at least a threefold greater sensitivity (*10*). Our findings therefore imply a risk to the human species.

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11. We thank Drs. G. Bentley and M. Cullen for valuable advice.

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Neurosecretory Granules: Evidence for an Aging Process Within the Neurohypophysis

Abstract. *When cysteine labeled with sulfur-35 is injected into the third ventricle of the rat brain, it is first incorporated into only one of the two populations of neurosecretory granules that can be isolated on an isosmotic gradient. The second population of granules is labeled much later. Stimulation of hormone release from isolated labeled neural lobes and subsequent isolation of neurosecretory granules at different times after the injection of labeled cysteine shows that the radioactivity decreases in only one population of granules. One of the fractions of the gradient represents the granules found near the release site; the second population is probably located deeper in the nerve endings or in the nerve swellings. Whereas neurophysins are found in both populations, smaller proteins can only be detected in one. Thus it appears that neurosecretory granules undergo an aging process and that isosmotic gradients can separate the aged granules from those newly formed.*

Neurosecretory granules (NSG) in the hypothalamo-neurohypophysial tract are formed in the magnocellular neuron of the supraoptic and paraventricular nuclei (*1*). Labeling with [^{35}S]cysteine indicates that the magnocellular neurons mainly contain a large precursor protein (~ 20,000 daltons), which presumably is packaged into granules. The contents of the granules mature during transport toward the neurosecretory nerve terminals. When the granules reach the neural lobe, almost all the precursor has been converted into proteins of 12,000 ± 2,000 daltons, neurophysins, and peptides that may include the hormones oxytocin and facopressin (*2*).

The neurosecretory axons in the neurohypophysis can be subdivided into three compartments, all of which contain NSG (*3*): undilated axons containing few NSG, nerve endings characterized by the presence of microvesicles, and nerve swellings containing secretory granules but few if any microvesicles. We previously showed that the mean diameter of NSG in the endings differs from that of NSG in the swellings (*4*). Furthermore, we showed that fractionation of NSG from the neural lobe of rats on an isosmotic gradient yields two populations of NSG, each of which contains neurophysins, oxytocin, and vasopressin. One population has an isopycnic density of 1.13 g/cm^3 and is insensitive to osmotic changes in the surrounding medium, whereas the other sediments at a density of 1.11 g/cm^3 and is much affected by the osmotic pressure of the surrounding medium. Furthermore, the mean diameter of osmotically insensitive NSG after fixation is very similar to that of the NSG in the nerve endings, whereas the mean diameter of the osmotically sensitive NSG resembles that of the granules in the swellings.

In this report we present evidence that the osmotically insensitive granules are newly formed NSG (NF-NSG) that have recently arrived at the neural lobe and that the osmotically sensitive NSG are probably derived from the former. Furthermore, we show that the NF-NSG are located in the nerve endings, whereas most of the aged granules (A-NSG) cannot immediately release their contents and are thus likely to be found in the swellings. Protein species of a molecular weight lower than that of neurophysins can be demonstrated in the A-NSG. This suggests possible postmaturational cleavage of intragranular proteins.

Albino (Wistar) male rats weighing 250 to 300 g were anesthetized with ketamine chlorohydrate (10 mg per 100 g of body weight) and given intracisternal injections of [^{35}S]cysteine that was obtained from the supplier (New England Nuclear, 500 Ci/mmole) or by reduction of [^{35}S]cystine (470 Ci/mmole) with 10 m*M* dithiothreitol in 0.1*M* phosphate buffer (*p*H 7.0). Each rat was injected with 10 μl of the final solution (50 μCi of radioactive cysteine). The rats were decapitated at different times after the injection and their neural lobes were dissected out in less than 1 minute. Immediately after their isolation, ten of the radioactive lobes were homogenized in 1.0 ml of 0.3*M* sucrose buffered at *p*H 6.8 with 10 m*M* Hepes, and mixed with 20 nonradioactive neural lobes homogenized under the same conditions. The NSG were isolated as described by Nordmann *et al.* (*4*). The gradient fractions were collected with a Buchler device, and a portion of each fraction was kept for a determination of density. Scintillation fluid was added to the fractions and their total radioactivity was measured with a

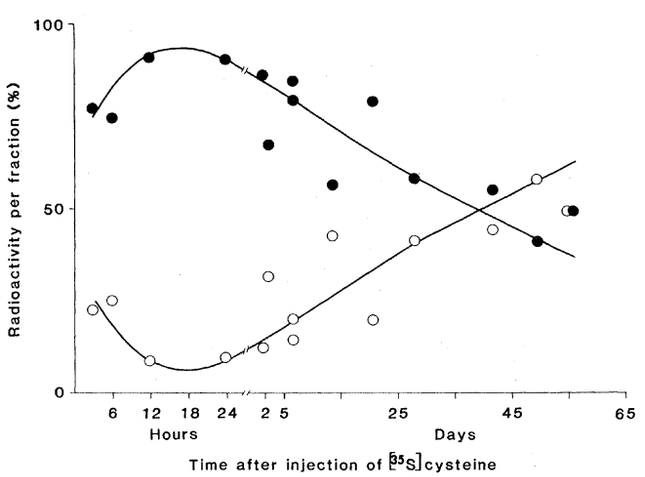

Fig. 1. Time course of the appearance of [^{35}S]cysteine in NSG fractions isolated on a sucrose-metrizamide isosmotic gradient at different times after the injection of the isotope. The radioactivity in NF-NSG (●) and A-NSG (○) was measured and the results are given as the percentage of the total radioactivity found in both fractions.

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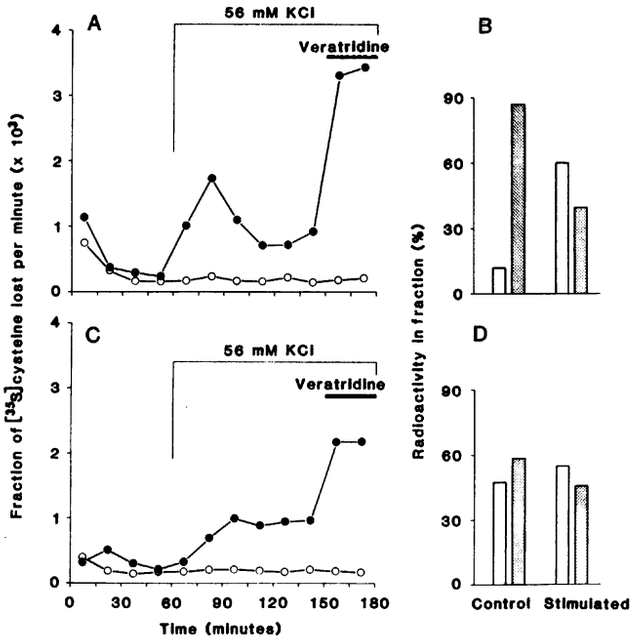

Fig. 2. Release of radioactivity from isolated neural lobes (A and C) and amount of radioactivity found in the two NSG fractions after prolonged stimulation of hormone release (B and D). (A and C) Lobes were incubated in normal saline (*12*) and stimulated by 56 m*M* KCl as indicated; veratridine (0.17 m*M*) was added during the last 30 minutes of incubation. Closed circles represent the loss of radioactivity from stimulated neurohypophyses, and open circles represent the rate constant for the efflux of ^{35}S (*13*) from unstimulated lobes. (B and D) Percentage of radioactivity found at the end of the incubation period in the NF-NSG fraction (shaded columns) and in the A-NSG fraction (open columns). The lobes were isolated 3 days (A and B) and 28 days (C and D) after the injection of [^{35}S]cysteine.

liquid scintillation spectrometer (Intertechnique).

Labeled granules were found in the neurohypophysis 3 hours after the injection of [^{35}S]cysteine; the arrival of radioactive molecules leveled off after 12 hours (Fig. 1). This time course is similar to that reported previously (*5*). More than 90 percent of the radioactivity incorporated by the two fractions was found in the NF-NSG fraction after 1 day, but after 2 months it was equally distributed between the fractions (*6*). Thus it seems likely that A-NSG are derived from NF-NSG. Alternatively, A-NSG may be transported to the posterior lobe more slowly than NF-NSG. However, since the amount of labeled material found in the neural lobe varies widely among rats after the intracisternal injection of an isotope, it is impossible to compare the exact amount of radioactivity in one fraction with that in others at various times after injection.

Neurohypophysial hormone is released only by the nerve endings (*7*). Furthermore, after the granule content is labeled, the specific radioactivity of the material released upon brief stimulation is higher than that of the tissue (*8*). Since the diameter of the isolated NF-NSG is very similar to that of the granules found in the endings, and since the NF-NSG are initially highly labeled, we speculate that these newly arrived granules are the first to release their content upon stimulation.

Three days after being labeled in vivo, neural lobes in vitro quickly released part of their radioactive content when depolarized with potassium and veratridine (Fig. 2A) (*9*). The material probably was released from the NF-NSG because the ratio of radioactivity between the NF-NSG and A-NSG fractions, which was ~ 10 in controls, was < 1 in stimulated neural lobes (Fig. 2B). Depolarized neural lobes isolated from rats that had been injected with [^{35}S]cysteine in 28 days previously released their radioactive content at a very different rate (Fig. 2C). During the first 30 minutes after the beginning of stimulation, less radioactivity was released, and this release was less rapid than in the animals injected only 3 days before stimulation. Moreover, the further release of radioactivity that occurred when the lobe was depolarized with veratridine represented only about 60 percent of that which was released from lobes isolated 3 days after labeling in vivo. Figure 2D highlights the main difference between the two experiments. After hormone release was elicited, the ratio of NF-NSG to A-NSG, which was markedly altered in lobes stimulated 3 days after labeling in vivo, was only slightly altered in lobes stimulated 28 days after labeling. We suggest that the labeled granules move from the release site (the nerve endings) deeper into the endings and eventually into the swellings, where they are less available for release upon stimulation.

It is, therefore, possible to characterize anatomically (*4*) and physiologically (Fig. 2) the two populations of granules that can be isolated from rat neural lobes. After their axonal transport, the granules appear in the endings and, if not released, move into the swellings. Autoradiographic determinations have shown that, shortly after the intracisternal injection of [^{35}S]cysteine, radioactivity is present in the neurosecretory endings and only later in progressively larger swellings (*10*). As shown previously (*4*), the movement of granules from the endings toward the swellings is associated with a change in their sensitivity to surrounding osmotic pressure. This change must be due to modifications of the granule membrane or the granule content or both. In view of the fact that neurophysins, oxytocin, and vasopressin originate from larger precursors, the granule must contain proteolytic enzymes that produce the necessary cleavages. Subsequent cleavages in the granule could be responsible for the change in the responsiveness of the granule to osmotic pressure. Biochemical analysis of the membrane composition

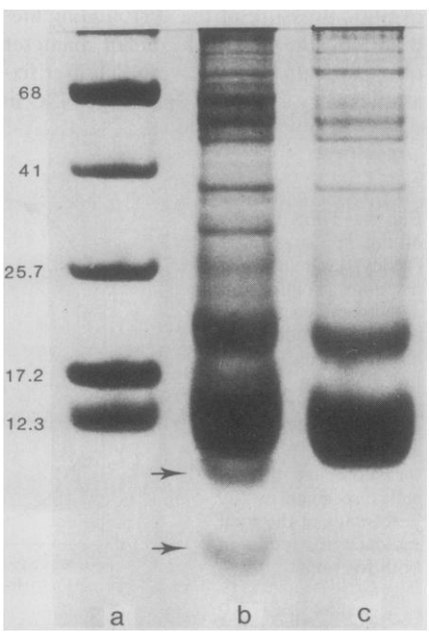

Fig. 3. Protein content of NSG electrophoresced on 12.5 percent polyacrylamide gel with 0.1 percent sodium dodecyl sulfate and 8*M* urea at *p*H 6.8 (*14*) (stain, Coomassie blue). The values on the left are the molecular weights ($\times 10^{-3}$) of the markers given in (a); (b) and (c) are the protein patterns of fractions A-NSG and NF-NSG, respectively. Note that smaller peptides than neurophysins (10,000 daltons) are only found in the A-NSG fraction (arrows). The gels were overloaded to reveal species of low molecular weight. Markers: 68, bovine serum albumin; 41, alcohol dehydrogenase (horse liver); 25.7, chymotrypsinogen; 17.2, myoglobin; and 12.3, cytochrome c.

and of the protein and peptide content of the granules should provide information about this aging process. Figure 3 shows the two different protein patterns that appear when the contents of both granule fractions are analyzed by slab gel electrophoresis. In the NF-NSG fraction, the smallest protein has a molecular weight of ~ 10,000; in the A-NSG fraction, proteins of 2,000 to 8,000 daltons appear. The peptide content of the A-NSG fraction may also be altered.

The aging of protein-containing secretory granules after maturation (*11*) might have two roles: providing a signal for the eventual lysosomal degradation of granules that are not released, and producing small peptides, whose physiological significance is yet unknown.

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6. The half-life of ^{35}S does not have to be taken into account because the results (Fig. 1) are given as radioactivity of one fraction times 100 divided by the sum of the radioactivity of both fractions. Furthermore, the amounts of hormones and neurophysins in both fractions are very similar (*4*).
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11. In this context, maturation is taken to mean the conversion of a putative precursor into neurophysin and neurohormone. Biochemical studies [H. Gainer *et al.*, in (*2*)] and morphological studies [M. A. Cannata and J. F. Morris, *J. Endocrinol.* **57**, 531 (1973); J. F. Morris and M. A. Cannata, *ibid.*, p. 157] suggest that NSG mature during their axonal transport from the hypothalamus to the neurohypophysis.
12. The normal saline contains 150 m*M* NaCl, 2.2 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 5.6 m*M* $KHCO_3$, and 10 m*M* glucose. The solution was maintained at 37°C and gassed with 5 percent CO_2 in O_2. During depolarization (56 m*M* K^+), the external Na concentration was reduced to maintain tonicity, and control glands were incubated in a medium in which 50 m*M* NaCl was replaced by 50 m*M* choline chloride.
13. The efflux of [^{35}S]cysteine per minute, calculated from the loss of radioactivity, was expressed as a fractional rate constant given by $\Delta x/\Delta t X_t$, where Δx represents counts of ^{35}S released in the time interval Δt and X_t the tissue content of ^{35}S at the midpoint of interval Δt.
14. The gels were made according to the method of R. T. Swank and K. D. Munkres [*Anal. Biochem.* **39**, 462 (1971)], except that the cross-linking agent ethylene diacrylate was used instead of *N,N'*-methylenebisacrylamide. The proteins were obtained after isolation of bovine NSG on an isosmotic gradient (*4*). The granule fractions were washed three times in 0.3*M* buffered sucrose, resuspended in 0.1*N* HCl containing 1 m*M* phenylmethane sulfonylfluoride, 10 n*M* soybean trypsin inhibitor (Sigma), Trasylol (Sigma; 160 kallikrein inhibitor units per milliliter), and frozen. After thawing, the resulting membrane fragments were centrifuged at 35,000*g*; the proteins of the supernatant were separated from the peptides by precipitation with trichloroacetic acid (final concentration, 10 percent). The protein precipitate was dissolved in the presence of 1 percent sodium dodecyl sulfate, 8*M* urea, 1 percent mercaptoethanol, and 0.01*M* H_3PO_4 adjusted to *p*H 6.8 with tris base.
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Lectins of Distinct Specificity in *Rhodnius prolixus* Interact Selectively with *Trypanosoma cruzi*

Abstract. *Lectins of different activities were found in the crop, midgut, and hemolymph of the insect* Rhodnius prolixus. *These were most specific for* N-*acetyl*-D-*mannosamine,* α-N-*acetyl*-D-*galactosamine, and* α- *and* β-D-*galactose, respectively. Lectin receptors were detectable in epimastigote but not in trypomastigote forms of* Trypanosoma cruzi, *a protozoan parasite of the insect and of humans.*

The parasitic protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease in man, is transmitted mainly by hematophagous reduviid insects. The insects become infected by ingesting trypomastigotes from the peripheral blood of infected mammals. In the lumen of the crop and midgut the parasites multiply as epimastigotes, and this multiplication leads to the development of metacyclic trypomastigotes that accumulate in the rectum of the insect (*1*, *2*). In general, *T. cruzi* does not develop in the insect's hemolymph (*3*). The mechanisms underlying differentiation of the parasite and the susceptibility of the insects to infection are largely unknown. Studies of specific recognition mechanisms suggest that host-parasite interactions could be influenced by lectins, a class of sugar-binding proteins of nonimmune origin (*4*, *5*). Lectins interact with cells through their carbohydrate-binding sites, thereby triggering a number of important biologic phenomena (*4*, *5*), such as lymphoblast transformation and cell division, activation of suppressor T cells, and insulin-like stimulation of fat cells; lectins also alter the movement of receptors on cell membranes, producing patching and capping. Membrane-bound lectins appear to play a role in both intercellular and intracellular recognition (*6*). Moreover, lectin receptors are characteristic markers of the developmental stages of *T. cruzi* and of morphologically similar stages from different milieus (*7*). Lectins are usually extracted from plants; they have also been detected in invertebrates, fish, birds, and mammals (*4*, *5*); yet not much is known about their occurrence in insects (*5*, *8*). We now report that lectin activities of distinct carbohydrate specificities are present in the crop (stomach), midgut (intestine), and hemolymph of *Rhodnius prolixus*, an intermediary host of *T. cruzi*, and that each lectin is highly specific in interacting with developmental stages of *T. cruzi*.

Insects were maintained in the laboratory at 25° ± 2°C in a relative humidity of 50 to 60 percent and were fed on human citrated blood every 25 to 30 days. Saliva and salivary glands, crop and crop contents, midgut, rectum, and hemolymph were obtained by procedures described previously (*9*, *10*). Materials were collected from fifth instar larvae or adult insects, from starved animals, or from animals that had had a blood meal 5 days earlier (crop washings were collected immediately after feeding the insects on 0.5*M* NaCl plus 0.001*M* adenosine triphosphate). Salivary glands (50 pairs per milliliter), crop (10 stomachs per milliliter), midgut (10 intestines per milliliter), and rectum (20 insects per milliliter) were homogenized in phosphate-buffered saline (0.1*M* PO_4 and 0.15*M* NaCl, at *p*H 7.2 to 7.4) and extracted in the cold for several hours. The volume of crop washings ranged from 0.1 to 0.2 ml per

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