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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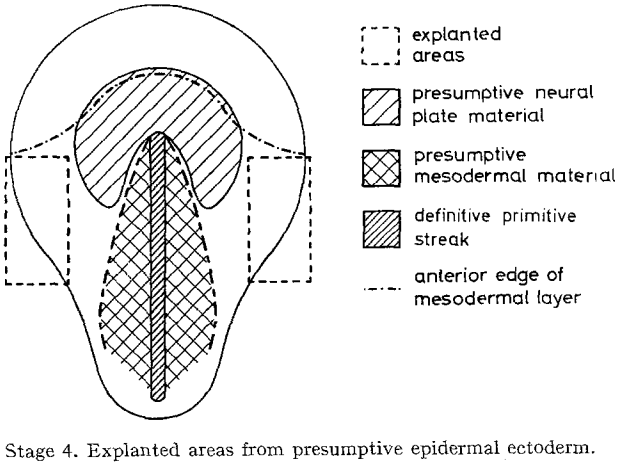
## A Neuralizing Influence of Dibutyryl Cyclic AMP on Competent Chick Ectoderm

$N^6, O^2$-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 $\chi^2$ 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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Explants made from presumptive epidermal ectoderm

| Addition to the culture medium | Concentrations (m$M$) | Results |
|---|---|---|
| None | — | 9/30 (30%) |
| DBcAMP | 0.005 | 5/25 (20%) |
| | 0.01 | 18/30 (60%) |
| | 0.05 | 16/30 (53%) |
| | 0.1 | 10/27 (37%) |
| 5'AMP | 0.01 | 15/31 (48%) |
| Sodium butyrate | 0.02 | 9/26 (35%) |
| Theophylline | 0.01 | 7/24 (29%) |
| | 0.1 | 5/15 (33%) |
| | 1.0 | 1/19 (5%) |
| 0.01 m$M$ DBcAMP + theophylline | 0.01 | 14/30 (47%) |
| | 0.1 | 19/33 (58%) |

On the first day of incubation, the various substances were added to the culture medium in order to obtain the final concentrations described. Controls were grown without any addition to the medium. Fraction of cultures producing neural antigens.

frequency of neural antigen-containing cultures to develop.

The effect of 5'AMP and sodium butyrate, degradative products of DBcAMP, on competent ectoderm was also tested by adding these substances to the culture medium in molar concentrations corresponding to 0.01 m$M$ DBcAMP. When compared with the control series, neither 5'AMP nor sodium butyrate resulted in a statistically significantly increased frequency of neural antigen-producing cultures. The sodium butyrate, but not the 5'AMP, series of cultures seemed to differ, however, from the DBcAMP (0.01 m$M$) series of cultures, but the statistical significance of the difference was uncertain.

Theophylline, which inhibits the cleavage of cyclic AMP by phosphodiesterase, did not increase the frequency of neural antigen-containing cultures when added separately to the culture medium. Nor did the addition of theophylline increase the effect of DBcAMP (0.01 m$M$). Rather, it was found that DBcAMP, at concentrations 0.01–0.1 m$M$, with or without the addition of theophylline, was equally effective on competent ectoderm ($\chi^2 = 2.7$ at 2 d.f., N. S.), resulting in a mean frequency of neural antigencontaining cultures of 51%. The results presented in the Table, however, suggest that DBcAMP alone is more effective at the concentrations 0.01 and 0.05 m$M$ than at the concentration 0.1 m$M$.

*Discussion.* Culturing ectoderm, neural plate, or neural tube pieces, Nord [10] showed that the present method gave an apparently fair record of neural differentiation when recorded as number of cultures containing neural antigens. The results presented here show a neuralizing influence of DBcAMP on competent chick ectoderm. The concentration of DBcAMP giving the maximally increased frequency of neural antigen-containing cultures in this system is low compared with other in vitro-systems showing effects by this substance; e.g., morphological differentiation of mouse neuroblastoma cells was induced by DBcAMP at about 50–100 times higher concentrations [1]. This probably reflects the general lability of competent ectoderm in the in vitro-situation. The lack of effects of theophylline, both alone and when added to the culture medium together with DBcAMP, do not give any further information about the way in which DBcAMP acts on the competent ectoderm. In the highest concentration tested, 1.0 m$M$, theophylline appears to have an inhibitory effect on the autoneuralization of the system. This is probably due to a toxic effect on the explanted ectoderm, reflected by the high percentage of necrotic explants found in the series of cultures with 1.0 m$M$ theophylline present in the culture medium.

As also 5'AMP seemed to increase the frequency of neural antigen-producing cultures in the present system, although not statistically significantly, it cannot with certainty be ruled out that the effect of DBcAMP on competent chick ectoderm is partly due to one of its degradative products. Anyhow, it would be interesting to investigate further the neuralizing influence of DBcAMP, particularly in relation to other specific neuralizing stimuli on competent ectoderm, for instance nerve growth factor. This protein, shown to act strongly neuralizing on competent chick ectoderm in the present system – probably by supporting the autoneuralization [8, 9] – was recently suggested as stimulating axonal maturation of embryonic sensory ganglia via cyclic AMP as a 'second messenger system' [12, 13].

*Zusammenfassung.* Mit einer Immunofluoreszenz-Technik wird gezeigt, dass DBcAMP, gemessen als Frequenz der neuralen Antigen-produzierenden Kulturen, neuralisierend auf kompetentes Kükenectoderm in vitro wirkt. 5'AMP und Natriumbutyrat, Abbauprodukte des DBcAMP, schienen teils einen gewissen, teils gar keinen Effekt zu haben. Teophyllin hatte keinen additiven Effekt auf DBcAMP allein.

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