tract corresponds neither to the CCK-PZ localization[11,16] nor to the localization of motilin[10,17].

The hormonal product of the D type cells has not yet been identified, the localization of these cells which have their maximum in the duodenum, corresponds to the CC-PZ[11,16] as well as to the motilin[10,17]. Our findings, after the L-phenylalanine stimulation, rather testify to the presence of the CCK-PZ in the D type cells. Following introduction of L-phenylalanine, a mass-scale release of the CCK-PZ takes place, without affecting the secretin, as has been proved by MEYER, SPINGOLA and GROSSMAN[14]. Following the same stimulation, we observed the release of the secretion granules, beside the above-mentioned EC cells, only in D type cells.

FUJITA and KOBAYASHI[15] have documented the release of hormones from the D cells of the pyloric antrum after stimulation with 0.1 *N* HCl. Solutions of extremely low pH value were conductive to a strong release of the CCK-PZ[18–20]. The FUJITA and KOBAYASHI hypothesis, stating that the D cells produce an inhibitor of the secretion of the stomach acid, may be correct, although the inhibitor is not the secretin but the CCK-PZ. The CCK-PZ, similarly to secretin, acts as an inhibitor of gastrin-stimulated secretion of the stomach acid in man and in dog[16,21–23]. The other substance, which we considered to be the possible product of the D cells, is the motilin. The motilin is released, however, after alkalinization of the duodenum[17], i.e. in conditions just contrary to those in which the secret of the D type cells is released. Consequently, it is probable that a cell type other than the D type cells is responsible for the production of motilin.

Zusammenfassung. Nach intraduodenaler Stimulation der Cholecystokinin-Pancreozymin Sekretion (CCK-PZ) beim Hund durch L-Phenylalanin finden sich Anhaltspunkte für die Ausschleusung von Sekretgranula bei 2 Typen von endokrinen Zellen. Es handelt sich einerseits um enterochromaffine Zellen (EC), die 5-Hydroxytryptamin produzieren und deren Verteilung im Gastrointestinaltrakt nicht dem CCK-PZ entspricht, andererseits um D-Zellen, deren Hormonalprodukt noch nicht identifiziert ist. Es wird angenommen, dass CCK-PZ von den D-Zellen des Gastrointestinaltraktes produziert wird.

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Compensatory Spawning Response After Unilateral Ovariectomy in the Skipper Frog, *Rana cyanophlyctis* (Schn.)

VIJAYAKUMAR[1] tried subtotal ovariectomy as an indirect method of determining the occurrence of compensatory hypertrophy in newly spawned toads. The reported increase in weight of the ovarian piece left after subtotal ovariectomy can be accepted only with some reservations, because the difference in weight between the two ovaries is not regular in frogs and toads. So an attempt was made to demonstrate compensatory hypertrophy through spawning induction.

Gravid female skipper frogs weighing 25–40 g, collected around Mysore City (India), were distributed into 5 groups as mentioned in the Table. Each group contained 6 frogs.

All the frogs were induced to spawn with a homogenate of 4 toad pituitaries in 1 ml of distilled water injected i.p. and were maintained in individual aerated aquaria containing 1 to 2 cm of spring water, at room temperature 25 ± 1 °C. 24 h after induction the number of eggs spawned per frog was counted and calculated results are given in the Table.

Group No.	Treatment	Eggs spawned/frog
1	Fresh intact controls induced on day 1	857.8 ± 94.0[a]
2	Unilaterally ovariectomized and induced on day 1	413.3 ± 73.6
3	Unilaterally ovariectomized maintained for 20 days and induced on day 21	644.3 ± 67.5
4	Unilaterally ovariectomized on day 21 and induced on the same day	446.5 ± 68.1
5	Fresh intact controls induced on day 21	803.0 ± 99.4

[a] Arithmetic mean ± standard error.

It is interesting to note that Group 1 and 5 intact control frogs spawned nearly 858 and 803 eggs respectively and the unilaterally ovariectomized frogs induced on day 1 or day 21 (Group 2 and 4) spawned almost half the number of eggs, namely 413 and 446 respectively. These results are in agreement with our previous report[2]. However, hemispayed frogs maintained in the laboratory for 20 days and induced on the 21st day to spawn (Group 3) showed an increase in the spawning rate by 55.9 and 44.3 % when compared to the controls of Groups 2 and 4 respectively, the percent increase being significant.

Hemispaying results in increased ovulation by the contralateral ovary in mammals[3–6]. VIJAYAKUMAR[1] reports

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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.