TABLE I.
Effect of Administration of Epinephrine and Refrigeration on Adrenal Ascorbic Acid Content.

	ascorbic acid, % of control value	
Age, days	Epinephrine	Refrigeration
2	93	
4	98	95
6	91	101
7		105
8	69	
10	66	
11		105
14		107
16		83
25		81
Adult	62	

TABLE II.
Effect of 2.5 Mg ACTH on Adrenal Ascorbic Acid Content.

Age, days	ascorbic acid, % of control value
4	66
6	64

the animals had reached 8 days of age. (Table I). Exposure of animals to refrigeration (5°C) for 2½ hours similarly did not result in a decline in the ascorbic content until the 16th day of life. With this stress the fall in ascorbic acid was 19% or approximately one-half of that with epinephrine (Table II).

Since very young rats do not respond to stress, it was necessary to further localize the point of insensitivity, *e.g.*, the pituitary or adrenal. Therefore, rats of 4 and 6 days of age were injected intraperitoneally with 2.5 mg† ACTH and killed 80-120 minutes later. Ascorbic acid analysis of the adrenals revealed a 34-36% decline in the vitamin C content under these conditions (Table II).

It would appear from these results that the fall in adrenal ascorbic acid in the newborn rat does not occur as a result of exposure to stress at it does in the adult animal. Since the adrenals are capable of responding to the administration of ACTH at this age, it is probable that the adenohypophysis is the organ which is not capable of responding to stress by secreting ACTH until the animal has reached a certain state of maturation.

Summary. The administration of epinephrine to the infant rat does not result in a fall in adrenal ascorbic acid until the animal reaches the eighth day of life. Under the conditions of the experiment, adrenal ascorbic acid does not decline as a result of refrigeration until the 16th day of life. The administration of ACTH, on the other hand, causes adrenal ascorbic acid depletion on the 4th and 6th day of life.

† Armour ACTH, kindly supplied by Dr. John R. Mote, Medical Director of Armour Laboratories.

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Labeling of Rat Hemoglobin with Radioactive Iron.* (17526)

KARLMAN WASSERMAN AND JOHN K. HAMPTON, JR. (Introduced by H. S. Mayerson)
From the Department of Physiology, Tulane University, School of Medicine.

In the studies of the biological aspects of iron metabolism, radioactive iron serves as a suitable means of following, *in vivo*, transformations of forms of iron. Especially valuable is its use in tagging hemoglobin molecules so that, under certain conditions, hemoglobin iron, hemoglobin molecules or red cells may be identified as they undergo certain chemical or physical changes. The current methods(1-7) used for incorporating

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radioactive iron as a heme constituent in hemoglobin often present major difficulties. Most iron compounds have a high toxicity and low stability which limits their use. The low specific activity and soft beta radiation of the available radioactive iron make it necessary to obtain a high percentage of tagging for most studies. Finally, since synthesis can be accomplished only in donor animals, preliminary production of anemic animals with drastically reduced iron stores is a time consuming and, at times, unpredictable procedure involving many serial bleedings and careful diet considerations.

In the course of iron studies in our laboratory, we have developed a procedure which we feel will provide an easier and, perhaps, a more satisfactory tagging of hemoglobin with radioactive iron than can be achieved with the methods which have been used. The iron used contained a mixture of Fe-55 and Fe-59 prepared by pile bombardment of enriched Fe-58. Concentrated hydrochloric acid, in amount approximately twice the molarity required for quantitative reaction with the iron sample was diluted with 3 parts of distilled water and poured over the powdered iron in a large flask. The reaction was allowed to continue, with heat supplied as the reaction slowed, until it was complete as indicated by a clear green solution without significant insoluble residue. This solution of ferrous chloride in excess hydrochloric acid was filtered, a sample analyzed by the Wong thiocyanate method(8) for iron and the remainder stored in a glass stoppered stock bottle. Working solutions were prepared by withdrawing from the stock the amount of iron desired and placing it in an appropriate volumetric flask. The HCl and water were removed by evacuation and heat. The green ferrous chloride powder was dissolved in 0.1 N lactic acid and the flask filled to the mark with this acid. The concentrations of ferrous chloride and lactic acid were considered in terms of volume which would be desirable for injection, the amount and concentration the animal could tolerate and the relative molarities of the iron and lactate which would provide a pH and reducing environment for the iron suitable for prolonged storage at room temperature. These conditions were met by preparing a 400 mg % Fe solution of ferrous chloride in 0.1 N lactic acid. The solution was sterilized by Seitz filtration and stored in a sterile rubber-capped bottle at room temperature. Such working solutions show no signs of deterioration after more than two months of storage.

Adult male and female white rats were used. Their weights ranged between 225 and 330 g. By cutting the tails and suspending them in graduated centrifuge tubes containing warm sodium citrate, the animals were bled about 1.5% of their body weight at 2-3 day intervals for 11 bleedings. Their hematocrit values dropped to an average of about 25%. At this point, iron injections were begun and during the course of the injections four more bleedings were performed. Also the usual Rockland Rat diet was supplemented by milk at this point. Response by weight gain, increased appetite and general vigor was apparent within one week. At no time were they maintained on a diet considered low in iron.

The working lactic acid-iron solution was injected into rats intraperitoneally in doses of 0.25 cc per 100 g of body weight, which provided 1 mg of iron per 100 g of body weight. These injections were given daily for the first half and every other day for the remainder of the series. A total of 14 injections were given, of which the third and tenth injections were at one-half dose. The total amount of iron thus administered was 13 mg/100 g of body weight. This is notably in excess of the animal's iron requirement.

The low toxicity of the injections is evident in animals in good health. Of 12 rats, 4 deaths occurred after iron injections were begun. One of the deaths occurred as a result of the 12th bleeding. The other 3 followed the 2nd, 7th and 9th injection of lactic acid-iron solution. Although peritonitis could not be excluded because of the irritation inflammation produced by the acid, death followed not

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TABLE I.
Percentage Tag of Hemoglobin with Radioactive Iron. The stock radioactive iron injected gave 3,300 counts/min./mg of iron. The values represent no duplicates since the blood was collected by exsanguination and pooled for each lot.

Sample	No. animals	Days since last inj.	Counts/min./mg Fe	% tag
4	3	—2	957	29.0
5	2	1	850	25.8
6	2	3	978	29.7
7	1	19	988	29.8

earlier than 12-24 hours after the injection.

Radioactivity determinations were made by counting desiccated protein-free filtrates and chemical iron was determined on such filtrates by the method of Wong.(8) Counts were sufficiently high under the geometric and sensitivity conditions employed to allow accurate comparison of samples.

In Table I are recorded the percentages of tagging achieved by our method. To be noted is the reasonable consistency of the values, each of which were obtained from a separate group of animals. Once bleedings were stopped, then no appreciable increase in tagging seemed evident over a 3-week period. After iron injections were begun, but before bleedings were stopped, a definite increase in tagging was apparent at weekly intervals.

Although we have demonstrated that a 25-30% tag may be accomplished in 48 days, in rats, we feel that the institution of milk supplement to the diet earlier in the experiment would have eliminated some of the emaciation. Furthermore, the distress observed in the animals with the first few injections might have been eliminated. We also feel that satisfactory tagging could be accomplished with smaller doses, thus minimizing adhesions and iron staining of the viscera which was noted at autopsy. It is believed that if a greater percentage of tagging was desired it could be accomplished by continuing the serial bleeding and iron injections for a longer period of time.

Conclusions and summary. The tagging of hemoglobin in the red cells of rats by the method described is efficient. The iron compound, ferrous chloride, is of low toxicity, the lactic acid in which it was dissolved provides a satisfactory stabilizing environment and the solution is well tolerated when injected. The data show a consistency in the percentage of tagging as evidence of the reproducibility of the experiment. In addition to the ease with which the materials are prepared, the short duration of the experiment is a desirable feature.

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Effects of Hemorrhagic Shock upon the Electroencephalogram. (17527)

H. A. DAVIS, W. R. GRANT, W. P. MCNEILL, R. F. WILKINSON AND C. MARSH
From the Departments of Surgery and Nervous Diseases, and Graduate School of Medicine, College of Medical Evangelists, Los Angeles, Calif.

The central nervous system is extremely sensitive to reduction of its oxygen supply, particularly the small pyramidal cells of the cerebral cortex.(1) In view of the general acceptance of this fact, it is surprising that studies have not been made of the effects of anemic anoxia produced by hemorrhage upon the electrical activity of the cerebral cortex as determined by the electroencephalograph.

Materials and methods. Rabbits weighing 5 to 11 lb were used in these experiments. General anesthesia was not used. Curare (In-

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