Correction. In the article, "Active Transport of Calcium in Inverted Vesicles of Escherichia coli," by Barry P. Rosen and John S. McClees, which appeared in the December 1974 issue of Proc. Nat. Acad. Sci. USA 71, 5042-5046, the authors have requested the following change. On page 5043, in the left-hand column, the concentration of calcium in transport assays was given as 1 mM. Subsequent analysis of the calcium solution showed that the actual concentration was 0.5 mM. Thus, the specific activities of calcium transport presented in the Results section must be divided by a factor of two. The kinetic parameters were determined with separate solutions and are correct as stated in the text.

Correction. In the article "Immunoreactive Somatostatin Is Present in Discrete Cells of the Endocrine Pancreas" by M. P. Dubois that appeared in the April 1975 issue of Proc. Nat. Acad. Sci. USA 72, 1340-1343, the structure of somatostatin was omitted by the printer. Line two of the left-hand column on page 1340 should be:

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH.
Immunoreactive Somatostatin Is Present in Discrete Cells of the Endocrine Pancreas
(hypothalamus/glucagon/insulin/immunofluorescence)

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ABSTRACT A discrete population of cells of the endocrine pancreas contains immunoreactive somatostatin as shown by immunofluorescence. These cells are different from those containing glucagon or insulin. This unexpected observation may be of physiopathological significance in the regulatory mechanisms involved in the secretion of glucagon and insulin.

Somatostatin is an oligopeptide with the primary sequence isolated from ovine hypothalamic extracts (1) on the basis of its activity to inhibit the secretion of adenohypophysial growth hormone (2, 3). Availability of the peptide in large quantities obtained by total synthesis (4) has shown it to be biologically active, both in the cyclized (oxidized) or linear (reduced) form, to inhibit the secretion of growth hormone in all the species studied so far. Recently, it has been shown that somatostatin also inhibits the secretion of glucagon and insulin (5) by acting directly at the level of the cells of the endocrine pancreas (5–7).

Furthermore, on the basis of bioassays, somatostatin or somatostatin-like substances have been shown to have a large extra-hypothalamic distribution in the central nervous system (6). This set of observations, combined with the evidence of a very short (<4 min) biological half-life for somatostatin upon intravenous injection, led to the hypothesis that the peptide might be delivered to the endocrine cells of the pancreas by peripheral nerves with peptidergic endings. This was explored by immunohistochemistry, a method which had previously led to localize (immunoreactive) somatostatin in the median eminence (9–11) as well as in discrete nerve fibers in the pars tuberalis (11). The results reported here will show that, contrary to expectations, no nerve fibers or endings containing immunoreactive somatostatin were found in the pancreas of the several species studied; unexpectedly, however, a definite population of cells in the islets of Langerhans was found to contain immunoreactive somatostatin.

MATERIALS AND METHODS

(a) Production of Antisera to Somatostatin. Somatostatin and its reduced form, H₂somatostatin, were coupled to human serum albumin with glutaraldehyde or bismellitotized benzidine. The complexed antigen was injected with adjuvant into rabbits. A detailed description of the immunization procedure has been published previously (11). Only animals injected with the oxidized form of somatostatin yielded detectable antibodies. The antiserum used here is our lot no. 1251.

(b) Other Antisera. Well-characterized antisera against polypeptides known or suspected to be present in the endocrine pancreas were obtained as follows: An antiserum to pancreatic glucagon (ref. no. GB 5667, courtesy of Dr. R. Assan, Hôtel Dieu, Paris); an antiserum to (bovine) insulin (ref. no. AIS III, courtesy of Dr. R. Unger, Dallas, Texas); an antiserum to gastrin (ref. no. 11, courtesy of Dr. W. Gepts, Brussels).

(c) Polypeptides. The following polypeptides were obtained in highly purified form and were used to ascertain the specificity of the various immunoreactions utilized here: oxytocin, [Arg⁸]vasopressin, neurophysin-A, luteinizing hormone releasing factor (LRF) (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂), thyrotropin releasing factor (TRF) (pGlu-His-Pro-NH₂), insulin, glucagon, secretin (Karolinska Institute G1H Research unit, lot no. 17281, courtesy of Dr. A. Renold, Geneva), somatostatin, H₂somatostatin, the tetrapeptide H-Thr-Phe-Thr-Ser-OH (the latter three peptides synthesized by Dr. J. Rivier, the Salk Institute, La Jolla, Calif.); this tetrapeptide represents a sequence common to somatostatin (Thr¹⁰...Ser¹³), glucagon (Thr⁴...Ser⁸) and secretin (Thr⁴...Ser⁸).

(d) Tissues Samples. Fragments of or whole pancreas were obtained from the following species: Man, sheep, ox, pig, rat, chicken. For control studies, the following tissues were obtained from rats, sheep, and pigs: (i) liver, gallbladder, salivary glands, stomach, duodenum; (ii) kidney, urinary bladder; (iii) testes, epididymis, vas deferens, seminal vesicles, prostate; (iv) thymus, spleen, lymph nodes; (v) pineal body, Gasser’s ganglion; (vi) thyroid. All tissues were fixed for 2–4 days in Bouin–Hollande fluid free of acetic acid and added with 10% saturated Hg-sublimate; they were then thoroughly washed in water, dehydrated, and included in paraffin. Sections (5 μm) were used after affixing on glass slides with 1% aqueous gelatin.

(e) Immunohistofluorescence. The various antisera described above were utilized in final dilutions of 1/2⁰ to 1/2⁰⁰ (as a function of the duration of exposure to the tissue slices) in isotonic pH 7.4 Veronal buffer added (0.4%) with the particular protein used in the coupling of the antigen studied (see above a and b). Following reaction with conjugated fluorescein
isothiocyanate (indirect or antiglobulin method) (12), the sections were counterstained with Evans' blue (0.01%) for improved contrast.

RESULTS

In all the fragments of pancreas studied, whatever the animal species of origin, each specific antiserum against glucagon, insulin, or somatostatin shows a unique population of cells as components of the endocrine islets; as shown by staining contiguous serial sections (see Fig. 1, a, b, and c), the three populations of cells are distinct from one another not only in their specificity for each antibody but also in their respective numbers and morphological appearance. Insulin-containing cells are the most numerous, somatostatin-containing cells, the least numerous. There is no constant, systematic distribution of the three types of cells from one endocrine islet to another, even in the same tissue fragment. The gross morphology of the cells demonstrating fluorescence with the somatostatin-antibody shows them to appear different from one animal species to the next, starlike or reticulated in the sheep, elongated or fusiform in the chicken, or smoothly ovular or round in man; frequently they appear with the nucleus on one (basal) side, the opposite (apical) side being strongly immunofluorescent and in contact with a sinusoidal capillary.

Neurones in the neuro-insular ganglia do not show immunofluorescence after incubation with the antiserum to somatostatin. Throughout the pancreas, no punctual nerve ending or nerve fiber was observed that would show fluorescence with the antiserum to somatostatin.

No immunofluorescence reaction was seen in the endocrine pancreas of any of the six species studied with the antiserum against gastrin, while the reaction was extremely intense in cells of the juxtapyloric regions of (rat) gastric mucosa.

Specificity of the immunofluorescence reactions

Somatostatin. It has been demonstrated and reported elsewhere (11) that the antiserum to somatostatin used here does not bind any of the following neuropeptides: neurophysin-A, oxytocin, [Arg*]vasopressin, luteinizing hormone releasing factor, and thyrotropin releasing factor. In the studies reported here, none of these peptides inhibit the immunocytochemical binding of the somatostatin-antiserum to the pancreatic cells described above. Similarly, glucagon, insulin, secretin, and the tetrapeptide Thr-Phe-Thr-Ser (common to somatostatin, glucagon and secretin) do not interfere with the immunocytochemical binding of the somatostatin-antiserum, in
concentrations up to 32 mg of peptide per ml of nondiluted antiserum. Only somatostatin in either the oxidized or reduced form inhibits the immunofluorescence reaction due to the somatostatin-antiserum.

**Insulin, Glucagon.** None of the polypeptides listed above inhibit the immunofluorescence reaction due to insulin-antiserum, except insulin itself. Glucagon and secretin (which share a large degree of homology in terms of charge and polarity of amino-acid sequences) (13) are the only two substances inhibiting the immunofluorescence reaction due to glucagon-antiserum. Actually, at very high concentrations, insulin and glucagon interfere reciprocally with the immunofluorescence reaction due to the other's antiserum, confirming the presence of a minor contaminant of the other peptide in each preparation.

**Specificity of the organ or tissue localization of immunoreactive somatostatin**

Specific immunofluorescence due to the somatostatin-antiserum (i.e., inhibited by competition with somatostatin only) is also observed in some discrete cells in the intestinal glands of Lieberkühn, and of the juxtapyloric gastric mucosa; the latter are different from those cells showing immunofluorescence with the antiserum to gastrin. A detailed study of these extrapancreatic localizations of immunoreactive somatostatin will be published elsewhere.

Immunoreactive somatostatin is not observed by the method described here in any of the other organs or tissues listed above.

**DISCUSSION**

None of the many peptides listed above, with the exception of somatostatin itself, inhibits the antigen–antibody reaction obtained with somatostatin-antiserum. Thus, with the caveat that must prevail when considering results obtained by immunocompetence, we will assume that the antigen characterized by the immunocytochemical procedure is somatostatin. In favor of this assumption is the recent observation of bioassayable somatostatin-activity in aqueous extracts of (rat) fetal pancreas (14).

The presence of cells containing somatostatin in the islets of the pancreas of all species studied is a totally unexpected observation, with possibly far-reaching significance in terms of the physiological mechanisms it evokes regarding secretion of glucagon and insulin. The statement relates to the now well-authenticated effect of exogenously administered somatostatin as an inhibitor of the secretion of both glucagon and insulin by direct action at some receptor site on both the α- and β-cells of the endocrine pancreas. Due to the absence of sensitive assay for measuring somatostatin in small volumes of body fluids, nothing is known at the moment of the dynamics of the secretion of hypothalamic somatostatin; nor is it known whether somatostatin is present in peripheral blood, and if it is at all, if it is in variable amounts as a function of corresponding physiological situations. In view of the very short biological half life of exogenously administered somatostatin, for somatostatin of hypothalamic origin to have a regulatory role at the level of the endocrine pancreas one can easily calculate that unusually high levels of the hypothalamic peptide should be secreted to maintain peripheral concentrations high enough to exert a regulatory effect at the level of the endocrine pancreas. Thus, observing the presence of cells containing somatostatin within the endocrine islets of the pancreas suggests that these may be part of a peripheral regulatory mechanism involving (pancreatic) somatostatin in the regulation of the secretion of glucagon and/or insulin. One now asks about the mechanisms that would regulate the secretion of pancreatic somatostatin; the obvious alternatives, are metabolic, endocrine or neural. Should a role of pancreatic somatostatin be demonstrated in the physiological mechanisms involved in the secretion of glucagon and/or insulin, alterations at any step of the system may be of significance in the physiopathology of diabetes.

What is the nature of the pancreatic cells containing somatostatin? They seem to be distinct from the α-cells containing and secreting glucagon, and from the β-cells containing and secreting insulin. Are the somatostatin-containing cells part of the population of the α or β cells? The nature of the secretion products of these cells has not been clearly established so far (15, 16) and their embryological origin is in question as possibly derived from neural crest anlage (17), a hypothesis that would explain how pancreatic cells would secrete a peptide observed originally in cellular elements of the central nervous system.

In view of the intensity of the immunofluorescence reaction observed in these cells and in view of the remarkable selectivity of the cells showing this reaction, it is unlikely that it be due to some sort of nonspecific adsorption of (circulating) somatostatin. The immunocytochemical reaction seen here is best explained by proposing that these cells synthesize and contain somatostatin; and that they probably release it in still undetermined circumstances.

Finally, the results reported here raise further the question of the relative ubiquity of somatostatin. Its presence as shown here in the endocrine pancreas of several species of mammals and of the one bird studied is in keeping with the demonstration of hypothalamic somatostatin in a large number of vertebrate species (11). In these various species, somatostatin now appears to be distributed in a series of tissues of neural crest origin, all with nearby effector. It certainly cannot be considered any longer as an exclusively hypothalamic hypophysiotropic peptide and may well have a much broader physiological significance than originally suspected.

I am indebted to each of the colleagues named in the text who graciously contributed aliquots of antisera or polypeptides used in the studies referred to here and grateful to Prof. Roger Guillemin, The Salk Institute, La Jolla, Calif., whose interest and cooperation made these studies possible and who assumed the task of putting this text in its final form. This work was supported by research Grant no. 74.4.443-35 Institut National de la Santé et Recherche Médicale (France).

hypothalamic inhibitor of the endocrine pancreas," Science 184, 482–484.