crystalline N-acetyl neuraminic acid (Sigma) as standard, a linear curve was obtained. The amount of sialic acid in the tissue sample was determined using the standard curve.

Results and discussion. Results documented in this study clearly indicate a change in the sialic acid content of both thymus and bursa of Fabricius of chicken during their normal age-involution. Thymic sialic acid is very high in 1-week-old and 16-week-old chickens (table). In the 4th and 24th weeks, there is a decline in the content of this component. Sialic acid concentration in the 1-week-old chicken bursa is also very high. It declines gradually during age, although the decrease is not so marked as that observed in the case of thymus. The thymus and bursa comprise the central lymphoid system in birds[6]. These organs are the sites of production of successive waves of lymphocytes which are transported to the peripheral lymphoid organs. It has been reported in chick thymus that these lymphocytes originate from a pool of immigrant stem cells, which are completely renewed once in the early postnatal period[8]. It is possible that, at this stage, there is a great demand for the polyanionic sialoglycoprotein, which is required to form a coating on the cell membrane for their attraction and final seeding into the lymphoid organs[9]. This mechanism justifies the presence of a very high content of sialic acid in the thymus and bursa of Fabricius of 1-week-old chicken. The growth of thymus in chicken reaches the peak on the 16th week of posthatching, after which involution is initiated[6]. A second rise of sialic in the thymus before involution, is perhaps directly associated with the outburst of mitotic activity and rapid transport of lymphocytes into circulation and other lymphoid organs. Before streaming out, these cells are coated at their surface with sialic acid, which aids in seeding to the proper peripheral lymphoid organs[9]. By contrast, sialic acid of the 4-week-old bursa did not show any decrease in its level. This is possibly due to the fact that the rate of growth of the bursa is faster than that of the thymus[6]. It is reported that in chicken the spontaneous age-involution of the bursa occurs long before the period of thymic involution[6]. In adult age, however, both these organs undergo involution. Our observations further reveal that sialic acid content is reduced to a minimal and constant level in the involuted thymus and bursa. It is known that the aging thymus of mammals continues its function at a basal level exhibiting renewal and seeding of lymphocytes at a very low rate[10]. These phenomena demand a supply of at least a small amount of sialic acid. Moreover, as thymic humoral factor in mammals has been found to be glycoprotein in nature, and as the reticuloepithelial cells of the thymic medulla (which proliferate greatly in the involuted thymus) are thought to be the origin of thymic humoral agent(s), the involvement of sialic acid in the biogenesis of such principles in young and aging thymus (and perhaps in bursa) cannot be ruled out.

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Radioimmunoassay of an invertebrate peptide hormone – the crustacean neurosecretory hyperglycemic hormone[1,2]

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Summary. Antibodies against hyperglycemic hormone (CHH) of *Carcinus* were raised in rabbits by injection of extract from sinus glands which contain high concentrations of CHH. The antiserum neutralizes the biological activity of CHH and binds ^{125}J-CHH. A RIA for CHH was established and was used to measure the hormone content of sinus glands.

In vertebrate endocrinology, the introduction of the radioimmunoassay has made possible the accurate determination of virtually all known hormones in physiological concentrations and has thus enormously extended our knowledge of the details of regulatory functions of hormones.

In invertebrates, RIAs have so far only been introduced for ecdysteroids[4] and juvenile hormone[5]. The ecdysteroid RIA, now established as a routine procedure, has greatly stimulated studies on the control of molting and metamorphosis in insects and crustaceans, and results have been obtained which were practically unattainable as long as the bioassay and gas chromatography were the only available techniques.

A wealth of evidence is available indicating the occurrence of a large variety of peptide hormones in invertebrates[6] which are vital for the regulation of many physiological processes. However, progress in this field has been slow, since determinations of hormones in tissues or blood still have to rely on sometimes laborious bioassays with limited accuracy and insufficient sensitivity. To our knowledge, no RIA has so far been reported for an invertebrate peptide hormone. This is mainly due to the fact that only very few hormones are available in pure form. Also, preparations are usually in short supply which makes it difficult to raise antibodies.

At present, isolation and complete structural elucidation, confirmed by synthesis, have been accomplished for 3 substances which may be regarded as hormones in the classical sense, namely crustacean red pigment concentrating hormone[7], retinal pigment activating hormone[8] and the insect adipokinetic hormone[9]. Another hormone which has been isolated and characterized in terms of amino acid composition is the neurosecretory hyperglycemic hormone from the eyestalk of decapod crustaceans[10,11] (CHH = crustacean hyperglycemic hormone). The CHH of *Carcinus* has recently been obtained in pure form from sinus glands and has been shown to be a 6700 dalton peptide containing 4 tyrosine residues[11]. It could therefore be expected to be susceptible to iodination with ^{125}J. It should be noted that none of

the known hormones mentioned above contains tyrosine. Furthermore, the size of the molecule suggested that it might be antigenic. Since it had been found that CHH is stored in surprisingly high concentrations in the sinus gland (about 20% of total protein[12]), we assumed that sinus gland extracts, instead of highly purified hormone, could be used as immunogen.

A water extract of 960 *Carcinus* sinus glands (SG) was mixed with Freunds complete adjuvant, and 3 rabbits received the equivalent of one-third of this amount each by multiple intradermal injections and by injections into the foot pads. Over a period of 8 months, each rabbit received 8 s.c. booster injections of 80 SG equivalents with incomplete Freunds adjuvant.

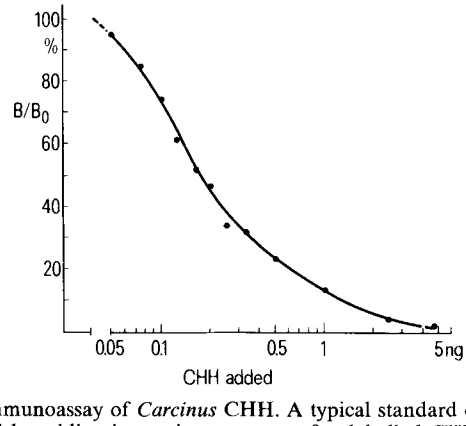
The antisera were first tested for CHH-neutralizing activity by bioassay. As shown in the table, hyperglycemic activity is significantly reduced by preincubation of *Carcinus* CHH with antiserum.

Iodination of CHH: 1.3 µg of pure *Carcinus* CHH[11] were iodinated by the chloramine T-method[13], using 1 mCi ^{125}J in a total volume of 130 µl. Separation of ^{125}J-antigen from free ^{125}J was accomplished by gel filtration on Sephadex G-25 and, subsequently, on Sephadex G-50. The specific activity of the hormone was 100–200 µCi/µg. The tracer retained its immunological activity for about 2 weeks. Whereas control serum bound only small amounts of ^{125}J-CHH (less than 5%), a binding of 73–76% was obtained with an excess of antiserum. For establishing a standard curve, a 1/50,000 antiserum dilution which bound 22–25% of added tracer was used. A typical standard curve is shown in the figure.

Neutralization of hyperglycemic activity of *Carcinus* CHH by antiserum

Treatment	n	mg glucose/ 100 ml hemolymph	p
a controls	9	15.2± 2.2	–
b CHH	9	73.4± 6.4	<0.0002
c CHH + antiserum	9	28.3± 5.6	0.035 (0.0027)
d CHH + control serum	9	76.8± 10.4	<0.0002

Fiddler crabs (Uca pugilator, live weight 1.3–1.7 g) were used as test animals. Per animal, 10 µl were injected and hemolymph glucose was determined 1 h after injection with the glucose oxidase method. The hormone dose was 115 ng/animal. a) controls; 1:10 dilution of antiserum in 60 mM phosphate buffer, pH 7.5; b) hormone in buffer; c) hormone preincubated in a 1:10 dilution of antiserum in buffer for 2 h at 25 °C; d) hormone identically preincubated in a 1:10 dilution of control serum. Statistical evaluation was performed by Student's t-test. p-value in parentheses refers to difference between c and b or d. Values are means ± 1 SEM.



Radioimmunoassay of *Carcinus* CHH. A typical standard curve as obtained by adding increasing amounts of unlabelled CHH and a constant amount of ^{125}J-CHH to the antiserum. Unlabelled CHH was incubated in a 1:50,000 dilution of antiserum in 50 mM phosphate buffer, pH 7.5; 0.9% NaCl; 1% bovine serum albumin and 0.03% EDTA for 5 h at 4 °C. Then labelled CHH was added and samples were incubated for another 24 h at 4 °C. Unbound hormone was adsorbed on dextran coated charcoal; after centrifugation, the precipitate was counted. Assays were performed in triplicates. Values have been corrected for nonspecific binding.

The assay was used to determine the amount of CHH stored in SG. Routinely, the complete standard curve was established along with each batch of samples to be assayed. An extract was prepared from pooled lyophilized SG with aqua bidist. Determinations were carried out with different dilutions, amounting to $5 \cdot 10^{-4}$, $2 \cdot 10^{-4}$ and $1 \cdot 10^{-5}$ SG-equivalents per sample. A hormone content of 1.2±0.3 µg per gland (mean ±SD, n=25) was calculated. Individual values were independent of the dilution. The results are basically consistent with former measurements of the CHH content of SG which were conducted by densitometry after electrophoresis of SG extracts on polyacrylamide gels[12].

It appears that the test is suitable for fast and accurate measurements of CHH, the current detection limit being about 50 pg. For comparison, in the bioassay about 30 ng of *Carcinus* CHH are required as a minimum dose to elicit a conveniently measurable increase in hemolymph glucose in Uca.

Further work, aimed at the improvement of the RIA, especially with regard to the minimal detectable concentration, is in progress.

The physiological function of CHH is still far from being understood. Crustacean blood glucose levels have been shown to increase in response to a variety of environmental stimuli, e.g. different kinds of stress, lack of oxygen, higher temperature etc. Circadian and circatidal rhythms of blood glucose have also been demonstrated. It has repeatedly been shown that, under these circumstances, hyperglycemia does not occur in animals without eyestalk which indicates that CHH is involved. It may be speculated that mobilization of carbohydrate adds to the ability of the animal to adapt to changes in the environment. This, however, has yet to be demonstrated. A method for the sensitive, rapid and accurate determination of circulating CHH under different physiological conditions would appear to be very useful for further studies. Currently, attempts are being made to use the RIA for the measurement of CHH in the hemolymph of crabs.

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