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Pathologic Anatomy of the Pancreas in Juvenile Diabetes Mellitus

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SUMMARY

1. Quantitative study of insular tissue has revealed that the number of B cells is greatly diminished in patients with acute juvenile diabetes from the time of clinical onset of the disease. The number of these cells is as a rule less than 10 per cent of normal. Such B cells as are still present show the cytological signs of marked activity.

2. The normal or supranormal insular activity that is usually found in juvenile diabetics in this stage of the disease cannot therefore be due to the presence of a normal insular tissue, but is produced by a small number of hyperactive B cells.

3. On the basis of histological findings (presence of islets of large size, signs of new islet formation), it may be assumed that during the preclinical phase of juvenile diabetes, an extrapancreatic factor has exerted a strong stimulant action on the insular tissue. In the long run this must lead to exhaustion of the islet-forming capacity on the pancreatic parenchyma and to a decrease in the number of the B cells. By the time the disease becomes clinically manifest only the latter stage of this process can be observed and the majority of islets consist of A cells or of atrophic tissue devoid of B cells.

4. Peri- and intra-insular inflammatory infiltrates have been found in 68 per cent of those patients with juvenile diabetes who died soon after the clinical onset of their disease. In other words, and contrary to the generally held view, this lesion is not uncommon. It is specific for diabetes and has never been observed in the chronic cases.

5. In patients with chronic juvenile diabetes, the B cells are completely absent, except in occasional cases. The islets consist of small, atrophic cells.

6. A valid assessment of the functional capacity of insular tissue can only be achieved if as much use as possible is made of quantitative technics and of cytological examination. DIABETES *14*:619-33, October 1965.

Clinical, biological and histological findings have led to the classical view that juvenile diabetes results from an absolute deficiency of insulin secretion, due to severe inadequacy of the insular tissue.

Nevertheless, recent investigations[1,2] have revealed that the serum of recent onset juvenile diabetics shows an insulin-like activity that is normal or even higher than normal. Furthermore, Maclean and Ogilvie[3] reported that the pancreas of juvenile diabetics who die soon after the clinical onset of their disease contains large islets and that in such patients both the relative and total mass of insular tissue are often only slightly less than normal.

Such observations are of obvious interest. They lead one to consider that the inadequacy of the insular tissue, which one is often inclined to regard as congenital, may not in fact be primary in nature, but only the late stage of a progressive deterioration under the influence of an extrapancreatic diabetogenic factor.

In view of this new aspect of the physiopathology of juvenile diabetes, we have resumed the study of the pancreas of the young diabetic patient. Most of the work previously done in this field was performed prior to the development of modern technics for the demonstration of the various types of insular cells, and at a time when our knowledge of the physiopathology of the disease was as yet rudimentary.

In collecting the material to be used in this study, we attempted to include as many cases as possible of juvenile diabetic patients who had died soon after the clinical onset of their disease.

MATERIAL AND METHODS

The material for our study consisted of fifty-four pancreases from young diabetic patients in whom the onset of the disease had occurred prior to the age of thirty-one. Of these patients, twenty-two had died less than six months after the onset of the first symptoms of diabetes (in most cases: weight loss, polydipsia and polyuria). In the remaining cases, the disease had been known from one to thirty-seven years. Most of these cases have been kindly provided by the Departments of Pathology of the principal hospitals of Boston and Philadelphia. Others, including twenty-six pancreases from nondiabetic patients serving as control material, came from the Departments of Pathology of the two University hos-

From the Department of Pathology of the University of Brussels, Brugmann University Hospital, and the Fondation Medicale Reine Elisabeth, Belgium.

pitals at Brussels. The origin of the cases, the age and sex of the patients, and the duration of the diabetes are shown in tables 1-3.

Because of the diverse origins of this pancreatic material, it is understandable that the quantity available for study and the methods of fixation were variable. In all but one case (Case 18) paraffin blocks were provided. Their number varied from one to five. In many cases routinely stained sections or pieces of wet tissue were also available. The fixative that had been used was: formalin (25 cases), Zenker (18 cases), Romeis (7 cases), Bouin (4 cases).

For Case 18, six Gomori stained sections of fairly well preserved pancreatic fragments were available. In all the other cases we stained the sections with hemalum erythrosin saffron and with the chromium hematoxylin phloxine method of Gomori. In those cases in which a satisfactory differential staining could not be obtained in newly made sections from paraffin blocks or from wet tissue, restaining of decolorized routine sections was tried. Decolorization was achieved by oxidation with a permanganate-sulfuric acid mixture, which represents the first step in the classical Gomori chromium hematoxylin method. This procedure yielded satisfactory results in two cases only (cases 9 and 16). In most of the acute and several of the chronic and control cases we also performed Dominici's method (toluidine blue erythrosine orange) or the methyl green pyronine method, with or without previous digestion in a 0.1 per cent solution of ribonuclease (120 min. at 37° C.).

In forty cases (twenty-two acute, eighteen chronic), the proportion of islet tissue in the pancreatic parenchyma and the size of the islets were assessed. Fourteen cases (26 per cent) (cases forty-one to fifty-four) were rejected from this part of the study because of unsatisfactory definition of the outlines of the islets. To determine the proportion of islet tissue and the size of the islets the sections were scanned systematically under a Leitz Panphot and all the islets encountered were drawn at a magnification of 250 X. The contours of

TABLE 1

Cases of acute juvenile diabetes

Case	Origin*	Age	Sex	Known duration of diabetes (days)	Weight of pancreas (gm.)	Number of islets per cm² of pancreatic tissue	Per cent of islet tissue in the pancreas	Number of B cells per cm² of pancreatic tissue
1	B.G. 55/145	11 mos.	F	3	—	76	0.33	172
2	E.C.H. 53/9	11 mos.	M	3	8	176	0.89	286
3	N.D.H. 43475	11 mos.	M	14	7.5	124	0.88	—‡
4	Ch.H.Ph. 1928	1 yr. 3 mos.	M	90	—	78	0.60	—‡
5	S.P. 57/130	2 yrs.	F	9	15	132	0.83	115
6	Ch.H.B. 60/184	2 yrs. 10 mos.	M	60	—	88	0.35	563
7	C.H. 61/43	3 yrs.	F	30	25	72	0.23	0
8	Ch.H.Ph. 56/85	3 yrs. 6 mos.	M	45	20	61	0.51	—‡
9	M.G.H. 9677	6 yrs.	M	15	25	38	0.52	199
10	M.G.H. 10938	9 yrs. 6 mos.	M	4	20	37	0.51	1,919
11	N.D.H. 197176	13 yrs.	F	14	40	36	0.46	378
12	M.G.H. 9381	15 yrs.	F	60	—	36	0.49	—‡
13	M.G.H. 14089	15 yrs.	F	90	49	58	0.33	296
14	M.G.H. 11301†	15 yrs.	F	21	80	(125)	(1.71)	(554)
15	M.G.H. 8275	15 yrs.	F	30	—	81	0.92	509
16	St.M.H. 56/103	15 yrs.	M	16	—	56	0.80	175
17	U.P.H. 59/200	15 yrs.	F	180	50	59	0.85	—‡
18	Ph.G.H. 60/397	16 yrs.	M	4	—	62	0.46	524
19	U.P.H. 55/99	17 yrs.	M	1	50	36	0.60	807
20	S.P. 56/237	21 yrs.	F	1	75	41	0.44	267
21	N.H. 48/91	22 yrs.	M	14	60	54	0.47	598
22	B.H. 58/112	30 yrs.	M	90	55	56	0.48	242

*N.D.H.: New England Deaconess Hospital, Boston.
Ch.H.Ph.: Children's Hospital, Philadelphia.
S.P.: Hôpital Universitaire St. Pierre, Brussels.
Ch.H.B.: Children's Hospital, Boston.
C.H.: Carney Hospital, Boston.
M.G.H.: Massachusetts General Hospital, Boston.
N.H.: Newton-Wellesley Hospital, Mass.
B.H.: Hôpital Universitaire Brugmann, Brussels.
Ph.G.H.: Philadelphia General Hospital, Philadelphia.
U.P.H.: University of Pennsylvania Hospital, Philadelphia.
B.G.: Bowman Gray School of Medicine, Winston-Salem, N.C.
E.C.H.: Elliot Community Hospital, Keene, N.H.
St.M.H.: St. Mary's Hospital, Montreal, Canada.

†Severe chronic pancreatitis.

‡Evaluation of the number of B cells proved impossible in these cases.

TABLE 2

Cases of chronic juvenile diabetes

Case	Origin*	Age	Sex	Known duration of diabetes (yrs.)	Weight of pancreas (gm.)	Number of islets per cm² of pancreatic tissue	Per cent of islet tissue in the pancreas	Number of B cells per cm² of pancreatic tissue
23	M.G.H. 10765	13	F	7	—	30	0.19	0
24	M.G.H. 10922	15	F	2	55	25	0.25	—†
25	N.D.H. 179.232	23	F	18	—	28	0.16	—†
26	N.D.H. 190.581	24	M	14	35	19	0.20	—†
27	N.D.H. 184.720	24	F	20	33	56	0.41	—†
28	N.D.H. 132.966	25	F	19	120	32	0.23	—†
29	B.H. 59/292	30	F	26	35	29	0.26	0
30	N.D.H. 197.521	31	M	14	60	19	0.14	0
31	N.D.H. 206.426	31	M	17	24	68	1.24	717
32	N.D.H. 183.253	31	M	23	40	45	0.88	—†
33	N.D.H. 174.414	31	M	23	90	45	0.36	285
34	N.D.H. 203.499	32	M	12	100	11	0.10	0
35	S.P. 57/424	33	F	6	70	26	0.17	107
36	F.H. 50/86	34	F	22	68	10	0.06	0
37	B.H. 62/228	37	M	17	16	34	0.36	0
38	N.D.H. 207.546	39	F	37	60	4	0.04	0
39	S.P. 57/135	43	M	23	85	57	0.65	0
40	N.D.H. 152.680	47	F	18	80	55	0.41	1,825

*See table 1.
†An evaluation of the number of B cells proved impossible in these cases.

TABLE 3

Nondiabetic controls

Case	Origin*	Age	Number of islets per cm² of pancreatic tissue	Per cent of islet tissue in pancreas	Number of B cells per cm² of pancreatic tissue
1	S.P. 60/479	9 mos.	587	3.09	8,576
2	S.P. 58/131	10 mos.	246	2.19	7,484
3	S.P. 60/81	1 yr.	264	1.26	5,448
4	B.H. 60/25	2 yrs.	172	1.06	— †
5	S.P. 59/30	2 yrs.	295	1.46	4,288
6	S.P. 59/437	3 yrs.	205	1.22	5,154
7	S.P. 58/269	4 yrs., 6 mos.	158	1.57	6,692
8	B.H. 59/154	4 yrs. 6 mos.	105	0.64	3,736
9	B.H. 62/93	5 yrs.	277	2.00	5,544
10	S.P. 60/82	9 yrs.	152	0.95	4,718
11	S.P. 59/130	10 yrs.	154	0.67	4,576
12	S.P. 60/182	11 yrs.	649	3.46	13,060
13	S.P. 58/52	13 yrs.	150	1.48	— †
14	B.H. 62/196	13 yrs.	78	1.02	2,378
15	B.H. 61/249	16 yrs.	55	0.69	1,248
16	B.H. 61/261	19 yrs.	122	0.55	987
17	B.H. 61/444	20 yrs.	183	1.73	3,104
18	B.H. 61/509	24 yrs.	124	0.99	1,940
19	B.H. 61/505	25 yrs.	133	2.04	2,844
20	B.H. 61/189	25 yrs.	48	0.52	1,400
21	B.H. 61/489	29 yrs.	69	0.51	950
22	B.H. 60/346	33 yrs.	93	0.72	2,114
23	B.H. 61/18	36 yrs.	53	0.66	1,038
24	B.H. 61/83	38 yrs.	59	0.61	1,058
25	B.H. 61/129	38 yrs.	202	2.69	2,900
26	B.H. 62/295	41 yrs.	121	2.04	2,688

* See table 1.
† An evaluation of the number of B cells proved impossible in these cases.

the section were drawn at magnification of 9 X, with deduction of the larger lacunae and the principal fibrous cords. The area of each islet and that of the section were measured with the aid of a planimeter.

The total area of insular tissue visible in the sections was calculated by adding up the area of the individual islets. The percentage of insular tissue in the pancreatic parenchyma was calculated with the aid of the formula: $\frac{s \times 100}{S}$ (s: total area of the islets, S: area of the section). From the number of islets present in the sections, the number per one square centimeter of pancreatic tissue was calculated.

Because, in juvenile diabetics, a considerable proportion of islets are composed of small cells which cannot be distinguished as A cells or undifferentiated cells, differential cell counts could not be carried out. The B cells, on the other hand, are clearly recognizable in most of the acute cases and in a small number of chronic cases. We therefore limited ourselves to counting the number of B cells in the sections, and we have calculated the number of B cells present in one square centimeter of pancreatic tissue. From this part of the study twenty-five cases (46 per cent) were rejected because the cytological preservation or the staining of the B cells proved unsatisfactory.

In view of the variability of the sample material, the crudeness of the quantitative methods employed, and the asymmetric distribution of the values, a statistical analysis appeared unwarranted and has therefore not been undertaken. In the cases which appeared unsuitable for quantitative estimation of the islet tissue or assessment of the number of B cells, only the qualitative changes were recorded.

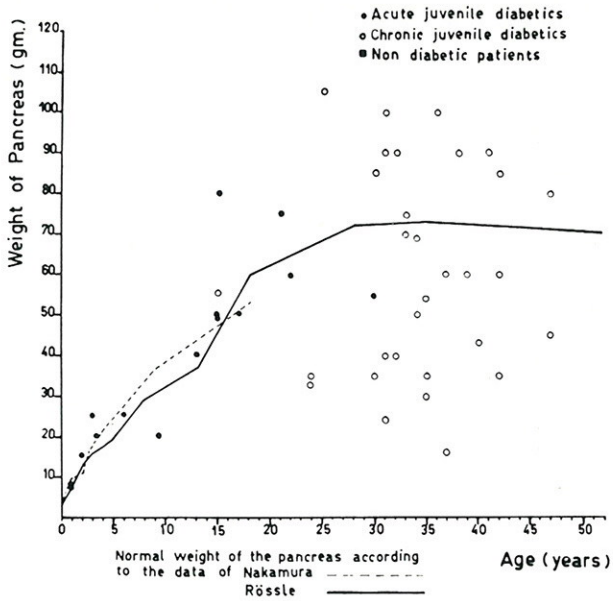

FIG. 1. Weight of the pancreas in acute and chronic juvenile diabetics, and in nondiabetics.

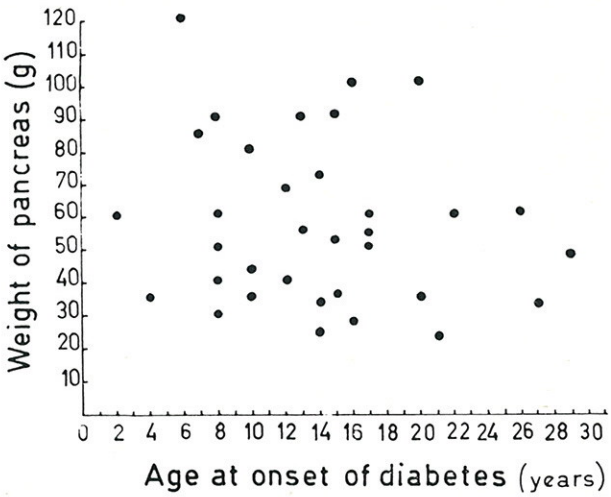

FIG. 2. Relationship of weight of pancreas to age at onset of diabetes (in chronic juvenile diabetics).

RESULTS

A. *Quantitative observations*

1. *Weight of the pancreas.* The weight of the pancreas was known in fifteen of the twenty-two acute cases (table 1, figure 1). The weight never deviated significantly from the normal figures, as listed in the studies of Rössle,[4] of Nakamura[5] and of Vartiainen.[6] In chronic cases, however, the weight of the pancreas was highly variable (table 2), and in a considerable proportion of such cases it was lower than normal (figure 1). This weight did not correlate with the age at onset of the diabetes (figure 2), the duration of the disease (figure 3) or the severity of the pancreatic fibrosis.

2. *Number of islets.* In both the acute and chronic cases, the number of islets per square centimeter of pancreatic tissue was, as a rule, smaller than that of the control cases of corresponding age (tables 1-3, figure 4). This numerical decrease was proportionally more pronounced in very young children belonging to the acute group, and in the chronic cases.

3. *Size of the islets.* As a rule, the islets of medium and of large size were proportionally more numerous in those young diabetics who had died after a brief period of disease than in the nondiabetic controls of corresponding age. This difference is less striking in the

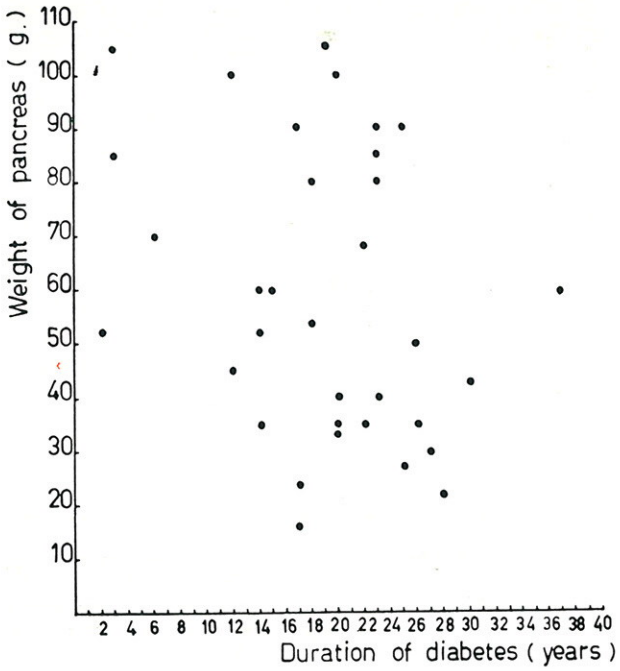

FIG. 3. Relationship of weight of pancreas to duration of diabetes (in chronic juvenile diabetics).

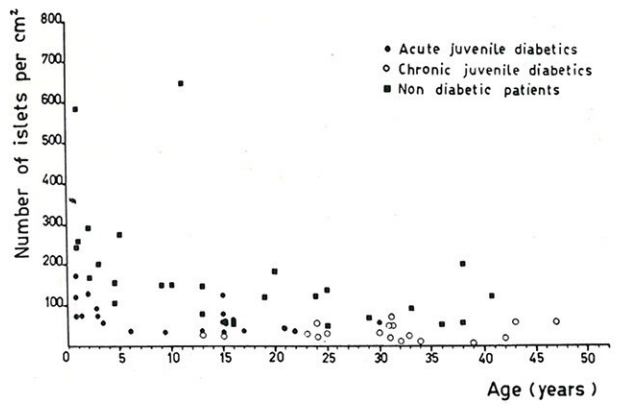

FIG. 4. Number of islets per square centimeter of pancreatic tissue in acute and chronic juvenile diabetics, and in nondiabetics.

diabetic children who had died prior to the age of three years since in these children the pancreas often contains very small islets. In the cases of chronic juvenile diabetes, the islets were variable in size; in some of these cases large islets were observed (figure 5).

4. *Proportion of insular tissue.* The proportion of insular tissue is as a rule smaller in the diabetic patients than in the controls (tables 1-3, figure 6). The difference is more marked in the chronic than in the acute cases.

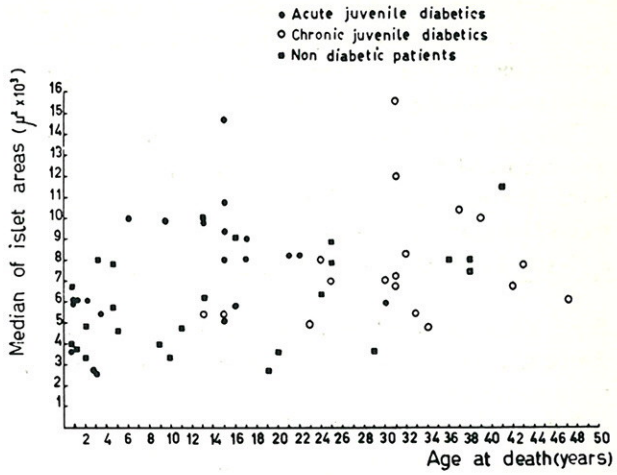

FIG. 5. Median (P50) of islet areas in acute and chronic juvenile diabetics, and in nondiabetics.

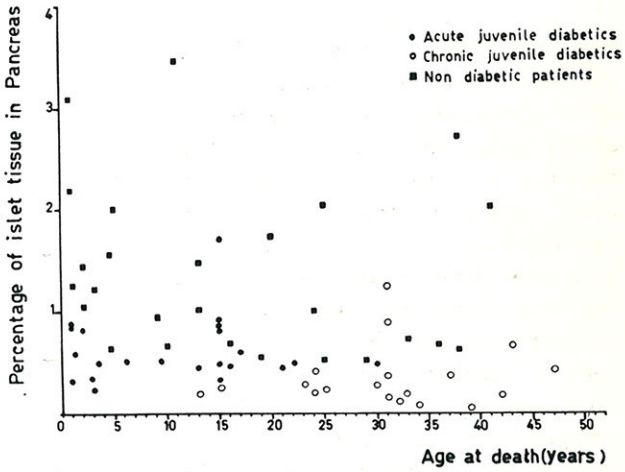

FIG. 6. Percentage of islet tissue in the pancreas of acute and chronic juvenile diabetics, and in nondiabetics.

5. *Number of B cells per square centimeter of pancreatic tissue.* The cytological preservation and the quality of the preparations were sufficient to allow evaluation of the number of B cells in seventeen of the twenty-two acute cases and in thirteen of the thirty-two chronic cases. In sixteen of the seventeen acute cases, B cells were found. Their numbers, which were always distinctly lower than in the controls of the corresponding ages, were nevertheless considerably higher than those in most of the chronic juvenile diabetics (tables 1-3, figure 7). In the latter category, not a single B cell could be found in nine of the thirteen cases evaluated. In the remaining four, they were present in small number in two cases, in moderate numbers in one case, and in relatively large numbers in the fourth case.

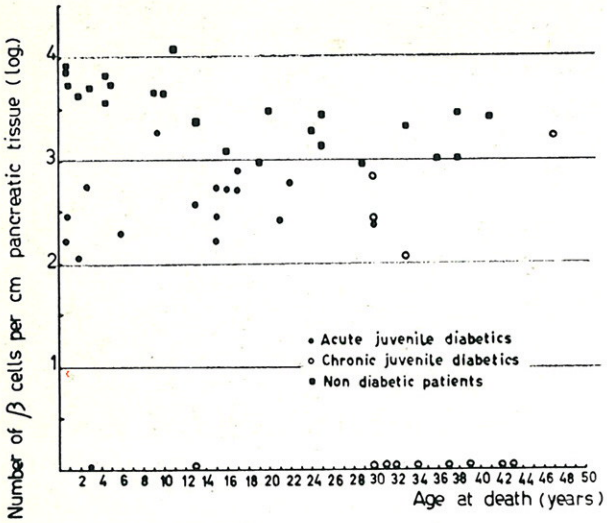

FIG. 7. Number of B cells (log. 10) per square centimeter of pancreatic tissue.

B. *Qualitative observations*

Appearance of the islets of Langerhans in the pancreas of young diabetic patients. The islets found in the pancreas of patients with acute or chronic juvenile diabetes can be classified into three groups, according to the cytological features of the cells of which they are composed:

1. *Islets of type I.* These islets are highly variable in size, mostly small, but sometimes very large. Some have a compact structure (figure 8); others, more numerous, consist of thin cords arranged in a fibrous stroma (figure 9) or separated by capillaries of varying diameter (figure 10). The cells forming these islets are small in size; the nucleus is small, with dense chromatin;

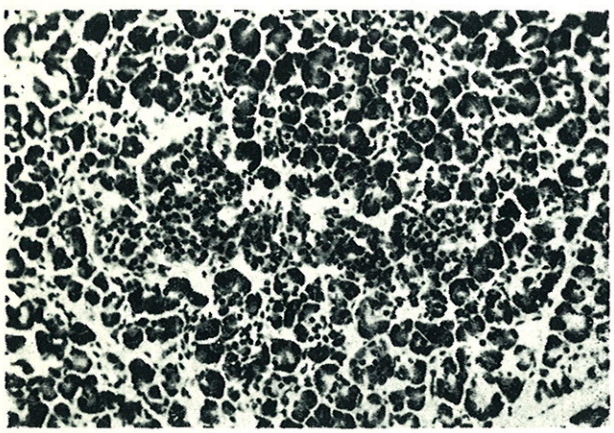

FIG. 8. Islet of Langerhans of type I, in a juvenile diabetic (Case 18, Ph.G.H. 60/397). The islet has a compact structure and is composed of small cells, which stain red with phloxine (A cells). Gomori's chromium hematoxylin-phloxine. X 150.

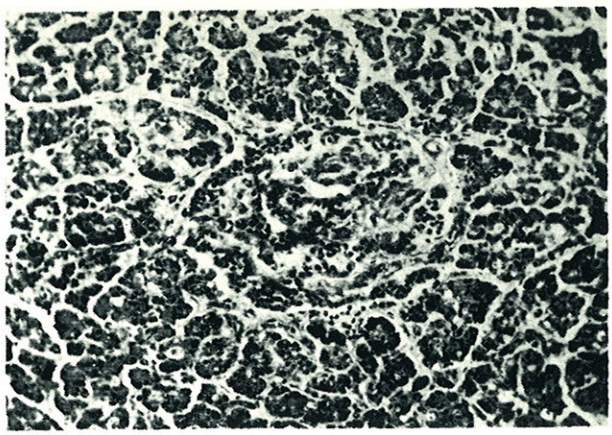

FIG. 9. Islet of Langerhans of type I in a juvenile diabetic (Case 11, N.D.H. 197.176). The islet is composed of narrow cords of small cells in a fibrous stroma. Gomori's chromium hematoxylin-phloxine. X 150.

the cytoplasm is not abundant, and shows no granulations; it may stain red with phloxine (A cells ?). These islets often have irregular outlines; continuity between the cords of insular cells and acinous tissue is frequently observed (figure 10).

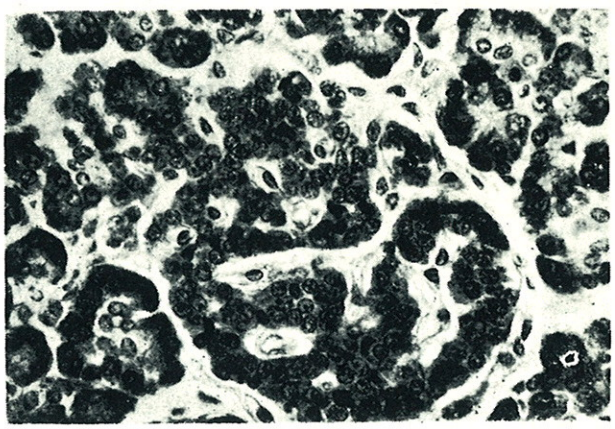

FIG. 10. Islet of Langerhans of type I, in a juvenile diabetic (Case 5, S.P. 57-130). The islet is composed of thin cords of small cells, separated by capillaries. Gomori's chromium hematoxylin-phloxine. X 500.

2. *Islets of type II.* These islets are also variable in size, but tend to be large. They have a more regular form than those of type I and are as a rule better defined (figure 11). Some of these islets show a central cavity (figure 12); we feel that such islets have been formed by a proliferation of cells from duct epithelium, of which flattened remnants are still visible along part of the contour (figure 13). Islets of type II consist to a considerable extent of large cells arranged in cords separated by capillaries. The nucleus of these cells is

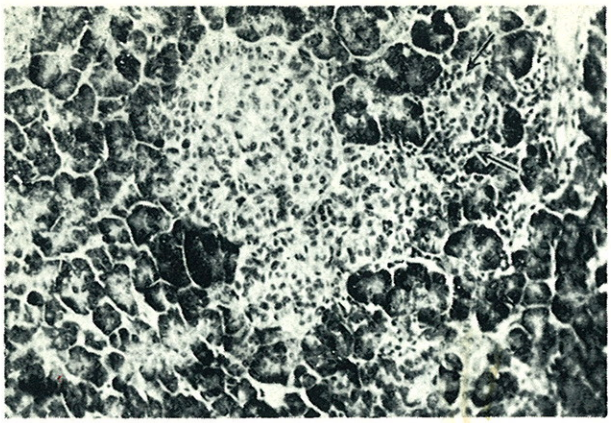

FIG. 11. Islet of Langerhans of type II, in a juvenile diabetic (Case 6, Ch.H.B. 60/184). The islet is composed of large cells, with a clear cytoplasm. The nuclei are slightly irregular. Lymphocytic infiltration (arrows). Gomori's chromium hematoxylin-phloxine. X 150.

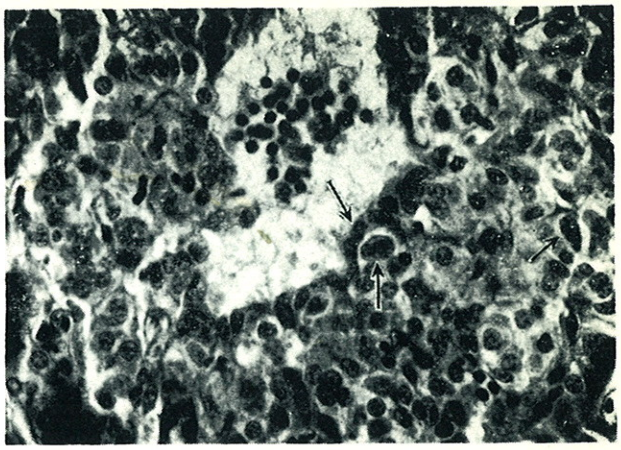

FIG. 13. Stronger magnification (X 450) of the same islet as in figure 12, showing the flattened epithelium (arrow) lining the dilated duct in the center of the islet. Also note the hypertrophy and the irregularity of the nucleus of some of the newly formed islet cells (arrows).

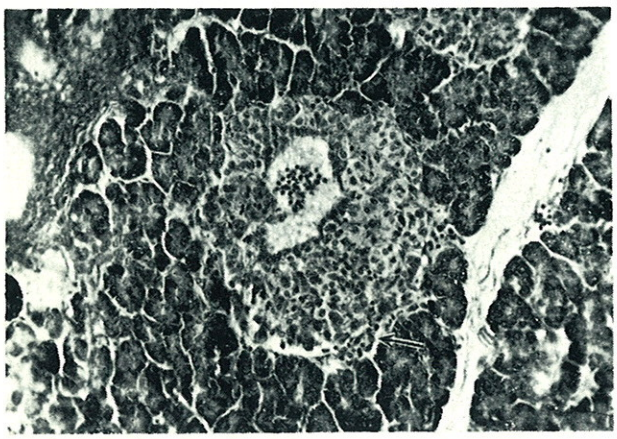

FIG. 12. Islet of Langerhans of type II, in a juvenile diabetic (Case 6, Ch.H.B. 60/184). Newly formed islet, developed around a dilated duct. Slight lymphocytic infiltration at the edge of the islet (arrow). Lymphocytes and histiocytes in the lumen of the duct. Gomori's chromium hematoxylin-phloxine. X 150.

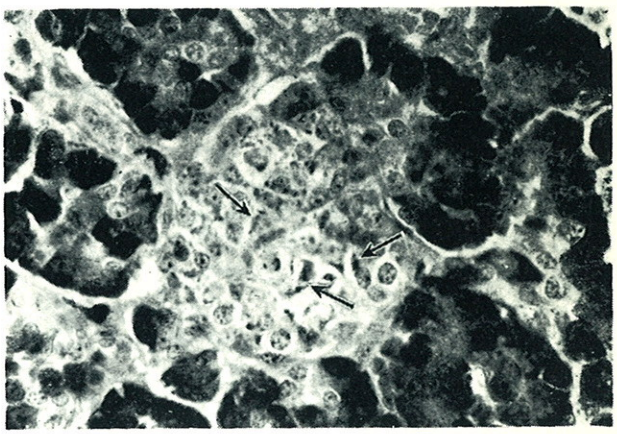

FIG. 14. Islet of Langerhans of type II, in a juvenile diabetic (Case 22, B.H. 58/112). "Kornchen" (arrows) in the cytoplasm of hypertrophic B cells. Dominici's toluidine blue-erythrosine-orange. X 450.

large, usually round. The cytoplasm is abundant, often completely free of granules or hydropic (figure 18); sometimes a few scattered granulations of B type can be found. Many of these cells contain irregular corpuscles (figure 14) with vague outlines, which stain gray with hematoxylin, blue with toluidine blue, and red with pyronin. These corpuscles have previously been described in the cytoplasm of insular cells by Weichselbaum[7] who called them "Körnchen." It should be stressed, however, that they are not secretion granules. Their affinity for toluidine blue or pyronin disappears, or is greatly lessened if the sections are subjected in advance to digestion with ribonuclease;[8] accordingly, they probably contain a significant quantity of ribonucleic acids. We regard these cells as hypertrophic and strongly hyperactive B cells. The islets of type II also contain a variable proportion of typical A cells, which stain well with phloxine; often they have a hypertrophic appearance, but they contain no "Körnchen."

3. *Islets of type III.* These as a rule resemble normal Langerhans islets, particularly if only routine staining methods are employed. They consist, in variable proportions, of typical A cells and of B cells that are slightly hypertrophic and more or less degranulated (figure 15). "Körnchen" were on no occasion observed in islets of this type.

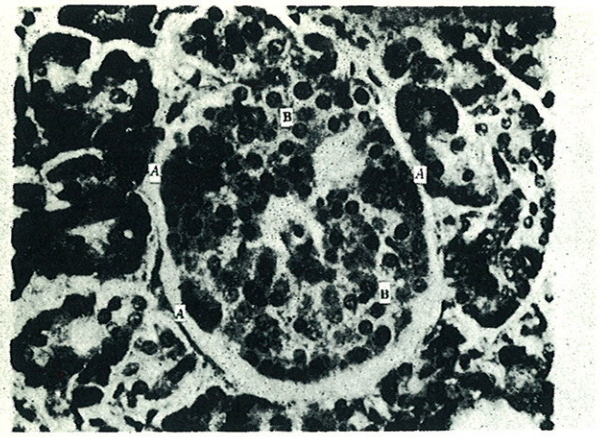

FIG. 15. Islet of Langerhans of type III, in a juvenile diabetic (Case 35, S.P. 57/424). The islet is composed of dark staining A cells, and partly degranulated B cells. Gomori's chromium hematoxylin-phloxine. X 450.

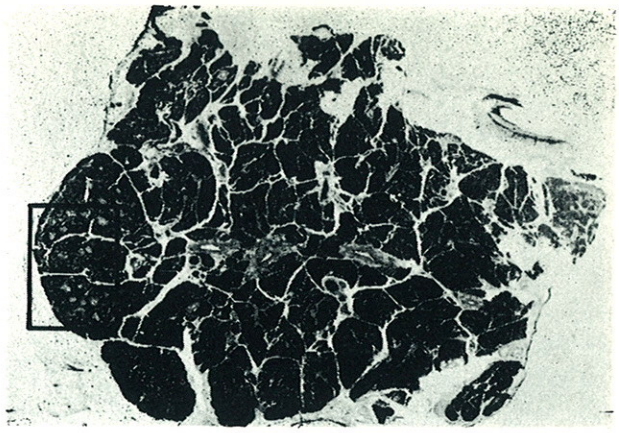

FIG. 16. Small magnification of a portion of pancreatic tissue of a juvenile diabetic (Case 6, Ch.H.B. 60/184). A focus of islet hyperplasia at left. Gomori's chromium hematoxylin-phloxine. X 6.

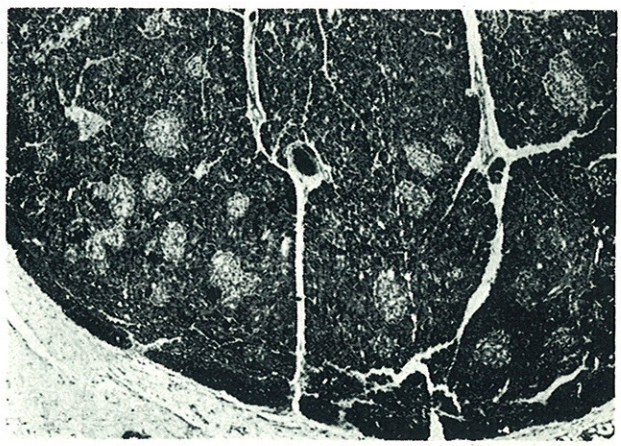

FIG. 17. A higher magnification of the area outlined in figure 16. Hyperplasia of islets (type II). Gomori's chromium hematoxylin-phloxine. X 35.

The respective proportions of these three types of islets differ considerably from one case to another. Type I is the dominant type in the pancreas of all the juvenile diabetics (table 4). In the juvenile diabetics in whom the disease ran an acute course, we also observed a highly variable number of islets of type II and transitional forms between types I and II. Their respective frequency differed not only from case to case, but even within the same case, from one area of the pancreas to another. In Case 6, for instance (Ch.H.B. 60/184) a few pancreatic lobules showed pronounced insular hyperplasia (figures 16-17), with islets of type II, whereas everywhere else in the pancreas only islets of type I could be found.

In the patients with chronic juvenile diabetes, on the other hand, virtually all islets were of type I. Islets of type II were nowhere found in the pancreas of these patients. Islets of type III were encountered only occasionally in the pancreases of juvenile diabetics; they were exceptional in the acute cases and in most chronic cases. On the other hand they are found in relatively large numbers in a few chronic cases, and, more precisely, in these cases in which B cells can still be found.

Hydropic transformation of the B cells. The hydropic B cells are swollen, and entirely degranulated; their cytoplasm appears to be empty (figure 18). These cells must be distinguished from those B cells which are hypertrophic and degranulated, but whose cytoplasm contains "Körnchen." If this definition is adhered to, genuine hydropic cells cannot be considered numerous in the pancreas of patients with juvenile diabetes; they have been seen in cases running an acute course and in only one of the chronic cases (tables 5, 6). We have been unable to demonstrate the presence of glycogen in these hydropic cells, but the conditions were not favorable for the histochemical detection of this substance.

Nuclear alterations in the insular cells. In the islets of type II and in the transitional forms between type I and II, the insular cells show large nuclei (figures 13, 18 and 19). In some of the cases (table 5) quite a

TABLE 4

Number of cases in which the different types of islets are present

	Type I	Type II	Type III
Young diabetics—short duration (22 cases)	22 (100%)	17 (77%)	3 (14%)
Young diabetics—chronic (32 cases)	32 (100%)	0 (0%)	5 (15%)

TABLE 5

Qualitative changes in the pancreas of acute juvenile diabetics

Case	Origin*	Age (yrs.)	Sex	Known duration of diabetes (days)	ISLET TISSUE						EXOCRINE TISSUE		
					Hydropic B cells	Nuclear Anomalies	Mitoses	Inflammatory infiltration	Hyalinosis	Fibrosis	Pancreatitis	Sclerosis parenchyma	Sclerosis vessels
1	B.G. 55/145	11 mos.	F	3	0	0	0	+++	0	+	+	0	0
2	E.C.H. 53/9	11 mos.	M	3	0	0	+	++	0	0	0	0	0
3	N.D.H. 43475	11 mos.	M	14	0	0	0	+	0	+	0	0	0
4	Ch.H.Ph. 1928	13 mos.	M	90	0	0	0	0	0	0	+	+	0
5	S.P. 57/130	2 yrs.	F	9	0	0	0	++	0	0	+	0	0
6	Ch.H.B. 60/184	2 yrs., 10 mos.	M	60	0	++	0	++	0	0	0	0	0
7	C.H. 61/43	3 yrs.	F	30	0	0	0	+	0	0	0	0	0
8	Ch.H.Ph. 56/85	3 yrs., 6 mos.	M	45	0	0	0	+	0	+	+	0	0
9	M.B.H. 9677	6 yrs.	M	15	0	0	0	+	0	0	+	+	0
10	M.G.H. 10938	9 yrs., 6 mos.	M	4	+	0	0	++	0	0	+	0	0
11	N.D.H. 197176	13 yrs.	F	14	0	0	0	++	0	++	0	+	0
12	M.G.H. 9381	15 yrs.	F	60	0	0	0	++	0	+	0	0	0
13	M.G.H. 14089	15 yrs.	F	90	++	+	0	0	0	0	0	0	0
14	M.G.H. 11301	15 yrs.	F	21	0	++	0	0	0	++	+++	+++	0
15	M.G.H. 8275	15 yrs.	F	30	+	+	0	+	0	+	0	0	0
16	St.M.H. 56/103	15 yrs.	M	16	0	0	0	+++	0	++	0	0	0
17	U.P.H. 59/200	15 yrs.	F	180	+++	+	0	+	++†	+	0	0	0
18	Ph.G.H. 60/397	16 yrs.	M	4	+	+++	0	0	0	+	+	0	0
19	U.P.H. 55/99	17 yrs.	M	1	+	+	0	+	0	+	0	0	0
20	S.P. 56/237	21 yrs.	F	1	+	0	0	0	0	+	0	0	0
21	N.H. 48/91	22 yrs.	M	14	+	0	0	0	0	+	0	0	0
22	B.H. 58/112	30 yrs.	M	90	+	+	0	0	0	+	0	0	0

*See table 1.

†The appearance of the hyaline substance is not typical.

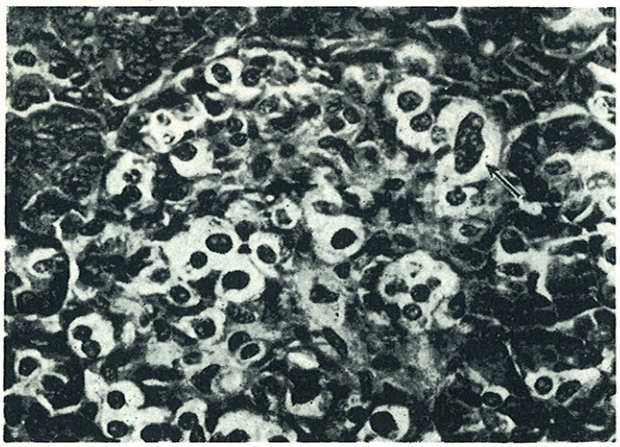

FIG. 18. Islet of Langerhans of type II, in a juvenile diabetic (Case 18, Ph.G.H. 60/397). Nuclear hypertrophy, irregularity and hyperchromatism. Gomori's chromium hematoxylin-phloxine. X 450.

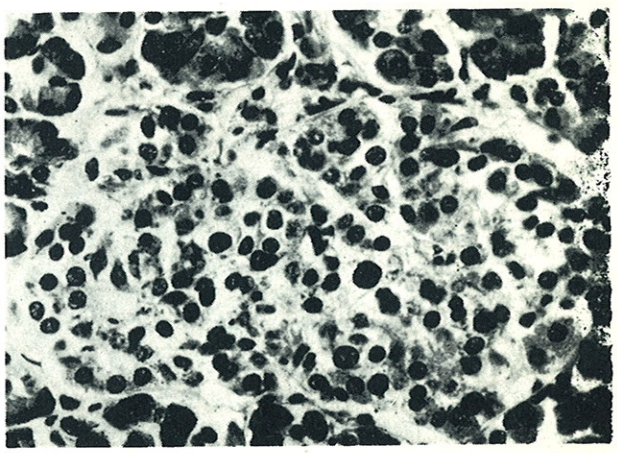

FIG. 19. Islet of Langerhans of type II in a juvenile diabetic (Case 17, U.P.H. 59/200). The B cells are swollen, hydropic; their nucleus is large. Note hypertrophic and irregular nucleus. Hemalum erythrosin saffron. X 450.

few nuclei appear irregular and/or hyperchromatic (figures 18 and 19). Other nuclei have vague contours and show only slight affinity for hematoxylin (figure 19). Pyknotic nuclei and nuclear debris were also observed in some of these islets. These nuclear anomalies have only been observed in acute cases. In chronic cases, the nucleus of the insular cells is small, as a rule round, and the chromatin is dense.

Mitoses in the insular cells. Although a systematic search was made for mitoses in the insular cells, they were found only in two cells in one of the acute cases (table 5).

Neoformation of islets. In many of the acute cases evidence of new islets formation has been found. This neoformation occurs either by proliferation of cells from

TABLE 6

Qualitative changes in the pancreas of chronic juvenile diabetics

Case	Origin*	Age (yrs.)	Sex	Known duration of diabetes (years)	ISLET TISSUE						EXOCRINE PANCREAS		
					Hydropic B cells	Nuclear anomalies	Mitoses	Inflammatory infiltration	Hyalinosis	Fibrosis	Pancreatitis	Sclerosis parenchyma	Sclerosis Vessels
24	M.G.H. 10922	15	F	2	0	0	0	0	0	+	+	0	0
41	M.G.H. 11212	17	F	13	0	0	0	0	0	0	+	++	0
42	U.P.H. 58/80	31	F	4	0	0	0	0	0	+	0	0	0
35	S.P. 57/424	33	F	6	+	0	0	0	0	++	++	++	+
23	M.G.H. 10765	13	F	7	0	0	0	0	0	0	0	+	0
43	B.H. 57/72	16	M	7	0	0	0	0	0	0	++	0	0
34	N.D.H. 203499	32	M	12	0	0	0	0	0	+	++	+++	++
26	N.D.H. 190581	24	M	14	0	0	0	0	0	+	0	+++	0
30	N.D.H. 199521	31	M	14	0	0	0	0	0	+	0	++	+
44	N.D.H. 198025	32	M	17	0	0	0	0	0	0	+	+	++
37	B.H. 62/228	37	M	17	0	0	0	0	0	+	+	+	+++
31	N.D.H. 206426	31	M	17	0	0	0	0	0	+	+++	++	+++
25	N.D.H. 179232	23	F	18	0	0	0	0	0	+	0	++	+++
45	N.D.H. 192150	35	M	18	0	0	0	0	0	+	0	+	++
40	N.D.H. 152680	47	F	18	0	0	0	0	0	++	+	+	++
28	N.D.H. 132966	25	F	19	0	0	0	0	0	++	0	++	+
46	N.D.H. 198586	35	F	20	0	0	0	0	0	+	0	+	++
27	N.D.H. 184720	24	F	20	0	0	0	0	0	+	0	+	++
47	N.D.H. 176898	36	F	20	0	0	0	0	0	+	0	+	++
48	N.D.H. 197730	32	F	20	0	0	0	0	0	+	0	+	+
36	F.H. 50/86	34	F	22	0	0	0	0	0	0	0	+	0
49	N.D.H. 206428	30	M	23	0	0	0	0	0	0	0	+	+++
39	S.P. 57/135	43	M	23	0	0	0	0	0	+	0	+	++
50	N.D.H. 189109	33	F	23	0	0	0	0	0	0	0	++	++
32	N.D.H. 183253	31	M	23	0	0	0	0	0	+	0	++	+++
33	N.D.H. 174414	31	M	23	+	0	0	0	0	++	0	++	+++
51	N.D.H. 188512	41	M	25	0	0	0	0	0	+	0	++	++
29	B.H. 59/292	30	F	26	0	0	0	0	0	+	0	+	+++
52	N.D.H. 191917	34	M	26	0	0	0	0	0	+	0	+	+++
38	N.D.H. 207546	39	F	37	0	0	0	0	0	+	0	++	+++
53	N.D.H. 181450	40	F	30	0	0	0	0	0	+	0	++	+++
54	N.D.H. 177827	38	M	25	0	0	0	0	0	++	0	+++	+++

*See table 1.

duct epithelium (figures 12 and 13), or more often by proliferation of centro-acinar cells. (figure 20). The insular nature of the proliferating cells is proved by the presence of scarce B granules and of "Körnchen." Similar images of neoformation of islets have never been found in the pancreas of any of the chronic or of the control cases.

Intra- and peri-insular inflammatory infiltrates. Inflammatory infiltrates were observed in and/or around certain islets in fifteen of twenty-two acute cases. They were numerous in eight cases, less common in the others (table 5). Often, they were more pronounced in the peripheral portions of the islets (figure 21). Sometimes, they also extended a small distance into the adjacent acinous tissue. Their cytological constitution was variable: lymphocytes, reticular cells, and a few scattered polynuclear cells. Plasmacytes were not observed. Such infiltrates were relatively more frequent around islets of type II, but could also be observed around islets of type I and the transitional forms between these two types. We have never seen them around islets of type III, or in the pancreas of chronic juvenile diabetics (table 6).

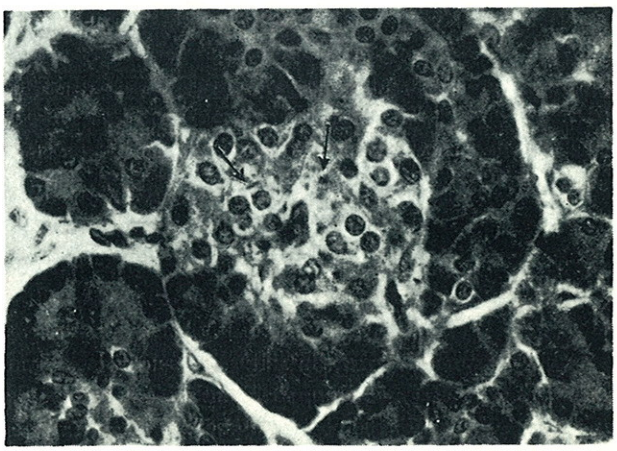

FIG. 20. Newly formed islet from centro-acinar cells. (Case 22, B.H. 58/112). The islet cells have a large round nucleus; their cytoplasm contains "Kornchen" (arrows). Gomori's chromium hematoxylin-phloxine. X 450.

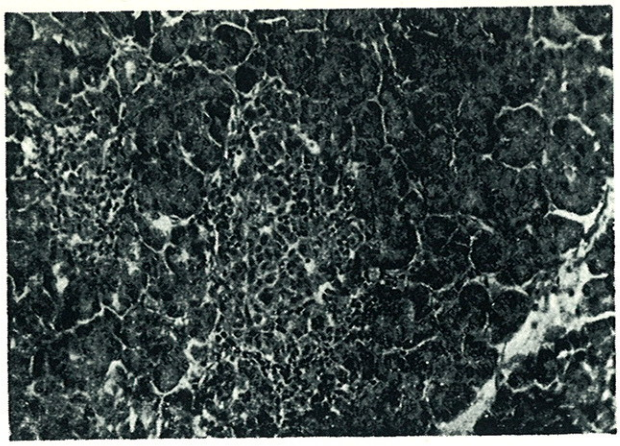

FIG. 21. Islet of Langerhans in a juvenile diabetic (Case 6, Ch.H.B. 60/184). Inflammatory infiltration inside and around the islets. Hemalum erythrosine saffron. X 130.

Insular fibrosis. This lesion is encountered very frequently in the islets of type I and is, indeed, rarely absent in the islets of patients with chronic juvenile diabetes; nor is it uncommon in the acute cases (tables 5 and 6).

Insular hyalinosis. This lesion, which is found frequently in older diabetics, was not observed in our cases of juvenile diabetes (tables 5 and 6). In a single pancreas (Case 17, U.P.H. 59/200), the islets showed hydropic B cells separated by a fibrillar substance, but the appearance was not typical of an insular hyalinosis.

Lesions of the exocrine pancreas. Lesions of the acinar tissue were frequent in the pancreases of juvenile diabetics (tables 5 and 6). In the acute cases, the findings comprised mostly focal or diffuse lesions of acute pancreatitis. These lesions were centered around the excretory canals, which were distended by the secretion product (dyschylia). In the chronic cases, these lesions were also present, but less frequent.

In one of the acute cases (Case 14, M.G.H. 11301), the pancreas presented severe lesions of chronic pancreatitis, with marked sclerosis and inflammatory infiltration. This patient also presented lesions of chronic thyroiditis.

Intra- and perilobular sclerosis was present in the great majority of the chronic cases. As a rule, these lesions were associated with mild atrophy of the acinous tissue. With the exception of the cases showing focal lesions of acute pancreatitis, inflammatory infiltrates were not numerous. Lesions of arterio- and arteriolosclerosis, on the other hand, were rarely absent; in the cases running a protracted course, they were also as a rule extremely severe.

DISCUSSION

A pathological study of the diabetic pancreas has essentially two purposes: (1) to establish a correlation between the clinical and biological features of the disease on the one hand and the histological changes on the other; (2) to determine the significance of these features with regard to the problem of the etiology of diabetes mellitus.

The principal findings of our study can be summed up as follows:

(1) In the patients who died shortly after the clinical onset of the disease, the islets of Langerhans were often large, but their number and the proportion of insular tissue were less than in nondiabetic subjects of corresponding ages. The number of B cells was greatly reduced: on the average it was only 10 per cent of normal. These cells showed cytological signs of intense secretory hyperactivity (cellular and nuclear hypertrophy, increase in the cytoplasmal ribonucleins). The insular cells often showed nuclear anomalies. Peri- and intra-insular inflammatory infiltrates were present in 70 per cent of the cases.

(2) In the patients with juvenile diabetes who died after a course of several years, the pancreas was often small, the islets of Langerhans were decreased in number and consisted of small atrophic cells. With the exception of a few cases, they no longer presented any B cells.

As regards the patients with chronic juvenile diabetes, our findings were entirely in accordance with the classical view that the metabolic disorder in such patients is due to an absolute deficiency of insulin, resulting from a severe inadequacy of the insular tissue. The decreased weight of the pancreas in juvenile diabetes has often been attributed to congenital hypoplasia. The fact that we did not observe this decrease in the cases that had run a brief course, whereas it was indeed present in 40 per cent of our chronic cases, constitutes an argument against this view. The decrease in weight of the pancreas in numerous patients with juvenile diabetes constitutes a secondary alteration which, however, cannot be attributed to an arrest of development or to sclerosis of the exocrine tissue.

The severe atrophy of the insular tissue and the almost complete absence of B cells in the majority of the chronic juvenile diabetics contrasts strikingly with the histological findings in diabetics in whom the disease has set in at a more advanced age. In these patients, the decrease in the quantity of insular tissue is much more moderate, and the B cells are on the average diminished

only to 40 or 50 per cent of normal.[9,10] It seems likely that the few cases of chronic juvenile diabetes in which persisting B cells were found constitute transitional forms between juvenile and adult diabetes.

In the patients with juvenile diabetes in whom the disease ran a brief, fatal course, the B cells were still present, but were markedly decreased in number. This finding deserves particular emphasis. From biological studies revealing normal or even supranormal insulin-like activity in the blood,[1-2] and from the histological studies of Maclean and Ogilvie,[3] various authors have concluded that the insular tissue must still be normal at this stage of the disease. Our investigation does not confirm this conclusion. If the cases we have studied are representative for acute juvenile diabetics as a group, they seem to indicate that the supranormal insulin-like activity or insulin content of the serum of these diabetics is produced by B cells which are strongly reduced in number but markedly hyperactive. The almost complete degranulation, the hypertrophy of the cells and of their nucleus, constitute classical signs of such hyperactivity. The increase in the cytoplasmic ribonucleins is clear evidence of an enhanced synthesis of protein, probably of insulin. We therefore believe that in these patients with juvenile diabetes, the B cells attempt, by intense hyperactivity, to maintain a satisfactory metabolic equilibrium. Up to the time of clinical onset of the disease, they have succeeded in this attempt for a longer or shorter period. However, this equilibrium grows more and more precarious. As soon as physiological or pathological circumstances lead to an increase in insulin requirements (growth, pregnancy, severe infection, etc.), decompensation appears in the form of an acidotic coma, this being the incident that reveals the existence of the disease in the majority of young diabetic patients.

Our study shows that exact comprehension of the role of the islets of Langerhans in the physiopathology of juvenile diabetes can only be achieved by a combination of cytological observations of the islet cells and quantitative estimates of the insular tissue. Neither the amount of islet tissue, nor the size of the islets furnishes an adequate evaluation of their functional value, because a large proportion of the islet cells appear atrophic. On the other hand the demonstration of B cells with cytological signs of hyperactivity in short duration juvenile diabetics and their absence in the majority of chronic cases allows a better correlation with the clinical and biological features of the disease than quantitative studies only. It could be objected that at least part of the islet cells which we have labeled as undifferentiated, are in fact B cells. We do not believe this to be the case. However, if these cells should be B cells, it is evident that they are quite abnormal, not only in respect to their morphological appearance but also by their absence of stimulation under conditions which have stimulated the other B cells. Our contention that the islet tissue is strikingly abnormal at the clinical onset of the disease already, does therefore not appear invalidated by this objection.

Although we have stressed the importance of the insular alterations early in the course of juvenile diabetes, we would definitely not wish to assert that these alterations are the primary cause of the disease. The further question arises whether the severe quantitative deficiency of the B cells observed in the juvenile diabetic patient is congenital or results from slow deterioration of the insular tissue under the influence of an extrinsic factor. From our morphological findings, no definite answer to this question can be obtained. On biological grounds it seems improbable that the intense hyperactivity of the B cells serves solely to compensate for their numerical inferiority; the often supra-normal insulin-like activity of the blood in cases of acute juvenile diabetes and of prediabetes[1,2] would be difficult to explain if this hypothesis were valid. On the other hand, the histological features of the insular tissue in these patients constitute a strong argument in favor of the existence of an extrinsic stimulatory factor. Maclean and Ogilvie[3] have already described the presence, in patients with acute juvenile diabetes, of large islets and our study confirms this finding. We have also observed evidence of new islet formation at the expense of centro-acinar and tubular cells; this leads to the formation of islets consisting largely of hyperactive B cells (islets of type II). For reasons that are not yet understood, the B cells disappear progressively from these islets, leaving only the A cells, which undergo atrophy, and a collapsed stroma which undergoes fibrosis. In this way, the islets of type I are formed, which constitute the greater part of the insular population in juvenile diabetics. It seems probable that in the pancreas of acute juvenile diabetics we had the opportunity to catch the final stages of a process which has been going for an indefinite time, perhaps from birth on.

In recent years, a number of factors have been described which might represent agents stimulating insular function; among these are physiological antagonists of insulin (growth hormone, adreno-cortical hormones), pathological antagonists ("synalbumin antagonist" of Vallance-Owen,[11] antibodies), disorders of

the mechanism of transport of insulin,[12-14] anomalies of fatty acid metabolism.[15] A discussion of these numerous factors would be outside the scope of the present paper, which is concerned only with morphological problems. We are of the opinion that none of these factors is capable of provoking permanent diabetes without severe alterations in the insular tissue being present.

The mechanism by which the B cells progressively disappear from the islets of type I has not yet been elucidated. We are confronted with an identical problem in the idio- and metahypophyseal diabetes of the dog. In the latter case, the destruction of the B cells has been attributed to a hyperactivity which leads to hydropic degeneration and to death of the cells. The studies of Lazarus and Volk[16,17] have revealed, however, that the glycogen overload which characterizes the hydropic transformation of the B cells, is not associated with any alteration in the cellular organelles and that it therefore cannot be regarded as a degenerative lesion. In dogs treated with growth hormone, on the other hand, the same authors observed a different type of cellular change: the "ballooning degeneration," which is associated with nuclear pyknosis and with other ultramicroscopic signs of cellular dysfunction. We have regularly found lesions of "ballooning degeneration" in the B cells of spontaneously diabetic dogs.[18] On the other hand we have been unable to find any corresponding changes in human diabetic patients. In a number of human cases, we have observed irregularity and hyperchromatism of the nuclei of the B cells. The exact significance of these nuclear changes is difficult to assess. We do not believe that they result from autolysis, because they have not been found outside the islets or in the islets of the control cases. They may represent a process of degeneration. The intense secretory stimulation indicated by the abundance of the cytoplasmic ribonucleins may in the long run lead to a disorder of the regulation of nucleic acid metabolism and to progressive extinction of the strain of B cells.

In connection with the etiology of diabetes, one finding deserves particular attention: the frequency of peri- and intra-insular inflammatory infiltrations in juvenile diabetics who had died soon after the clinical onset of the disease. Such infiltrates were observed as early as the beginning of the century by a number of pathologists (see Kraus).[19] Warren and Root[20] and Stansfield and Warren[21] again called attention to this lesion. So far, all authors are in agreement that this finding is specific for diabetes and that it is only found in young diabetic patients in whom the disease has run a rapid course. LeCompte[22] has recently made a study of this lesion and advances the hypothesis that it may not be so uncommon as has hitherto appeared. Our findings support this view: we have been able to find such infiltrates in fifteen of the twenty-two acute cases, i.e., in 68 per cent. This high frequency is the more remarkable since we had only routine material at our disposal, so that fully detailed examination was possible only in a proportion of the cases.

What significance is to be attached to the inflammatory infiltrates? In LeCompte's study[22] a number of possibilities are reviewed: an inflammatory lesion of viral or microbial origin, an alteration secondary to lesions in the insular cells due to hyperactivity, an inflammatory reaction following the destruction of insular cells by an unknown toxic agent, and an immunological reaction.

None of these hypotheses appears to be entirely satisfactory. The possibility of an infectious etiology is suggested by the fact that in certain juvenile cases, infectious phenomena coincide with the onset of the diabetes.[23] The development of diabetes following mumps has been described.[24] Gundersen[25] has observed an increased incidence of diabetes during the years immediately following epidemics of mumps in Norway. Barboni and Manocchio[26] have reported the onset of diabetes in cows shortly after an epidemic of aphthous fever; in the islets of the diabetic animals these authors observed inflammatory infiltrates. However, in the great majority of cases of human diabetes, no infectious etiology appears to be present.

Inflammatory infiltrates of the islets of Langerhans are usually not observed in idio- or metahypophyseal diabetes, after alloxan, or in other forms of experimental diabetes. However, Toreson et al.[27] have recently reported such infiltrations in rabbits made diabetic by immunization with beef insulin.

The view that the insular lesions might have an immunological origin has very recently found support in a number of interesting observations. In rats that have been rendered diabetic by intravenous administration of anti-insulin serum from guinea pigs, Lacy et al.[28] found inflammatory infiltrates with eosinophil polynuclears and lymphocytes around and within the islets of Langerhans. Toreson et al.[27] recently reported infiltrations of the islets and interlobular stroma with mononuclear cells in rabbits that have developed a diabetic state, following immunization against beef insulin. After protracted administration of homologous insulin to cows, Renold et al.[29] observed very pronounced and extensive

lymphocytic infiltrates in the islets. With the aid of fluorescence technics, Mancini et al.[30] were able to demonstrate antibodies against human insular tissue in the serum of a young diabetic who had never received insulin. In this connection, it may be worthwhile to recall that one (Case 14, M.G.H. 11301) of the young diabetic patients whom we have studied concurrently presented lesions of thyroiditis. These observations pointing to a possible immunologic derangement are of the greatest interest in view of the high frequency with which we have found inflammatory infiltrates in our recent onset juvenile diabetics. Further investigation is however required before it will be possible to evaluate their significance in regard to the etiology and the pathogenesis of human diabetes.

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