SEX VARIATIONS IN THE UTILIZATION OF IRON BY ANEMIC RATS

THE finding that all compounds of iron are not equally effective in the remission or prevention of nutritional anemia even in the presence of ample copper has led to an increased interest in the determination of iron availability.[1,2] The accepted method for measuring the amount of iron in a foodstuff which is utilizable for hemoglobin formation consists, briefly, of feeding the food of analyzed iron content, with copper, to anemic rats upon a whole milk diet and comparing the hemoglobin response with that obtained from anemic rats given the same amount of iron as ferric chloride.

In the course of investigation in this laboratory of the hematopoietic value of foodstuffs by this method, a marked difference in response between male and female rats has been consistently noted. As it has been customary to use males and females interchangeably in other laboratories, the effect of a sex difference upon the accuracy of the results obtained seemed worthy of investigation.

EXPERIMENTAL PROCEDURE

The technique used for the preparation of the anemic rats used in the test was essentially that of Elvehjem and Kemmerer.[3] When young rats were two weeks of age the stock colony ration provided for the mothers was replaced by whole-milk powder (Klim). During the third week of age, therefore, the young had access to milk only. The mothers were separated from their young for several hours each morning and allowed to eat as much of the stock colony whole wheat and milk ration as they desired. They were then carefully brushed and returned to their young. When three weeks of age the young were weaned and continued on whole-milk powder as sole food.

Blood samples were taken from the tail at weekly intervals, and the hemoglobin content was determined by comparison with a standard Newcomber plate in a Dubosecq colorimeter. By the fourth or fifth week the hemoglobin level of rats in this laboratory prepared in the fashion described above fell to an average of 3.9 (range 2.9 to 4.5) grams per 100 cc of blood. At this time the animals were placed in individual galvanized iron cages with raised screen bottoms. The whole-milk powder which served as the basal diet and distilled water were provided *ad libitum* in glass containers.

The iron supplements or foods under test were fed separately for a subsequent test period of six weeks duration. Throughout the test period, .05 mg Cu as $CuSO_4$ and .04 mg Mn as $MnCl_2$ were given daily. The course of hemoglobin regeneration was followed by hemoglobin measurements made at two-week intervals. The comparative hemoglobin responses of males and females to iron supplements of the same magnitude, given as $FeCl_3$ or a food, appear in Table I,

COMPARATIVE HEMOGLOBIN REGENERATION IN MALE AND FEMALE RATS

Supplement fed daily	6 weeks gain in Hb (gms per 100 cc)	
	Males	Females
.014 mg Fe as $FeCl_3$	1.0	2.3
.050 " " " "	2.9	4.2
.1 " " " "	5.5	6.5
.2 " " " "	9.1	10.9
.25 " " " "	10.4	10.7
.3 " " " "	10.1	10.2
3 gms whole wheat	7.7	9.1
3 " rolled oats	5.7	7.0
1.5 gms dried lima beans	4.8	6.1

which records the gains in hemoglobin concentration during the six weeks test period. At least 10 males and 10 females were used on each level of iron.

It may be seen that hemoglobin regeneration was greater in the females than in the males fed the same amount of iron or given the same amount of an iron-bearing food. This difference between the sexes was consistently noted among litter mate rats except at levels of iron feeding greater than .2 mg daily. At daily iron levels of .25 mg and above, the iron intake was sufficiently high to promote maximum hemoglobin development in all the rats of this age so that a difference between the sexes in the rate of gain of hemoglobin could not be expected. That the observed differences are significant is indicated by the fact that the experimentally obtained differences are from three to six times greater than the probable errors of these differences.

The explanation of this difference in response may lie in the greater store of iron in the female[4] which becomes available for hemoglobin formation upon the giving of copper. This view is substantiated by the finding that the difference between male and female response was not observed after two weeks of the supplemental feeding and also by the fact that the difference is approximately the same at all levels of iron supplementation. The original hemoglobin level of all the males discussed in this paper was 3.8 gms per 100 cc of blood as compared with a level of 3.9 gms in the females at the beginning of the test period. Thus a difference in reserve supply of iron in the males and

[1] C. E. Elvehjem, E. B. Hart and W. C. Sherman, *Jour. Biol. Chem.*, 103: 61–70, 1933.

[2] W. C. Sherman, C. A. Elvehjem and E. B. Hart, *Jour. Biol. Chem.*, 107: 383–394, 1934.

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

[4] Mary Swartz Rose and Lan-Chen Kung, *Jour. Biol. Chem.*, 98: 417–437, 1932.

females was not indicated by a significant difference in hemoglobin concentration of the blood at the beginning of the test period. Elvehjem[3] has stressed the necessity of exhaustion of body iron stores in preparation of test animals, and the question arises as to what evidence of exhaustion can be accepted. Allowing the hemoglobin level to fall too low results in animals which are sickly and not capable of a normal response to the iron supplement given subsequently.

Whatever is the explanation of this greater hemoglobin regeneration in anemic female rats as compared with males, it is the authors' belief that ignorance of this fact may explain some of the discrepancies of the same magnitude in the findings in various laboratories relative to the availability of iron in foodstuffs.

MARGARET CAMMACK SMITH
LOUISE OTIS
UNIVERSITY OF ARIZONA

A CATALYTIC METHOD FOR THE PREPARATION OF α-PYROABIETIC ACID

THE preparation of the so-called pyroabietic acids by the usual prolonged high temperature treatment of rosin[1, 2] entails very considerable pyrolytic decomposition, with consequent contamination of the resulting product. In connection with recent experiments on dehydrogenation of rosin products (rich in pimaric acids), by way of palladium charcoal,[3] it was noted that an appreciable proportion of a positive rotating acid survived the high temperature (300–325° C.), a region normally well above that at which decarboxylation of rosin acids takes place. The isolated acid did not give a crystalline sodium salt characteristic of α-pimaric acid, nor did it have its optical properties.[4] Its melting point (171–172° C.), rotation ($[\alpha]_d^{20} + 54°$; $[\alpha]_j^{20} + 58°$) and other properties agree well with those of α-pyroabietic acid described by Dupont-Dubourg, Fanica and others. Subsequent experiments with the palladium charcoal catalyst showed that the isomerization can be carried out at much lower temperatures (250° C.) and completed in about two or three hours. The yield at the lower temperature is excellent, the product quite uniform and apparently unaccompanied by the usual intermediate isomers. Acids with the same properties were obtained with this catalytic procedure from α-pimaric acid, l-abietic acid (Schulz), mixed rosin acids and rosins from longleaf and slash pines (*Pinus palustris* and *Pinus caribaea*) and French gum (*Pinus pinaster*). This finding, which would indicate highly selective isomerizing action for the catalyst, is in marked contrast with results obtained by the usual 100-hour heating without a catalyst when applied to rosin acids and rosins from different sources.[2]

Preliminary experiments showed that palladium charcoal catalyzes the isomerization even at 200° C., but not as effectively as at higher temperatures. Platinum charcoal, nickel charcoal and, to a lesser extent, activated charcoal itself also catalyzed the formation of pyroabietic acid.

This laboratory is at present engaged in a systematic study of the application of various catalysts and different types of carriers to the primary rosin acids, as well as the rosin acids or partially isomerized acids. Publication of more comprehensive data is contemplated in the near future.

ELMER E. FLECK
S. PALKIN
BUREAU OF CHEMISTRY AND SOILS
U. S. DEPARTMENT OF AGRICULTURE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

PRESERVING THE NATURAL COLOR OF GREEN PLANTS[1]

IMPROVED teaching methods of botanical subjects demand better demonstration materials. Living specimens should be preferred to non-living ones. However, increased difficulties in obtaining living specimens forces the use of more preserved forms. Better methods of preservation are needed to increase attractiveness of dead specimens. Several methods have been published which are more or less useful. Keefe's[2] method is outstanding among these. The writer has experimented with older formulae as well as new combinations for a period of about three years. Out of this work success with one new, general method seems to justify publication.

Formalin-acetic acid-alcohol solutions (5 cc of commercial formalin, 5 cc of glacial acetic acid and 90 cc of 50 per cent. ethyl alcohol; or 10 cc of commercial formalin, 5 cc of glacial acetic acid and 85 cc of 70 per cent. ethyl alcohol) are in general excellent preservatives. They are being used extensively for museum and histological materials. By adding 0.2 gram of copper sulfate to 100 cc of either of these F.A.A. formulae, a preservative results which will bring about an almost normal green color in nearly all

[1] Dupont and Dubourg, *Bull. Inst. Pin.*, 51: 181, 1928.
[2] Fanica, *Bull. Inst. Pin.*, 44: 155, 1933.
[3] Method of Ruzicka and Waldman, *Helv. Chim. Act.*, 16: 842, 1933.
[4] S. Palkin and T. H. Harris, *Jour. Am. Chem. Soc.*, 55: 3683, 1933.

[1] Papers from the Department of Botany, the Ohio State University, No. 383.

[2] Keefe, Anselm Maynard. SCIENCE, 64: 331–332, 1926.