appears that they must bind to separate, but related, epitopes in order to cooperatively inhibit ADH activities. These results complement the report of Lad et al.[11], who isolated inhibitory anti-rat liver ADH monoclonal antibodies. Their antibodies, in contrast to ours, were species specific. TA, TB and TN-1 are all cross-reactive between horse, hamster and mouse and thus must be considered as auto-antibodies produced in response to the hyperimmunization of the mice by the original antigens. It is interesting to note that V79 cells and brain tissue express only ADH class II and III respectively, these tissues could be used in conjunction with antibodies specific for the two types of enzyme for the study of ADH gene regulation. It is expected that our antibodies will be useful to purify ADH in its native form (they do not bind to the denatured ADH) and also to help distinguish between the isozymes of the different classes since they are unreactive with class III ADHs.

Acknowledgment. This work was supported by the National Cancer Institute of Canada.

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0014-4754/87/040426-03$1.50 + 0.20/0

Co-localization of glucagon and pancreatic polypeptide in testudine pancreas

W.B. Rhoten[1]

Department of Anatomy and Cell Biology, UMDNJ-New Jersey Medical School, Newark (New Jersey 07103, USA), and Cell Biology Unit, Medical School, University of Cape Town, Observatory 7925, Cape (South Africa), 28 May 1986

Summary. Immunocytochemistry was carried out on sections of pancreas from the gopher tortoise, *Gopherus polyphemus*. Combined immunofluorescent and peroxidase-anti-peroxidase techniques showed unequivocally that some of the cells were immunoreactive for both glucagon and pancreatic polypeptide (PP). Antibodies directed against avian PP, bovine PP, and human PP all have a positive reaction. Co-localization of glucagon and PP in the pancreas of the gopher tortoise indicates that the occurrence of these hormones in the same cells is widespread in higher vertebrates.
Key words. Antibodies; hormones; pancreatic; peptides; localization; immunocytochemical.

The four major hormones of the vertebrate pancreas, insulin, glucagon, pancreatic polypeptide (PP), and somatostatin, are generally regarded as being found in separate cell-types which may[2], or may not[3,4], have particular spatial relationships with one another. However, cells positive for both glucagon and PP have been found in a lower vertebrate, the frog *Rana pipiens*[5], and more recent studies have found cells reactive for both glucagon and PP in mammalian pancreas, including human islets[4,6,7]. I report here the occurrence of glucagon and PP in the same cells in a reptilian pancreas.

Materials and methods. Gopher tortoise, *Gopherus polyphemus*, pancreas was fixed overnight in Bouin's fluid and processed for embedding in Paraplast-Plus (Lancer, St. Louis, MO). Sections of pancreas were reacted with the peroxidase-antiperoxidase (PAP) immunocytochemical technique of Sternberger et al.[8], with one recycling[9]. Sequential reactions were carried out using indirect immunofluorescence followed by PAP. Immunocytochemical controls consisted of both methodological and antisera controls. Controls for specificity of the method consisted of replacing the primary antisera with normal (non-immunized) rabbit serum and omitting coupling immunoglobulin or PAP, or including an excess of unlabeled second antibody with indirect immunofluorescence. Controls for antibody specificity consisted of absorption of the primary antisera with an excess of the antigen for which the particular antiserum was known or believed to be selective. The antisera absorptions were carried out with the following hormones at a concentration of hormone expressed as mass per microl of undiluted serum: glucagon (bovine-porcine, lot number 258-V016235, Lilly Research Laboratories), 5 µg/µl; pancreatic polypeptide (bovine, lot 615-D63-295, Lilly Research Laboratories, 50 µg/µl; or turkey, HPLC grade from H. Gail Pollock, 10 µg/µl). All of the controls gave the appropriate negative result.

Immunocytochemical reagents were obtained from the following sources. Rabbit anti-bovine pancreatic polypeptide serum (RABPP), used at a concentration of 1:4000–1:20,000, was a gift from Dr Ronald E. Chance, Lilly Research Laboratories, Eli Lilly and Co. (lot 615-R110-146-16). RABPP was routinely absorbed with glucagon and insulin, 10 µg and 2 µg respectively per µl of undiluted serum (see above). Rabbit anti-avian pancreatic polypeptide, prepared in the laboratory of the late Dr Joe R. Kimmel and used at a concentration of 1:1000–1:10,000, was the gift of H. Gail Pollock and the Medical Research Service, Medical Center, Veterans Administration. Rabbit anti-human pancreatic polypeptide (lot No. 0010), used at a concentration of 1:500–1:2000, was purchased from Miles Scientific, Naperville, IL. Rabbit antiserum to glucagon, used at a concentration of 1:5000–1:20,000, was generously provided by Dr Howard Tager (R-1, 11–20)[10]. Ovine anti-rabbit immunoglobulin G (heavy and light chains), soluble horseradish peroxidase-rabbit anti-peroxidase complex, and normal ovine serum were obtained from CooperBiomedical, Inc., Malvern, PA. Donkey anti-rabbit immunoglobulin conjugated to fluorescein was purchased from Amersham Int., Buckinghamshire, England.

Results and discussion. Endocrine cells of the gopher tortoise pancreas which were immunoreactive by the PAP method for PP were widely distributed, including duct epithelium (fig. 1). The PP-positive cells occurred singly or in small groups and were relatively abundant (fig. 1). Glucagon-containing cells generally had a distribution similar to the PP-containing cells, but were less abundant. Many of the PP-positive cells were not reactive for glucagon and glucagon-positive cells were absent from some

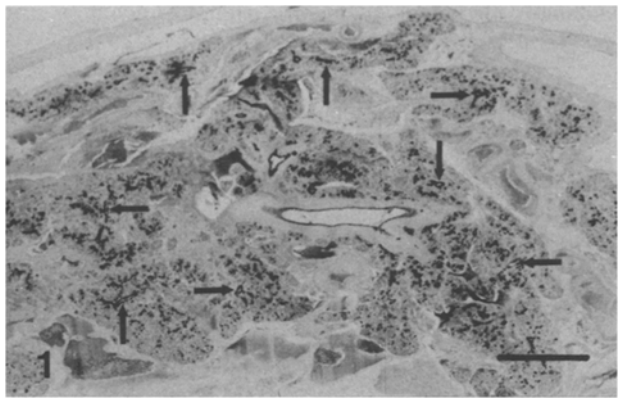

Figure 1. Gopher tortoise pancreas reacted for avian PP. PP-positive cells (black) are abundant including many cells that line ducts (arrows). Scale marker = 500 μm.

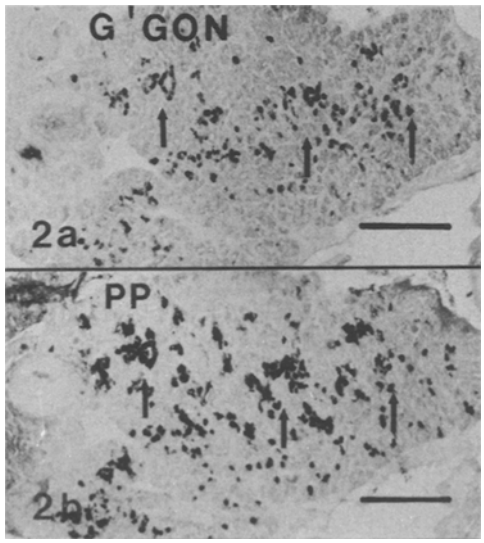

Figure 2. Adjacent sections of pancreas reacted for bovine PP or glucagon. Glucagon-positive cells (G'GON) (*a*) are not as numerous as PP-positive cells (PP) (*b*) Similar areas are indicated by arrows. Scale marker = 200 μm.

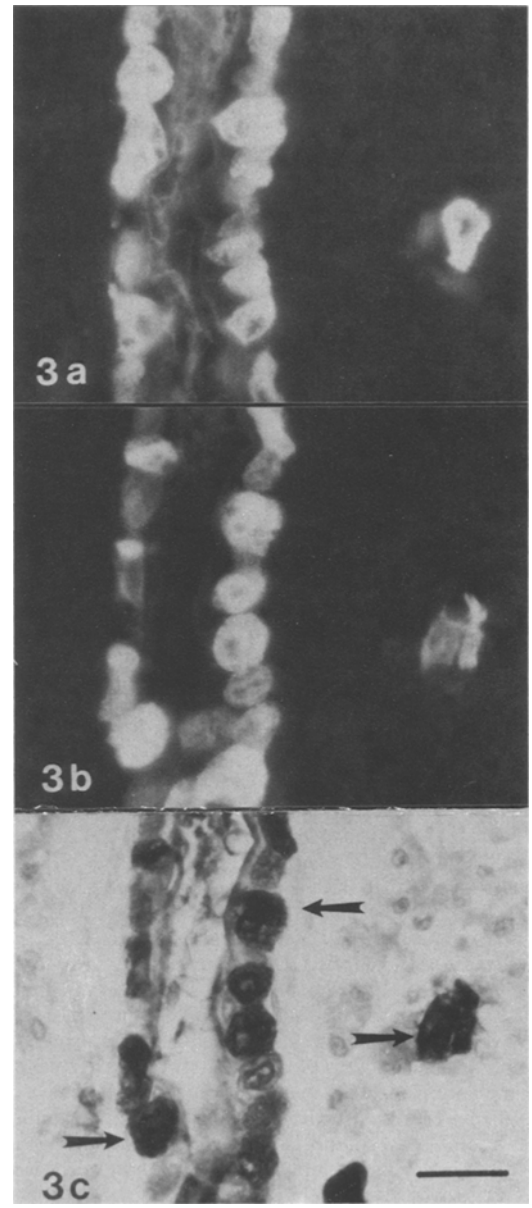

Figure 3. Sequential staining for PP and glucagon. PP was localized by immunofluorescence (*a*, human PP; *b*, avian PP) in serial sections. After elution of immunofluorescent reactants, glucagon was localized on the same sections by the PAP technique (*c*, same section as *b*). Note that many identical cells are positive for both and glucagon (arrows in *c*) (compare *b* and *c*). Scale marker = 20 μm.

lobules which had numerous PP-positive cells (fig. 2). Even at low magnification it appeared that some of the glucagon-positive cells and some of the PP-positive cells were present in the same locations (fig. 2). The glucagon- and PP-immunoreactive cells were distinct from insulin-positive and somatostatin-positive cells (not illustrated). When three different antisera to PP were tested by indirect immunofluorescence, they all gave a positive reaction and this reaction was present in the same cells (fig. 3). After elution of antigen-antibody complex with 0.1 N HCl and reaction for glucagon by the PAP method, some of the cells which were now positive for glucagon, previously were positive for PP (fig. 3).

Absorption of the antisera to PP with the peptide, eliminated completely specific staining, as did absorption of the antiserum to glucagon with glucagon. Conversely, incubation of PP-antisera with glucagon, or glucagon-antiserum with PP, had no apparent affect on immunostaining. Therefore, the antigenic determinants with which the PP-antisera were reacting and those with which the glucagon-antiserum were reacting are evidently different. This is not particularly surprising since glucagon and PP are considered to belong to separate 'families' of peptides[11–14]. Glucagon itself is highly conserved[11], but preproglucagon exhibits considerable variation[12] thus allowing the possibility of many cross-reactivities within the 'glucagon family'. Avian PP differs markedly from the mammalian peptides[11,14], yet cross-reactivity was seen with all three antisera to PP used in the present study.

The cross-reactivity between glucagon-positive cells and PP-positive cells seen in the pancreas of the gopher tortoise does not appear to be typical for the tortoises or for reptiles in general. We found no specific crossreactivity between glucagon and PP in our previous studies with saurian[15,16], including developing pancreas[17], ophidian[18], and crocodilian[3] pancreas. Furthermore, the pancreas of the tortoise *Terapene carolina*, which has PP-positive cells with all three anti-PP sera used in this study, has a separate cell-population reactive for glucagon (Rhoten, unpublished observations). A recent study of turtle pancreas also reported independent cell populations for glucagon and PP[19]. The

reptilian endocrine pancreas appears to be even more complex with regard to peptide localizations than the islets of mammals. Thus, the elucidation of the functional significance of different pancreatic hormones occurring in the same cells is an exciting challenge for the future.

1 Acknowledgments. This work was supported in part by grants from the University of Cape Town, the M. Crossley bequest, the N. Atkinson bequest, and Herman and Caporn bequests. The generous gifts of antisera by Dr Chance and the late Dr Kimmel, and the gift of hormones by the Lilly Research Laboratories are gratefully acknowledged. The excellent technical assistance of J. Thompson and J. B. De Haan is also gratefully acknowledged. Please send all correspondence to the USA address.

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0014-4754/87/040428-03$1.50 + 0.20/0

Encephalomyocarditis (EMC) virus-induced diabetes mellitus prevented by *Corynebacterium parvum* in mice

E. Kounoue, S. Nagafuchi, M. Nakamura, S. Nakano, T. Koga, M. Nakayama, M. Mituyama*, Y. Niho and R. Takaki**

*First Department of Internal Medicine and *Department of Bacteriology, Faculty of Medicine, Kyushu University, Fukuoka 812 (Japan), and **Department of Medicine 1, Medical College of Oita, Oita 879-56 (Japan), 10 February 1986*

Summary. Corynebacterium parvum prevented the development of encephalomyocarditis virus-induced diabetes in mice, when it was given 3–14 days before the virus infection. This treatment inhibited virus replication in the pancreas of the infected mice at an early stage of the infection.
Key words. EMC virus; *Corynebacterium parvum;* diabetes mellitus; immunity.

Accumulating evidence suggests that some cases of insulin-dependent diabetes mellitus (IDDM) are triggered by viral infection, acting either alone or in concert, with an autoimmune response[1,2]. Viruses have been isolated from patients with IDDM and these viruses are diabetogenic in experimental animals[3,4]. Therefore, it is of interest to investigate mechanisms which may afford protection against the viral infection.

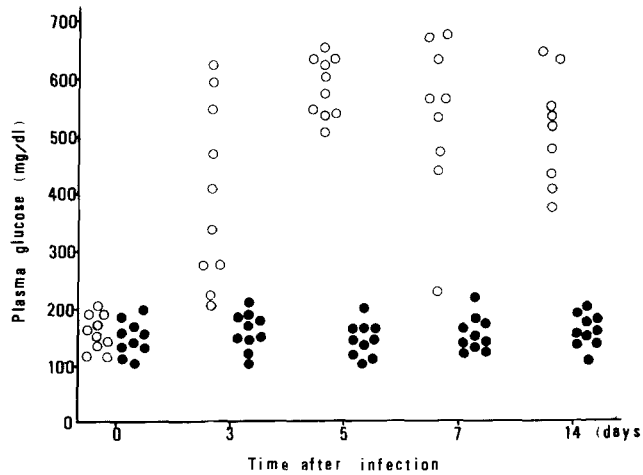
Encephalomyocarditis (EMC) virus strain D causes diabetes in certain strains of mice and is an excellent model for studying IDDM in humans[5,6]. We report here that *Corynebacterium parvum* (CP) provided complete protection for EMC-D virus infected mice and diabetes did not occur.

Materials and methods. EMC virus strain D was kindly provided by Dr A. L. Notkins, NIH, USA, and was propagated and titrated on a secondary culture of CD-1 mouse embryonic cells. Five hundred plaque forming units (p.f.u.) of the virus were inoculated intraperitoneally (i.p.) into each mouse on day 0. Six to eight-week-old male DBA/2 mice were purchased from Charles River Inc, Kanagawa, Japan and were fed food and water ad libitum, in an SPF environment. Plasma glucose levels were determined by glucose oxidase methods. Blood samples were obtained by retroorbital plexus puncture from non-fasted mice on 0, 3, 5, 7, 14 days after virus challenge. *Corynebacterium parvum* (CP) was purchased from Institut Merieux, Lyon, France. Each preparation, containing 4 mg of heat-inactivated dried CP (Lot No. 151) and 400 µg of formaldehyde was dissolved in 2 ml of phosphate buffered saline (PBS) and used throughout the study. For solvent controls, 400 µg of formalde-

Table 1. Protective effect of CP administered at various times before and after infection against EMC virus-induced DM

CP treatment (days)	Incidence of DM 5 days after infection
−42	4/4 (100%)
−28	3/6 (50%)
−21	5/6 (83%)
−14	0/7 (0%)
−7	0/7 (0%)
−5	0/7 (0%)
−3	0/7 (0%)
−1	3/6 (50%)
0	6/6 (100%)
+1	5/6 (83%)

Any mouse with a glucose level exceeding 250 mg/dl was defined as diabetic.



Protection against EMC-D virus-induced diabetes by *Corynebacterium parvum* (CP). One half milligram of CP was given to each mouse. Seven days after this treatment, the mice were infected with 500 p.f.u. of EMC-D virus. ○, control infected; ●, CP-pretreated and infected.