

16597

Failure of Splenectomized Rabbits to Respond to Liver Extract and to Pteroylglutamic Acid.*

EDGAR JONES, J. L. WYATT, AND E. W. EWERS.

From the Department of Medicine, Vanderbilt University School of Medicine.

Jacobson and Williams^{1,2} reported that splenectomized rabbits responded to the administration of several materials with an increase in reticulocytes. Liver extracts effective in the treatment of pernicious anemia, synthetic xanthopterin, leucopterin and the pigment derived from fused uric acid caused reticulocytosis in their animals. Clinically inactive liver extracts as well as a number of unrelated materials failed to evoke a response. Substances inducing reticulocyte increases were effective when given either intramuscularly, intravenously or orally. Based on a large number of control counts it was concluded that any reticulocyte value exceeding 3.5% following administration of a trial material indicated hemopoietic activity of that material. It is to be noted that none of their animals was anemic at any time during the period of observation.

The nature of the materials found effective by these authors stimulated us to test in a similar fashion the newly synthesized pteroylglutamic acid (PGA)³ which had been found to be effective in the treatment of the macrocytic anemia of sprue⁴ and in pernicious anemia.⁵

Procedure. Nine adult white rabbits were used. The animals were fed Purina rabbit chow exclusively throughout the period of experimentation. Eight were males. The spleen was removed from 6 of these animals; the remaining 3 served as normal controls. Of the 6 splenectomized rabbits 4 were treated and 2 were followed as splenectomized controls. There was very little loss of blood from operation. However, to avoid reticulocyte increases owing to blood loss, 3 weeks were allowed to pass following operation before any trial material was given. Pre-operative and post-operative red blood cell counts and determinations of hemoglobin indicated no anemia by standards reported for rabbits.⁶

Rabbits No. 1 and 2 (splenectomized) were given 5 mg of PGA intramuscularly on 4 occasions over a period of 8 days. Rabbits No. 3 and 10 (splenectomized) were given 5 units of liver extract (Wilson) intramuscularly on 3 occasions over a period of 7 days. No reticulocyte increases having occurred by 10 days following the last injection of a series the animals were re-treated after a rest period of 10 days. Animals No. 1 and 2 were given 20 mg of PGA intramuscularly on 3 occasions within a 5 day period. Rabbits No. 3 and 10 were given 20 units of liver extract (Wilson) intramuscularly on 3 occasions over a period of 5 days. No reticulocyte responses having followed, liver extract and PGA were then given into the stomach by means of a tube. Animals No. 1 and 3 were given 50 units of liver extract (Wilson) by tube on 3 occasions over a period of 7 days. Animals Nos. 2 and 10 were given 10 mg of PGA by tube on 3 occasions over a 7 day period.

* This work was supported by a grant from The National Vitamin Foundation.

¹ Jacobson, W., and Williams, S. M., *J. Path. Bact.*, 1945, **57**, 101.

² *Ibid.*, 1945, **57**, 423.

³ Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., Subbarow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr., *Science*, 1945, **102**, 227.

⁴ Darby, W. J., and Jones, E., *Proc. Soc. Exp. Biol. and Med.*, 1945, **60**, 259.

⁵ Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D., *J. Lab. and Clin. Med.*, 1945, **30**, 1056.

⁶ Wintrobe, M. M., Shumacker, H. B., Jr., and Schmidt, W. J., *Am. J. Physiol.*, 1936, **114**, 502.

With few exceptions daily reticulocyte counts were made on the 4 splenectomized animals throughout the entire periods following the first treatment. These periods covered approximately 7 weeks. Splenectomized and normal controls were followed with practically daily reticulocyte counts over a period of 3 weeks from the beginning of the experiment. The wet technic for counting reticulocytes was employed using brilliant cresyl blue dye. One thousand cells were counted on each determination. The same 3 observers made all counts. The liver extract used was known to be potent in the treatment of pernicious anemia.

Results. No significant increase in reticulocytes was noted in any of the animals which has been splenectomized and adequately treated with either liver extract or PGA. The normal controls showed reticulocyte ranges from .6 to 5.3%; the splenectomized controls showed ranges of 1.3 to 4.8%; the animals treated showed ranges of .7 to 5.2%, the values bearing no relation to material administered. Chart 1 shows the reticulocyte range of a normal control animal. Chart 2 shows the range of a splenectomized control. No charts are shown for rabbits treated since the reticulocyte ranges of these animals differed in no respects from those shown. The reticulocyte ranges given were experienced

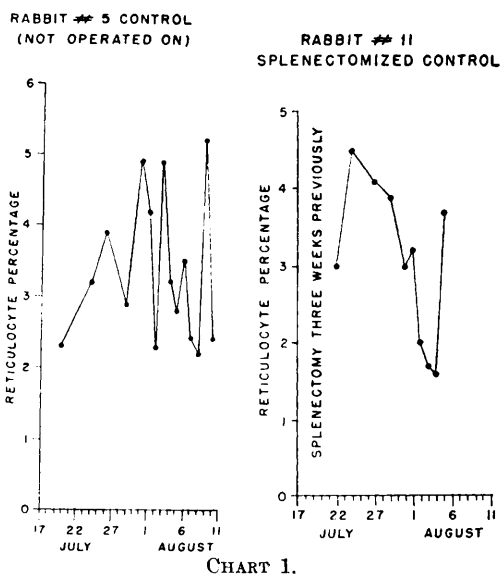


CHART 1.

by practically all of the animals used, regardless of group. No trend in any direction was noted in the case of a single animal.

Conclusions. Splenectomized white rabbits showed no significant change in percentage of reticulocytes following oral and parenteral administration of either pteroylglutamic acid or of liver extract effective in pernicious anemia. Greater variations were found in daily reticulocyte counts, both in controls and in splenectomized animals, than were reported by Jacobson and Williams.

16598

Observations on Isosensitization to the M Agglutinin in Man.

ALEXANDER S. WIENER, EVE B. GORDON, AND C. A. MAZZARINO.

From the Serological Laboratory, Office of the Chief Medical Examiner, New York City.

It is generally considered that the agglutinogens M and N can be disregarded when selecting donors for blood transfusion therapy. While this is true for patients requiring only a single transfusion, or multiple transfusions within a short space of time, in cases where several transfusions spaced over a long period of time (months or years) are required, one must take into account the possibility of isosensitization to the M agglutinin as well

as the Rh factor.

Only a relatively small number of cases have been reported of type N individuals with natural anti-M agglutinins in their sera.^{1,2} In addition, a few cases have been encoun-

¹ Wiener, A. S., *Blood Groups and Transfusion*, 3rd ed., p. 219, C. C. Thomas, Springfield, Ill., 1945.

² Unger, L. J., Wiener, A. S., and Sonn, E. B., *Am. J. Clin. Path.*, 1946, **16**, 45.