyclic phosphate, the 6-amino and the 2′-hydroxyl groups, remain unmodified, should prove useful tools for the investigation of the effects of cAMP on platelet function[16].

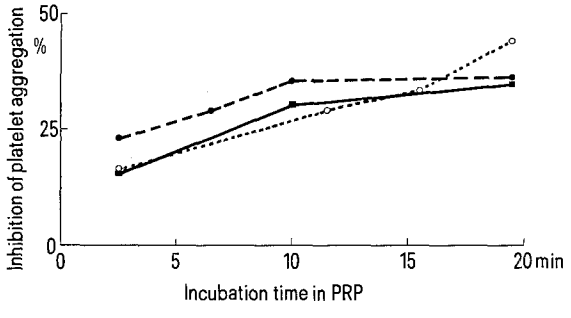

Fig. 2. Effect of incubation time on the inhibition of ADP-induced platelet aggregation by cAMP (■), c2-MeSAMP (●) and c2-ClAMP (○). 1.8 mM cAMP, 0.19 mM c2-MeSAMP and 0.9 mM c2-ClAMP were incubated in stirred PRP for the times indicated prior to the addition of 0.67 μM ADP. Inhibition is expressed as described in the legend to Figure 1.

Zusammenfassung. Nachweis, dass die zyklischen Nukleotide schwächere Inhibitoren der ADP-induzierten Plättchenaggregation sind als die analogen 5′-Phosphate und Nukleoside, wobei es sich um eine direkte Wirkung und nicht um den Effekt einer Umwandlung in 5′-Phosphate und Nukleoside handelt.

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Influence of Emulsified Fat on Chlorpromazine Availability in Rabbit Blood

Previous studies have shown that in simplified blood the bulk of the lipophilic chlorpromazine (CPZ) is bound to erythrocytes and to albumin; only about 3% of CPZ are free in solution[1–3]. The distribution of CPZ between the storage depots and the aqueous phase could be influenced by various drugs, as well as by relatively low concentrations of free fatty acids[3]. It was suggested that fat, too, could change the distribution of CPZ in blood. Therefore, in the present paper the influence of a commercially available fat emulsion on the fraction of free CPZ in rabbit blood, as well as its influence on the acute toxicity of CPZ in rabbits, has been investigated.

Material and methods. Animals: rabbits of mixed breed and either sex weighing 1.8–2.2 kg were used. Drugs: chlorpromazine·HCl was obtained from Bayer, Leverkusen. Lipofundin S 10®, containing 100 g soybean oil, 7.5 g soybean phospholipides and 50 g xylitol in 1000 ml of water (Braun, Melsungen) was used as fat emulsion. An aqueous solution of 5% xylitol was purchased from Pfrimmer, Erlangen.

In vitro experiments: 1 ml of the fat emulsion was added to 4 ml of heparinized rabbit blood to yield a final concentration of 25 mg fat/ml and 10^{-4} *M* CPZ. In control experiments 1 ml of 5% xylitol solution was added to 4 ml blood. Furthermore, control experiments were carried out using 5 ml of normal rabbit blood containing 10^{-4} *M* CPZ. These samples were dialysed in Visking tubings against 5 ml of phosphate buffer (0.02 *M* phosphate containing 0.15 *M* NaCl, pH = 7.4). The fraction of free CPZ in the buffer solution and the total CPZ content of the dialysed blood were extracted with *n*-heptane containing 1.5% isopentanol (v/v) and were measured colorimetrically in 5 *N* H_2SO_4 containing $FeCl_3$ (0.1 mg/ml)[4]. Additional dialysis experiments were performed using the phosphate buffer solution containing 10^{-4} *M* CPZ (Table I).

In vivo studies: 3 days before the experiments were started 4 silver screw electrodes were placed into the skull of the rabbits for EEG recording[5]. The fat emulsion or the xylitol solution were infused i.v., the perfusion rate was 1 ml/min. After infusion of 15 ml, CPZ·HCl (25 mg/kg and 30 mg/kg, respectively) was given. CPZ was dissolved in isotonic saline solution and was always injected intravenously in a volume of 2.5 ml/kg within 55–65 sec. The perfusion was continued without interruption to a final volume of 50 ml if the animals had not died after CPZ injection.

Table I. Influence of emulsified fat (25 mg/ml) on free CPZ content of rabbit blood in vitro and on free CPZ content of phosphate buffer solution

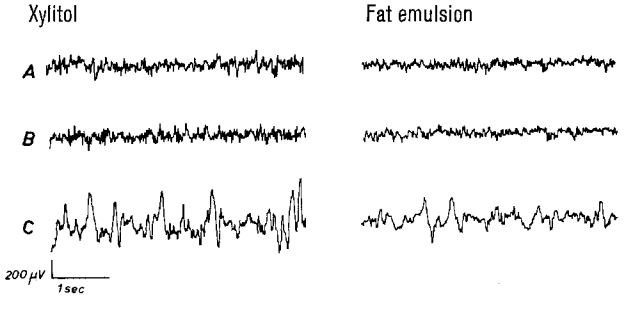
Added drug	Percentage of free CPZ	
	Rabbit blood	Buffer solution
—	2.05 ± 0.20 (*n* 4) [b]	
Xylitol solution [a]	2.23 ± 0.19 (*n* 4) [b]	96.2 ± 2.0 (*n* 6) [c]
Fat emulsion [a]	0.87 ± 0.11 (*n* 4) [b]	0.88 ± 0.23 (*n* 6) [c]

The initial CPZ concentration was 10^{-4} *M*. Values are given as means ± S.E.M. [a] 1 ml of xylitol solution and fat emulsion, resp. was mixed with 4 ml of rabbit blood or buffer solution. [b] *n*, number of animals. Dialyses were made in triplicate. [c] *n*, number of dialyses.

Table II. Survival of rabbits after a toxic dose of CPZ

Infusion	25 mg/kg CPZ (number of animals)		30 mg/kg CPZ (number of animals)	
	Survived	Died	Survived	Died
—	0	6		
Fat emulsion	7	0	7 [a]	0
Xylitol solution	6	1	0	6

The animals were treated with an infusion of either fat emulsion or xylitol solution (1 ml/min) up to 50 ml. 15 min after starting the infusion a single dose of CPZ was injected i.v. [a] Two animals died 2 and 3 h after CPZ application. Different from the xylitol treated group with $P < 0.01$ calculated by the Fisher-test[13].



EEG of rabbits before (A), 14 min after 1 ml/min infusion of xylitol solution or fat emulsion (B), and 5 min after 25 mg/kg CPZ application, i.e. 20 min after the infusion was started (C).

Results and discussion. As demonstrated in Table I, the fat emulsion added to rabbit blood containing 10^{-4} *M* CPZ caused a significant decrease of the fraction of free CPZ, whereas in control experiments in which only a 5% xylitol solution was added, a significant change in the free CPZ concentration was not apparent. In comparison with normal blood there was no significant change of free CPZ concentration by the xylitol solution added. It may be suggested that CPZ as a lipophilic drug was taken up by the emulsified fat and thus fraction of free CPZ was reduced. As even the low concentration of 2% free CPZ is still reduced, the emulsified fat obviously has a great capacity for CPZ. This idea is supported by the almost complete transfer of CPZ from the buffer solution to the added fat emulsion (Table I).

These in vitro findings suggested that emulsified fat represented an additional storage depot for CPZ in blood. If such a storage depot occurs in vivo, too, the acute toxic effects of CPZ should be decreased.

In in vivo studies the survival of rabbits after administration of high doses of CPZ was chosen to investigate the effect of fat on acute toxicity of this drug. The results are summarized in Table II. A dose of 25 mg/kg CPZ i.v. was lethal; the LD_{50} in rabbits is 15 mg/kg i.v.[6]. However, all the animals survived after this lethal dose after prior infusion of the fat emulsion, yet 6 of 7 animals also survived after prior infusion of the xylitol solution. Presumably, under these conditions the hypotensive effect of CPZ was reduced by expanding plasma volume and thus the animals survived. When the dose was increased up to 30 mg/kg CPZ all the animals pretreated with the fat emulsion survived, whereas the animals pretreated with the xylitol solution died towards the end or shortly after the CPZ injection. Obviously central effects of CPZ are involved

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in its toxic action, as may be suggested from the EEG-changes (Figure), which seem to be more pronounced in rabbits pretreated with the xylitol solution than with the fat emulsion. The survival of animals would be due to an uptake of CPZ by the emulsified fat as the i.v. infusion of a fat emulsion leads to a temporary lipemia[7,8]. Furthermore, additional effects of the fat emulsion might decrease the acute toxicity of CPZ. Surfactants like the phospholipids of the fat emulsion used can reduce the diffusion of drugs into the tissue[9]. In addition, it could be demonstrated that the capillary flow in the mesentery of rabbits is lowered in the presence of emulsified fat[10]; a decrease of the capillary flow in brain would reduce the CPZ uptake into the CNS.

Infused fat emulsions hardly enter the brain[11] but can be taken up by phagocytosis by the liver[12]; this means a further pathway of elimination for CPZ. The latter effect, however, is probably of less importance to the acute toxicity of CPZ. From the present results it might be concluded that a fat emulsion in blood can take up lipophilic drugs, reduce their fraction dissolved in plasma water and thus decrease their actual availability at the sites of action. Whether this effect may be used for therapeutical management of poisoning due to CPZ or other lipophilic drugs remains to be shown.

Zusammenfassung. Kaninchen überlebten die letale Dosis von 30 mg/kg Chlorpromazin (i.v.) nur zusammen mit einer Fettinfusion (0,5 ml/kg/min Lipofundin S 10®). Es konnte in vitro gezeigt werden, dass der Zusatz einer Fettemulsion (Lipofundin S 10®) zu Kaninchenblut (25 mg Fett/ml) den Anteil an freiem Chlorpromazin (Gesamtkonzentration 10^{-4} *M*) von 2,05% auf 0,87% herabsetzt.

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The Effects of Nicotinamide on Mouse Sleep

We have previously reported[1] that methionine administered to rats or mice induced behavioral disruption and that the administration of nicotinamide simultaneously with methionine[2] did not prevent disruption of mouse sleep/wake cycles. However, we did observe that nicotinamide when administered alone appeared to increase the amount of paradoxical or rapid eye movement sleep (REM) which the mice had in a 10 h period. This present study describes the effects of nicotinamide on mouse sleep.

Methods. 30 adult, male, random bred Swiss mice weighing between 27 and 35 g were used. The mice were housed randomly 5 per cage in a sound-attenuated room, with continuous diffuse lighting. Half of the animals were injected daily with saline and the other half with 250 mg/kg nicotinamide. The volume injected was 0.01 ml/g body weight, given s.c. in the back. The pH of the nicotinamide solution was adjusted to approximately 7.4. The injections were administered at 09.00 h. On day 19 of injection the animals were anesthetized with diabutal and implanted with 4 cortical electrodes, 2 over the frontal and 2 over the occipital regions of the cortex. A small surgical clip was also fixed to the skull of each mouse. Dental acrylic was built up around the clip and electrodes to hold them in place. The mice were then placed individually in small cages. They were allowed to recover on day 20, but were injected at the normal times. On day 21 they were injected and habituated to the small recording leads which were connected from the implanted electrodes to a polygraph. On day 22 after injection the polygraph was started at 09.30 h and run at a speed of 6 mm sec continuously until 700 pages of electroencephalogram (EEG) record had been collected (this was approximately 10 h later).

Results. VAN TWYVER[3] and others[4–6] have shown that mouse EEG can be classified into 3 categories: awake-low amplitude, high frequency waves; sleep – high amplitude, low frequency waves; and paradoxical or REM

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The effects of nicotinamide on the percentages of the total recording time for slow wave sleep and rapid eye movement sleep (REM) and the percentage of REM of total sleep time

Compound	Slow wave sleep (%)		REM (%)		REM of total sleep time (%)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Saline	49.8	6.91	6.8	1.35	12.0	2.39
Nicotinamide	43.1	7.73	7.6	1.50	14.9	2.37