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Prevention of Diabetes on Forced Fed Rats Under Prolonged Diethylstilbestrol Administration. (17668)

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Ingle(1) observed the appearance of glycosuria in normal rats or its increase, if already present, in partially pancreatectomized rats, under prolonged administration of diethylstilbestrol. This diabetogenic effect took place in forced fed animals. Diethylstilbestrol had no effect on the glycemia or glycosuria of partially pancreatectomized male rats, providing the animals were allowed to eat *ad libitum*(2,3). Ingle believes that the reason of this failure is the diminished food intake due to the anorexia produced by that estrogen. In previous works(4,5) we showed that the estrogens had a protective and androgens an aggravating action on the onset and course of diabetes of partially pancreatectomized rats (surgical ablation of 95% of pancreatic mass) when feeding was *ad libitum*.

The present paper shows that, in forced fed rats, a protective action followed the initial—although not constant—diabetogenic action of diethylstilbestrol.

Methods and material. White rats from

1. Ingle, D. J., *Endocrinology*, 1941, v29, 838.
2. Janes, R. G., and Dawson, H., *Endocrinology*, 1946, v38, 10.
3. Ingle, D. J., *J. Clin. Endocrin.*, 1947, v7, 449.
4. Foglia, V. G., Schuster, N., and Rodríguez, R. R., *Rev. Soc. argent. Biol.*, 1947, v23, 202; *Endocrinology*, 1947, v41, 428.
5. Lewis, J. T., Foglia, V. G., and Rodríguez, R. R., in press.

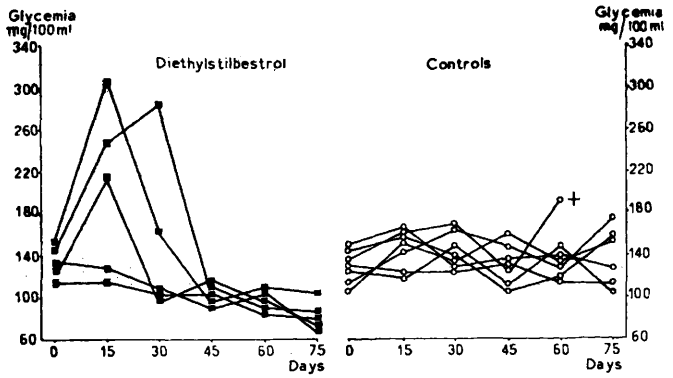

FIG. 1.

Variation of the glycemia of castrated and partially pancreatectomized female rats (ablation of 95% of the pancreas) under forced feeding, injected with 50 μg/day of diethylstilbestrol. Controls.

the Institute strain were used throughout the investigation. In the first experiments female rats of 130 to 150 g of body weight were castrated, followed a week later by removal of 95% of the pancreas using Foglia's technic(6). Ten days later, after recovery from the 2 surgical procedures, forced feeding was started. After 5 weeks under the special diet (Fig. 1) the entire group was divided in 2, the animals of the control group receiving 25 mg of lactose daily, subcutaneously, in 0.5 ml of 0.9% ClNa solution; the animals of the second group were injected daily with the same dose of lactose in the saline solution and with 5μg of diethylstilbestrol dipropionate in addition.

The diet used in the forced feeding had the following composition:

	g
Cellu-flour	120
Salt mixture (McCollum-Davis)	40
Yeast	100
Dry milk	600
Wheat germ oil	12
Mazola oil	220
Cod liver oil	12
Water	1400

The final % composition of the diet was:

	g %
Water	53.00
Dry residue	47.00
Carbohydrate	15.00
Protein	8.30
Fat	17.35
Cellulose	3.00
Ash	3.35

6. Foglia, V. G., *Rev. Soc. argent. Biol.*, 1944, v20, 21.

The animals received a total daily dose of 25 ml, divided in 2 rations. They were fed from 8 to 8:45 a.m. and from 4:30 to 5:30 p.m. The mixture was warmed up to 35°C immediately before feeding. It was observed that the animals remained during 2 or 3 hours in a state of sopor after the feeding. In order to avoid alimentary shock a total daily ration of 10 ml was given at the beginning, increasing it in 1 ml daily, in such a manner that 2 weeks later the animals were receiving the full daily dose of 25 ml. The animals were kept under such constant diet and without any treatment during the follow-

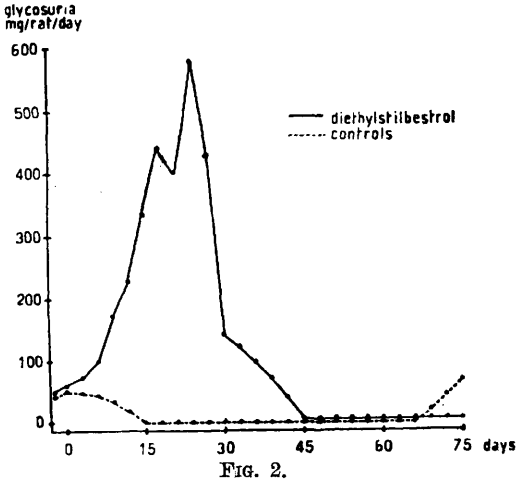

FIG. 2.

Variation of the glycosuria of castrated and partially pancreatectomized female rats (ablation of 95% of the pancreas) under forced feeding, injected with 50 μg/day of diethylstilbestol. Controls (average of 5 rats injected and 7 controls).

ing 3 weeks, in order to allow the glycemia and glycosuria to reach a constant level.

Each animal was kept separately in a single cage, at a temperature from 22°C to 23°C. They drank water at will. The urine of each animal was collected in the usual manner, preserved under toluol and every 48 hours determination of its glucose content was made by the Benedict method. Blood samples were drawn from the tail 7 hours after the morning forced feeding and glycemia was determined by the Hagedorn and Jensen method.

Results. The data of Fig. 1 and 2 show that pancreatectomized rats, without hyperglycemia up to the moment when diethylstilbestrol at a 50 μg daily dose was started, showed an initial raise of the glycemia and glycosuria. This increase in the glycemia and glycosuria took place in most but not all of the animals. In the others the glycemia and glycosuria variations were only temporary as they dropped to their initial level approximately 1 month after the diethylstilbestrol treatment, and while still under it. One of the non-diabetic animals died, probably due to the toxic effect of the drug. At the moment when diabetes appeared in the control group, injected with lactose, glycosuria and blood sugar level were normal in all the diethylstilbestrol injected animals. Table I shows less increase of weight in the diethylstilbestrol injected animals as compared with the control series at the end of the experiment.

Autopsy findings (Table I) of the diethylstilbestrol group consisted in enlargement of the pituitary and adrenal glands.

Histology. The microscopic study of the pancreas was performed by Dr. A. F. Cardeza. Marked hyperplasia of the insular tissue was found in the diethylstilbestrol series (Fig. 3a).

TABLE I.
Pituitary and Adrenal Weight (mg % of Body Weight) of Castrated and 95% Pancreatectomized Female Rats Under Forced Feeding. Average of 6 Injected Animals and 7 Controls

	Lactose	Diethylstilbestrol 50 μg/day
Hypophysis	4.7	17.9
Adrenals	28.5	46.9
Final body wt, g	234	203

Confluent masses of newly formed islets could be observed of a polylobulated and ramified appearance; transformation of the centro-acinous cells in beta cells, in different stages of the process could be seen in some small islets. Bands of sclerosis connective tissue were seen in the periphery of some insular masses. At times, these bands penetrated and dissociated the beta cells trabecules. Other islets, clearly differentiated from the exocrine part did not show evidence of sclerosis; cytologically, the beta cells and the typical granules were the ones which almost exclusively could be demonstrated. The beta cells were hypertrophic, sometimes globulous or spherical, with a clearly visible perinuclear negative image of the Golgi apparatus when stained with Gomori's stain. The pancreas of the non-diabetic controls (Fig. 3b) showed foci of less intense hyperplasia of the insular tissue than the animals injected with diethylstilbestrol. Collagenous tissue was present around and within the islets. The granuli of most of the beta cells were well stained. The diabetic control rats showed islets with very scant hyperplasia of the insular tissue. The beta cells showed degenerative lesions with vacuolization of the cytoplasma, nuclear atrophy and pyknosis. The pituitary glands of the diethylstilbestrol-injected rats were enlarged, with sinusoid dilatations, blood lakes and necrobiotic zones. The cytology showed an equal number of cromphobic and basophilic cells, the latter with a large intraprotoplasmatic acidophilic vacuola. These elements were smaller than in the castration cells, the vacuolar content was more acidophilic and similar to the colloid substance. The pituitary gland of the control animals had the typical appearance of a castration gland, with hypertrophic basophilic cells, excentric nuclei, the cytoplasma occupied by a large vacuola and a decrease in the eosinophilic cells. Loss of hair as previously described (5) was prominent in the diethylstilbestrol injected rats.

Discussion. The increase in glycosuria observed by Ingle after diethylstilbestrol administration persists for a variable period, usually less than a month and is accompanied by hyperglycemia, which follows the same chronological order. During this period, some

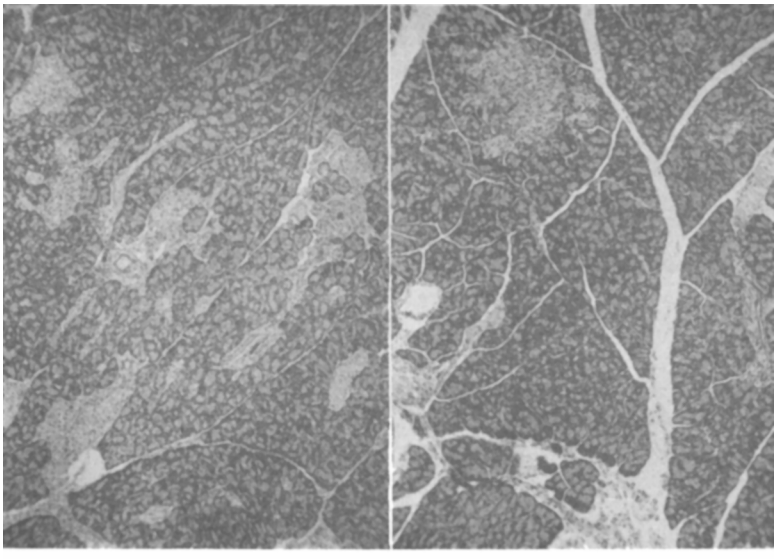

FIG. 3.
Pancreas of non-diabetic partially pancreatectomized rats (removal of 95% of the pancreas), under forced feeding, injected with: (a) diethylstilbestrol, showing marked hypertrophy and hyperplasia of the islets of Langerhans; (b) lactose, showing sclerosis inside and outside the islets. Hematoxylin-eosin. × 60.

animals, both with and without diabetes, died probably due to a toxic action of the drug. Some factors have been published which agree with our own findings. Ingle's Fig.(1) show that stilbestrol does not maintain the increase in glycosuria initially produced by it in normal and pancreatectomized rats. The same author(7) later states: "When stilbestrol was administered hyperglycemia and glycosuria were induced in over two-thirds of a large series of animals studied. Daily doses as small as 50 gamma were effective. Adaptation occurred so that the diabetic state disappeared after being continually present for periods of 2 to 36 days". After the first month of injecting diethylstilbestrol all the surviving animals remained normoglycemic and aglycosuric, a persistent condition of the treated animals even when their controls became diabetic.

The mechanism of the effect of diethylstilbestrol might be: a) a probably direct hypertrophy of the Langerhans islets, because in unpublished experiments we found that hypertrophy is produced by the local administration of estradiol in the pancreas of rats, guinea pigs and cats; b) probable hypofunction with increase in weight of the pituitary and of the adrenal glands, in spite of the fact that Ingle(8) showed that the initial action of diethylstilbestrol took place even in the absence of these two glands; c) a direct effect on the liver; d) a peripheric action.

The protective effect upon diabetes and hyperplasia of the Langerhans islets was observed in normal rats and also in those deprived of pancreas and adrenals. Further details on these last experiments will appear in a future publication.

Summary. Castrated female rats, with surgical ablation of 95% of the pancreas and maintained under forced feeding were injected with stilbestrol.

In some of these animals, immediately after stilbestrol administration, a transitory stage lasting for about one month, with an increase of the blood sugar level and glycosuria values was observed, followed by a per-

7. Ingle, D. J., *Am. J. Physiol.*, 1941, v133, 337.

8. Ingle, D. J., *Endocrinology*, 1944, v34, 361.

manent protective action in all the stilbestrol treated rats with normal values in blood sugar and glycosuria, in contrast with the increase of these values observed in non-injected controls.

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Some Effects of Protein Depletion and 2-Acetylaminofluorene on Activity of Rat Plasma Pseudocholinesterase.* (17669)

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McCance, Widdowson and Hutchinson(1) demonstrated that starvation resulted in a considerable decline in the pseudocholinesterase activity of human plasma. Mendel, Hawkins and Nichikawara(2) reported similar results in rats. The following experiments were planned to determine whether or not this decrease in pseudocholinesterase activity was associated with depletion in protein stores. The activity of this enzyme was also studied in animals fed 2-acetylaminofluorene, where depletion in protein and other signs of inanition have been noted incident to the development and growth of tumors.

Methods. A. *Enzyme Assay.* The Warburg manometric method of pseudocholinesterase determination as described by Ammon(3) and modified by Mendel and Mundell(4) was used to estimate enzyme activities. All determinations were done in duplicate; 3 readings were taken at 20 minute intervals and, after summing, the activities were expressed as μl CO_2/ml plasma/hr. The buffer system was bicarbonate-CO_2 at pH 7.4. The substrate was benzoylcholine chloride (Hoffmann-LaRoche) at a final concentration of 0.006 M. Blood was obtained from ether anesthetized animals by heart puncture with a lightly oxalated syringe.

B. *Liver Protein Assay.* Liver protein was determined by applying the factor of 6.25 to micro-Kjeldahl N determination. The liver was removed from the animal immediately upon sacrifice and a sample homogenized for analysis.

C. *Diets and Animals.* Male Wistar rats were used throughout. Control animals were fed a semi-synthetic diet compounded as follows, the values being expressed as percentages. Casein 18, sucrose 13.4, glucose 20.2, dextrin 18.2, lard 24.2, Wesson's salt mixture(5) 1.7, agar 3.3, cod liver oil 1, water added equal to 1.4 times the dry weight. Vitamin supplements, as mg/kg wet weight of diet were, choline chloride, 1000 mg; inositol, 100; riboflavin, 3.2; thiamin, 2; ascorbic acid, 4; pyridoxine HCl, 1.6; 40 mg each of Ca Pantothenate, niacin, p-aminobenzoic acid and α-tocopherol and 0.2 each of 2-methyl-1, 4-naphthoquinone biotin and folic acid. Water was added so that the diet might be fed as a gel, thus reducing scattering and allowing a more accurate estimation of uneaten food. For animals to be fed the protein-free diet, glucose was substituted isocalorically for the casein of the control diet. For animals to be fed the "drug" diet, 0.05% 2-acetylaminofluorene was added to the control diet.

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† Major, USAF. Studying at Rutgers University under the provisions of Air Force Letter 50-21, dated August 6, 1947.

1. McCance, R. A., Widdowson, E. M., and Hutchinson, A. O., *Nature,* 1948, v161, 56.

2. Mendel, B., Hawkins, R., and Nichikawara, M., *Am. J. Physiol.,* 1949, v154, 495.

3. Ammon, R., *Pflug. Arch. ges. Physiol.,* 1933, v233, 486.

4. Mendel, B., and Mundell, D. B., *Biochem. J.,* 1943, v37, 64.

5. Wesson, L. G., *Science,* 1932, v75, 339.