THE EFFECTS OF CONTINUOUS INTRAVENOUS INJECTION OF DEXTROSE IN INCREASING AMOUNTS ON THE BLOOD SUGAR LEVEL, PANCREATIC ISLANDS AND LIVER OF GUINEA PIGS

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ONE PLATE (THREE FIGURES)

In an earlier paper, the effects of continuous intravenous injection into guinea pigs of approximately 1 gm. of dextrose per kilogram of body weight per hour for varying periods of time were described (Woerner, '38). These studies have been extended with the administration of 2 to 3 plus grams of dextrose per kilogram of body weight per hour for varying periods of time. In this series twenty male guinea pigs were used; nine animals injected with 2 plus grams per kilo per hour, eleven animals with 3 plus grams.

The method of continuous intravenous injection was the same as that described in the earlier series with the exceptions that the sugar was preceded by a recovery period of 24 hours, during which time normal saline solution was injected at the rate of 2 cc. per hour, a 50% solution of glucose was used in some cases instead of a 30% solution and in some cases the rate was increased from 2 to 4 cc. per hour. The methods for obtaining tissue for cytological studies were the same as in the earlier series. By employing the micro-method of Miller and Van Slyke ('36) for quantitative blood and urine sugar analyses instead of the Somygi modification of the Shaffer-Hartman method, many more determinations could be made during the course of an experiment.

Animals receiving 3 plus grams per kilo per hour continuously did not often survive much longer than 2 days. For this reason the injection was interrupted for 24 hours in the case of one animal (no. 68), in order to study the effects of more prolonged injection.

Following are given the protocols of selected animals from each group where the tissue was adequate for cytological studies and the sugar analyses were successfully made. The physiological description of the alpha and beta cells is made on the basis of the correlation of cytological changes with functional states of the cell described in an earlier paper (Bensley and Woerner, '38).

GROUP A. ANIMALS RECEIVING 2 TO 2 PLUS GRAMS OF DEXTROSE PER KILOGRAM OF BODY WEIGHT PER HOUR

I. *Animal no. 78. Twenty hours of 2.0 gm.*

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
20 hours	800 plus	8.0 (bladder urine—8.3%)

1. Pancreas:

Beta cells.

1) Many almost agranular, some with rod-like mitochondria, suggesting active exhaustion; a few with globular mitochondria, suggesting beginning degenerative changes.
2) Some have polar distribution of granules. Most of these have rod-like mitochondria, suggesting active secretion. Some have granular mitochondria.
3) A few are filled with granules, their mitochondria being oval to globular, suggesting secretory inactivity. No evidences of increase in the number by mitosis or transformation were found.

Alpha cells.

1) The majority are well filled with granules and contain tiny vacuoles, their mitochondria not visualized. Apparently these cells were not actively secreting.
2) A few have polar distribution of the granules and contain rod-like mitochondria, suggestive of actively secreting cells.
3) A few are agranular and shrunken, their mitochondria not visualized, suggesting atrophic cells.

2. Liver:

Cells well filled with glycogen, contain small round vacuoles but no osmic combining fat.

II. *Animal no. 59.* Four days of 2.44 gm. (buffered).

Time day	*Blood sugar mg./100 cc.*	*Urine sugar %*
First		2.72
Second		3.29
Third		8.00
Fourth	1670	9.55

1. Pancreas (fig. 1):

Beta cells.

1) Many are completely agranular. In most of these the mitochondria are globular, suggesting degenerative changes. In some the mitochondria are rod-like. A few mitoses are found in agranular cells with rod-like mitochondria.
2) Some contain polar distribution of granules with rod-like mitochondria, suggesting secretory activity.
3) Rarely a cell filled with granules, containing globular mitochondria, suggesting secretory inactivity.

Alpha cells.

Almost all are well filled with granules (their mitochondria not visualized). Apparently these cells were not actively secreting.

2. Liver:

Cells are distended with glycogen and contain a moderate amount of osmic combining fat. Almost all Kupffer cells contain some fat.

III. *Animal no. 62.* Eight days of 2.0 gm. (buffered).

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
Before injection	120	
After 5 hours	220	1.9
After 1 day	253	2.0
After 2 days	248–258	2.0
After 3 days		2.75
After 4 days	167	
After 8 days	247	0.30

1. Pancreas (fig. 2):

Beta cells.

1) Many have polar distribution of granules. Most of these have mitochondria which are long large rods. A few have globular mitochondria.
2) Quite a few are agranular; some with rod-like and some with globular mitochondria.
3) A few filled with granules, some containing rod-like mitochondria, some with globular mitochondria.

Many evidences of increase in number by mitosis and transformation were found.

Alpha cells

Very few typical alpha cells to be found.

1) Some almost agranular with rod-like mitochondria.
2) Some with polar distribution of granules with rod-like mitochondria. One has the impression that there are more undifferentiated cells than are usually found in the islands.

2. Liver:

Cells moderately enlarged and filled with glycogen. Very little osmic combining fat in liver cells. Kupffer cells contain some small black droplets.

GROUP B. ANIMALS RECEIVING 3 TO 3 PLUS GRAMS OF DEXTROSE PER KILOGRAM OF BODY WEIGHT PER HOUR

I. Animal no. 56. Forty-eight hours of 3.2 gm.

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
Before injection	78	
After 22 hours	118	
After 2 days	213	2.54

1. Pancreas:

Beta cells.

1) Many have polar distribution of granules. Most of these contain rod-like mitochondria, some contain globular mitochondria.
2) A few filled with granules and contain globular mitochondria. No evidence of increase in number by mitosis or transformation.

Alpha cells

1) The majority are well-filled with granules and contain mitochondria that are tiny rods and granules.
2) Some with polar distribution of granules containing rod-like mitochondria.

2. Liver:

Cells large and well filled with glycogen. Large droplets of osmic combining fat occur toward the central vein; clusters of small black droplets are found at the periphery of the lobule.

II. Animal no. 60.

1 day of 1.1 grams
1 day of 2.2 grams
1 day of 3.3 grams

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
After second day	260	
After third day	1316–1348	9.42

1. Pancreas:

Beta cells.

1) Many with polar distribution of granules with mitochondria varying from rods to globules.
2) Some filled with granules and containing globular mitochondria.
3) A few agranular with globular mitochondria.

Alpha cells

The majority are well filled with granules, containing tiny vacuoles and thick rod-like mitochondria.

2. Liver:

Cells large and filled with glycogen. Not much fat in the liver. A few cells contain rather large osmic combining fat globules.

III. Animal no. 52.

9 days of 1.2 gm.
42 hours of 3.6 gm.

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
Ninth day	190	
90 minutes of 3.6	280–320	
13 hours of 3.6	560–720 (640)	
18 hours of 3.6	440–460	
24 hours of 3.6		3.44
36 hours of 3.6	840–860	
42 hours of 3.6	1260–1300	4.70

1. Pancreas:

Beta cells.

1) Some with polar distribution of granules. Some of these contain rod-like mitochondria, more contain globular mitochondria.
2) Some agranular with globular mitochondria.
3) Some filled with granules and with globular mitochondria. Some evidence of increase in number by mitosis.

Alpha cells

The majority are well filled with granules, contain tiny vacuoles and filamentous mitochondria.

2. Liver:

Cells large and well filled with glycogen. Large osmic-combining fat globules occur in cells toward the central veins.

IV. Animal no. 68.

2 days of 3.17 grams
1 day of rest
2 days of 3.17 grams

Time	*Blood sugar mg./100 cc.*	*Urine sugar %*
Before injection	108	
After 24 hours	121–117	4.8
After 48 hours	363	9.8
Injection stopped		
in 30 minutes	254	
in 60 minutes	188	
in 90 minutes	141	
in 2 hours	138	
After 1 day of rest		0.19
Injection resumed		
After 24 hours		7.74
After 48 hours	595	11.20
Injection stopped		
in 90 minutes	333	
in 2 hours	323	

1. Pancreas (fig. 3):

Beta cells.

1) The majority are agranular. In some the mitochondria are globular, in most they are rod-like.
2) Some filled with granules and contain small granular mitochondria.
3) Occasional mitoses are found in agranular cells with rod-like mitochondria.

Alpha cells

1) The majority are loaded with granules and contain filamentous mitochondria.
2) A few have polar distribution of granules with rod-like mitochondria.
3) A few shrunken and agranular; the mitochondria not visualized.

2. Liver:

Cells distended with glycogen. Some cells contain masses of fat that partially or completely blacken with osmic acid. Osmic combining fat globules appear to be even in the central vein.

DISCUSSION

From these experiments it is apparent that the degree of hyperglycemia produced by increased amounts of intravenous dextrose is very variable. But the correlation between the degree of hyperglycemia and the condition of the beta cells is more uniform. Where the response of the beta cells to the intravenous dextrose is maximal and there is no evidence of

hyperplasia the level of the blood sugar has been progressively increased. When, however, the secretory activity of the beta cells is accompanied by mitotic activity there appears to be some cyclic evidence of partial adjustment of the blood sugar level. Animal no. 52 is an apparent partial exception to this generalization.

The condition of the alpha cells is in sharp contrast to that found after the injection of 1 gm. of dextrose per kilo per hour. In the latter case the alpha cells were exhausted and in some instances, hydropic. With increasing amounts of sugar they appear to be actively secreting and finally suppressed.

In this series the liver cells are well filled with glycogen in contrast to the liver cells of the animals receiving only 1 gm. per kilo per hour in which case they contained very little glycogen.

The relationship of the secretory activity of the alpha cells to the accumulation of fat in the liver is not as clear. However, it is apparent that when the alpha and beta cells are both actively secreting there is little osmic combining fat to be found in the liver. It also appears that with suppression of secretion of the alpha cells osmic combining fat occurs in the cells chiefly toward the central veins and in the Kupffer cells. But from this series it is not clear which precedes and which follows, or whether they are coincident.

It is interesting to note that in the case of animal no. 68 the blood sugar level obtained after the second 48 hours of injection was 1.6 plus times that of the level after the first 48 hours of injection although the same amount was being injected and there was a recovery period of 24 hours between, during which time glycosuria almost completely disappeared. This would suggest either that during the first 48 hours of intravenous injection some of the exhausted beta cells were damaged and that any proliferative response in the remaining beta cells was not adequate to control the blood sugar in the second period to the extent of that in the first, or that the liver glycogen was not removed during the 24-hour recovery

period, or that liver function was impaired and therefore sugar was not as effectively removed from the blood by this organ.

The appearance of mitoses in the exhausted beta cells may be correlated with the fact that the animal had a 2-hour recovery period, during which time blood sugar determinations were made, before the animal was killed. This would suggest that although the beta cells were exhausted they were not all damaged.

Although most of the alpha cells are loaded with granules the appearance of a few actively secreting cells may be correlated with this premortem period of recovery. The appearance of the fat in the liver is suggestive of its removal and perhaps may be correlated with the activity of some of the alpha cells.

The results of continuous intravenous injection of varying amounts of sugar into guinea pigs are not entirely in accord with those found by Jacobs and Colwell ('36) in dogs. These authors state: ". . . . The continuous administration of glucose even at rates just within the limits of tolerance, that is, not causing glycosuria, is always fatal. . . . . higher injection rates, causing glycosuria, are more rapidly fatal. The mechanism leading to death would appear to be a non-ketogenic acidosis associated with profound congestive and hemorrhagic changes, particularly involving the pancreas and anterior hypophysis."

In guinea pigs the administration of dextrose was not always fatal. In over sixty animals examined, frank hemorrhage in the pancreas was never found. Higher injection rates were not always more rapidly fatal.

The results are in accord in that the individual variation of animals to intravenous glucose was considerable, the storage of glycogen was remarkable with the higher rates of injected sugar, and that, in general, greater amounts of glucose were more often fatal.

The differences in the results may be due in part to differences in the character of the experiments. The sugar injected into dogs was dissolved in water whereas that injected

into guinea pigs was dissolved in isotonic salt solution. The dogs were deprived of food whereas the guinea pigs were fed. The concentration of the sugar solution injected was different except in the case of the higher rates. In addition, it may be that compensation in the guinea pig is more readily effected than in the dog.

The data obtained from guinea pigs corroborate the findings of Felsher and Woodyatt ('24) and Jacobs and Colwell ('36) in that the tolerance of normal animals to glucose varies somewhat with the individual animal and is approximately 1 gm. per kilogram of body weight per hour.

CONCLUSIONS

From these eperiments it appears that although there is considerable individual variation in the reaction of the islands of different guinea pigs to the amount of dextrose continuously injected intravenously, there is an apparent correlation between the blood sugar level and the condition of the island cells. There is also some evidence that the reaction of the island cells to the sugar in the blood is a phasic or cyclic one.

The results indicate that when the intravenous injection of dextrose is accompanied by considerable hyperplasia and secretory activity of the beta cells and the blood sugar level is raised only slightly above the feeding level the alpha cells may be exhausted and even hydropic, little glycogen may be stored in the liver and no osmic combining fat is found there (Woerner, '38).

When the injection is accompanied by actively secreting and exhausted beta cells with some mitotic activity and the blood sugar level is considerably raised (i.e., two to three times the normal level in 24 to 48 hours), the alpha cells may be actively secreting and somewhat suppressed, the liver cells may be filled with glycogen and very little osmic combining fat found in that organ.

When the blood sugar level is excessively high, the beta cells exhausted and degenerating or suppressed, the alpha

cells may be almost completely suppressed, the liver cells distended with glycogen and increasing amounts of osmic combining fat found in the Kupffer cells and in the liver cells toward the central vein.

The cytological studies suggest that the functional state of the island cells may not be always accurately determined by the granule content of the cells alone. An agranular cell may be either temporarily functionally exhausted, or degenerating either from exhaustion or suppression. Whereas a cell filled with granules may be in the resting state, suppressed, or in the stage of granule formation. Simultaneous studies of the mitochondria and Golgi nets suggest means of differentiating functional phases in the granular and agranular cells. However, more experimental material is needed before these correlations may be made absolute.

SUMMARY

With the continuous intravenous injections of 2 to 3 plus grams of dextrose per kilogram of body weight per hour into guinea pigs for varying periods of time, the following results were observed.

1. The liver cells were filled or distended with glycogen. The distention of the cells with glycogen may have impaired liver function and this may account for the fact that animals injected continuously with 3 plus grams did not often survive much longer than 2 days.

2. The increase in blood sugar level was very variable and in some cases showed fluctuations during the course of the experiment when frequent determinations were made.

3. There appeared to be some correlation between the terminal blood sugar level and the condition of the cells in the islands of Langerhans. Where the blood sugar was high, usually the majority of the beta cells were exhausted or showed beginning degenerative changes and the majority of the alpha cells appeared to be suppressed. Where the blood sugar level was only moderately elevated the majority of

the beta cells and the alpha cells appeared to be actively secreting.

4. There appeared to be some correlation between the liver glycogen and fat and the condition of the island cells. When the majority of both beta and alpha cells appeared to be actively secreting, the liver cells were well filled with glycogen and not much osmic combining fat was found in them. When the majority of beta cells were exhausted or showed beginning degenerative changes, and the alpha cells appeared inactive or suppressed, the liver cells were distended with glycogen and osmic combining fat occurred in the cells toward the central vein and in the Kupffer cells.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

The optical system used for figures 1 to 3 consisted of a Zeiss apochromatic oil immersion lens H.I. × 35, 0.85 aperture and a Leitz Homel ocular, with a bellows length which gave a magnification of × 645.

The line drawings were made by tracing outlines in the photomicrographs, the details being checked by microscopic observation of the cells photographed.

1 and 1 a Four-micra section of pancreas of guinea pig no. 59, injected for 4 days with 2.44 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublimate, stained in neutral gentian. Cells in a large peripheral island.

α, alpha cells; β, beta cells with polar distribution of granules; a-β, agranular beta cells; r, red blood corpuscles.

2 and 2 a Four-micra section of pancreas of guinea pig no. 62, injected for 8 days with 2.0 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublimate, stained in neutral gentian. Cells in a large interlobular island.

α, alpha cells; a-β, agranular and vaculated beta cell; ?, undifferentiated cells or modified alpha cells?

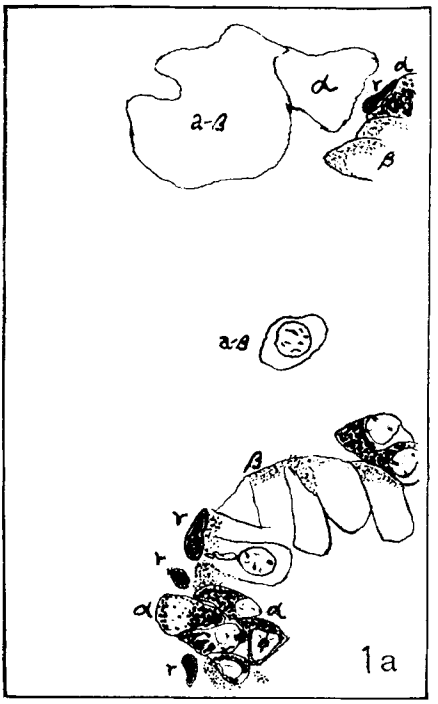
Note the scarcity of typical alpha cells.

3 and 3 a Four-micra section of pancreas of guinea pig no. 68, injected for 4 days with 3.17 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublmate, stained in neutral gentian. Cells in a large peripheral island.

All the dark-staining areas are alpha cells loaded with granules except for those regions indicated as βg (beta granules) in figure 3a.

r, red blood corpuscles; v, vacuolated cells.

Note the extensive Golgi nets in the central group of agranular beta cells.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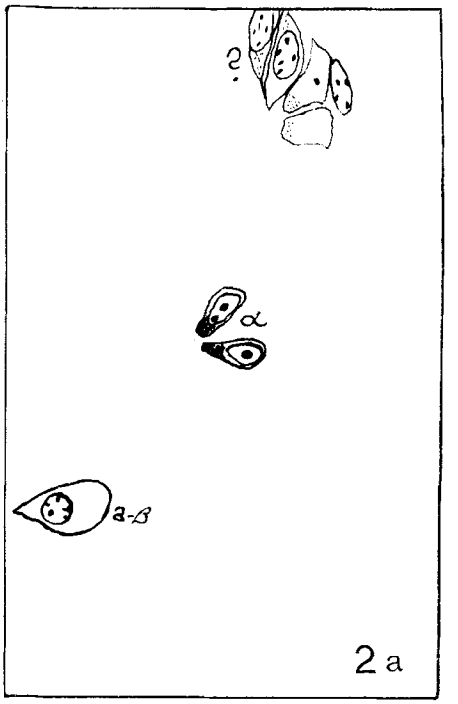
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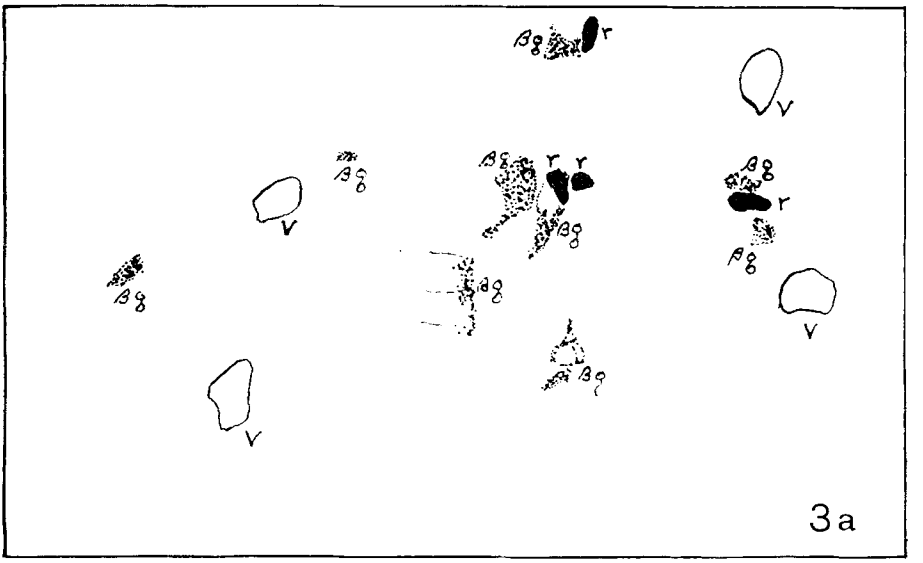
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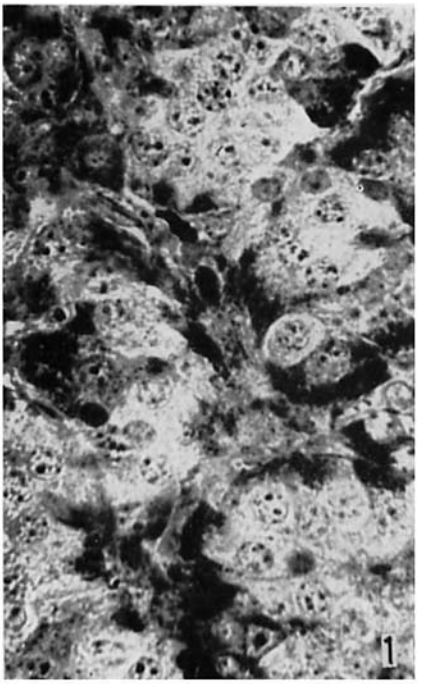
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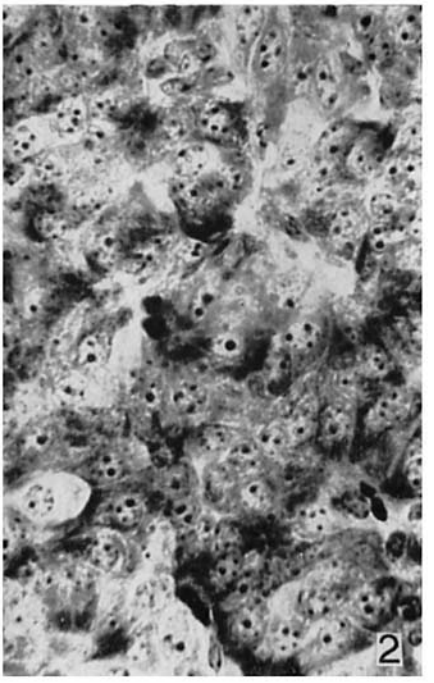
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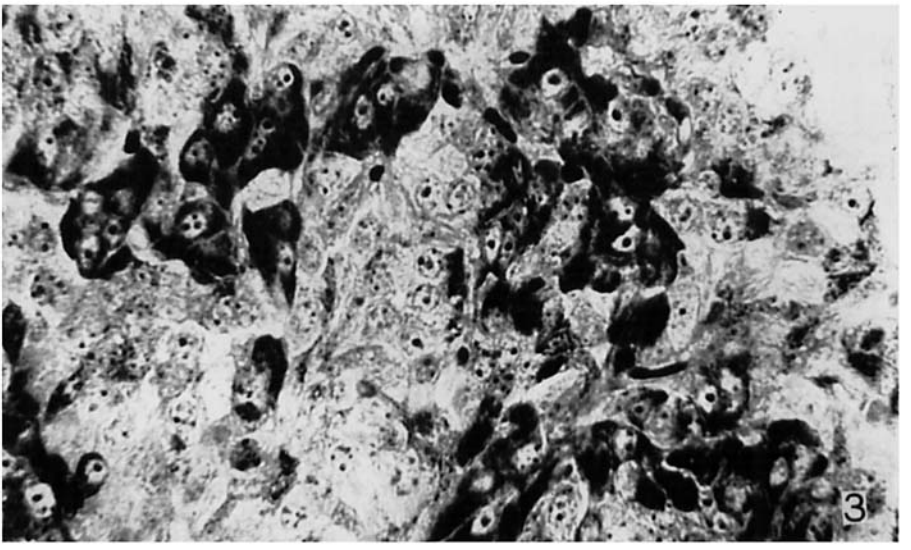
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