

hours and a veritable honey-comb design is outlined by the vessels, a small number only of which seem to be completely open to the circulation. There are very many developing red blood-cells, all within strands of endothelium. In some places it appears almost as though the endothelium were being actually replaced by a strand of developing red blood-cells, though the regularity and continuity of outline is nowhere broken. There are numerous small groups of from five to eight young granulocytes in various stages of development, all located extravascularly in the parenchyma. Succeeding stages increase the complexity of the picture. However, insofar as we have been able to observe, the red blood-cells have appeared only intravascularly, and the white blood-cells extravascularly. No analyses of hyperplastic marrows have as yet been attempted.

124 (2084)

On the intravascular development of erythrocytes in the bone marrow of the adult rabbit.

By R. S. CUNNINGHAM and C. A. DOAN.

[From the Department of Anatomy, Johns Hopkins University, Baltimore, Maryland.]

Bone marrow has been one of the most difficult tissues to understand because it has proved so hard to reduce it to a sufficiently simple state for analysis. The older methods, consisting chiefly in classification of cell types, have led to the almost universal acceptance of the monophyletic theory, and in general to the conclusion that the developing red blood cells are formed in parenchymal spaces outside the vascular system, hence differing from the manner of development found in the embryo. Since it has been accepted that the red cells develop extravascularly, it has been obviously necessary to determine their mode of entry into the circulation. The two principal explanations offered have been: (1) That the endothelial lining of the vascular bed was incomplete, as in the spleen, and consequently the young cells could be forced through these openings; and

(2) that the growing clumps of red cells cause erosion of the endothelial lining by pressure, and hence obtain entrance to the vascular bed. Drinker, Drinker and Lund¹ were able to demonstrate that the endothelial lining in normal marrow is quite complete, but that in hyperplastic marrows this is probably not the case. They found that the injection mass was not so evenly outlined by the walls of the sinusoids in the hyperplastic as in the normal marrows. This difference they explained as due to young red cells which had eroded the endothelial wall by pressure, while, at the same time, these cells were so closely packed together that they prevented the injection mass from extravasating freely into the parenchyma.

Our concept of the normal structure of the vertebrate bone marrow has been considerably modified by the demonstration by one of us² of a very elaborate capillary bed in the marrow of the adult pigeon. These capillaries were first demonstrated by injection, but it was found that they could only be satisfactorily injected after the marrow had been reduced to a very simple hypoplastic state by starvation. The demonstration of a similar vascular pattern in the mammalian marrow has been very difficult, and yet such a study is essential since, if these capillaries occur in mammals as well as birds, it will be of the greatest importance to hematology. The vast number of investigations of the bone marrow which have been carried out on anemias produced by various toxic agents, as *e.g.*, Thorium-X and other radioactive substances, have caused too much injury to the marrow to allow analysis of the normal structure. Starvation has so far proved relatively ineffective in the mammal and no other satisfactory method has yet been found for reducing the marrow to the hypoplastic state which has been obtained in the pigeon.

We have been able, however, by a very simple procedure, to obtain a marrow in the rabbit in which the myelocytic cells are very largely removed and only the developing reds retained; in such a marrow it has been immediately possible to see that there are large numbers of young red cells definitely within capillaries which are not part of the ordinary blood filled sinuses

¹ Drinker, Drinker, and Lund, *Amer. Journ. Physiol.*, 1922, lxii, 1.

² Doan, *Contributions to Embryology*, 1922 xiv, 29, Carnegie Inst. of Washington, Publication No. 277.

and which are running from sinus to sinus as shown by Doan in the pigeon. With endothelium, as delicate as this is, it is obviously impossible in sections to always determine the exact location of the red blood cells, but a large proportion of them appear in rows extending from sinus to sinus, with here and there parallel, investing, endothelial nuclei. The histological picture obtained is strikingly similar to that described for the pigeon's marrow during recovery from starvation,³ and in view of such a similarity our conclusion that most of the red cells arise intravascularly seems wholly justified.

The method that we have used for this purpose has been the administration of large doses of dead typhoid bacilli intravenously. Our doses have been increasingly large, usually beginning with $\frac{1}{2}$ of a 24 hour agar slant, planted in a standard manner, and increasing to 1, 2 and 3 cultures to the dose. Very large outpourings of leucocytes have immediately occurred and after 3 doses of this character the bone marrow has been depleted to the maximum of its myelocytes and has not begun to form new white cells to any appreciable extent.

The conclusion that the red cells arise by proliferation of endothelial cells which remain in the bone marrow in a relatively undifferentiated state seems justified from the observations of Doan on the pigeon and those reported here for the rabbit. That large clumps of developing red cells do occur in the marrow has been observed many times, and though an endothelial margin can not always be demonstrated, this may be either the difficulty of demonstrating so delicate a structure in sections, or else an overgrowth of the young cells into a blood island. Either conclusion obviously explains the method of entry of the adult erythrocytes into the circulation through the original openings of the capillaries into the sinusoids. It has been determined that endothelium gives rise to the erythrocytes of the embryo in a large number of species; and suggestive evidence is presented here for the mammal, and elsewhere for the pigeon, that a similar relationship exists in the adult marrow. This seems to us to indicate an additional and most important physiological significance for endothelium in the adult vertebrate.

³ Doan, *Proc. Soc. Exp. Biol. and Med.*, 1923, **xx**, 5.