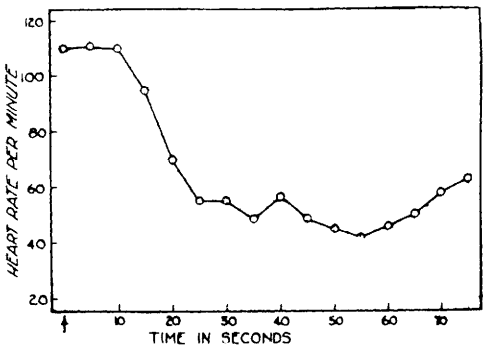

FIG. 4.

mine does not alter the cardio-inhibitory responses produced by the injection of pitressin and angiotonin. This would indicate that, as in the anesthetized animals, Dibenamine does not impair the vasoconstrictor actions of these compounds.

Summary and Conclusions. The actions of Dibenamine on the cardiovascular adjustments caused by epinephrine, acetylcholine, pitressin, and angiotonin have been studied in unanesthetized dogs. Dibenamine produced an increase in heart rate which persisted for one to 3 hours.

A test dose of epinephrine which regularly caused reflex cardiac slowing in normal unanesthetized dogs produced severe cardiac acceleration after administration of Dibenamine. This acceleration is attributable to the direct stimulatory action of epinephrine on the sino-auricular node and to reflex acceleration from a fall in blood pressure.

The compensatory cardiac acceleration produced by injection of a test dose of acetylcholine was undiminished and prolonged in unanesthetized animals under the influence of Dibenamine. This result is compatible with the interpretation that the vasoconstrictor mechanisms are impaired while the cardioaccelerator mechanisms are relatively intact.

The severe cardiac inhibition produced by pitressin and angiotonin in unanesthetized dogs, which is attributable to reflexes elicited by the rise in arterial blood pressure subsequent to vasoconstriction, was not prevented by Dibenamine.

The results of these experiments on unanesthetized dogs are in accord with the interpretations of Nickerson and Goodman[1] concerning the sites of action of Dibenamine.

16051

Diabetogenic Effect of Two Synthetic Estrogens in Force-Fed, Alloxan-Diabetic Rats.

DWIGHT J. INGLE AND JOHN A. HOGG.

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Ingle[1] has reported on the diabetogenic effect of diethylstilbestrol, dihydrostilbestrol, estradiol and equilin in the force-fed, partially depancreatized rat. More recently it was shown by Ingle, Nezamis and Prestrud[2] that diethylstilbestrol will intensify the glycosuria of rats having alloxan diabetes when the food intake is kept constant by forced feeding. The present study was a partial test of the hypothesis that compounds which are estrogenic are also diabetogenic in the rat. Substance I (2,4-di[p-hydroxyphenyl]-3-ethyl hexane) was described by Blanchard, Stuart and Tallman[3] and Substance II (1,2-dimethyl-2-carboxy-7-methoxy - 1,2,3,4,9,10-hexahydrophenathrene) was described by Hogg.[4]

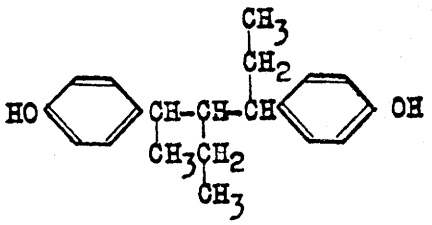
Each of these two compounds belongs to a chemically different series of synthetic estrogens than any of the substances which have previously been examined for diabetogenic

1 Ingle, D. J., *Endocrinology*, 1941, **29**, 838.

2 Ingle, D. J., Nezamis, J. E., and Prestrud, M. C., *Endocrinology*, 1947, **41**, 207.

3 Blanchard, E. W., Stuart, A. H., and Tallman, R. C., *Endocrinology*, 1943, **32**, 307.

4 Hogg, J. A., *J. Am. Chem. Soc.*, in press.



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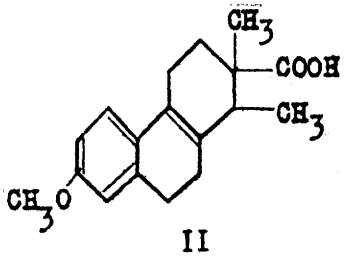

II

activity. Each compound was found to intensify the glycosuria of the alloxan-diabetic rat.

Methods. Male rats of the Sprague-Dawley strain were maintained on Purina Dog Chow until they reached a weight of 310 g. They were then fed a medium carbohydrate diet made according to Table I. During the administration of alloxan all of the rats ate the diet *ad libitum*. Alloxan was injected subcutaneously in doses of 25 mg every other day until glycosuria appeared. After a diabetic state was established the animals were placed in metabolism cages and were force-fed by stomach tube each morning (8:30 to 9:15 a. m.) and afternoon (4:15 to 5:00 p. m.). The techniques and the diet were modifications of those described by Reinecke, Ball and Samuels.[5] During the period of adaptation to

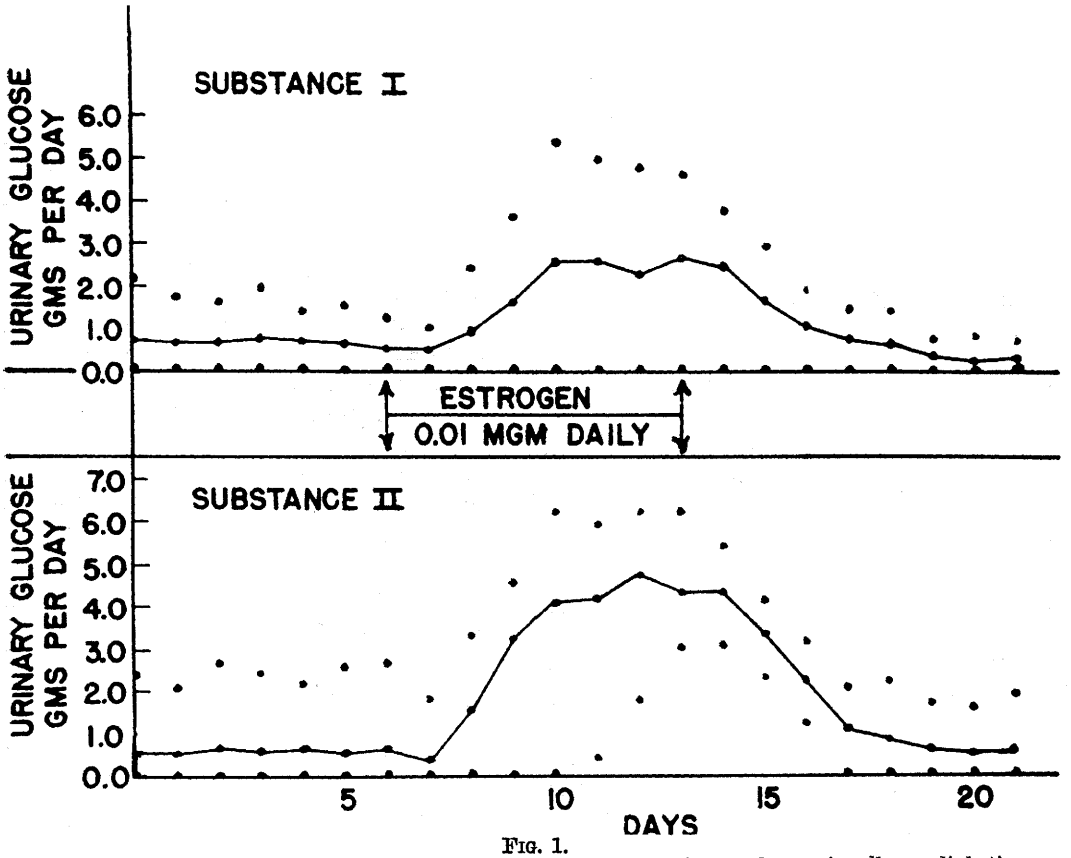

FIG. 1.

The effect of two synthetic estrogens upon the level of urinary glucose in alloxan-diabetic rats. Averages (solid line) and range of individual values. Six rats were tested in each of the two experiments.

TABLE I.
Composition of Fluid Diet.

Constituent	
Cellu flour (Chicago Dietetic Supply)	120 g
Osborne and Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Mazola oil	200
Vit. K (2-methyl-1-4-naphthaquinone)	100 mg
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Egg albumin (Merck)	
Water to make total volume of	2000 cc

forced feeding, the amount of diet was increased gradually to prevent the development of "food-shock." The animals were brought to a full feeding of 26 cc of diet per rat per day on the fifth day.

The animals were maintained in an air-conditioned room in which the temperature was maintained at 74-78°F and the humidity at 30-35% of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 a. m.) and were preserved with thymol. Urine glucose was determined by the method of Benedict.[6]

The estrogens were made up in vegetable oil solution and were each given by subcutaneous injection once daily in a volume of 0.1 cc. Substance I (Schieffelin) was purchased on the market. Substance II was prepared by one of us (JAH) by laboratory synthesis.

Experiments and Results. Twelve rats having mild alloxan diabetes were used in these experiments. The animals were observed for a period of 3 to 4 weeks before the tests of the estrogens were started. In experiment 1, 6 rats were injected with 0.01 mg of Substance I per day for 7 days. Two of the rats were free from glycosuria during the control period. Five of the 6 rats responded to the injections of Substance I by an increase in the level of urinary glucose. When the injections were stopped the level of glycosuria decreased. The sixth rat did not excrete glucose during any phase of the experiment.

In Experiment 2, 6 rats were injected with 0.01 mg of Substance II per day for 7 days. Three of the rats were free from glycosuria during the control period, but all of the animals showed a significant increase in urinary glucose during the injection period. When the injections were stopped the glycosuria decreased in all of the animals and disappeared entirely in 4 of the 6 animals.

The data on urinary glucose are summarized in Fig. 1.

Discussion. The results of these experiments support our tentative hypothesis that compounds which are estrogenic also have the property of intensifying the glycosuria of mildly diabetic, force-fed rats. Other hormonal substances, especially those of the adrenal cortex and the anterior pituitary, are diabetogenic in the rat although they lack estrogenic activity. Of the polycyclic compounds which have been tested, only those having estrogenic activity or those having adrenal cortical activity have been found to be diabetogenic. The mechanism of diabetogenic activity in these compounds and its relationship to the role of the hormones in body economy are unknown.

These data are relative to the conclusion of Janes and Dawson[7] that estrogens are not diabetogenic in the rat and support the findings of Ingle, Nezamis and Prestrud[2] who found that diethylstilbestrol does intensify the glycosuria of the alloxan-diabetic rat just as in the partially depancreatized rat (Ingle[1]). There are two essential conditions for the exacerbation of diabetes by estrogens in the rat. First, the test animal should be mildly diabetic rather than severely diabetic. Second, the food intake must be sustained by forced feeding, otherwise the diabetogenic effect of the estrogen may be partially or completely masked by the concomitant decrease in voluntary food intake. These essential conditions were not observed in the study by Janes and Dawson.[7]

Summary. Male rats having mild alloxan

[5] Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

[6] Benedict, S. R., *J. A. M. A.*, 1911, **57**, 1193.

[7] Janes, R. G., and Dawson, H., *Endocrinology*, 1946, **38**, 10.

diabetes were used as test animals in this study. They were force-fed a medium carbohydrate diet. Two synthetic estrogens were tested, each of which represents a chemically different series of compounds than have previously been tested for diabetogenicity. Substance I was 2,4-di(p-hydroxyphenyl)-3-ethyl hexane, and Substance II was 1,2-dimethyl-2-carboxy-7-methoxy - 1,2,3,4,9,10-hexahydrophenathrene. Substance I caused exacerbation of the diabetes in 5 out of 6 test animals. The sixth rat did not have a spontaneous glycosuria and failed to respond to the estrogen. Substance II intensified the glycosuria in all 6 test animals and appeared to be the more potent of the two compounds. When the injections were stopped the glycosuria decreased to its pre-injection level or below.

16052

BAL Inhibition of Mercurial Diuresis in Congestive Heart Failure.*

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From the Medical Service of Beth Israel Hospital, New York City.

This study was undertaken to evaluate the effect of 2,3 dimercaptopropanol[1] (dithiopropanol),—commonly known as British Antilewisite or BAL—upon the diuresis induced by the organic mercurials with the view of gaining further information regarding the mechanism of mercurial diuresis, as well as controlling some of the untoward sensitivity reactions[2,3] encountered when these substances are employed. 1 - (methoxy - oxymercuri - propyl) - 3 - succinylurea—generally known as Mercuhydrin—which contains 39 mg of mercury in organic combination with 48 mg of theophyllin per cc of aqueous solution, was the sole mercurial studied. The preparation known as BAL in oil (10% 2,3 dimercaptopropanol in benzyl benzoate and peanut oil) was used.

The subjects selected were cardiac patients in chronic congestive failure who were known to respond to organic mercurial injection with diuresis and weight loss. The experience with 7 patients comprises this report. Five patients had hypertensive arteriosclerotic heart disease; one was a rheumatic cardiac and the other suffered from heart failure of undetermined etiology. Regular sinus rhythm was present in 6 of 7 of these subjects at the time of the study.

So far as feasible, the patients were maintained on the usual therapeutic cardiac regimen consisting of incomplete bed rest, salt-poor diet, containing less than 2 g of NaCl in 24 hours, no fluid restriction, and in most instances the administration of some digitalis preparation.

When it appeared that they had attained a relatively constant weight under such management, BAL in oil was introduced for a period of 24 to 36 hours. It was administered intramuscularly every 6 hours in doses of 2.5 mg per kilo of body weight. After receiving BAL in oil for 24 hours, Mercuhydrin was injected intramuscularly in 2 cc doses. The BAL in oil was then continued for a period of 12 hours.

Table I contrasts the weight changes occurring in patients receiving BAL in oil for about 24 hours, prior to the administration of Mercuhydrin with those who received the mercurial only.

The individaul weight loss 48 hours after

* Work done under a grant from the Joseph and Helen Yeamans Levy Foundation in memory of Miriam Levy Finn.

1 Peters, R. A., Stocken, L. A., and Thompson, R. H. S., *Nature*, 1945, **156**, 616.

2 Brown, G., Friedfeld, L., Kissin, M., Modell, W., and Sussman, Ralph M., *J. Am. Med. Assn.*, 1942, **119**, 1004.

3 Long, W. K., and Faran, A., *Science*, 1946, **104**, 220.