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Some observations concerning chicken bone marrow in living cultures.

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Tower and Herm[1] presented recently before the Society for Experimental Biology and Medicine some ideas concerning the origin of the mammalian (cat) and avian (chicken) blood cells. These authors were led by their observations on bone marrow in living cultures to the following conclusions:

1. The mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell.

2. The mammalian normoblast and the red corpuscle of the bird are the product of intranuclear activity and are phylogenetically identical.

3. Phagocytosis of red cells by the giant cells (megakaryocytes) in normal blood-forming tissues is by no means common. The true process is undoubtedly the manufacture of red cells and not the destruction of them.

My own observation of *chicken* bone marrow in living cultures led to some conclusions which are not in harmony with the quoted statement. I studied the bone marrow of *chicken*; therefore, my remarks are based only on observations on the bone marrow cells of this animal.

[1] Tower and Herm, 1916, PROCEEDINGS OF THE SOCIETY OF EXPERIMENTAL BIOLOGY AND MEDICINE, 1917, Vol. XIV, pp. 61–52.

I could observe budding of red blood corpuscles after the first day of cultivation. Buds with and without nuclei appeared, also the rapid division of erythroblasts could be noted. The budding off of either small nucleated or non-nucleated cells cannot be a progressive process in the chicken because the avian red blood corpuscle is nucleated. Therefore the analogous observation of Tower and Herm with its conclusion that the mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell, loses its convincing power; probably the budding is a reaction of the normoblast to the change of its media, as it is also observed in amebae as soon as they are under unfavorable conditions and can be produced experimentally. The mono- and polynuclear eosinophil leucocytes show in living cultures the same tendency to divide rapidly into nucleated *small* cells or non-nucleated components in living cultures. The bud-forming capacity and the tendency to divide rapidly seems to be a general behavior of blood cells in living cultures.

Another phenomenon to produce non-nucleated cells observed in living cultures is the following: the nucleated blood corpuscle loses its nucleus by *ejection* of chromatin, this process resembles the formation of Cabot's bodies in experimental anemia or the anemia of man (Juspa[2]).

These two processes seem to be *degenerative in the chicken.* If these two phenomena are observable in the chicken the question arises, can the mammalian normoblast be capable of losing either its nucleus by the budding off process or by ejection of the condensed original nucleus. The authors identify strongly in their second thesis the mammalian normoblast and the red corpuscle of the bird, it may be therefore possible that they have not observed the ejection of the mammalian normoblast nucleus.

I can agree with the authors that phagocytosis of true *megacaryocytes* (giant cells) is by no means common in normal bone marrow tissues. But still the most striking feature in *my cultures* was the phagocytosis of a kind of "Riesenzellen." But these "Riesenzellen" are not the usual multinucleated cells of the bone marrow; (megacaryocytes) this name includes cells which have

[2] Juspa, 1913–14, *Folia Haemat.*, Bd. XVII, II Teil, pp. 429–441.

first been observed in the bone marrow by Foot[3, 4] and thought by him in his first publication to be of mesenchymal origin and in the second publication of lymphocytic origin. These "giant cells" can be either changed fat cells or elongated vacuolized connective tissue cells, or even enlarged myelocytes. They are able to phagotize, to store fat, to divide into smaller forms—the so-called cell culture type, small forms with nuclei, the chromatin of which is arranged on a fine network. The formation of "giant cells" characterizes the first period in the history of bone-marrow in living cultures. After five or eight days, cultivation, the "giant cells" have cleaned up the debris of the dying cells (blood corpuscles, fat cells, and large mononuclear lymphocytes). In the second period the remaining cells adjust themselves to the continued life in tissue cultures. Cell types of the small lymphocyte type with vesicular nuclei appear, which later are transformed into different types of connective tissue cells—not exactly resembling the connective tissue cells in the outgrown animal—but closely resembling the mesenchymal cells of the embryo.

The production of the cells of the second period can be accelerated by washing the original bone marrow particle in plasma. After the plasma has been renewed three or four times, blood corpuscles and the lymphocytes which had been from the beginning in the meshes of the bone marrow, have been left in the media and only those cells close to the bone marrow network are transferred to the new culture medium. In cultures prepared in this way, we can observe small cells of lymphocytic character which can store fat, phagotize and adopt all shapes of connective tissue cells—but no formation of *blood* corpuscles or large mononuclear lymphocytes can be observed. This proves clearly that after the already preformed "ripe cells" are disposed of, no new formation of blood corpuscles takes place in the living cultures. It may be that the lack of oxygen prevents the appearance of red blood corpuscles. The conditions in tissue cultures do not seem to allow the stem cell to show its dualistic character. It does not form blood corpuscles, but forms only the different elements of *connective* tissue.

[3] Foot, *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, Bd. 53, pp. 446–447.

[4] Foot, *Jour. of Exp. Med.*, 1913, Vol. XVII, pp. 44–60.

The above mentioned authors do not state in their preliminary paper how old their cultures were when they made their observations and if they have distinguished between cultures of fat containing bone marrow, nearly fatless bone marrow and bone marrow with large amounts of red blood corpuscles. All these facts may alter the conclusions because if the bone marrow contains a large amount of fat then many "Riesenzellen" are present and phagotization can be observed in a very considerable degree. If fatless bone marrow is used, phagotization appears to go on in a remarkable degree only in the second outlined period of culture life, because only then the cells near the bone marrow network begin to migrate in the plasma clot, to phagotize and to assume different types of connective tissue cells.

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On the isolation of streptococci from rabbits.

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In our work for the purpose of investigating the relation of streptococci to poliomyelitis as claimed by Dr. E. C. Rosenow, we have made cultures of the brains of 6 monkeys and 20 rabbits. Our technique was as follows:

The animals were etherized just before death and, when anesthetized, were fastened to an autopsy-board—abdomen downward. With animals that died during the night this part of the procedure could not, of course, be carried out. Our purpose was to make sure, whenever possible, that any organisms cultivated from the brains were not post-mortem invaders, but were present during the life of the animal. A median incision was made through the skin over the skull running from the tip of the nose to the back of the neck, and the skin dissected back on both sides of the head. The skull was disinfected with tincture of iodine, and the head and body covered with 3 layers of gauze soaked in lysol. A small hole was made in the gauze so as to expose the upper