suggesting the likelihood of incomplete equilibration within the circulation. The very short half life is similar to circulation time suggesting a high extraction rate on passage through the lungs and peripheral tissues.

Despite the identical systemic and portal infusion rate in the anesthetized pig there was a considerable difference in the plateau levels of VIP achieved. The lower systemic levels after portal infusion presumably reflect considerable clearance of exogenous VIP by the liver.

Previous studies, in dogs with completely transposed portal vein and superior vena cava, failed to show a significant hepatic inactivation of VIP[15]. The possible explanations for the different results include altered hepatic function, or development of a portal systemic collateral circulation after transposition. Previous data from an anesthetized pig preparation demonstrated a significant porto-systemic difference in endogenous VIP[16].

The pharmacokinetic data exogenous VIP obtained in dog and pig thus demonstrate rapid clearance and are consistent with a postulated neurotransmitter role.

1 Reprint requests to S.R.B., Francis Fraser Labs, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12OHS.
2 S.I. Said and V. Mutt, Science *169*, 1217 (1970).
3 M.G. Bryant, S.R. Bloom, J.M. Polak, R.H. Albuquerque, I. Modlin and A.G.E. Pearse, Lancet *1*, 991 (1976).
4 S.I. Said and R.N. Rosenberg, Science *192*, 907 (1976).
5 L.I. Larsson, L. Edvinsson, J. Fahrenkrug, R. Hakanson, C.H. Owman and O. Schaffalitsky de Muckadell, Brain Res. *113*, 400 (1976).
6 G.O. Barbezat and M.I. Grossman, Science *174*, 422 (1971).
7 S.R. Bloom and M.G. Bryant, Gut *14*, 823 (1973).
8 I.M. Modlin, S.R. Bloom and S.J. Mitchell, Experientia *34*, 535 (1978).
9 A.V. Edwards, P.M.M. Bircham, S.J. Mitchell and S.R. Bloom, Experientia *34*, 1186 (1978).
10 J. Fahrenkrug, H. Galbo, J.J. Holst and O. Schaffalitzky de Muckadell, J. Physiol, Lond. *280*, 405 (1978).
11 S. Domschke, W. Domschke, S.R. Bloom, P. Mitznegg, S.J. Mitchell, G. Lux and U. Strunz, Gut *19*, 1049 (1978).
12 S.J. Mitchell and S.R. Bloom, Gut *19*, 1943 (1978).
13 A. Goldstein, L. Aronow and S.M. Kalman, Principles of drug action, 2nd edn, p.311. Eds Goldstein, Aronow and Kalman. Wiley, New York 1974.
14 S. Domschke, W. Domschke, S.R. Bloom, P. Mitznegg, S. Mitchell, G. Lux, U. Strunz and L. Demling, in: Gut hormones, p.475. Ed. S.R. Bloom. Churchill Livingstone, Edinburgh 1978.
15 V.T. Strunz, J.H. Walsh, S.R. Bloom, M.R. Thompson and M.I. Grossman, Gastroenterology *73*, 768 (1977).
16 I.M. Modlin, S.J. Mitchell and S.R. Bloom, in: Gut hormones, p.470. Ed. S.R. Bloom. Churchill Livingstone, Edinburgh 1978.

Regional distribution of pancreatic polypeptide cells in the 21-day fetal rat pancreas

Judith L. Shevell and M. R. Schweisthal[1]

Department of Anatomy, School of Medicine, Oral Roberts University, Tulsa (Oklahoma 74171, USA), 30 September 1981

Summary. 21-day fetal rat pancreata were stained with the unlabeled antibody peroxidase-antiperoxidase technique using bovine pancreatic polypeptide as the primary antibody. Total counts of pancreatic polypeptide cells were made over the entire pancreas. It was found that the head region contained the greatest number of pancreatic polypeptide cells with the body next and the tail having the smallest number. The pancreatic polypeptide cells of the body were concentrated in the portion closest to the distal duodenum. This distribution pattern seems to support the suggested role of pancreatic polypeptide on the physiological function of the digestive tract.

Pancreatic polypeptide (PP) is a recently discovered pancreatic hormone found in birds as well as mammals. As has been reported by several workers[2-5] PP has been shown to have an effect on various physiological functions of the digestive tract. Floyd et al.[6] have shown that basal plasma PP levels are significantly elevated in insulin treated diabetic patients compared to healthy subjects. These studies are in agreement with those of Gingerich et al.[5] and correlate with the increased PP cell population that Sundler et al.[7] found in alloxan induced diabetes. Lundquist et al.[8] suggested that PP could have a possible role as a local regulator of glucose induced insulin secretion.

Many studies[7-12] have been done on adult animals to determine the distribution of PP cells. Few if any studies have been performed on neonatal or fetal animals. It was the purpose of this study to examine the PP cell distribution in the 21-day fetal rat pancreas to determine whether there is any substantial difference from the pattern in the adult. If there is such a difference it would merit a study of the neonatal pancreas to determine when the adult pattern is established and possibly by what mechanism(s).

Materials and methods. Intact pancreas, spleen and duodenum were removed from 12 fetal rats of 21 days gestation. 6 preparations were made using 3 procedures: 2 were fixed en bloc and subsequently divided into 3 regions; 2 were divided prior to fixation, and 2 were pinned en bloc to lead lined redwood blocks. Pins stabilizing the head region were placed in the duodenum, those for the tail pierced the spleen, additional pins were placed in the mesentery. Tissues and blocks were submerged in Bouin's fixative for 20 min, then pins and blocks were removed and the straight, flattened tissue bloc returned to the fixative. The latter was the method of choice. Regardless of method used all tissues were fixed for 16 h in Bouin's fluid.

Following routine histological procedures the tissues were embedded flat in paraplast and serially sectioned at 4 μm. The slides were stained with the unlabeled antibody peroxidase-antiperoxidase technique[13]. A 1:100 dilution of bovine pancreatic polypeptide (BPP) was used as the primary antibody (lot number 615-R110-146-16, graciously

Regional distribution of pancreatic polypeptide cells of 21-day fetal rat pancreata*

Number of PP cells per region Region	Fetus I	Fetus II
Head	1134	1030
Body	639	612
Tail	112	390

*As determined by qualitative methods over 10 slides for each of 2 pancreata.

supplied by Dr R.E. Chance, Eli Lilly Co.) with 3-3′ diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) as the chromagen. All slides were then counterstained with alcoholic eosin Y.

A series of titration experiments was performed with antiBPP ranging from a 1:100 dilution to a 1:10,000 dilution. Although we did get positive results with the 1:10,000 dilution, we found that a 1:100 dilution gave more consistent results in staining when length of time in stain, density of the stain, and photographability were considered. We have found that staining procedures often have to be altered when going from adult to fetal tissue and/or to tissues grown in organ culture. The dilution of 1:100 worked best in the fetal rat tissue used in this study and for this particular lot of antiBPP.

Controls for staining were as follows: 1. Phosphate buffered saline with bovine serum albumin was used in place of the primary antibody; 2. the 1:100 antiBPP was absorbed with a 2.5, 5, and 10 µg/ml dilution of the antigen (BPP lot number 615-D63-166-7); 3. Dr R.E. Chance, Eli Lilly Co., suggested that preabsorption tests be performed on each new lot of antiBPP to help determine its cross reactivity. Thus, a series of titration type absorption experiments using concentrations of antiBPP from 1:50 to 1:10,000 and glucagon (lot number 109-0331, Sigma Chemical Co.) from 1.1, 2.5, 5.0, and 10 µg/ml was performed with these 2 lots of chemicals. No cross reactivity was found and we proceeded accordingly.

For purposes of evaluation, 6 sections were placed per slide and 3 were selected for counting BPP positive cells and for photography. For each pancreas selected, only 10 consecutive slides which contained all 3 regions were observed and photographed with a Zeiss photomicroscope III.

The pancreas was visually divided into regions. Everything within the curvature of the duodenum was considered head. The portion in immediate contact with the spleen was considered tail and the remainder of the tissue was termed body (fig. 1). Specific configurations of the tissue on the slide were utilized to further define the 3 regions.

Positive cells were determined to be those in which the cytoplasm stained brown. In the few instances of questionably positive cells, every other such cell was counted. 3 sections per slide were chosen for counting. For each section counted on a slide totals were determined for each region. An average was calculated for each region per slide. The slide averages were totaled and that determined the totals for each region.

Point counting methods were attempted, but due to the small numbers of pancreata used and because of the sparseness of PP cells not enough data could be collected to produce statistically significant results.

Results. The method of placing the tissues on wooden blocks for fixation proved to be invaluable for studying the 3 regions of the fetal pancreas. The result was a flat preparation which could be consistently embedded, sectioned and stained with ease. The wood block also facilitated regional determinations since the entire pancreas was on a slide, and the duodenum and spleen could be used as markers for the regions.

When considering numbers of BPP (which will be referred to as PP cells) positive cells per region the head was found to contain the greatest number of PP cells and the body second. The tail contained the smallest number of positive cells (table).

The PP cells in the head region were found to be scattered throughout (fig. 2). The numbers of PP cells in the body appeared to be localized around the distal duodenum with a few cells that were less darkly stained scattered towards the splenic portions (compare figs 3 and 5–7). The numbers of PP cells in the tail were evenly distributed throughout the region with no portion having a greater concentration than any other (fig. 4).

Discussion. The tissues that were fixed and then divided were too brittle to yield reasonably intact regional divisions. The tissues that were divided and then fixed gave inconstant results, indicating the difficulty in making dupli-

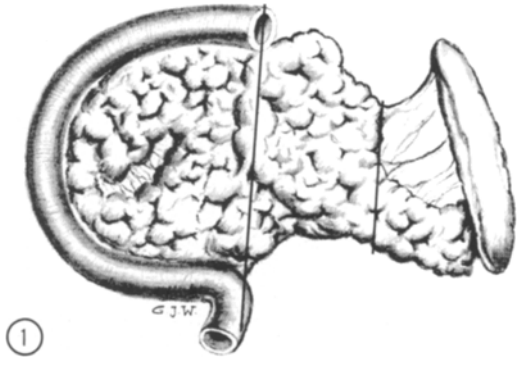

Figure 1. Schematic drawing of a 21-day fetal rat pancreas. Lines indicate regional divisions; left to right: head, body, and tail.

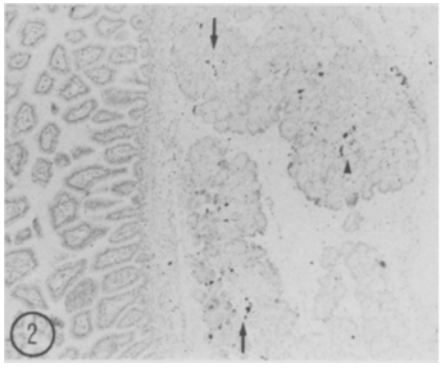

Figure 2. Low power of a portion of the head region. Several islets with positive PP cells are located between the arrows. Clusters of other PP cells can be seen; 1 is indicated by an arrowhead. × 80.

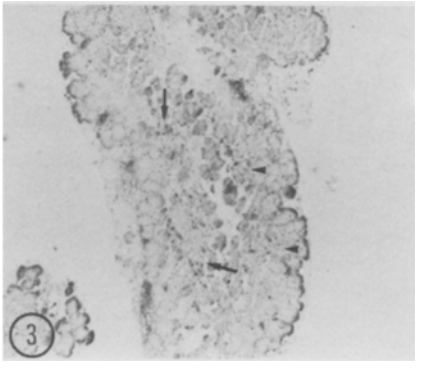

Figure 3. Low power of a portion of the body closer to the splenic end. Islets with positive PP cells are indicated between the arrows. Clusters of other PP cells can be seen; 2 are indicated by arrowheads. A dark border can be seen (e.g., right margin of larger tissue). This border is due to an uptake of the eosin Y counterstain and does not indicate a positive reaction product of the PAP technique. The same staining reaction is present in figures 4, 5, and 7. × 48.

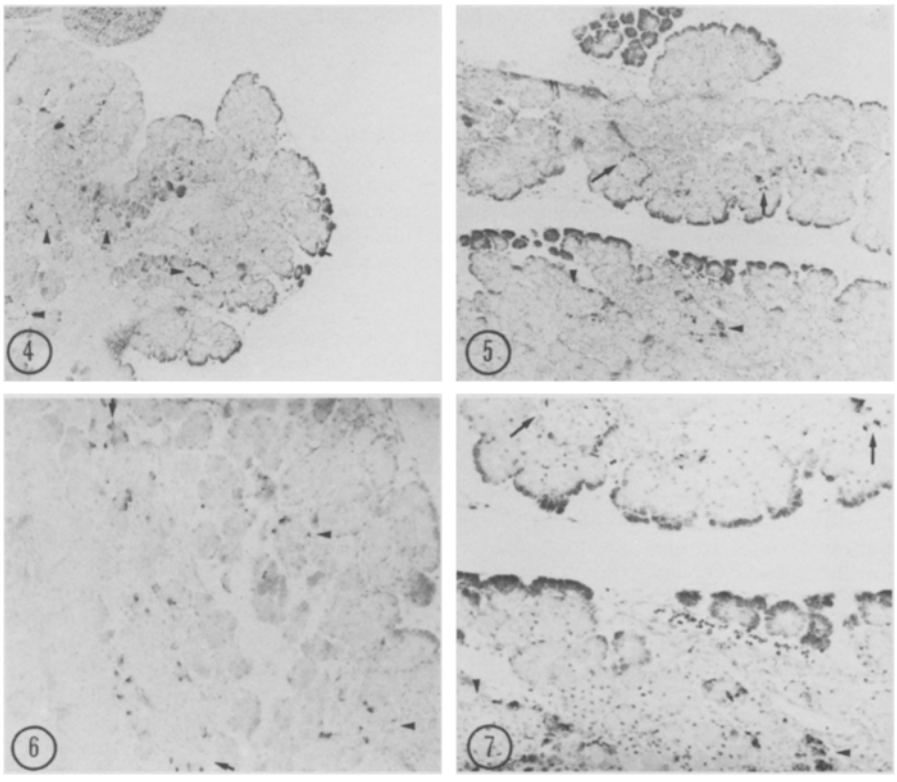

Figure 4. Low power of a portion of the tail region. Some islets containing positive staining PP cells are indicated by arrowheads. × 64.

Figure 5. Low power of a portion of the body closest to the distal duodenum. Islets with positive PP cells are indicated between arrows and arrowheads. × 48.

Figure 6. Higher magnification of figure 3 showing the same groups of islets (arrows and arrowheads). Note the lesser degree of positive staining PP cells. × 80.

Figure 7. Higher magnification of figure 5 demonstrating the same islets as indicated. Note the degree of positive staining PP cells. × 80.

cate cuts in delicate fetal tissue. The tissues that were pinned en bloc to blocks and then fixed yielded a flat preparation that could be consistently embedded, sectioned and stained. In these preparations the division into regions was facilitated by having both duodenum and spleen attached to the pancreas.

Our results demonstrate that the majority of PP cells are localized in the head. This is in agreement with results reported for the adult rat[8], mouse[7], dog[12,14], and human[12]. Larsson et al.[11] found that PP cells in the adult rat were about equally numerous in the tail of the pancreas and in the duodenal lobe. Sundler et al.[7] found that PP cells were somewhat more numerous in islets of the duodenal portion than the splenic lobe. Larsson et al.[11] also found PP cells to be less numerous in the body. Orci et al.[9] found the PP cells of adult rats to be in the lower part of the head in islets with very few or no glucagon containing cells as opposed to islets in the body which had very few or no PP cells and the 'normal' rim of glucagon cells. These observations by Orci et al.[9] and the fact that in our study PP cells were found to be concentrated in the head region and in that part of the body closest to the distal duodenum may offer morphological support for the proposed physiological functions of PP on the digestive tract.

PP cells have been shown to increase in quantity in obese-hyperglycemic mice[5], in alloxan treated rats[7], and in spontaneously diabetic mice[10]. Because of this relationship, care should be taken in selecting regions of the pancreas for islet donation and ultimately for transplantation. It should be determined beforehand whether an increased population of PP cells is advantageous or detrimental to the organism receiving the transplant. To our knowledge no one has ever reported what combination of cell types is best for transplantation, or whether the pure beta cell transplant is the only consideration. Perhaps this needs to be reconsidered now that there are 4 known hormones for the pancreatic islet.

That our results appear to be in agreement with the proportional distribution of PP cells in the adult seems to support the premise that the fetal pattern sets the plan for what follows in the adult. This, however, does not mean that the neonatal pancreas should not be studied at all, for it may be important to compare the normal pattern at this period with results under experimental conditions.

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2 R.F. Beers and E.G. Bassett, Miles international symposium series No. 12, p. 423. Raven Press, New York 1979.
3 G.R. Greenberg, R.F. McCloy, V.S. Chadwich, T.E. Adrian, J.H. Baron and S.R. Bloom, Dig. Dis. Sci. *24*, 11 (1979).
4 I.L. Taylor, Ann. intern. Med. *90*, 817 (1979).
5 R.L. Gingerich, D.J. Gersell, M.H. Greider, E.H. Finke and P.E. Lacy, Metabolism *27*, 1526 (1978).
6 J.C. Floyd, S.S. Fajans and S. Pek, Rec. Prog. Horm. Res. *33*, 519 (1977).
7 F. Sundler, R. Håkanson, I. Lundquist and L.-I. Larsson, Cell Tissue Res. *178*, 307 (1977).
8 I. Lundquist, F. Sundler, B. Ahrén, J. Alumets and R. Håkanson, Endocrinology *104*, 832 (1979).
9 L. Orci, D. Baetens, M. Ravazzola, Y. Stefan and F. Malaisse-Lagae, Life Sci. *19*, 1811 (1976).
10 D. Baetens, Y. Stefan, M. Ravazzola, F. Malaisse-Lagae, D.L. Coleman and L. Orci, Diabetes *27*, 1 (1978).
11 L.-I. Larsson, F. Sundler and R. Håkanson, Diabetologia *12*, 211 (1976).
12 D.J. Gersell, R.L. Gingerich and M.H. Greider, Diabetes *28*, 11 (1978).
13 L.A. Sternberger, P.H. Hardy, Jr, J.J. Cuculisand and H.G. Meyer, J. Histochem. Cytochem. *18*, 315 (1970).
14 W.G. Forssmann, V. Helmstaedter, J. Metz, J. Greenberg and R.E. Chance, Histochemistry *50*, 281 (1977).