PGA,[9] behaves similarly to chlorguanide in *P. gallinaceum* infections. It potentiates sulfadiazine to about the same degree as chlorguanide and its activity is partially inhibited by the same amounts of PGA as are required to inhibit chlorguanide. If chlorguanide should prove to be a PGA antagonist, this fact would be highly significant in the development of chemotherapeutic agents for malaria and other diseases as well as for the study of the metabolism of the malaria parasite.

Summary. The antimalarial activity of chlorguanide against *Plasmodium gallinaceum* in the chick could be significantly but not completely inhibited by pteroylglutamic acid *in vivo*. Pteroylglutamic acid had no significant effect on the antimalarial activity of quinine, chloroquine or pamaquine, but completely inhibited the effect of sulfadiazine.

Received May 18, 1949. P.S.E.B.M., 1949, **71**.

9 Daniel, L. J., and Norris, L. C., *J. Biol. Chem.*, 1947, **170**, 747.

17174. Pancreatic Islet Hyperplasia in Rats Force Fed High Carbohydrate Diets.

ROBERT W. WISSLER, JOHN W. FINDLEY, JR. AND LAWRENCE E. FRAZIER.
From the Departments of Pathology and Medicine, University of Chicago.

Although both the cat and the dog may develop islet degeneration and irreversible diabetes after prolonged periods of hyperglycemia,[1-6] permanent diabetes has not been produced in the rat by methods short of partial pancreatectomy or alloxan treatment. After noting the ease with which Ingle produced chronic glycosuria in the rat by force feeding a high carbohydrate diet,[7] it occurred to us that it might be of interest to perform a similar experiment and examine the islets of Langerhans.

Plan of experiments. Two groups of 6 male albino rats of Sprague-Dawley strain were subjected to force feeding of gradually increasing quantities of high carbohydrate rations until glycosuria was sustained. Weights and urinary glucose excretion were determined at frequent intervals. Rats which died during the experiment as well those which survived for the entire experimental period of 56 days were examined grossly and histologically, especial attention being given the appearance of the pancreatic islets.

Rations, method of feeding and urine glucose determinations. A liquid ration containing about 50% carbohydrate was force fed to each group. The ration fed Group I (Table I) was slightly modified from that described by Ingle.[7] The ration fed Group II was similar except that the carbohydrate was all glucose. The latter diet was employed with the thought that intestinal absorption might be faster and more complete, thus making it possible to obtain higher levels of blood glucose. The rations were force fed 2 times per day at about 8 a.m. and 5 p.m. by means of the technic described by Shay and Gruenstein.[8] Starting with 6 ml per rat the daily portions administered were increased by 4 ml per day until 26 ml were being given. This quantity was maintained for a week to accustom the rats to the method of feeding and to avoid "food-shock".[7] Following this the quantities of ration were gradually in-

1 Homans, J., *J. Med Res.*, 1914, **30**, 49.

2 Homans, J., *J. Med. Res.*, 1915, **33**, 1.

3 Allen, F. M., *J. Metab. Res.*, 1922, **1**, 75.

4 Copp, E. F. F., and Barclay, A. J., *J. Metab. Res.*, 1923, **4**, 445.

5 Dohan, F. C., and Lukens, F. D. W., *Science*, 1947, **105**, 183.

6 Dohan, F. C., and Lukens, F. D. W., *Endocrinol.*, 1948, **42**, 244.

7 Ingle, D. J., *Endocrinol.*, 1946, **39**, 43.

8 Shay, H., and Gruenstein, M., *J. Lab. and Clin. Med.*, 1946, **312**, 1384.

TABLE I.
Composition of Rations for Force Feeding.

Constituents	Units	Ration I (Group I)	Ration II (Group II)
Egg albumin	g	8	8
Brewers yeast	"	5	5
Corn starch	"	25	—
Dextrin	"	12.5	—
Glucose	"	12.5	50
Ruffex	"	6	6
Salt mixture	"	2	2
Wheat germ oil	"	5	5
Corn oil	"	10	10
Oleum percomorphum			
Vitamin A	U.S.P. units	200	200
" D	" "	29	29
" K	mg	5	5

Water to make total of 100 ml added to each ration.

creased as tolerated until 68 ml were being given daily. Each group of rats was housed in wire bottomed cages and allowed water *ad libitum*. Beginning on day 7 and on alternate days thereafter the rats of each group were placed in individual metabolism cages and their urine was collected under toluol for a 24-hour period for qualitative and quantitative glucose determinations. Qualitative determinations were performed with 0.5 ml of urine and 5 ml of Benedict's solution which were mixed and heated in a boiling water bath for 5 minutes. Quantitative urine glucose determinations were performed using Benedict's method when the qualitative test showed 2+ or more.

Histologic preparations. All tissues were fixed in Bouin's solution and imbedded in paraffin. The sections of pancreas were prepared in Dr. Gomori's laboratory and stained with the chrome hematoxylin and phloxine stain.[9] Hematoxylin and eosin stained sections of liver, stomach, kidney, thymus, and adrenal were examined from all animals and lung, skeletal muscle, mediastinum and testis were studied from selected animals.

Experimental results. The quantity of diet administered daily, the average body weights and the urinary glucose values for Group I are summarized in Fig. 1. It is apparent that increasing the diet intake from 26 ml per day to 68 ml per day resulted in a steady increase in body weight amounting to an average weight gain of almost 200 g for the 8 week period. Urinary glucose became apparent on the 15th day and was excreted by the majority of the animals of this group in quantities varying from 0 to 1.48 g per day for the remainder of the experiment. The animals of this group appeared in moderately good health and only one rat succumbed before the termination of the experiment, due to suffocation from aspirated diet.

The response of the rats of Group II to the ration containing only glucose as the carbohydrate source differed from those of group I, which received the mixed carbohydrate ration in the following respects: the animals tolerated this ration much more poorly and developed signs of food-shock on several occasions, necessitating the interruption of the steady increase of daily rations. Three of the 6 rats succumbed early in the experiment, 2 from food-shock and 1 from false passage of the stomach tube. Two of the remaining 3 rats died before the end of the experiment, 1 of food-shock and 1 of aspiration of diet. The 2 rats which survived beyond the 35th day of the experiment consistently showed more urinary glucose than the rats of Group I. Acetone was noted on 3 occasions in the urine of these rats.

At necropsy the only consistently noted gross change was marked obesity in rats which had been force fed either ration for 30 or more days.

The significant histologic findings in these

[9] Gomori, G., *Am. J. Path.*, 1941, **17**, 395.

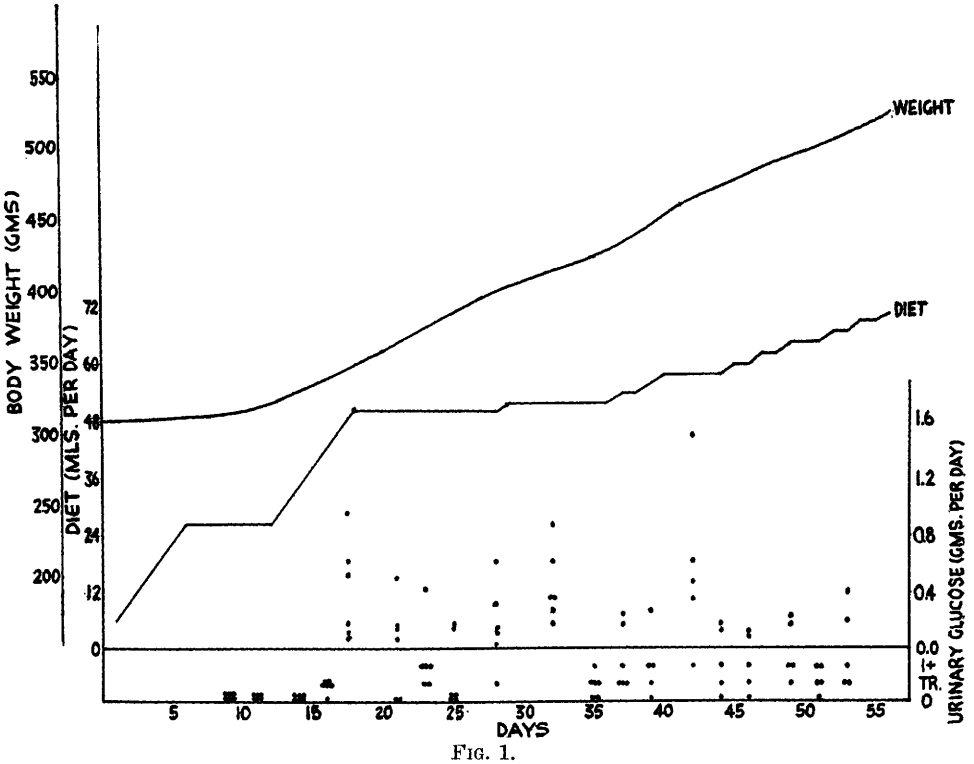

FIG. 1.
Weights, dietary intakes and urinary glucose excretion of rats force fed Diet I.

rats are summarized in Table II. The most striking changes were apparent in the islets of Langerhans. All animals which had received either ration for 32 days or longer showed marked hyperplasia of these structures, accompanied by a consistent decrease in granulation of the beta cells (Fig. 2 and 3). These changes were well developed in the rats which succumbed at 32 and 38 days and tended to increase as the rations were fed for longer intervals. In most instances the average islet diameters were 2 to 4 times greater than those of control animals. There was no evidence that the rats of Group II showed more marked

TABLE II.
Histological Findings in Rats Force Fed High Carbohydrate Diets.

Group No.	Rat No.	Time force fed, days	Pancreas		Adrenal cortical hyperplasia	Thymic atrophy	Liver	
			Islet hyperplasia	Degranulation of beta cells			Glycogen	Fat
I	1	55	+++	+++	+	0	+++	+
	2	55	++++	+++	++	0	++++	0
	3	55	++++	+++	+	+	++++	0
	4	55	+++	+++	++++	0	+++	+
	5	38	+++	+++	++	0	+	0
	6	55	++++	+++	0	+	+++	++
II	1	55	+++	+++	++	0	++++	0
	2	14	+	++	0	0	+	0
	3	10	0	+	0	0	++	0
	4	14	+	++	+++	0	+	+
	5	32	++	+++	0	+	+	0
	6	51	+++	+++	0	0	++++	0

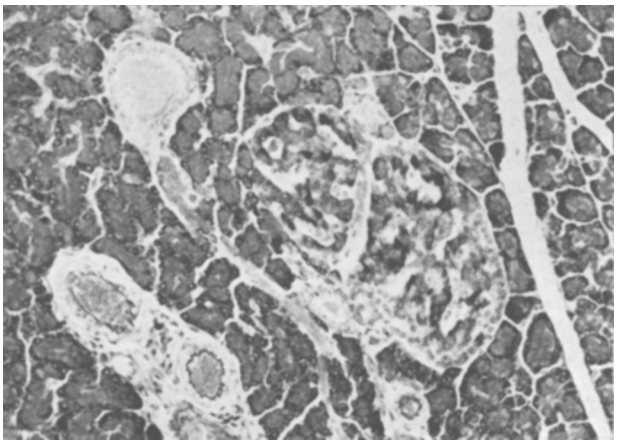

FIG. 2.
Typical pancreatic islet from rat II-3 which died after only 10 days of force feeding. Note the almost normal appearance of the islet cells. Chrome-hematoxylin and phloxine stain. × 150.

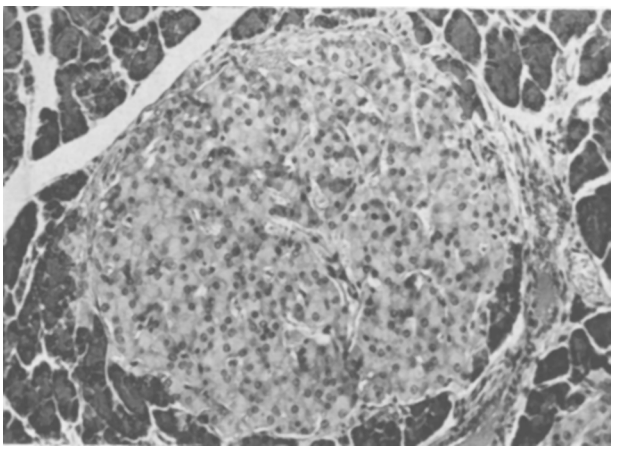

FIG. 3.
Typical pancreatic islet from rat II-1 which was sacrificed after 56 days of force feeding. Note the marked hyperplasia of the islet cells with almost complete degranulation of the beta cells. Chrome-hematoxylin and phloxine stain. × 150.

changes. Although the pitfalls of evaluating islet volume from examination of a single section through the pancreas are well established,[10] these hyperplastic changes were so uniform and conspicuous that there is little doubt of their significance. Furthermore, the recent study of Tejning[11] gives us added confidence in the validity of our interpretation. After detailed studies of pancreatic islet volumes, this investigator concluded that if one finds in a section of pancreas a number of islets that are larger than normal, one can conclude that the total volume of islet tissue is also greater than normal. No evidence of necrosis, hydropic change, hemorrhage, inflammation or absolute decrease in beta cells

[10] Richardson, K. C., and Young, F. G., *J. Physiol.*, 1937, **91**, 352.

[11] Tejning, S., *Acta Med.* Scandinav. Supp., 198, 1947.

was apparent in any of the sections.

Other significant histologic findings consisted of a somewhat variable but often marked increase in glycogen in the liver cord cells, sometimes accompanied by slight large droplet fatty change. The stomachs of the rats which had been force fed for a period of 30 days or more showed a spectacular hypertrophy of the muscularis but no other abnormality. The thymus glands showed microscopic evidence of atrophy in only 3 rats, and this was in no instance well developed. The other organ showing rather consistent alteration was the adrenal. Here there was a moderate cortical hyperplasia which was evident in 7 rats. There was no apparent correlation between the adrenal cortical hyperplasia and the thymic atrophy.

The kidneys as well as the skeletal muscle and the structures of the mediastinum revealed no significant histologic changes in the instances in which they were examined. Unfortunately, the testes were studied microscopically in only one rat (number 4, Group I). Here the tubules were of normal size and cellularity and spermatogenesis was apparently active.

Discussion. Our data are not complete enough to warrant conclusions to be drawn regarding the adrenal cortical hyperplasia. The similarly treated rats of Ingle[7] uniformly showed grossly enlarged, dark red. adrenal cortices. The findings may be due to the very large amounts of carbohydrate administered, to the stress incident to the acts of force feeding, or to some other factor. High protein diets have been reported to cause adrenal cortical enlargement,[12] but the protein contained in the diets fed to our rats probably was not sufficient at any time to cause such changes.

The concentration of sugar in the blood has repeatedly been found to affect islet structure and volume. The islet cell degeneration produced by partial pancreatectomy and/or anterior pituitary extract injections in the dog and cat can be prevented or, in certain instances, reversed by a low carbohydrate intake, insulin, or phloridzin.[1-4,13] Rabbits treated with alloxan develop hydropic degeneration of the islets after about one month of marked hyperglycemia.[14] Corpaci believed that he had increased islet volume and produced damage of islet cells in rabbits by repeated parenteral injections of glucose and adrenalin, but the evidence is not conclusive.[15] Similar experiments in guinea pigs by Meneghini, using glucose alone, are reported to have caused islet hyperplasia.[16] Careful studies have shown that rats fed a high carbohydrate diet *ad libitum* develop pancreatic islet volumes that are significantly greater than those fed *ad libitum* other types of diets.[11] Evidence has been obtained of both hyperplasia and exhaustion of guinea pig islets after continuous infusions of large amounts of glucose.[17,18] In experiments lasting not more than 100 hours, dogs killed by the continuous intravenous administration of glucose were found to have specific intense hemorrhages into and destruction of the pancreas and hypophysis.[19] Special studies of islets were not reported. Finally, clinical diabetes has been produced in an intact cat after 39 days of intraperitoneal glucose injections, and part of a series of cats treated in this way developed marked hydropic degeneration of the islets.[5,6] Verzár and von Kúthy produced heavy glycosuria, mild hyperglycemia, and, on rare occasions, ketonuria in dogs by force feeding solutions of glucose and sucrose for several weeks.[20] Glucose tolerance was thereby impaired but soon returned to normal. Postmortem examinations are not recorded.

Degeneration of rat pancreatic islets has not been produced by hyperglycemia. Lukens

12 Tepperman, J., Engel, F. L., and Long, C. N. H., *Endocrinol.*, 1943, **32**, 403.

13 Lukens, F. D. W., Dohan, F. C., and Wolcott, M. W., *Endocrinol.*, 1943, **32**, 475.

14 Duff, G. L., McMillan, G. C., and Wilson, D. C., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 251.

15 Corpaci, A., *Sperimentale, Arch. di biol.*, 1932, **86**, 129.

16 Meneghini, T., *Ginecologia*, 1939, **5**, 539.

17 Woerner, C. A., *Anat. Rec.*, 1938, **71**, 33.

18 Woerner, C. A., *Anat. Rec.*, 1939, **75**, 91

19 Jacobs, H. R., and Colwell, A. R., *Am. J. Physiol.*, 1936, **116**, 194.

20 Verzár, F., and von Kúthy, A., *Pflueger's Arch. f. d. gesam. Physiol.*, 1930, **225**, 606.

and Dohan examined the pancreatic remnants of rats that had had pronounced hyperglycemia for from 2 weeks to several months following partial pancreatectomy and found almost no damage.[21] In similarly treated animals hyperplasia instead of damage was reported by Friedman and Marble.[22] The same type of change was observed after the administration of anterior pituitary extract.[10] In order to produce glycosuria consistently in the rat with anterior pituitary extract, partial pancreatectomy is necessary,[23] although adrenal steroids cause temporary insulin resistant diabetes.[24] However, Peterson has recently reported complete degranulation of rat islet beta cells within 15 minutes of the intracardiac injection of 3.0 g of glucose/kg.[25] This lasts 48 hours and is associated with temporary impairment of glucose tolerance. Similar but less consistent degranulation was found after one intraperitoneal injection of glucose in the guinea pig by Gomori *et al.*[26]

In canine anterior pituitary diabetes, proliferation of the islet cells may precede the exhaustive or degenerative changes which accompany the onset of permanent diabetes.[27] Our observations indicate that the carbohydrate tolerance increased with each increase in feeding, glycosuria decreasing when the diet was maintained at a level which had initially caused moderate sugar excretion. This adaptation is evidently implemented by islet hyperplasia. Although it might be possible to prevent hyperplasia and produce islet damage by increasing the volume of food more rapidly this would probably be difficult without causing fatal "food-shock." It is conceivable that some relatively small additional stimulus, perhaps the administration of anterior pituitary extract or adrenal steroids, might have produced exhaustion of the islets of our rats. Older rats may be more susceptible.[28]

Summary. Degranulation of beta cells of the pancreatic islets and islet hyperplasia have been observed in rats which were force-fed large amounts of high carbohydrate, otherwise balanced diets for periods of as long as 8 weeks. Glycosuria and, in rare instances, ketonuria occurred.

[21] Lukens, F. D. W., and Dohan, F. C., *Endocrinol.*, 1942, **30**, 175.

[22] Friedman, N. B., and Marble, A., *Endocrinol.*, 1944, **29**, 577.

[23] Long, C. N. H., *Harvey Lect.*, 1936-37, **32**, 194.

[24] Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H., *Endocrinol.*, 1945, **37**, 341.

[25] Peterson, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 352.

[26] Gomori, G., Friedman, N. B., and Caldwell, D. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 567.

[27] Richardson, K. C., and Young, F. G., *Lancet*, 1938, **1**, 1098.

[28] Martinez, C., *Rev. Asoc. med. argent.*, 1946, **60**, 666.

Received May 16, 1949. P.S.E.B.M., 1949, **71**.

17175. Inorganic Aging of the Plasma Layer of Tissue Cultures.

JOHN H. HANKS.

From the Leonard Wood Memorial, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

The problem of maintaining mammalian cells *in vitro* for long intervals in an original or lightly patched plasma base may be broken down into two aspects: (a) the avoidance of calcification during the usual transfer intervals of two to four weeks and (b) the problem of an inorganic aging which complicates more prolonged maintenance. Though the control of calcification difficulties during the usual transfer intervals seems feasible by the measures summarized in the preceding paper,[1] solution of the long-term problems

[1] Hanks, J. H., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 328.