Combinations of ascorbic and nicotinic acid are often, but not always, additive. The combined effect is rarely as high as that of peptone. It is of interest to note that in the presence of ascorbic plus nicotinic acid, adenine and cysteine increase the rate while they show little or no effect when alone. This seems to be a simple case of limiting factors.

The increased fermentation by injured cells makes it seem probable that ascorbic acid is a regular constituent of all cells, but is not needed by normal cells because they can supply their own demand. The present results do not indicate whether the injury is due to increased permeability of the membranes, to autolysis of important constituents, or to other causes. After injury, fermentation depends, at least partly, upon the ascorbic acid diffusing into the cells from the surrounding medium. Peptone and nicotinic acid do not show this increased effect with injured cells.

Summary. The formation of lactic acid by washed cells of *Streptococcus lactis* is increased by the presence of ascorbic acid or nicotinic acid. The two compounds act differently. Ascorbic acid stimulates mostly (perhaps exclusively) injured or exhausted cells while nicotinic acid affects normal and injured cells equally. The combined action of the two acids is usually, but not always additive, and approximates the effect of peptone. When both nicotinic and ascorbic acid are present, other compounds like cysteine or adenine may increase the rate of fermentation while these compounds alone have no such effect.

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Nature of the Reticulocytosis in Pernicious Anemia Following Liver Therapy.

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Since the original publications by Minot, *et al.*,[1] it has been constantly observed that, in uncomplicated cases, pernicious anemia patients during relapse exhibit a reticulocytosis in the peripheral

[1] *a.* Minot, G. R., Murphy, W. P., and Stetson, R. P., *Am. J. Med. Sci.*, 1928, **175**, 581; *b.* Minot, G. R., Cohn, E. J., Murphy, W. P., and Lawson, H. A., *ibid.*, 1928, **175**, 599.

blood following the administration of liver extract which is, in general, inversely proportional to the degree of anemia. This has been interpreted to indicate a reactivation of megaloblasts to mature to later stages of normal erythropoiesis.[2] Moreover, Doan[2a] has considered it quite identical, both qualitatively and quantitatively, with that found in hypochromic anemias after iron therapy. These interpretations have been based upon Doan, Cunningham and Sabin's theory[3] that megaloblasts are the earliest recognizable stage in normal erythropoiesis. Consequently, this has given rise to the concept advocated by Peabody, Doan and Isaacs[4] that the marrow lesion in pernicious anemia is a hyperplasia of cells at the megaloblast level which have failed to mature to later stages and elaborate hemoglobin.

Contrary to these opinions, there is an ever-increasing amount of evidence that genuine pernicious anemia megaloblasts are not present in normal bone marrow and are not related to normal erythropoiesis.[5] Also, in pernicious anemia, there is not a failure of cells to differentiate beyond a certain level of maturation but rather, the development of a pathologic red cell series. The ultimate products of this series are reticulated and mature megalocytes.[5a, 6] The latter is the most obvious answer to the query raised by Persons.[7] Furthermore, Kirschbaum[8] has shown that pernicious anemia megaloblasts are similar to the primitive erythroblasts of the embryonic yolk-sac but not identical with them.

The present investigation is concerned chiefly with a study of pernicious anemia bone marrows before, during and at the peak of the reticulocyte response following the administration of massive doses

[2] *a.* Doan, C. A., *Medicine,* 1931, **10**, 323; *b.* Isaacs, R., *Phys. Rev.*, 1937, **17**, 291; *c.* Osgood, E. E., and Ashworth, C. M., *Atlas of Hematology,* J. W. Stacey, Inc., San Francisco, 1937; *d.* Kracke, R. R., and Garver, H. E., *Diseases of the Blood and Atlas of Hematology,* J. B. Lippincott Co., Phila., 1937.

[3] Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Carnegie Contributions to Embryology,* 1925, **16**, 163.

[4] *a.* Peabody, F. W., *Am. J. Path.*, 1927, **3**, 179; *b.* Doan, C. A., *J. Lab. and Clin. Med.*, 1932, **17**, 887; *c.* Isaacs, R., *Am. J. Med. Sci.*, 1937, **193**, 181.

[5] *a.* Jones, O. P., *Handbook of Hematology* (Ed. by Hal Downey), Paul Hoeber, N. Y., in press; *b.* Dameshek, W., and Valentine, E. H., *Arch. Path.*, 1937, **23**, 159; *c.* Rohr, K., *Knochenmarksmorphologie des menschlichen Sternal punktates.*, Urban and Schwarzenberg, Berlin, 1937; *d.* Schulten, H., *Die Sternalpunktion als Diagnostiche Methode,* G. Thieme, Leipzig, 1937; *e.* Segerdahl, E., *Über Sternalpunktionen,* Appelbergs, Upsala, 1935; *f.* Nordenson, N. G., *Studies on Bone Marrow from Sternal Puncture,* Börtzells, Stockholm, 1935; *g.* Mallarmé, J., *Le Myélogramme,* G. Doin & Cie, Paris, 1937.

[6] Schartum-Hansen, H., *Folia Hematol.*, 1937, **58**, 145.

[7] Persons, E. L., *J. Clin. Invest.*, 1929, **7**, 615. See top of p. 628.

[8] Kirschbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 542.

of liver extract. Material for this investigation consisted of 20 sternal marrows obtained before therapy and 25 secured from 10 hours to 18 days after therapy.* All dry smears and imprints were stained with May-Grünwald-Giemsa combination of Pappenheim. Reticulocytes were studied in preparations made by smearing freshly aspirated sternal marrows upon a slide previously treated with an alcoholic solution of brilliant cresyl blue. Smears thus prepared were allowed to remain in a moist chamber for 5 minutes and then dried rapidly. Some of these preparations were counterstained. At least one thousand cells were examined in each marrow.

During relapse, megaloblasts constitute from one-fifth to one-half the total marrow cells. Our averages show that promegaloblasts and basophilic megaloblasts form 6% of the total cells while polychromatic and orthochromatic megaloblasts constitute 24%. All transitional stages exist between the latter and reticulated and mature megalocytes. Cells of the normal series, which are in a quiescent state, constitute about 3%. We have recently shown that in addition to the development of a pathologic red cell series there is also a pathologic regeneration of the neutrophils.[9]

After liver therapy, normal erythropoiesis is reëstablished by heteroplastic development from the myeloblast and by homoplastic development of the dormant normoblastic series. During this activity there is not a wholesale transformation of the megaloblastic series into normoblasts, but rather, megaloblasts complete their maturation as reticulated and mature megalocytes.[10, 11, 12] The fate of megaloblasts after liver therapy parallels that of the primitive erythroblasts of the embryo following the appearance of the liver anlage, in that they complete their maturation and finally die out.[10, 12, 13] Naegeli[12] has reported megaloblasts disappear from the marrow 8 days after therapy and believes that it is a gross error to assume that they have matured to normal cells. In general, our material supports this view, but in some cases orthochromatic megaloblasts were present 18 days after therapy. In a few instances, 8 to 12 days after therapy, hemoglobin-bearing megaloblasts appeared to be undergoing a peculiar granular degeneration while in the marrow.

* This material was made available by the generous cooperation received from Dr. C. J. Watson, Dr. I. J. Pass, and other members of the staff of the Department of Medicine, University of Minnesota Medical School.

9 Jones, O. P., *Arch. Int. Med.*, 1937, **60**, 1002.

10 Schwarz, E., *Wien. klin. Wchnschr.*, 1928, **41**, 192.

11 Rohr, K., *Praxis, Schweiz. Rdsch. Med.*, 1935, No. 26.

12 Naegeli, O., *Wien klin. Wchnschr.*, 1935, **48**, 225.

13 Kirschbaum, A., and Downey, H., *Anat. Rec.*, 1937, **68**, 227. See figs. 3-6.

TABLE I.

Patient	Days After Liver	Blood Reticulocyte %	Marrow Erythroblasts % of all cells		
			Normal Series	Polychrom. Megaloblasts	Orthochrom. Megaloblasts
W.M.	4	26	33.0	—	3.5
W.K.	4	22	33.6	2.4	8.5
J.B.	4	18	40.5	1.0	3.8
P.L.	5	17	25.0	1.3	8.2
M.H.	5	18.8	44.9	0.5	4.9
B.P.	4 (peak)	21	43.8	1.3	2.9

Table I shows that during the rise of reticulocytes in the blood, there are still cells in later stages of megaloblastic development in the marrow which have not yet completed their differentiation to reticulated and mature megalocytes. All intermediate stages between the latter and the remaining megaloblasts are present. It is obvious, therefore, that the total reticulocyte population is composed of cells derived from 2 entirely different sources, *i. e.*, the pathologic series and the normal series. Hence, the reticulocyte response after liver therapy in pernicious anemia not only indicates a renewed activity on the part of normal erythropoiesis but it also indicates that the marrow is being purged, so to speak, of its pathologic red cell series, the megaloblasts. In all probability, this purging effect of liver extract upon the bone marrow explains the rise in the mean reticulocyte diameter as well as the subsequent decrease to normal values which were observed by Fitzhugh and Persons.[14] It has also been reported that a few reticulated megalocytes remain in the blood until the erythrocyte count has reached between 2 and 3 million.[15]

This interpretation of the dual nature of the reticulocytosis is in agreement with certain mathematical formulæ[16] devised to predict the height of a response from the initial erythrocyte count. Furthermore, it can now be more fully appreciated why the reticulocytosis should be inversely proportional to the degree of anemia. For, in general, the more severe the anemia the more hyperplastic[4a] and more megaloblastic[6] the marrow. Accordingly, there would be more cells of the pathologic series available to be transformed into reticulated megalocytes for the response, in addition to the normal reticu-

[14] Fitzhugh, G., and Persons, E. L., *J. Clin. Invest.*, 1929, **7**, 631.

[15] Davidson, L. S. P., and McCrie, J. G., *Lancet*, 1928, **215**, 1014.

[16] $R = \frac{0.73 - 0.2\ E_0}{0.73 + 0.8\ E_0}$; Riddle, M. C., *Arch. Int. Med.*, 1930, **46**, 417.

locytes. For practical purposes discrimination of the two reticulocyte types is unnecessary.

Wintrobe[17] has shown that the increased mean corpuscular volume during liver therapy is due to an outpouring of reticulocytes from the marrow. This marked increase in corpuscular volume can also be more fully appreciated when it is considered that part of the cells are reticulated megalocytes which are larger than normal reticulocytes.[15]

The participation of reticulated megalocytes in the reticulocytosis of pernicious anemia patients following liver therapy makes this response qualitatively distinct and different from that in hypochromic anemias after iron therapy, since it is well established that marrows from the latter do not possess megaloblasts. In like manner, the reticulocyte response in guinea pigs after the administration of liver extract[18] is qualitatively different from that in pernicious anemia patients since these animals do not have megaloblasts in their marrow.[19] Reports to the contrary by Gall[20] are due to the fact that he has adopted Sabin's definition of the term megaloblast which indiscriminately includes primitive erythroblasts, promegaloblasts and pronormoblasts (proerythroblasts).

Conclusion. After liver therapy megaloblasts complete their maturation as reticulated and mature megalocytes. The resultant reticulocytosis is composed of reticulated megalocytes and normal reticulocytes. This not only indicates the reëstablishment of normal erythropoiesis but also, that the marrow is being purged of its pathologic red cell series, the megaloblasts. This response is qualitatively distinct from the reticulocytosis of hypochromic anemias following iron therapy and the one in normal laboratory animals after liver treatment.

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Gonadotropic Hormones in the Hereditary Dwarf Mouse.

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Snell[1] described dwarfism in the mouse which was inherited as a

17 Wintrobe, M. M., *J. Clin. Invest.*, 1934, **13**, 669.

18 Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 664.

19 Jones, O. P., *J. Lab. and Clin. Med.*, 1936, **21**, 335.

20 Gall, E. A., *Am. J. Path.*, 1937, **13**, 575.

1 Snell, G. D., *Proc. Nat. Acad. Sci.*, 1929, **15**, 733.