vitro at an abnormally high rate in response to glucose. Twenty-four hours after induction of the insulin-deficiency, the absolute rate of insulin secretion is subnormal, but is still increased in relation to the low insulin content of these islets. This sequence of changes is seen in pancreatic tissue removed from spontaneously diabetic animals and suggests that this system could be used as a model for the study of islet function during the development of insulin-deficiency.

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Received June 26, 1967. P.S.E.B.M., 1967, v126.

Effects of Ovariectomy-Hypophysectomy and Adrenalectomy-Ovariectomy-Hypophysectomy on Feed Intake and Mammary Gland Growth as Measured by DNA.* (32482)

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An extensive study is under way in our laboratory concerning the effect of endocrine gland removal on feed intake because of interest in the role of feed intake on mammary gland growth and milk secretion. In previous studies, the effect of thyroparathyroidectomy and of adrenalectomy separately and together(1), of pinealectomy(2), and hypohysectomy(3) on feed intake has been reported. In addition, the effect of hypophysectomy on mammary gland DNA has been observed(4).

The present study was undertaken to determine the effect of the combined operations of ovariectomy (OVAR-X), hypophysectomy (HYPO-X), and of adrenalectomy (ADRE-X) on feed intake, and to present quantitative data on mammary gland development in the triply operated rats using DNA as a measure of mammary gland tissue.

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 5190. Approved by the Director. Aided in part by a research grant from USPHS.

Materials and methods. Hypophysectomized virgin female rats of the Sprague-Dawley strain were purchased from Hormone Assay Laboratory, Chicago, Ill. Animals were housed individually in metabolism cages and fed Purina Lab Chow with an energy value of 4.41 cal/g and 23.4% total protein, in an animal room maintained at a constant temperature of 78 ± 1°F. All the hypophysectomized rats were allowed at least 7 days of rest from the date of operation. Following this, the feed intake of these animals was determined and the animals in the study were ovariectomized or ovariectomized and adrenalectomized. The triply operated rats were given a 1% NaCl drinking water replacement therapy. A 7-day feed intake determination was begun at least 7 days later by the method previously described(5). For the study of the mammary gland DNA, animals were killed 26 days after the final operation and the 6 posterior mammary glands

TABLE I. Effect of Double Operations and Triple Operations on Feed Intake in Rats.

Group	Type of rats	Treatment	No. of animals	Before treatment (mean of 7 days)			7-14 days after treatment (mean of 7 days)					
				Mean bw, g	Feed, g/day	Intake, g/100 g bw	Mean bw, g	Change, %	Feed intake, g/day	Change, %	Feed intake, g/100 g bw	Change, %
I	HYPO-X	None	21	227.3 ± 5.9	13.48 ± .27	5.99 ± .21	228.1 ± 4.9	+ .4	12.90 ± .37	— 4.3	5.63 ± .21	— 6.0
II	HYPO-X	OVAR-X	15	218.2 ± 2.1	13.75 ± .34	6.29 ± .13	214.8 ± 2.2	—1.6	12.83 ± .35	— 6.7	5.97 ± .15	— 5.1
III	HYPO-X	OVAR-X +ADRE-X	13	199.3 ± 7.3	12.16 ± .47	6.10 ± .14	193.1 ± 6.9	—3.1	9.85 ± .38	—19.0	5.10 ± .20	—16.4*

* Significance of t probability, pre-treatment *vs* treatment, $P < .001$.

were removed for DNA determination as previously described(6).

Results. In Group 1, consisting of 21 hypophysectomized control rats, the mean body weight and daily feed intake were 227.3 g and 5.99 g/100 g bw, respectively, during the first 7 days (Table I). It will be noted that the mean daily feed intake decreased 6% whereas the mean body weight was unchanged during the experimental period.

Group II, consisting of 15 hypophysectomized rats, showed a mean body weight of 218.2 g and a mean daily feed intake of 6.29 g/100 g bw. Seven days following ovariectomy, the mean body weight and daily feed intake were 214.8 g and 5.97 g/100 g bw, a reduction of 1.6% and 5.1%, respectively, as compared to a mean preoperative body weight and feed intake. However, these decreases were statistically insignificant.

Group III, including 13 hypophysectomized rats weighing a mean of 199.3 g showed a mean feed intake of 6.10 g/100 g bw. Seven to 10 days following ovariectomy and adrenalectomy, the mean body weight and daily feed intake declined to 193.1 g and 5.10 g/100 g bw, a reduction of 3.1% and 16.4%, respectively. The decrease in feed intake was statistically significant. It may be noted that both body weight and feed intake decreased much faster in Group III as compared to Group II. However, it was noted that the mean daily feed intake in Group III gradually increased from the 15th day after ovariectomy and adrenalectomy, and returned to the preoperative level 10 to 14 days later even though body weight remained at the reduced level.

Mammary glands of rats sacrificed 26 days after ovariectomy had DNA content of 2.89 mg/100 g bw as opposed to 2.95 mg DNA in a virgin control group. Difference in the two groups was non-significant (Table II). Mean total DNA of 25 hypophysectomized rats sacrificed 33 days after the operation was 2.01 mg/100 g bw, while mammary glands of triply operated rats sacrificed 33 days after hypophysectomy and 26 days after ovariectomy and adrenalectomy had DNA content of 1.84 mg/100 g bw. Difference between these two groups was not

TABLE II. Effect of Endocrine Gland Removal on Mammary Gland DNA.

Group	Treatment	No. Rats	Final bw, g	DFFT, g	DNA, μg/100 g bw DFFT	Total DNA, mg/100 g bw (mean ± S.E.)
I	Virgin control	22	246.0	381.1	18.46	2.95 ± .23
II	OVAR-X	22	289.7	445.4	17.90	2.89 ± .28[1]
III	HYPO-X	25	211.2	213.0	19.79	2.01 ± .11[2]
IV	HYPO-X + OVAR-X + ADRE-X	10	175.5	166.9	19.65	1.84 ± .09[3]
Student's "t" probability			1-2 $P < .001$	1-3 $P < .001$	2-3 $P < .20$	

significant. However, the DNA value of these groups was significantly different from the DNA value of normal or ovariectomized rats.

Discussion. In a previous study of the effect of hypophysectomy on feed intake in rats, it was observed that the mean daily feed intake was reduced about 30% and the mean body weight 11.2% during a post-operative period of 10 to 17 days. In the present study, it was shown that ovariectomy in addition to hypophysectomy had no effect on feed intake compared with pretreatment level. In contrast to the stimulating effect of ovariectomy in normal rats on feed intake and body weight as reported by Holt *et al* (7), the present study showed a slight depressing effect on both body weight and feed intake; however, this depressing effect is not statistically significant.

Despite the presence of 1% NaCl in the drinking water, the triple operation caused a profound initial decrease in feed intake. Although the mean feed intake/100 g bw was reduced significantly, it was interesting to observe that the feed intake of the triply operated rats gradually returned to the control level at longer intervals while body weight remained at reduced level. It is possible that the presence of 1% NaCl was unable to compensate for the lack of aldosterone which might have caused the initial decrease in feed intake. It is not clear whether the subsequent increase in feed intake might be due to a high secretion of other hormones not controlled by the pituitary or its target glands, or gradual replacement of aldosterone by NaCl, or the presence of an antidiuretic hormone in the hypothalamus after hypophysectomy to influence the maintenance of the water balance.

In the previous study, using DNA as a measure of mammary gland tissue, a significant involution of the duct system from the ovariectomized level following hypophysectomy was observed(4). Although the adrenals secrete several steroids which can influence mammary growth(8), there is no significant difference between the group hypophysectomy alone and the group having combined operations of hypophysectomy, ovariectomy and adrenalectomy. This suggests that the ovaries and adrenals are of secondary importance on maintenance of mammary glands after hypophysectomy. However, in view of the fact that the mean DNA value of the group after hypophysectomy alone or the group having triple operations showed a highly significant difference from that of ovariectomized rats, the results suggest that a pituitary factor or factors are involved in the maintenance of the mammary duct system.

Summary. Combined effects of OVAR-X and HYPO-X (double operations) on feed intake were studied. In addition, the effect of triple operations on mammary gland DNA was reported. While OVAR-X in addition to HYPO-X had little or no effect, OVAR-X and ADRE-X in addition to HYPO-X caused a marked initial decrease in feed intake. However, the feed intake gradually returned to the preoperative control level at longer intervals while body weight remained at a reduced level. It was suggested that the presence of 1% NaCl in the drinking water was unable to compensate for the lack of aldosterone which might have caused the initial decrease in feed intake.

With DNA as a measure of mammary gland tissue, a significant involution of the duct system from the ovariectomized level

was observed following HYPO-X and triple operations. Although triple operations caused a lower DNA value than that of HYPO-X alone, there was no significant difference between the two groups. This suggests that the ovaries and adrenals are of secondary importance on maintenance of mammary glands after hypophysectomy.

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Received June 26, 1967. P.S.E.B.M., 1967, v126.

Electrophoresis of Human Hexokinases in Acrylamide Gel.* (32483)

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Recent reports have established that hexokinase exists in multiple forms with distinct structural and kinetic properties in tissues of the rat(1). It has also been shown that the activity of some isoenzymes of hexokinase alter under conditions such as fasting and insulin deficiency(2,3). A systematic survey of the pattern of isoenzymes of hexokinase in human tissues would therefore be of value toward an understanding of the changes occurring in pathological conditions. This paper presents a study of the isoenzyme pattern of hexokinases in normal human tissues using acrylamide gel electrophoresis, and includes a variation of pattern observed in a patient with hemolytic anemia.

Materials and methods. Human tissues were obtained during surgical operation from 13 patients. After removal the tissue was transferred to the laboratory in gauze soaked in saline, and, after washing, the tissue was homogenized in an equal volume of buffer containing: KCl 150 mM; 2-mercaptoethanol 10 mM; EDTA 5 mM; and $MgCl_2$ 5 mM and adjusted to pH 7.4 with $KHCO_3$. The homogenate was centrifuged at 15,000 $\times$ *g* for 20 minutes in a refrigerated International Centrifuge (angle head: 856) at 4°C. After centrifugation equal parts of the supernatant and spacer gel were mixed and 100 to 150 µl were applied to the columns of acrylamide gel. Electrophoresis was performed at 0-4°C using a 4% acrylamide running gel according to the method of Davis(4) except that in place of the spacer and sample gel a sephadex-sucrose-trisCl solution was used(5). Electrophoresis required approximately 50 minutes maintaining 2.5 to 3 m amps per column.

On termination of electrophoresis the acrylamide columns were stained for hexokinase in a solution similar to that described by Katzen and Schimke(1). Each experiment was performed at least in triplicate. Control tubes without ATP were run for each sample to distinguish nonenzymatic bands. Bands additional to those observed in controls were assumed to be due to the activity of hexokinase.

Results. Fig. 1 presents the pattern of isoenzymes of hexokinase in a variety of normal human tissues. The controls for each are included to show that non-specific bands develop in addition to those caused by hexokinase. In conformity with other observers employing starch gel electrophoresis, the bands of hexokinase are numbered in order

* This work was supported by USPHS Grant No. AM-612-15 and F2-AM-29, 650-02.