moiety in the type **B** β-adrenergic drugs, owing to the conjugation of oxygen with the naphthyl ring, can electronically and sterically simulate a portion of an aromatic ring and therefore take the place of the aryl group directly linked to the C_2 atom in the type **A** drugs in the interaction with the receptor. This hypothesis is supported by the fact that the relative orientations of the least-square planes of the C_3–O_2–C_4–C_5 group and the aminoethanol side chains are very similar in **2**, **2**·HCl and **3**·HCl, and that this same kind of orientation has been found between the aryl and aminoethanol groups in the majority of type **A** (**1**) drugs which have been examined by X-ray crystallography. The least-squares plane angle data reported in the Table show that there is a clear relationship between the Ar–CH(OH) and Ar–O–CH_2CH(OH) moieties: the angles are all in the 56–87° range, with the one exception of adrenaline tartrate (2.8°). Small differences observed in the angles are probably due to crystal packing forces[15].

Zusammenfassung. Mittels Röntgenstrahlen wurde die Kristallstruktur des β-Blockers Propranolol und seines Chlorhydrats vermessen und beim letzteren gewisse Unterschiede zu früheren Resultaten gefunden. Auf Grund dieser Vermessungen wird erklärt, weshalb auch der Typus B Antagonisten liefern kann, und eine neue Hypothese für die Wirksamkeit von Typ B aufgestellt.

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[15] We thank the University of Maryland Computer Science Center for a grant of computer time.

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Properties of Ca^{2+}, Mg^{2+}-Dependent Endonuclease from Sea Urchin Eggs of *Arbacia punctulata*

A Mg^{2+}-dependent endonuclease is present in embryos of sea urchin (*Paracentrotus lividus*)[1], in testis of the crabs (*Neptunas astalus*)[2] and *Cancer pagurus*[3] and in the hepatopancreas of *Octopus vulgaris*[4] which requires Ca^{2+} for maximal activity. In the present study a deoxyribonuclease was demonstrated in eggs of the sea urchin *Arbacia punctulata* which required both Ca^{2+} and Mg^{2+} for activity. It was capable of stimulating the template activity of sea urchin sperm chromatin for DNA synthesis.

Materials and methods. Eggs and sperms were obtained from sea urchin by an established method of injecting 0.5 ml of 0.55 *M* KCl. The semen was suspended in a small amount of artificial sea water and centrifuged at a low speed. The supernatant was designated as seminal plasma. Sperm chromatin was prepared as described by OZAKI[5] with slight modifications.

Endonuclease was isolated from eggs which were washed several times with artificial sea water (MBL) and suspended in a cold medium containing 25 m*M* *Tris*-HCl (pH 8.0), 2 m*M* $MgCl_2$. The eggs were homogenized in a Dounce homogenizer. The homogenate was centrifuged at 15,000 *g* for 15 min. The supernatant was fractionated by precipitation with solid ammonium sulfate (50–80% saturation). The precipitated proteins were collected by centrifugation at 15,000 *g* for 15 min and dissolved in a medium containing 0.01 *M* *Tris*-HCl (pH 8.0), 30% glycerol.

Egg nuclei were prepared according to PIKO, TYLER and VINOGRAD[6]. The endonuclease was extracted from the isolated nuclei as described for rat testis nuclei[7]. The procedure for the determination of acid and alkaline endonuclease activities by measuring the amount of radioactivity solubilized from [^{3}H]DNA gel and the method for measuring the template activity of sperm chromatin with DNA polymerase and [^{3}H]TTP were described in previous reports[8–10].

DNA was prepared from sea urchin sperm chromatin as described by SMITH[11]. The endonucleolytic property of the egg enzyme was determined by incubating a mixture containing 100 μg of sea urchin sperm DNA, 10 m*M*

Table I. Effect of bivalent cations and EGTA on endonuclease activity in sea urchin eggs

Assay systems	Activity (10^{-3} units/mg protein)
Control	0
+ 10 m*M* $MgCl_2$	0.06
+ 2 m*M* $CaCl_2$	0.18
+ 10 m*M* $MgCl_2$ 0.6 m*M* EGTA	0
+ 10 m*M* $MgCl_2$ 2 m*M* $CaCl_2$	8.88
+ 10 m*M* $MgCl_2$ 2 m*M* $CaCl_2$ 0.6 m*M* EGTA	8.22

Control assay system contained 100 μl [^{3}H] DNA gel (2×10^4 cts/min) 50 m*M* *Tris*-HCl (pH 7.5), 10 m*M* 2-mercaptoethanol, appropriate amount of enzyme preparations and with or without 10 m*M* $MgCl_2$, and 0.6 m*M* EGTA in a total vol of 0.2 ml. The mixture was incubated at 37 °C.

[1] E. PARISI and B. DE PETROCELLIS, Biochem. biophys. Res. Commun. *49*, 706 (1972).
[2] J. G. GEORGATSOS and O. ANTONOGLU, Biochim. biophys. Acta *95*, 544 (1965).
[3] G. SABEUR, P. J. SICARD and G. AUBEL-SADRON, Biochemistry *13*, 3203, 1974.
[4] J. G. GEORGATSOS and O. ANTONOGLU, J. biol. Chem. *241*, 2151 (1966).
[5] H. OZAKI, Devel. Biol. *26*, 209 (1971).
[6] L. PIKO, A. TYLER and J. VINOGRAD, Biol. Bull. *132*, 68 (1967).
[7] E. OHTSUKA, Y. TANIGAWA and S. S. KOIDE, Experientia *31*, 175 (1975).
[8] K. YOSHIHARA, Y. TANIGAWA and S. S. KOIDE, Biochem. biophys. Res. Commun. *59*, 658 (1974).
[9] L. BURZIO and S. S. KOIDE, Biochem. biophys. Res. Commun. *40*, 1013 (1970).
[10] L. BURZIO and S. S. KOIDE, Biochem. biophys. Res. Commun. *53*, 572 (1973).
[11] M. C. SMITH, Methods Enzymol. *12* (Part A), 545 (1967).

Table II. Ca^{2+}, Mg^{2+}-dependent endonuclease activities in eggs, seminal plasma and sperm extract

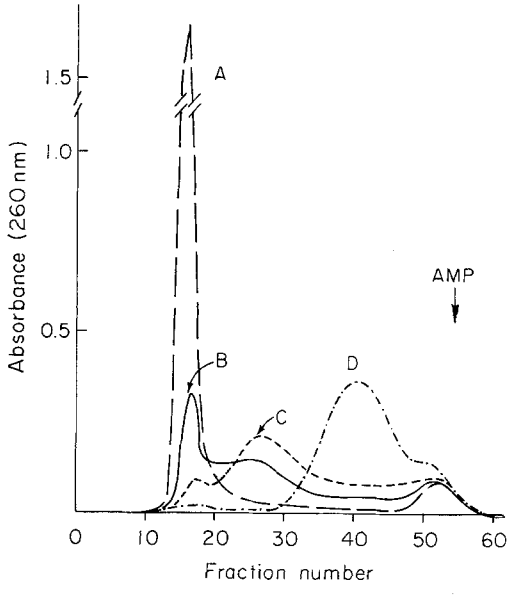
	Activity (units/mg of proteins)
Semen	1.3
Seminal plasma	40.8
Sperm extract	16.9
Crude egg supernatant	3.8×10^3

The assay system is described in the legend to Table I. Incubation was carried out at 37 °C for 10 min.

Table III. Requirement of bivalent cations for the activation of sperm chromatin template for DNA synthesis by egg endonuclease

Cations	[^{3}H]TMP incorporated (*p*moles)
Control, not activated	6
Control, activated	8
+ 1 m*M* Mg^{2+}	14
+ 1 m*M* Ca^{2+}	20
+ 1 m*M* Ca^{2+} + 10 m*M* Mg^{2+}	86
+ 1 m*M* Mn^{2+}	13

The control incubation system contained egg endonuclease preparation (135 units/12.5 µg of protein/25 µl), 2 m*M* 2-mercaptoethanol. The systems were incubated at 37 °C for 30 min to activate the template except the control, not activated, sample.



Gel filtration of sea urchin sperm DNA treated with sea urchin oocyte endonuclease through Agarose column. The reaction mixtures were incubated at 0 min (A); 30 min (B); 60 min (C); and 150 min (D). AMP was eluted in fraction No. 54.

$MgCl_2$, 2 m*M* $CaCl_2$, 10 m*M* 2-mercaptoethanol and 51 units of Ca^{2+}, Mg^{2+}-dependent endonuclease in a total volume of 1.0 ml at 37 °C. The reaction was stopped by the addition of 5 µl of 5 *M* NaCl and 2 µl of 0.1 *M* trisodium citrate. The mixture was heated for 10 min at 100 °C. The samples were cooled in an ice-bath and filtered on an Agarose column (Bio-gel A-5m, 100–200 mesh, Bio Rad Laboratories, Calif., 1.4 × 37.0 cm) according to PARISI and DE PETROCELLIS[1]. Fractions of 30 drops per tube were collected. Absorbance of each fraction was measured at 260 nm. Protein and DNA were determined by the methods of LOWRY et al.[12] and BURTON[13], respectively.

Results and discussion. The fraction precipitated between 50–80% saturation of ammonium sulfate contained most of the endonuclease activity (1.13×10^4 units/mg of protein). The enzyme activity was maximal in the presence of both Ca^{2+} and Mg^{2+} in 50 m*M* *Tris*-HCl (pH 8.5) (Table I). The optimal temperature for activity was 37 °C. Since the assay does not discriminate endonuclease from exonuclease activity, the amount of exonuclease activity in the preparation was estimated by incubating the reaction mixture for varying periods and by analyzing the products formed by gel filtration on Bio-gel A-5m column (Figure). The major products formed were oligonucleotides associated with a small amount of mononucleotides. The present findings suggest that the enzyme preparation possessed low exonucleolytic activity which was estimated to be less than 10% of the total activity.

Nuclei prepared from eggs of *Arbacia punctulata* possessed high Ca^{2+}, Mg^{2+}-dependent alkaline endonuclease activity but was devoid of Mg^{2+}-dependent acid endonuclease activity. The total Ca^{2+}, Mg^{2+}-dependent endonuclease activity was slightly higher in the cytosol than in the nuclear fraction; whereas the specific enzymic activity was significantly higher in the nuclear fraction. It should be noted that the egg supernatant fraction possessed high activity while the activity was low in semen, seminal plasma or sperm extract (Table II).

The ability of egg endonuclease to stimulate the template of sperm chromatin for DNA synthesis was investigated (Table III). The protein and DNA content of sperm chromatin was 6.11 mg/ml and 3.9 mg/ml, respectively, with a ratio of protein to DNA of 1.57. The template activity of sperm chromatin for DNA synthesis was low which increased slightly by the addition of 1 m*M* Ca^{2+} and 10 m*M* Mg^{2+} to the assay system (Table III), suggesting that sperm chromatin contained minimal, if any, endonucleolytic activity. On the other hand, the template activity was markedly stimulated by the addition of egg endonuclease to the assay system (Table III). The template activity increased with the duration of the incubation and reached a maximum level after incubation for 20 min.

The present findings of low endonucleolytic activity in sperm, poor template activity of sperm chromatin for DNA synthesis, and stimulation of the template activity of sperm chromatin on incubation with egg endonuclease suggest that the Ca^{2+}, Mg^{2+}-dependent endonuclease present in eggs might participate in the dispersion of the tightly coiled sperm DNA, influence the association of DNA of sperm and egg and/or activate sperm and egg chromatin for DNA synthesis subsequent to fertilization.

[12] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[13] K. BURTON, Biochem. J. *62*, 315 (1956).

Zusammenfassung. Eine Endodesoxyribonuclease wurde aus den Eiern des Seeigels *Arbacia punctulata* isoliert. Das Enzym braucht sowohl Ca^{2+} als auch Mg^{2+} um maximale Aktivität mit *Tris*-HCl bei pH 8.5 zu erreichen. Die Matrizenaktivität von Samenchromatin für DNA-Synthese konnte durch Ei-Endonuclease stimuliert werden.

[14] Acknowledgements. The present study was conducted as part of the 1974 Fertilization and Gamete Physiology Training Program, Marine Biological Laboratory. The work was supported in part by grant No. 1 PO1 HDO5671 from the National Institute of Child Health and Human Development, USPHS.

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18 February 1975.

Effect of Territorial Conditions on the Maintenance of Pair Contact in Duetting Birds

Many species of tropical birds communicate by antiphonal duets. In some species more than two individuals may sing together. Thereby they perform trios, quartets and quintets. The analysis of the temporal patterning of these performances has shown that the vocal contributions of individuals living in pair contact occur in a coordination which is more clear and precise than that between the contributions of not mated birds. There is evidence that a well developed vocal coordination will support the maintenance of pair contact between the duetting birds[1–5]. The vocal coordination is developed by social learning and improved by repeated exercise[6].

Based on field observations, we worked out an experimental program which allowed us to test the function of well coordinated antiphonal songs. The program was carried out on the behaviour of the central African thrush, *Cossypha heuglini* H., captured and now living under controlled conditions in our laboratories (6 individuals). Like other species duetting antiphonally, these thrushes are extremely territorial[7,8]. In general, mated *Cossyphas* claim and defend their territories together and predominantly by singing 'counter-duets'[4]. When separated from its mate for more than 2 weeks, a *Cossypha* may start to develop antiphonal duets performed with new song partners. Our question: Would a bird, which 1. has learned to answer song patterns of more than one partner with antiphonal responses, and 2. is exposed to two of these partners simultaneously, demonstrate a clear preference for that one of the partners which shows the better coordination with the bird's own singing behaviour?

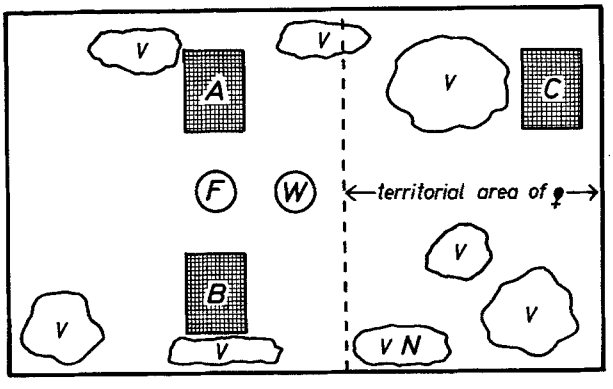

Fig. 1. Sketch of the habitat of our *Cossypha heuglini*. 'Territorial area': region wherein the ♀ spends 93% of its time. A) B) and C), places of cages for ♂ (= *m1*) and ♂ (= *m2*). W, water; F, food; N, nest; V, dense vegetation (coffee tree a.o.). Dimension of the room in meters: 5 × 3 × 2.5.

In order to investigate this question, we removed male *Cossyphas* (symbol: *m1* = ♂) out of aviary rooms wherein they had lived in normal pair contact: each of them with 1 female and sometimes (additionally) with 2 youngsters of their own. Each removed male (*m1*) was replaced by another adult and already mated *Cossypha* male (symbol: *m2* = ♂). For avoidance of aggressive attacks, these birds were kept in cages (1m × 0.5m × 0.5 m). Almost 3 weeks after such a replacement, the unremoved female started to accompany the songs of the *m2* male by the same vocal patterns which it had uttered formerly in the duets with its *m1* male. They did so inspite of the following facts: 1. the song patterns vocalized by the *m2* were different from those uttered by the *m1*; 2. the duets performed by the *m2* and the female did not show the regular timing (coordination) between the duet contributions which was observed in the duets performed by the *m1* and the female. Like these, the duets between the *m2* and the female, however, occurred predominantly in consequence of certain auditory or visual stimulations[6,7].

Another 2 weeks later, when the antiphonal duets between the *m2* and the female could be released with high probability ($p > 0.9$; stimulation with stuffed bodies of *Cossyphas* = dumies of rivals), we removed the *m2* male out of the females rooms. Then we started 3 types of experiments. Each of them was carried out to examine the vocal behaviour of the *Cossyphas* when again brought together: The female, its mate (*m1*) and the *m2* male. Again the male birds were kept in cages, both of identical construction.

Experiment 1. The cages with the males were installed simultaneously in the female's room but outside of its territorial area (Figure 1; position A and B). Immediately after the start of the exposure of the 2 males to the female, the vocal activity of the birds (measured individually in the number of the vocalized duet contributions) increased to a maximum value (100%). About 7 min later, the female, which had first approached the cages of the males, retired to its territorial area. In parallel, the vocal activity of the birds began to decline. The activity of *m2*

[1] T. HOOKER and B. I. HOOKER (LADE), in *Bird Vocalization* (Ed. R. A. HINDE; Cambridge University Press, 1969), p. 185.
[2] D. VON HELVERSEN and W. WICKLER, Z. Tierpsychol. *29*, 301 (1971).
[3] W. H. THORPE, Behaviour Suppl. *18* (1972).
[4] D. TODT, J. Ornith. *111*, 332 (1970).
[5] W. WICKLER, Z. Tierpsychol. *30*, 464 (1972).
[6] D. TODT, in Vorbereitung.
[7] T. FARKAS, Ibis *111*, 281 (1969).
[8] D. TODT, Behaviour *38*, 146 (1971).