the same iron level. It is interesting to note also that at the lowest levels of iron fed—when most economical use of the iron is of vital importance—the females actually retained more iron than the males, in spite of their smaller size.

TABLE I

ANALYSES OF RATS AFTER RECEIVING IRON SUPPLEMENTS

Mg Fe fed daily	Sex	No. of rats	Avg. 6 wks. gain in wt. grams	Avg. 6 wks. gain in HB Hb/100 ml blood	Avg. total mg Fe/rat	Avg. mg Fe/gm
.05	♂	3	90	1.9	2.3666	.017
.05	♀	3	71	5.8	3.3083	.026
.10	♂	3	100	5.3	3.5335	.020
.10	♀	4	72	8.7	4.2765	.029
.20	♂	5	120	8.9	5.0328	.032
.20	♀	6	83	10.4	4.3545	.035
.30	♂	1	153	11.0	7.2250	.039
.30	♀	3	83	11.5	5.2798	.045

To check this point further, male and female rats were compared that were fed the same iron supplement and whose gain in weight during the six weeks' test period was the same. When fed 0.05 mg Fe daily, the male rat gained 74 grams in weight and 2.3 gms Hb/100 ml blood and retained 2.1229 mg Fe or 0.018 mg Fe/gm, while the female rat gained 75 grams in weight and 5.1 gms Hb/100 ml blood but retained 3.7500 mg Fe or 0.027 mg Fe/gm. When the iron supplement was 0.20 mg Fe daily the male rat gained 100 grams in weight and 7.5 gms Hb/100 ml blood and retained 3.5707 mg Fe or 0.027 mg Fe/gm, while the female rat gained 102 grams in weight and 10.3 gms Hb/100 ml blood and retained 4.6832 mg Fe or 0.037 mg Fe/gm.

Thus, from a study of the total iron content of rats, it has been shown that at the end of the iron depletion period the iron content of male and female rats is very nearly the same. However, after feeding iron supplements to anemic rats, the bodies of female rats were found to contain more iron than those of males comparably fed. These results support the previous findings of this laboratory that there is a true sex difference in the way male and female rats utilize iron and that it can not be explained by a difference in the weights of the two sexes.

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ON THE ORIGIN OF UROGASTRONE[1]

THE presence of a substance in human and canine urine which inhibits the gastric secretory response to histamine and a meal has been previously demonstrated.[2,3,4,5] This substance has been called urogastrone[5] because of its resemblance to enterogastrone, which inhibits gastric secretion and motility.[6] The latter principle is liberated from the upper intestinal mucosa in response to contact with fat.

The experiments reported here were designed to answer two questions: Will urogastrone disappear from the urine following combined subtotal gastrectomy and total enterectomy? Is the output of urogastrone increased by feeding a high fat diet?

From four female dogs prepared and trained for catheterization, urine was collected successively under the following conditions: (*a*) daily for a 5-hour period after the ingestion of 75 cc of corn oil with a mixed meal; (*b*) for a 4-day period during which they received daily approximately 1,200 cc of a modified dextrose-free Locke's solution subcutaneously, but no food; and (*c*) for a period of a week after an operation in which the pyloric antrum and the entire small intestine were removed, the pancreatic ducts tied, the pancreas separated from the duodenum by a subserous resection of the latter, and external gastric and biliary fistulas constructed. During this post-operative period, modified dextrose-free Locke's solution was administered. With one exception, the animals survived considerably longer than one week. The catheterized urine was treated by the method of Katzman and Doisy,[7] and the resulting extracts were assayed in six Heidenhain-pouch dogs injected with histamine. The results are shown in Table 1.

TABLE 1

Experiment	No. of assays	Dose				Inhibition of gastric secretion of free acid in per cent.
		Mgms.	Urine equiv. in cc	Equiv. in hours	Equiv. in kg. hrs.	
Dogs 1, 2, 3, 4 (urine pooled)						
Fat fed ..	29	4.2	207	7.64	128	44
Fasted ...	18	5.3	393	12.03	205	32
Enterectomized ..	24	7.5	199	13.29	208	3

The results clearly demonstrate, first, that the ingestion of a high fat diet augments the excretion of urogastrone; and second, that urogastrone disappears from the urine following surgical removal of the small intestine. In these respects urogastrone behaves exactly as would be expected if it represents excreted enterogastrone. Accordingly, our experiments provide strong circumstantial evidence in favor of this inter-

[1] Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

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pretation. However, they do not permit the unreserved conclusion that urogastrone is formed by the small intestine, inasmuch as the effect of control surgical procedures on the excretion of urogastrone is as yet unknown. It should be pointed out that the selection of a proper control experiment is inseparable from the more general question of why urogastrone should be excreted in the fasting urine. Experiments are now in progress which are designed to provide at the same time an answer to this general question as well as controls for the experiments reported here.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A ROLLER BOTTLE TISSUE CULTURE SYSTEM[1]

IN 1936 Gey and Gey[2] described procedures which they employed in maintaining *in vitro* tissue cultures of normal and malignant cells in roller tubes. Cultures are grown in a thin layer of clotted plasma on the walls of ordinary or specially designed glass tubes, and by revolving them horizontally they are alternately in contact with a nutrient medium and with air or any gaseous mixture that may be desired. The rotation gives a constant, slow washing action which seems to promote an adequate exchange of food materials and waste products between the supernatant fluid and the cells. The Gey roller tube method has several advantages over the more usual hanging drop, Maximow slide or Carrel flask techniques, but has certain disadvantages and limitations which we believe to be partially eliminated by the modifications in technique described below.

It is difficult and time-consuming to prepare the sterile human normal and placental sera as described in the original articles.[2,3] For experiments of a few weeks' duration we believe the substitution of the "synthetic medium," described by Baker[4] in 1936, is helpful. In roller tube cultures of various human and animal normal and malignant tissues we have obtained very vigorous and healthy outgrowths, even when this medium has been simplified by omission of hemin, insulin, thyroxine and vitamins A and D. Directions for the preparation of this and other media are given in a recent book by Parker[5] and by Baker and Ebeling[6]. Synthetic media have the great advantage that their components can be varied at will.

The danger of contamination during feeding and transfer of roller tube cultures is a serious one, since contact of the nutrient fluid with the stopper is difficult to avoid. We have found it advantageous to employ an arrangement shown in Fig. 1. A short length of

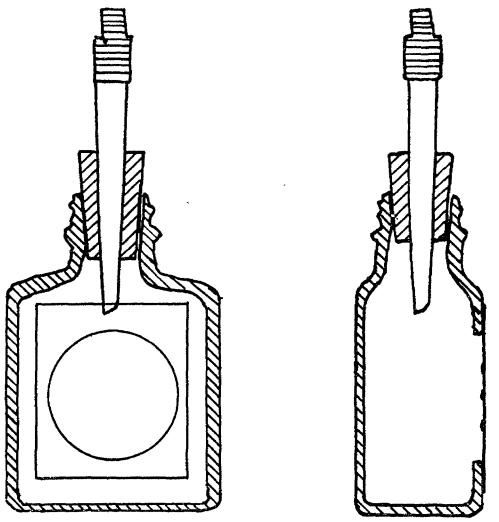

FIG. 1. Cross section views of roller bottle, with stopper and coverslip in place.

Pyrex tubing having a slight constriction at one end is introduced through a hole in a tightly fitting rubber stopper. The two parts are sterilized as a unit by autoclaving in large Pyrex test-tubes. A sterilized, inverted serum vial cap may be used to seal the end of the small tube after flaming it. For ordinary procedures this cap is removed, and the end of the small glass tube flamed. Fluid transfers can then be made by means of sterile syringes and long hypodermic needles.

Observations of the colonies under highest magnification is impossible in ordinary glass roller tubes, due to the thickness and curvature of the walls. Gey[3] in 1933 described the construction of special hexagonal tubes having flat sides of thin glass which were better suited to higher power observations (*i.e.*, 45× objectives). Even with these, however, satisfactory permanent stained specimens would be difficult to prepare and store and would involve sacrifice of the tubes.

[1] Aided by a grant from the National Research Council. From the Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University.

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[6] L. E. Baker and A. H. Ebeling, *Proc. Soc. Exp. Biol. and Med.*, 39: 291, 1938.