Kohlenhydratkonzentrationen in der Hämolymphe von *Orconectes* nach Injektion von Augenstielextrakt

Versuchszeit	Versuchsgruppe	Zeitpunkt der Messung (h nach Injektion)	*n*	Glukose (mg Glukoseäquivalent/100 ml Hämolymphe)	Fruktose	Trehalose
Dezember	Kontrollen	–	3	6,0 ± 0,6	3	2 ± 1
	augenstielextrakt-injizierte Tiere	7½	6	131 ± 5	15 ± 4	16 ± 7
Februar – März	Kontrollen	–	3	22 ± 7	7 ± 3	16 ± 3
	augenstielextrakt-injizierte Tiere	2	5	45 ± 9	14 ± 6	39 ± 20
		4	5	100 ± 19	25 ± 8	71 ± 33
		5	9	100 ± 38	–	119 ± 48
		6	5	144 ± 21	46 ± 15	65 ± 40
		8	5	127 ± 27	33 ± 17	30 ± 24
		10	5	97 ± 23	29 ± 11	17 ± 11
April	Kontrollen	–	6	2,0 ± 0,5	–	0,7 ± 0,1
	augenstielextrakt-injizierte Tiere	4	8	68 ± 21	–	40 ± 23

Pro Tier wurde die einem halben Augenstiel entsprechende Extraktmenge (ca. 0,4 mg Augenstielprotein) in van Harreveldlösung injiziert. Kontrollen mit van Harreveldlösung. Die Versuchstiere wogen im Mittel 20 g. Die Konzentrationen der einzelnen Zucker sind als Mittelwerte ± Standardabweichung aufgeführt.

mit Eisessig-Methanol-Methyläthylketon 1:1:3 (v/v), Bestimmung der Glukose mit Glukoseoxydase (Testkombination Boehringer), der übrigen Zucker mit Anthron. Die Kontrollwerte liegen in den Versuchen vom Frühjahr möglicherweise für Glukose etwas zu hoch, für den Restzucker etwas zu niedrig, da hier infolge längerer Manipulation bei der Blutentnahme stressbedingte Veränderungen anzunehmen sind[5].

Ergebnisse. Die von SPECK[4] nachgewiesenen jahreszeitlichen Schwankungen in Menge und Zusammensetzung der Kohlenhydrate in der Hämolymphe von *Orconectes* spiegeln sich auch im vorliegenden Fall wieder in sehr unterschiedlichen Ausgangswerten in den verschiedenen Monaten. Im Februar–März, zu einer Zeit, in der die Normalwerte besonders hoch liegen, wurden auch bei den augenstielbehandelten Tieren maximale Werte für Glukose, Fruktose und Trehalose gemessen. Im April, zur Zeit eines allgemein steilen Abfalls der Zuckerkonzentrationen, erhöht das hyperglykämische Hormon Glukose und Trehalose nur noch auf etwa halb so hohe Konzentrationen wie im Februar–März. Fruktose war im April nicht messbar. Zu dieser Zeit kann sich auch eine allgemeine Abnahme der Empfindlichkeit der Krebse, die sich jetzt der Häutungsperiode nähern, gegenüber dem hyperglykämischen Hormon bemerkbar machen, wie sie auch KELLER[6] beobachtete. Die relativ niedrigen Fruktose- und Trehalosewerte nach Hormoninjektion im Winter korrespondieren mit Erfahrungen von KELLER[6], der zu dieser Zeit eine starke Wirkung des hyperglykämischen Hormons auf die Glukose, aber nur eine geringe auf den Restzucker fand.

Es ist deutlich, dass Augenstielextrakte nicht nur die Glukose erhöhen, sondern zu bestimmten Zeiten in vergleichbarem Masse auch Fruktose und Trehalose. In wieweit die Vermehrung der einzelnen Fraktionen auf wechselseitiger Umwandlung der Zucker beruht, ist eine noch offene Frage. Bemerkenswert ist, dass besonders die Trehalose zu gewissen Zeiten hohe Werte annehmen kann. Man kann somit vermuten, dass die Trehalose auch bei den Crustaceen eine bedeutende Rolle im Stoffwechsel spielt.

Summary. Quantitative changes of glucose, fructose and trehalose in the hemolymph of *Orconectes limosus* were measured after injection of eyestalk extracts. Especially in February and March high levels of trehalose were found after hormone injection.

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[6] R. KELLER, unveröffentlicht.

Spontaneous Thyroiditis in Laboratory Rats

Experimental allergic thyroiditis has been used as a model for studying similar human conditions, particularly Hashimoto's thyroiditis. Successful production of the disease has been reported in several animal species, including the rabbit, guinea-pig, dog and rat[1–6]. Spontaneous thyroiditis, however, has rarely been observed in experimental animals. This lesion was reported to occur in beagle dogs[7] but to our knowledge spontaneous thyroiditis has never been reported in laboratory rodents. During comparative endocrine pathology studies in our laboratories a high incidence of thyroiditis has been noted in a particular strain of rats. The present report briefly describes this unusual finding and summarizes the morphological characteristics of the lesion of the thyroid gland.

For the initial study the following strains of rats were used: Sprague-Dawley (Charles River), BUF, F344, Wistar, LEW (Microbiol. Associates Inc., Bethesda, Md.),

Strain	Age (weeks)	Sex	No. of rats examined	No. of rats exhibiting thyroiditis
Sprague-Dawley	19	♂	20	0
Sprague-Dawley	19	♀	17	0
Sprague-Dawley	30	♀	40	0
Sprague-Dawley	82	♂	13	0
Sprague-Dawley	82	♀	19	0
BUF	4	♂	5	0
BUF	4	♀	5	0
BUF	10	♂	5	0
BUF	36	♂	11	6
BUF	52	♀	5	0
F344	35	♂	6	0
Wistar	33	♂	16	0
LEW	34	♂	9	0
RAH	34	♂	15	0
Long Evans	33	♂	8	0

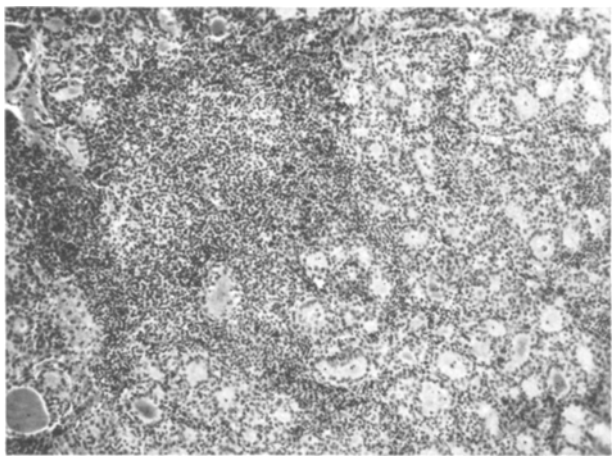

Fig. 1. Lymphoreticular stroma, containing follicles with germina centers, separates the acini (HE. X 125).

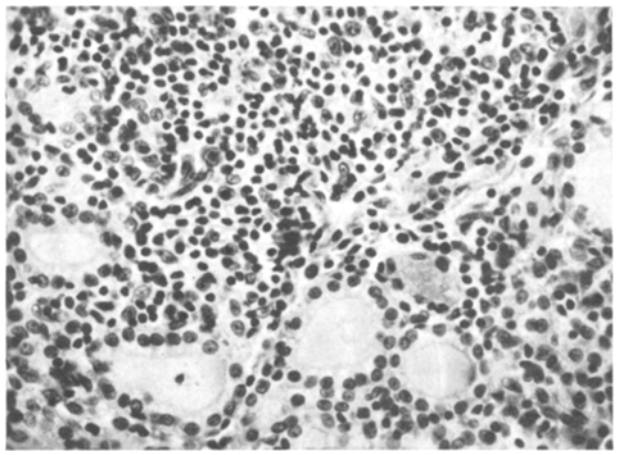

Fig. 2. The follicles are lined by granular cuboid cells and contain pale colloid. There is abundant lymphoreticular stroma (HE. X 250).

RAH, Long Evans (Research Animals Inc., Braddock, Penna.). The number, sex and age of the rats are indicated in the Table. The animals were observed from 2 to 80 weeks in our laboratories and were fed Purina powdered food. The rats were killed by chloroform. The thyroid gland, adrenals and the pituitary were weighed. All organs were fixed and processed for histological examination. Slides were stained with hematoxylin eosin. For selected thyroid sections a modified trichrome stain, silver impregnation and Prussian blue reaction were also applied.

A high incidence (6/11) of thyroid change was observed in a group of BUF ♂ rats (36 weeks old), while the thyroid glands of all the other groups appeared to be normal (Table). At autopsy the thyroid glands of the affected animals showed marked enlargement: 45.0 ± 7.3 mg, versus the values obtained in the unaffected rats of the same group, 14.6 ± 0.9 mg ($P \leqq 0.01$).

Histological examination revealed wide bands of lymphoreticular tissue separating the acini apart. There was a characteristic infiltration of the stroma by lymphocytes, plasma cells and macrophages. In the majority of cases distinct lymphoid follicles were observed with germinal centers (Figures 1 and 2). Perivascular cuffing by lymphocytes was also noted. No neutrophils or eosinophils were among the infiltrating cells. In the majority of animals the lymphocytic infiltration was so extensive that large portions of the acinar structure were obscured, replaced or sequestrated by the lymphoreticular tissue. The acinar tissue showed only insignificant primary alterations. The lining cells were low columnar or cuboid with pale, granular cytoplasm. No definite Hürthle cell metaplasia was demonstrable. The lumen of the acini was reduced and was filled with scanty, pale colloid. There were some desquamated lymphocytes or epithelial cells intermingled with the colloid. By silver impregnation method it was possible to demonstrate a great deal of irregularities (wrinkling, discontinuity, etc.) of the basement membranes. Reaction for iron revealed a few hemosiderin containing macrophages.

The thyroid changes described in BUF rats showed close resemblance to Hashimoto's thyroiditis[8–10], lymphocytic thyroiditis of dogs[7] and experimental allergic thyroiditis of laboratory animals[1–6]. The fact that the lesions occurred in a group of 36-week-old males while the thyroids of very young animals or older females of the same strain were normal, might indicate some age and sex relationships. Further studies on larger groups are in

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progress in order (1) to establish an exact incidence rate and (2) to elucidate the pathogenesis and functional significance of this interesting lesion with particular emphasis on the possible role of immunological factors.

Zusammenfassung. Spontane Schilddrüsenentzündung wurde in einer Gruppe von BUF Ratten beobachtet. Das histopathologische Bild der Schilddrüse wies grosse Ähnlichkeit mit Hashimoto's Schilddrüsenentzündung sowie experimentell hervorgerufener allergischer Thyroiditis in Laboratoriumstieren auf.

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Calcium Dependent Corticosteroid Release from the Perfused Cat Adrenal Gland

Calcium plays a crucial role in the secretion of many hormones and neurohumoral substances[1,2]. The intimate mechanism by which calcium regulates the secretory process is, at present, still a matter of conjecture; however, in most systems the secretory products are stored in granular or vesicular structures, and the release of the secretory products from these membrane-bound structures is thought to occur by the process of exocytosis[1]. It has been postulated that the action of calcium might be to facilitate an interaction between the granular and the cell membranes[2-4].

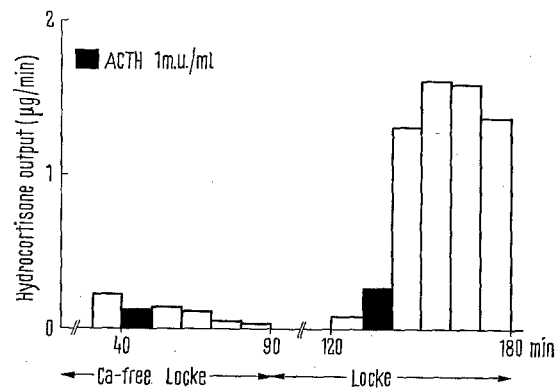
In the adrenal cortex, electron microscopic evidence indicates that secretory products do not appear to be stored in granular structures[5]. It is thought that during stimulation of the cortex by pituitary corticotrophin (ACTH), the steroids are synthetized de novo and then diffuse out to the cell exterior[6,7]. Therefore, an in vivo study to determine the importance of calcium on this secretory system might give further insight into the means whereby this divalent cation influences the secretory process.

Cat adrenal glands were perfused in situ through an aortic cannula at room temperature with normal or modified Locke's solution according to the method previously described[8]. The perfusate, collected from a cannula in the adrenolumbar vein, was assayed for 11-hydroxycorticosteroids according to the fluorometric method of MATTINGLY[9]. Since the major corticosteroid produced by the cat is hydrocortisone[10], outputs were calculated as µg hydrocortisone (alcohol) per minute.

When an adrenal gland was perfused for 40 min with Locke's solution from which calcium was omitted, no significant response to ACTH was observed (Figure). When the same concentration of ACTH was added some 60 min later but now in the presence of calcium, the response to ACTH was readily observable (Figure). The total steroid output during this 10 min exposure to ACTH plus the next 40 min in the calcium-free medium in the absence of ACTH was less than 10% of that observed in the presence of calcium (Table). When the calcium concentration was reduced to 0.2 m*M* (10% of normal), the response to ACTH was approximately one-half that of the control (2.0 m*M* calcium; Table).

Substitution of an equimolar concentration of strontium for calcium in the perfusion medium maintained the secretory response to ACTH (Table). However, magnesium (5 m*M*) was not able to replace calcium (Table). In fact, magnesium (15–20 m*M*) inhibited the ACTH-evoked steroid output in the presence of the normal calcium concentration (Table).

The present study indicates that the adrenal cortex and medulla manifest certain similarities in regard to their ionic requirements for secretion[8,11,12]. Thus, acetylcholine-evoked catecholamine output from the medulla is also drastically reduced in the absence of calcium; and strontium, but not magnesium can replace calcium. Furthermore, magnesium also antagonizes the effects of calcium in the medulla. These parallels are very interesting in light of the fact that the medulla contains a large quantity of preformed hormone which is sequestered within the membrane-bound chromaffin granules and is immediately released upon the initiation of the stimulus[8]. On the other hand, the action of ACTH on the cortex is thought to involve mainly an increase in steroidogenesis,



The release of hydrocortisone from the perfused cat adrenal gland by ACTH and its dependence on calcium. A gland was perfused initially with calcium-free Locke's solution for 90 min and ACTH (1 m.u./ml) was added during the 40th–50th minute of perfusion. Perfusion was then switched to Locke's solution containing calcium for an additional 90 min and ACTH (1 m.u./ml) was again added during the 130th–140th minute of perfusion. Each vertical column indicates the rate of steroid release in a 10 min period both in the presence and absence of ACTH.

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