An experimental investigation into the possible neural crest origin of pancreatic APUD (islet) cells

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SUMMARY

It has recently been contended that pancreatic APUD cells are neural crest derivatives. In an experimental investigation, isotopic grafts of neural tube containing neural crest cells were transplanted from chick and quail embryos labelled with tritiated thymidine, and from unlabelled quail embryos, to host chick embryos at the same stage of development. Transplantations were performed at various levels between somites 5 and 24 in embryos at 6- to 24-somite stages. In operated embryos at 3½ days of incubation, the pancreatic APUD cells were not labelled; nor did their nuclei show quail features. Migration of cells from the graft was evidenced by the presence of quail nuclei and/or radioactive label in autoradiographs, in spinal and sympathetic ganglia in the operated region.

It is concluded that the pancreatic APUD cells of the 3½-day-old chick embryo are not derived from the trunk neural crest up to the level of somite 24. It is unlikely that more caudal levels contribute, because APUD cells are already concentrated in the dorsal pancreatic bud region at the 24-somite stage, by which time no migration of crest cells has occurred caudal to somite 24. This conclusion probably concerns A, B and D pancreatic endocrine cells.

INTRODUCTION

It has long been generally accepted that the cells of pancreatic islets are of endodermal origin (see for instance, Liegner, 1932): they and the exocrine cells are thought to differentiate from common 'protodifferentiated' endoderm cells (Pictet & Rutter, 1972). Descriptions abound of the differentiation of islet cells from primitive cell cords, ductules, acini and/or centro-acinar cells in the endodermal pancreatic buds in fish, amphibians, birds and mammals (Liegner, 1932; Villamil, 1942; Hard, 1944; Bencosme, 1955; Frye, 1958; Robb, 1961; Hellman, 1966; Przbylski, 1967; Like & Orci, 1972; Pictet, Clark, Williams & Rutter, 1972; Epple & Lewis, 1973; Schweisthal & Frost, 1973; Belsare, 1974).

The only suggestion that islet cells may be mesodermal has come from Wessels (1968) who, in the mouse, identified islet cells first at the endoderm–mesoderm

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interface of the pancreatic anlage. He commented that it was not clear whether they originated in the endoderm or the mesoderm.

Other workers have postulated a developmental relationship between islet cells and endoderm cells of the gastro-intestinal tract. Feyrter (1943) suggested that islet cells and enterochromaffin cells might share similar progenitor cells; Adelson (1971) regarded the protein-secreting ability common to islet and gut endocrine cells as supporting a common endodermal origin. Recognizing the same similarity, Pearse and his co-workers have proposed that the neural crest is the source (Pearse, 1969; Pearse & Polak, 1971; Polak, Rost & Pearse, 1971; Pearse, Polak & Bussolati, 1972), though leaving open the possibility that not all the pancreatic endocrine cells are so derived (Pearse & Polak, 1971; Pearse, 1973; Pearse & Takor Takor, 1975). This proposal is contributory to the concept that APUD cells in general are neural crest derivatives (Pearse, 1966; Pearse & Welsch, 1968; Pearse & Takor Takor, 1975). That pancreatic islet cells of one or other type belong to Pearse's APUD (Amine Precursor Uptake and Decarboxylation) cell series (Pearse, 1966) has been well established (Falck & Hellman, 1963; Cegrell, 1967, 1968; Cegrell, Falck & Rosengren, 1967; Legg, 1968; Trandaburu, 1972).

Like & Orci (1972) have pointed out that already in the pancreatic anlage there may be cells committed to become islet cells, but they commented that there was no evidence to justify claims that these were of neural crest or even of endodermal origin. Epple & Lewis (1973), though favouring the neural crest as the source, also recognized that no satisfactory evidence was yet available. So far, except in the recent work of Phelps (1975), the grounds for the various proposed sources of islet cells have been morphological observations made at successive developmental stages. Clearly, experimental evidence is more convincing.

The present study, of which a brief account has already appeared (Andrew, 1976a), is an experimental investigation into the question of whether or not islet cells arise from the neural crest. Lengths of neural tube and neural crest were removed from chick embryos, and replaced by neural tube containing neural crest marked so that it could be recognized at later stages; the differentiated pancreatic APUD (islet) cells were subsequently examined for the neural crest marker.

MATERIALS AND METHODS

Two methods were used to render neural crest cells distinguishable after their migration away from the neural tube. Grafts of neural tube together with neural crest were prepared from chick embryos labelled with tritiated thymidine (a method used by Weston, 1963) or from quail embryos. Le Douarin (1971) has shown that the nuclei of cells of the Japanese quail, Coturnix coturnix japonica, carry large Feulgen-positive nucleoli and are thus easily distinguished from chick cells.
Origin of pancreatic APUD (islet) cells

Following in general the procedures used by Weston (1963) and Le Douarin & Teillet (1973), grafts from labelled Black Australorp chick embryos or from labelled or unlabelled embryos of the African quail, Coturnix coturnix africana, were transplanted to host Black Australorp embryos in which the corresponding segment of the neural tube had been removed. In each case, the donor and the host embryo were at the same stage of development. The operated embryos were incubated until 3½ days old, and then treated with the amine precursor dihydroxyphenylalanine (DOPA) in preparation for the demonstration of the APUD reaction in sections. After photographing the APUD cells in the dorsal pancreatic bud, autoradiographs were prepared and/or staining by the Feulgen method was carried out, so that cells of grafted neural crest origin could be recognized by means of radioactive label and/or quail nuclear features. Evidence for migration of neural crest cells from the graft was sought in the presence of marked spinal and sympathetic ganglia in the operated region.

Various preliminary and control procedures were necessary to ascertain that (1) the African quail also has nuclei clearly distinct from chick nuclei in the structures to be examined; (2) at the stage of sacrifice of the operated embryos, radioactive labelling is still adequate in the grafted neural tube, spinal and sympathetic ganglia and pancreatic APUD cells, of chick and quail embryos; (3) at this time, cells giving the APUD reaction have differentiated in the pancreas of both species; (4) pancreatic APUD cells do not show autofluorescence; (5) none of the structures to be examined in autoradiographs show either positive or negative chemography; (6) the techniques for the demonstration of the APUD reaction, of radioactive label and of nuclear characteristics do not interfere with one another; and (7) the operation does not affect the normal development of the pancreas, including its ability to synthesize dopamine from DOPA (APUD reaction). To serve these purposes, operated embryos which had received unlabelled chick grafts, unlabelled quail grafts, labelled chick grafts or labelled quail grafts, as well as unoperated embryos, chick and quail, labelled and unlabelled, were subjected to the various procedures severally or jointly.

Eggs were incubated at 38.5 °C. All micro-surgical procedures were carried out under sterile conditions. Chick Ringer's solution contained antibiotics as described previously (Andrew, 1963).

Labelling of embryos

Preliminary tests showed that a dose of 10 μc of tritiated thymidine (thymidine-6-H³, TRA 61, specific activity 5 Ci/mmol; Amersham) had no deleterious effect on development. The isotope was injected in 100 μl Ringer's solution through a small window in the shell, into the air-space of eggs incubated for 24 h, blunt end upwards. The window was sealed with Sellotape. Survival of 24-h embryos was better than if the isotope was injected directly on to the blastoderm. Undoubtedly some embryos were not centrally situated under the
air-space: these probably took up less isotope, as found by Mawhinney, Austin & Riley (1972).

Isotope was administered 14 to 24 h before donor embryos were used for transplantation: practically all nuclei became labelled overnight. Labelled unoperated chick and quail embryos showed well-labelled nuclei until 5 days of incubation.

**DOPA administration and the formaldehyde-induced fluorescence (FIF) procedure**

DOPA was administered to embryos incubated for $3\frac{1}{2}$ days. A dose of 150 μg DL-DOPA (B.D.H.) dissolved in 150 μl warm Ringer's solution was injected through a window in the shell on to the surface of the embryo. The window was sealed and the egg returned to the incubator. (Some control embryos received no DOPA.)

A portion of the trunk was excised, 1–1 ½ h later, to include the pancreas and the operated region of the neural tube. This was left in pre-warmed Ringer's solution containing 100 μg/ml DOPA in the incubator for up to 15 min. The specimens were then washed in fresh pure Ringer's solution, quenched, freeze-dried and fixed in hot formaldehyde vapour as described elsewhere (Andrew, 1975); the duration of fixation was 1–1 ½ h. (Some control specimens were not subjected to vapour fixation.) The specimens were embedded in paraplast (m.p. 58 °C) in vacuo, serially sectioned at 8 μm and dry-mounted on lightly albumenized slides. Wax was allowed to drain off the slides overnight in an oven at 60 °C. Slides were stored as previously (Andrew, 1975).

Sections were examined in xylol under blue light bright field illumination on a Reichert Fluoropan microscope equipped with an HBO 50 mercury burner. A 6 BG 12/h exciter filter and appropriate absorption filter were used.

**Autoradiography**

Originally, de-waxed sections were hydrated before dipping in emulsion: subsequently it was found that a more even layer of emulsion was produced on slides allowed to air-dry immediately before dipping. They were de-waxed, transferred to absolute ethanol and then exposed to the air. The drying procedure had no deleterious effects on the sections.

The emulsion used was Ilford K2; the method followed that of Rogers (1967). In a test for negative chemography, several slides in each batch were exposed to light after dipping; dipped slides of unlabelled tissue served as tests for positive chemography. Both procedures are essential for valid interpretation of autoradiographs (Rogers, 1967). All the slides (including the chemography controls) were exposed in the dark at 4 °C. An exposure of 17 days was usually adequate. The autoradiographs were developed in D 163 (Kodak); the method was that recommended by Rogers (1967).
Origin of pancreatic APUD (islet) cells

Staining

Autoradiographs of sections of control chick embryos and of operated embryos which had received chick grafts of labelled tissue were lightly stained with haematoxylin. Autoradiographs of sectioned control quail and some chick embryos, and of operated embryos which had received quail grafts, were examined before staining with the Feulgen method for DNA (Pearse, 1960). This was found necessary because the hydrolysis involved, caused removal or re-distribution of some of the developed silver grains, as was shown on clean slides (without sections) dipped, exposed to light and then developed and subjected to hydrolysis.

Photography

Photographs of fluorescent cells in the dorsal pancreatic bud were taken on the fluorescence microscope with Kodak Tri-X Pan film (ASA 400) at an exposure of 10 secs. As a rule, every second or third section showing the fluorescent cells was photographed.

Transplantation

Segments of neural tube with accompanying neural crest cells were transplanted from radioactively labelled chick or quail embryos, unlabelled quail embryos, and occasionally from unlabelled chick embryos, to the same somite levels of chick embryos at the same stage of development. (Unlabelled grafts from chick embryos served as controls for positive chemography.) The levels of the transplant were determined on the basis of morphological studies of the stage of neural crest formation at different somite levels in Black Australorp chick embryos of relevant stages (Andrew, 1963). The levels selected for transplantation were those at which the crests had not yet formed as such, or at which no migration from the crests proper had yet taken place. In a 12-somite embryo for example, levels caudal to somite 8 fulfil these criteria; in a 16-somite embryo, levels caudal to somite 13. In practice a safety margin of two or more somites’ width was usually allowed. In order to vary the levels transplanted, embryos were used as donors at stages between 6 and 20 somites. The most likely level of origin of pancreatic islet cells seemed that at which the duodenum develops, i.e. somites 8 to 15 (Le Douarin, 1961). The levels of neural transplants were therefore at first concentrated on these and then on adjoining levels (see Table 2).

The grafts varied from 4- to 8-somites’ length: where they extended into the post-somitic region, the future somite level at their caudal end was estimated. The estimates were checked on sacrifice whenever the caudal end of the graft could be identified, and were found to be accurate.

The desired length of neural tube was excised together with underlying notochord and endoderm. It was transferred to 0.1% trypsin made up in calcium- and magnesium-free Ringer’s solution containing neutral red (2 x 10^{-4} mg/ml)
for 2 min at 37.5 °C, and then to warm calcium- and magnesium-free Ringer’s solution with neutral red for 2 min. Radioactively-labelled tissue was well rinsed in ‘cold’ thymidine in Ringer’s solution (4 mg/ml). The neural tube was dissected clear of adherent tissue. More latterly, a transverse strip of blastoderm bounded by the desired rostral and caudal levels of the graft was transferred to a 0.15 % trypsin solution otherwise made up as above, at room temperature, and placed in a refrigerator at 2 °C for 15 mins, according to the method used by Le Douarin & Teillet (1973). Further steps were carried out as above. The notochord is tightly adherent to the neural tube, so its removal sometimes results in slitting of the neural tube. Therefore, occasionally small fragments of notochord were left on the graft.

Likewise, in preparing the graft site in host embryos, tiny tags of the floor of the neural tube were sometimes left behind. The graft was manoeuvred into position in the prepared site by means of a very fine glass thread with a rounded tip. After the window in the shell of the host egg had been sealed, it was returned to the incubator. At one time, the eggs were placed straight away on turntables in the incubator (see below). However, when it was discovered that grafts were sometimes dislodged, operated eggs were left stationary in the incubator for an hour before being placed on the turntables. They remained there overnight. During further incubation to reach a total incubation age of 3½ days, the eggs were stationary.

Rotation of operated eggs

Survival of operated embryos was vastly improved by incubating the eggs horizontally on individual turntables which rotated alternately clockwise and anticlockwise through 90° around a vertical axis at 8 cycles per minute. These were a modification of the apparatus designed by Silver (1960). Probably the movement was effective because it prevented adherence of the blastoderm to the edges of the window.

RESULTS

Large Feulgen-positive nucleoli were evident in the cells of the neural tube, spinal and sympathetic ganglia, and all cells of the dorsal pancreatic bud in three 3½-day-old embryos of the African quail to which DOPA had been administered and in all of which the FIF procedure revealed fluorescent pancreatic APUD cells. Fig. 2 illustrates this nuclear feature in the same pancreatic cells shown to be fluorescent in Fig. 1. The cells in chick embryos, including those of the pancreas and the others to be examined in this study, show only small Feulgen-positive karyosomes, rather indistinct in the freeze-dried tissue. Comparison of the relevant chick and quail cells confirmed the reliability of the nuclear marker for this experiment.

Two chick and two quail embryos each labelled with 10 μCi H³-thymidine in the same way as donor embryos, each given DOPA at 3½ days of incubation
Sections through the dorsal pancreas of normal (unoperated) 3½-day-old embryos.

Figs. 1, 2. Adjoining sections of a quail embryo.
Fig. 1. DOPA-provoked FIF in pancreatic APUD cells. × 420.
Fig. 2. Large Feulgen-positive nucleoli in the pancreatic APUD cells. × 420.

Figs. 3, 4. The same section of a labelled quail embryo.
Fig. 3. DOPA-provoked FIF in pancreatic APUD cells. × 420.
Fig. 4. Autoradiograph showing label in the pancreatic APUD cells × 420.

Fig. 5. DOPA-provoked FIF in pancreatic APUD cells of an unlabelled chick embryo. × 210.
and submitted to the FIF procedure followed by autoradiography, showed adequate isotope labelling in the neural tube, spinal and sympathetic ganglia and pancreatic APUD cells (Figs. 3, 4), provided observations on embryos with quail grafts were made prior to Feulgen staining. Labelling of pancreatic cells was particularly intense. Development of all these structures was normal in the labelled embryos.

Under the same conditions, none of the relevant structures showed negative chemography in the normal chick and quail embryos tested; nor did positive chemography occur over sections of comparable unlabelled embryos. Examination of autoradiographs of operated embryos, four of which had received chick grafts and two, quail grafts, confirmed that positive chemography was no problem. Nor were any signs of negative chemography present in the test slides of operated embryos with labelled grafts, except for one specimen, which was discarded.

The necessity for administration of DOPA for demonstration of the APUD reaction of pancreatic cells at 3½ days was confirmed for chick embryos (see Andrew, 1975) and shown for quail embryos. Five embryos of each species treated with DOPA showed fluorescence of the cells, whereas in three chick and two quail unoperated embryos, and one operated chick embryo, none of which was given DOPA, fluorescence was lacking. In three chick and two quail unoperated embryos and two operated (chick) embryos all treated with DOPA, but not subjected to formaldehyde vapour fixation, there was no autofluorescence in any pancreatic cells. The presence of isotope did not affect the above results.

Pancreatic APUD cell development (Figs. 1, 3, 5) in five unoperated chick and five unoperated quail embryos showed 3½ days of incubation to be a suitable time for sacrifice in relation to the duration of radioactive label. APUD cells are present in the chick dorsal pancreatic bud considerably earlier, at the time of its evagination at or shortly before the 27-somite stage (Andrew, 1975); in quails they are already present at the 31-somite stage. Even if surgical intervention had retarded development, pancreatic APUD cells should therefore still have been demonstrable, though in smaller numbers.

Operated embryos

Transplantations were performed on 128 embryos, of which 73 % survived. Three were discarded due to poor or abnormal development, one due to negative chemography, and a few were spoilt during technical procedures. Of the rest, labelled chick grafts had been transplanted to 40, labelled quail grafts to 26, unlabelled quail grafts to 12 and unlabelled chick grafts to 6.

In most cases, the grafts healed well in the host embryos. Small fragments of donor notochord were occasionally identified in sectioned operated embryos, and in two cases only, a little donor mesoderm adjacent to the graft. More often, remnants of host neural tube were present in the operated region, generally ventral to the grafted tube. The latter was usually well-formed, though junctions
Table 1. *The absence of graft-derived cells among pancreatic APUD cells*

<table>
<thead>
<tr>
<th>Presence of ganglia in operated region</th>
<th>Grafts</th>
<th>Pancreas</th>
<th>Labelled and/or quail nuclei in APUD cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal and sympathetic</td>
<td>13</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Spinal or sympathetic</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total no. of embryos</td>
<td>14</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

The figures represent numbers of successfully operated embryos.

Figs. 6, 7. Autoradiograph of a 3½-day-old embryo which had received a labelled chick graft at the levels of somites 16–23 at the 17-somite stage. In order to show the silver grains, the tissue is slightly out of focus.

Fig. 6. Labelled grafted neural tube (NT) and labelled spinal ganglion (SG). × 620.

Fig. 7. Labelled sympathetic ganglion (SyG). × 620.
Figs. 8–11. Sections through the dorsal pancreas of 3½-day-old operated embryos.

Figs. 8, 9. The same section through an embryo which had received a labelled chick graft at the levels of somites 16–23 at the 17-somite stage.

Fig. 8. DOPA-provoked FIF in pancreatic APUD cells. ×420.

Fig. 9. Autoradiograph showing no label in the pancreatic APUD cells. Only a few background silver grains are present. ×420.

Figs. 10, 11. The same section of an embryo which had received an unlabelled quail graft at the levels of somites 17–22 at the 17-somite stage. ×420.

Fig. 10. DOPA-provoked FIF in pancreatic APUD cells.

Fig. 11. Lack of large Feulgen-positive nucleoli in the pancreatic APUD cells. ×420.

with the host tube were not always neat. In all but a few cases, development of the pancreas was normal (Tables 1, 3; Figs. 8–11). The general appearance and stage of development of successfully operated embryos (for criteria see below) at 3½ days of incubation were almost always normal.

In eight embryos with well-formed chick grafts and six with well-developed quail grafts, the intensity of radioactive labelling in autoradiographs of the donor neural tube was considered inadequate. Those which bore quail grafts are considered in Table 1, together with the embryos having unlabelled quail grafts. The embryos with chick grafts could not be used.
Table 2. Successful transplantation or deletion of neural tube and crest at various levels

<table>
<thead>
<tr>
<th>Somite level</th>
<th>No. of times transplanted</th>
<th>No. of times deleted</th>
<th>Somite level</th>
<th>No. of times transplanted</th>
<th>No. of times deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>—</td>
<td>16</td>
<td>13</td>
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<td>13</td>
<td>4</td>
<td>30</td>
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<td>1</td>
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</table>

Table 3. Pancreatic APUD cells in embryos lacking neural crest at various levels

<table>
<thead>
<tr>
<th>Total no. of embryos</th>
<th>Ganglia present in operated region</th>
<th>Pancreas</th>
<th>Fluorescent APUD cells present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spinal</td>
<td>Sympathetic</td>
<td>Well developed</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

The figures represent numbers of embryos.

Operations were regarded as successful if there was good evidence of migration of graft cells from the transplant. Of the 40 such embryos, at least some spinal ganglia and/or sympathetic ganglia were present in the operated region (Table 1); they showed the markets of the grafted tissue, i.e. radioactive label (Figs. 6, 7) and/or quail nuclei. In many of these, migration was normal or almost normal. In most cases, neurilemmal (Schwann) cells of graft origin were identified in the spinal nerve roots. In a further eight operated embryos there was little or no evidence of cell migration from the graft. These are not included among the successful operations and are not shown in Table 2. In 36 of the 40 successful cases, FIF was demonstrated in normal numbers of cells in the dorsal pancreatic bud (Figs. 8, 10). In none of these did any pancreatic APUD cells show radioactive label (Fig. 9), nor did any have nuclei with quail features (Fig. 11; Table 1).

The number of times neural tube and neural crest of each somite level were
included in a transplant is recorded in Table 2. Levels from somite 5 to somite 24 are well-represented.

The grafted pieces of neural tube escaped from the site of transplantation in eight embryos. These may be regarded as embryos from which the neural crest was deleted at the operated levels. The somite levels affected by such deletions are listed in Table 2. Spinal and sympathetic ganglia were very seldom present in the operated regions. FIF revealed normal development of APUD cells in all except one of these embryos (Table 3).

**DISCUSSION**

In this experiment, it was vital that any migration of host neural crest cells from operated levels to the pancreas should be forestalled. Previous experiments showed that the grounds for the selection of levels for transplantation in embryos at the various stages (see Materials and Methods) was sound: chorio-allantoic grafts of blastoderm designed – on the same basis – to exclude neural crest, were shown to be free of crest derivatives (Andrew, 1963).

In operated embryos in which a radioactively labelled graft was recognizable at the time of sacrifice, label was nevertheless absent from the pancreatic APUD cells. This was not attributable to dilution of label in these cells as they are well-labelled in normal embryos; it was not due to negative chemography, nor to interference with latent image formation by the APUD–FIF procedure. Since quail cell nuclei are markedly different from chick nuclei in pancreatic APUD cells of normal embryos, the absence of quail features was a reliable indication that no graft cells had entered the pancreas in those operated embryos bearing quail grafts. The development of the operated embryos, and of the pancreas and the pancreatic APUD cells in particular, were unaffected by the experimental procedure in all but a few cases, and the cells appeared to be present in normal numbers.

In operated embryos, normal migration of neural crest cells did not always occur. Only embryos were scored which showed evidence of migration of marked cells to form known derivatives of the neural crest and perhaps neural tube, i.e. spinal and sympathetic ganglia, and neurilemmal cells. As judged by these criteria, the migration of neural crest cells from quail grafts was as extensive as from chick grafts. It is apparent from many similar experiments of Le Douarin and her co-workers, that isotopic quail to chick grafts are prolific in the formation of migratory neural crest cells (Le Douarin & Teillet, 1970, 1973, 1974; Le Lièvre & Le Douarin, 1975).

In the operated embryos, the transplants represented the levels between somites 5 and 24 well enough to justify the conclusion that the pancreatic APUD cells present on sacrifice were not derived from these levels of the neural crest.

The presence of apparently normal numbers of pancreatic APUD cells in the embryos lacking segments of trunk neural crest is in line with the above
Origin of pancreatic APUD (islet) cells

It seems unlikely that levels of the crest caudal to somite 24 give rise to pancreatic APUD cells. At the 24-somite stage (about 2 days of incubation), when the evagination of the dorsal pancreatic bud is imminent, APUD cells are already present in the region which will evaginate (Andrew, 1975). No migration of crest cells occurs at post-somitic levels (see Andrew, 1963). Therefore, at least those APUD cells present at the 24-somite stage could not have arisen from the neural crest of levels caudal to somite 24. From previous morphological observations, it seems that APUD cells appearing in the primitive gut groove from the 16-somite stage are the progenitors of pancreatic APUD cells, since they appear to aggregate in the presumptive dorsal pancreatic region (Andrew, 1975). It is therefore more likely that the APUD cells in the dorsal bud when it evaginates will proliferate to form the full complement of pancreatic APUD progenitor cells, than that others arrive there later from caudal levels of the neural crest.

Rhombencephalic levels of the neural crest are still to be tested. Pearse (1973) on one occasion mentioned these levels of the crest as the source of pancreatic APUD cells, but gave no reason for doing so. Certainly neural crest cells from hindbrain levels do reach the gastro-intestinal tract caudal to the pancreas (where they become enteric ganglion cells (Andrew, 1969; Le Douarin & Teillet, 1973)).

The APUD cells present in the chick pancreas at 3½ days of incubation probably include A, B and D cells. In different species, different islet cell types are APUD (see Andrew, 1975): in chick embryos of between 9 and 18 days of incubation all three types are APUD (Andrew, unpublished observations). A and B cells have been identified in the dorsal pancreatic bud shortly before 3½ days by electron microscopy (Dieterlen-Lievre, 1965; Przbylski, 1967) and D cells at the 31-somite stage (2½ days of incubation) by light microscopy (Andrew, unpublished observations). It is therefore likely that A, B and D cells may be included among the APUD cells of the present study, but direct evidence is still required.

Completely in line with the results of this investigation are the findings of Phelps (1975). He has shown experimentally that B cells in the rat pancreas are not derived from the neural crest. He cultured endoderm and mesoderm from which ectoderm had been removed before formation of the neural crests. Insulin was detected in the differentiated pancreas; B cells were identified ultrastructurally.

Pearse & Polak (1971) proposed that APUD cells in the mouse pancreas are derived from the neural crest, because they saw cells showing DOPA-provoked conclusion. Alone, such evidence would be inconclusive, because neural crest from adjoining areas is known to migrate into levels from which the crests have been deleted (see Andrew, 1971). The rare occurrence of spinal and sympathetic ganglia at levels deprived of neural crest, however, suggests only limited penetration from adjoining crests.

The APUD cells present in the chick pancreas at 3½ days of incubation probably include A, B and D cells. In different species, different islet cell types are APUD (see Andrew, 1975): in chick embryos of between 9 and 18 days of incubation all three types are APUD (Andrew, unpublished observations). A and B cells have been identified in the dorsal pancreatic bud shortly before 3½ days by electron microscopy (Dieterlen-Lievre, 1965; Przbylski, 1967) and D cells at the 31-somite stage (2½ days of incubation) by light microscopy (Andrew, unpublished observations). It is therefore likely that A, B and D cells may be included among the APUD cells of the present study, but direct evidence is still required.

Completely in line with the results of this investigation are the findings of Phelps (1975). He has shown experimentally that B cells in the rat pancreas are not derived from the neural crest. He cultured endoderm and mesoderm from which ectoderm had been removed before formation of the neural crests. Insulin was detected in the differentiated pancreas; B cells were identified ultrastructurally.

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FIF first in a position attributed to the central stream of the neural crest cells, in the mesenchyme between the neural tube and the pharynx, then in the pharynx, and later in the dorsal pancreatic bud. The earliest APUD cells illustrated seem to the author to be in too large a mass, and to lie too far laterally, to be neural crest cells. Also, although observations made on cells with the same features at successive stages of development are highly suggestive evidence that the cells follow the route of migration mapped out in this way, such evidence alone is not conclusive.

Later, Pearse, Polak & Heath (1973) apparently abandoned the idea that crest cells invade the dorsal pancreatic bud directly, and traced pancreatic APUD cells in the mouse from the ‘primitive endocrine cell’ in the gut wall, claimed by Pearse & Polak (1971) to be of neural crest origin. Granules (described as pleomorphic), characteristic of these non-argentaffin APUD cells, were found later on in development in pancreatic cells, together with round granules regarded as characteristic of islet cells. The latter were also shown to be APUD cells. These findings tie in with the observations of the present author on the occurrence of APUD cells successively in the gut wall of chick embryos, the presumptive dorsal pancreas and the bud itself (see above) and are in accord with the suggestion that the differentiation of the islet cells in chick embryos may begin before evagination of the pancreatic bud (Przbylski, 1967). Pearse et al. (1973) conclude that the islet cells (all three types according to a later statement of Pearse, 1973), are derived from the neural crest. The present study does not support this contention for the trunk neural crest at least.

The pancreatic APUD cells referred to in the present investigation are localized in the dorsal pancreatic bud. None are present in the ventral buds before these fuse with the dorsal bud (Andrew, 1975). (Fusion occurs at a stage later than the time of sacrifice in this experiment.) Many workers maintain that islets arise only from the dorsal bud in vertebrates (see Gianelli, 1908; Wolf-Heidegger, 1936 – cited by Frye, 1962; Frye, 1962), though some attribute islet formation to ventral as well as dorsal buds (Hard, 1944, in the rat). From the distribution in the adult, Bencosme & Liepa (1955) contend that derivatives of both buds in the dog and cat form islets. However, islet cells arising from one bud could easily pass into and proliferate in the other after fusion. Extirpation and transplantation of buds, or of presumptive regions from which the buds arise, have produced evidence that the islets arise from the dorsal bud only, in amphibians (Frye, 1962) but from both dorsal and ventral buds in chicks (Sandstrom, 1934; Dieterlen-Lièvre, 1970).

The lack of APUD cells in chick ventral pancreatic buds seems to be at variance with participation of ventral buds in islet formation. If, however, the ventral buds do indeed contribute to the definitive islets, then it is clear that the present study has not dealt with their origin. It would be strange, though, if their source were different from those of the dorsal bud.

The conclusion reached from this experiment is that the pancreatic APUD
Origin of pancreatic APUD (islet) cells

(islet) cells present in the chick embryo at 3 days 18 h of incubation are not derived from the neural crest of the levels of somites 5 to 24, and probably not from more caudal levels. It is likely that these pancreatic APUD cells include A, B and D cells. The pathological significance of the embryological origin of APUD cells is discussed elsewhere (Andrew, 1976b).

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