

**Zoology.** — *On the function of the Golgi-apparatus in the exocrine pancreas cell. II. Experimental quantitative study on the relationship between Golgi-vacuoles and pro-enzyme granules.* By J. W. SLUITER. (From the Zoological Laboratory, University of Utrecht.) (Communicated by Prof. CHR. P. RAVEN.)

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It is generally accepted that the Golgi-apparatus is participating in some way in the process of formation of pro-enzyme granules in the exocrine pancreas cell. This has been concluded from the marked topographical connection, which can be observed between Golgi-apparatus and pro-enzyme granules but it has never been definitely proved for instance by experimental or quantitative data.

In a previous paper (SLUITER, 1948) it could be shown that special small vacuoles are lying in close connection with the Golgi-apparatus, which vary considerably in number.

The aim of the present paper has been to find out, whether this phenomenon could be a structural manifestation of the functional activity of the Golgi-apparatus.

#### *Experiments.*

The pancreas cells of a white mouse do not function synchronously under normal conditions. Sections of the pancreas of this animal show, at any given moment, cells in several different stages of their activity. This wellknown circumstance is not favourable for a statistical investigation into the physiological sequence of these stages and of the functional changes, which may go on in the Golgi-apparatus. Therefore, the method of pilocarpine injections has been used for this investigation.

Although several of the previous authors on the subject (NASSONOV 1924, MORELLE 1927) have used this method, the most important results have been obtained by HIRSCH (1932). Applying his "Stufenzählmethode" on living pancreas material, partly after injection of pilocarpine, this author could show the following facts. Under normal conditions the cells of one pancreas are functioning asynchronously (i.e. less than 30 % of the cells have reached the same functional stage). In starving animals the cells work hemisynchronously (i.e. 30—60 % of the cells are at the same stage). Injection of pilocarpine after a starvation period forces most of the cells to extrude their accumulated products and to begin at the same moment with the restitution of pro-enzyme granules. This causes the hemisynchronous activity to be altered in a synchronous one (i.e. more than 60 % of the cells are at the same stage).

This state of affairs is much more favourable for the aim of the present

investigation. Therefore, a number of white mice, with body weights varying from 5,3 to 25,5 gr, were injected with 0,3 to 0,7 mgr pilocarpine. At different moments after the injection two injected animals (one juvenile and one adult) were killed for autopsy at the same time. Pieces of their pancreas were fixed, impregnated with osmic acid, sectioned and coloured as has been described in a recent paper (SLUITER 1948).

Of each pancreas 100 median cell sections were selected and classified according to the "Stufenzählmethode". This number can be considered to be sufficient, since HIRSCH (1932) actually observed that data, which are obtained from a little part of the pancreas, are representative for the organ as a whole. Objectivity in selecting the cell sections is sufficiently guaranteed by the condition that they must contain the median plane of the cell. This can be easily recognized as the cells are conical and as the positions of the nucleus, the mitochondria and other cell components in the sections are very characteristic.

Moreover, the "Stufenzählmethode" has been applied here for the first time to cell components by counting the Golgi-vacuoles and by estimating the number of pro-enzyme granules in the 100 cell sections mentioned above.

Considering the reliability of the method used here, it must be taken into account that according to HIRSCH (1932) circumstances like food, general condition, age and other ones may influence the results. Moreover, it was not possible to observe the Golgi-vacuoles in living cells. Fixation of the material implies in this case that the data of several different mice have to be used to reconstruct processes, which are supposed to go on in one pancreas cell, while HIRSCH (1932) studying the living pancreas could use the same animal during 10 hours after pilocarpine injection.

In order to test the reliability of material and method, used for the present paper, the mode of functioning of the pancreas cells has been reinvestigated on fixed sections and compared with the results, which HIRSCH (1932) obtained by counting living total cells.

### *Results.*

It is impossible to count all stages of restitution which may occur, separately, because these stages are passing gradually into each other. Therefore, HIRSCH (1932) selected 4 *stages* which are lying far enough apart to be distinguished and to be counted separately. They are characterized by the amount and the position of the pro-enzyme granules to be found in one cell.

In the present paper the restitution period will be divided into 4 *phases*. These phases are defined by the following properties of the cell sections:

I. One cell section contains from 0 to 10 pro-enzyme granules, which are mostly lying in the extreme apex of the cell (cf. fig. 1). They originate, probably, from a previous restitution period.

II. One cell section contains from 10 to 30 pro-enzyme granules, which form little groups in the region of the Golgi-apparatus (cf. fig. 2).

III. One cell section contains from 30 to 50 pro-enzyme granules. They form a compact mass with a convex base line, which extends downwards to about half the height of the cell (cf. fig. 3).

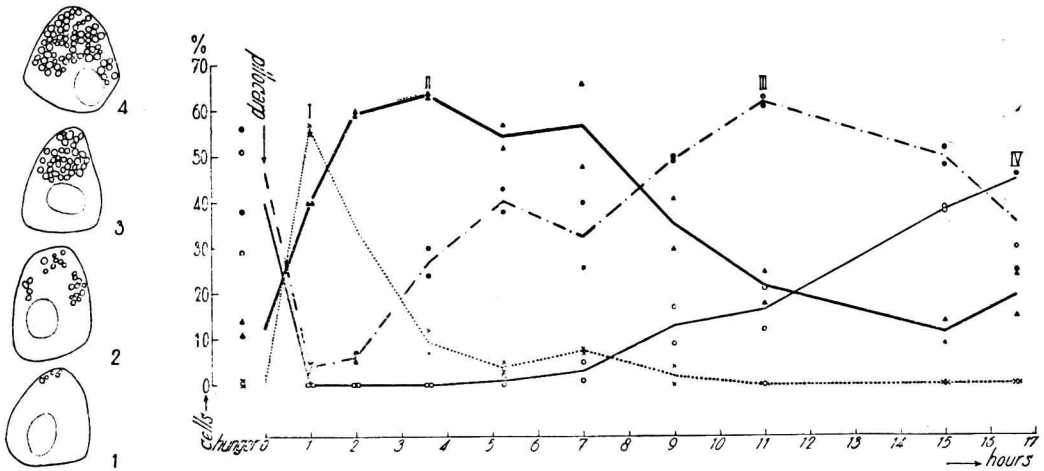


Fig. 1—4. Exocrine pancreas cells in different phases of the restitution period.  $\times 900$ . Only the outlines of pro-enzyme granules and nuclei have been drawn. Fig. 1: phase I; fig. 2: phase II; fig. 3: phase III; fig. 4: phase IV. (cf. the definitions on page 504).

Fig. 5. Graph showing extrusion and restitution after pilocarpine injection in starving animals. Abscissae: time. Ordinates: percentages I, II, III, IV; phases cf. text. During the first hour the extrusion prevails causing a drop of III and IV and a rise of I. Thereafter, restitution begins causing a drop of I and a rise of II, III and IV, successively; II and III obtain a maximum at about  $3\frac{1}{2}$  and 11 hours after the injection, respectively.

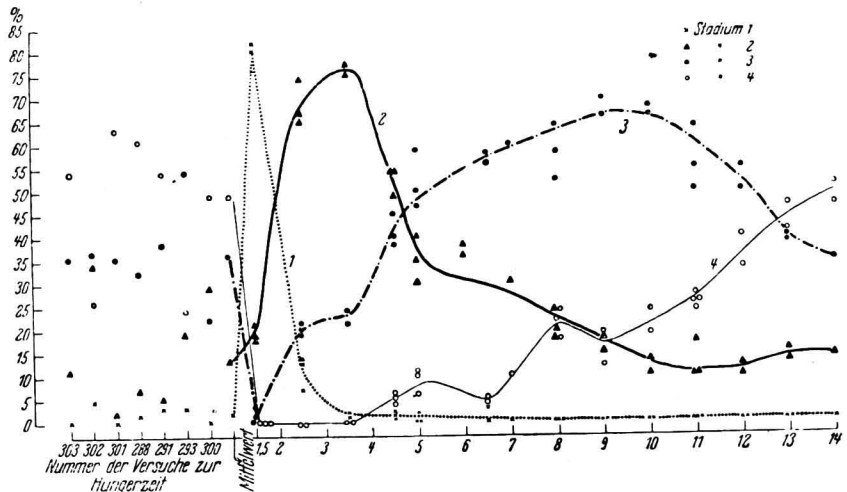


Fig. 6. Reproduction of HIRSCH' Abb. 4 (1932, p. 300) showing similar results (cf. fig. 5), obtained by counting of living cells.

IV. One cell section contains from 50 to 70 pro-enzyme granules forming a mass, whose often straight or concave base line is situated further downwards than half the height of the cell (cf. fig. 4).

a) Counting of stages on fixed material.

Fig. 5 demonstrates the results, which were obtained by counting 100 cell sections in the pancreas of 20 animals killed at different moments after pilocarpine injection, and by classifying these sections according to the restitution phases I to IV, as defined above.

Comparing these graphs with those obtained by HIRSCH (1932, Abb. 4, p. 300) which are reproduced here in fig. 6, the following similarities can be observed.

1) The mean values found in starving animals differ very little. This causes the curves to begin at about the same points.

2) In consequence of the sudden extrusion caused by pilocarpine the curves III and IV reach their lowest and the curve I reaches its highest point between 1 and 2 hours after the injection.

3) In consequence of the beginning of the restitution of pro-enzyme granules the curve I reaches a very low and the curve II reaches its highest point between 3 and 4 hours, followed by a peak of curve III between 9 and 11 hours after the injection; the peak of curve IV is, probably, not yet reached between 14 and 16 hours after the injection.

The principal differences are:

1) The peaks of the curves in the present paper (fig. 5) are lower than those obtained by HIRSCH (fig. 6). This difference is not essential; it may be caused by the dose of pilocarpine in the present investigation being lower than that administered by HIRSCH (1932).

2) The way of crossing of the curves II and III in this paper (fig. 5) differs from that of HIRSCH' curves 2 and 3 (fig. 6). But this also may be a fact of less importance, as it is caused, probably, by the phases II and III being delimited in not quite the same way as has been done by HIRSCH (1932, Abb. 2, p. 296) with respect to his "Stadium" 2 and 3.

It may be concluded, then, that it is actually possible to reconstruct the restitution process, as it is going on in the pancreas cells of *one animal*, with the aid of data, obtained from the 20 *white mice*, which have been used here. Therefore, it will also be allowed to reconstruct quantitatively the changes in number of Golgi-vacuoles and pro-enzyme granules during the restitution process by counting them in the pancreas material of these 20 mice.

b) Numbers of Golgi-vacuoles during the restitution period.

The Golgi-vacuoles have been counted in each of the 100 cell sections of each of the pancreas glands, which have been used for the composition

of fig. 5; in fig. 7 the means of these numbers have been plotted against the times after pilocarpine injection.

This graph shows the following quantitative variations of the Golgi-vacuoles in the pancreas as a whole.

1. With the sudden extrusion of pro-enzyme granules some vacuoles disappear too.

2. From the beginning of the restitution period on the mean number of Golgi-vacuoles begins to rise and reaches a maximum at about 7 hours after the injection. This is a point, where nearly all the cells are in phase II or III of the restitution period (cf. fig. 5).

3. From 7 hours after the injection on the mean number of Golgi-vacuoles begins to decrease and reaches a lower level at a time, when most cells are in phase III and IV of the restitution period (cf. fig. 5).

In order to analyse these phenomena more in detail, the numbers of Golgi-vacuoles have been counted separately in each phase of the restitution period and at different points of time after pilocarpine injection, as is shown in fig. 8. From this figure the following conclusions can be drawn:

ad 1. The disappearing Golgi-vacuoles have been extruded not only from cells, which were in phase III and IV, but also from cells, which were in phase II of the restitution period at the moment of the injection.

ad 2. Already during phase I there is a distinct increase in number of the Golgi-vacuoles, which is continued during the first part of phase II. At 7 hours after the injection this increase ends and during the remaining part of phase II the number of Golgi-vacuoles is kept nearly constant on a high level.

This means that the number of Golgi-vacuoles begins to increase *before*

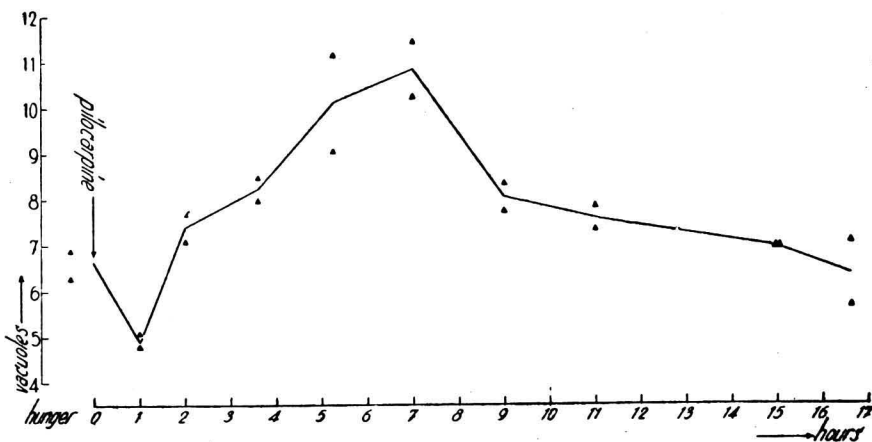


Fig. 7. Graph showing the changes in average number of Golgi-vacuoles during extrusion and restitution period after pilocarpine injection in starving animals.

any pro-enzyme granules worth mentioning can be observed in a cell; the vacuoles are most numerous at the time, when the first granules have appeared in the cell.

ad 3. The decline of the curve of fig. 7 is caused according to fig. 8 by a rather sudden decrease in number of the Golgi-vacuoles on the moment that the cell passes over from the restitution phase III to phase IV (in a cell, reaching this last phase, the restitution of granules has come to an end).

This means that the number of Golgi-vacuoles in a cell is maintained on a high level, as long as the restitution of pro-enzyme granules in the cell is going on, but decreases as soon as the end of the restitution period is reached.

Conclusion. The points ad 2 and ad 3 are strong arguments for the hypothesis that the formation of pro-enzyme granules in the exocrine pancreas cell depends on the formation of vacuoles by the Golgi-apparatus of this cell.

These processes are going on in one cell simultaneously, but not at the same speed at every moment. In the following chapter this phenomenon will be investigated more closely.

Numbers of Golgi-vacuoles and pro-enzyme granules directly compared.

The numbers of pro-enzyme granules were estimated by multiplying the mean numbers of granules, which are present during phase II, III and IV respectively (cf. the definitions on page 504) with the numbers of the cells, which have reached these phases at a given moment (cf. the

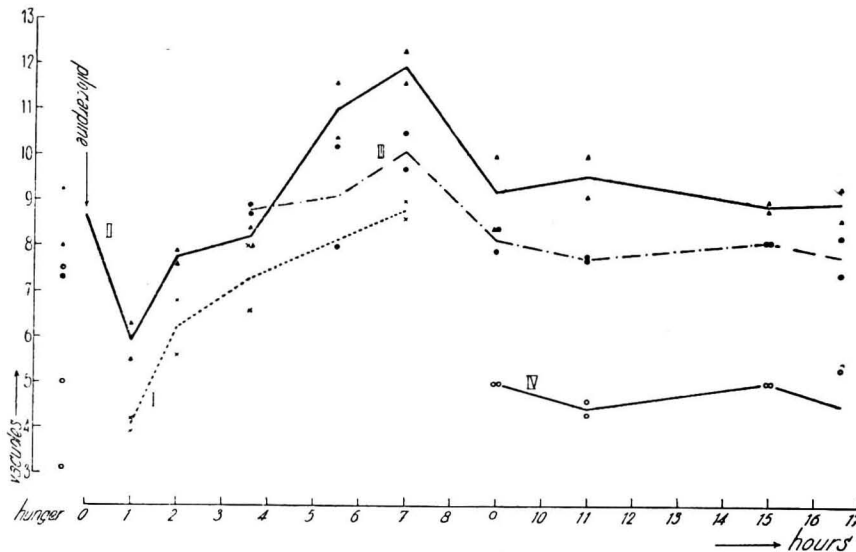


Fig. 8. Graph showing the changes in number of the Golgi-vacuoles, when counted in each restitution phase separately, and at different moments after pilocarpine injection in starving animals.

numbers of fig. 5). By summing up the 3 products, obtained in this way, the total number of granules, which is present at this moment in 100 cells can be calculated.

In fig. 9 the mean numbers of granules of cells at different moments of the restitution period have been plotted against the mean numbers of Golgi-vacuoles, which are present in the same cells at the same moments. These numbers of vacuoles have already been used before for fig. 7. Each point of fig. 9 represents the data of two animals which have been killed at the same time after pilocarpine injection.

Considering the curve on fig. 9 one must realize that each new-formed granule is probably remaining in the cell during the entire restitution period, whereas it is most probable that each vacuole does exist much shorter. Therefore, the values for the granules represent *the total* numbers, which have been formed on a given moment, whereas the values for the vacuoles represent, probably, only the *difference* between the total numbers, which have been formed by the Golgi-apparatus, and the numbers, which have been consumed for the formation of granules.

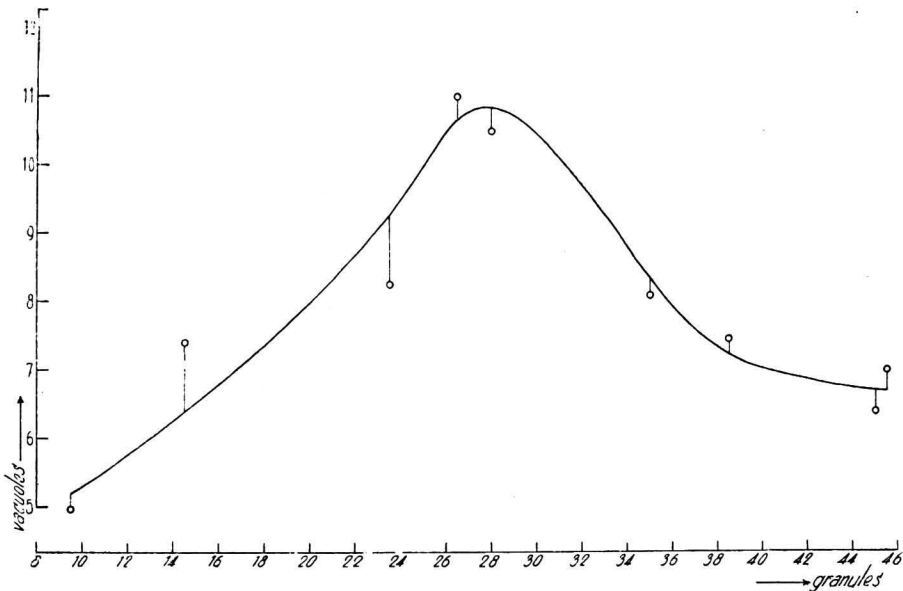


Fig. 9. Graph showing that the quantitative relationship between Golgi-vacuoles and pro-enzyme granules during the restitution period can be expressed by an optimum curve.

From fig. 9 it may be concluded that the quantitative relationship between Golgi-vacuoles and pro-enzyme granules can be expressed by an optimum curve dividing the restitution process into two parts. During the first part, the regeneration of vacuoles by the Golgi-apparatus is going on at a higher rate than the consumption of vacuoles in behalf of the process of granule formation; this causes the curve to rise. However, during the second part the reverse is happening, causing the curve to decline.

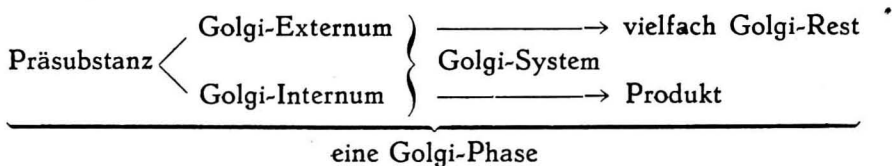
From the results of fig. 9 this conclusion may only be drawn for the pancreas gland as a whole. But considering the numbers of Golgi-vacuoles in the 4 restitution phases separately (cf. fig. 8) and the frequency of the cells, which are in these phases on different moments after pilocarpine injection (cf. fig. 5), it may be concluded that the optimum curve of fig. 9 is valid also for each pancreas cell separately.

#### Discussion.

Hitherto nothing has been said about a physiological interpretation of the phenomena described.

According to the "Systemtheorie" of HIRSCH (1939, 1940) the function of the Golgi-apparatus in the exocrine pancreas cell would be to attract and to concentrate the raw material, which the cell has taken up from the blood, and to transform the molecules of this material into the "Golgi-Produkte", which are, in this case, the pro-enzyme granules. These processes should be reflected in the following structural phenomena (cf. fig. 10). Inside the Golgi-substance ("Golgi-Präsubstanz") vacuoles ("Golgi-Interna") are formed; originally they are very small but they grow gradually and become visible microscopically. In this phase of the process HIRSCH (1939, 1940) calls the Golgi-substance, which can be blackened with osmic acid "Golgi-Externum". One "Internum" forms together with the surrounding "Externum" a "Golgi-System". The "Externum" attracts raw material, which is transformed into the "Golgi-Produkt" in the "Internum". This produces a condensation of substances in the "Internum", which causes the original vacuole to be transformed into a granule. The "Golgi-Produkt" releases itself from the Golgi-substance and is identical, then, with a pro-enzyme granule.

JÄRVI (1940)<sup>3</sup> is fundamentally in accord with the hypothetic reconstruction of HIRSCH (1940) as far as the structural changes are concerned, which are reproduced in fig. 10 of the present paper. However, JÄRVI considers conceptions as used by HIRSCH (1940, p. 378) in the following scheme:



to be incorrect and deceptive. For a discussion of the further conflicting points of view of both authors on these questions I may refer to my recent paper (SLUITER 1948).

As I pointed out before (SLUITER 1948) it is doubtful whether either HIRSCH or JÄRVI have actually seen the vacuoles, which I have counted here, or even whether they have seen any real vacuoles at all in connection with the Golgi-apparatus in the exocrine pancreas cell.



Considering the diagrammatic reconstruction by HIRSCH (1940) (cf. fig. 10 of the present paper) with respect to the results of my own

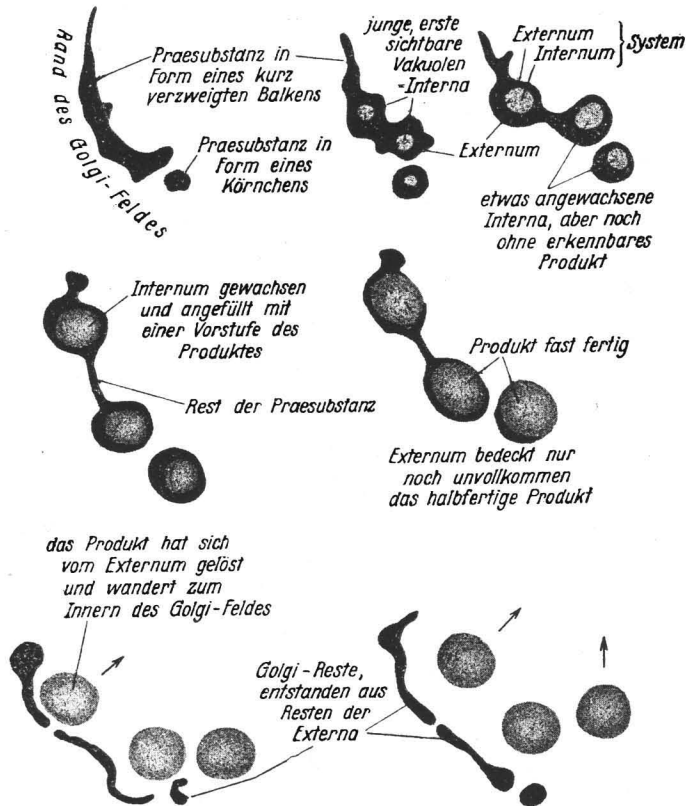


Fig. 10. Reproduction of fig. 1—7 of HIRSCH (1940, p. 382) showing a hypothetical reconstruction of the functional changes in the Golgi-apparatus of the exocrine pancreas cell according to the "Systemtheorie".

investigations on the subject, only the following conclusions can be definitely drawn:

- 1) There are indeed vacuoles, which are in close connection with the Golgi-apparatus.
- 2) The formation of pro-enzyme granules depends, actually, on the formation of these vacuoles by the Golgi-apparatus.
- 3) The quantitative relationship between vacuoles and granules is not in conflict with the assertion of HIRSCH that each vacuole would be a pro-enzyme granule in statu nascendi.
- 4) The pro-enzyme granules, which appear first, are indeed lying in the region of the Golgi-apparatus.

However, in accepting this diagrammatic reconstruction, the following restrictions must be made:

ad 1) The vacuoles cannot be observed lying *in the centre* of the Golgi-threads and growing gradually (SLUITER 1948).

ad 3 and 4) A gradual condensation of substances within the vacuoles cannot be observed; I have never seen pro-enzyme granules to be surrounded as completely and as closely by the blackened Golgi-substance as has been drawn by HIRSCH (1940).

### *Summary.*

The following results have been obtained by counting of exocrine pancreas cells of the white mouse in different phases of their functional activity and of the Golgi-vacuoles and pro-enzyme granules in these cells according to the "Stufenzählmethode" (HIRSCH 1932) after pilocarpine injection.

It is actually possible to reconstruct the restitution process, as it is going on in the pancreas cells of one mouse, with the aid of data, obtained from 20 mice, which have been killed at different times after pilocarpine injection (cf. fig. 5).

The formation of pro-enzyme granules depends on the formation of vacuoles by the Golgi-apparatus (cf. fig. 7 and 8).

The number of Golgi-vacuoles, which can be observed in one cell on a given moment, is the resultant of two processes: regeneration of vacuoles by the Golgi-apparatus and their consumption by the process of granule formation. In the first part of the restitution period the regeneration surpasses the consumption. In the second part the reverse is happening (cf. fig. 9).

The "Systemtheory" of HIRSCH (1939, 1940) could be confirmed in some respects, but not in other ones, as far as the exocrine pancreas cell is concerned (cf. fig. 10).

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- SLUITER, J. W., On the function of the Golgi-apparatus in the exocrine pancreas cell. I. Structural variability of the Golgi-apparatus. *Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam*, **52**, 353—357 (1948).

For the other papers cited I may refer to the list of references in my previous paper (SLUITER 1948).