Meat infusion*	300 cc
Casein hydrolysate†	17.5 g
Starch paste‡	100 cc
Water	100 "
Adjust pH to 7.4-7.6.	

Mix and distribute at once either into test tubes (about 20 cc each for pours, 5 cc for slants) or flasks of 120-200 cc. Autoclave *not more than 10 minutes at 10 pounds.* Over-autoclaving spoils the medium. The flasks can be used to pour plates at once. The tubes may be melted in boiling water and used as needed. The medium seems to give best results when not too moist, either with gonococcus or meningococcus. For either organism a candle jar must be used, and the cultures incubated at about 36° for 24 to 48 hours in the case of gonococcus, or 16 to 24 hours for meningococcus.

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Mechanism of Action of Estrogens on Insulin Content of the Rat's Pancreas.*

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The action of estrogens on the pancreas of rats has been demonstrated in a twofold manner. Griffiths, Marks and Young[1, 2] have shown the insulin content of rat pancreas to be increased after implantation of tablets of various estrogens for periods of 2-4 weeks;

* Meat Infusion—1 pound of meat (chopped lean beef or beef heart), 500 cc water. Suspend meat in water, bring to active boiling, strain through cheese-cloth and filter through paper. For routine use, this infusion may be preserved in the cold room in stoppered bottles containing a few cc of chloroform.

† Casein Hydrolysate—Quantity specified for Difco preparation, Lot S-64123 of "Casamino Acids, Technical."

‡ Starch Paste—Suspend 1.5 g ordinary starch (corn starch or laundry starch, not "soluble starch") in 10 cc cold water. Pour slowly into 90 cc boiling water, while stirring.

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1 Griffiths, M., and Young, F. G., *Nature,* 1940, **146**, 266.

2 Marks, H. P., and Young, F. G., *Lancet,* December 7, 1940.

Vasquez-Lopez[3] demonstrated marked enlargement of the Golgi apparatus of the islets of Langerhans in similarly treated mice. More recently, the English group of workers[4] also reported an increase in liver weight and glycogen content of such estrinized rats. The authors do not discuss the mechanism of these effects beyond suggesting the possibility that the increase in pancreatic insulin might be secondary to the increase in liver glycogen storage.

In view of the well known overgrowth and cytological changes provoked in the pituitary by the prolonged action of estrogens, it appeared possible that the effect of these compounds on the pancreas might be mediated by the pituitary. This working hypothesis was tested by two approaches. In the first experiment pituitaries of estrogen treated rats were compared with those of untreated controls, regarding their ability to increase upon implantation, the pancreatic insulin content of hypophysectomized rats. In the second experiment, the effect, if any, of estrogens on the pancreatic insulin content of hypophysectomized rats was investigated.

1.† Normal rats (donors) were implanted 3 times (once weekly) with 10 mg pellets of alpha estradiol dipropionate. After 18 days' treatment they were sacrificed and their pituitaries as well as those of untreated control rats were immediately implanted into hypophysectomized rats (recipients, each receiving 9 pituitaries in 19 days, with autopsy on the 21st day) and the insulin content of the pancreas of donors as well as of the two types of hypophysectomized recipients compared with their respective controls.‡

2. Hypophysectomized rats were implanted 3 times (once week-

[3] Vasquez-Lopez, E., *Nature*, 1940, **146**, 589.

[4] Griffiths, M., Marks, H. P., and Young, F. G., *Nature*, 1941, **147**, 359.

† For a preliminary account of this experiment see 5,6.

‡ The pancreas of each group of rats were pooled, minced and extracted according to the method of Fisher and Scott.[7] The solutions of crude insulin prepared by this procedure were then assayed by the convulsion method in mice. (For details see 8.) Pancreas extracts which were to be compared were always assayed parallel in groups of 10-15 mice, these assays being repeated from 3 to 6 times. While the insulin content of the pancreas of control groups was found quite variable from one experiment to another (in the earlier experiments possibly owing to an insufficient use of standard insulin solutions), the error inherent in the mouse assay, using 30 to 60 mice per group, was, in many experiments, never found to exceed $\pm 17\%$. In agreement with others,[2] however, we are not inclined to attribute much significance to differences in the pancreatic insulin of 2 groups of rats of less than 50% unless confirmed by repetition of the experiment. A comparison of the insulin content of normal female and hypophysectomized rats revealed no significant difference (5 groups of the former averaging 0.84 units, and 8 groups of the latter 0.79 units of insulin per 100 g rat).

ly) with alpha estradiol dipropionate—also similar rats receiving at the same time daily injections of a crude pituitary (globulin) fraction. They were autopsied on the 18th day p.o. and of estrogen treatment. The insulin content of the pancreas of these groups was compared with two groups of controls (globulin fraction alone, and untreated).

Results. Experiment 1: The finding of the English investigators[1, 2] has here been clearly confirmed, as shown in the first 2 insulin assays reported on Table I, 1a. Increases in pancreatic insulin of 54% and 90% respectively were obtained in normal rats treated with estrogen pellets.

Comparing the pancreatic insulin content of hypophysectomized rats repeatedly implanted with the pituitaries of estrinized and untreated rats with that of hypophysectomized controls, it was found that the pituitaries of the estrinized rats increased to a moderate extent (39%) the pancreatic insulin, while untreated pituitaries had no 'pancreatrophic' effect (Table I, 1b).

Experiment 2: In hypophysectomized rats no increase in pancreatic insulin content was produced by implants of estrogen pellets. This was true for rats which received the estrogen alone as well as

TABLE I.

Exp. No.	Type of rat	Treatment	No. of rats	Pancreatic insulin U/100 g/rat	Pancreatic insulin Change
1a	Normal ♀, 78-79 days at autopsy	Estrogen pellets*	6	0.8	+54%
		Controls (untreated)	6	0.52	—
	Normal ♀, 84-86 days at autopsy	Estrogen pellets*	6	1.35	+90%
		Controls (untreated)	6	0.70	—
1b	Hypophysectomized ♀, 54-56 days old, 28-30 days p.o. at autopsy	Pituitaries of above *estrinized* rats implanted	6	1.8	+39%
		Pituitaries of above *untreated rats* implanted	6	1.3	0%
		Controls (untreated)	4	1.3	—
2	Hypophysectomized ♀, 72-76 days old, 18 days p.o. at autopsy	Estrogen pellets*	4	0.62	—28%
		Estrogen pellets* and pituitary globulin fraction†	8	0.62	—28%
		Globulin fraction alone†; no implants	9	1.04	+21%
		Controls (untreated)	9	0.86	—
	Normal ♀, 75-84 days at autopsy	Controls	7	0.56	—35%

*10 mg pellets of alpha-estradiol dipropionate (Ciba), implanted once weekly.

†Globulin fraction of beef anterior pituitary extract, containing growth hormone, thyrotrophic hormone, adrenocorticotropic hormone, lactogenic hormone and ICSH (0.2 mg daily intraperitoneally).

TABLE II.
Hypophysectomozed Rats.*
(See Table I, Exp. 2.)

Type of treatment	Body wt (g)		Liver† % of body wt
	Initial‡	Change	
Estrogen alone	156	—34	3.3
Estrogen + pituitary fraction	149	—15	3.4
Pituitary fraction alone	151	+ 8	3.3
Hypophysectomized controls	142	—16	3.5
Normal controls	156	+26	3.5

*Fasted 18 hours before autopsy.
†All liver glycogens were very low (between 0.03 and 0.05%).
‡On day of hypophysectomy and onset of treatment.

for rats receiving pituitary substitution therapy with the estrogen.§ Both groups contained slightly less insulin in their pancreas (28%) than the untreated hypophysectomized controls (Table I, 2).

Table II summarizes the body weights and liver weights and liver glycogens of these groups. The body weight changes appear of interest in connection with the mechanism of the antagonism of estrogens and "growth"; the liver data in view of Griffiths'[4] finding of an increase in liver weight and glycogen content in normal estrinized rats.

Discussion and Conclusions. The finding of an increase in pancreatic insulin produced by prolonged estrinization of normal rats has been confirmed. It appeared possible that this effect might be mediated by the pituitary which in such estrinized rats is greatly enlarged. A stimulation of production and secretion by the pituitary of greater than usual amounts of a 'pancreatrophic hormone' might account for the observed facts. The pancreatrophic potency of the pituitaries of such estrinized rats was therefore tested, in comparison with that of untreated animals. No pancreatrophic action was evident upon implantation of 9 pituitaries of control rats.‖ The same number of pituitaries of treated rats caused a slight increase in pancreatic insulin content, which by itself, cannot be regarded as sufficient evidence for the increased pancreatrophic potency of

§ The pellets of alpha estradiol dipropionate were not well tolerated by hypophysectomized rats. At autopsy after 18 days' treatment, only 4 of 10 rats were alive, 2 of these moribund. Daily administration of 0.2 mg globulin fraction (prepared from an alkaline extract of beef anterior pituitary) counteracted the toxic effect of the estrogen satisfactorily (8 rats alive and healthy at autopsy). These animals resembled normal rats treated with estrogen pellets.

‖ The lack of pancreatrophic activity in rat pituitaries is attributed not to the absence of the pancreatrophic factor but to a neutralization of its action by another hormone. This will be discussed elsewhere.[8]

the glands. The hypothesis that the effect of the estrogen is mediated by the pituitary is, however, supported by the outcome of the second experiment, *i. e.*, the failure to produce pancreatrophic effects in hypophysectomized rats with the same estrogen treatment which had marked effect in normal rats. That this failure was not due to the pronounced toxic action of the estrogen in such rats was demonstrated by its lack of effect also in rats receiving pituitary substitution therapy besides the implanted estrogen.

It is also noteworthy that estrinization does not lead to increases in liver weights or liver glycogen storage in hypophysectomized rats, as reported for normal rats.[4] It would thus appear very probable that the effect of estrogens on pancreatic insulin content as well as on liver weight and glycogen is mediated by the pituitary gland. Evidence will be discussed elsewhere[8] which indicates that the lactogenic hormone exerts a pancreatic insulin increasing action in normal and hypophysectomized rats. The recent finding[5, 6] that the lactogenic hormone content of and excretion by the pituitary appears to be increased in estrinized rats stands thus in good agreement with the reported observations of an increased 'pancreatrophic' function of the pituitaries of estrinized rats.

Summary. Two types of evidence favor the assumption that the activity of estrogens in elevating the pancreatic insulin content is mediated by the pituitary. (1) The pituitaries of estrinized rats when implanted elevate the pancreatic insulin content of the host animals while the pituitaries of untreated rats do not show this activity. (2) Estrogen which raises pancreatic insulin content in normal rats does not produce this effect in hypophysectomized rats.

5 Simpson, M. E., and Evans, H. M., *Anat. Rec.*, 1941, **79**, Suppl. 2.

6 A detailed account of this work is in preparation for press.

7 Fisher, A. M., and Scott, D. A., *J. Biol. Chem.*, 1934, **106**, 305.

8 Fraenkel-Conrat, H. L., Herring, V. V., Simpson, M. E., and Evans, H. M., *Am. J. Physiol.*, in press.