

Zusammenfassung

Das Männchen des dreistachligen Stichlings zeigt unmittelbar nach Balz und Befruchtung der Eier kein sexuelles Verhalten mehr. Die Erniedrigung der sexuellen Motivation wird nicht durch Balzhandlungen oder Befruchtung verursacht, sondern durch die Wahrnehmung der Eier im Nest (Beispiel einer «Endsituation»).

The Response of Costal Cartilage to Changes in Hormonal Environment

The effects of testosterone propionate (T. P.) and hydrocortisone (F) on anabolic and catabolic processes, insofar as nitrogen metabolism is concerned, are well documented. Since nitrogen balance studies are measurements of general metabolism and as such do not give any direct information of hormonal effects on any one tissue, we have focused our interest on metabolic alterations induced by these steroids in a specific tissue.

Of the various tissues studied, we have observed, as have others^{1,2,3} that costal cartilage has a strong affinity for radiosulfate and that this tissue is sensitive to changes in hormonal environment. The present report describes some of our observations on the effect of T. P. and F on the radiosulfate uptake by costal cartilage in the intact or hypophysectomized male rat.

Methods. A. Animal treatment. Albino rats of the Holtzman strain were hypophysectomized⁴ at 21 ± 2 days. Ten days post-hypophysectomy (8 days after arrival in the laboratory) the animals were dosed subcutaneously $1 \times$ /day/7 days. T.P. was given in cottonseed oil, F as a suspension in carboxy-methylcellulose vehicle and growth hormone, intraperitoneally, in saline solution. When T.P. was administered concurrently with F, the injection site was in an area removed from the site of F injection. S^{35} ⁵, as sodium sulfate in saline, at a dose of $3 \mu\text{c}$ was injected intraperitoneally daily. (For exceptions see Tables.)

24 h after the last injection, the animals were sacrificed, VII rib cartilage removed, cleaned of adhering tissue, weighed and digested. Body weight was taken prior to and at the end of the experiment. Animals showing abnormal growth curves or any other gross indication of incomplete hypophysectomy were discarded.

B. Analytical methods. Individual rib cartilage specimens were digested in a water bath at $80-85^\circ\text{C}$ in covered test tubes containing 1 cm^3 0.2 M Na_2SO_4 and 1 cm^3 conc. HCl. Following digestion, 10 cm^3 distilled water was added to each tube, and after the solution reached water bath temperature, the sulfate was precipitated as a barium salt by addition of 10 cm^3 1% BaCl_2 in $1-2 \text{ cm}^3$ aliquots at 10 min intervals. The supernatant and precipitate were quantitatively transferred to a tared filter disc (S & S No. 586, 59/64 in. diameter) using a Tracerlab metal filter. The tube and precipitate were washed with distilled water followed by 95% ethanol. The filter paper with precipitate was then transferred to a tared planchet

¹ S. ELLIS, J. HUBLE, and M. E. SIMPSON, *Proc. Soc. exp. Biol. Med.* **84**, 603 (1953).

² C. W. DENKO and D. M. BERGENSTAL, *Endocrinology* **57**, 76 (1956).

³ W. R. MURPHY, W. H. DAUGHADAY, and C. HARTNETT, *J. lab. clin. Med.* **47**, 715 (1956).

⁴ The Endocrine Laboratories, Inc., Madison, Wisc.

⁵ The assistance of N. A. DRAKE, of the Upjohn Co., for procuring and handling the isotope is appreciated.

Table I

Radiosulfate Uptake by Costal Cartilage (cpm/mg) in Intact and Hypophysectomized Male Rats Following Administration of Hydrocortisone and Testosterone Propionate

Group	Hypophysectomized cpm/mg*	Body Weight (g)		Intact** cpm/mg	Body Weight (g)	
		Intact	Final		Intact	Final
Control	39.3 ± 2.5	60	70	15.8 ± 1.9	65	85
T.P., 5 μg	46.1 ± 2.2	60	73	17.8 ± 0.7	63	82
25 μg	54.5 ± 1.4	59	70	21.7 ± 1.0	60	87
50 μg	55.4 ± 2.9	58	60	17.2 ± 1.0	66	89
100 μg	57.9 ± 2.8	60	75	21.2 ± 0.7	61	84
Control	38.6 ± 5.2	60.9	71.8	43.1 ± 2.2	47.4	71.6
F, 25 μg	29.8 ± 3.3	61.9	72.2	40.5 ± 2.8	47.0	71.0
50 μg	28.2 ± 1.3	60.5	70.0	42.1 ± 2.7	45.0	66.6
100 μg	21.8 ± 1.3	60.6	64.3	40.4 ± 1.6	47.4	65.0
200 μg	14.7 ± 1.1	61.1	59.6	32.7 ± 2.0	48.2	62.0
500 μg	12.9 ± 1.0	59.6	55.6	45.0 ± 1.4	47.6	49.2

Group	Hypophysectomized cpm/mg	Body Weight Intact	Change Final
Control	38.3 ± 2.9	63	70
T.P., 50 μg	51.5 ± 3.4	63	73
F, 25 μg	31.7 ± 3.5	65	73
50 μg	21.7 ± 2.7	63	70
500 μg	12.6 ± 2.7	66	60
F, 25 μg + T.P. 50 μg	40.7 ± 2.4	67	77
50 μg + T.P. 50 μg	30.1 ± 1.9	62	70
500 μg + T.P. 50 μg	13.0 ± 1.7	63	59

* S^{35} -3 μc /day/7 days, I. P. ** S^{35} -1 μc /day/7 days, I. P.

and dried under infrared light. The samples were counted using an end window gas flow counter. Self-absorption was calculated to infinite thinness and the counts reported as counts per min per mg wet weight (cpm/mg tissue).

All experiments were repeated 2-3 times with 8-10 animals per group. The results presented are those of a representative group and not the average of all the experiments.

Table II

Effect of Age at Hypophysectomy on Radiosulfate Uptake (cpm/mg Tissue) by Cartilage in Male Rats

Age (Days)	Control*	50 μg T. P.	100 μg T. P.
26	24.9 ± 3.8	27.8 ± 4.0	—
31	13.6 ± 3.0	16.3 ± 2.8	19.5 ± 1.0
36	10.5 ± 1.0	11.7 ± 1.4	—
41	5.9 ± 0.9	7.7 ± 0.7	10.5 ± 1.2
		20 μg G.H.**	100 μg G. H.
26	20.2 ± 2.7	48.4 ± 6.0	68.9 ± 4.7
31	13.0 ± 3.1	30.1 ± 3.0	41.6 ± 2.8
36	8.8 ± 6.0	34.3 ± 4.0	46.8 ± 3.8
41	5.6 ± 0.8	—	30.4 ± 2.5

* S^{35} -3 μc /day/7 days, I. P.

** Growth Hormone - $1 \times$ day/7 days, I. P.

Results. Radiosulfate retention by costal cartilage in the intact or hypophysectomized rat is recorded in Table I.

Hypophysectomy has a marked effect on affinity of cartilage for radiosulfate. Incorporation of the isotope in hypophysectomized animals is decreased $1/2-1/3$ when compared to normal rats weighing 85 g. T. P. and growth hormone are both effective in stimulating retention of radiosulfate cartilage. The response is qualitatively similar to but not of the same order of magnitude as response to growth hormone (Table II).

In contrast, T. P. at the same dose level has no apparent effect in the intact animal in that the steroid does not increase isotope retention significantly above that observed for uninjected animals (Table I).

The most sensitive to T. P. appears to be the animal hypophysectomized at 21 days of age. Animals operated at a later age demonstrated a decreased sensitivity to the steroid (Table II, note 36-day group). The lower counting rates observed in control and treated animals are most likely a reflection of the dose of S^{35} /g body weight. Growth hormone has a stimulating effect on radiosulfate incorporation by cartilage regardless of age at hypophysectomy (Table II).

Hydrocortisone accentuates the effects of hypophysectomy on cartilage activity. Incorporation of the isotope into cartilage cells is inhibited by doses of F ranging from 25 to 500 μ g. This dosage level was without effect in the intact rat. Simultaneous treatment with T. P. and F partially corrected the effects obtained when F alone was used (Table I).

We have observed that the hypophysectomized rat gains about 10–15 g in body weight during the 7 day experimental period. Animals operated at 36 days of age gain very little weight. In this respect it is interesting to note that the stimulating effects of T. P. as well as the inhibitory effects of F (at 25 and 50 μ g) occur without any observable influence on body weight.

Discussion. The foregoing data illustrate the susceptibility of costal cartilage to changes in hormonal environment. The decreased metabolic activity of the cartilage cell following hypophysectomy is in agreement with the reports of others^{1,2,3}. Consistent with their findings, we have observed that growth hormone, while having no effect in the intact animal, stimulates incorporation of S^{35} sulfate in the costal cartilage of the hypophysectomized rat.

From the present study it appears that a similar effect, insofar as costal cartilage is concerned, can be ascribed to T.P., since response of the intact or hypophysectomized animal following administration of the steroid is qualitatively similar to response following growth hormone. Nevertheless, the question arises as to whether the response of cartilage can be considered as a direct effect or reflects general improvement of the operated animal.

The fact that T. P. stimulates retention of radiosulfate without apparent effect on body weight suggests a specific effect on the cartilage cell activity rather than a general improvement of the metabolic state of the animal. Further, it was demonstrated that hydrocortisone accentuates the inhibitory effects of hypophysectomy at dosage levels that are without any observable influence on body weight. Consequently it is suggested that the stimulatory effect of T.P. is a direct anabolic effect on cartilage.

Incorporation of radiosulfate by cartilage has been shown to be influenced by hormones other than growth hormone. It has been reported that thyroxine⁴ stimulates

cartilage metabolism while cortisone^{7,8} and large doses of hydrocortisone³ reduce rate of sulfate fixation. We have shown in the hypophysectomized rat that T. P. also influences incorporation of radioactive sulfur. However, since we are measuring total sulfate it is only assumed that the increased concentration of isotope reflects synthesis of chondroitin sulfate, as has been postulated for growth hormone^{3,9}. It is yet to be proven that radiosulfate uptake by cartilage, in response to hormones, is a direct metabolic effect on the tissue or an alteration in turnover rate.

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Résumé

1° L'hypophysectomie diminue l'incorporation de S^{35} dans le cartilage costal de jeunes rats. L'hydrocortisone renforce l'action de l'hypophysectomie. Au contraire, le propionate de testostérone stimule la fixation de S^{35} dans le cartilage costal. Un traitement à l'hormone de croissance donne une réponse qualitativement identique à celle obtenue avec le propionate de testostérone. 2° Le propionate de testostérone, l'hydrocortisone et l'hormone de croissance n'influencent pas l'incorporation de radiosulfate chez le rat normal.

¹ L. L. LAYTON, *Proc. Soc. exp. Biol. Med.* 76, 596 (1951).

⁸ H. BOSTROM and E. JORPES, *Exper.* 9, 392 (1954).

⁹ H. BOSTROM, *Ark. Kemi* 6, 43 (1953).

Der elektrophysiologische Nachweis der propriozeptiven Muskelafferenz und des monosynaptischen Reflexbogens der inneren Kehlkopfmuskeln

Der physiologische Nachweis propriozeptiver Muskelafferenz und des monosynaptischen Reflexbogens der Kehlkopfmuskeln ist bisher weder beim Menschen noch im Tierversuch erbracht^{1,2}. Der histologische Nachweis von Muskeldehnungsrezeptoren in Kehlkopfmuskeln ist bis heute umstritten^{3,4}.

Bei Reizversuchen an Menschen, bei denen wegen eines Kehlkopfkrebse eine Totalexstirpation des Larynx durchgeführt werden musste, konnten wir das Vorhandensein propriozeptiver Muskelafferenz nachweisen und den monosynaptischen Reflexbogen darstellen.

Methodik: Sorgfältige Präparation von Kehlkopf und Nn. laryngici superiores und recurrentes. Nach Freilegung werden diese Nerven etwa 1 cm vor ihrem Eintritt in den Larynx in die Gabeln bipolarer Elektroden eingehängt und diese soweit angehoben und fixiert, dass kein Kontakt von Elektrode und gereizter Nervenstrecke mit dem umgebenden Gewebe möglich ist. Die bipolaren Elektroden können wahlweise zur Reizung wie zur Ableitung benutzt werden. Zur elektromyographischen Ableitung vom M. vocalis wurden koaxiale Nadelelektroden verwandt. Registriert wurde mit einem Dreikanal-DISA-

¹ H. LULLIES, in O. F. RANKE und H. LULLIES, *Lehrbuch der Physiologie. Gehör, Stimme, Sprache* (Springer, Berlin 1953).

² K. MÜNDNICH, *Klin. Wschr.* 35, 802 (1957).

³ K. PAULSEN, *Z. Zellforsch.* 47, 363 (1958).

⁴ D. D. DZIEWIATKOWSKI, *J. biol. Chem.* 189, 717 (1951).