operation. In nuc. accumbens, however, (figure 1) there was no significant difference in DA fluorescence between the denervated and innervated side. However, a significant reduction of DA fluorescence compared with the control group was observed 3, 7 and 15 days following the 6-OH-DA injection. As seen in figure 2, there was a significant correlation between the degree of degeneration in nuc. caudatus and the degree of degeneration of dotted and diffuse DA fluorescence in the nuc. accumbens. The Pearson's product moment correlation coefficient between caudatus and dotted accumbens was 0.579 ($p<0.05$) and between caudatus and diffuse accumbens 0.582 ($p<0.05$). In contrast to this there was no correlation between the degree of degeneration of DA terminals in the nuc. caudatus and in tuberculum olfactorium ($r_{y/x} = 0.389$). The results of the GABA measurements are summarized in the table. As can be seen from the table, there was no significant difference between the innervated and denervated side regarding the degree of GAH induced GABA accumulations. Furthermore, there is no significant change in the degree of GAH-induced GABA accumulation when the GAH + H 44/68-treated groups are compared with the GAH alone group. Furthermore, when intraindividual correlations were made between the degree of DA fluorescence disappearance and the GAH-induced GABA accumulation, no significant correlations were found when using the Pearson's product moment correlation coefficient.

The present findings show that, inspite of a depletion of DA stores with the help of combined treatment with 6-OH-DA and the tyrosine hydroxylase inhibitor H 44/68, it was not possible to change GABA accumulation in the nuc. caudatus, in subcortical limbic areas and in the DA cell body rich region of the midbrain. These findings agree with the results obtained using the DA receptor blocking agent pimozide. Thus, treatment with pimozide was found not to change GABA accumulation in the 3 regions mentioned above (see[2] and unpublished data). In contrast, when drugs are given such as apomorphine which increases DA receptor activity, a clearcut increase in GABA accumulation is observed in these 3 regions[2,3]. Thus, it seems as if increases of DA receptor activity will result in increases of GABA accumulation, but only in DA nerve terminal and cell body regions[6]. A reduction of DA receptor activity, on the other hand, will have less influence on GABA accumulation in DA nerve terminal and cell body rich regions. These studies will now be continued by making lesions which produce a far more complete degeneration of the DA systems and by the use of other types of DA receptor blocking agents.

It should be mentioned that the degree of 6-OH-DA-induced disappearance of DA stores in the nuc. caudatus and in the 2 parts of the nuc. accumbens studied was significantly correlated, whereas this was not true for the disappearance of DA stores in nuc. caudatus and tuberculum olfactorium. These findings may suggest that the DA pathways to the nuc. caudatus and nuc. accumbens run close together in the ventral tegmental area.

11 E. M. Scott and W. B. Jacoby, J. biol. Chem. *234*, 932 (1959).
12 P. Einarsson, H. Hallman and G. Jonsson, Med. Biol. *53*, 15 (1975).
13 K. Fuxe, L. Agnati, P. Bolme, B. J. Everitt, T. Hökfelt, G. Jonsson, Å. Ljungdahl and A. Löfström, Neuropharmacology *14*, 903 (1975).
14 L. Olson, Å. Seiger and K. Fuxe, Brain Res. *44*, 283 (1972).

Immunohistochemical demonstration of an SRIF-like system in the brain of the reptile: Lacerta muralis Laur.[1]

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Summary. The distribution of an SRIF-like substance in the brain of normal lizards (Lacerta muralis Laur.) has been determined. SRIF is shown to be present in neural cell bodies in the hypothalamic paraventricular nucleus, the hypothalamo-hypophysial tractus and the median eminence.

In the Poïkilotherms, cytoimmunological research on polypeptidic neurosecretion essentially concerns gonadotropic LHRH hormone[2–5]. We only know of one study on the SRIF factor (somatostatin-release-inhibiting hormone) in the Amphibian[6]. One of us (Dubois) prepared antibodies against this substance and checked immunoreactive specificity towards the antigene. In this study SRIF antisera were used to trace the SRIF-like system in the brain of a reptile: Lacerta muralis, by immunofluoroscence.

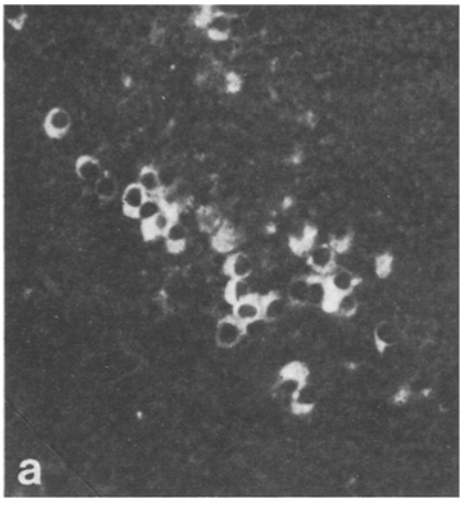
Techniques. We used SRIF and neurophysin antisera as described in earlier publications[6–8]. Indirect immunocytological reactions were examined in 12 lizard brains fixed with Bouin Holland sublimate without acetic acid, dehydrated and embedded in paraffin and then cut into 7 µm sections along the frontal, sagittal and transversal planes. Controls were carried out on contiguous sections treated respectively with non-inhibited antibodies, and with the same serum saturated with 200 µg of antigen per ml of undiluted serum. SRIF antiserum is then completely inhibited as regards the antigen present in the brain, and no immunofluorescent reaction is detected on the sections. In addition, inhibition reactions with other peptides present in the brain and median eminence

1 Contrat INSERM, No. 74.1.444.35.
2 J. Doerr-Schott and M. P. Dubois, C. r. Acad. Sci. *278*, 1397 (1974).
3 J. Doerr-Schott and M. P. Dubois, C. r. Acad. Sci. *280*, 1285 (1975).
4 H. J. Th. Goos, P. Ligtenberg and P. G. W. J. van Oordt, Cell Tissue Res. *168*, 325 (1976).
5 J. Doerr-Schott and M. P. Dubois, Cell Tissue Res. *172*, 447 (1976).
6 M. P. Dubois, J. Barry auf J. Leonardelli, C. r. Acad. Sci. *279*, 1899 (1974).
7 M. P. Dubois, Proc. Nat. Acad. Sci. USA *72*, 1340 (1975).
8 M. P. Dubois and J. Barry, Ann. Endocr. *35*, 663 (1974).

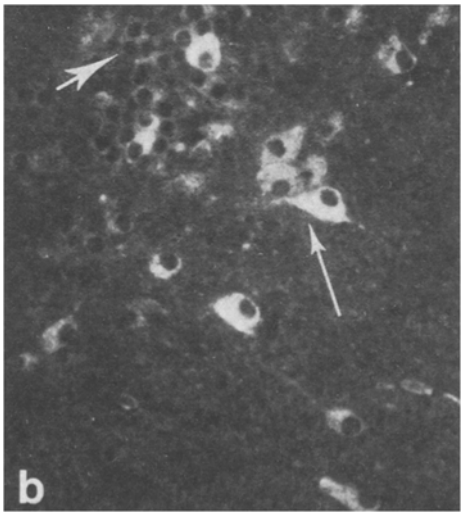
(LHRH, TSHRH[9], oxytocin, vasopressin, neurophysin) were performed. None of these factors suppress the immunocytological SRIF-anti-SRIF reaction. Neurophysin antibodies were similarly checked for their reactions on serial sections using either an antibody not saturated with the same antigene or the antibody inhibited with the same antigene or with the other substances tested. Reaction specificity was also checked by applying: 1. normal instead of specific rabbit serum; 2. fluorescein labelled globulins in the absence of the specific serum. No immunological reaction occurred under these conditions.

Results. On sections studied, SRIF antiserum is localized in the paraventricular nucleus (PVN), the hypothalamo-hypophysial tractus and the median eminence (ME).

1. Paraventricular nucleus. Our immunocytological observations show that lizard PVN seems to comprise 3 types of neurons: one which reacts simultaneously with SRIF and neurophysin antisera, a second which reacts only with neurophysin antiserum; while the third, as yet to be defined, does not react with either.



a Sagittal section of the PVN region. Small SRIF immunoreactive pericarya in line near the third ventricle. × 310.



b Sagittal section treated with neurophysin-antibodies. Large neurophysin-like pericarya dispersed in the PVN region and small SRIF-neurophysin positive cells. × 310.

a) SRIF-like cells (figure a). These pericarya bind SRIF and neurophysin antisera. They located exclusively in the PVN and are particularly numerous close to the third ventricular wall, near the ependymal cells. The SRIF-like cells are small and generally rounded with more or less fluorescent cytoplasm. In certain cells the whole cytoplasm is intensely fluorescent, while others show only more peripheral halo. They form a subependymal row, generally with a single layer, but which can double or triple, and is about 100 μm long. On many SRIF-like cells axonal and dendritic extensions are seen as fluorescent points. Such cells are often near the capillaries which irrigate this part of the brain.

b) Neurophysin-like cells (figure b). In the PVN this cell group comprises: 1. SRIF-neurophysin-positive cells; 2. cells only reacting with neurophysin antiserum; in the supraoptic nucleus (SON) cells reacting only with anti-neurophysin serum. The latter two groups comprise large irregular cells which react intensely with neurophysin antiserum. Their highly fluorescent axons are easy to follow over a considerable distance. From these immunocytological observations it would appear that the PVN contains at least 2 types of cells. On one hand SRIF-neurophysine-positive cells; on the other neurophysine positive-SRIF-negative ones. As regards the latter, it has yet to be shown for reptiles if neurophysin is associated solely with vasopressin or with oxytocin[10], or, as Watkins[11] envisages in the rat, with another neurohormonal factor (CRF).

c) Non-immunoreactive cells. Among the fluorescent pericarya there are always small non-immunofluorescent cells which closely resemble SRIF-like cells. They may be pericarya not containing enough antigene (SRIF or neurophysin) to be revealed by immunofluorescence, or neuroglandular cells producing another factor, or non-glandular cells.

2. Hypothalmo-hypophysial tractus. On parasagittal sections, SRIF fibre pathways are easily visible as fine fluorescent dots or as short immunofluorescent chains along the hypothalamo-infundibular tractus. The neurosecretory fibres coming from the SRIF-neurophysin-positive PVN cells run ventro-laterally and join the supraoptic hypophysial tractus behind the optic chiasma. These fibres run along the ventral region of the diencephalon floor. The SRIF fibres terminate on the capillaries of the portal system which irrigates the ME. Some neurophysin-positive-SRIF-negative fibres follow the same pathway, but most of them end in the neural lobe of the hypophysis.

3. The median eminence. Fluorescent SRIF substances are observed throughout the median eminence. In the internal zone fine reactive fibres are rather rare whereas in the external zone fibres and SRIF-like endings are abundant, particularly around the blood capillaries. Reactive fibres and nerve endings are absent in the neural lobe.

Discussion. Use of a highly specific SRIF antiserum suggests that the immunoreactive paricarya and axons revealed in the lizard brain contains a substance immunologically related to SRIF. Since the antiserum used is

9 LHRH: luteinizing hormone releasing hormone; TSHRH: thyreotropin releasing hormone, CRF: corticotropin releasing factor.

10 F. Vandesande and K. Dierrickx, Cell Tissue Res. *164*, 153 (1975).

11 W. B. Watkins, P. Schwabedal and R. Bock, Cell Tissue Res. *152*, 411 (1974).

12 J. Doerr-Schott, personnal observations.

13 M. P. Dubois and F. Kolodziejczyk, C. r. Acad. Sci. *281*, 1737 (1975).

inhibited by SRIF, but not by other similar synthetic peptides, we can consider that the substance revealed by the immunological reaction corresponds to SRIF, or at least to a very closely related peptide. In the lizard, the system which reacts specifically with SRIF antiserum originates in a group of PVN cells whose axons partially join the hypothalamo-hypophysial tractus, but terminates almost exclusively in the external zone of the median eminence. It would therefore seems that somatostatin, which intervenes in regulating the hypophysial somatotropic function, posses through the capillaires of the hypophysial portal system to the reactive sites (STH cells) of the anterio lobe.

In the lizard, the immunocytological reactions with SRIF and neurophysin antisera reveal that the PVN comprises at least 2 immunologically distinct cells. These observations tally with those carried out, under the same conditions, on an amphibian, Xenopus laevis[12], revealing a cross-reaction with SRIF antiserum in a small group of neurophysin positive cells of the preoptic nucleus. They also agree with those of Dubois and Kolodziejczyk[13], who observed cells reacting with both SRIF and neurophysin antibodies in the PVN of the rat. The results for the lizard, as previously for the amphibian, demonstrate the existence, in the Poïkilotherms, of an SRIF-like regulatory system, and give a very clear idea of its localization.

Binding of ferritin-lectin conjugates to C-type virus in intact cells[1]

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Summary. Ferritin-Ricin II and Ferritin-Concanavalin A bound to budding as well as mature C-type viral particles. No differencies in binding between the viral coat and adjacent plasma membrane were detected with either lectin conjugate. Aggregation of viral particles by lectin conjugates was observed, and linking of virus to the plasma membrane resulted in phagocytosis of viral particles.

C-type particles budding from the surface of a cell characteristically display an outer electron dense coat arranged in spikes whereas the adjacent plasma membrane of the cell is uncoated or smooth. The viral coat presumably contains unique glycoprotein(s) that distinguishes it from the rest of the cell membrane. After infection by C-type viruses new glycoproteins have been found on the cell membranes[2]. The question of whether there are restricted sites on the cell membrane that will permit viral budding, and if such sites are associated with specific glycoproteins is still largely unsolved. Plant lectins interact with specific saccharide residues and thus might be useful for earmarking glycoproteins containing such residues. In the present study we have used the plant lectins Ricinus communis agglutin, specific for β-galactopyranosyl-like residues[3] and Concanavalin A, which is specific for α-D mannopyranosyl-like residues[4,5], in order to find a) if there are restricted or clustered binding sites on the cell membrane and b) if the membrane of the viral bud or of the mature virus can be distinguished from the rest of the cell membrane. Ferritin-lectin conjugates purified by affinity chromatography[6] were used for high resolution studies of binding sites in intact cells producing C-type particles[7]. Very recently, affinity chromatography of membrane fragments of C-type virus infected cells on Ricinus communis columns has shown that 2 viral glycoproteins were bound and largely purified from many other membrane proteins[8]. Concanavalin A was studied because it has been used as the basis for a high yield isolation of infectious C-type particles from culture media[9].

Materials and methods. Ricin II was prepared from beans as described[10] and was stored at a concentration of 25 mg/ml in 50 mM $NaPO_4$ pH 7.0. Concanavalin A (Con A) was purified by affinity chromatography on Sephadex G-75 or G-100[4] and stored under similar conditions. Conjugates of ferritin-Ricin II and ferritin-Con A were cross-linked with glutaraldehyde essentially as described[6]. 1 ml of stock Ricin II or Con A was mixed for 2 min with 1 ml (25 mg) of 6 × recrystallized ferritin using a magnetic stirrer. 0.1 ml of 0.5% (v/v) glutaraldehyde in 50 mM $NaPO_4$ pH 7.4 was added dropwise over a period of 5 min and stirred 45 min at 22 °C. The conjugate was dialyzed against 1 l of 50 mM NH_4Cl at 22 °C for 3–4 h followed by 1 l of 50 mM $NaPO_4$ pH 7.2 at 4 °C overnight. Prior to affinity chromatography the solution was clarified at 3,000 rpm for 30 min and the precipitate discarded. For ferritin-Ricin II affinity chromatography, supernatant was added to a 2.2 × 20 cm column of Sepharose 6B in 25 mM $NaPO_4$ pH 7.2. The column was washed with 50 ml of 25 mM $NaPO_4$ pH 7.2 and the conjugate of ferritin-Ricin II then eluted from the column with 0.1 M galactose in 25 mM $NaPO_4$ pH 7.2. 5.5 ml fractions were collected (figure 1, top) and the second peak eluted by galactose (Fx 25–28) was concentrated using a XM 50 Amicon filter to about 2 ml final. The concentrate was diluted, refiltered 1–2 × and centrifuged for 15 min at 10,000 rpm. The supernatant contained almost pure ferritin-Ricin II conjugate and the small amount of aggregated ferritin-Ricin II it contained was removed by gel

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2 L. J. Old and E. A. Boyse, Fed. Proc. *24*, 1009 (1965).
3 G. L. Nicolson, J. Blaustein and M. E. Etzler, Biochemistry *13*, 196 (1974).
4 B. B. Agrawal and I. J. Goldstein, Biochim. biophys. Acta *133*, 376 (1967).
5 G. L. Nicolson, Int. Rev. Cyt. *39*, 89 (1974).
6 G. J. Nicolson and S. J. Singer, J. Cell Biol. *60*, 236 (1974).
7 L. Manuelidis and E. E. Manuelidis, Int. J. Cancer *17*, 659 (1976).
8 J. E. Smart and N. Hogg, Nature *261*, 314 (1976).
9 M. L. Stewart, D. F. Summers, R. Soeiro, B. N. Fields and J. Maizel, Proc. nat. Acad. Sci. *70*, 1308 (1973).
10 M. Tomita, T. Kurokawa, K. Onozaki, N. Ichiki, T. Osawa and T. Ukita, Experientia *28*, 84 (1972).