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ON ENDOCRINOLOGY

Vol. 9  Internal Secretions of the Pancreas
A leaflet giving details of available earlier volumes in this series, and also of the Ciba Foundation General Symposia and Colloquia on Ageing, is available from the Publishers.
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PREFACE

Between January 1950 and July 1955 the Ciba Foundation has organized and held on its premises in London, 36 small, international conferences. In the early days the majority of these were on subjects within the field of endocrinology, partly because the Director was a novice in his task and it was easier to concentrate his efforts within fairly well-defined limits, and partly because research on hormones entered almost every other field of biological activity and so afforded an opportunity for the Foundation's promotion of co-operation, not only internationally, but also, and of at least equal importance, between workers in different disciplines.

The range of the Foundation's conferences has spread much further in recent years, but at least one opportunity is found each year to maintain our interest in endocrinological research. A colloquium on "Hormonal factors in carbohydrate metabolism", held in 1952, had defined many unsolved problems and left an impression of advances being made to some purpose in this field. The Director therefore readily agreed to Prof. Young's suggestion, partly stimulated by Prof. de Duve, for a follow-up colloquium on "The nature and actions of the internal secretions of the pancreas". Both in the arrangements for the meeting and for his chairmanship of the proceedings, the Director is happy to acknowledge his real debt to Prof. Young.

To those to whom this book serves as an introduction to the activities of the Ciba Foundation it should be explained that it is an international centre, which is established as an educational and scientific charity under the laws of England. It owes its inception and support to its founder, CIBA Ltd., of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific
workers who visit London from abroad, organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiène) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussions, awards an annual lectureship, has initiated a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

Leading research workers from different countries and in different disciplines are invited to attend the symposia or colloquia. The size of the group is, however, very strictly limited in order to obtain a free conversational manner of discussion—although the basic time-table of the programme is strictly observed. The smallness of the groups means the exclusion of many workers active and interested in the subjects discussed, and therefore the proceedings of these conferences are published and made available throughout the world.

It is hoped that the papers and discussions in this book will prove not only informative and stimulating, but will also give to readers a sense of participation in an informal and friendly occasion.
# CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chairman's opening remarks</td>
<td>1</td>
</tr>
<tr>
<td>F. G. Young</td>
<td></td>
</tr>
<tr>
<td>Morphological studies of the destruction of α-cells by chemical means</td>
<td>2</td>
</tr>
<tr>
<td>by H. Ferner</td>
<td></td>
</tr>
<tr>
<td>Discussion: Best, Cavallero, Ferner, Foà, Goldner, von Holt, Mosca, Sutherland, Young</td>
<td>7</td>
</tr>
<tr>
<td>Metabolic effects of α-cell destruction</td>
<td>14</td>
</tr>
<tr>
<td>Discussion: Behrens, Best, C. F. Cori, de Duve, Ferner, Foà, Goldner, von Holt, Kerly, Lawrence, Mosca, Randle, Schulze, Young</td>
<td>26</td>
</tr>
<tr>
<td>Pituitary growth hormone and blood insulin activity</td>
<td>35</td>
</tr>
<tr>
<td>by P. J. Randle</td>
<td></td>
</tr>
<tr>
<td>Discussion: Best, R.-Candela, G. Cori, Foà, Lawrence, Park, Randle, Waugh, Young</td>
<td>51</td>
</tr>
<tr>
<td>The control of the secretory activity of the islets of Langerhans</td>
<td>55</td>
</tr>
<tr>
<td>by P. P. Foà</td>
<td></td>
</tr>
<tr>
<td>Discussion: Best, G. Cori, de Duve, Foà, von Holt, Mosca, Randle, Young</td>
<td>72</td>
</tr>
<tr>
<td>The effects of hypophysectomy and of prolonged growth hormone administration on the pancreatic α-cells of the rat</td>
<td>75</td>
</tr>
<tr>
<td>by M. G. Goldner and B. W. Volk</td>
<td></td>
</tr>
<tr>
<td>Discussion: Behrens, Best, R.-Candela, Desaulles, de Duve, Ferner, Foà, Goldner, von Holt, Mosca, Sutherland, Young</td>
<td>85</td>
</tr>
<tr>
<td>Fractionation of insulin</td>
<td>89</td>
</tr>
<tr>
<td>by E. Fredericq</td>
<td></td>
</tr>
<tr>
<td>Discussion: Behrens, Craig, Fredericq, Hodgkin, Sanger, Waugh, Young</td>
<td>108</td>
</tr>
</tbody>
</table>

Vii
## CONTENTS

<table>
<thead>
<tr>
<th>The chemical structure of insulin</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by F. SANGER</td>
<td></td>
</tr>
<tr>
<td><em>Discussion: Craig, Fredericq, Porter, Sanger, Waugh</em></td>
<td>110</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A concept of the three-dimensional structure of insulin</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by D. F. Waugh</td>
<td></td>
</tr>
<tr>
<td><em>Discussion: Behrens, Best, C. F. Cori, G. Cori, Craig, Fredericq, Hodgkin, von Holt, Lens, Porter, Sanger, Sutherland, Waugh</em></td>
<td>122</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A speculation on insulin</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by Dorothy Crowfoot Hodgkin and Beryl Oughton</td>
<td>133</td>
</tr>
<tr>
<td><em>Discussion: Behrens, Best, C. F. Cori, G. Cori, Waugh</em></td>
<td>138</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin and glucagon</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by W. Schulze</td>
<td>147</td>
</tr>
<tr>
<td><em>Discussion: Behrens, Best, C. F. Cori, de Duve, Ferner, Foà, Goldner, Schulze, Young</em></td>
<td>163</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical and biological characteristics of glucagon</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by O. K. Behrens, A. Staub, Mary A. Root and W. W. Bromer</td>
<td>167</td>
</tr>
<tr>
<td><em>Discussion: Behrens, Best, R.-Canadela, de Duve, Foà, Goldner, von Holt, Randle, Schulze, Sutherland, Young</em></td>
<td>174</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The action of glucagon on liver phosphorylase</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by E. W. Sutherland, W. D. Wosilait and T. W. Rall</td>
<td>179</td>
</tr>
<tr>
<td><em>Discussion: C. F. Cori, de Duve, Sutherland, Young</em></td>
<td>191</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitory effect of glucagon on the insulin glucose uptake of the isolated diaphragm of the rat</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by J. L. R.-Canadela and R. R.-Canadela</td>
<td>194</td>
</tr>
<tr>
<td><em>Discussion: Behrens, R.-Canadela, C. F. Cori, G. Cori, de Duve, Foà, Park, Randle, Sutherland, Young</em></td>
<td>198</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The hepatic action of insulin</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by C. de Duve</td>
<td>203</td>
</tr>
<tr>
<td><em>Discussion: Behrens, Best, C. F. Cori, de Duve, Kerly, Lawrence, Randle, Schulze, Sutherland, Young</em></td>
<td>223</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Some problems of permeability of tissue cells to sugars</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>by E. Helmreich and C. F. Cori.</td>
<td>227</td>
</tr>
</tbody>
</table>
CONTENTS

The transport of glucose and other sugars across cell membranes and the effect of insulin


*Discussion: R.-CANDELA, C. F. CORI, PARK, ROSS, SCHULZE, YOUNG* 260

Pancreatic islets and growth

*R. CAVALLERO* 266

*Discussion: BEHRENS, BEST, CAVALLEBO, G. CORI, DESAULLES, FERNER, GOLDNER, RANDLE, SUTHERLAND, YOUNG* 280

Chairman's closing remarks

*F. G. YOUNG* 285
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21st–23rd June 1955

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CHAIRMAN'S OPENING REMARKS

This meeting is a mixture of physicists, chemists and biologists which might at first sight seem to be a somewhat immiscible one. I am sure that we shall quickly prove the contrary and that all of us will find interest in the subjects discussed by specialists in other fields. I hope that those who are chemists or physicists will not hesitate to enter into discussions concerning biological matters, and conversely. All may have ideas that can usefully be discussed on an occasion such as this.

I hope we are going to discuss without inhibitions, and perhaps in some detail, the control of the secretion of some of the hormones with which we are concerned. This is a subject that has developed considerably since the last Ciba Foundation Colloquium on today's topic, and some interesting measures of disagreement in this field are foreshadowed by the abstracts before us. Though we may not eliminate these differences of opinion as a result of this meeting, I believe that we shall certainly find a great deal of fruitful discussion on matters such as these and indeed many others.
MORPHOLOGICAL STUDIES OF THE DESTRUCTION OF $\alpha$-CELLS BY CHEMICAL MEANS

Helmut Ferner
Department of Anatomy, University of Hamburg

There is no doubt that only the $\beta$-cells of the islets of Langerhans produce insulin. However, as you know, we have good reason to consider the $\alpha$-cells as the source of glucagon. Both cell types of islets are characterized by the difference in staining ability of their granules. In adult mammals and in adult man the $\alpha$-cells amount to approximately 20 per cent and the $\beta$-cells to 80 per cent of the islet epithelial cells. In newborn infants the islet organ consists of approximately equal numbers of $\alpha$- and $\beta$-cells. The normal $\alpha:\beta$-relationship is dependent upon the age of the individual. One obtains this relationship rather precisely in counting the $\alpha$- and $\beta$-cells in 30–50 islets and taking an average. The $\alpha:\beta$-relationship is a significant index. A sharp shift of the $\alpha:\beta$-relationship to one side or the other is linked with blood sugar changes. Predominance of the $\beta$-cells, for example, after destruction of the $\alpha$-cells or in $\beta$-cell adenoma, results in hypoglycaemic conditions. In all types of permanent diabetes mellitus we find a predominance of the $\alpha$-cells. This may be a relative or an absolute predominance of the $\alpha$-cells, as, for example, after the destruction of the $\beta$-cells by alloxan (Ferner, 1952).

In rats and rabbits, which are the subject of our studies, we found the $\alpha$-cells always in the peripheral zone of the islets. In rats they form an incomplete covering around the $\beta$-cells (Fig. 1). In rabbits the $\alpha$-cells are found as individual cells or in small groups on the periphery of the islets.

An insight into the metabolic significance of the $\alpha$-cells may be gained if the $\alpha$-cells can be selectively destroyed and eliminated. Such experiments have been carried out by a
number of investigators with different chemicals (Campenhout and Cornelis, 1951; Campenhout et al., 1954; Davis, 1952; Goldner, Volk and Lazarus, 1952; Runge, 1954; v. Holt et al., 1955).

Extreme care must be exercised during microscopic examination, because often as a result of handling of tissues an unspecific shrinkage is found on the periphery of the islets, where the \( \alpha \)-cells are located. These artifacts might mislead one into believing that the \( \alpha \)-cells have been destroyed. Moreover, the entire pancreas must be examined. If part of the \( \alpha \)-cells are injured and the others remain intact, a functional impairment cannot be expected, because the islet system has a great functional reserve. On the other hand, if one considers the morphological picture of the actual destruction of the \( \alpha \)-cells it is not as impressive as the destruction of the \( \beta \)-cells by alloxan, because the \( \alpha \)-cells comprise only one fifth of the islets.

We have microscopically examined the pancreas of many rats and rabbits which have been treated with synthalin A or with \( p \)-aminobenzenesulphonamidoisopropylthiodiazole (IPTD). The dose of synthalin administered was 15 mg./kg. intraperitoneally to rats and 6 mg./kg. intramuscularly to rabbits. IPTD was given in doses of 1–2 g./kg. intraperitoneally to rats and orally to rabbits. For the differentiation of the \( \alpha \)- and \( \beta \)-cells Gomori’s chromhaematoxylin-phloxin method was used.

Both \( \alpha \)-cytotoxic drugs act rapidly on the \( \alpha \)-cells. In from 1–3 hours after a single dose we observed the first changes in the \( \alpha \)-cells, which as a rule after 24 hours have reached a maximum. The first indication of injury is the clumping of the fine \( \alpha \)-granules. The \( \alpha \)-cells are filled with large, dirty red-stained clumps, between which the pink cytoplasm is visible. After approximately 3–6 hours only a few clumps are discernible. The cytoplasm loses its affinity for phloxin and appears transparent and very clear (Fig. 2). The \( \alpha \)-cells now resemble vacuoles with nuclei whose cell bodies are swollen and enlarged (Fig. 3). This is not a question of true
degranulation. However, it is a progressive dissolution of the cell contents with the enlargement of the cell bodies by absorption, apparently of water.

The nuclei of the α-cells remain unchanged for a longer time. Then in the hydropic stage many nuclei are deformed, their outlines are angular and the chromatin structure is coarser and indistinct. Some of the nuclei are pycnotic.

In rats the hydropic state of the α-cells persists for many days after a single dose. It is not evident that the α-cells undergo necrosis and that they disappear in great numbers. However, no signs of a regeneration of α-cells have been observed.

In rabbits it is quite different. If they survive, the majority of treated animals demonstrate a resorption of the injured α-cells. In a few rabbits the α-cells actually disappeared completely. If one examines, microscopically, the pancreas of an animal 1–2 days after treatment, one finds on the periphery of the islets a cleft, where the α-cells were located previously (Fig. 4). The cytologically intact β-cell complex is now separated from the exocrine parenchyma. In the clefts are visible a few red-stained fragments and débris, which are the remnants of the α-cells (Fig. 5).

It is surprising that sometimes 6 hours after the single dose of synthalin or IPTD the α-cells of the rabbit pancreas can actually be made to disappear, and it is interesting to note that this phenomenon occurs only in some of the treated animals in spite of equal dosage and administration.

Later the gap between the exocrine parenchyma and the β-cell complex is closed and the islets present a normal picture in unspecifically stained preparations.

Especially after large doses of synthalin or IPTD the destruction of the α-cells can be demonstrated in a somewhat different manner, which is reminiscent of the destruction of the β-cells by alloxan. The α-cell groups break up into individual cells, which are separated from one another by gaps. They become small, shrunken and hyperchromatic. They stain intensively red or reddish violet. Even these injured
FIG. 1. One half of an islet of Langerhans from a normal adult rat. The \( \alpha \)-cells on the periphery, indicated by arrows, contain fine granules. Gomori's stain.

FIG. 2. Islet of a rat, 28 hours after a single dose of IPTD; dropic \( \alpha \)-cells on the periphery. Low magnification.

*Facing page 4*
FIG. 3. Marked hydropic degeneration and swelling of the $\alpha$-cells of a rat, 24 hours after IPTD. High magnification.

FIG. 4. An islet from a rabbit, 6 hours after 2 g./kg. IPTD. Destruction and elimination of the $\alpha$-cells. Clefts with $\alpha$-cell fragments and débris; $\beta$-cells are intact.
FIG. 5. An islet from a rabbit, 28 hours after administration of 2 g./kg. IPTD. Destruction and complete disappearance of the α-cells. Clefts are visible where α-cells were located previously; β-cells are uninjured.

FIG. 6. Rabbit treated with IPTD and alloxan. A destroyed islet containing the remnants of injured β-cells. On the periphery are spindle-shaped remnants of the α-cells. Coagula in the intermediate spaces.
**Fig. 7.** Pancreas of a rat treated with alloxan and synthalin. Loose connective tissue as an islet scar.

**Fig. 8.** Pancreas of a rat after combined use of alloxan and synthalin. The remnant of the islet consists of connective tissue with an $\alpha$-cell plasmodium without signs of injury.
Fig. 10. Left: $\alpha$-cell undergoing mitosis in a 5-day old rat (control). Right: $\alpha$-cell undergoing mitosis after administration of 10 mg./kg. synthalin to a 4-day old rat. The mitotic figure is pycnotic.
α-CELL DESTRUCTION BY CHEMICAL MEANS

α-cells disappear and in their place one finds a cleft with cell fragments.

Concerning the β-cells one cannot demonstrate any lesions after administration of synthalin or IPTD. If the animal does not receive glucose, the β-cells are as a rule small, dense and uniformly granulated. As a consequence of the administration of glucose we observed a degranulation of the β-cells. Glucose was given to offset hypoglycaemic shock, which occurs after administration of synthalin or IPTD.

The combined use of alloxan on the one hand and synthalin or IPTD on the other, brings about the destruction of both islet cell systems with the result that the islets of Langerhans completely disappear (Fig. 6). Such a nesectomy will be most completely accomplished by chemical substances, if one commences with a diabetogenic dosage of alloxan and then administers synthalin or IPTD from 5 to 10 days later. When we speak of a complete nesectomy, we mean that in the entire pancreas neither intact islets nor intact islet cells can be observed.

For a short period prior to the complete elimination of the islets one finds scars (Fig. 7). These scars consist of loose connective tissue with a rich capillary network. They exist for a short period of time and then they too disappear completely.

Furthermore, we suppose that the metabolic difference between a total pancreatectomy and a chemical nesectomy, apart from the exocrine pancreas, is related to the existence of a gastro-intestinal α-cell system.

In rats plasmodial α-cell complexes develop after alloxan treatment in the region of the islets. In the duct system, as we know, one observes the outgrowth of α-cell complexes. It is surprising that these newly developed α-cells of alloxan diabetic rats were not injured by α-cytotoxic substances (Fig. 8). Therefore we investigated the α-cells in young rats. In young rats we have found another effect of synthalin on α-cells (Ferner and Runge, 1955). We gave one subcutaneous injection of synthalin (10 mg./kg.) to rats in the first days of
FIG. 9a. A curve showing the mitotic rate of the α-cells in 100 islets of rats from the second to the fifth day of life. 
A = controls, A' = rats after synthalin.

FIG. 9b. A curve showing the mitotic rate of the β-cells in 100 islets of rats from the second to the fifth day of life. 
B = controls, B' = rats after synthalin.
life. It was not possible to produce any type of injury to the \( \alpha \)-cells in young rats. However, the mitotic frequency of the \( \alpha \)-cells in synthalin-treated rats was sharply reduced in comparison with normal animals of the same age. At the fifth day of life, for example, the mitotic rate in treated rats was only 25 per cent of the mitotic rate of normal animals (Fig. 9a). In normal rats the mitotic rate of the \( \alpha \)-cells steadily increases from the second to the fifth day of life. In synthalin-treated rats of the same age the curve remains at a low level. The mitotic frequency of the \( \beta \)-cells and of the exocrine cells is the same in treated and in untreated animals (Fig. 9b). A further result was that the existing mitosis of the \( \alpha \)-cells in synthalin-treated young rats was, without exception, pathological. The chromosomes of the prophase and the metaphase were clumped and swollen. The entire mitotic figure was pycnotic (Fig. 10).

We conclude that synthalin acts as a selective mitotic poison on the \( \alpha \)-cells in young rats during the first days of life without causing noticeable injuries to the \( \alpha \)-cells which are not dividing.

REFERENCES


DISCUSSION

Young: Prof. Ferner, you said that in all types of diabetes mellitus there is a predominance of \( \alpha \)-cells in the islets. Does that mean that the \( \alpha : \beta \)-cell ratio changes from 1:4 to something greater than 1:1 in all cases?
DISCUSSION

_Ferner_: There is a relative predominance of α-cells in permanent diabetes mellitus. A ratio of $\alpha : \beta = 2 : 3$, that means a relative predominance of α-cells, is sufficient, it is not necessarily a ratio greater than 1:1.

_Young_: Do you know if the gastro-intestinal α-cell system to which you referred is also sensitive to your agents?

_Ferner_: We do not yet know, because it is very difficult to find the single spread α-cell in the gastro-intestinal mucosa and to be sure that this is one α-cell. This is only possible by silver impregnation methods, and we have no results on it.

_Fodor_: In a seminar at the University of Illinois a year or two ago, Dr. A. A. Godlowski showed that intestinal α-cells may be destroyed by cobalt chloride.

_Goldner_: I would like to raise two questions with regard to Prof. Ferner's observation of an α-cell preponderance in the pancreatic islets of all types of diabetes. Is it not correct that such α-cell predominance may be found occasionally also in the pancreatic islets of patients with diseases other than diabetes, and that many investigators find it only in the minority of cases with diabetes? I have in mind particularly Gomori's latest statement that he finds a normal α : β-cell ratio in more than 60 per cent of his human material. What is the explanation for this discrepancy? This leads to my second question: is it possible that it is due to differences in staining techniques? Creutzfeldt (1953, _Beitr. path. Anat._, 113, 183) has only recently re-emphasized the capriciousness of the Gros-Schultze silver stain; in animal experiments, Dr. Volk, Dr. Lazarus and I, like many other investigators, find quite a difference in the α-cell count in the islet of the rat, depending on the staining technique which is applied. The Gomori chrom-alum-haematoxylin-phloxin technique, for instance, permits us to identify as α-cells about 36 per cent of the islet cells, while our silver impregnation method identifies only about 6 per cent as α-cells. This silver technique is a modification of the Davenport method which Dr. Volk has developed and which appears to give more reliable and reproducible results than the Gros-Schultze method. I shall refer to this method later in our paper.

_Ferner_: This is a question of method and of animal. In man, a good silver impregnation method brings out nearly the same number, in my opinion, as does the Gomori stain technique. I think the Gomori stain technique brings out more α-cells than really exist, because degranulated β-cells may be stained red by phloxin and appear to the eye like α-cells. In normal human pancreas I always found 20 per cent of α-cells with the silver impregnation method. In the pancreas of rats the silver impregnation method is not useful for the determination of the α : β-ratio.

_Cavallero_: Prof. Ferner, have you any information about the damaging effect of IPTD on the α-cells of the pancreatic ducts?

_Ferner_: Newly developed α-cells are not destroyed by α-cytotoxic substances. It is surprising, and this is why we investigated young rats.

_Cavallero_: The same thing happens with alloxan, as young animals appear to be resistant to this substance.
DISCUSSION

Fernen: Quite right.

Goldner: Prof. Ferner did not mention the $\alpha$-cytotoxic action of cobalt chloride—possibly because his experiences with this agent have not been satisfactory. I feel, however, that it is in place here to call your attention to the fact that cobalt chloride can produce in the pancreatic islets of the rabbit and the dog histological changes which are almost identical with those shown by Prof. Ferner and produced with IPTD and with synthalin. Shortly after Van Campenhout and Cornelis had demonstrated the selective destructive action of cobalt chloride in the islets of the guinea pig, Drs. Volk and Lazarus and I succeeded in showing that the rabbit and the dog are equally susceptible to the $\alpha$-cytotoxic action of cobalt chloride (1952, *Metabolism*, 1, 544; 1953, *Metabolism*, 2, 518).

Some laboratories, like the group in Hamburg, had difficulty in reproducing our findings, but others have readily been able to do so. Confirmation of our histological findings has been reported by Fodden in the United States (1953, *Amer. J. clin. Path.*, 23, 1002), Kadota in Japan (1955, *Metabolism*, 4, 387), Stahl and Dorner in France (1954, *C. R. Soc. Biol. Paris*, 148, 590), and Rao in India (1955, *Acta Endocr.*, 18, 253), to name only a few. If cobalt chloride is given in adequate doses, one obtains pancreatic islets which are entirely void of $\alpha$-cells, as shown in Fig. 1, a and b. If cobalt chloride is given to alloxan diabetic rabbits or dogs, one can obtain histological pictures showing a chemical nesidectomy. This is shown in Fig. 1, c and d. Thus, the morphological effect of cobalt chloride appears to be quite similar to that of the $\alpha$-cytotoxic agents used by Ferner, von Holt and their associates. A most important difference, however, becomes evident if we look at the functional changes in blood sugar homeostasis which accompany the administration of cobalt chloride. There occurs immediately a transitory hyperglycaemia. This was interpreted initially, and as we now know erroneously, as evidence of release of a hyperglycaemic factor from the disintegrating $\alpha$-cells. We have been able to demonstrate that this transitory hyperglycaemia is of extrapancreatic origin (1953, *Proc. Soc. exp. Biol., N.Y.*, 82, 408). To give only one piece of evidence: it occurs also in the pancreatectomized animal. Our finding has been confirmed by other investigators with other methods. Thus, Elrick (1953, *Proc. Soc. exp., Biol., N.Y.*, 82, 76) demonstrated by means of adrenergic blocking agents that cobalt hyperglycaemia is not due to glucagon, since the former is inhibited by dihydroergotamine, while glucagon hyperglycaemia is not.

Now except for this transitory hyperglycaemia which has nothing to do with the $\alpha$-cells, there occur no other changes in the blood sugar level of the animals whose pancreatic $\alpha$-cells have been destroyed or damaged by cobalt chloride. The normal animals do not develop a hypoglycaemia, nor do the alloxan diabetic animals show a decrease of their hyperglycaemia. These findings led us to conclude that the $\alpha$-cells do not play a significant role in blood sugar homeostasis and that glucagon, if it is produced by $\alpha$-cells, has little significance for the aetiology of diabetes (1954, *Arch. intern. Med.*, 93, 87).

von Holt: We have also tried to destroy the $\alpha$-cells by using cobalt
chloride in doses of 12.5 and 25 mg./kg., and were not successful; but after repeated injections of cobalt chloride we found in rabbits a hyperplasia of the \( \alpha \)-cell system, and after about 14 days an impaired sugar tolerance. It was possible to change the proportion of the \( \alpha \): \( \beta \)-cells to 1:1. (Holt, C. v., and Holt, L. v., 1954, *Z. Naturf.*, 9b, 319).

*Cavallero*: In our laboratory a dosage of 40–50 mg. of cobalt chloride has been injected daily into rats for 7 days without any alteration in the \( \alpha \): \( \beta \)-cell ratio and without cytological changes of the \( \alpha \)-cells (Lesca, S., and Malandra, B., 1954, *Biol. latina*, 7, 781).

*Best*: I wonder if the histologists have any further information regarding similarities or differences between the pancreatic \( \alpha \)-cells and the silver-staining cells of the gastric mucosa.

*Goldner*: Although I am not a histologist, I shall try to answer Prof. Best's question. There are some staining differences which indicate functional differences between these cells. The intestinal argentaffin cells reduce silver salts directly, while the pancreatic cells and those in the gastric mucosa need a reducing agent (formalin) to become impregnated by silver. I believe Fodden (1953, *Amer. J. clin. Path.*, 23, 994) has done a comparative study of these cells and described still other differences.

*Young*: Has it been demonstrated in a depancreatized animal that the \( \alpha \)-cytotoxic substance will significantly diminish the intensity of the diabetes?

*Ferner*: That has not been investigated.

*Goldner*: If one considers cobalt chloride as an \( \alpha \)-cytotoxic agent, then one can say that the diabetes of a depancreatized dog is not ameliorated by it. Of course there remains the problem of the so-called \( \alpha \)-cells in the gastro-intestinal tract. But there are species which have these cells in their gastro-intestinal tract, but no glucagon can be extracted from them, for instance, the hog. Is that right?

*Sutherland*: Yes. However, it is possible that some species contain glucagon-secreting cells in the intestine.

*Goldner*: I do not know whether diabetes by alloxan or pancreatectomy has ever been produced in a hog. If that were successful, one could then give an \( \alpha \)-cytotoxic agent and would not have to contend with the intestinal \( \alpha \)-cells. A report on amelioration of diabetes mellitus following subtotal gastrectomy was published recently (1955, *Surg. Gynec. Obstet.*, 100, 201). The authors describe three cases with rather impressive results. I am familiar with them, and wonder whether the improvement of the diabetes was due to the removal of a specific hyperglycaemic factor in the gastric mucosa, or simply to the change in the patient's general well-being, relief of pain, change in diet, etc. This is very difficult to decide.

*Young*: The findings may be all right, but this particular interpretation could easily be wrong.

*Fod*: The interpretation is difficult. I was fortunate enough to hear Dr. Ellison of Ohio State University report on nine cases of "Intractable peptic ulceration associated with \( \alpha \)-cell tumours of the pancreas" (Illinois Section of Soc. exp. Biol. Med., meeting of May 17, 1955).