THE RELATIONSHIP BETWEEN THE ALPHA AND BETA-CELLS IN THE ISLETS OF LANGERHANS OF THE ALBINO RAT *

MORPHOLOGY AND CYTOGENESIS

A. C. ESTERHUIZEN, M.Sc., *Department of Physiology, University of Stellenbosch*

'In spite of the voluminous literature on the morphology of the pancreas, there is as yet no agreement on such a fundamental point as the recognition of cell types in the islets of Langerhans' (Thomas[27] 1937). During the past 21 years a considerable additional number of papers dealing with this subject have been published; the problem, however, remains largely unsolved.

Thus, according to Thomas, 'A, B and D cells have been identified by their differential coloration and constant nuclear qualities in the islets of all forty-one species of mammals examined' (including 10 species of rodents), whereas Jewell and Charipper[17] could find no D-cells in the islets of the golden hamster. Gomori[8] stated that 'there is no accepted routine procedure by which the cell types in the pancreatic islets can be identified with invariable certainty' and proposed a new modification of the Mallory-Heidenhain-azan stain suitable for the study of the islet cells. This was followed (Gomori[9]) by an improvement of the chromium-haematoxylin phloxin method with which, according to Gomori, 'D-cells are indistinguishable from alphas.'

This lack of agreement on the recognition of cell types in the islets of Langerhans is overshadowed by the existing controversy on the *origin* of the cell types of the islets and the possible *transitions* between the different cell types found in the pancreas as a whole. The present investigation was undertaken in the hope that it would throw some light on these controversies.

MATERIAL AND METHODS

The animals used in this study were of the Wistar strain *Rattus norvegicus* bred in the Department of Physiology, University of Stellenbosch. For studying the different cell types in the islets of the albino rat, female animals weighing between 170-200 g. were used, except for 6 animals which were very young and weighed between 15-19 g. Some of these animals received injections of alloxan while others were

* Paper presented at 2nd anniversary of the Stellenbosch Medical School, Bellville, 5 September 1958.

made diabetic by repeated injections of dextrose solution over a period of 21 days.

Regarding the study of the histogenesis and cytogenesis of the islets, embryos were collected at 6 different stages of development. Females in oestrus were placed with male animals at 10 a.m. and separated into individual cages at 2 p.m. The age of the embryos were arbitrarily computed from 12 noon.

Animals were killed by a sudden blow on the head and the pancreas or embryos quickly removed. The embryos were immediately decapitated and placed in the fixing solution. All materials were fixed in Bouin-solution for 10-12 hours, washed in running water for 8 hours and imbedded in paraffin wax. The adult material was sectioned serially at 3μ and stained according to Gomori's modification of the Mallory-Heidenhain-azan stain. The embryos were sectioned serially at 5μ and stained according to Gomori's improvement of the chromium-haematoxylin-phloxin method.

RESULTS

Experiment I. An Investigation of the Different Cell Types found in the Islets of Langerhans of the Albino Rat.

In this experiment 30 rats were used. They were divided into 3 groups: Group A was the normal control group. The animals of group B were injected with a freshly prepared 3% solution of alloxan in distilled water. The animals of group C were injected with a 30% solution of chemically pure dextrose. The protocols of these animals are listed in Table I.

TABLE I. PROTOCOLS OF THE 30 RATS

Group	*Rat No.*	*Body Weight (g.)*	*Injection and Dose*	*No. of Islet Sections Investigated*	*State of Islets*	*Cell Types*
A	I	180	—	200	Normal	A & B
	II	176	—	200	,,	A & B
	III	170	—	200	,,	A & B
	IV	16	—	200	,,	A & B
	V	17	—	200	,,	A & B
	VI	170	—	18,206	,,	A & B
	VII	175	—	19,107	Some degenerated	A, B & D
	VIII	180	—	17,983	Normal	A & B
B	IX	175	Alloxan-	200	Some degenerated	A, B & D
	X	180	20 mg./kg.	200	,,	A, B & D
	XI	190	body weight	200	,,	A, B & D
	XII	185	3X daily	200	,,	A, B & D
	XIII	175	for 21 days	21,288	,,	A, B & D
	XIV	180	,,	25,180	,,	A, B & D
	XV	180	,,	21,906	,,	A, B & D
	XVI	170	,,	22,679	,,	A, B & D
	XVII	175	,,	21,469	,,	A, B & D
	XVIII	180	,,	24,302	,,	A, B & D
C	XIX	201	Dextrose-	200	Some degenerated	A, B & D
	XX	187	0·75 g./kg.	200	,,	A, B & D
	XXI	19	body weight	200	,,	A, B & D
	XXII	18	3X daily	200	,,	A, B & D
	XXIII	15	for 21 days	200	,,	A, B & D
	XXIV	18	,,	200	,,	A, B & D
	XXV	180	,,	23,741	,,	A, B & D
	XXVI	180	,,	22,730	,,	A, B & D
	XXVII	175	,,	22,139	,,	A, B & D
	XXVIII	190	,,	25,652	,,	A, B & D
	XXIX	175	,,	23,469	,,	A, B & D
	XXX	180	,,	22,804	,,	A, B & D

Two distinct cell types could be distinguished in the normal islets with the aid of Gomori's method (Fig. 1), except in rat No. VII (Table I) where a third type (which will be referred to as type D) was found. Although rat No. VII was included in this series as normal, some of the islets of Langerhans showed an advanced state of degeneration which was, however, only detected after the total number of islets in the pancreas was investigated (Fig. 2).

In the normal islet-section the central part is occupied by cells of irregular form with an average cross-section of 14 μ.

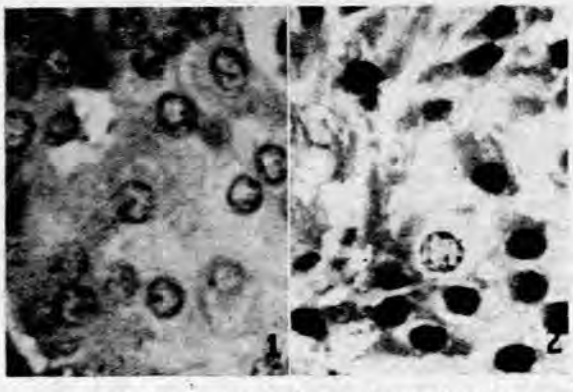

Fig. 1. Islet of Langerhans of normal rat. β-cells occupy the central part of the islet, while α-cells can be seen in the upper left-hand corner. X970. *Fig.* 2. Islet of rat No. VII (Table I). One 'D-cell' is shown among a number of degenerated β-cells with pyknotic nuclei. X970.

The cytoplasm stains a light orange-grey and is packed with fine dustlike cytoplasmic granules. These are considered to be β-cells. The second type is invariably found on the periphery of the islets, sometimes forming a complete ring encircling the β-cells, but often clumped together to form isolated groups on the periphery. They are on the average smaller than the β-cells, the cytoplasm stains somewhat more darkly and their cytoplasmic granules are more distinct with a faint red colour. These are considered to be α-cells. No difference in the structure of the nuclei of the α- and β-cells could be observed.

The 'D-cells' are characterized by being larger than both the α- and β-cells with an average cross-section of 18 μ. The nuclei are also larger with apparently less chromatin-substance, which is usually arranged in irregular clumps against the nuclear membrane. In some of these cells the nuclear membrane itself is damaged and karyorrhexis has taken place. The cytoplasm appears to be very faintly stained and not filled with granules. Some cells of this type were found with large portions of the cytoplasm totally devoid of granules. However, where present, these granules could not be distinguished from those of the normal β-cells. They were therefore considered to be β-cells which had undergone hydropic degeneration.

In some islets of the animals in groups B and C, as well as in some of rat No. VII (group A), a considerable number of cells with pyknotic nuclei was found. These cells were smaller than the normal α- or β-cells and were considered to be β-cells which had undergone a marked degree of *pyknotic degeneration*, in contradistinction to the *hydropic degeneration* of the 'D-cells'.

From these observations it must be quite clear that the *normal* islets of Langerhans of the albino rat are constituted of *two cell types* only, while in those made diabetic, either by injection of alloxan or by a persistent elevation of the blood sugar by repeated intraperitoneal injections of dextrose, a third cell type ('D-cells') is invariably encountered apart from the fully degenerated β-cells showing pyknotic nuclei (Figs. 3 and 4).

Experiment 2. *A Study of the Origin and Cytogenesis of the Islets of Langerhans*

Normal healthy female animals were paired with males and killed at different times after successful copulation had

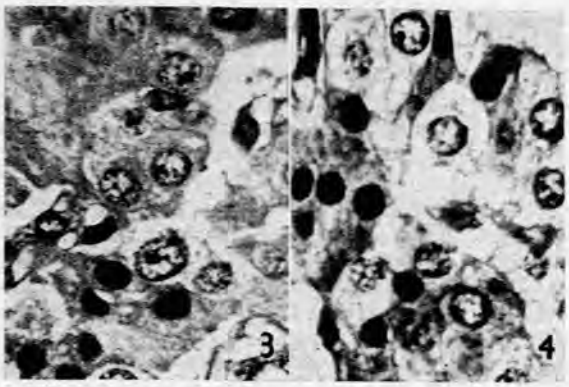

Fig. 3. Islet of alloxan-diabetic rat. Normal β-cells, 'D-cells' and degenerated β-cells with pyknotic nuclei can be distinguished. X970. *Fig.* 4. Islet of rat made diabetic by repeated injections of dextrose-solution; cells similar to Fig. 3. X970.

TABLE II. FINDINGS IN RAT EMBRYOS KILLED AT DIFFERENT TIMES

Rat No.	*Killed after*	*No. of Islets*	*Total No. of Islet-cross-sections*	*Average Islet-Vol. c.mm.* $\times 10^{-2}$	*Total Islet Vol c.mm.* $\times 10^{-2}$
XXXI	10¼ days	None	—	—	—
XXXII	14¼ days	,,	—	—	—
XXXIII	17¼ days	Islet-anlagen	—	—	—
XXXIV	19¼ days	178	3,557	0·52	93·0
XXXV	21 days	233	4,787	0·57	132·3
XXXVI	22¼ days	344	8,779	1·09	374·6

taken place (Table II). The embryos (one from each age-group) were serially sectioned at 5 μ and all the sections, after having been stained with Gomori's modification of the chromium-haematoxylin-phloxin method, were microscopically examined with the aid of the oil-immersion objective. The total number of islets as well as the total number of cross-sections through these islets were counted. From this the average volume of the islets was calculated from the formula $4/3\pi r^3$. The diameter (2·r) was obtained by dividing the number of islet cross-sections by the number of islets (assuming that the islets are all spherical) and multiplying by 5 μ i.e. the thickness of the sections. The following observations were made in this experiment:

At 10¼ *days.* At this stage the ventral and dorsal pancreatic anlagen have developed and grown to such an extent that they have come in contact with each other, although complete fusion has not yet occurred. The cells are arranged in cords, some of which show distinct lumina (tubules) while others are nothing but solid masses of undifferentiated cells. Mitotic figures are very conspicuous, especially in the cells bordering the lumina of the tubules or those situated centrally in the solid cell cords. At this stage no structures are present that can properly be designated *islets of Langerhans.*

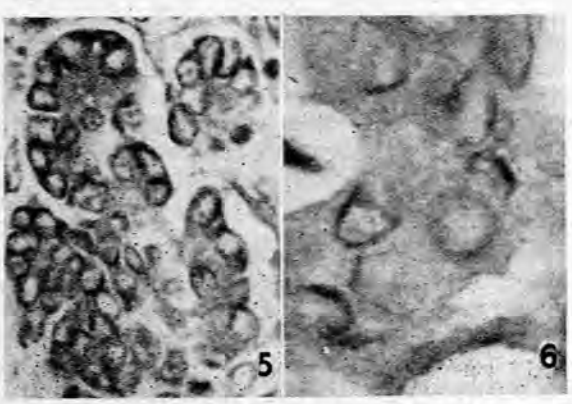

Fig. 5. Portion of pancreas of 17¼-day embryo. An acinus-like structure is seen with centrally-situated cells similar to the centro-acinar cells of the adult pancreas. X450. *Fig.* 6. 'Primitive islet' from 17¼-day embryo. It is seen in close relation to a pancreatic tubule. X970.

At 17¼ *days.* The pancreas at this stage consists chiefly of tubules and solid cell cords embedded in embryonic connective tissue. Occasionally a tubule (now a secretory duct) ends in an acinus-like structure with one or more centrally-situated cells similar to the centro-acinar cells of the adult pancreas (Fig. 5). Mitotic figures are often present in these cells. Dispersed among these structures are found a few irregular masses of cells that are considered anlagen for future islets of Langerhans. Fig. 6 shows one of these *primitive islets* in close relation to a pancreatic tubule. Some of the cells can best be described as medium-sized with irregular shape and finely granular cytoplasm resembling that of the β-cells of adult islets of Langerhans.

Apart from these 'islets' a few cell masses are present that could easily be erroneously designated *primitive islets.* They are, however, nothing but conglomerates of connective tissue cells, as judged by their cytoplasmic processes. As seen from Table II the 'islets' were not counted because they were considered in no wise comparable to adult islets of Langerhans.

At 19¼ *days.* Some of the 'islets' at this stage have developed to such a degree that they can be enumerated as distinct units. They should, however, not be considered 'islets of Langerhans' because they do not yet possess the two types characteristic of the mature islets—alpha cells are still absent.

At 21 *days.* More 'islets' are present, and the larger ones are more numerous (17% greater than 150 μ in cross-section as compared to 12% at 19¼ days). The average cross-section of the islets is, however, not appreciably greater than at 19¼ days. This is due to the presence of a larger percentage of small, newly-formed 'islets'.

A very interesting phenomenon was noticed at this stage: several cells, showing beta granules, were found embedded among the cells of the pancreatic ducts, especially the intercalated ducts (Fig. 7). These are considered to be β-cells which differentiate directly from the duct-epithelium, in contrast to the β-cells previously encountered at the 17¼ and 19¼-days stage. These latter were invariably present among massas of undifferentiated cells (the primitive islets) and are considered to develop from these.

At 22¼ *days.* Thirty-one per cent of the islets have now reached a size of 150 μ or more in cross-section, and the total number of 'islets' has nearly doubled since the 19¼-days stage. The total 'islet-volume' has more than doubled during this period. This enlargement of the islets must be attributed to mitotic activity within the islets—not only were mitoses observed in the undifferentiated cells of the primitive islets but mitotic figures are also found in the well-differentiated β-cells. The increase in the total 'islet-volume' is effected by enlargement of the existing islets as well as the development of new islets from the anlagen.

At this stage a *new cell type* also differentiates from the tubule-epithelium. Fig. 8 shows a cell of this type with characteristic alpha granules arranged in such a way as to

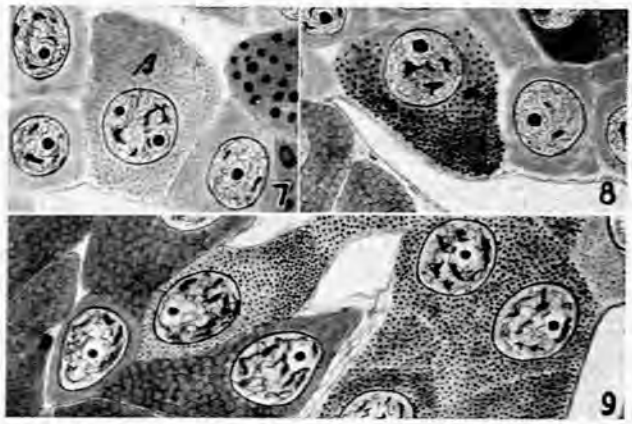

Fig. 7. Section through pancreatic tubule from 21-day embryo. A fully differentiated β-cell is shown. *Fig.* 8. Section through pancreatic tubule from 22¼-day embryo. An α-cell with distinct polarity towards the capillary is shown.
Fig. 9. Section of pancreas taken from rat treated with $CoCl_2$. An acinus (left) adjacent to an islet (right) is shown. An α-cell, situated among the acinar cells and in contact with the centro-acinar cell, can be distinguished.
(*Figs.* 7-9 were drawn at a uniform magnification; oil immersion.)

give it a distinct polarity towards the capillary and away from the lumen of the intercalated duct.

Addendum

During the course of an investigation, under way, on the destruction of the alpha cells by injection of $CoCl_2$ it was noticed that new α-cells are regenerated by differentiation from the centro-acinar cells. Fig. 9 shows an alpha cell situated between the cells of an acinus and still in contact with a centro-acinar cell, presumably the sister cell of the one under investigation. The polarity of this cell is similar to that of the α-cell described at the 22¼-days stage.

DISCUSSION

The cell types in the islets of Langerhans. Laguesse[20] (1894) was the first to observe the existence of more than one cell type in the islets of Langerhans. Since then, different investigators paid attention to these different cell types and Lane[21] succeeded in identifying two kinds of granules in the islet cells which differed chemically from each other and from the zymogen of the acini. These are now generally known as alpha and beta granules (present in the α-cells and β-cells, respectively). In addition to the α- and β-cells three other cell types were distinguished by different authors in different animal species: Bensley[1] described cells in the islets of the guinea pig and called them C-cells (gamma cells; Gomori[8]) and Bloom[2] (1931) described the so-called D-cells in the human islets of Langerhans. D-cells were found in 41 species of mammals (including the albino rat) by Thomas,[27] who also identified a fifth type of cell (E-cells) in the opossum islets.

With the staining methods of Gomori[8, 9] it was invariably possible to distinguish *two cell types* in the normal islets of the rat, except in some of the smaller islets in which only β-cells could be found. The differentiation of at least two cell types in adult pancreatic islets is further supported by their differential phosphatase activity (Gomori,[10] Jacoby[15] and McAlpine[22]) and the electron-microscopic investigation by Lacy.[19]

Concerning the C-cells conflicting evidence appears to exist: Cowdry[4] stated that 'A, B and C cells can be accepted without reservation as fundamental components of the islets'. According to Gomori,[8] however, gamma cells (C-cells) are merely beta cells which are very poor in, or possibly devoid of specific granules. Bremer and Weatherford[3] stated that 'the C-cell may represent a younger form of the A-cell'. E-cells were described in the islets of the opossum only. The D-cells, in my opinion, are nothing but cells in a certain phase of activity or degeneration, comparable to the β-cells undergoing hydropic degeneration. This opinion is in partial agreement with the statement of Gomori[9] that 'D cells are probably aged alpha cells'.

The origin of the islets of Langerhans. The embryonic islets differentiate from the entoderm and do not show any genetic relationship to the mesodermal tissue in which they become embedded. The first 'islet-anlage' could be distinguished in the 17¼-day embryo. No 'islets' were encountered in the 14¼-day embryo, a finding which conflicted with Hard's statement[12] that the first 'islets' differentiated in the 13-day embryo. It must, however, be stressed that in the present investigation only those structures with a marked cellular compactness comparable to the adult islets of Langerhans, were counted as 'islets'.

From 17¼ days onward 'islets' differentiated and increased in size at such a rate that at 22¼ days the total volume reached the value of 374×10^{-3} c. mm. (Table II). This volume, however, constituted only a very small part of the islet-tissue of the adult as estimated by Haist and Pugh.[11] One might therefore conclude that only a small percentage of the future islets of Langerhans originate during intra-uterine development.

The cytogenesis of the β-cells. Gomori[9] stated that 'the origin of beta cells is unknown'. Hard[12] was able to distinguish granules in the islet cells at the 19-day stage of embryonic development, while McAlpine[22] described 'a new cell type' at 18 days and identified this type as the beta cell. In my material specific granules were first seen at 17¼ days, which is in good agreement with the findings of McAlpine.

It was concluded that these first recognizable β-cells originated from undifferentiated cells which budded from the pancreatic ducts. Later on β-cells differentiated directly from the duct-epithelium, while some cells originated as a result of mitosis of existing β-cells. These results suggested a threefold origin of the β-cells. This conclusion conflicted, however, with that of Ferner and Stoeckenius[6] who also claimed that three possibilities existed concerning the cytogenesis of the β-cells, but according to whom β-cells might originate *inter alia* by the transformation of α-cells into β-cells.

The cytogenesis of the α-cells. According to Hard[12] 'the beta cell is the only islet cell type to differentiate during embryonic development', and 'alpha cells are first recognized during the second day of postnatal life'. This opinion was not substantiated by the present study because occasional α-cells (with specific granules) were distinguished at 22¼ days. Although Hultquist and Thorell[14] believed that 'α-cells . . . differ with respect to their ultraviolet cytology in budding germs and in islands of the adult type', I was unable to make this distinction.

In contrast to the suggested threefold origin of the β-cells, the α-cells showed a monophyletic origin *viz.* differentiation

from the duct-epithelium. Mitotic figures were never encountered in the α-cells of my material. This is supported by Jaffe,[16] but conflicts with the finding of Mosca[23] who could see mitotic figures in both the α- and β-cells. Even after $CoCl_2$-treatment the proliferation of α-cells in the adult occurred from duct-epithelium (the centro-acinar cells).

The relationship between the different cell types of the pancreas. A number of authors (Dale,[5] Woerner,[30] and Johnson[18]) described transition-cells which showed characteristics of both islet- and acinar cells. From this they suggested that acinar cells could be transformed into islet-cells. Vincent,[29] Otani[24] and Sergeyeva[25] claimed that transformation of acinar cells into islet-cells and *vice-versa* occurred even in adults. On the other hand Tschassownikow,[28] working on the axolotl, stated that even the *possibility* of islets being transformed into glandular acini was excluded in the light of his observations. He therefore did not accept the 'theorie de balancement Laguesse'.

In the present study no transition-cells which showed characteristics of both acinar and islet-cells were found; it was concluded, therefore, that the islets of Langerhans were morphological entities *sui generis*. This conclusion is supported by Freise,[7] Hirata[13] and Gomori.[9]

Ferner and Stoeckenius' statement[6] that α-cells can be transformed into β-cells is mistaken in my opinion. It is also in marked contrast to the findings of Terbrüggen,[26] Gomori[9] and others. If the α-cells were to be taken as the precursors of the β-cells there is no possible explanation for the fact that the β-cells invariably differentiated during the embryonic development several days before there was any sign of specific α-granules (and thus of α-cells). Also, it will be very difficult to explain the observation that destruction of the β-cells with alloxan (Esterhuizen, 1948—unpublished results) is permanent, whereas destruction of the α-cells with $CoCl_2$ is followed by marked regeneration.

SUMMARY

1. The cellular composition of the islets of Langerhans in normal and diabetic rats was investigated. Rats were made diabetic by (*a*) injections of alloxan and (*b*) repeated injections of dextrose solution. The normal islets of Langerhans of the albino rat are constituted of two cell types, while in those made diabetic a third type ('D-cells') was invariably encountered apart from the fully degenerated β-cells showing pyknotic nuclei.

2. Regarding the origin and cytogenesis of the islets it was found that the first 'islet-anlage', as well as specific beta-granules, could be distinguished in the 17½-day embryo. Alpha-cells were first distinguished at 22½ days. It is suggested that the β-cells have a threefold and the α-cells a monophyletic origin.

3. No transition-cells which showed characteristics of both acinar and islet-cells were found. It is concluded, therefore, that the islets of Langerhans are morphological entities *sui generis*.

I wish to thank Prof. H. E. Brink for his interest in this work and Dr. M. W. P. Nienaber for his help in preparing the manuscript.

All photomicrographs were taken through a Wratten K_1 filter.

REFERENCES

1. Bensley, R. R. (1911): Amer. J. Anat., **12**, 297.
2. Bloom, W. (1931): Anat. Rec., **49**, 363.
3. Bremer, J. L. and Weatherford, H. L. (1944): *A Text-book of Histology*, p. 394. Toronto: The Blakiston Company.
4. Cowdry, E. V. (1934): *A Text-book of Histology*. Philadelphia: Lea and Febiger.
5. Dale, H. H. (1905): Philos. Trans. Roy. Soc. London Ser. B., **197**, 25.
6. Ferner, H. and Stoeckenius, W. (1950): Cit. by Hultquist and Thorell.
7. Freise, G. (1929): Z. Mickr.-anat. Forsch., **17**, 185.
8. Gomori, G. (1939): Anat. Rec., **74**, 439.
9. *Idem.* (1941): Amer. J. Path., **17**, 395.
10. *Idem.* (1941): J. Cell. Comp. Physiol., **17**, 71.
11. Haist, R. E. and Pugh, E. J. (1948): Amer. J. Physiol., **152**, 36.
12. Hard, W. L. (1944): Amer. J. Anat., **75**, 369.
13. Hirata, K. (1934): Sci. Rep. Res. Inst. Tohoku Univ., **9**, 159.
14. Hultquist, G. T. and Thorell, B. (1953): Acta Path. Microbiol. Scand., **32**, 245.
15. Jacoby, F. (1946): Nature (Lond.), **158**, 268
16. Jaffe, F. A. (1951): Anat. Rec., **111**, 109.
17. Jewell, H. A. and Charipper, H. A. (1951): *Ibid.*, **111**, 401.
18. Johnson, D. D. (1950): Endocrinology, **47**, 393.
19. Lacy, P. E. (1957): Anat. Rec., **128**, 255.
20. Laguesse, E. (1894): C.R. Soc. Biol. (Paris), **10**, 667.
21. Lane, M. A. (1907): Amer. J. Anat., **7**, 409.
22. McAlpine, R. J. (1951): Anat. Rec., **109**, 189.
23. Mosca, L. (1957): Quart. J. Exp. Physiol., **42**, 267.
24. Otani, S. (1927): Amer. J. Path., **3**, 123.
25. Sergeyeva, M. A. (1940): Anat. Rec., **77**, 297.
26. Terbrüggen, A. (1947): Klin. Wschr., **24**, 434.
27. Thomas, T. B. (1937): Amer. J. Anat., **62**, 31.
28. Tschassownikow, N. (1934): Z. Zellforsch., **20**, 619.
29. Vincent, S. (1924): Lancet, **1**, 947.
30. Woerner, C. A. (1938): Anat. Rec., **71**, 33.