Our observations showed that there was no difference in the growth rate between eyeless and normal forms in either species studied. Throughout the entire period, eyeless axolotls, reared in the light, were pale; normals were decidedly dark. The one-eyed axolotls, with respect to color changes, behaved like the normal 2-eyed animals. This situation agrees with the findings of Parker[4] on Fundulus and is in contrast with his findings on Ameiurus.

Pale eyeless axolotls 9 months old when injected with intermedin,* became dark but never as dark as the normal 2-eyed controls. The effect wore off gradually and at the end of a week the original light color was restored.

Removal of the second eye from a one-eyed axolotl at 9 months, caused the animal to become pale, but not as pale as those rendered eyeless in the embryonic stage. Enucleation of a 12-year-old axolotl had no effect upon coloration. The animal remained in a permanently dark state.

Summary. Embryonic enucleation of *A. punctatum, A. tigrinum* and *A. mexicanum* (axolotl) has no significant effect upon the growth rate. The influence of the eyes upon color changes and possibly upon metamorphosis seems not to be related to any eye substance (hormone) developed within the eye itself.[1] No effect of embryonic enucleation upon the development of the gonads could be found in *A. tigrinum* up to 170 days after the embryonic stage nor in axolotls two years of age.

4 Parker, G. H., *Proc. Nat. Acad. Sci.*, 1939, **10**, 499.

* The preparation which we used was made from the anterior lobe of the whale, and was kindly supplied by Dr. Geiling of the University of Chicago.

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Serial Bone Marrow Studies in Pernicious Anemia. I. Fluctuation in Number and Volume of Nucleated Cells.

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A number of authors[1-14] who studied the bone marrow at intervals after the beginning of liver-induced remission emphasized the rapid disappearance of megaloblasts and the appearance of normoblasts. These authors obtained by biopsy[1,2,7] or aspiration technic[3-5, 7-14] specimens from patients with pernicious anemia before liver treatment and one or two specimens at varying intervals during liver therapy. Some authors aspirated the same patient 2 or 3 times at different intervals during liver therapy,[5,12,14] or in groups of from 4 to 10 patients, performing the aspiration in different phases of their re-

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mission.[4,5] In one study[11] the marrow of one patient had been studied 17 times at frequent intervals and in other studies[5,8,13] the aspiration was performed at regular 48-hr intervals 5 or 6 times. These authors studied the morphological and proportional changes of the different types of cells. Only 3 reports have included quantitative data.[4,6,9] One study concerned cases of pernicious anemia on which bone marrow studies were performed at necropsy. These patients died at varying intervals after the institution of liver therapy.[6] More recently 2 authors attempted to follow the bone marrow changes by frequent serial marrow punctures. Their studies included both qualitative and quantitative data.[4,9]

In the following study, an attempt was made to follow the quantitative cellular changes of the sternal marrow during liver-induced remission.

Materials. In this study 16 patients with Addisonian pernicious anemia were observed. These cases were divided into 3 groups according to the dose of liver extract administered during the first 10 days of hospitalization. The first group included 9 patients receiving more than 60 USP units. The second group included 5 patients, 4 of whom received 30 units and the fifth, 40 units. The third group included 2 patients, each of whom received one unit daily.

Technic. The marrow samples from the same patient were obtained on one or 2 occasions before therapy had been instituted and then at 24-hr intervals. In 3 instances the first puncture was obtained 6 hr, in one 9 hr, and in two 12 hr after the first liver injection.

The subsequent aspirations were performed at about 24-hr intervals for an average of 9 times. Different portions of the sternum were aspirated with a 17-18 gauge needle, and one cc of marrow was obtained. The total nucleated elements and reticulocyte counts were performed instantly after aspiration of the marrow. For the total nucleated elements the marrow was diluted 1:20 with 1% acetic acid using the standard white cell pipette and counting all nucleated elements in 80 small squares under high dry objective, and to the number obtained 3 ciphers were added. This gave the number of nucleated cells of all types per cubic mm of aspirated marrow. The volume percentage of the total nucleated elements together with the volumes of fat, plasma and erythrocytes were determined according to the Limarzi technic,[15] using as an anticoagulant a dried mixture of ammonium and potassium oxalates. Smears for the differential count were made from the mixed buffy coat after centrifugalization. Five hundred cells were counted and the percentage of megaloblasts, pronormoblasts, normoblasts and other myeloid cells was computed.

Daily peripheral blood studies on oxalated blood included the determination of hemoglobin (Hellige), the number of red and white blood cells, hematocrit (Wintrobe), and percentage of reticulocytes, using brilliant cresyl blue counterstained with Wright's stain.

To ascertain the homogeneity of different portions of the sternum, the marrow was aspirated from two sites 5 cm. apart in 7 control patients. These double marrow punctures were repeated at 24-hr intervals using again different sites.

Observations. Preliminary studies revealed that marrow samples taken simultaneously at 2 different sites of the sternum exhibited comparable quantities of nucleated cells of all types. We found also that the number of cells varied proportionately with the amount of the buffy coat. The normal value of these cells ranged between 30,000 and 50,000 per mm^3 while the volume of buffy coat ranged between 3 and 8%. These two data together usually reflected quite satisfactorily the functional status of the marrow.

Table I illustrates marrow samples obtained from 16 patients in relapse. Twelve samples exhibited hyperactivity, and 4 were within normal limits, as judged by the number of cells. Twenty-four hr after the beginning of liver treatment, there was a drop in the number of nucleated cells in 13 instances. In 2 instances there was a marked rise in the number of nucleated cells which

[15] Limarzi, L. R., *Illinois Med. J.*, 1939, **75**, 38.

TABLE I.

Shows the Fluctuation in the Total Number of Nucleated Marrow Cells Following the Injection of Liver Extract in 16 Patients.

Case No.	Red blood cells*	Liver dose†	Nucleated marrow cells in thousands: Before therapy	Hr after 1st dose: 6-24	48	72	Fluctuation: Min.	Max.	Time and peak of blood reticulocytes: Hr	%
1	2.00	60	268	160	197	45	20	197	72	24.6
2	1.30	75	250	121	66	68	10	157	120	20.0
3	0.75	210	145	40	41	225	38	245	—	—
4	0.77	315	93	298	48	357	48	357	120	40.0
5	1.70	180	107	222	129	239	15	239	144	30.4
6	0.76	180	40	38	40	73	38	129	144	30.0
7	1.15	135	176	35	87	165	35	165	96	35.0
8	1.70	100	162	72	98	140	5	140	120	16.0
9	1.80	60	185	136	109	100	30	136	120	28.0
10	1.20	40	202	107	267	282	17	282	120	27.0
11	1.30	30	170	53	201	117	12	201	96	32.8
12	1.50	30	49	105	40	—	40	105	96	33.0
13	1.00	30	112	92	114	63	31	114	96	37.0
14	1.70	30	42	19	21	105	18	105	96	39.0
15	1.30	10	159	99	212	38	7	256	144	26.0
16	1.70	10	32	31	37	60	31	60	144	18.0

*In millions.
†U.S.P. units.

did not decrease until 72 hr later. In one instance there was no change in the number of cells. Within 48 to 72 hr after the first administration of the liver extract, there was, in all cases, a periodic fluctuation in the number and volume of nucleated cells. The majority of the cases showed 3 irregular fluctuations in cellularity during the 10-day period following the institution of liver therapy. This was followed by a period in which more constant numerical data were obtained.

These changes in the total number of nucleated cells presented a marked individual variation. The extent of the fluctuations was independent of the amount of liver administered. No direct correlation existed between the degree of fluctuation and the number of reticulocytes. An overdose of liver did not accelerate the maximum response of the reticulocytes.

The morphological study of these marrow samples revealed a rapid disappearance of the megaloblasts within 24 to 48 hr and the presence of an increased number of early normoblasts. Further analysis showed that the fluctuations of the nucleated elements were almost entirely due to the periodic increase and decrease in the number of these cells of the normoblastic series (Fig. 1). The increase of reticulocytes in the marrow preceded or was simultaneous with the increase of reticulocytes in the peripheral blood.

Comment. In this study an attempt was made to distinguish between the megaloblast and pronormoblast only on the basis of the chromatin distribution in their nuclei and the degree of basophilia of their cytoplasm.[16] A rapid increase of basophilic cells with a tendency for chromatin condensation in their nuclei was noted, and was interpreted as normoblastic stimulation. These latter cells frequently showed mitotic and amitotic forms. The prompt decrease of cellularity soon after the beginning of liver administration in the majority of the cases suggested a "purging" process of megaloblasts in the marrow while the subsequent increase in cellularity indicated an erythroid stimulation.

The specimens obtained during the period from 96 to 168 hr after the administration of

[16] Jones, O. P., *Cytology of Pathologic Marrow Cells with Special Reference to Bone-Marrow Biopsies.* Downey, H., *Handbook of Hematology,* Hoeber, New York, 1938, 3, 2045.

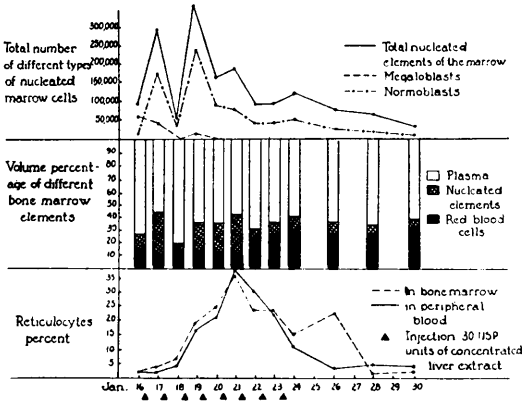

FIG. 1.

Illustrates the changes in the number and volume of the nucleated elements of the marrow following large doses of liver extract.

the first liver dose showed again a marked decrease in the number and volume of the total nucleated cells which was due to an almost complete depletion of the normoblastic cells in the marrow. This was again followed by marked normoblastic proliferation.

With these data we confirmed and elaborated the observations made by others using a similar technic.[4,8,9] These authors also noticed a fluctuation of the total number of the nucleated marrow cells.

Summary. Serial sternal marrow studies at 24-hr intervals before and after liver induced remission in 16 patients with Addisonian pernicious anemia, revealed a marked fluctuation of the total number and volume of the nucleated elements. Six to 24 hr after the first injection of liver the majority of the cases showed a marked decrease in the total number of nucleated cells. This was followed by a periodic increase and decrease of the absolute number of the normoblasts. During the period from 96 to 168 hr after the institution of liver therapy there was at times an almost complete depletion in the marrow of these normoblasts.

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Serial Bone Marrow Studies in Pernicious Anemia. II. Nucleated Cells and Blood and Urine Uric Acid.

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Because of an increase of the uric acid levels in the blood in pernicious anemia, early observers assumed that a primary purine disturbance was present in this disease.[1-3] Increased amounts of uric acid in the blood and urine simultaneous with a rise in the blood reticulocytes has been observed in pernicious anemia during liver induced remission,[4,5] in iron deficiency anemias,[5] and in experimentally produced anemias.[6,7] Few authors reported acute attacks of gout following liver administration in patients with pernicious anemia.[8-10] The increased amount of uric acid in these conditions was regarded as a disintegration product of the nucleic acid portion of the extruded nuclei of the normoblasts.

In the previous communication there was evidence of marked fluctuation in the number of nucleated marrow cells during the early phases of liver induced remission. This fluctuation was due to irregularly

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