whatever axon growth did occur was almost completely obscured by other cells.

In the rat, neither biotin deficiency[7] nor administration of amounts of the vitamin far in excess of normal intake[8] has been found to affect the rate of regeneration of crushed sciatic nerves significantly. These findings in the living animal in conjunction with those reported here in tissue culture do not support the contention that biotin is a stimulant of axon growth.

Summary. In a total of 236 tissue cultures, no evidence was found for a growth stimulating action of biotin on embryonic chick axons, neuroblasts, spindle cells, or macrophages. In view of the close resemblance between axon regeneration *in vivo* and *in vitro*,[9] these results discourage the view that biotin might be clinically useful as a stimulant of nerve regeneration, at least during the crucial outgrowth phase.

[7] Fischer, E., *Fed. Proc. Am. Soc. Exp. Biol.*, 1943, **2**, 13.

[8] Lazere, B., Thomson, J. D., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 81.

[9] Weiss, P., *Growth*, 1941, **5**, 163.

14362

Depression of Experimental Polycythemia by Stomach U.S.P.; Presence of Choline in Stomach U.S.P.*

JOHN EMERSON DAVIS

From the Department of Physiology and Pharmacology, University of Arkansas School of Medicine, Little Rock.

It was reported that the daily administration of 5 g of Stomach U.S.P. (Ventriculin†) to polycythemic animals had no effect on the red blood cell count, although the feeding of raw liver produced a reduction of erythrocytes.[1] Subsequently, we showed[2] that choline chloride depressed experimental polycythemia in the same manner as raw liver, and later[3] the probable mechanism of action was reported.

The present communication reports the results of the administration of a higher (10 g) daily dose of Ventriculin to polycythemic dogs, and the proof that Ventriculin contains choline which is probably the substance responsible for the depression of polycythemia.

Procedure. Two normal and 2 splenectomized dogs were maintained on a constant adequate diet. Polycythemia was produced in 2 of the animals by the daily oral administration of cobalt chloride according to a method described previously,[4] and the other 2 dogs were made polycythemic by the daily injection of posterior pituitary solution.[5] After the development of the polycythemias, each dog was fed 10 g of Ventriculin daily in addition to the erythropoietic-stimulating substances.

To determine the possible existence of choline in Ventriculin, a chemical analysis was made by the method of Johnson, Irvine, and Walton.[6] In addition, a pharmacological test was made by acetylating a deproteinized aqueous extract of Ventriculin according to the method of Mentzer *et al.*[7] and determining its effect on the blood pressure of a dog.

Results. Fig. 1 shows the effect of the daily oral administration of 10 g of Ventriculin upon the red blood cell counts of 4 polycythemic dogs. It will be noted that this

* Research paper No. 539, journal series, University of Arkansas.

† Supplied by Parke, Davis & Co., Detroit, Mich.

[1] Davis, J. E., *Am. J. Physiol.*, 1938, **122**, 397.

[2] Davis, J. E., *Am. J. Physiol.*, 1939, **127**, 322.

[3] Davis, J. E., *J. Pharm. and Exp. Therap.*, 1940, **70**, 408.

[4] Davis, J. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 96.

[5] Davis, J. E., *Am. J. Physiol.*, 1942, **137**, 699.

[6] Johnson, C. G., Irvine, J. L., and Walton, C., *J. Biol. Chem.*, 1939, **131**, 425.

[7] Mentzer, C., Corteggiani, E., and Carayon-Gentil, A., *Bull. soc. chim. biol.*, 1939, **21**, 503.

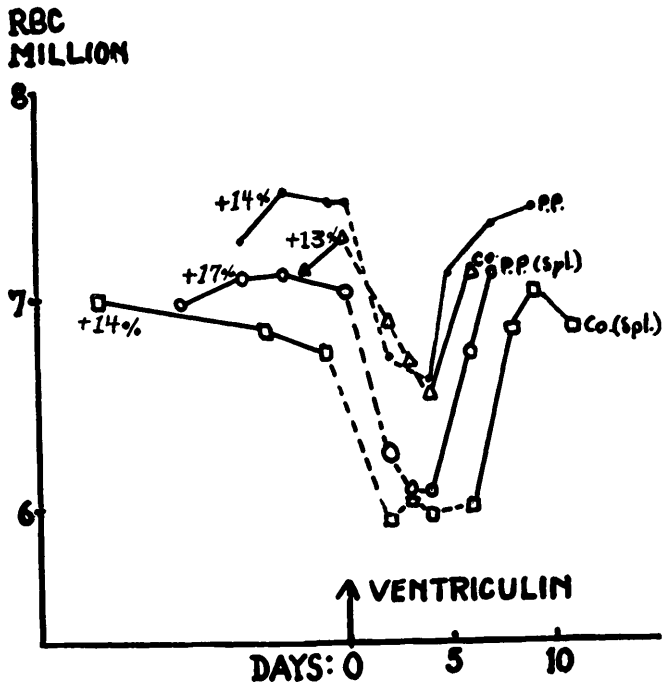

FIG. 1.
The Effect of the Daily Oral Administration of 10 Grams of Stomach, U.S.P., to 4 Polycythemic Dogs.
Figures at left of each line indicate degree of polycythemia in terms of percentage increase of red cell count above normal. Dashed lines show time during which Ventriculin was fed. P.P. = dog receiving posterior pituitary injections; C = cobalt-fed dog; Spl. = splenectomized animal.

procedure caused marked reductions in the erythrocyte numbers of all of the animals. Hemoglobin percentages (Hellige) were diminished proportionately, but leukocyte counts showed no uniform change and remained fairly constant. Upon cessation of stomach administration, the erythrocyte numbers and hemoglobin percentages returned to their polycythemic values.

The intravenous injection of acetylated extracts of ventriculin into anesthetized dogs caused precipitate decreases in blood pressure, amounting to more than 90 mm Hg. Lowering of the blood pressure did not occur when the acetylated extract was given after atropinization.

Discussion. The depression of polycythemia by stomach U.S.P. in these experiments appears to be similar to that produced by liver, choline,[2,3] and other vasodilator drugs.[8] Jacobs[9] has shown that a certain liver extract contains at least 1% of choline.

We believe that choline is the active constituent in liver that is responsible for the depression of erythropoiesis in polycythemia.[2] The possibility that choline is the common denominator in liver and stomach, as far as polycythemia depression is concerned, motivated us to search for choline in the antianemic stomach preparation. Our chemical extraction and determination yielded a choline reineckate product giving a value of about 1% choline in Ventriculin. Although this percentage of choline accounts very well for the effectiveness of Ventriculin in polycythemia, we doubt the purity of the reineckate compound obtained in the analysis. However, the vasodepressor action of acetylated extracts of Ventriculin, and its inhibition by atropine, would seem to prove conclusively that choline

[8] Davis, J. E., *J. Pharm. and Exp. Therap.*, 1941, **73**, 162.

[9] Jacobs, H. R., *J. Lab. and Clin. Med.*, 1938, **24**, 128.

is present in Ventriculin.

Conclusions. The daily oral administration of 10 g of Stomach U. S. P. to 4 polycythemic dogs caused depressions of their red blood cell counts. This preparation contains choline which is probably the constituent responsible for the depression of experimental polycythemia in these experiments.

The author wishes to thank Mr. Ralph Houghton for technical assistance rendered in the course of this work.

14363

Effect of Ephedrine Sulfate on the Red Blood Cell Count of Humans.*

A. M. HARRIS AND J. E. DAVIS.

From the Department of Physiology and Pharmacology, University of Arkansas School of Medicine, Little Rock.

In 1941, the production of experimental polycythemia in dogs, rabbits, and man by the daily administration of ephedrine sulfate was reported by one of the authors.[1] Although the data on animals was adequate, it was possible to report data on only one human subject who received a daily dose of 50 mg of ephedrine sulfate. Erythropoiesis was apparently stimulated by the drug in this single individual, but the desire to have more data on more human subjects led us to make the investigation herein reported.

Procedure. Seven normal men who were leading a physically active life were used as subjects in this investigation. Red cell counts, leukocyte counts, and hemoglobin percentages (Sahli) were determined at frequent intervals before and during administration of the drug. After the normal blood cell values were obtained, each subject was given 50 mg of ephedrine sulfate daily by mouth. Fingertip blood samples were taken with the subject at *rest* and *before* the administration of the daily dose of the drug. Blood cell counts were made by an impartial technician who was unaware of the experimental procedures.

Results. Fig. 1 shows the effects of ephedrine sulfate administration for 3 to 4 weeks upon the red blood cell counts of all 7 subjects. It will be noted that the drug caused a rise of about one-half million (on the average) in the erythrocyte numbers. Hemoglobin percentages increased correspondingly, but leukocyte counts showed no uniform or constant change (not shown). In the subjects who could be observed for longer than 4 weeks, the erythrocyte counts fell back toward normal in spite of continued ephedrine administration.

Discussion. Although only one of the human subjects in these experiments showed a rise of 0.8 million (Fig. 1) which was comparable to the erythrocyte elevation from ephedrine in the subject reported previously[1] by Davis, it appears that ephedrine, in the daily dose of 50 mg produces a mild polycythemia. This polycythemia seems to be only temporary, a fact which we may perhaps attribute to the phenomenon of tachyphylaxie. It is also possible that these subjects, because of their physically active mode of life, showed weaker cardiovascular responses to ephedrine in the 50 mg dose. The mechanism of the ephedrine induced increase of erythropoiesis was postulated[1] as depending upon vasoconstriction and a curtailed blood and oxygen supply to bone barrow.

Conclusions. The daily oral administration of 50 mg of ephedrine sulfate to 7 normal men caused a mild but appreciable polycythemia within 3 weeks. The elevation of the red cell count appeared to be only temporary, since in some subjects it disappeared at some time after the fourth week of drug administration.

* Research paper No. 540, journal series, University of Arkansas.

[1] Davis, J. E., *Am. J. Physiol.*, 1941, **134**, 219.