CHANGES IN OUTLYING BONE MARROW ACCOMPANYING A LOCAL INCREASE OF TEMPERATURE WITHIN PHYSIOLOGICAL LIMITS*

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Plates 13 to 16

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This report is concerned with the functional reasons for the distribution of red and yellow bone marrow in the extremities of mammals and birds. Bone marrow is largely confined to the interior of the skeleton in these species. Exceptions are the physiological occurrence of hemopoietic tissue in the liver and spleen during fetal and neonatal life and in certain diseases such as myelogenous leucemia and the rare heterotopic hemopoietic foci seen in the adrenal gland (1), pelvis (31), and elsewhere. The reasons for this predilection of blood cell forming tissue for cavities in bone are still unknown. In the production of bone by ligation of the renal pedicle in rabbits, caused by the action of renal pelvis epithelium on adjacent fibroblasts (2), hemopoietic marrow is observed (3).

At birth, in mammals and birds, all of the bones which contain marrow contain red marrow (4, 6), which is similar in all loci, suggesting that the erythropoietic stimuli are of a general nature; in an examination of more than 50 newborn mammals we found no exception. Soon after birth, a replacement of red marrow by fat takes place, greater in certain regions than in others, but present in normal animals in all bones. In adult life, marrow containing enough chromogens to produce a red color is found in skull, clavicles, scapulae, sternum, ribs, vertebrae, pelvis, and in the proximal portions of the extremities,

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and fat is present in these areas, mostly in amounts only visible microscopically. This fat is associated in part with a surplus of blood forming tissue in the normal organism at sea level, since under a low O_2 tension the fat recedes and marrow becomes hyperplastic in these central areas. A different situation exists in the distal regions of the extremities, where blood cell formation stops and the bone marrow is yellow with fat in the gross. An examination of the literature shows topographical variations in normal distribution in the extremities which is the subject of the present investigation.

The Distribution of Fatty and Red Bone Marrow in the Extremities of Normal Animals

Exact data on the anatomical distribution of fatty and hemopoietic marrow are not extensive. Certain technical difficulties are potentially present in that sawing of bones is apt to distribute blood-stained bone dust over the surface of yellow marrow, and secondly that unless the bones are examined soon after death hemolysis and staining of yellow marrow from gravitation of blood pigment and infiltration is apt to occur; both difficulties are surmounted by histological examination.

The evidence substantiates the observations of Neumann (5) concerning the centripetal regression of red bone marrow in these regions. The data in mammals may be summarized as follows.

Man: Neumann (5) in his classical contribution to the subject reported: (*a*) In none of the cases (mostly adults) was there found yellow marrow in the shaft of long bones with red marrow in both epiphyses. (*b*) Either the bones of the extremities contain fatty marrow exclusively or red marrow is confined to the upper parts of the arm and thigh bones (upper epiphysis and more or less of the adjacent diaphysis) while the more peripheral bones contain only yellow marrow. (*c*) Yellow marrow occurs in a centripetal manner and marrow hyperplasia after hemorrhage develops centrifugally. (*d*) There is a congruence with respect to yellow marrow distribution in upper and lower extremities. Piney (6) examined the marrow distribution in 91 subjects between the ages of 3 days and 83 years. At birth the marrow had a rich red color. At age 12 to 14 years fatty metamorphosis was complete in tibia, fibula, and lower femur, but not in proximal femur; this is the adult condition. In tarsal and carpal bones, fatty metamorphosis was complete before the whole of the diaphysis of tibia, fibula, and lower femur, but not in proximal femur; this is the adult condition. In tarsal and carpal bones, fatty metamorphosis was complete before the whole of the diaphysis of

tibia, fibula, radius, and ulna was fatty. Litten and Orth (7), Hedinger (11), Askanazy (4), and Fahr (8) reported on the adult femur marrow and agreed that in the majority of cases the red marrow in this bone is localized in the proximal end and that it is unusual to find the proximal end yellow and the distal end red. Peabody (9) reported that the marrow of the long bones is normally in greater part fatty and hypoplastic as opposed to the active extremely complex marrow of vertebrae and flat bones. Williams (28) studied the bone marrow from lumbar vertebrae, sternum, and junction of the lower and middle thirds of the humerus, femur, and tibia in 100 unselected necropsies in adults, and found that red marrow occurs in the long bones in orderly combinations. When hyperplasia was present in tibia it was also present in femur and humerus; this combination occurred in 2 cases. In 31 cases hyperplasia was present only in femur and humerus. In 25 cases hyperplasia was present in humerus alone.

Horse: Ackerknecht (10) examined the bone marrow of 120 horses of various ages and states of nutrition and found that red marrow persisted throughout life in the proximal and middle thirds of the femur with some variation in pattern. Red marrow was not found except in disease, in the radius, tibia, and more distal bones after 3 years of age. In the series of pathological horses the proximal epiphysis of tibia contained red marrow 4 times, the distal never. *Dog*: Oehlbeck, Robscheit-Robbins, and Whipple (12) studied the red marrow spread in normal dogs and found that in young animals it occupied nearly all the cancellous bone in ribs, vertebrae, and long bones. As the dog matured, red marrow occupied all of the cancellous bone in ribs and vertebrae but much less in the long bones, where femora and humeri might show a third of the marrow cavity filled with fat instead of red marrow, while tibiae, radii, and ulnae were two-thirds fatty. Bock (13) stated that the marrow is similar to other great mammals as found in the horse (10).

In the *pigeon*, Muller (14) reported that radius marrow is moderately fatty and femur is usually red, and Doan, Cunningham, and Sabin (15) stated that the bone marrow of the radius and ulna in the normal adult pigeon was similar to normal mammalian marrow of long bones.

The present authors sawed the femur and tibia of 17 mature normal dogs and found: (*a*) In all cases there was yellow bone marrow corresponding to the epiphyses of the femur and tibia bordering on the knee joint. (*b*) Femur: all dogs had red marrow in this bone; in 6 it was completely red as far as the knee epiphysis; in 10 there was a yellow island of fat occupying the middle third of the femur surrounded by a red peripheral bark and with red marrow above and below; in 1 the red marrow occupied the proximal third of this bone and all distal bones had fatty marrow. (*c*) Tibia: 1 dog had completely red marrow except at the epiphyses; 5 had completely fatty marrow; 8 had red

marrow in the proximal quarter to third of the diaphysis; 3 had red marrow in proximal and distal areas of metaphysis with a fatty island in the middle third. *Rabbit*: In this laboratory in 8 adult rabbits the marrow of pelvis, femur, and the proximal two-thirds of tibia was found to be very red; rather abruptly a change took place in the distal third of the tibia where the marrow changed from red hemopoietic marrow to yellow marrow, nearly or quite fatty. The metatarsals were always yellow in grown rabbits. It was found possible to recognize topography from microscopic sections of the marrow from the proximal and distal regions of the tibia in adults judging purely from fat content. *Albino rat*: A situation similar to the rabbit was found in 40 adult animals in this laboratory; the marrow of the leg was always red as far as the proximal two-thirds of the tibia; the metatarsal marrow was always fatty.

It will thus be seen that the distribution of red marrow spread in the extremities is not entirely an anatomical problem of fatty diaphysis and red epiphysis as will be discussed below, and it is certainly not one wholly of age as was early demonstrated by Litten and Orth (7). The evidence clearly shows that fat accumulates in the limbs in a centrifugal direction with a corresponding decrease of hemopoietic tissue.

No one save Whipple and his associates has offered an opinion as to the reasons for red-yellow marrow distribution. These investigators (12) felt that the red marrow does not expand as fast as does the mass of cancellous bone in the growing skeleton and that the liver might be the limiting factor in red marrow spread in setting the top limit for maximal red cell and hemoglobin production. Piney (6) did not believe that the proximal-distal variations of marrow distribution could be explained by gravity. Ackerknecht postulated that the centripetal development of fat was a physiological process and Askanazy referred to it as a secondary physiological lipomatous atrophy or involution.

McMaster and Haessler (32) studied the marrow in rabbits rendered anemic from blood loss, and found a greater red marrow spread in a group where a concentrated hemoglobin solution was injected intramuscularly than in uninjected animals.

An important observation was made by Ranvier (16) that the vertebrae of the tail of adult cattle, dog, and rabbit contain yellow fatty marrow. This was found true for more than 100 normal adult albino rats examined in this laboratory. In this animal all of the trunk verte-

brae have lateral bony processes and contain red marrow. Yellow marrow begins in the tail, one or two vertebrae beyond the last trunk segment equipped with a lateral process, and extends to the tip in adults. The transition is exceedingly sharp, indeed often in the proximal end of one vertebra the marrow will be mostly red, while the distal end contains mostly fat and all distal vertebral marrows are fatty.

The problem may then be posed: Why is bone marrow in the distal region of limbs and tail at a disadvantage from the standpoint of formation of blood as compared with marrow in the bones of the body trunk?

The sharpness of transition so frequently observed between red and yellow marrow suggests that a physical agent is the responsible factor. One of the fundamental differences between proximal and distal tissues is the higher temperature of the former. It has recently been shown (29) that the bone marrow participates in the thermal decrement occurring in the extremities. The following experiments were devised to learn if an increase of temperature would affect the distribution of red and yellow marrow in the extremity. Since the rat tail is always fatty after an early age, most of the present experiments were done on this structure which was found to serve as an excellent indicator for red marrow spread.

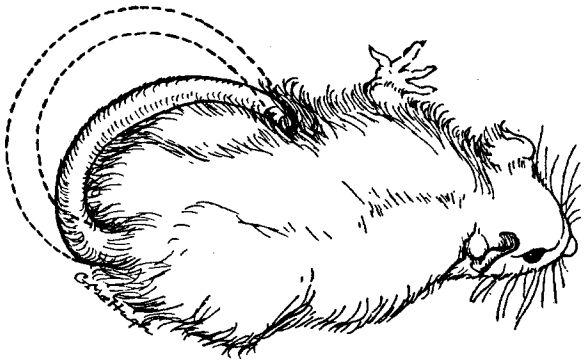
Effect of an Increase of Temperature on the Intact Adult Rat Tail

One of the simplest methods found to produce a chronic elevation in temperature in bone marrow is to denude the skin and surgically anastomose the otherwise intact structure to the abdominal wall so that the tail is inserted and maintained in the peritoneal cavity (Text-fig. 1).

Series 1.—Under ether anesthesia,[1] 35 albino rats between 27 and 200 days of age were operated upon aseptically. The skin of the tail was circumcised at about its middle and the distal skin removed and discarded. A 1 cm. incision was then made in the lateral abdominal wall and the denuded tail inserted into the peritoneal cavity and anchored by a suture on either side between a longitudinal tendon bundle and the loin muscles. The skin of the tail was then sutured at its cut margin to the abdominal incision. No dressing was applied and it was found that

[1] All operations were performed under ether anesthesia.

prompt healing usually took place leaving an *outside loop* to be compared as a control area with the intra-abdominal portion. In some of the experiments a few terminal segments were amputated for additional control observations; this did not appreciably affect the experiment. In all of the present experiments the tail was permitted to follow a gentle curve from sacrum to peritoneal cavity to avoid the effects of sharp angulation. The animals were killed at 5 to 7 day intervals between the ages of 17 and 111 days. At autopsy a part of the outside loop was compared with the intra-abdominal portion. All of the histological preparations described in this report were made from paraffin sections of tissue fixed in Zenker's fluid with 10 per cent formalin added, and decalcified in 5 per cent nitric acid.



TEXT-FIG. 1. In the experiments of series 1 and 2 the distal portion of the tail was skinned and surgically inserted in the abdomen, forming an outside tail loop. Differences in effect were observed when the outside loop was short or long (dotted lines).

An approximately similar experiment devised for a different purpose was reported by Ribbert (17), Matsuoka (18), Müller (19), and Mau (20, 21). These investigators studying the problem of spinal curvature denuded the skin of the tail in rabbit or rat and anchored it beneath the skin of the trunk to produce marked angulation of the bones. The effect of this procedure on the bone marrow was not reported.

It was thus possible to produce a chronic elevation of temperature of the distal intraperitoneal portion of the tail and to compare it with a more proximal outside loop of the same tail at a lower temperature without compromising blood, nerve, or lymphatic supply; the excised tip, moreover, in certain experiments served as further control tissue. Distinct changes in the bone marrow were observed as a result of this

operative procedure. In each instance, increased hemopoiesis was found in the segment of increased temperature as compared with the outside loop. In two experiments of the shortest duration the increase was slight, but in all the others obvious and unequivocal. More striking results were obtained in animals operated on between the ages of 27 to 50 days than in the oldest animals.

Several typical protocols will be given; the initial figures in each case signify the laboratory number, the age at the beginning, and the duration of the experiment.

Rat 8-78, old rat, 17 days: *Intra-abdominal portion* shows wide dilatation of blood vessels, increase of protein framework, and slight clumping (+) of hemopoietic cells in metaphyseal regions. *Outside loop*: normal vessels, very rarely a small cluster of cells is seen.

Rat 8-85, old rat, 38 days: *Intra-abdominal portion* (Fig. 3) shows a much greater dilatation of vessels and moderate hemopoiesis (++) with many red and white cell precursors, and megacaryocytes as compared with the almost completely fatty *outside loop*.

Rat 8-88, old rat, 65 days: *Intra-abdominal portion* shows much gelatinous protein material and vascular dilatation with moderate hemopoiesis (+), especially in the neighborhood of the metaphyses; the *outside loop* shows less gelatinous groundwork, slight capillary dilatation, no hemopoiesis.

Rat 8-98, 28 days, 46 days: *Intra-abdominal* portion shows an increase of protein framework in the diaphysis, with moderate hemopoiesis (+++) in the metaphyseal areas; *outside loop* is very fatty and has no hemopoietic foci.

Rat 8-80, old rat, 111 days, weight 260 gm.: *Intra-abdominal portion* shows hemopoiesis (++) with many red cell precursors; *outside loop* contains only normal fatty marrow without hemopoiesis.

Rat B, 27 days, 86 days: *Intra-abdominal portion*, much hemopoiesis (+++), many megacaryocytes, slight vascular dilatation (Fig. 1); *outside loop*, entirely fatty, no sign of hemopoiesis anywhere in the sections (Fig. 2).

An increase of vascularity, protein framework, and hemopoiesis was seen in that portion of the rat's tail which had been subjected to a chronic elevation of temperature, both as compared with the cooler outside loop and the normal tail. In most of the outside loop segments, the normal fatty marrow of the adult rat tail was seen; in some, however, there were observed increased framework and vascularity, with slight hemopoiesis; in no case did the magnitude of these changes approach that of the inside portion. Variations in temperature of the outside loop occurred depending on whether a small or large portion

of the tail was in the peritoneal cavity. In the former case, the long redundant loop was cooler than when this structure was short and closely applied to the trunk and thus influenced by apposition to warm structure.

The hemopoietic cell increase in the area subjected to temperature elevation concerned chiefly the granular leucocyte series, although there was a definite increase of hemoglobin carriers. A typical field showed stem cells, many eosinophile and heterophile myelocytes, a great many reticular and endothelial cells, and somewhat fewer erythrocyte precursors, polychromatic erythroblasts, and normoblasts. Megacaryocytes were frequently observed.

One of the deviations from the normal conditions in these tail loops was an interference of motion in the tail. It was at once observed that motion was as much restricted in the outside loop as it was in the intra-abdominal portions, so that the effect of immobilization on marrow was balanced and thus controlled in each animal.

Effect of Elevation of Temperature on the Intact Rat Tail in Anemia

None of the changes described in the preceding experiment approaches in amount the changes found in similar rats provided in addition with a stimulus to hemopoiesis as in anemia. The anemia was produced by splenectomy and by repeated bleedings.

Series 2.—Anemia through blood loss was produced by heart puncture under ether anesthesia every second day in 12 albino rats in which tail loops had previously been made as described above. It was possible to remove very large quantities of blood in this way, thus 47 cc. were bled from a 204 gm. rat in 23 days. The circulating hemoglobin was reduced to 25 to 40 per cent; the usual ground meal diet (purina fox chow) was supplemented by 1 per cent ferrous carbonate.

Splenectomy was done in another series of 16 rats, 8 of which had had fixed tail loops made. Confirming the observations of Lauda (30) the usual splenectomized laboratory albino rat in this region (22) promptly develops an anemia as a result of infestation with *Bartonella.*

Several typical protocols are given.

Rat 9-40, 150 days, 200 gm.: Skin from distal half of tail denuded and tail sutured in peritoneal cavity, Mar. 27, 1935. Heart puncture every second day with removal of 31 cc. of blood between Apr. 1 and 15. caused hemoglobin to decrease to 25 per cent. No further bleeding until the rat was killed on Apr. 30, when weight was 188 gm., hemoglobin 70 per cent. *Intra-abdominal portion*: There is a very dense band of hemopoietic marrow at the metaphysis (Fig. 4).

The epiphyseal spaces are filled with the same tissue and fat has completely receded from them. In the diaphysis there is still fat but this has been markedly reduced; the vessels are engorged and there are many islands of hemopoietic cells but fewer than in the solid bands at the epiphyses. *Outside loop*: almost completely fatty, there are a few scattered clumps of erythropoietic cells in the region of the epiphysis (Fig. 5).

Rat 9-29, 100 days, weight 112 gm., hemoglobin 15 gm.: Splenectomy and formation of fixed tail loop Apr. 24, 1935. Hemoglobin 5 gm. on May 3, Rat killed May 28, when hemoglobin was 9 gm. In the gross the marrow was yellow in the *outside loop* and microscopic examination showed fat with no signs of hemopoiesis. The diaphyseal regions of the *intra-abdominal portion* were also yellow, but there was a 2 mm. broad band of bright red hemopoietic tissue at each metaphysis. Histologically the bright band was seen to be composed of an uninterrupted sheet of hemopoietic cells. In the diaphysis, the fat cells were interspersed with numerous clusters of hemoblasts.

These experiments show very much more hemopoiesis in the portion of the tail which had been situated in the peritoneal cavity than in the outside loop. The effect of this operation on the formation of red marrow is enhanced by the anemia produced regardless of production by hemolysis or blood loss, and in all cases the gross and histological picture was unequivocal in demonstrating an increased medullary activity in the warmer bone marrow.

A further control series of 20 normal rats has been studied where anemia was produced by cardiac puncture, as much as 76 cc. of blood being removed in 47 days, and will be presented in a subsequent report. In 10 of these animals, red marrow formed in the tail in varying amounts and in a similar number no changes could be detected in the fatty marrow, showing that red marrow can form in the tail of old rats under certain conditions, the causes of variation in response being at present unknown. Similar variations in response to anemia were found in red marrow spread in the extremities of dogs (12) and rabbits (32). We wish to emphasize here the greater formation of bone marrow in the locus where the temperature had been elevated as compared with control areas of the tail.

Free Autogenous Transplantation of the Rat Tail (and Foot) to a Warmer Environment

At birth and for some 12 hours after, the tail consists of a series of vertebral elements interspaced with well developed notochordal remains; most of the vertebral mass at the poles consists of flattened

cells of undifferentiated mesenchyma; the equatorial region consists of precartilage, a short row of more swollen hypertrophic cells with small nuclei, but bone and cartilage are absent. At 12 to 24 hours the cartilage becomes swollen, hypertrophic, and calcifies. On the 3rd day the equatorial region consists of calcified cartilage which is infiltrated with hemopoietic bone marrow cells and surrounded at the periphery by bone; proceeding toward the poles there is found hypertrophic cartilage, small flat cartilage cells, and mesenchyma. The 6 day tail has its center filled with a dense accumulation of bone marrow, otherwise bone and cartilage have increased, mesenchyma has decreased. At 13 days the distal vertebrae have lost most of the red bone marrow cells, which have been replaced by fat. The red marrow then retreats centripetally almost to the sacrum. The adult condition in the distal caudal vertebrae, consisting of fat, macrophages, vessels and nerves is reached at 70 to 90 days after birth.

The idea behind the present experiment was that if a decrease of temperature in the outlying tail were responsible for the atrophy of the red marrow, then the tail when transplanted at an early stage to a region where the temperature was persistently warmer than its normal environment, should retain its hemopoietic marrow. Accordingly the tail was amputated as early as possible in neonatal life and transplanted to the peritoneal cavity as a free graft.

Series 3.—Free tail transplants had been made by Paul Bert (26) who amputated 2 mm. of a young rat's tail and transplanted it beneath the skin of another; 2 months later it was twice the original size. Marchand (27) observed growth in free autogenous subcutaneous transplants of the tail in 2 rats.

In the present experiments 19 rats were operated upon 3 to 6 hours after birth. It was helpful to wait until the rat had the stomach distended with milk for technical reasons, because when an incision was made in the peritoneal cavity it was exceedingly difficult to successfully replace the prolapsed viscera. The full stomach, however, may be seen through the abdominal wall; wherever it presented itself a 2 mm. incision was made, usually in the lateral abdominal wall; the tendency of the stomach to herniate obturated the hole so that small viscera could not protrude. The mesenchymal tail was amputated, skinned, grasped by its butt end and pushed inside the peritoneal cavity, the skin was coapted with the fingers and closed with two fine silk sutures.

Unless the skin was removed, the tail died and sebaceous cyst formation was found at autopsy.

The tail quickly became adherent to adjacent tissue; at times a mesentery was developed in which the delicate vessels supplying it were seen; in one instance the experiment failed due to lack of attachment of the graft which was found as a series of pearly calcified cartilaginous beads devoid of soft tissue. The rats were killed at first at 4 day intervals, later weekly and monthly.

The free grafts otherwise survived and went through in normal time relationships the functional changes up to the point of development of red marrow; growth occurred and the evidence is presented in x-rays (Fig. 6). An important difference was thereafter observed in that there was less infiltration of fat, and the red marrow persisted in large amounts for at least 1 year, far past the time when it has entirely receded in the normal tail position.

The significant data observed in the development in these free grafts may be summarized as follows:

4 day graft: Hemorrhage was seen in the connective tissue surrounding the vertebrae; areas of the cartilage showed impairment of nutrition by staining palely but most of the graft was alive and calcified cartilage was seen in the middle of the segment.

8 day graft: There was a size increase in the surrounding connective tissue. Small necrotic areas were seen, but occupied proportionately less of the graft than at 4 days; the necrosis occurred usually along one side of the graft. The notochordial remains appeared normal, but were distorted by the cartilage necrosis so that they did not occupy the midline. Bone marrow cells infiltrated the center of the calcified cartilage as in the normal 6 day tail. A slight amount of bone was seen.

15 day graft: This showed almost complete disappearance of the intervertebral disc fibers; in this and all subsequent sections there was resulting fusion of the cartilage masses of adjacent vertebrae which in later stages became replaced by bone. Extensive hemopoietic bone marrow was found partially infiltrated with fat; the cellular bone marrow consisted of nucleated red cells, megacaryocytes, and all other hemoblast precursors. The bone was extensively developed, trabeculae had appeared.

24 day graft: In addition to the findings at 15 days no necrotic elements were seen. Development of the epiphyses had occurred and these contained slight cellular marrow. There were, however, much more extensive deposits of hemopoietic marrow in the main vertebral body, most extensive at the metaphyses, but also in the shaft where they were somewhat diluted by fat infiltration.

60 day graft: The vertebrae were united by bone where there had been carti-

laginous fusion. There was greater infiltration of fat than at 24 days. Much hemopoietic tissue was still present, although there was some fat in the diaphyses.

100 day graft: There has been a great increase in growth in the tail vertebrae. Dense hemopoietic aggregates fill the epiphyseal regions and a part of the diaphysis. There has been a slight increase in the amount of fat present in the marrow, mostly in the diaphysis as compared with the 60 day specimen, but the fat content is much less than in a normal adult rat tail.

205, 256, 311, 359 day grafts: These specimens resemble each other and the 100 day graft. Bone, cartilage and notochordal remains persist with living cells. The metaphyses are filled with hemopoietic tissue chiefly of the granulocytic series but with many red cell forms and megacaryocytes (Fig. 7). The epiphyseal marrow spaces contain a slight amount of fat but much cellular marrow. The diaphysis contains mostly fat with some red marrow cell clusters.

In this experiment then, the tail was amputated at a period before bone or bone marrow had developed and transplanted to the peritoneal cavity where the temperature greatly exceeded the temperature of the tail. The graft survived and developed essentially normally, aside from an initial minor necrosis. The bone marrow maintained in very large part its red marrow in the epiphyseal and metaphyseal regions where it would have undergone complete fatty substitution in the normal location. Fatty marrow developed in the middle of the diaphysis just as it would have in the normal position. There was thus found a fundamental difference in the development and retention of hemopoietic marrow of the tail which developed in the peritoneal cavity as compared with the marrow of the tail developed in its normal site.

The circulation in the rat tail at birth is very slight and this structure could be amputated with loss of only a tiny drop of blood. The circulation of the grafts was obtained from any neighboring structure and the avascular cartilage developed a complex sinusoidal circulation, showing that the capacity to form sinusoids is determined by the cartilaginous mass rather than the ingrowing endothelium. In other words, the character of the tissue determines whether the ingrowing endothelium is sinusoidal or not.

Since the foot is an outlying region in which hemopoiesis yields to a fatty marrow during development, as a variant to the experiment just reported, in 18 rats at birth one foot was amputated, skinned, and inserted in the peritoneal cavity. An adequate control area was provided in the unamputated foot. In the transplant usually the phalanges disappeared but tarsus and metatarsus survived.

After 60 days, a great difference was observed in the hemopoietic bone marrow of the graft (Fig. 8) as compared with the fatty marrow of the normally developed foot (Fig. 9).

Free Transplantation of the Rat Tail to Other Rats in Warm and Cooler Loci

Series 4.—In this experiment 16 litters of rats were used within 6 hours after birth, when the tail of each rat was amputated, denuded of skin, and transplanted to either the peritoneal cavity or the dorsum of the foot of an adult rat under ether anesthesia. Both the mother and another rat were used in each case. It must be emphasized that the stock had been greatly inbred in this rat colony. The histological results in 35 grafts are reported, 20 to 150 days after transplantation.

The results may be divided into three groups. Nine grafts, all placed in the peritoneal cavity of the mothers, developed exactly like autogenous grafts in this location; they formed well organized vertebrae with cartilage, bone, and hemopoietic bone marrow which was retained in the epiphyses and metaphyseal regions as long as the experiment lasted. The red marrow (Fig. 10) consisted of erythroid and granulocytic elements with many megacaryocytes. The diaphysis was filled with fat for about one-half of its extent. Those grafts where red marrow formed were characterized by good anatomical organization. Nine grafts, all implanted in the feet of mothers developed equally well and contained completely fatty marrow (Fig. 11) after 5 months when the abdominal grafts of litter mate origin all had red marrow.

Five grafts with equally good cartilage and bone developed a decidedly different marrow from these consisting of a diffuse protein framework with many similar nonphagocytic mononuclear basophilic cells without differentiation into erythroid or granulocyte cells. There was a slight fatty infiltration in this marrow.

Twelve grafts resembled each other and were quite different from the groups just described in that cartilage and calcified cartilage formed with slight or no bone formation. The anatomical organization was poor and epiphyseal cartilage separated by rather large masses of fibroblasts was seen where bone marrow is normally present. While some of the cartilage and calcified cartilage was dead (absence of cells) much of the graft survived (Fig. 12). In these areas there were seen clusters of a very primitive myelocytic type of marrow with macrophages, but without erythroid elements.

In one-half of the grafts not developing this bizarre non-bony tissue, or primitive marrow, the differences in content of red and yellow marrow were related to abdominal and foot position and were exactly predictable on a thermal basis. The causes of the difference in reaction between grafts forming organized chambers and grafts forming disorganized cartilage remnants without bone could not be predicted. The disorganized state was seen when grafts were made to the mother as well as to some other animal, and it occurred in the abdomen as often as in the foot. Moreover it occurred in some of the tails of members of a litter grafted in a given host, while those of other litter mates in the same host formed normal appearing units. It is clear that cartilage was successfully grafted and grew in every instance; the defect seems to be in subsequent development and may be related to tissue groups somewhat analogous to the Landsteiner blood group effect, since red marrow makes erythrocytes.

Influence of a High Environmental Temperature on Bone Marrow

In order to exclude all operative procedures, 40 albino rats were kept at an elevated environmental temperature (33–36°C.) for 21 to 70 days. It was impossible to keep rats at 38°C., since all died in 2 to 4 days with lung infections. Bone marrow of feet and tail were studied at the conclusion of the experiment. Control rats for each age and sex were kept.

Method.—The rats were placed in an incubator, consisting of a wood box 4 x 2 x 2 feet in dimensions, equipped with an electric heating coil built in the roof and insulated with asbestos. Temperature control was obtained by an aneroid thermostat. The box had glass windows on three sides, and electric lighting was uninterruptedly maintained. Temperature was measured by a thermo-electric couple and a mercury thermometer. For ventilation one of the glass windows was replaced by a perforated fiber board. The temperature range was 34.2–1.8°C. The air was agitated continuously with an induction motor fan. No attempt was made to control humidity, which was probably low; the drinking water, always adequate in amount and changed twice daily, was kept in large inverted stoppered bottles furnished with a glass tube as outlet. The box was kept in a very small room without windows or heat, used for no other purpose. Food consisted of a mixture of dried meat, milk, and grain with cod liver oil (purina fox chow). Hemoglobin determinations were made on blood removed by heart puncture at the beginning and end of the experiment.

Series 5.—Typical protocols of three groups with controls are furnished in

Table I. All of the older rats lost weight while in the incubator, while young rats placed in the box after weaning, gained weight, but at a slightly slower rate than litter mate controls. There was marked polydipsia.

In many rats the testes showed profound atrophy involving all of the spermatogenic series of cells with giant cell formation in the testis tubules. In approximately one-third of the rats, either slight cytolysis or no change was detected in the testes.

TABLE I

*The Effect of Elevated Temperature on Bone Marrow of Feet, Tail, and on Testis of Rats Kept in an Incubator**

No.	Sex	Age	Time in incubator	Environmental temperature variation	Histological estimate of hemopoiesis	Carbon deposition in tail marrow	Carbon deposition in foot marrow	Histological estimate of testis atrophy	Weight change	Remarks
		days	*days*	*°C.*					*gm.*	
1	♂	163	33	32.8–35.5	++	+++	++	No atrophy	−6	
2	♂	163	33	32.8–35.5	+	++	++	Partial	−34	
3	♂	163	33	32.8–35.5	++	+++	+	Partial	−20	
4	♂	163	—	16–26	None	None	None	None	+24	Control for 1, 2 and 3
5	♂	270	24	33–36	Trace	++	++	Profound	−30	
6	♂	270	24	33–36	Trace	Trace	+	Profound	−56	
7	♂	270	24	33–36	+	+	+	Profound	−46	
8	♂	270	24	33–36	Trace	+	+	Profound		
9	♂	270	24	33–36	+++	+++	+++	Partial	−45	
10	♂	270	—	16–26	None	None	None	None	+18	Control for 5 to 9
11	♂	>365	23	33–36	++	+++	++++	Profound	−126	
12	♂	>365	22	33–36	+	++	++	—	−158	
13	♀	>365	23	33–36	++	++	++++	—	−77	Ovariectomized
14	♀	>365	—	18–26	None	None	None	—	+18	Control for 11 to 13

* ++++ indicates maximal hemopoietic response.

The bone marrow of the feet showed hyperplasia in all of the animals. The marrow of the tail showed slight or moderate hyperplasia in each case but in no case did it approach the large amounts seen in operations where marrow was maintained at peritoneal temperature. The changes consisted of formation of clumps of cells at the metaphysis, mostly of the myelocytic and macrophage series; slight erythropoiesis and megacaryocyte formation was observed. In young animals inserted in the box after weaning, infiltration of fat occurred in the

diaphysis as in the previous experiment; the metaphyseal marrow cells were well preserved.

DISCUSSION

The evidence presented has dealt with the formation and maintenance of hemopoiesis in bone marrow normally yellow after the developmental period. The experimental procedures were designed to and indeed did produce an elevation of temperature of yellow marrow chambers. An effect occurred in each case resulting in blood cell formation in fatty marrow. We feel therefore that the results support the hypothesis that temperature variations of the order present in the extremities of normal mammals and birds (29) significantly affect marrow activity.

In addition to the experiments several supporting facts can be adduced which derive from previous findings. The observation that all bone marrow in newborn mammals is red supports the suggestion that the abdominal warmness is a factor in determining marrow distribution, especially since red marrow regresses soon after birth in cooler skeletal areas. Knipping (34) found in 2 dogs that recovery from the hemolytic anemia caused by pyrodine was more rapid in tropical than in temperate climates. Barcroft and coworkers (36) found an increase of reticulated red cells in the circulating blood of two men kept for several days in a glass chamber at 32–35°C. The observations of Sasybin (37) on animals and man exposed to very high temperatures are not comparable with the present experiments; red marrow spread occurred in all of the bones during the period of disturbed compensation to excessive heat, and an anemia was present.

In general there are two ways in which a physiological elevation of temperature may affect the bone marrow, namely a primary effect on tissue metabolism and a secondary vasomotor effect; while both effects are presumably operative, no evidence could be derived from these experiments as to the mechanism by which an elevation of temperature facilitated hemopoiesis.

In the first place, it is chemically reasonable to suppose that tissue fabricating large molecular compounds like hemoglobin, complex nucleic acids, and antibacterial agents would be greatly affected by fluctuations of temperature of the order of 6–10°C., as occur in normal

bone marrow (29). Changes in temperature in an organism are in general very important: "on thermo-dynamical grounds this fact is not difficult to understand and its biological implications are manifest" (38). In blood itself, with variations of temperature there are appreciable changes in the masses of the components as well as in the solubilities of gases and in chemical affinities; there occurs on lowering of temperature a marked decrease of dissociation of oxyhemoglobin (23) as well as changes in carbonic acid-bicarbonate concentrations and pH (39, 40). The van't Hoff (43) law concerning the increased velocity of chemical reactions with temperature increase apparently applies to general metabolism in man with increased temperatures (35). Tipton (33) found an increased metabolism of nucleated vertebrate red cells with temperature increases.

Other complex structures are known to be affected by thermal differences of this order. Moore (24, 25) found that the testis is adversely affected by thermal increase to the body-trunk level; the spermatic cells of the testis atrophy or fail to develop when this structure is exposed to a climate as warm as the peritoneal cavity, as was also found in our experiments at a slightly lower thermal level. Schultz (41, 42) found that the black winter pigmentation of the body tips in the Russian rabbit could be produced by experimental application of cold.

It is common knowledge that vascular tonus responds to increase of temperature by relaxation and vasodilatation. In the warm environments in the present experiments, red marrow formed in the free grafts in connection with a newly formed circulation, and in the other experiments it formed or was preserved with the original circulation of the marrow intact. It was found that red marrow was located chiefly in the epiphyseal and metaphyseal regions, undoubtedly because of circulatory effects since the circulation of tail marrow (Fig. 13) shows vastly greater capillary accumulations here than in the diaphysis, even when the whole chamber contained only fatty marrow. The reason for this vascular accumulation in these regions needs further analysis. The retention of red marrow longest in the epiphyseal regions of marrow chambers where capillaries are most concentrated confirms the widespread view of many earlier writers, but it must be emphasized that the normal distribution of red marrow is not purely a question of

epiphysis *versus* diaphysis; for example, while the diaphysis contains red marrow the lowest epiphysis of the mature dog femur usually contains yellow marrow. Epiphyseal predilection accounts for the fact that red marrow often is held longer in these regions than in the shaft, but it in turn yields to segmental regression as the extremity matures.

It is our conception, based on the experimental evidence, that there are two types of fat infiltration in marrow. The first type present in outlying regions of the skeleton, is chiefly a local topographic effect, and was overcome in these experiments presumably due to the elevation of temperature. The second type is generalized and involves the central bones in healthy adults, and is probably related to advantageous or disadvantageous local sinusoidal effects (Fig. 13), the physiology of which is unknown. This is the effect by which fatty infiltration in the diaphysis of free tail grafts in warm loci is produced, as well as the epiphyseal location of hemopoiesis in tail loop experiments. This is the effect on which the transitory retention of red marrow in the epiphyses as well as fat infiltration in deep bones may be explained. The epiphyseal predilection accordingly is explainable on the basis of a more favorable vascular effect in this region than in the diaphysis. The fatty infiltration in normal bones of the body trunk is due to a less favorable vascular effect than in the non-fatty regions. The second or generalized type of fatty infiltration depends to some extent on the state of nutrition of the animal, especially with respect to the *Bausteine* of blood (32, 47), since in starvation fatty marrow is replaced by a gelatinous non-fatty condition. It also depends on the oxygen tension of the blood, since bone marrow fat decreases in anemia and on mountains. It is possible on an experimental basis to produce differences as demanded by this theory. (*a*) In local tail loop, incubator, and free abdominal grafts, red marrow is produced or maintained in epiphyseal regions but not in diaphysis, since the generalized vascular mechanism is not favorable. (*b*) In anemia in many cases (12, 44, 45) and under diminished oxygen tension as at high altitude (46), red marrow spread does not uniformly involve all outlying bones, since local conditions (thermal decrement) interfere. (*c*) In the present experiments in anemia in animals where a portion of the outlying marrow is at an elevated temperature, all of the warm outlying bone is involved in marrow hyperplasia.

Referring again to the testis as affected by heat, it may be seen that the bone marrow presents a converse situation to this gland. Under physiological conditions a *maximal* temperature exists for the testis and increase beyond this abolishes gametogenesis, whereas in the bone marrow a *minimal* temperature affects hemopoiesis adversely.

The difference in response to homogenous grafting of rat tails to other rats deserves a word of comment. Cartilage apparently was always successfully grafted to non-donors, but in many animals further differentiation into bone is absent, and poorly organized cartilaginous remains with an exceedingly primitive marrow result. If this effect is due to tissue cell groups, obviously the defect comes in differentiation rather than in cartilage growth.

SUMMARY

A great difference exists in the adult bone marrow of central bones as compared with outlying bones of the mammalia and avia, the distal bones being at a great disadvantage from the standpoint of blood cell production. Several experimental procedures are reported by which this disadvantage is overcome and in consequence fatty marrow of outlying bones is replaced by red marrow occurring chiefly at the epiphyseal regions, unless a low oxygen stimulus is also provided when marrow of the diaphysis becomes involved. A common factor in all of the experiments was an elevation of temperature beyond that prevailing in these distal regions, and it is felt that the evidence warrants the opinion that the cause of improvement is thermal. In some experiments, blood cell formation was increasing while the heat was adversely affecting the testis. The experiments permit construction of a general theory of fat distribution in bone marrow.

In certain grafts of precartilage to other rats, normal differentiation into bone, cartilage, and marrow occurred, while in others cartilage and very small amounts of primitive marrow developed with slight, or no bone formation. Cartilage was always successfully engrafted.

The capacity to form sinusoids in bone marrow is determined by the nature of the tissue rather than by the ingrowing endothelium.

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EXPLANATION OF PLATES

Photographs of sections stained with hematoxylin-eosin. Except in Figs. 8 and 9, a portion of epiphyseal cartilage (C) is shown to provide comparable loci. All specimens were decalcified with 5 per cent HCl, except Figs. 6 and 12.

PLATE 13

FIG. 1. Rat B, series 1, tail loop operation: Portion of the tail inserted in peritoneal cavity for 86 days. × 220.

FIG. 2. Rat B, series 1, tail loop operation: Outside loop region of the tail of same rat as Fig. 1, a completely fatty marrow. × 220.

FIG. 3. Rat 8-85, series 1, tail loop operation: Portion of the tail inserted in peritoneal cavity for 38 days, showing many dilated capillaries and moderate hemopoiesis. × 220.

FIG. 4. Rat 9-40, series 2, tail loop operation in anemia: Portion of the tail inserted in peritoneal cavity for 34 days; 31 cc. of blood removed by heart puncture in first 14 days after operation. × 310.

PLATE 14

FIG. 5. Rat 9-40, series 2, tail loop operation in anemia: The outside loop region of the same rat as Fig. 4 (short loop). × 390.

FIG. 6. X-rays of end-results in series 3, following free transplantation of tail to peritoneal cavity with a control specimen (½ day). The x-rays shown were made at the same focal tube distance of specimens removed 9 to 100 days after grafting; no calcification was present in the control tissue and the development and growth of the bony units may be seen.

FIG. 7. Series 3, autogenous free transplantation of tail to peritoneal cavity after 359 days. This rat had been injected with India ink 4 and 2 days before necropsy, and carbon granules can be seen in some of the macrophages. × 640.

PLATE 15

FIG. 8. Series 3, autogenous free transplantation, 4 hours after birth, of foot to peritoneal cavity after 167 days, showing dense marrow accumulation in heel bone. × 110.

FIG. 9. Series 3, showing marrow of heel bone, of the control unamputated foot of the same rat as in Fig. 8. × 110.

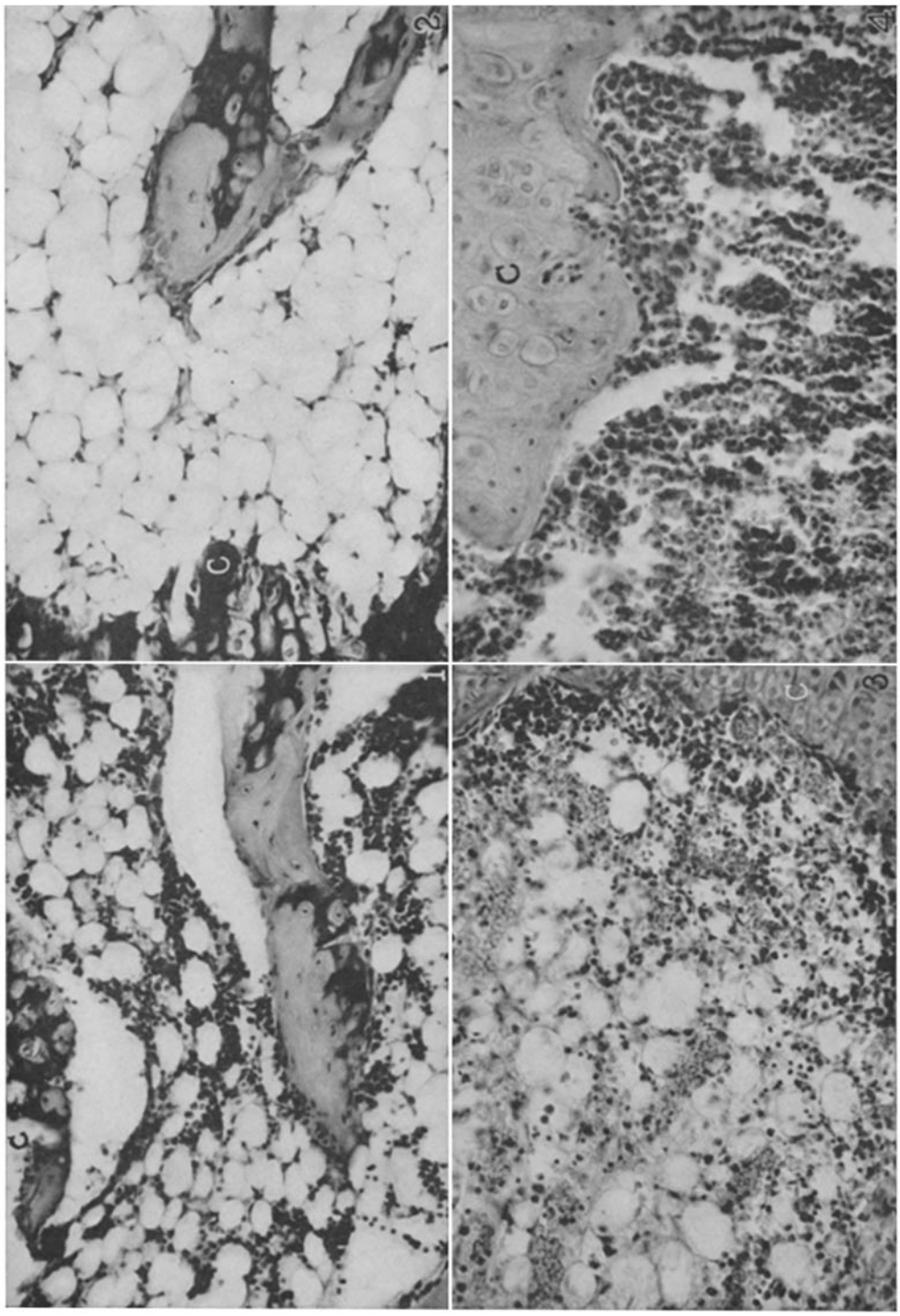
FIG. 10. Series 4, homogenous transplantation of tail at birth to the peritoneal cavity of mother, after 182 days. × 110.

PLATE 16

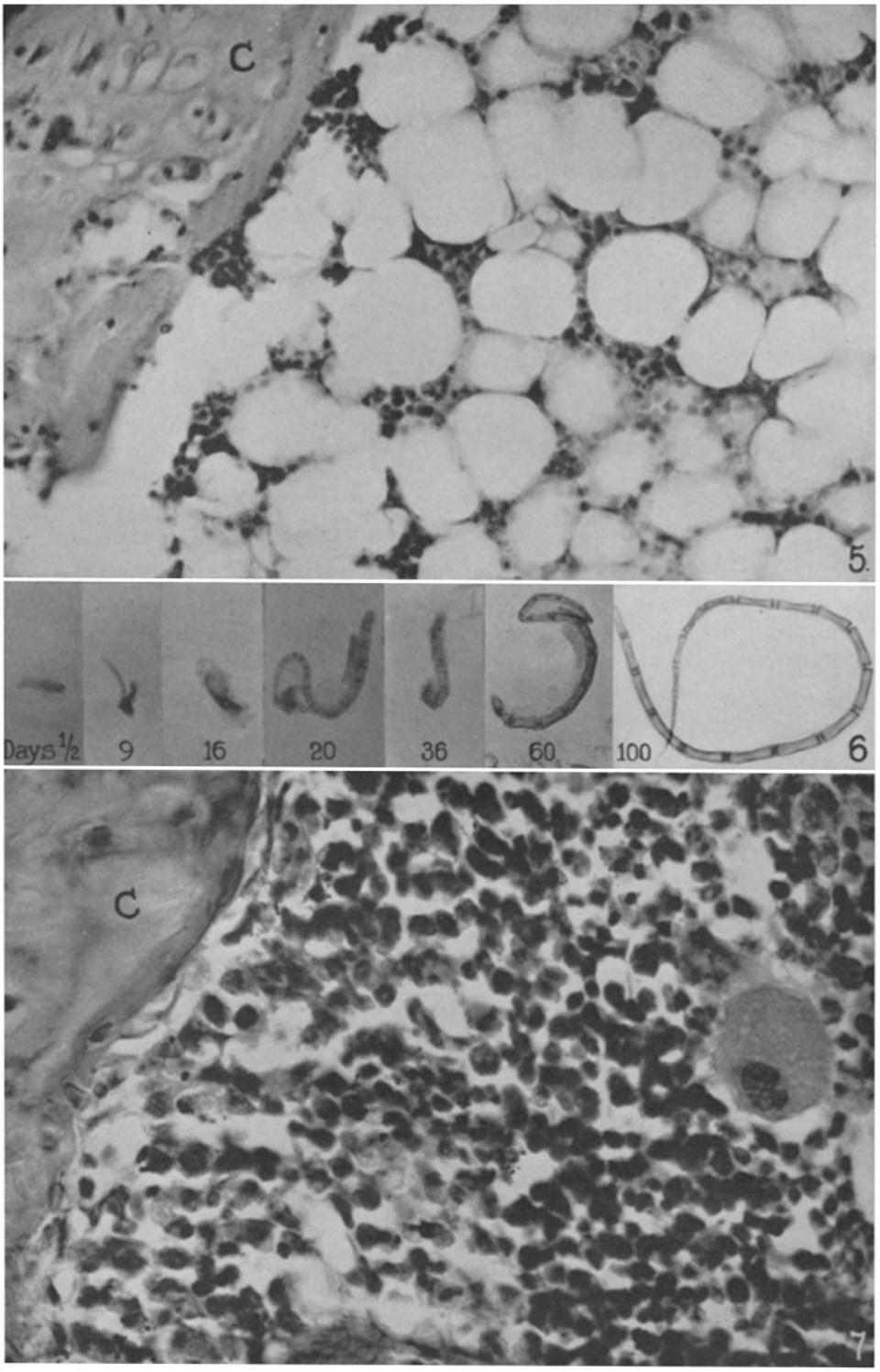
FIG. 11. Series 4, homogenous transplantation of tail at birth to foot of mother after 182 days: The tail was transplanted from a sibling of that illustrated by Fig. 10, to the same recipient. × 690.

FIG. 12. Series 4, homogenous transplantation of tail at birth to abdomen of mother after 150 days: This shows the atypical formation of cartilage (C), calcified cartilage (CC), and dead calcified cartilage (DCC). No traces of bone were found.

FIG. 13. Cleared specimen showing circulation of tail in an old rat. This rat was injected with 17 cc. of 20 per cent ink during the period until death occurred, 5 minutes after beginning the injection. Although the marrow of these vertebrae was yellow, much greater capillary accumulations of ink are evident in the metaphysis and epiphysis than in the diaphyseal region.



(Huggins and Blocksom: Temperature increase affecting bone marrow)



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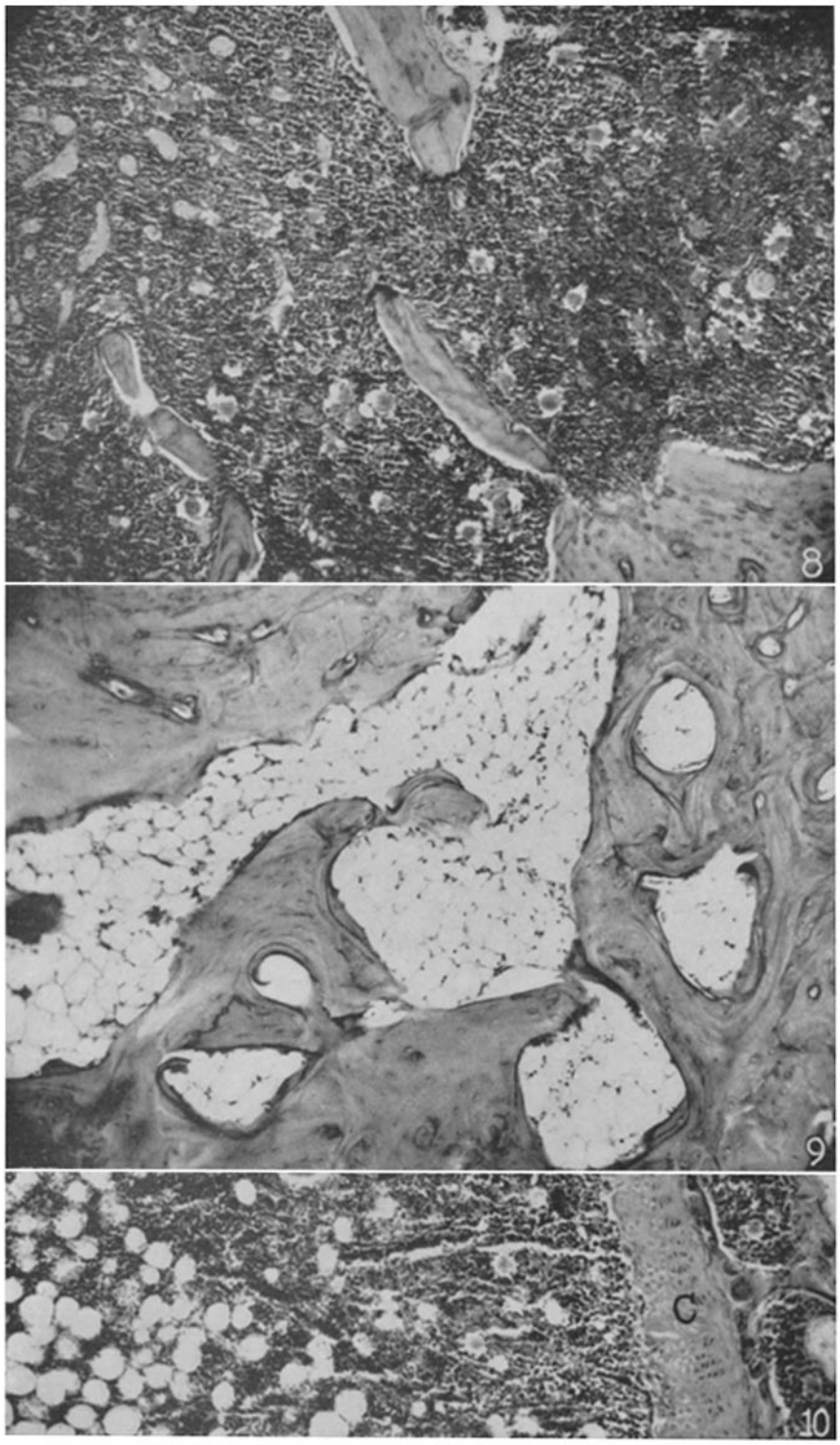

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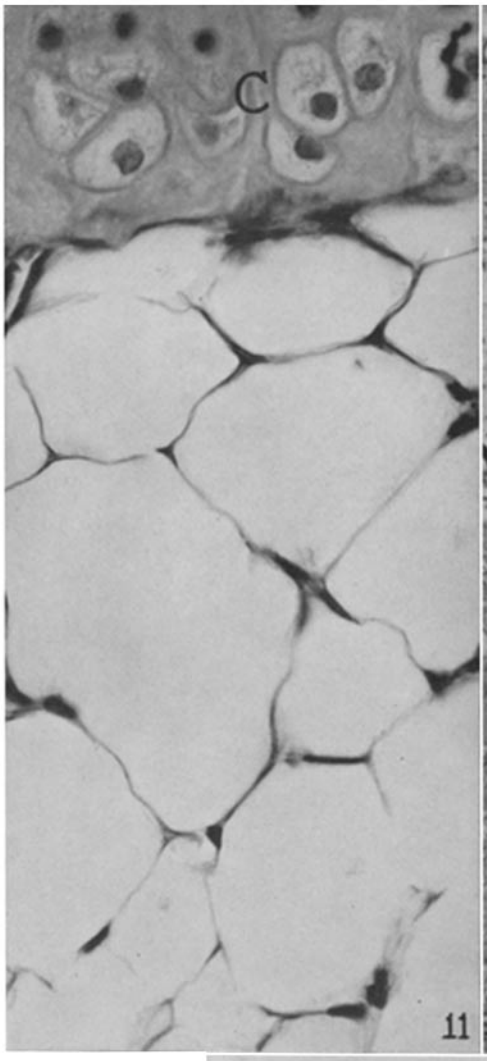

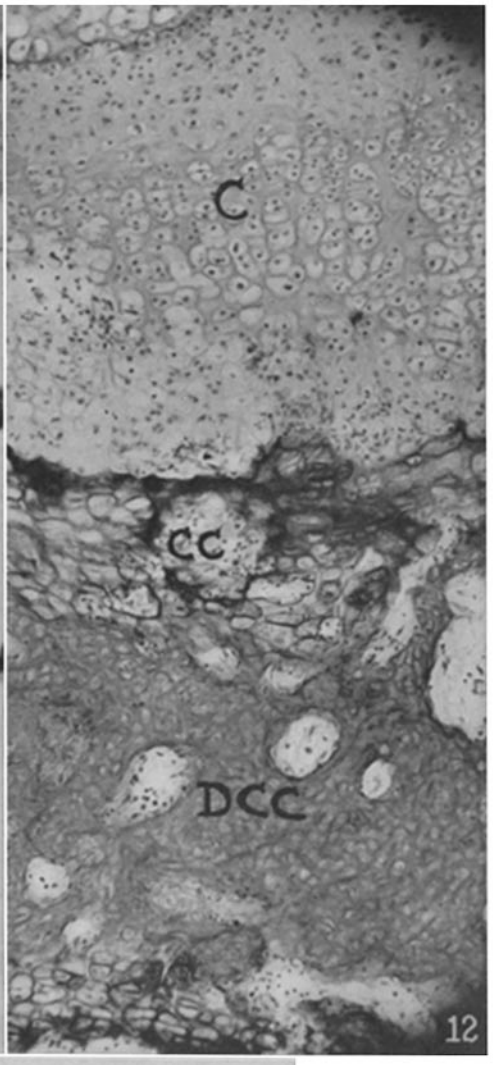

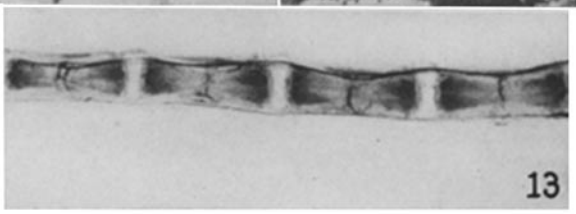

(Huggins and Blocksom: Temperature increase affecting bone marrow)