preceded the administration of the thymidine, even though relatively large proportions of the interphase cells had become labeled. Even when the agent was given 30 minutes after the addition of nucleoside and the usual normal proportions of interphase cells were labeled, there was a remarkable inhibition of the appearance of the grains in the mitotic cells.

Since it was not known whether MCM might exert its effects directly or indirectly on the nuclear apparatus, it was of interest to see if there was any preferential accumulation of MCM in a particular region of the cell. In the present experiment, 3 hours after the injection of MCM-H^3, grains began to appear around the nucleus and increased in number. No grains were observed within the nucleus. Therefore, it can be concluded that the MCM-H^3 became associated with cytoplasmic and not nuclear components. These components into which MCM-H^3 was incorporated may play a significant role in the formation of the spindle fibers. Experiments are under way in which an attempt is being made to isolate the H^3-labeled cytoplasmic constituents.

Summary. The intraperitoneal injection into mice with Ehrlich ascites tumor of trace amounts of H^3-methyl N-carbamyl maleamate, a substance which in larger amounts produces destruction of spindle fibers, resulted in the appearance on autoradiography of grains chiefly in the cytoplasm and only rarely in the nucleus. In a number of instances the grains appeared to be distributed in a perinuclear fashion. Somewhat similar results were obtained when H^3-methanol was administered. The administration of cold methyl N-carbamyl maleamate (1 mg) together with the labeled substance resulted in a marked enhancement of the uptake of the labeled material into the cells, while a similar experiment with nonradioactive methyl N-carbamyl maleamate and H^3-methanol showed no increase in uptake of isotope by the tumor cells. It was concluded that H^3-methyl carbamyl N-maleamate enters the tumor cells as an intact molecule and associates with cytoplasmic components. The relationship of some of these components to spindle fiber structures or precursors is suggested.

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Insulin Secretion *in vitro* by Islets from Insulin-Deficient Rats.* (32481)

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Following intravenous or intraperitoneal injection of guinea pig anti-insulin serum, transient hyperglycaemia induces rapid secretion of insulin(1) and more rapid depletion of pancreatic insulin than can be induced by hyperglycaemia due to intravenous administration of glucose(2). On the other hand, glucose induces the same rate of insulin secretion from isolated islets whether guinea pig anti-insulin serum is present in the incubation medium or not(3). An explanation for this discrepancy was sought in the present experiments by incubating islets obtained from normal rats and from rats treated with guinea pig anti-insulin serum for different times.

Materials and methods. Well fed male albino rats (250-350 g, Holtzman, Wisconsin) were injected either intravenously (1.5 ml) or intraperitoneally (2-5 × 5 ml) with guinea pig anti-insulin serum (GPAIS, Lots 401 or 404; binding 2.5 to 3.0 units bovine insulin/ml). Rats were anesthetized 60 or 150 minutes after a single intravenous injection and

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24 or 40 hours after the first 12-hourly intraperitoneal injection of GPAIS. From the pancreas of each animal, 70 to 90 individual islets were isolated, according to the method of Lacy and Kostianovsky(4). Islets (40 to 60) were incubated in groups of 10 for 90 minutes at 36°C in bicarbonate-buffered media (1.0 ml) containing glucose (300 mg/100 ml) and albumin (0.5%, w/v; bovine albumin, Fraction V, Sigma Chemical Co., St. Louis, Mo.); after incubation secreted insulin was measured by addition of GPAIS to an aliquot of the medium, using a method described in detail elsewhere(3,5). Between 25 and 30 islets from each animal were homogenized in acid-alcohol and their insulin content measured(6).

Results. As shown in Fig. 1, the absolute rate of insulin secretion evoked by glucose in islets removed 1 hour after a single injection

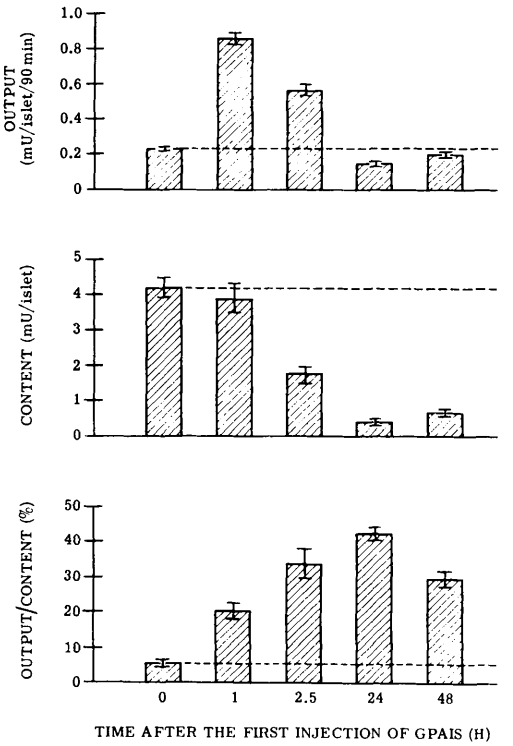

FIG. 1. Mean values (± SEM) for the rate of insulin secretion (mU/islet/90 min) induced by glucose (300 mg/100 ml) *in vitro*, the insulin content (mU/islet), and the ratio of insulin output to content (per cent) in isolated islets removed before and at intervals after the induction of insulin deficiency by administration of GPAIS into normal rats.

of GPAIS was 3- to 4-fold greater than normal. After 150 minutes the absolute rate of secretion was still elevated but the insulin content of the islets had decreased. After 24 hours the absolute rate of secretion was significantly less than normal ($p<0.001$) but, in relation to the reduced insulin contents of the islets, it was 7 to 8 times greater than the rate observed in normal islets. Between 24 and 48 hours, only minor changes occurred.

Comments. Despite the delay of about one hour between the removal of the pancreas from the rat and the beginning of incubation, insulin secretion was significantly increased in islets removed 1 and 2½ hours after the induction of insulin deficiency with GPAIS. During this time, therefore, the insulin secretory mechanism must undergo a marked change which increases its sensitivity to glucose, as was demonstrated *in vitro* in the complete absence of GPAIS. This change could be due either to a direct or to an indirect effect of the injected antibodies upon the function of the islets. The first possibility is unlikely, since GPAIS has no direct effect *in vitro* upon glucose-induced insulin secretion (3). It appears, therefore, that some metabolic change secondary to the insulin-deficiency induced by GPAIS *in vivo* could be responsible. After exposure *in vivo* to fasting (7), adrenalectomy or treatment with cortisone(8), changes in response of pancreatic tissue to glucose have been observed, but the present changes were induced much more rapidly. The sequence of changes is reminiscent of those seen in tissue from spontaneously diabetic animals(9,10); at first excessively responsive to glucose, the tissue later secretes at a low absolute rate, but relative to its reduced insulin content, at an abnormally rapid rate. Whatever the explanation, the results suggest that islets removed from rats treated with GPAIS could be used to study the acute effects of insulin deficiency upon the insulin synthetic and secretory mechanisms, effects which over a longer period may also be operative during the development of insulin deficiency in spontaneous diabetes.

Summary. Isolated islets obtained 1 to 2.5 hours after injection of guinea pig anti-insulin serum into normal rats secrete insulin *in*

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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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.

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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