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-

1. Gomez, L., and Pike, F. H., *J. Exp. Med.*, 1909, v11, 257.

tocostrin) was employed to abolish skeletal muscular movements which affect the electroencephalogram. Curare was used in a dose of 0.7 unit per lb. of body weight. The 4 electrodes of the electroencephalograph were placed in similar positions on the heads of the animals throughout these studies. The right femoral artery was exposed under local anesthesia (5 cc of 1% novocaine solution), cannulated and attached to a blood pressure recording manometer. Shock was produced by graded hemorrhage. The blood which was withdrawn was prevented from clotting by the addition of heparin solution. The heparinized blood was returned to the animals in many of the experiments.

Results. In control runs before curare was given, the electroencephalograms revealed a basic frequency of 6 to 7 cycles per second with an amplitude averaging about 75 microvolts. Numerous variations in the amplitude and frequency of the waves occurred through the record. There were short flurries of low voltage and of 20 to 30 cycle activity, and occasional sharp waves and spikes of 100 microvolts were observed. Following injection of curare, the rapid activity disappeared leaving a fairly symmetrical rolling type of wave, varying in frequency between 4 to 7 cycles per second and having an average amplitude of 50 microvolts.

Moderate to deep shock produced by bleeding resulted in slowing of the wave frequency to an average of 2 to 4 cycles per second. At the same time the amplitude of the waves increased to 150 to 200 microvolts and in some animals reached 400 microvolts. This was the basic pattern. There were, however, many irregular waves, sharp waves, rolling waves and occasional dicrotic or notched waves. During recovery from shock the slow waves with high amplitude were replaced intermittently by rapid waves (10 cycles) of lower amplitude (100 microvolts).

If shock were progressive and transfusions were not given, the slow rolling waves persisted but their amplitude decreased. The voltage diminished and finally the wave pattern disappeared corresponding with the death of the animal. In those animals which survived without transfusion (reversible shock) the basic pattern of slow rolling waves of increased amplitude was maintained until the conclusion of the experiment. In shock reversible by blood transfusion the wave pattern returned (sometimes within 10 minutes) to the type present before bleeding. The blood pressure returned to normal before the electroencephalogram. In shock irreversible by blood transfusion, the waves decreased in amplitude and frequency despite the administration of blood. It was noted in these experiments that the response of the electroencephalogram was a better prognostic criterion than was the response of the blood pressure. A persistent decrease in the frequency and amplitude of the waves was an ominous sign even though the blood pressure rose following blood transfusion. The longer the duration of shock, the more difficult was it to restore the electroencephalogram to normal with blood transfusions.

Discussion. The mechanism of production of the electroencephalographic changes in hemorrhagic shock may be briefly considered. Slow waves of increased amplitude have been observed in hypotension due to carotid sinus syncope,(2) insulin shock,(3) in anoxemia,(4) and after ligation of the carotid arteries for an intracranial saccular aneurysm.(5) The effect of low blood pressure due to stimulation of the vagus in depressing cortical electrical activity has been observed to be almost identical with that due to anoxemia.(6) It is suggested, therefore, that the electroencephalographic changes in hemorrhagic shock are due, in part at least, to cerebral anoxia.

Summary and conclusions. Hemorrhagic shock in rabbits produces definite changes in the electroencephalogram. These are characterized basically by the appearance of slow

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5. Yeager, C. L., and Walsh, M. N., *J.A.M.A.*, 1940, v114, 1625.

6. Bailey, P., and Bremer, F., *J. Neurophysiol.*, 1938, v1, 403.

waves of increased amplitude. In shock irreversible to transfusion there occurs a progressive decrease in cortical electrical activity which is shown on the electroencephalogram as a continued reduction in the frequency and voltage (amplitude) of the wave pattern. The effects on the electroencephalogram of blood transfusion in reversible and irreversible hemorrhagic shock are discussed. It is tentatively suggested that the electroencephalographic response to blood transfusions in shock may be used as a means of differentiating the reversible and irreversible forms.

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Vitamin B_{12b}: Some Properties and its Therapeutic Use.* (17528)

H. LICHTMAN, J. WATSON, V. GINSBERG, J. V. PIERCE, E. L. R. STOKSTAD, AND T. H. JUKES

From the Department of Medicine, Kings County Hospital, Brooklyn, N. Y., and Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

The preparation of two red fractions from liver extract,(1) both clinically active in pernicious anemia, was reported by Smith who suggested that one fraction might arise from the other by proteolysis. In later publications(2,3) it was reported that paper chromatography enabled 2 red factors to be recognized in liver extract. Both of these were active for *L. lactis* Dorner. In our investigations it was found that concentrated liver extract yielded a pink fraction by chromatography with silicic acid. Upon purification, this fraction yielded a red pigment with an absorption spectrum differing from that of vitamin B_{12}. An apparently similar fraction was obtained from cultures of *Streptomyces aureo-*

FIG. 1.
Crystals of vitamin B_{12b} ×425.

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