ing colchicine to hexaploid wheat which possessed an isochromosome. Isochromosomes are distinctive in that two homologous arms are joined to a common centromere.

Pollen mother cells of the variety Chinese Spring, monoisosomic for the long arm of chromosome 5D, were scored for the frequency with which the homologous arms of the conventional chromosomes entered chiasma formation and the frequency with which the homologous arms of the isochromosome entered chiasma formation. These frequencies were determined in untreated material and in material which had been treated with 0.03 percent aqueous colchicine 7 days before fixation. Colchicine was injected into the uppermost leaf sheath with a hypodermic syringe.

The length of the long arm of chromosome 5D (3.1 μm at metaphase I) is quite similar to the average length of wheat chromosome arms (2.9 μm), as calculated from Sears (*3*).

In the untreated monoisochromosome $5D^L$, the mean number of homologous arms of the conventional chromosomes that entered chiasma formation per cell was 38.55 (Table 1). If differences in lengths of chromosome arms are disregarded and it is assumed that these 40 pairs of homologous arms enter chiasma formation with equal probabilities, the average frequency with which one of these pairs of arms forms a chiasma is 0.96. The infrequent instances of a second chiasma in the same pair of chromosome arms is ignored in this study, as emphasis is placed on whether two homologous arms enter at least one chiasma.

The isochromosome in the untreated material formed an interarm chiasma with about the same frequency (Table 1). Thus, being attached to the same centromere does not normally afford two homologous arms a greater chance of entering chiasma formation.

In the monoisochromosome $5D^L$ material treated with colchicine, the homologous arms of the conventional chromosomes behaved differently to the homologous arms of the isochromosome. In this case the mean number of arms of the conventional chromosomes that formed a chiasma per cell was 17.59. On the same assumption as above, the frequency with which one of these pairs of arms forms a chiasma equals 0.44. This is much less frequent than in the control. In these same cells the homologous arms of the isochromosome formed a chiasma with a frequency of 0.96. Thus, they were unaffected by the treatment.

The $5D^L$ isochromosome can be considered representative of isochromosomes in general as similar results were obtained with monoisochromosome 7D (standard arm) after colchicine treatment, that is, almost complete formation of interarm chiasmata between arms in the isochromosome in otherwise highly asynaptic pollen mother cells. Thus, in the case of colchicine-treated material, being attached to a common centromere does afford two homologous arms a greater chance of entering chiasma formation.

The immunity of the homologous arms of the isochromosome to colchicine is due to the fact that the arms bear a close spatial relationship to each other, at least in the regions close to the centromere. Once this requirement is satisfied, colchicine does not affect the remaining stages of chromosome pairing.

This establishes at least two separable stages of chromosome pairing. The first, which results in a closer spatial relationship of homologs, is susceptible to colchicine; the second, which results in synapsis and chiasma formation, is immune to colchicine. Colchicine is known to destroy microtubules (*4*), thus perhaps the attainment of a close spatial relationship of homologs requires the participation of microtubules.

Although excess dosage of chromosome $5B^L$ of hexaploid wheat and colchicine have similar effects on chromosome pairing, there are, however, obvious differences. With excess $5B^L$ the majority of bivalents are rod bivalents, and interlocking of bivalents occurs frequently (*1*). After colchicine treatment there are fewer bivalents per cell, and the majority are ring bivalents. In this material interlocking of bivalents was not induced by colchicine.

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Bone Marrow Histogenesis: A Comparison of Fatty and Red Marrow

Abstract. *Histogenesis of fatty marrow in extramedullary sites was compared to histogenesis of the red marrow. Both tissues undergo a similar sequence of events leading to reconstruction of hemopoietic marrow nodules. Histogenesis of yellow marrow then continues; such implants become nodules of fatty marrow, whereas implants of red marrow remain hemopoietically active. This observation supports the concept that histogenesis of the bone marrow recapitulates the ontogeny of this organ and indicates an intrinsic difference between the stroma of red and yellow marrow.*

Substantial evidence indicates that the specialized stroma of the marrow is essential to hemopoietic proliferation. Without this stroma, hemopoiesis does not exist (*1*, *2*). A distinction should therefore be made between the marrow's stroma and the product of its function, hemopoietic proliferation. The marrow, in essence, is two organs, one dependent upon the other. Characteristic of this stroma is its ability to repair itself after it has been damaged (*2–4*). The repair takes place in a series of well-defined steps resulting in reconstruction of the stroma, followed by reestablishment of hemopoiesis. This process, histogenesis of bone marrow, appears to recapitulate the embryogenesis of this tissue (*3*).

During embryogenesis of bone marrow (*5*) cells originating in a layer of mesenchyme invade the primordial marrow cavity. The mesenchymal cells differentiate into osteoblastic elements and give rise to a lattice of osteoid tissue and trabecular bone. Within the bony interstices marrow develops as fixed mesenchymal cells and very wide sinusoids. Soon precursors of various blood cells begin to appear and proliferate.

Several models have been used to

study postnatal histogenesis of marrow. Removal of marrow and injury to marrow in situ are examples of such models (*3*). We implanted marrow into extramedullary sites in order to study its histogenesis in different environments (*2*).

Histogenesis of marrow has been described (*2*). During the first 24 hours, an implanted fragment of marrow becomes depleted of hemopoietic elements leaving behind a network of marrow stroma. At the same time this stroma becomes surrounded and invaded by blood vessels. By day 4, stroma cells proliferate, giving rise to a monotony of cells similar to mesenchymal cells (Fig. 2). These cells, which originate in the implanted tissue and not in supporting tissue (*6*), differentiate into osteoblastic elements and begin to produce osteoid substance. By 2 weeks, the implant consists of a lattice of trabecular bone. Within the interstices of this lattice, a sinusoidal microcirculation appears, consisting of large, thin-walled, highly anastomosing vascular channels (Fig. 3). At this stage, the marrow stroma is ready to support the proliferation of hemopoietic elements which now appear. Resorption of the trabecular bone follows. By 5 weeks the implant consists of a hemopoietic nodule surrounded by a thin shell of bone (Fig. 4).

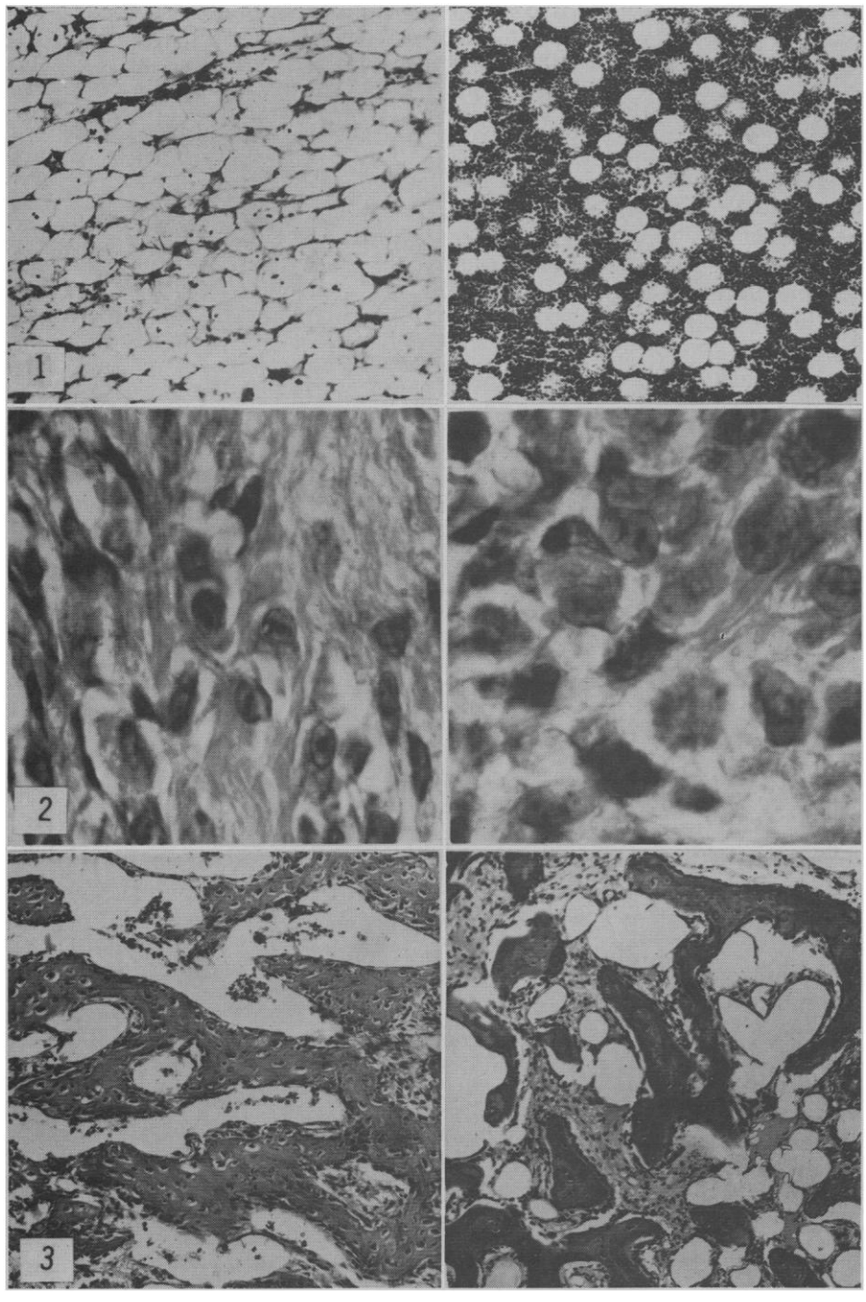

Fig. 1. Rabbit marrow before implantation. (Left) Fatty marrow obtained from distal tibia; (right) red marrow obtained from proximal femur (× 100). Fig. 2. Nine days after implant at the stage of proliferation of stromal cells. (Left) Fatty marrow; (right) red marrow; subcutaneous sites (× 1000). Fig. 3. Twelve days after implant. Sinusoidal structure of the marrow is being formed within the interstices of trabecular bone. (Left) Fatty marrow; (right) red marrow; splenic sites (× 120).

To test whether the marrow's ontogeny is being recapitulated we studied the postnatal histogenesis of fatty marrow. In distal bones, marrow ontogeny continues after birth (*7*). Hemopoietic tissue in these bones undergoes fatty involution. In the adult human, for example, hemopoiesis is limited almost entirely to bones of the torso; bones of the extremities contain fatty marrow as do the distal bones of the rabbit's extremities (*8*).

Mature New Zealand white rabbits (3 kg) were maintained under standard laboratory conditions, with ambient temperature at 25°C. Fragments of red marrow were obtained from the proximal femoral cavity as previously described (*9*). Implants were made on the splenic surface and in the subcutaneous tissue of the abdominal wall. Fragments of yellow marrow were obtained from the tibia and implanted in the subcutaneous tissue of the abdominal wall and on the surface of the spleen, often side by side with hemopoietically active marrow implants from the proximal femur. The relative intensity of hemopoiesis in the specimens for transplantation was evident from gross appearance; however, smears were stained, and the cellularity of the specimens was evaluated microscopically. Only specimens full of hemopoietic cells were included in this study as representative of red marrow. Conversely, only those specimens devoid of recognizable hemopoietic cells were used as fatty marrow (Fig. 1). The implants were removed periodically, decalcified and studied in histological sections stained with hematoxylin and eosin. In the histogenesis of both the fatty and the red implants the sequence of events is similar (Figs. 2 and 3). After 5 weeks the implants of both have become marrow nodules containing hemopoietic tissue (Fig. 4). During the subsequent weeks, however, in the nodules which derive from fatty marrow, the cytoplasm of some stromal cells undergoes

a gelatinous transformation and begins to accumulate droplets of fat. These droplets coalesce, forming larger droplets, and the fat cells increase in girth and number. At the same time hemopoiesis diminishes (Fig. 5). Sixty to 90 days after implantation, an implant of yellow marrow consists of a nodule of fatty tissue (Fig. 6, left). At this stage no hemopoiesis is distinguishable in these nodules. The implant of red marrow, however, remains hemopoietically active (Fig. 6, right). We have observed these nodules for up to 6 months and they remain unchanged. In hundreds of experiments, so long as the site of implantation was well vascularized, the implants were uniformly successful and similar to one another (*9*). The appearance of implants within the spleen and those beneath the skin, at any stage of the reconstruction, was the same; hemopoietic marrows always produced hemopoietic nodules, whereas fatty marrow produced fatty nodules.

Our observations support the concept that the histogenesis of bone marrow implanted in the postnatal period recapitulates the marrow's ontogeny. Furthermore, the behavior of implants of red and fatty marrows in a common environment indicates an intrinsic difference between these two types of tissue. Differing environmental conditions, which may affect the hemopoietic activity of the marrow (*10*), cannot account for our observations. The varied product of the two transplanted tissues represents a fundamental difference of the proliferating cells which reconstruct the nodules. Each represents a different epigenetic system or epigenotype (*11*).

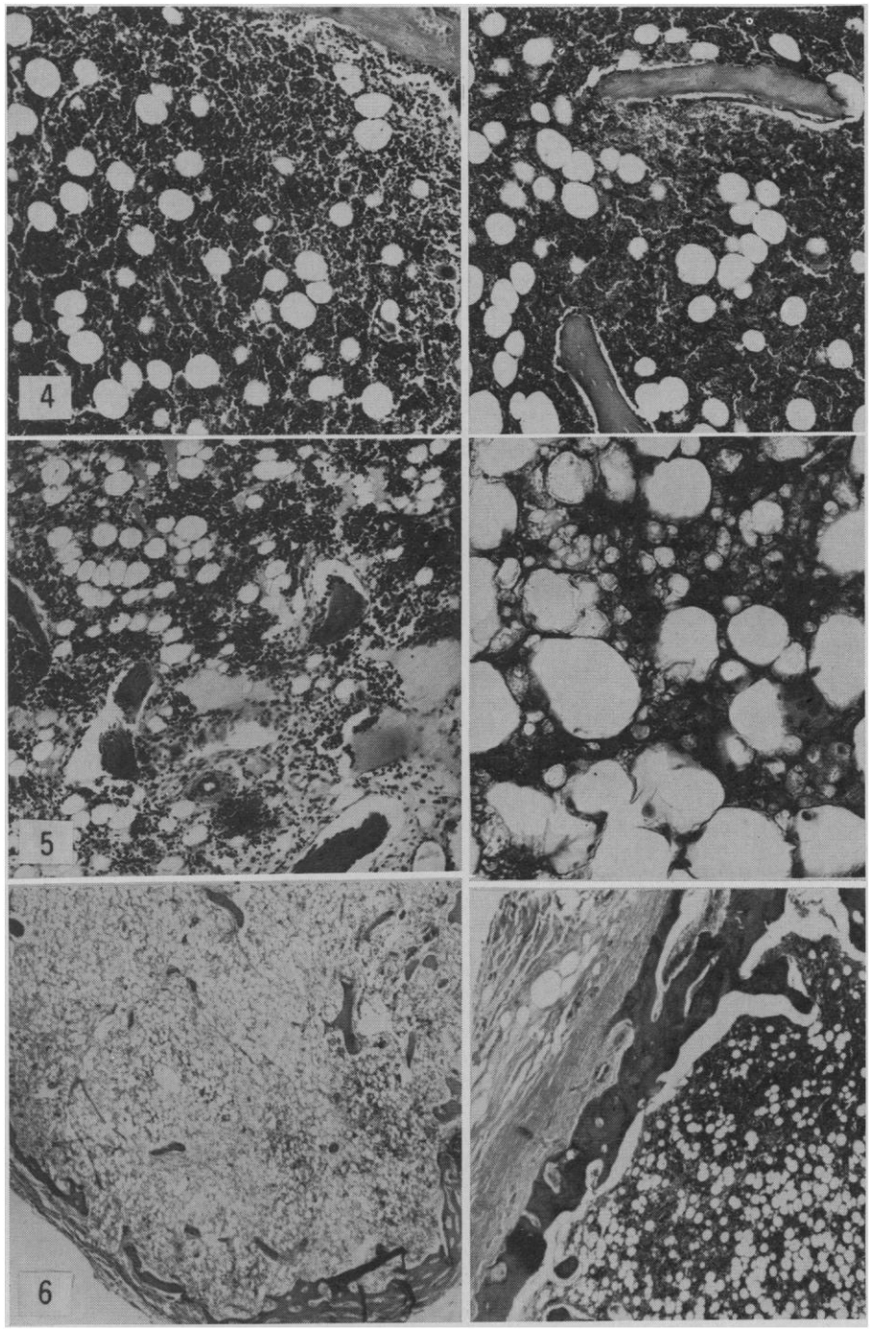

Fig. 4. Five weeks after implant. Hemopoiesis is established within bony capsules. (Left) Fatty marrow; (right) red marrow; subcutaneous sites (× 80). Fig. 5. (Left) Six weeks after implantation of fatty marrow. Hemopoiesis is diminishing, gelatinous transformation and accumulation of fat droplets are seen in the marrow stroma; subcutaneous site (× 80); (right) 7 weeks after implantation of fatty marrow. Fatty involution in a more advanced stage with droplets of fat coalescing to form larger droplets; subcutaneous site (× 200). Fig. 6. Two months after implantation. A thin bony shell surrounds each nodule. (Left) A nodule of fatty tissue is established from implant of fatty marrow; splenic site (× 16); (right) a nodule of hemopoietic tissue results from implantation of red marrow; subcutaneous site (× 16).

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