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The development and application of a method for the specific localization of vasopressin and oxytocin in the rat brain by immunofluorescence is described. The topographic distribution of these neuropeptides is given not only in the classical neuroendocrine cells of the supraoptic and paraventricular nuclei, but vasopressin was also found in the suprachiasmatic nucleus. [The SC* indicates that this paper has been cited in over 175 publications.]

Immunofluorescence of Vasopressin and Oxytocin
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This paper and reference 1 deal with the development and the application of a procedure for the specific immunocytochemical localization of vasopressin and oxytocin in the rat brain, at the Netherlands Institute for Brain Research. I wrote the first draft of this paper during a short Christmas holiday in Paris in 1974, exactly two years after J.L. Touber (University Clinic for Internal Medicine, Amsterdam) suspected his animal keeper had killed a few of his rabbits that were immunized against vasopressin for an economical Christmas dinner: Investigation of their hypothalami, blood, and urine revealed, however, that these animals suffered from a severe diabetes insipidus due to excellent antibodies against vasopressin.

In August 1973 I went to T.E.W. Feltkamp of the Central Laboratory of the Red Cross with far too many of these antibodies in a Dewar vessel and followed the procedure they routinely used to determine autoantibodies in serum of patients. The next day I found for the first time (by immunofluorescence) vasopressin in cells of the supraoptic nucleus (SON).

Following improvements of the fixation procedure, I wrote this proposal on this line of work, but our director at that time, J. Ariens Kappers, decided to give priority to his own interest in the pineal gland. In 1975, as a consolation, I was allowed to employ C.W. Pool as a part-time student for the development of immunocytochemistry. He has contributed enormously to the immunocytochemical research in our institute. In spite of—or because of—Ariens Kappers's decision, we dedicated our paper to him on his 65th birthday. Later he told me he had enjoyed this present very much.

Touber's group showed by radioimmunoassay that our antibodies were specific for vasopressin. Yet, we got strong staining in the hypothalamus of a homozygous diabetes insipidus rat. This "Brattleboro" mutant is not capable of producing vasopressin. Using peptides on agarose beads (a model system that P.L.A. Capel presented during the Fifth International Conference on Immunofluorescence and Related Staining Techniques), it was shown that this staining was due to cross-reactivity with the related peptide oxytocin, and a solid phase procedure for antibody purification was developed.

Our findings on the topographic distribution of vasopressin and oxytocin in the SON and paraventricular nucleus (PVN) showed that each cell contained one hormone (i.e., vasopressin or oxytocin) and that vasopressin cells were localized more caudally and oxytocin cells more rostrally; and our findings contradicted the "classical" view that the SON would predominantly or entirely synthesize vasopressin and the PVN, oxytocin. These results are mentioned in 66 percent of the citations to this paper. In addition, vasopressin was found in neurons that were not neurosecretory in nature, i.e., in the suprachiasmatic nucleus. This new and important aspect is only mentioned in 10 percent of the citations. Yet, our paper thus became the start of a number of well-cited papers from our group on extra- and hypothalamic sites of production of these neuropeptides, their transport by nerve fibers to other brain areas, and the necessity to use solid-phase adsorption for purification of antibodies against peptides, did not get across sufficiently. Papers overlooking these problems are still published regularly. It is remarkable that these aspects have low citation scores (2 percent and 4 percent of all the citing papers, respectively). It is hard to believe that this is because the message was not brought out sufficiently clearly in our paper, especially since our group has since then repeated this message over and over again in courses, reviews, and other papers with only limited practical success. It is probably just more convenient and attractive to apply an antibody and describe the results instead of carrying out painstaking work to find out what is the substance one has actually stained using a combination of separation techniques and immunocytochemistry.