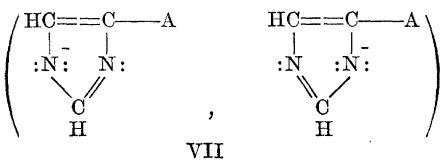
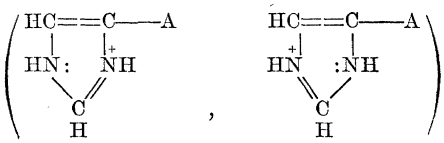
and abnormally acidic. (3) The third nitrogen, resembling in the classical structure the nitrogen of pyridine, possesses a net charge of $-1/5$. It is double bonded 3/10 on one side and 1/2 on the other. On the other hand, the resonance in pyridine gives the nitrogen a relatively small negative charge and makes it slightly less than half double bonded on each side. As a result the histidine nitrogen is much more basic than that of pyridine.

It is doubtful that the negative charge of this "pyridine" nitrogen can entirely compensate for its doublebondedness, and it is therefore probably less basic than the nitrogen in the alanine residue. The formation of salts of histidine indicates two basic nitrogens, $pK_b = 4.85$ and $pK_b = 7.97$, of which the first corresponds to the nitrogen of an amino acid, and the second to a nitrogen much more basic than that of pyridine.

A similar treatment of the nitrogens of histidine could have been accomplished by a consideration of the alternative resonances VII and VIII,[1] in which the ring has a net charge.



VII



VIII

Substitutions that depend on the basicity of the nitrogen occur at the amino group, *e.g.*, $C_3N_2H_3 \cdot CH_2 \cdot CHNH_2 \cdot COOH + C_6H_5 \cdot SO_2Cl \rightarrow C_3N_2H_3 \cdot CH_2 \cdot CHNH\,(C_6H_5SO_2) \cdot COOH + HCl$. Those that depend on removing a hydrogen by a metal, which is afterwards replaced by a group, occur on an acidic nitrogen of the ring, *e.g.*, $AgN_2C_3H_2 \cdot CH_2 \cdot CHNH_2 \cdot COOAg + 2CH_3I \rightarrow CH_3N_2C_3H_2 \cdot CH_2 \cdot CHNH_2 \cdot COOCH_3 + 2AgI$.

TERRELL L. HILL
GERALD E. K. BRANCH

UNIVERSITY OF CALIFORNIA,
BERKELEY

FURTHER EVIDENCE OF SEX VARIATION IN THE UTILIZATION OF IRON BY ANEMIC RATS

PREVIOUS work in this laboratory has indicated that female anemic rats utilize iron more efficiently than male anemic rats. This conclusion was based on the greater gain of the hemoglobin per 100 milliliters of blood shown by female rats when both male and female rats were fed the same iron supplement.[1] The question has been raised as to whether this is really a characteristic of the female organism or is simply due to the difference in the size of the two sexes and therefore different food intakes.[2] In the hope of shedding further light on this question, the total iron content of anemic male and female rats which have been given supplemental iron feedings has been investigated.

Anemia was produced in young rats using the Elvehjem-Kemmerer technique,[3] modified only by the feeding of copper sulfate supplements, as is customary in this laboratory.[4] When the rats were eight weeks old, the hemoglobin level was constant or falling, and ranged from 2.9 to 5.4 gms Hb/100 ml. of blood for the six litters used in this work. At this time representative rats from each litter were killed, while the rest of the rats were placed on $FeCl_3$ supplements varying from 0.05 mg Fe to 0.3 mg Fe daily. At the end of the six weeks' test period and twenty-four hours after the feeding of the last iron supplement, the hemoglobin concentration was determined by the acid-hematin method and the rats killed. After the removal of the digestive tract, the rats were ashed and the hydrochloric acid solution of the ash analyzed for iron, using the method of Farrar.[5]

Results of the iron analyses of the rats given comparable treatment showed remarkable agreement. Six male and seven female rats were killed at the end of the five weeks' iron depletion period on the basal milk diet. Although the hemoglobin concentration ranged from 2.9 to 5.4 gms of Hb/100 ml. blood, the iron content varied only from 0.013 to 0.017 mg Fe/gm of rat with an average of 0.015 mg Fe/gm for both male and female rats. However, as the females weighed a little more than the males, the total iron content of the females (0.8073 mg Fe) was slightly higher than that of the males (0.7065 mg Fe). In another series of experiments the iron depletion period was continued for eleven weeks and although there was a definite decrease in both the hemoglobin concentration and the iron content, again no sex difference in the iron content of rats which had their reserves depleted on a milk diet régime could be detected.

However, Table I shows the results obtained when litter mate rats rendered anemic by the usual procedure were fed varying amounts of iron as $FeCl_3$ solution. It can be seen that the mg Fe/gm of rat was always higher for the females than for the males fed

[1] Jukes and Branch, SCIENCE, 80: 228, 1934.

[1] M. C. Smith and L. Otis, SCIENCE, 85: 125–126, 1937.

[2] H. H. Mitchell and T. S. Hamilton, SCIENCE, 85: 364–366, 1937.

[3] C. A. Elvehjem and A. R. Kemmerer, *Jour. Biol. Chem.*, 93: 189–195, 1931.

[4] M. C. Smith and L. Otis, *Jour. Nutr.*, 14: 365–369, 1937.

[5] G. E. Farrar, Jr., *Jour. Biol. Chem.*, 110: 685–694, 1935.

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.

LOUISE OTIS
MARGARET CAMMACK SMITH
UNIVERSITY OF ARIZONA

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.

[2] C. U. Culmer, A. J. Atkinson and A. C. Ivy, *Endocrinology*, 24: 631, 1939.

[3] J. S. Gray, E. Wieczorowski and A. C. Ivy, SCIENCE, 89: 489, 1939.

[4] J. S. Gray, E. Wieczorowski and A. C. Ivy, *Am. Jour. Physiol.*, 126: 507, 1939.

[5] J. S. Gray, C. U. Culmer, E. Wieczorowski and J. L. Adkison, *Proc. Soc. Exper. Biol. and Med.* In press.

[6] A. C. Ivy and J. S. Gray, *Cold Spring Harbor Symposia on Quant. Biol.*, 5: 405, 1937.

[7] P. A. Katzman and E. A. Doisy, *Jour. Biol. Chem.*, 98: 739, 1932.