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.

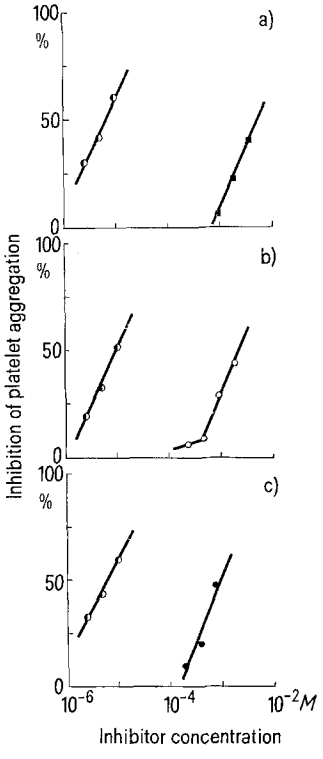

Fig. 1. Log-dose response curves for the inhibition of ADP-induced sheep platelet aggregation by cAMP (■), c2-MeSAMP (●), c2-ClAMP (○) and adenosine (◐). Inhibition is expressed as percentage inhibition of the initial rate of aggregation induced by 0.67 μ*M* ADP. a), b) and c) are response curves obtained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