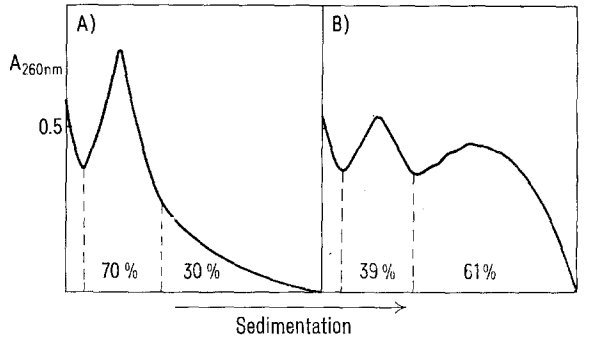
Results and discussion. As shown in Table I, cystamine prevents the polysomal disaggregation caused by the i.p. administration of CCl_4. As can be observed, cystamine largely prevents the polysomal disaggregation when administered at the doses of 20, 40 and 100 mg/100 g body wt. ($P < 0.001$ in respect to controls). At the doses of 5 and 10 mg/100 g body wt. cystamine did not protect significantly.

The preventive effect of cystamine is shown also in the Figure, where we have reported the polysomal patterns of a single experiment.

As shown in Table II when cystamine is given 2 and 12 h before CCl_4, at the dose of 60 mg/100 g body wt. it clearly prevents CCl_4-induced polysomal disaggregation ($P < 0.001$).



Sedimentation patterns of liver polysomes from a rat treated with CCl_4 (A) and from a rat pretreated with cystamine (100 mg/100 g body wt.) and poisoned by CCl_4 (B).

At 16 and 24 h cystamine pretreatment did not significantly prevent CCl_4-induced polysomal disaggregation.

Our findings that cystamine inhibits CCl_4-induced polysomal disaggregation are in accordance with results of CASTRO et al.[1,2], however our results do not show how cystamine could act. Since the damage of polyribosomes caused by CCl_4 has been correlated to free radicals arising during the homolytic scission of CCl_4[6], the protective effect of cystamine may be attributed to the inhibition of CCl_4-activation to free radicals, as also suggested by CASTRO et al.[1,2]. This mechanism could be mediated by inhibition of the drug-metabolizing enzyme system that metabolizes CCl_4 and/or by an inhibition of microsomal lipid peroxidation, that could be responsible for the polyribosomes damage, although this last mechanism is unlikely, since it has been shown that cystamine does not prevent the increase in microsomal lipid peroxidation caused by CCl_4[2]. Further studies are therefore necessary to obtain a better understanding of this problem.

Riassunto. Il pretrattamento di ratti con cistamina impedisce la disaggregazione dei polisomi di fegato indotta dal tetracloruro di carbonio.

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Effect of PGE_2 on the Turnover of Calcium in Rat Uterus

It has been demonstrated on several muscle preparations that prostaglandins (PGs) affect the turnover of cellular calcium[1-8]. However, it has not yet been clarified whether PGs act primarily on the calcium transmembrane flux or on the release of calcium from intracellular storage sites; the first process seems to be important in the slow-contracting smooth muscle, the second one in the rapid-contracting cardiac and skeletal muscles[9-14].

The present investigations have been undertaken on rat uterus in order to clarify the site of action of PGs on the turnover of calcium in smooth muscles; for this purpose the extent of the intracellular exchangeable calcium and the wash-out curves of the ion have been investigated. PGE_2 has been used because it does not induce tachyphylaxis[15,16].

Methods. The mechanical stillstand of rat uterus has been obtained in estrogen-treated ovariectomized animals; the organs were incubated in Krebs solution containing 0,69 mM Ca^{++} at 32 °C and pH 7.4; the bathing fluid was aerated with 5% CO_2 in O_2. PGE_2 (kindly supplied by Upjohn Co. Kalamazoo) was used at the concentration of 0.1 μg/ml, $^{45}CaCl_2$ at the concentration of 0.1 μC/ml. Extracellular spaces have been determined with an inuline method[17]: no differences were found in controls and in PGE_2 contracted uteri (43.43 ± 2.38% and 42.46 ± 1.76% respectively).

Table I. Effect of PGE_2 (90 min contact) on the cellular calcium content of rat uterus

Treatment	No. of determinations	Ca(μEq/g fresh tissue)	P
—	11	2.89 ± 0.38	—
PGE_2	12	2.78 ± 0.33	>0.80

Evaluation of exchangeable calcium. Uteri incubated in labelled Krebs solution were withdrawn after different times of incubation (5, 15, 30, 45, 60, 90 min), dipped in cold bathing fluid, blotted on filter paper and heated at 200 °C with HNO_3-$HClO_4$ 1:1; the residue was dissolved in 0.1 *N* HCl and used for the determination of total calcium by atomic absorption spectometry, and for the radioassay by liquid scintillation counting. The percent of intracellular exchangeable calcium was calculated as the ratio between the specific radioactivity (cpm/μEq Ca) of the organ and of the bathing fluid, taking into account the amount of calcium and of radioactivity present in the extracellular spaces.

Wash-out curves. After 60 min incubation in labelled Krebs solution, the wash-out curves were performed by changing the bathing fluid (containing or not the usual dose of PGE_2) after 2, 4, 6, 8, 10, 15, 30, 45, 75, 90 min. A sample was taken at each time fo radioassay. Differences between control and PGE_2-treated organs have been evaluated by the Duncan's test.

Results and discussion. The influence of PGE_2 on the calcium exchangeability in rat uterus is shwon in Figure

Table II. Effect of PGE_2 on the wash-out of radioactivity from Ca^{45}-loaded rat uterus

Time (min)	Released Ca^{45}		P
	Control	PGE_2	
2	3467.0 ± 314.2	6166.0 ± 585.6	<0.05
4	1230.0 ± 103.3	1950.0 ± 109.5	<0.05
6	891.3 ± 81.2	832.0 ± 78.7	n.s.
8	645.7 ± 65.6	832.0 ± 79.8	n.s.
10	467.7 ± 44.2	660.7 ± 65.3	n.s.
15	331.1 ± 37.3	436.5 ± 62.8	n.s.
30	186.2 ± 19.2	195.5 ± 18.3	n.s.
45	117.5 ± 10.1	117.5 ± 9.4	n.s.
60	42.7 ± 7.4	104.7 ± 10.3	<0.02
75	39.8 ± 5.9	77.6 ± 6.9	<0.05
90	30.9 ± 4.8	70.8 ± 10.3	<0.05
Total released Ca^{45} cpm	21409 ± 631.2	31548 ± 740.5	<0.01

Figures are the average of 12 experiments and are expressed as cpm per 100 mg fresh tissue/min of time interval. Significance has been evaluated by the Duncan's test.

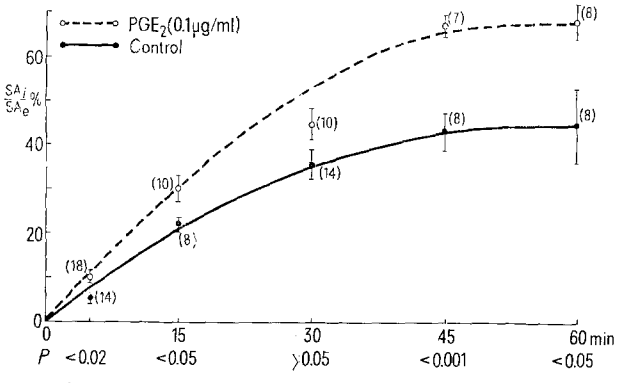

Fig. 1. Effect of PGE_2 on the exchangeable calcium in rat uterus. Figures are given as ratio between the intracellular specific activity (SA_i) and the extracelluar specific activity (SA_e). Number of determinations in brackets.

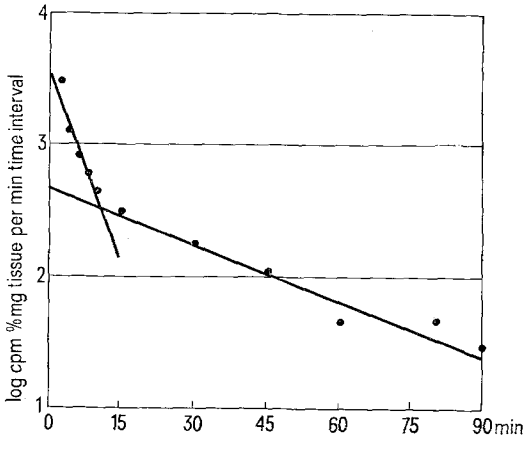

Fig. 2. Pattern of release of radioactivity from Ca^{45}-loaded rat uterus. Experimental values are expressed as cpm per 100 mg fresh tissue and per min time interval.

1. The steady-state values demonstrate that the drug induces an enlargement of the pool of intracellular exchangeable calcium. Since the total calcium content is not modified (Table I), it can be suggested that PGE_2 releases a portion of the calcium which is bound to the subcellular organelles; this is in agreement with the findings obtained on sarcoplasmatic reticular vescicles isolated from bovine heart[18], where PGE_2 lowered the calcium binding capacity.

The above-mentioned data demonstrate that PGE_2 acts at intracellular level, as proposed by COCEANI[6] for the rat stomach muscle, but do not give any information about the possibility that PGE_2 could affect also the transmembrane flux of calcium.

A PGE_2 action at membrane level is suggested by the observation that the steady state of calcium exchange in the presence of PGE_2 is reached within the same time as in control experiments, although the absolute value is considerably higher in treated organs than in controls. In order to comply with this situation, calcium must be transferred from the extracellular compartment at higher rate in PGE_2 treated uteri than in controls. This is demonstrated also by the acceleration of calcium exchange induced by PGE_2 in earlier contact times.

The wash-out curves confirm this assumption. Calcium moves from control uteri (Figure 2) at 2 different rates, the rapid one ($t\ ^1/_2$ = 6 min) probably corresponding to the release of the ion from the extracellular spaces and the cell membranes, the slower one ($t\ ^1/_2$ = 53 min) corresponding to the efflux of calcium from the intracellular compartment.

The effect developped by PGE_2 on the membrane flux of calcium is demonstrated by the enhancement of output from the rapid releasing compartment (Table II); moreover, in agreement with the findings of COCEANI in rat stomach muscle[6] and with our Ca^{45} uptake experiments, PGE_2 reveals the presence of a slow-releasing compartment which is affected only after a long contact time. This pool of calcium, however, could hardly be related to the contraction induced by PGE_2, which begins within sec

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after the drug is administered. These data are consistent with the findings obtained by EAGLING[19] with different experimental methods, and could suggest that PGE_2 is able to affect not only the calcium storage capacity of intracellular organelles, but also the flux of calcium across cell membranes. Both mechanisms lead to a larger availability of free intracellular calcium and could be proposed to explain the stimulant actions of PGs[20].

Riassunto. La PGE_2 determina nell'utero di ratto un aumento della frazione scambiabile del calcio cellulare, un aumento della cessione del calcio dal compartimento a rapida liberazione, e mette in evidenza la esistenza di un compartimento dal quale il calcio può venire liberato dopo oltre un'ora di contatto. I dati sperimentali avvalorano l'ipotesi che il meccanismo d'azione della PGE_2 possa essere sostenuto dall'azione da essa svolta sul turnover del calcio.

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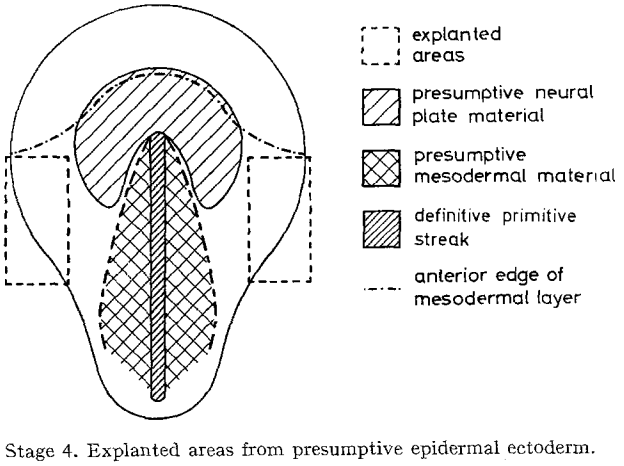
A Neuralizing Influence of Dibutyryl Cyclic AMP on Competent Chick Ectoderm

N^6, O^2-dibutyryl adenosine 3′:5′-cyclic monophosphate (dibutyryl-cyclic AMP – DBcAMP –) has been shown to induce morphological differentiation and biochemical changes of mouse neuroblastoma cells in vitro[1–3]. This study was made to investigate whether DBcAMP could have a neuralizing influence on competent chick ectoderm, which is known to react to various neural inductive and neural supportive stimuli[4–9]. The immunofluorescence method was used for the identification of neural differentiation, as neural antigen production in cultures made from chick ectodermal explants could be used as an indication of such a differentiation[10].

Method. Explants were taken from the presumptive epidermal region of the chick ectoderm at stage 4 (stage according to HAMBURGER and HAMILTON[11]) as shown in the Figure and wrapped in a piece of vitelline membrane prepared from an unincubated egg. The vitelline membrane with its content was put on a millipore filter strip, and placed on a piece of gel-foam previously set inside a Leighton tube containing 1 ml culture medium. The culture medium was composed of 3 parts human serum, 3 parts 50% chick embryo extract, and 7 parts Tyrode. The substances tested were added to the culture medium on the first day of incubation. The Leighton tubes were incubated at 37 °C for 8–10 days; thereafter the cultures were freed, crushed, and processed for immunofluorescence investigation. Antisera specific to antigens present in the chick central nervous system were used for the first of the 4 steps in the immunofluorescence process (for further details of the immunofluorescence method and its specificity, see ref.[8]).



Stage 4. Explanted areas from presumptive epidermal ectoderm.

Results. 421 cultures were examined with different additives to the culture medium; 101 of them, being necrotic, were discarded. Thus, 76% were useful. The necrotic explants were approximately evenly distributed between the different experimental groups, with one exception. In the series of explants grown in the highest concentration of theophylline (1.0 m*M*), more than 50% were necrotic at the end of the culture period. Generally, the surviving cultures showed good growth, and – when present – cells containing neural antigens could be seen as large sheets of fluorescent tissue.

The Table shows the number of neural antigen-containing cultures in the different experimental groups. The control series demonstrates a fairly high capacity of autoneuralization (30%) for the competent ectoderm in the present system. When the 4 groups of experiments with DBcAMP (0.005–0.1 m*M*) present in the culture medium are compared with the control cultures, however, a statistically significant increase in neural antigen-containing cultures is noted. A χ^2 analysis for heterogeneity between the control group and the 4 experimental groups gives $\chi^2 = 12.6$ at 4 d.f., $0.01 < P < 0.02$. This is not only due to a difference between the control group and the experimental groups, as exclusion of the controls still gives a significant heterogeneity ($\chi^2 = 10.6$ at 3 d.f., $0.01 < P < 0.02$). The significant heterogeneity between the 4 treated groups is completely due to the lowest concentration of DBcAMP (0.005 m*M*); exclusion of this gives $\chi^2 = 3.1$ at 2 d.f., not significant (N. S.). Thus, a concentration of 0.005 m*M* DBcAMP seems to have no effect, but concentrations of 0.01–0.1 m*M* have a statistically significant effect on competent ectoderm, causing an increased

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