an increase in the number of oocytes passing into the vitellogenic phase as an effect of subtotal ovariectomy in the toads. Our results confirm this, because hemispayed frogs of the experimental group show 55.9 and 44.3% increase in spawning when compared to hemispayed frogs of Group 2 and 4 (Table). Laboratory maintenance of frogs for 20 days has no inhibitory effect on spawning, as could also be seen from these results. In frogs where gravimetric analysis of the ovaries is not reliable, compensatory hypertrophy in spawning response after hemispaying seems to be the true portrayal of the phenomenon of compensatory hypertrophy.

Zusammenfassung. 20 Tage nach der einseitigen Entfernung eines Eierstockes wurde bei *Rana cyanophlyctis* eine Zunahme der Laichmenge beobachtet. Das Resultat des induzierten Ablaichens zeigt, dass eine ausgleichende Hypertrophie des verbliebenen Eierstockes stattgefunden hat.

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Some New Aspects of a Sheep Pineal Gonadotropic Inhibiting Activity in in vitro Experiments

Previously we have reported that an epiphyseal-hypothalamic interaction of some sheep pineal fractions could be revealed in an in vitro study. From that study we concluded that the pineal contains active principles, other than melatonin, capable of acting via the hypothalamus[1]. In this paper we will give the results of experiments studying the influence of low molecular weight sheep pineal fractions on the gonadotropic activity of anterior hypophysis in vitro.

Material and methods. Column chromatography of aqueous extracts of sheep pineals (ERSCO, San Mateo, California) was carried out on Sephadex G-25 columns (56 × 4.2 cm) as described previously, EBELS et al.[2]. The localization of the excitation and fluorescence maximum of the eluate has been carried out as published in detail before[3,4].

Ultrafiltration of the low molecular weight Sephadex G-25 fractions F2 and F3 were performed as described by EBELS et al.[5]. The UM-05 filtrate (Diaflo membrane UM-05 will generally partition mixtures of solutes above and below the 500 mol wt. range. Thus the UM-05 filtrate contains substances below the 500 mol wt. range) was separated on Sephadex G-10 columns (142 × 1 cm) as reported in[5] Sephadex G-10 fractions were separated further by paper electrophoresis on Whatman 3 MM-paper in a pyridine-acetate buffer at pH 6.5. For details see[5].

Bioassays. A) Incubation experiments with half-anterior hypophyses of mice or rats. In the experiments with mice we have used 6-halves anterior hypophyses and in the experiments with rat-anterior hypophyses we have used 4-halves anterior hypophyses. For the study of each pineal fraction anterior hypophyses of mice or rats were incubated with a pineal fraction for 3 h in a Krebs-Ringer solution at 37 °C, aerated with 95% O_2 and 5% CO_2 and anterior hypophyses of mice or rats incubated alone served as control. After centrifugation of this incubation liquid, the supernatant was injected s.c. in 5 injections into immature 21-day-old female Swiss mice, to determine the gonadotropin-releasing activity. Autopsy was carried out 18 h after the last injection. We compared the average value of the ovary weights and the average value of the uterine weights of the groups. For detail of the method, see MOSZKOWSKA and EBELS[6].

B) Radioimmunological determination of LH in the incubation liquid of anterior hypophyses of rats was carried out according to the method described by NISWENDER et al.[7].

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Table I. Reaction of immature mice after injection of each with the incubation liquid of 6-halves anterior hypophyses of male mice, with (A) or without (B) a sheep pineal Sephadex G-10 fraction

Sephadex G-10 fraction	Elution volume of the fraction (ml)	Group of test mice	No. of mice per group	Mean ovary weight (mg) [a]	*P* value	Mean uterus weight (mg) [a]	*P* value
1	0–70	A	6	3.65 ± 0.14	ns [b]	52.25 ± 3.38	ns [b]
		B	4	3.40 ± 0.28		45.72 ± 4.92	
2	70–120	A	6	3.73 ± 0.19	ns	49.06 ± 5.32	ns
		B	4	3.40 ± 0.38		45.72 ± 4.92	
3	120–140	A	5	3.52 ± 0.30	ns	60.83 ± 7.03	ns
		B	5	4.08 ± 0.20		74.50 ± 6.26	
4	140–165	A	5	4.19 ± 0.29	ns	43.62 ± 5.14	< 0.05
		B	5	4.08 ± 0.20		74.50 ± 6.26	
5	165–195	A	5	3.82 ± 0.23	ns	39.05 ± 2.62	< 0.01
		B	5	4.08 ± 0.20		74.50 ± 6.26	
6	195–255	A	5	3.87 ± 0.25	ns	51.96 ± 4.27	< 0.05
		B	5	4.08 ± 0.20		74.50 ± 6.26	

[a] Mean value ± standard error of the mean value for 4–6 mice. [b] ns, not significant at 5% level.

Table II. Localisation of sheep pineal gonadotropic inhibiting activity by paper electrophoresis

Experiment	Elution volume of the Sephadex G-10 fraction	Band of the strip (cm) [a]	Group of test mice	No. of mice per group	Mean ovary weight (mg) [b]	P value	Mean uterus weight (mg) [b]	P value	ng NIH LH SI/ rat hypophyse (λ standard curve = 0.04)	Inhibition (%)
I	75–200	$-2^1/_4 \rightarrow -^1/_4$	A	3	4.66 ± 0.16	< 0.01	47.83 ± 3.97	ns [c]	243	56%
			B	5	6.97 ± 0.46		74.57 ± 8.61		558	
		$-^1/_4 \rightarrow +2$	A	5	5.64 ± 0.18	< 0.01	75.81 ± 10.93	ns	315	57%
			B	5	9.88 ± 0.95		74.44 ± 12.29		738	
II (1)	52–137	$-2^1/_4 \rightarrow +1^1/_4$	A	5	4.24 ± 0.40	< 0.01	45.44 ± 5.12	ns	169	77%
			B	5	9.88 ± 0.95		74.44 ± 12.29		738	
II (2)	137–222	$-1^1/_2 \rightarrow +^3/_4$	A	5	5.54 ± 0.43	< 0.01	56.30 ± 5.41	ns	272	63%
			B	5	9.88 ± 0.95		74.44 ± 12.29		738	

Reaction of immature mice after injection of each with the incubation liquid of 4 halves anterior hypophyses of male rats with (A) or without (B) a sheep pineal fraction and determination of LH in the incubation liquid of anterior hypophyses of rats. [a] Start = 0; (−) = cathode side; (+) = anode side. [b] Mean value ± standard error of the mean value for 3–5 rats. [c] ns, not significant at 5% level.

Results and discussion. Separation of aqueous sheep pineal extracts on Sephadex G-25 gave rise to several distinct peaks detected by spectrofluorometry. We have chosen the low molecular weight Sephadex G-25 fractions F2 and F3 for the experiments described in this paper (for details of these fractions, see EBELS et al.[5]. These Sephadex G-25 fractions (F2 and F3) separated through the diaflomembranes UM-2 and UM-05 gave 3 fractions: UM-2 residue, UM-05 residue and UM-05 filtrate. The UM-05 filtrate separated on Sephadex G-10 gave rise to several peaks detected by Spectrofluorometry. The activity of the lyophilized Sephadex G-10 fractions are presented on Table I.

From comparable experiments, the fractions eluted from the Sephadex G-10 column, with approximately the same elution volume as the active fractions, presented in Table I, were used for paper electrophoresis studies. The activity of the lyophilized fractions, obtained after elution of the paper electrophoresis strips with distilled water, are presented in Table II. Our usual incubation technique gave only an impression of the inhibition of FSH-activity of the anterior hypophyses of rat and mice. In this paper we also used a radioimmunological assay for LH-secretion and from these data, it is clear that we could detect also an inhibition of the LH-secretion of rat anterior hypophyses.

A gonadotropic inhibiting activity in incubation experiments with rat-anterior hypophyses has been seen in crude pineal extracts and later in Sephadex G-25 and Sephadex G-10 fractions[8–10]. However, that activity, which seems to be fairly specific for the pineal body, seemed to be unstable under the experimental conditions which we used at that time [11]. Now it is possible to isolate by rather mild and simple extraction and separation methods a gonadotropic-inhibiting activity from low molecular weight fractions of Sephadex G-25 columns, followed by ultrafiltration and separation on Sephadex G-10 columns.

As we could detect the inhibitory activity in UM-05 filtrate fractions (after gelfiltration on Sephadex G-10), it may be concluded, with some reservations, that the active substance(s) has a molecular weight less than 500. As we were able to determine the elution volume of melatonin from comparable Sephadex G-10 columns, we can say that the elution volume of the inhibiting activity, described in this paper, differs from that of synthetic melatonin applied to a comparable column[5].

We can conclude that it is possible to isolate and localize by paper electrophoresis a gonadotropic-inhibiting activity from low molecular weight sheep pineal fractions. Experiments are in progress to purify and characterize the active substance(s).

Résumé. Par des méthodes simples et bien déterminées: gelfiltration sur Sephadex G-25, G-10 et l'ultrafiltration par des diaflomembranes UM-02 et UM-05, on a pu isoler à partir d'un extrait aqueux d'épiphyses de mouton, des fractions capables d'inhiber l'activité gonadotrope hypophysaire. Cette activité inhibitrice a pu être localisée par l'électrophorèse sur papier. Les fractions ainsi obtenues sont capables d'inhiber in vitro l'excrétion hypophysaire de FSH et de LH.

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[15] Acknowledgment. The authors gratefully acknowledge Dr. Reichert and Dr. Niswender for the generous supply of antigens and antibodies for the radioimmunoassay. Our thanks are also due to Mrs. A. E. M. Horwitz-Bresser for her skilful assistance in the isolation procedures.