Tableau II. Dénombrement des cellules germinales en division (MGm) et en dégénérescence dans les ébauches gonadiques d'embryons de poulet et de caille issus d'œufs témoins et d'œufs traités au DDT

	Poulet Témoins	DDT	Caille Témoins	DDT
N° moyen de gonocytes dans les 2 gonades (MG)	670	415	633	445
MGm (mitoses)	24	7	26	12
MGm/MG	1/27	1/59	1/24	1/57
% de MG en dégénérescence	2	10	4	20
	0,3	2,4	0,6	4,5

Les résultats des dénombrements de cellules germinales en cours de mitose ainsi qu'en dégénérescence sont donnés dans le Tableau II. Dans le premier cas, après simple immersion des œufs de poule et de caille dans une suspension aqueuse de DDT, l'index mitotique des gonocytes contenus dans les ébauches gonadiques est deux fois plus faible que celui des embryons témoins analysés au même stade de développement.

Quant aux cellules germinales pycnotiques, chez les embryons témoins, elles se rencontrent à des pourcentages très faibles, voisins de ceux obtenus pour des embryons plus âgés[2]. Par contre, après traitement au DDT, les taux de gonocytes en dégénérescence sont nettement plus élevés mais néanmoins légèrement inférieurs à ceux déterminés à un stade ultérieur du développement.

Conclusions. Aux jeunes stades étudiés ici, il existe un décalage sensible entre la population germinale des embryons témoins et celle des embryons traités. Ceci montre que le pesticide agit déjà à un stade plus précoce du développement, peut-être avant ou lors de la migration des gonocytes du croissant germinal vers les ébauches gonadiques. Des dénombrements à des stades plus précoces, nous permettrons de conclure sur ce point. Cependant, le fait que le déficit au stade 29 pour le poulet et 20 pour la caille soit deux fois plus élevé qu'aux stades 24 (poulet) et 18 (caille), indique que l'effet du pesticide sur la population germinale des gonades doit s'exercer progressivement, peut-être par blocage des mitoses (phénomène important aux jeunes stades, sinon à un âge plus avancé) ainsi que par dégénérescence d'une partie des gonocytes déjà en place dans les gonades.

Summary. In consequence of treatment of bird's eggs with DDT, before the incubation, a strong reduction of gonadic germ stocking is observed. However, at the 24 (chick) and 18 (quail) stage of embryonic development, the germ deficit is lower than at older stages: 29 (chick) and 20 (quail). The pesticide provokes a significant decrease in the mitotic activity and some pycnotic hypertrophy of several gonocytes. DDT acts already at the earliest stages of development.

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Effects of the 3′,5′-Cyclic Phosphates of 2-Methylthioadenosine, 2-Chloroadenosine and Adenosine on Platelet Aggregation

Exogenous adenosine 3′,5′-cyclic phosphate (cAMP) has been reported to be a weak inhibitor of the ADP-induced aggregation of human platelets[1-3]. Derivatives of ADP having methylthio or chlorogroups in position 2 of the purine ring possess greatly enhanced platelet aggregating properties compared to ADP[4], and 2-methylthioadenosine 5′-phosphate and 2-chloroadenosine are more potent inhibitors of platelet aggregation than, respectively, AMP and adenosine[5-7]. In view of the potency enhancing effects of these two substituents on the activity of certain adenine nucleotides in platelet systems, we prepared 2-methylthioadenosine 3′,5′-cyclic phosphate (c2-MeSAMP) and 2-chloroadenosine 3′,5′-cyclic phosphate (c2-ClAMP)[8] and investigated their effects, and those of cAMP, on the ADP-induced clumping of platelets in sheep platelet-rich plasma (PRP).

Synthetic procedures. c2-MeSAMP and c2-ClAMP were obtained by the cyclization of 2-methylthioadenosine 5′-phosphate[5] and 2-chloroadenosine 5′-phosphate[7] respectively, using a modification of the method of SMITH et al.[9]. A solution of 0.5 mmole of the appropriate AMP analog as its 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt in anhydrous pyridine (50 ml) was added to a refluxing solution of dicyclohexylcarbodiimide (412 mg, 2 mmoles) in 50 ml pyridine over a period of 2 h, and refluxing was continued for a further 1.5 h. The solution was evaporated and the residue was treated with H_2O (40 ml). Dicyclohexylurea was removed by filtration and washed with H_2O, and the combined filtrate and washings were concentrated to about 3 ml and passed through a column (1 × 5 cm) of Bio-Rad AG2 (formate form). The column was washed with H_2O (in the preparation of c2-ClAMP a by-product which had a yellow fluorescence in UV-light was eluted at this stage), and the cyclic nucleo-

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Table I. Rf values, electrophoretic mobilities and spectral properties of c2-MeSAMP and c2-ClAMP

Compound	Rf Solvent I	Rf Solvent II	M_{AMP}	λ_{max} 0.1 *N* HCl (nm)	$\varepsilon \times 10^{-3}$
c2-MeSAMP	0.46	0.72	0.76	268	15.9
c2-ClAMP	0.49	0.68	1.03	263.5	13.9

Solvents: I, i-PrOH — 0.25 *M* NH_4HCO_3 (2:1); II, isobutyric acid — 1 *M* NH_4OH (5:3). M_{AMP} = electrophoretic mobility relative to that of AMP in 0.025 *M* citrate buffer, pH 4.8, at 200 V.

tides were displaced with 4 *M* formic acid. Evaporation of the eluates gave the cyclic nucleotides as chromatographically homogeneous white solids.

c2-MeSAMP (89% yield), Anal. Calc. for $C_{11}H_{14}N_5O_6PS \cdot HCOOH$: N, 16.62%. Found: N, 16.64%. *c2-ClAMP* (81% yield) was recrystallized from H_2O, Anal. Calc. for $C_{10}H_{11}ClN_5O_6P$: C, 33.03; H, 3.05; N, 19.25; P, 8.51%. Found: C, 33.03; H, 3.13; N, 19.43; P, 8.28%. Rf values, electrophoretic mobilities and UV spectral properties are summarized in Table I. Chromatograms and electropherograms were visualised under UV-light. The analogs were stored at −10°; samples used for platelet aggregation studies were checked for homogeneity prior to use by paper chromatography with markers of the appropriate parent AMP and adenosine analogs.

Platelet aggregation. The aggregation of sheep platelets in citrated PRP induced by 0.67 μ*M* ADP was studied by a photometric technique[6,10,11]. Aggregation was measured in the presence and absence of the cyclic nucleotides and the effects observed were compared to the effects of adenosine on each batch of PRP. The cyclic nucleotides and adenosine were routinely incubated in the stirred PRP for 2.5 min before the addition of ADP. Initial rates of aggregation were used to plot log-dose response curves for the inhibitors, from which molar potency values were obtained; these were expressed relative to that of adenosine.

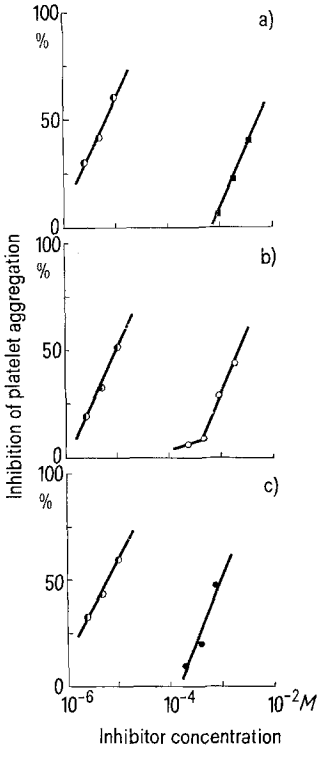

Fig. 1. Log-dose response curves for the inhibition of ADP-induced sheep platelet aggregation by cAMP (■), c2-MeSAMP (●), c2-ClAMP (○) and adenosine (◐). Inhibition is expressed as percentage inhibition of the initial rate of aggregation induced by 0.67 μ*M* ADP. a), b) and c) are response curves obtained from different batches of PRP.

Stability of cyclic nucleotides in PRP and PFP. Platelet-free plasma (PFP) was prepared by centrifuging PRP in a Hettich II Universal centrifuge for 20 min at 2,500 rpm. PRP was sonicated with a Biosonik II Ultrasonic Probe for 25 sec at setting 40 W followed by 25 sec at 80 W. Samples of PRP, PFP or sonicated PRP (0.45 ml) containing 2 m*M* cAMP or c2-ClAMP in a total volume of 0.5 ml were incubated for 2 h at 37 °C and cooled to 0 °C. 6% $HClO_4$ (0.2 ml) and H_2O (0.3 ml) were added to each assay, protein was spun down and the supernatants were neutralized and evaporated in vacuo; zero time control assays were processed similarly. Each residue was triturated with H_2O (0.15 ml) and a 50 μl aliquot was applied to Whatman No. 1 chromatography paper, together with markers of the appropriate 5′-phosphates and nucleosides. Chromatograms were developed in solvent I (Table I) and in *n*-BuOH – H_2O (86:14). The sensitivity of the method was such as to detect a 0.8% conversion of cyclic nucleotide to 5′-phosphate or nucleoside.

Results and discussion. c2-MeSAMP, c2-ClAMP and cAMP inhibited ADP-induced platelet aggregation in a concentration dependent manner, giving log-dose response

Table II. Molar potency values of cAMP, c2-MeSAMP, c2-ClAMP and related AMP and adenosine derivatives as inhibitors of ADP-induced sheep platelet aggregation

Compound	Molar potency
Adenosine	1.0
cAMP	0.0009 (0.0007–0.011, $n = 5$) [a]
c2-MeSAMP	0.009 (0.0016–0.015, $n = 3$) [a]
c2-ClAMP	0.005 (0.0013–0.0097, $n = 4$) [a]
AMP	0.1 [5,7]
2-MeSAMP	2.5 [7]
2-ClAMP	0.1 [5]
2-Methylthioadenosine	0.1 [7]
2-Chloroadenosine	4.0 [5]

[a] Range of values and number of determinations.

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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].

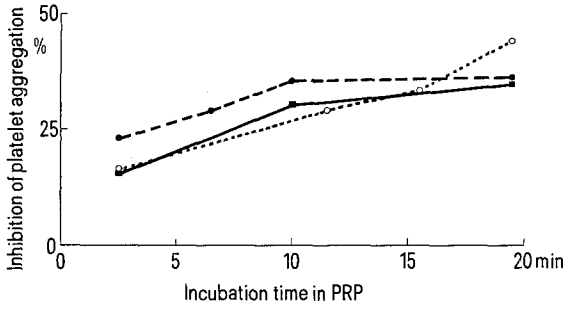

Fig. 2. Effect of incubation time on the inhibition of ADP-induced platelet aggregation by cAMP (■), c2-MeSAMP (●) and c2-ClAMP (○). 1.8 mM cAMP, 0.19 mM c2-MeSAMP and 0.9 mM c2-ClAMP were incubated in stirred PRP for the times indicated prior to the addition of 0.67 μM ADP. Inhibition is expressed as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