THE EFFECT OF CERTAIN EXPERIMENTAL PROCEDURES ON THE ISLANDS OF LANGERHANS.*

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Although it may truthfully be said that at the present time the so-called "island theory" of diabetes is regarded with favor by the majority of those who have studied the subject, it must also be admitted that its more general acceptance has been retarded by the persistent opposition of certain investigators who maintain that the islands of Langerhans are not independent structures, but are formed by certain changes in the arrangement and properties of the acinar cells.

Of this school, Laguesse is perhaps the most zealous. In a series of exhaustive histological studies he (1) has attempted to establish his so-called "balance theory," which holds that there is a constant transformation in the pancreas of acini into islands and of islands into acini. In spite of this view, however, Laguesse believes that the islands are especially concerned in carbohydrate metabolism, and that they increase or diminish in number according to the needs of the body. Statkewitsch (2), Lewaschew (3), Dale (4), Vincent and Thompson (5) and other investigators of this school look upon the islands as merely phases of acinar life, exhausted alveoli, which may in time resume their normal appearance.

In the study of the islands the experimental procedures that have been most frequently employed are: (1) inanition experiments, (2) exhaustion of the pancreas with secretin, (3) the production of phlorizin and adrenalin diabetes, (4) ligation or partial extirpation of the pancreas.

The method last mentioned has been the most popular mode of approach, and by means of it a number of important contributions have been made to our knowledge of the islands. The present study, however, has to do with only the first three of these methods.

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It has been claimed that after inanition and after exhaustion of the pancreas with secretin, there is a marked increase in both the number and size of the islands of Langerhans at the expense of the secreting parenchyma, and it has been argued from this that the islands are transitory structures without independent function. Such deductions are perfectly sound, since the islands could hardly be considered an organ of internal secretion if their existence were proved to be ephemeral. The claims, however, of these observers have been based largely on mere observation of sections; and inasmuch as the islands are known to vary greatly in number and size in apparently normal pancreases, a convincing demonstration of an increase in these properties after inanition or injection of secretin must rest on comparative numerical counts and measurements.

The work of Lazarus (6) on phlorizin and adrenalin diabetes supports the island theory. Lazarus maintains that there is a well marked hypertrophy and hyperplasia of the islands in these conditions, but his work is also open to criticism, for he gives no counts or measurements to verify this statement.

The experiments reported in this paper are repetitions of those performed by Dale, Vincent and Thompson, and Lazarus. In every case, however, the islands have been counted and measured, and the averages compared with those from controls. The counting and measuring of islands is exceedingly tedious, but the question involved is of such importance in determining the nature of the islands that the undertaking has seemed justifiable.

METHODS.

I have used dogs for the inanition and secretin experiments, while the phlorizin experiments were carried out on guinea pigs. In the dogs the sections were taken from the tail of the pancreas, about one cubic centimeter from the tip. In the phlorizin series, sections from the head, body, and tail of the pancreas were studied and an average was obtained from the three different parts.

In order that I might make use of Lane's (7) differential stain for the islands, I employed his chrome sublimate solutions for fixation. It is rather hard to obtain constantly good results with this stain, but when it is successfully used the differentiation of islands

from the secreting parenchyma is quite sharp. I have been able to demonstrate the granules in the insular cells of both dogs and guinea pigs. For counting and measuring the islands, however, the hematoxylin and eosin preparations have been invariably used. Dale's toluedin-blue and eosin stain has also been tried, but has not proved reliable.

A Leitz No. 3 objective and No. 2 ocular were employed for the counting. At this magnification the islands are easily recognized, and this is the highest magnification at which one square millimeter of the section can be seen at one time in the field. By means of a camera lucida the islands were plotted off on heavy smooth square cards which corresponded to one square millimeter of the section. Then the islands were counted and their long diameters measured. Fifty square millimeters were counted in each dog's pancreas, and approximately the same number in each guinea pig's pancreas. In order to determine the ratio of island tissue to pancreatic tissue, I followed the technique of Heiburg (8) and of Vincent and Thompson, cutting out the islands and comparing their weight with that of the remaining cardboard.

THE ISLANDS OF LANGERHANS AFTER INANITION.

A number of experimenters have studied the islands after inanition. As far back as 1894, Statkewitsch found that after starvation the acini were considerably reduced in size and were finally converted into homogeneous masses, dotted with small pyknotic nuclei. These latter structures he considered to be identical with the islands of Langerhans. Jarotsky (9), in 1899, compared the pancreases of normal white mice with those of others that had been starved to death. He found no difference in the size or number of the islands and concluded that they were independent structures.

Ssobolew (10) studied the islands in starved dogs and in dogs overfed with carbohydrates and found the insular cell protoplasm much richer in granules in the starved series than in the overfed series. He repeated Statkewitsch's inanition experiments and observed a very slight increase of islands, which he attributed to either the activity of the islands or to atrophy of the non-functionating acini. In 1904 Dale subjected toads to inanition for several months

and observed what he considered a widespread conversion of pancreatic alveoli into islands of Langerhans. Dale also found a considerable increase in the number and size of the islands of a stray cat which had apparently been without food for some time. Vincent and Thompson repeated Dale's experiments on dogs and pigeons and obtained similar results. Dale concluded from his study that the islands of Langerhans are not independent structures, but are formed by certain changes in the arrangement and properties of the acinar cells, the process constituting a reversion to the embryonic type. Vincent and Thompson maintained that when starved dogs were again subjected to a normal diet, the number and size of the islands returned to normal.

Dewitt (11) examined, measured, and counted the islands in (1) normal guinea pigs and in guinea pigs (2) after starvation, (3) after a pure carbohydrate diet, and (4) after a pure meat diet. Dewitt found the variations in size and number of the islands for the different series so slight that she considered them insignificant. She concludes that the islands are not affected by any of these procedures.

The most recent and thorough study of the pancreas after inanition is that of Bensley (12), who used guinea pigs and dogs for his experiments. Bensley made careful estimates of the number of islands after injecting neutral red into the blood-vessels of the fresh pancreas. He was unable to confirm the work of Dale so far as any increase in islands was concerned, but he demonstrated by means of his vital staining methods that the islands are in most cases in direct continuity with the ducts and acini.

In my own experiments, I first enumerated and measured the islands from the pancreases of six normal dogs, all of them on regular meat diet. The results are shown in table I. On examining this table, the most noticeable feature is the wide variation in the size and number of the islands in different pancreases. The average number of islands varied from 1.9 to 4.2 to the square millimeter; the average island diameter, from 79.1 to 99.4 micromillimeters; and the ratio of insular tissue to secreting parenchyma, from 0.8 : 100 to 1.8 : 100.

In table II I have determined the averages for all six dogs. The

TABLE I.

Starvation and Secretin Experiments.

Dog.	Duration of experiment.	Number of sq. mm. counted.	Long diameter of largest island.	Per cent. of islands with long diameters greater than 100μ.	Average long diameter of islands.	Average number of islands per sq. mm.	Ratio of island tissue to secreting parenchyma.
Control 1...		50	220μ	41	97.9μ	4.2	1.8 : 100
Control 2...		50	235μ	27	87.6μ	2.8	1.0 : 100
Control 3...		50	225μ	39	96.3μ	2.1	0.9 : 100
Control 4...		50	225μ	40	99.4μ	2.9	1.6 : 100
Control 5...		50	210μ	20	79.1μ	2.7	1.0 : 100
Control 6...		50	230μ	37	88.8μ	1.9	0.8 : 100
Starved dog 1.....	6 days	50	365μ	38	96.6μ	4.5	2.0 : 100
Starved dog 2.....	6 days	50	360μ	40	102.8μ	3.9	2.1 : 100
Starved dog 3.....	6 days	50	280μ	48	105.6μ	3.5	1.9 : 100
Starved dog 4.....	6 days	50	245μ	49	105.0μ	2.8	1.6 : 100
Starved dog 5.....	10 days	50	200μ	37	92.4μ	4.1	1.6 : 100
Starved dog 6.....	16 days	50	170μ	37	92.6μ	2.5	1.0 : 100
Secretin dog 1.....	9¾ hrs.	50	260μ	38	93.7μ	2.9	1.4 : 100
Secretin dog 2.....	7⅙ hrs.	50	265μ	44	100.5μ	3.3	1.5 : 100
Secretin dog 3.....	16½ hrs.	50	355μ	47	107.7μ	3.7	2.6 : 100
Secretin dog 4.....	6½ hrs.	50	255μ	50	108.5μ	2.7	1.5 : 100
Secretin dog 5.....	10¼ hrs.	50	295μ	51	111.9μ	3.9	2.5 : 100
Secretin dog 6.....	16 hrs.	50	240μ	40	94.1μ	3.4	1.6 : 100

average number of islands was 2.8 to the square millimeter. The average island diameter was 91.5 micromillimeters. The ratio of island tissue to the rest of the pancreas was 1.2 : 100. According

TABLE II.

Starvation and Secretin Experiments. Comparison of Averages.

Series.	Average duration of experiment.	Average long diameter of largest island.	Average per cent. of islands over 100 μ in long diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Average ratio of island tissue to secreting parenchyma.
Control dogs.....		224μ	34	91.5μ	2.8	1.2 : 100
Starved dogs.....	8.4 days	270μ	41	99.2μ	3.5	1.7 : 100
Secretin dogs.....	11 hrs.	278μ	45	102.7μ	3.3	1.8 : 100

to this estimate, then, the islands constitute a little more than 1 per cent. of the entire pancreas of the normal dog, a ratio, however, that is subject to wide variations.

In all these cases the acinar cells were filled with zymogen granules, which were particularly well brought out by Bensley's neutral gentian violet. The islands in a dog's pancreas are rather irregular in shape, and sometimes appear to be directly continuous with adjacent acini.

Having obtained these control estimates from normal dogs, I next undertook to determine the effect of inanition on the dog's pancreas. Six dogs were subjected to inanition for various lengths of time (table I) and estimates were made of the number and size of the islands, as in the control series.

In this series one is again impressed with the wide variations between the different cases. The number of islands per cubic millimeter varied from 2.5 to 4.5; the average island diameter, from 92.4 to 105.6 micromillimeters; the ratio of island tissue to parenchyma, from 1 : 100 to 2.1 : 100. Moreover, these variations bear no relation to the duration of inanition.

In table II the general averages obtained from the inanition series are compared with the general averages obtained from the control series. On comparing these figures, the average number of islands is found to be slightly increased in the inanition series. The difference in the average island diameter is negligible. The ratio of island tissue to parenchyma is slightly larger in the inanition than in the control series.

A histological study of the pancreas after inanition reveals no striking changes. In some places the acinar cells, as well as their nuclei, appear definitely smaller, a shrinkage which seems to be due in large part to a diminution in size of the inner granular zone. By the use of Lane's stain, however, zymogen granules are found to be present even after sixteen days of inanition. The islands are somewhat irregular in outline, but are sharply defined from the acini, and differ in no way from those in the normal dog's pancreas. In sections stained by Lane's method, the insular cells show their characteristic granulation.

The results of these experiments are in accord with those of

Jarotzky, Dewitt, and Bensley. According to my observations, the pancreas of a dog subjected to inanition does not show any active transformation of acini into islands, and the increase in the number and size of the islands after inanition is so insignificant that in consideration of the normal variations it may be ignored.

THE ISLANDS AFTER EXHAUSTION OF THE PANCREAS WITH SECRETIN.

Lewaschew was the first to study the effect of secretory activity of the pancreas on the islands of Langerhans. In 1886 he injected dogs with large doses of pilocarpin and found what he considered a well marked increase of the island tissue after this procedure. Opie (13), however, repeated these experiments, making careful counts of the islands and could find no such increase. von Hansemann (14), Ssobolew, and Vincent and Thompson have also repeated Lewaschew's experiments with negative results.

In this connection the experiments of Launoy have an important bearing. Launoy (15) showed that if the gastric juice were prevented from flowing into the duodenum, the injection of pilocarpin would not stimulate the flow of pancreatic juice. He concludes that pilocarpin is unable, of itself, to produce a pancreatic secretion.

The secretin experiments of Dale have excited considerable interest and have been widely quoted, especially by English writers, as furnishing evidence against the independent existence of the islands. Dale injected secretin at frequent intervals into the jugular vein of dogs and cats until the pancreas showed signs of exhaustion, and found a great increase in the number and size of the islands and signs of active formation of islands from acini. The same changes were found in toads injected with secretin. Dale did not count or measure the islands.

Dale's experiments have been repeated by Vincent and Thompson, who found some increase in the islands after the secretin injections, but in their experience the increase was not so marked as after inanition. Vincent and Thompson counted the islands, but do not give their figures.

The experiments of Dale have recently been repeated by Bensley with a modified technique. He substituted guinea pigs for dogs and cats, and stained the fresh pancreas *in toto* by injecting neutral

red into the blood-vessels. Bensley's secretin experiments, like his inanition experiments, were very carefully carried out, the number of islands in every case being estimated and compared with control animals. The counts in his secretin series showed a wide range of variation similar to that noted in the control series, but there was no evidence of an increased number of islands in the secretin animals. Strangely enough, the maximum number was found in one of the control animals and the minimum in one of the secretin animals. Bensley also found that the injection of secretin into toads had no influence on the islands.

I have performed Dale's secretin experiment on six dogs. In every case the secretin was prepared from the intestines of sheep or pigs according to the directions of Bayliss and Starling (16) and always produced active secretion of the pancreas. Morphin and A.C.E. were used for anesthesia. Previous to the injections, the duodenum was opened and a cannula tied into the pancreatic duct. The shortest of the six experiments lasted a little over six hours; the longest, sixteen and a half hours. The average duration was eleven hours. Although in some cases I varied both the doses of secretin and the intervals for injecting it, I was always able to maintain a constant flow of pancreatic juice. For instance, in dogs 2 and 4, I desired to maintain a maximum secretion during the entire experiment. I found that the largest flow ensued when two cubic centimeters of concentrated secretin were allowed to flow into the vein every minute. The experiment under these conditions lasted only six or seven hours. I was never able to exhaust completely the dog's pancreas, even with bleeding toward the end of the experiment. The cause of death in most of the dogs appeared to be pulmonary edema.

In the histological study of the pancreas from these dogs, the technique was similar to that for the inanition experiments and the method of counting and measuring the islands was the same. Table I shows the results obtained from the counts and measurements. In none of the secretin dogs is the number of islands per square millimeter as large as that of the first control dog. They appear in the table to be somewhat larger than the normal island, but the average island diameter in some of the control dogs is

larger than that in some of the secretin dogs. The average island diameter for the entire secretin series is 102.7 micromillimeters, about 10 per cent. greater than the average diameter for the control series (table II). The average number of islands per square millimeter was 3.3; *i. e.,* slightly larger than that for the controls. The ratio of insular tissue to parenchyma was 1.8 : 100; that is, about the same as that for the starved dogs and somewhat greater than that for the control series.

There were wide variations among the dogs subjected to the secretin experiments. The average island diameter varied from 93.7 micromillimeters (dog 1) to 111.9 micromillimeters (dog 5); the average number of islands varied from 2.7 to 3.9 per square millimeter; the ratio of island tissue to parenchyma, from 1.4 : 100 to 2.6 : 100.

If one considers the great variations in the size and number of the islands in the normal pancreas, the slight increase noted in the secretin series can hardly be given any weight; for this difference would probably have been neutralized if a larger series had been studied.

My experiments, then, have failed to demonstrate any notable increase in either the size or the number of the islands after overstimulation of the pancreas with secretin. The islands differ in no respect from those in the resting pancreas, showing, with Lane's differential stain, the same granules that are seen in the normal island. In some places the islands appear to be continuous with adjacent acini, but there is no evidence of a transition from the one form into the other. I have noted, however, that prolonged stimulation of the pancreas with secretin produces definite qualitative changes in the glandular acini. The acinar cells are noticeably shrunken, vacuolated, and granular, and their nuclei are small and pyknotic. The most striking change is the great diminution in zymogen granules. In several of my cases, they were almost entirely absent.

THE ISLANDS IN ADRENALIN AND PHLORIZIN DIABETES.

In 1907 Lazarus reported a study of the pancreas of guinea pigs in adrenalin and phlorizin diabetes. In both conditions he found

the pancreas hypertrophied and the islands increased in number and size.

Herxheimer (17) has recently repeated the adrenalin experiments, continuing the injections for five months. A careful comparison of normal guinea pigs with those injected with adrenalin showed no difference in either the number or the size of the islands.

TABLE III.

Phlorizin Experiments.

Guinea pig.	Duration of experiment.	Number of sq. mm. counted.	Diameter of largest island.	Per cent. of islands over 100μ in diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Ratio of island tissue to parenchyma.
Control 1...		50	385μ	35	105.8μ	2.2	1.4 : 100
Control 2...		46	345μ	38	107.3μ	2.0	1.3 : 100
Control 3...		57	290μ	32	96.1μ	2.5	1.5 : 100
Control 4...		52	345μ	38	110.4μ	2.5	2.0 : 100
Control 5...		59	425μ	45	103.8μ	2.4	1.8 : 100
Control 6...		50	470μ	41	108.5μ	4.2	3.2 : 100
Phlorizin guinea pig 1.	35 days	64	324μ	28	91.5μ	3.3	1.9 : 100
Phlorizin guinea pig 2.	39 days	52	234μ	32	93.9μ	1.8	1.0 : 100
Phlorizin guinea pig 3.	66 days	58	330μ	42	103.0μ	2.1	1.7 : 100
Phlorizin guinea pig 4.	103 days	52	260μ	34	83.8μ	2.2	1.2 : 100
Phlorizin guinea pig 5.	202 days	45	540μ	40	107.3μ	2.3	2.1 : 100
Phlorizin guinea pig 6.	215 days	81	420μ	38	104.9μ	2.7	2.2 : 100

In view of these negative findings of Herxheimer, it seemed to me desirable to repeat the phlorizin experiments also. Six guinea pigs were injected with one centigram of phlorizin subcutaneously every day for various periods. Guinea pig 1 received only thirty-five injections, but guinea pig 6 received two hundred and fifteen injections. In the case of guinea pig 6 the dose was gradually increased until at the time of its death it was receiving three centigrams daily.

Small sections of tissue were taken from various parts of the pancreas, were stained with hematoxylin and eosin, and the islands counted and measured in the manner already described.

In table III the results are recorded. The variations among the

individual cases in both series are just as wide as those observed in the dog's pancreas in the inanition and secretin experiments. The average island diameter in the control series varied from 96.1 to 108.5 micromillimeters; the average number of islands per square millimeter, from 2 to 4.2; the ratio of island tissue to parenchyma, from 1.3 : 100 to 3.2 : 100. In the phlorizin series, the average island diameter in the pancreas of guinea pig 4 was only 83.8 micromillimeters, while in guinea pig 5 it was 107.3 micromillimeters. The average number of islands per square millimeter was 1.8 in guinea pig 2, and 3.3 in guinea pig 1. The ratio of island tissue to parenchyma was 1 : 100 in guinea pig 2, while in guinea pig 6 it was 2.2 : 100.

In table IV the averages obtained for the entire series of phlorizinized guinea pigs are compared with those obtained in the control

TABLE IV.

Phlorizin Experiments. Comparison of Averages.

Series.	Average duration of experiment.	Average diameter of largest island.	Average per cent. of islands over 100μ in diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Average ratio of island tissue to parenchyma.
Control guinea pigs		373μ	38	105.3μ	2.6	1.8 : 100
Phlorizin guinea pigs	110 days	351μ	36	97.4μ	2.4	1.7 : 100

animals. There is surprisingly little difference in the figures. The average island diameter is eight micromillimeters greater in the control series than in the phlorizin series. This difference can be largely accounted for by the very low average diameter obtained in the case of phlorizin guinea pig 4,—only 83.8 micromillimeters. The average number of islands and the average ratio of island tissue to parenchyma are practically the same for the two series.

When sections of pancreas from the phlorizinized animals were stained by Lane's method, the granulation of both the insular and acinar cells was found to differ in no way from that observed in normal guinea pigs. The coarse zymogen granules of the acini as well as the two types of granules in the insular cells could, in either case, be well made out. There was no evidence of any kind of a transition from acinus into island or *vice versa*.

From the study of the phlorizin experiments, one is forced to the conclusion that in phlorizin diabetes, as in adrenalin diabetes, the islands of Langerhans are not affected.

DISCUSSION.

The wide variation in the number and size of the islands of Langerhans in normal pancreases is probably in large part responsible for the erroneous conclusions which have been reached by Dale, Vincent and Thompson, Lazarus, and others. Such mistakes were all the easier to make when no comparative counts and measurements were made. Bensley points out the fact that areas of post-mortem degeneration fail to take up toluidin-blue, and he suggests that Dale may have mistaken these areas for large islands. It is undoubtedly true that prolonged stimulation of the pancreas with secretin produces a certain amount of self-digestion, caused probably by the greatly increased amount of pancreatic juice; and in some of Dale's photographs (particularly figure 5) the areas displayed are very suggestive of autolysis.

Dale speaks of "exhausting" the pancreas with secretin, but admits that exhaustion of the mammalian pancreas is almost impossible. I have found it so, even after the injection of active secretin for sixteen hours. I can see no reason why it should become completely exhausted as long as the animal is alive, and blood and secretin continue to circulate through it. The theories of Laguesse and of Dale which assume that acini may be rapidly converted into islands, or islands into acini, have for their support the pictures sometimes seen of islands in continuity with acini. But, as Bensley says, in order to prove this point, it is necessary "to show not only the phases of the disappearance of the specific elements at one end of the series, but also the appearance of the specific elements at the other end." The same writer argues that it is reasonable to suppose that this change could not go on with a rapidity too great to permit the discovery of intermediate phases, because the anatomical structure of an island necessitates a considerable degree of anatomical rearrangement in constructing islands out of acini. As a matter of fact, however, these intermediate phases are lacking and by the use of Lane's granule stain, it can

be shown that many of the structures which have the appearance of transitional forms are entirely free from the characteristic granulations of island cells.

These continuations between acini and islands can be explained in a much more satisfactory way. By the cutting of serial sections, it has been shown by Laguesse, Weichselbaum and Kyrle (18), Cecil (19), and Bensley that the islands in both health and disease may often be directly connected with the ducts or acini, from which they have developed. Bensley's work on this subject is one of the most important contributions to our knowledge of the islands that has appeared in recent times, and in conjunction with certain new pathological data, it would seem to have established beyond doubt the anatomical independence of the islands of Langerhans. By means of new vital staining methods he has been able to demonstrate an intricate anastomosing network of small tubules, which are connected on the one hand with the pancreatic ducts, and on the other with acini and the islands of Langerhans. In rare cases only does the lumen of the tubule acually penetrate the substance of the island, so that, notwithstanding its direct connection with the tubule, the island is unquestionably a ductless gland. One of the most important points brought out by Bensley is the fact that the majority of the islands are connected in this manner with the ducts.

As far as the phlorizin experiments are concerned, my results serve to substantiate still further the prevalent belief that phlorizin diabetes is not of pancreatic origin.

CONCLUSIONS.

Although the results of this study have been of a negative character, the conclusions that may be drawn from it have an important bearing on the true anatomical character of the islands.

1. Neither inanition[1] nor the prolonged injection of secretin has any noteworthy effect upon the number, size, or structure of the islands of Langerhans in the dog's pancreas.

[1] The inanition experiments were carried out partly at The Rockefeller Institute for Medical Research, and partly at the Cornell University Medical School. For the privileges of these institutions I am indebted to Dr. Simon Flexner and to Dr. James Ewing.

2. The islands of Langerhans in the guinea pig's pancreas are in no way altered in phlorizin diabetes.

3. The islands of Langerhans are not formed out of exhausted or degenerated acini, but develop from the ducts or acini with which they are often in direct continuity.

PROTOCOLS.

Control Dogs 1 to 6.—Normal dogs on normal diet. Anesthetized with ether. The pancreas in every case appeared normal. Small bits of pancreas were preserved in Lane's chrome sublimate solutions.

Starved Dog 1.—Mongrel male; weight 9,000 gm. Subjected to inanition for 6 days and then bled to death. Stomach and intestines empty. Pancreas firm and pale.

Starved Dog 2.—Fox terrier, female; record of weight lost. Subjected to inanition for 6 days, then killed by ether. At autopsy, fairly well nourished. Stomach and small intestine empty. Small amount of feces in rectum. Pancreas pale.

Starved Dog 3.—Mongrel, female; record of weight lost. Subjected to inanition for 6 days, then killed by ether. Fairly well nourished animal. At autopsy, the stomach contained a little brownish mucus with shreds of hay in it. Small intestine empty. Small quantity of feces in rectum.

Starved Dog 4.—Mongrel, male; record of weight lost. Subjected to inanition for 6 days, then killed by ether. At autopsy, the stomach contained a little hay and mucus. The small intestine contained a tapeworm. There was a small quantity of feces in the rectum.

Starved Dog 5.—Male; weight 12,500 gm. Subjected to inanition for 10 days, then bled to death. Weight at time of death, 10,200 gm. Stomach and intestines empty. Pancreas normal.

Starved Dog 6.—Male; weight 10,700 gm. Subjected to inanition for 16 days and then bled to death. Weight at time of death, 8,250 gm. Stomach and intestines empty. Pancreas normal.

Secretin Dog 1.—Male; weight 4,944 gm. The first injection of secretin produced active secretion from the pancreatic duct, seven or eight drops to the minute. After six hours the responses to these injections were much less marked, only three or four drops to the minute being counted. Duration of experiment, $9\frac{3}{4}$ hours, the dog receiving 8 c.c. of secretin every five minutes except during the last two hours of the experiment, when he received 5 c.c. of secretin every three minutes. During the last two hours the dog was bled several times. The pancreas responded to secretin to the last.

Secretin Dog 2.—Female; weight 9,300 gm. During the first hour and a half of the experiment, the dog received 1 c.c. of secretin every minute. After that she received 2 c.c. every minute. There was a flow of two or three drops of pancreatic juice to the minute. Duration of experiment, $7\frac{1}{6}$ hours. The dog was bled toward the end of the experiment. Although the secretion was not so active toward the end, there were no signs of pancreatic exhaustion.

Secretin Dog 3.—Male; weight 6,420 gm. During the first thirteen hours

of the experiment, the dog received 5 c.c. of secretin every five minutes. After that he received 1 c.c. every minute. The pancreas responded actively, especially to the latter method of administration. Duration of experiment, 16½ hours. The dog died of pulmonary edema. He was not bled at any time during the experiment. The flow of pancreatic juice continued to the end.

Secretin Dog 4.—Female; weight 6,550 gm. The secretin was more concentrated than that used in previous experiments. During the first two hours, the dog received 1 c.c. of secretin every minute. The pancreas responded well (five to six drops of juice a minute). During the remainder of the experiment, the dog received 2 c.c. of secretin every minute. Duration of experiment, 6½ hours. Toward the end the flow of pancreatic juice diminished to one drop a minute, but there was never complete exhaustion. The cause of death was apparently pulmonary edema. The dog was not bled at any time during the experiment.

Secretin Dog 5.—Male; weight 21,000 gm. The dog received 10 c.c. of secretin intravenously every five minutes. The flow of pancreatic juice was fairly active. Duration of experiment, 10¼ hours. No signs of pancreatic exhaustion. The animal stopped breathing rather suddenly, after receiving a small dose of morphin.

Secretin Dog 6.—Mongrel, male; weight 8,500 gm. The dog received 5 c.c. of secretin every five minutes for 16 hours. In this experiment the efficacy of the secretin was tested on a control dog, but in the case of the subject for the experiment the abdomen was not opened. The cause of death was respiratory failure. At autopsy there were small fat necroses scattered over the pancreas which was dark red and edematous.

Control Guinea Pigs 1 to 6.—Normal guinea pigs fed on vegetable diet. The pancreas in every case appeared normal macroscopically and microscopically.

Phlorizin Guinea Pig 1.—Weight 210 gm. Daily subcutaneous injections of phlorizin for 35 days. Dose, 1 cg. of phlorizin (dissolved in warm water). Loss of weight, 40 gm. Urine gave a positive Fehling test for glucose. The fermentation test indicated 0.2 to 0.4 per cent. glucose. At autopsy the pancreas appeared normal.

Phlorizin Guinea Pig 2.—Weight 260 gm. Daily subcutaneous injections of phlorizin for 39 days. Dose, 1 cg. Loss of weight, 110 gm. Urine gave a positive Fehling test for glucose (0.2 to 0.4 per cent.). At autopsy the pancreas appeared normal.

Phlorizin Guinea Pig 3.—Weight 200 gm. Daily subcutaneous injections of phlorizin for 66 days. Dose, 1 cg. The weight remained about stationary. Urine gave a positive Fehling test (0.2 to 0.4 per cent.). At autopsy the pancreas showed no gross changes.

Phlorizin Guinea Pig 4.—Weight 335 gm. Daily subcutaneous injections of phlorizin for 103 days. Dose, 1 cg. Slight gain in weight. Urine for twenty-four hours contained from 0.25 to 0.48 gm. of glucose. At autopsy the pancreas was rather large but otherwise normal.

Phlorizin Guinea Pig 5.—Weight 345 gm. Daily subcutaneous injections of phlorizin for 202 days. Dose, 1 cg. Moderate gain in weight. Twenty-four hour specimens of urine examined from time to time contained from 0.18 to 0.9 gm. of glucose. At autopsy the pancreas was infiltrated with fat.

Phlorizin Guinea Pig 6.—Weight 445 gm. Daily subcutaneous injections of phlorizin for 215 days. The dose was 1 cg. during the first 120 days; during the following 47 days, 2 cg.; and for the last 12 days, 3 cg. Moderate gain in weight. Twenty-four hour specimens of urine contained from 0.24 to 1.2 gm. of glucose. At autopsy the pancreas was infiltrated with fat.

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