confirm the negative results of the external measurements of plutonium content made with the proportional counter.

The urine samples were also analyzed for ^{90}Sr by chemical separation and β-counting. The mean excretion rate of the 16 subjects was 0.63 ± 0.33 pCi/day, whereas that of seven control subjects was 0.66 ± 0.30 pCi/day. We thus infer that both groups were drawn from the same population; that is, the ^{90}Sr excreted by the test subjects arose from exposure to global fallout rather than from their presence at weapon tests.

In summary, we were unable to detect internal deposits of radioactivity in any of these 16 men in excess of normal levels found in the general population. We could only test for long-lived isotopes, but, because of their solubility in body fluids, ^{137}Cs and ^{90}Sr contribute the major portion of the dose from internally deposited fallout (*11*). Thus we have no evidence that these subjects received any significant internal dose from their participation in the "Smoky" weapon test.

R. E. TOOHEY
J. RUNDO
M. A. ESSLING
J. Y. SHA
R. D. OLDHAM
Center for Human Radiobiology, Argonne National Laboratory, Argonne, Illinois 60439
J. SEDLET
J. J. ROBINSON
Occupational Health and Safety Division, Argonne National Laboratory

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Pituitary Gastrins Occur in Corticotrophs and Melanotrophs

Abstract. *The gut hormone gastrin was identified in pituitary cells containing adrenocorticotropic hormone and α-melanocyte–stimulating hormone by region-specific immunocytochemistry and radioimmunoassays. Smaller amounts of gastrin were found in nerve fibers of the neural lobe and pituitary stalk. Since adrenocorticotropic hormone–like peptides occur in antropyloric gastrin cells, these data indicate a considerable similarity in peptide composition of pituitary and gastrointestinal endocrine cells and reinforces questions of multiple hormone production.*

Adrenocorticotropic hormone (ACTH)–related peptides were recently shown not to be confined to the pituitary but to also occur in central neurons (*1*, *2*) and in gastrointestinal gastrin-secreting cells (*2*). Immunoreactive gastrins have also been detected in pituitary extracts (*3*). By using region-specific immunocytochemistry and radioimmunoassays on microdissected pituitaries and isolated pituitary cells, we have determined that pituitary gastrins occur in cells containing ACTH and α-melanocyte–stimulating hormone (MSH) and in nerve fibers of the pituitary stalk and neural lobe.

Pituitaries from pentobarbital-anesthetized cats were dissected into anterior lobes, neurointermediate lobes, and pituitary stalks. Pig pituitaries, from a local abattoir, were microdissected into anterior, intermediate, and neural lobes as well as pituitary stalks and hypothalami. Porcine pars intermedia cells were also isolated by the method of Crine and coworkers (*4*). Specimens were frozen in liquid nitrogen and extracted by boiling for 20 minutes in redistilled water, then homogenized and centrifuged (*3*). Each pellet was reextracted in 0.5*M* acetic acid as described (*3*). Extracts were tested by radioimmunoassays specific for different sequences of gastrin-17 and of the related hormone cholecystokinin-33 (CCK-33) (*5*). Gel filtration was performed on Sephadex G-50 SF columns (10 by 1000 mm) with 0.02*M* barbital (*p*H 8.4) containing 0.1 percent bovine serum albumin being used for elution (4 ml per hour at 4°C). The fractionations were monitored by the gastrin and CCK assays (*5*). Protein was determined according to the method of Lowry *et al.* (*6*).

For immunocytochemistry, pentobarbital-anesthetized cats were perfusion-fixed with 4 percent paraformaldehyde in 0.1*M* sodium phosphate buffer, *p*H 7.4 (1.0 to 2.0 liters). Pituitaries from formaldehyde-perfused cats and from nonperfused pigs were immersion-fixed in the formaldehyde solution for 24 hours at

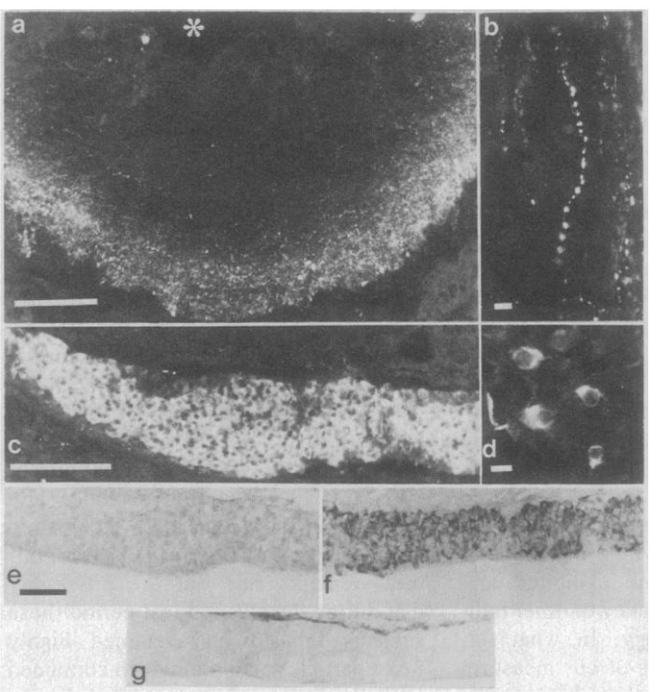

Fig. 1. Sections of cat pituitary. (a) Median eminence (scale bar, 100 μm); (b) pituitary stalk (scale bar, 10 μm); (c) intermediate lobe (scale bar, 150 μm); (d) anterior lobe (scale bar, 15 μm); and (e–g) three adjacent sections of intermediate lobe (scale bar, 100 μm). Sections (a) to (d) were stained with the COOH-terminus–specific antiserum 4562, which reacts with both gastrin-17 and CCK-33 (indirect immunofluorescence). For reasons specified in the text, the nerves seen in the median eminence (a) are chiefly CCK nerves (asterisk indicates the third ventricle), whereas the nerves detected in the pituitary stalk [note the varicose fiber in (b)] and posterior lobe and in the endocrine cells of the intermediate lobe (c) and the anterior lobe (d) store gastrin. The gastrin-storing cells and nerves also react with gastrin(6–13) antiserum 4710 but not with CCK(25–30) antiserum 4698, as illustrated for the intermediate lobe in (e) (antiserum 4698) and (f) (antiserum 4710), by means of the PAP technique; (g) is stained by gastrin-17-absorbed antiserum 4710.

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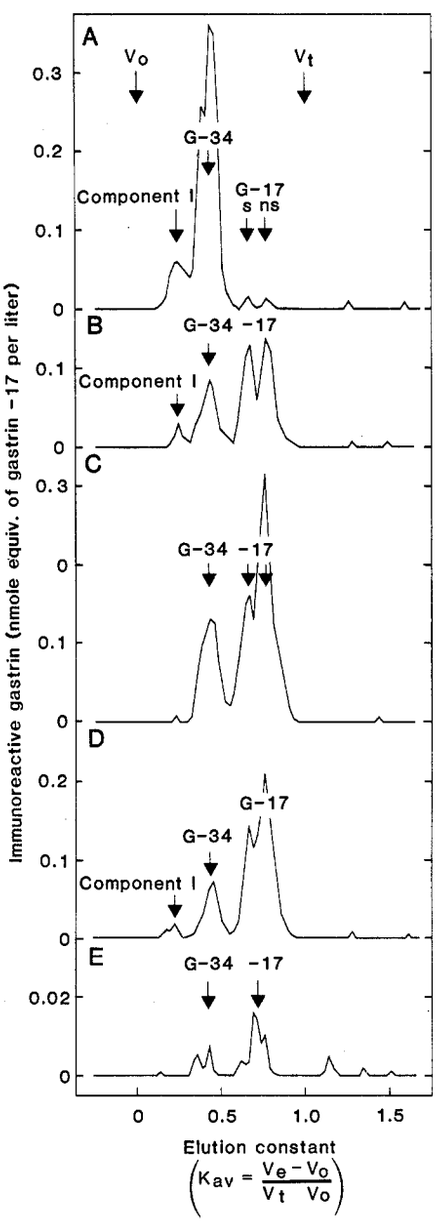
4°C and prepared for cryostat sectioning (*7*). Sections were reacted for 24 hours at 4°C with region-specific antiserums to gastrin and CCK as described (*2, 7, 8*). The antiserums used included the COOH terminus–specific antiserums 4562 and 2717, which recognize both gastrin and CCK; the gastrin-specific antiserum 4710, prepared against the synthetic (residues 6–13) portion of human gastrin-17; and the CCK(25–30)-specific antiserum 4698 (*8*). These antiserums react with feline and porcine gastrin or CCK (*8*). Antiserum to ACTH(1–24), αMSH, and ACTH(18–39) were also used (*2*). The site of antigen-antibody reaction was detected by the peroxidase-antiperoxidase (PAP) method (*9*) or by indirect immunofluorescence with the use of fluorescein isothiocyanate-conjugated goat antiserum to rabbit immunoglobulin G (*7, 9*). We used conventional staining controls (*9*) as well as absorption controls against synthetic human gastrin-17, porcine CCK-33, synthetic human ACTH(1–39), and αMSH (*10*).

Immunocytochemistry revealed specific gastrin immunoreactivity in scattered cells of the anterior lobe and all cells of the intermediate lobe of both cat and pig pituitaries (Fig. 1). In addition, a variable number of immunoreactive nerve terminals were detected in the pituitary stalk and neural lobe (Fig. 1). No staining could be obtained with the CCK-specific antiserum (Fig. 1). All staining controls were negative and absorptions against gastrin-17 inactivated staining, whereas absorptions against ACTH(1–39) and αMSH did not. Absorptions against CCK-33 inactivated the staining by antiserums 4562 and 2717, which react with the COOH-terminal tetrapeptide sequence common to gastrin and CCK (*8*), but did not affect staining with antiserum 4710, which recognizes region (6–13) of gastrin-17, which is not present in CCK (*8*). The gastrin immunoreactive cells of the anterior lobe reacted more weakly than the intermediate lobe cells. They corresponded to the ACTH(1–24) and ACTH(18–39) immunoreactive cells.

Significant amounts of gastrin were measured in all regions of the pituitary (Table 1). Cholecystokinin immunoreactivity was, in contrast, only detected in extracts of the pituitary stalk, hypothalamus, and, in very low amounts, in the anterior lobe extracts. Gastrin concentrations were highest in isolated pars intermedia cells and in microdissected intermediate lobes. Slightly lower concentrations were detected in neural lobes and in specimens from the pituitary stalk (Table 1). Still lower gastrin concentrations were measured in anterior lobe extracts and very low gastrin concentrations were detected in hypothalamic extracts. The predominant gastrin components of the pituitary corresponded to component I ($K_{av} \simeq 0.24$), gastrin-34, and gastrin-17 (Fig. 2). As previously described (*3, 5, 11*), mild trypsin treatment converted component I and the gastrin-34–like component into the gastrin-17–like component. The anterior lobe contained mainly component I and gastrin-34, whereas gastrin-17 predominated in the intermediate and neural lobes (Fig. 2). Component I and gastrin-34 are believed to be precursors to gastrin-17 (*11, 12*).

Table 1. Concentrations of gastrin in regions of the pituitary. The concentrations were measured as picomole equivalents of porcine gastrin-17 per milligram of protein.

Region	Mean concentration	Range
Anterior lobe	0.71	0.69 to 0.72
Intermediate lobe	9.02	7.50 to 10.53
Neural lobe	7.86	7.86
Pituitary stalk	9.01	6.36 to 11.67



The distribution of immunoreactive gastrins and CCK's in pituitary and hypothalamus accords well with the immunocytochemical data. Thus, the relatively weak staining of anterior lobe ACTH-containing cells by gastrin antiserums, as well as the fact that these cells are disseminated between nonimmunoreactive cells, agrees with the lower concentrations of gastrins measured in anterior lobe extracts. From both the immunocytochemical and radioimmunoassay results we conclude that the intermediate lobe contains the highest concentration of pituitary gastrins. Therefore, some of the gastrin measured in the neural lobe could be due to contamination by intermedia cells. As shown by parallel assays of αMSH, such contamination was present. However, posterior lobe gastrin concentrations were only slightly lower than intermediate lobe concentrations and the presence of gastrin immunoreactive nerves in the neural lobe and of immunoassayable gastrins in pituitary stalks and in hypothalami argues that nerve fibers also contain gastrins. This is in accordance with reports of gastrins in the vagal nerve (*13*). Thus, the two related peptides, gastrin and CCK, can both be identified in neurons as well as endocrine cells. Of these two, however, CCK represents the main brain peptide. Our finding of high CCK concentrations in hypothalami accords with previous studies in which CCK was identified in nerves of the hypothalamus and median eminence (*7, 14*).

Previously, ACTH- and αMSH-related peptides were found in antropyloric gastrin-containing cells (*2*). Thus, not only do pituitary ACTH- and αMSH-containing cells contain gastrin-like pep-

Fig. 2. Gel chromatography of gastrins in boiling-water extracts of pools of (A) porcine anterior, (B) intermediate, and (C) posterior lobes as well as (D) pituitary stalks and (E) hypothalami. Fractions were tested with a gastrin-specific assay (*5*) with the use of antiserum 2604. The cross-reaction of this assay with CCK-8 is below 0.1 percent. The assay measures gastrin component I, gastrin-34, and gastrin-17 with equimolar potency (*5*). Parallel monitoring with CCK-specific assays and similar experiments with acetic acid extracts confirmed the absence of measurable CCK's in the regions reported in the text.

tides, but gastrin-containing cells also contain ACTH- and αMSH-related peptides. Adrenocorticotropic hormone is biosynthesized as a large precursor molecule in both the anterior and intermediate lobe (*15*). Selective cleavages of this precursor give rise to ACTH(1–39) in anterior lobe cells and αMSH [= *N*-acetylated and amidated ACTH(1–13)] in intermediate lobe cells (*4*, *15*, *16*). Recently, the sequence of the messenger RNA coding for the ACTH (αMSH) precursor was determined (*17*) and, subsequently, the structure of its corresponding gene was elucidated (*18*). The transcribed regions of this gene do not code for gastrin. Therefore, the mechanism underlying the frequent coproduction of gastrin- and ACTH-related peptides by endocrine cells remains to be elucidated.

Note added in proof: Recently, Vanderhaeghen (*19*) has also reported that COOH-terminal gastrin and CCK immunoreactivity occur in ACTH and αMSH cells of dog and human pituitaries.

L.-I. LARSSON
J. F. REHFELD
Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

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Methylation of Trimethyltin Compounds by Estuarine Sediments

Abstract. *Both biologically active and autoclaved sediments convert trimethyltin hydroxide to the volatile tetramethyltin. Larger amounts of tetramethyltin were formed in the bioactive sediments than in the sterile sediments. No volatile tin compounds were detected in the absence of trimethyltin hydroxide or from trimethyltin hydroxide in seawater or in seawater containing bentonite. The formation of tetramethyltin is slow, taking over 80 days at 16°C to reach a maximum. The extent of conversion, although significant, is not extensive. The formation of tetramethyltin occurs in estuarine sediments by both abiotic and biologically enhanced pathways. A redistribution mechanism accounts for at least the abiotic pathway and possibly both formation pathways.*

Over 25,000 metric tons of organotin compounds are used annually throughout the world (*1*). A major portion of these includes the triorganotin biocides, whose use for the prevention of the fouling of ship hulls and other marine structures is continually increasing. Such use represents an important anthropogenic input of tin into the marine environment. The fate of organotins is just beginning to receive attention (*2*, *3*).

The environmental significance of tin methylation is uncertain at present. Laboratory studies have suggested the potential for biomethylation of tin. Dizikies *et al.* (*4*) have reported that methylcobalamin, a vitamin B_{12} derivative that is involved in bacterial methanogenesis, will transfer a methyl group to inorganic Sn(III). However, the tin compounds introduced into the environment are in the Sn(IV) state. It is conceivable, but not likely (*5*), that in highly reducing environments such as anoxic estuarine sediments some Sn(III) may be produced by the reduction of Sn(IV). Then methylation of Sn(III) species might occur. It has also been reported that methylcobalamin is capable of methylating trimethyltin acetate in a manner analogous to mercuric acetate (*6*), but no evidence for the formation of tetramethyltin [$(CH_3)_4Sn$] was presented. Huey *et al.* (*7*) observed the methylation of inorganic and monomethyltin by a *Pseudomonas* sp. from Chesapeake Bay. Recently inorganic tin(IV), mono-, di-, and trimethyltin compounds have been detected in the water of Tampa Bay (*8*), the water of Lake Michigan (*9*), and human urine (*8*). These compounds could have been produced by an environmental methylation of tin, as suggested by Craig (*3*). To our knowledge, no evidence regarding environmental methylation of trialkyltin compounds has been presented.

Our investigations were carried out to determine the potential for environmental methylation of trialkyltin(IV) species. Trialkyltins, including hexabutyldistannoxane and tributyltin fluoride, are the most frequently used organotins for the control of biofouling and represent major inputs of organotins into estuarine eco-

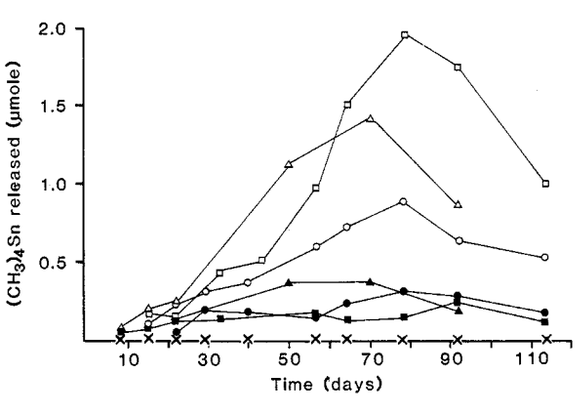

Fig. 1. The amount of $(CH_3)_4Sn$ in the headspace, as a function of time, formed during the incubation of $(CH_3)_3SnOH$ in sediment (○), sediment plus Na_2S (□), sediment plus sodium acetate (△); solid symbols represent the corresponding autoclaved controls; *x* represents seawater, bentonite, and all flasks without added $(CH_3)_3SnOH$.

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