

250
Det Kgl. Danske Videnskabernes Selskab.

Biologiske Meddelelser. **XIII**, 8.

THE PRODUCTION
OF MATING INSTINCTS IN THE RAT
WITH CHEMICALLY WELL-DEFINED
OESTROGENIC COMPOUNDS

BY

AXEL M. HEMMINGSEN AND NIELS B. KRARUP



KØBENHAVN

LEVIN & MUNKSGAARD

EJNAR MUNKSGAARD

1937

Det Kgl. Danske Videnskabernes Selskab udgiver følgende
Publikationer:

Oversigt over Det Kgl. Danske Videnskabernes
Selskabs Virksomhed,
Historisk-filologiske Meddelelser,
Filosofiske Meddelelser,
Archæologisk-kunsthistoriske Meddelelser,
Mathematisk-fysiske Meddelelser,
Biologiske Meddelelser,
Skrifter, historisk og filosofisk Afdeling,
Skrifter, naturvidenskabelig og matematisk Afdeling.

Selskabets Kommissionær er *Levin & Munksgaard*, Nørre-
gade 6, København.

Det Kgl. Danske Videnskabernes Selskab.

Biologiske Meddelelser. **XIII**, 8.

THE PRODUCTION
OF MATING INSTINCTS IN THE RAT
WITH CHEMICALLY WELL-DEFINED
OESTROGENIC COMPOUNDS

BY

AXEL M. HEMMINGSEN AND NIELS B. KRARUP



KØBENHAVN

LEVIN & MUNKSGAARD

EJNAR MUNKSGAARD

1937

Printed in Denmark.
Bianco Lunos Bogtrykkeri A/S

1. Introduction.

Recent years have seen the discovery of a number of chemically well-defined oestrogenic compounds (see e. g. DODDS, 1935; DODDS and LAWSON, 1936). The vaginal smear method has made this possible; but, on the other hand, it seems to have been the object of scanty interest, if any, to what extent these compounds are capable of inducing the essential oestrous phenomena, the mating instincts; let alone the rise in spontaneous muscular activity characteristic of oestrus. Both these groups of oestrous phenomena can be induced with more crude follicular extracts (see HEMMINGSEN, 1933, p. 164—183, and p. 203—214). One cause of the omission has been the nocturnal occurrence of the heat symptoms in the laboratory species employed for these studies. In the previous communication (HEMMINGSEN and KRARUP, 1937) we have shown how to shift the occurrence of heat in the rat to the natural day-time by exposing the animals to light in the night and to dark in the day.

On spayed rats under such "reversed" conditions we have tested three of the compounds in point for ability to induce mating instincts and enhanced spontaneous muscular activity, viz. the crystalline preparations theelin (keto-hydroxyoestrin, or estrione) and theelol (trihydroxyoestrin, or estriol) of Parke, Davis & Co., and the synthetic oestrogenic compound 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6 dibenzanthracene of COOK, DODDS, HEWETT,

and LAWSON (1934), which was kindly placed at our disposal through Professor DODDS and Dr. HAGEDORN. According to WOLFE (1936) the latter substance prevents the structural changes which occur in the anterior hypophysis of the female rat following castration.

2. Technique.

The technique of reversing the illumination and of recording the vaginal smears, the mating instincts, and the spontaneous muscular activity, was exactly as in the previous communication, to which the reader is referred. Vaginal smears and mating tests were made daily every 3 hours from 9 to 21 o'clock (dark period from 6—18) both before the injections from the day of ovariectomy, and after the injections.

3. Description of experiments.

4 of the normal female rats used in the previous investigation were spayed. After ovariectomy 10 days were allowed to pass in order to obtain assurance that all the oestrous phenomena were abolished.

Two of the rats were then injected one day every hour from 9—22 o'clock with 0.4 c. c. of a solution of theelol containing 50 rat units per c. c. It was prepared by dissolving commercial theelol Parke, Davis & Co., into 0.9 p. c. NaCl, containing 0.025 n NaOH (according to MELCHIONA, 1931, p. 653). Each of these two rats thus received 20 rat units of theelol per injection, i. e. 280 rat units in total.

These large amounts were given because in earlier experiments (see HEMMINGSEN, 1933, p. 181) 100—200 times the dose giving vaginal effect in some insensitive rats only give mating responses of type 3.

Both rats reacted normally as regards the vaginal smear, but only one of them showed mating reactions above No. 2 (responses of types 5, 6, and 7, were observed on the day after the injection).

The two others were injected in the same way with the same number of theelin units. Theelin was available in commercial vials containing 50 rat units per c. c. The result was similar. Both rats showed vaginal response, but only the one showed mating reactions above 2 (types 5 and 6 were observed on the two days following the injections). 3 days afterwards when the vaginal effects had vanished in all 4 animals, those two which had not shown mating responses, were again injected with the same total dose of 280 rat units with the modification that double the amount was injected every two hours from 10—22 o'clock. The result was still negative, a vaginal response being obtained without any mating reactions. Obviously these two animals were less sensitive than the two others. The positive mating responses in the other two rats showed that both theelin and theelol are capable of producing mating instincts.

We wished to confine ourselves to the sole object of deciding this, and therefore no further experiments were made with theelin and theelol.

On the same day as these two insensitive animals were injected the second time with theelin and theelol, those two which had shown positive mating responses, were now injected with the synthetic oestrogenic compound obtained through Prof. DODDS, and at the same hours, i. e. every second hour from 10—22 o'clock; each time with 0.5 c. c. of a sesame oil solution containing 4.3 mg per c. c., i. e. 30 mg in total. A rest, which would not readily dissolve, was

mixed thoroughly with the oil in a mortar. According to Professor DODDS oil is the only medium in which the substance can be taken up. The sites of injection of these oily injections were closed with collodium.

It is impossible to state in any exact way the strength of the oily solution in terms of rat units. We can only say that in a preliminary assay experiment a total amount of 0.1 mg in one spayed rat and 0.05 mg in two spayed rats injected over 48 hours at 9, 18, 9, 15, 22 and 9 o'clock (method of ALLAN, DICKENS, and DODDS, 1930) gave a positive vaginal response, whereas 0.025 mg injected in the same way in one spayed rat, was negative.

Smears were taken in these preliminary tests at frequent intervals (every four hours) on the day when an effect was to be expected. By the same method DODDS and his coworkers have seen full vaginal cornification in 50 p. c. of the injected rats with 0.025 mg (personal communication).

The total dose injected in the two rats which received 0.5 c. c. every two hours from 10—22 o'clock, thus was approximately 600 times as large as the lowest dose observed in the preliminary tests to give vaginal response. The previous experience quoted above and the negative results with 280 rat units of theelin and theelol in two of the 4 injected rats, dictated these large doses.

These two rats came into constant heat varying from types No. 6 to 11. After some days when heat had been observed to be continuous, the inspections were confined to noon, i. e. in the middle of the dark periods. When it had lasted one month, the two animals were killed. Their uteri were not distended.

The two other rats which had shown negative mating responses to the injections of theelin and theelol, were in-

jected 10 days after the last of these injections, when the vaginal smear had been D for about a week, with a total amount of 15 mg of the synthetic compound, given in three injections at 10, 13 and 16 o'clock. The one came into constant heat varying between types 4 and 11, until it was killed after this condition had lasted for 3 weeks (inspections daily at noon). Its uteri were enormously distended. The other one, however, only reached mating response of type 3, but not until on the fifth day after the injection. It was, therefore, 8 days after the injection, again injected with about double this amount, i. e. 30 mg, in three injections at 10, 13 and 16 o'clock. Thereafter it exhibited heat of type 4 every day (inspections at noon) until it was killed 11 days after the last injection. Its uteri were moderately distended. This animal obviously was less sensitive than the others.

No doubt the protracted effect of the synthetic product must be attributed to its slow absorption from the oily medium in which it is dispersed, and which in itself remains for long periods under the skin.

About the capability of the synthetic product to produce mating instincts there can be no doubt.

In all four animals the vaginal smear during the continuous heat was often invaded by numerous leucocytes.

In conformity with previous observations (e. g. HEMMINGSEN, 1933, p. 200—203) the spontaneous muscular activity was considerably reduced by ovariectomy, and its normal oestrous periodicity vanished. The injections of theelin and theelol affected the muscular activity only in the two positive cases, where small peaks in the activity curves were observed. The injections of the synthetic product, however, caused a considerable rise in all four animals as the fol-

owing table shows. There was no regular periodicity in this enhanced activity, which showed a gradual rise during the continuous heat lasting until the animals were killed.

Table 1.

Spontaneous muscular activity.

(Average daily number of revolutions of activity wheel).

Rat No.	From ovariectomy to injection of synthetic compound.	From injection of synthetic compound until killed (i. e. during constant heat).
3947	1090	3620
3949	497	5060
3950	1190	6400
3951	1230	3870

Discussion.

The existence of a great number of chemically well-defined compounds known to influence the genital tract of females, has raised the question of the specificity of the female sex hormones (see for instance DODDS, 1934—35).

It is natural to ask whether this relative lack of specificity extends beyond the mere anatomical effects.

The work involved in testing all the compounds known to produce the vaginal effect, also for mating instincts and enhanced spontaneous muscular activity would be large, but the present successful induction of mating instincts and enhanced spontaneous muscular activity with three rather widely differing compounds shows that actually the relative lack of specificity in regard to the vaginal effect, applies also to phenomena of primary biological significance in regard to oestrus and mating. In our opinion this should be the object of as much attention, or still more, than the lack of specificity of the mere vaginal reaction.

Summary.

Not only the vaginal smear changes, but also reliable mating instincts of high degree, and enhanced spontaneous muscular activity, are produced by injections into spayed female rats of theelin and theelol, and the synthetic oestrogenic compound 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene of COOK, DODDS, HEWETT, and LAWSON (1934).

Beside Dr. H. C. HAGEDORN, Mr. BØRGE CLAUSEN, Mr. ANKER NIELSEN, and Miss I. L. BISGAARD, who have helped us in the same respects as mentioned in the preceding paper, we wish to thank Professor E. C. DODDS for providing samples of the synthetic di-n-propyl-benzanthracene compound.

(From Nordisk Insulinlaboratorium, Gentofte, Denmark.)

References.

- ALLAN, H., DICKENS, F., and DODDS, E. C. (1930): Observations on the standardization of the water-soluble, oestrus-producing hormone. *J. Physiol.* **68**. 348—362.
- COOK, I. W., DODDS, E. C., HEWETT, C. L., and LAWSON, W. (1934): The oestrogenic activity of some condensed-ring compounds in relation to other biological activities. *Proc. Soc. Ser. B.* **114**. 272—286.
- DODDS, E. C. (1934—35): A study of specificity in relation to hormone and other biological relations. *The Harvey Lectures*. — (1935): A consideration of the structure of certain sex hormones, vitamins, and carcinogenic agents in relation to their biological activity. *Ergebnisse der Physiologie.* **37**. 264—272.
- and LAWSON, W. (1936): Synthetic oestrogenic agents without the phenantrene nucleus. *Nature.* **137**. 996.
- HEMMINGSEN, A. M. (1933): Studies on the oestrus-producing hormone. *Skand. Archiv. Physiol.* **65**. 99—250.
- and KRARUP, N. B. (1937): Rhythmic diurnal variations in the oestrous phenomena of the rat and their susceptibility to light and dark. *Det kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser.* XIII, No. 7.
- MELCHIONA, R. (1931): The effect of theelol on the blood pressure, heart rate, and respiratory rate. *J. Biol. Chem.* **91**. 653.
- WOLFE, J. M. (1936): The action of a synthetic oestrogenic agent on the anterior pituitary of the castrated female rat. *Amer. J. Physiol.* **115**. 665—669.

BIOLOGISKE MEDDELELSER

UDGIVNE AF

DET KGL. DANSKE VIDENSKABERNES SELSKAB

BIND X (KR. 23,25):

Kr. ø.

1. JENSEN, AD. S.: Der grosse europäisch-sibirische Kreuzschnabelzug 1927. 1930 1.00
2. KOLDERUP ROSENINGE, L.: The Reproduction of *Ahnfeltia Plicata*. 1931 1.75
3. WEIS, FR.: Fortsatte fysiske og kemiske Undersøgelser over danske Hedejorder og andre Podsoldannelser. With an English Summary: Further investigations on danish Heath Soils and other Podsols. Med 2 Tavler. 1932 9.25
4. ENGELBRETH-HOLM, J.: Undersøgelser over den saakaldte Erytroleukose hos Høns. 1932 2.75
5. JENSEN, AD. S.: Studier over *Incurvaria Koernerella* Zell (Lepidoptera, Incurvariidae). Med 32 Figurer i Texten. Deutsche Zusammenfassung. 1932 2.90
6. BOAS, J. E. V.: Der Hinterfuss von *Caenolestes*. Ein Supplement zu der Abhandlung über den Hinterfuss der Marsupialier. Mit einer Tafel. 1933 1.00
7. HAGERUP, O.: Zur Organogenie und Phylogenie der Koniferenzapfen. 1933 3.20
8. BØRGESEN, F.: On a new Genus of the Lophotalieæ (Fam. Rhodomelaceæ). 1933 0.90
9. MORTENSEN, TH. and KOLDERUP ROSENINGE, L.: Sur une nouvelle Algue, *coccomyxa astericola*, parasite dans une Astérie. 1933 0.50

BIND XI (KR. 23,50):

1. ASMUSSEN, ERLING und LINDHARD, J.: Potentialschwankungen bei direkter Reizung von motorischen Endplatten. 1933 1.50
2. LIND, J. Studies on the geographical distribution of arctic circumpolar Micromycetes. 1934 4.50
3. BOAS, J. E. V.: Über die verwandtschaftliche Stellung der Gattung *Antilocapra* und der Giraffiden zu den übrigen Wiederkäuern. Mit 3 Tafeln. 1934 2.40
4. O. HAGERUP: Zur Abstammung einiger Angiospermen durch *Gnetales* und *Coniferæ*. 1934 3.20
5. JENSEN, AD. S.: The Sacred Animal of the God Set. 1934 1.00
6. BØRGESEN, F.: Some Marine Algæ from the northern part of the Arabian Sea with remarks on their geographical distribution. With 2 Plates. 1934 3.50
7. MORTENSEN, TH. et KOLDERUP ROSENINGE, L.: Sur une Algue Cyanophycée, *Dactylococcopsis Echini* n. sp., parasite dans un Oursin. 1934 0.70
8. GABRIELSEN, E. K. und LARSEN, POUL: Über den Kohlenstoffhaushalt der terrestrischen Halophyten. 1935 2.20
9. HAGERUP, O.: Zur Periodizität im Laubwechsel der Moose. Mit 4 Tafeln. 1935 4.50

Bind XII (Kr. 23,55):

	Kr. Ø.
1. JESSEN, KNUD: The Composition of the Forests in Northern Europe in Epipalæolithic Time. With the assistance of H. JOHANNSEN. With 3 Plates. 1935.....	3.75
2. BØRGESEN, F.: A list of Marine Algæ from Bombay. With 10 Plates. 1935.....	4.25
3. KRABBE, KNUD H.: Recherches embryologiques sur les organes pariétaux chez certains reptiles. Avec 19 planches. 1935.....	7.00
4. NIELSEN, NIELS: Eine Methode zur exakten Sedimentationsmessung. Studien über die Marschbildung auf der Halbinsel Skalling. Mit 16 Tafeln. 1935.....	5.50
5. BØRGESEN, F. and FRÉMY, P.: Marine Algæ from the Canary Islands especially from Teneriffe and Gran Canaria. IV. Cyanophyceæ. 1936.....	1.80
6. SCHMIDT, S., OERSKOV, J. et STEENBERG, ELSE: Immunisation active contre la peste aviaire. Avec 1 planche. 1936.....	1.25

Bind XIII (under Pressen):

1. BOYSEN JENSEN, P.: Über die Verteilung des Wuchsstoffes in Keimstengeln und Wurzeln während der phototropischen und geotropischen Krümmung. 1936.....	1.50
2. FRIDERICIA, LOUIS SIGURD and GUDJÓNSSON, SKULI V.: The Effect of Vitamin A Deficiency on the Rate of Growth of the Incisors of Albino Rats. 1936.....	1.00
3. JENSEN, AD. S.: Den kinesiske Uldhaandskrabbe (<i>Eriocheir sinensis</i> M-Edw.) i Danmark. Med 3 Tavler. Deutsche Zusammenfassung. 1936.....	1.50
4. KROGH, AUGUST and SPÄRCK, R.: On a new Bottom-Sampler for Investigation of the Micro Fauna of the Sea Bottom with Remarks on the Quantity and Significance of the Benthonic Micro Fauna. 1936.....	0.75
5. SPÄRCK, R.: On the Relation between Metabolism and Temperature in some Marine Lamellibranches, and its Zoogeographical Significance. 1936.....	1.50
6. HAGERUP, O.: Zur Abstammung einiger Angiospermen durch <i>Gnetales</i> und <i>Coniferae</i> . II. <i>Centrospermae</i> . 1936.....	3.00