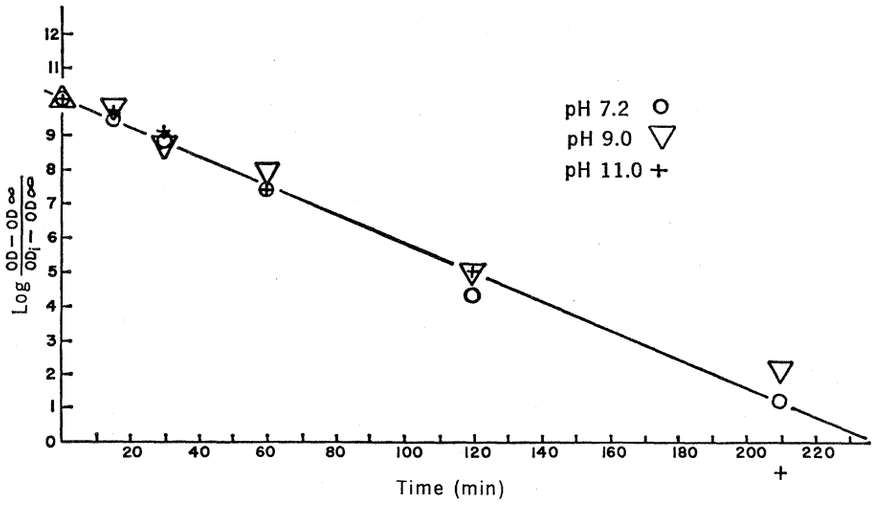

Fig. 1. Kinetics of the hydrolysis of cyanovinyl phosphate at 100°C.

cyanovinyl phosphate) by heating 0.7 ml of 0.75*M* sodium cyanovinyl phosphate and 1.6*M* uridine (containing 0.1 curie of C^{14}-uridine) at 60°C for 18 days. The UMP was detected by the coincidence of the radioactivity with that of an authentic UMP sample on paper chromatography (*10*). The ultraviolet spectrum of a sample eluted from the paper chromatogram was identical with that of UMP (λ_{max} 260 mμ).

To assess the prebiotic significance of cyanovinyl phosphate as a phosphorylating agent, the relative ease with which it transfers orthophosphate to uridine and water must be determined. If the selectivity for uridine is high then cyanovinyl phosphate may have prebiotic significance. If it is low then cyanovinyl phosphate will only undergo hydrolysis in dilute solution without effecting any phosphorylation. This selectivity factor, which was first defined by Lohrmann and Orgel (*11*), may be formulated as shown, with M_u the molarity of uridine, X the percent yield of UMP, and assuming the molarity of water to be 55:

$$\text{selectivity}\left(\frac{\text{uridine}}{\text{water}}\right) = \frac{55}{M_u}\left(\frac{X}{100 - X}\right)$$

This selectivity factor is only 1.3 for uridine, suggesting that very little UMP was formed prebiotically in homogeneous solution from uridine and cyanovinyl phosphate. This result is in agreement with the usual observation that water and alcohols are phosphorylated at about the same rate (*11, 12*).

However, orthophosphate is phosphorylated 4.5 to 9.2 times as efficiently as water, suggesting that cyanovinyl phosphate may have been the prebiotic source of the pyrophosphate bond. For example, 1 percent and 0.1 percent conversions to pyrophosphate would be obtained starting with 0.1*M* and 0.01*M* phosphate, respectively, and assuming a selectivity factor of 5.5.

A potential unified synthesis of pyrimidines, amino acids, and a high-energy phosphate compound from one source makes cyanovinyl phosphate attractive as a potential prebiological phosphorylating agent.

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10. Descending paper chromatograms on Whatman 3 MM paper, using 7 parts by volume of 95 percent ethanol and 3 parts of 1*M* ammonium acetate, *p*H 7.5.
11. I thank Dr. R. Lohrmann for his assistance with the UMP analyses. The analytical procedures will be described in detail in a fourthcoming publication (R. Lohrmann and L. E. Orgel, *J. Am. Chem. Soc.*, in press).
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Transplantation of Marrow to Extramedullary Sites

Abstract. *Autologous fragments of transplanted marrow have survived in various extramedullary sites in the rat, rabbit, and dog. Survival of the fragments occurs with a complete reconstitution of the hemopoietic and adventitial structures. The process originates from a network of surviving reticular cells which proliferate and differentiate into osteoblasts and give rise to trabecular bone. Later, the reticular cells reconstruct the marrow's microcirculation. Hemopoietic repopulation of the marrow implant takes place only after its sinusoidal microcirculation has been established.*

The failure of implants of autogenous bone marrow to survive in extramedullary sites has led to a conclusion that hemopoiesis cannot be sustained except in the marrow cavity. This concept is supported by indications that hemopoiesis requires the unique microcirculation of the marrow. The work of Knospe, Blom, and Crosby (*1*) demonstrates the relation between the two major elements of bone marrow, the blood forming elements and the adventitial elements which comprise the microcirculation of marrow. Hemopoiesis cannot be sustained except when sinusoidal blood vessels are present and functioning normally. In earlier attempts to transplant fragments of marrow or aspirates, with one exception (*2*), this specialized adventitia was evidently not retained (*3, 4*). Our success in transplanting the bone marrow of rat, rabbit, and dog into extramedullary tissues evidently requires the use of a relatively large piece of undisrupted marrow to permit a reconstruction of the sinusoids.

More than 200 Wistar albino rats (300 to 500 g) of both sexes were used. A window was cut by a dental drill in the anterior plate of the tibia, the sequestrum was removed, and the marrow was lifted out on the tip of a spatula; by this method we obtained a relatively large piece of marrow without disrupting the tissue. These fragments were implanted into splenic, renal, subcutaneous, hepatic, muscular, and omental tissues. They were removed periodically; after obtaining touch imprints on slides for cytological and cytochemical examinations, we fixed the tissue in 10 percent buffered formalin for at least 3 days and decalcified it in Prenny's solution. Sections were stained

with hematoxylin and eosin and examined by light microscopy.

During the first 2 days, necrosis and phagocytosis of the hemopoietic elements occurred, leaving behind a reticular network of adventitial cells (Fig. 1). At the same time, capillaries penetrated inward from the surrounding live tissues. Vascularization of the implant, at this stage, lacked the character of the marrow sinusoidal system. During the next 3 days marrow reticular cells proliferated. These cells were star-shaped or spindle-shaped, having a delicate chromatin pattern and two or more nucleoli (Fig. 2). Developmental capacity of such cells has been shown to include both osteoblastic and osteoclastic elements (*5*). This developmental capacity was substantiated by our observation that these proliferating cells acquired a Golgi net and by day 5 showed distinct morphological features of osteoblasts. By day 6 development of osteoid tissue was noted; small foci of amorphous substance joined to form a lattice of trabecular bone throughout the implant (Fig. 3). For the next 3 days a new phase of vascularization in the interstices appeared resembling the marrow sinusoidal system. Proliferating reticular cells which had given rise to osteoid tissue in an earlier phase now were developing into the lining layer of sinusoids (Figs. 4 and 5). The proliferating reticular cells of day 4 in these implants apparently have more than one potential for differentiation. They may differentiate into osteoblastic elements giving rise to a lattice of trabecular bone, and, later, they reconstruct the marrow sinusoids.

It was not until day 10, upon completion of this specific phase of vascularization, that the first recognizable hemopoietic elements appeared around the sinusoids in the interstices of the lattice, indicating that hemopoietic proliferation requires this unique microcirculation. The origin of the stem cells which give rise to hemopoietic elements remains unclear. These cells may derive from the same reticular cells which became osteoblasts and sinusoidal cells, or they may enter the implant via the circulating blood and repopulate bone marrow after the microcirculatory requirement for hemopoiesis has been met.

During the next several weeks, hemo-

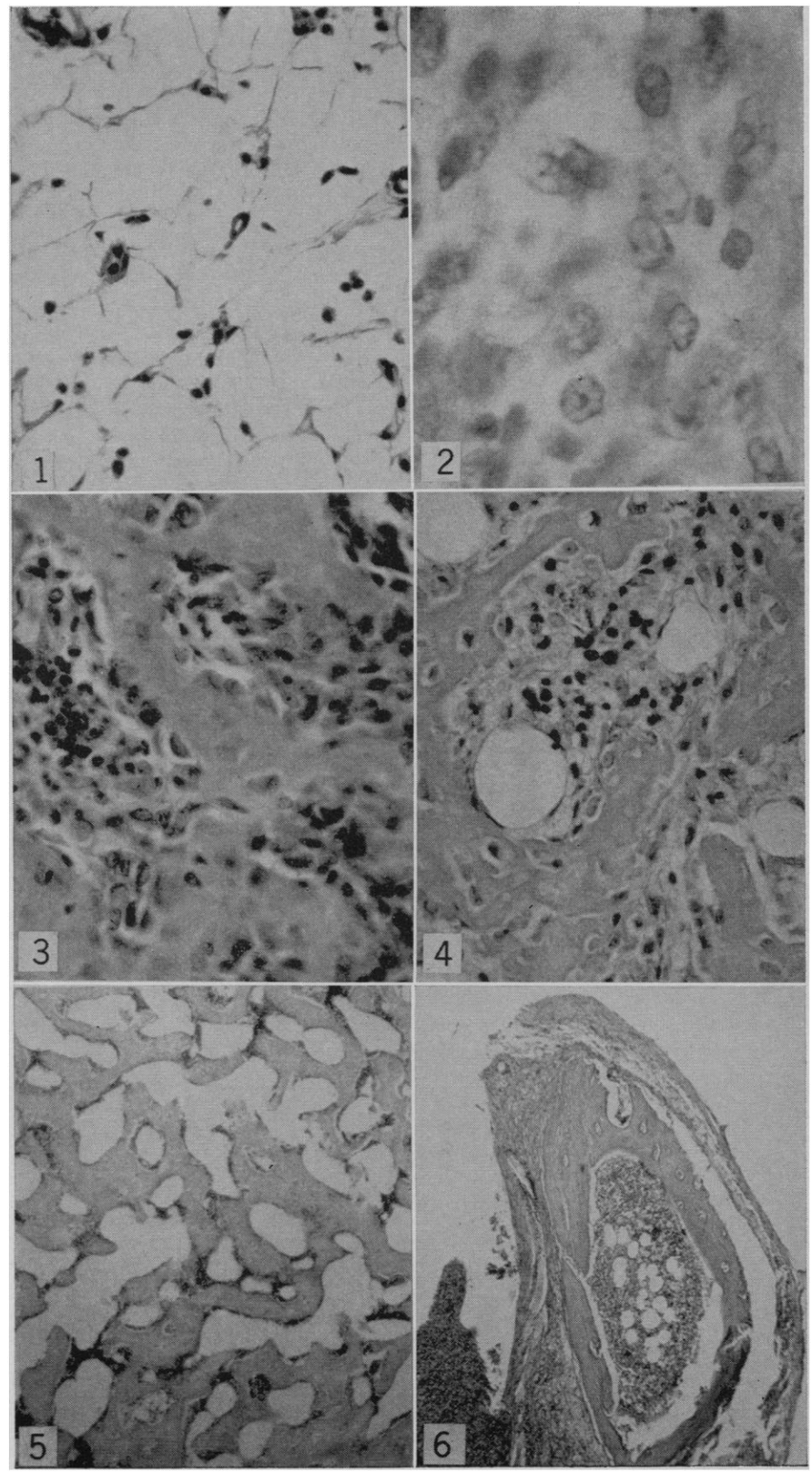

Fig. 1 (right). One day after implant. Reticular network of bone marrow after hemopoietic elements have disappeared. Rat, subcutaneous site (× 225). Fig. 2. Five days after implant. The implant is a monotonous sheet of proliferating reticular cells. Rat, subcutaneous site (× 500). Fig. 3. Seven days after implant. Reticular cells are differentiating into osteoblasts with formation of osteoid tissue. Rat, subcutaneous site (× 225). Fig. 4. Nine days after implant. The reticular cells in the interstices of osteoid tissue are developing into an endothelial layer of sinusoidal structure. Rat, subcueaneous site (× 225). Fig. 5. Ten days after implant. Sinusoidal structure is completely formed; darker areas in between represent beginning of hemopoietic activity. Rat, subcutaneous site (× 50). Fig. 6. Five weeks after implant. Hemopoiesis is fully established within a shell of bone separating it from the supporting tissue. Rat, spleen (× 25).

poietic activity became even more evident, and, through the process of remodeling of the bone, the lattice spaces grew wider and the bony trabeculae narrower, until by the 5th week there was no bone in the center and only a shell of bone surrounded the new marrow (Fig. 6).

All normal hemopoietic elements were present on the imprints of the tissues, and normal ratios were maintained. Mitotic figures indicated that the proliferation of blood cells was occurring in the reconstituted marrow. These nodules of ectopic marrow have been recovered as long as 6 months after implantation.

The ultimate size of the reconstituted tissue depends on the size of the implanted tissue. When the implant is too small, it may not survive at all; we have been unable to obtain survival and growth of marrow pieces smaller than 3 mm in diameter. When the implanted tissue was of adequate size, the implants in well-vascularized areas were uniformly successful. This indicates that in reorganization of marrow a critical size is involved; this fact may account for the failure of earlier attempts to grow the marrow in rabbit ear chambers (*3*).

The best supportive tissues for the growth of these marrow implants have been the spleen and kidney; their suitability may be related to the intense vascularity of these tissues. Regeneration of the marrow evidently requires a rather intensely vascular tissue, although the bone formation can take place with less vascular support. Fragments implanted in adipose tissue, for example, did not always survive. When regeneration did occur, the ratio of bone to marrow was greatly increased.

Implantation of autogenous marrow in seven New Zealand white male rabbits and in one female beagle dog has resulted in the same reconstitution of the hemopoietic tissue at the ectopic sites.

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5 April 1968

Falciparum Malaria Transmissible from Monkey to Man by Mosquito Bite

Abstract. Anopheles freeborni *mosquitoes were infected by feeding on a New World monkey,* Aotus trivirgatus, *infected with the Malayan IV strain of* Plasmodium falciparum. *After a normal incubation period, the infection was passed to a human volunteer through the bites of these mosquitoes, demonstrating for the first time the practicability of using a simian host as a donor for the infection of mosquitoes with this species of human malarial parasites.*

It was recently reported that the New World monkey *Aotus trivirgatus* (owl or night monkey) can be experimentally infected with the human malarial parasites *Plasmodium vivax* (*1*, *2*) and *P. falciparum* (*3*). These reports were indeed welcome since they offered, for the first time, an experimental animal well within the means of most laboratories interested in research on human malaria. Before these discoveries were made, the chimpanzee (*4*) and the gibbon (*5*) were the only suitable experimental animals that could be infected with human malarial parasites.

In our research program on human malaria, whenever infected mosquitoes are required, it has been necessary to subject volunteers to prolonged, though modified, clinical illness while waiting for gametocytes to be produced. Infection of small, inexpensive monkeys with human malarial parasites would preclude the necessity of employing volunteers for infecting mosquitoes.

We began to study, in owl monkeys, several strains of *P. falciparum* obtained from colleagues (Camp strain, Uganda strain). To date, all attempts to infect mosquitoes with these strains have failed. Recently, we have not only infected owl monkeys with one of the strains of *P. falciparum* resistant to chloroquine, which we maintain for study (Malayan IV), but have also transmitted this strain from the owl monkey back to man by mosquito bite.

On 13 February 1968, blood from volunteer M.I. infected with the Malayan IV strain of *P. falciparum* was inoculated into owl monkey AO-23. Patent infection obtained 27 days later. The infection was passed by blood to a second owl monkey, AO-25. *Anopheles freeborni* mosquitoes fed on this monkey became infected and exhibited sporozoites in the salivary glands 14 days later.

Nine mosquitoes from this lot, four of which had sporozoites in the salivary glands, were allowed to bite volunteer B.R. on 15 April 1968. Eleven days later, patent infection obtained in this volunteer. On day 12, the first paroxysm, with shaking chills and fever of 101.2°F (38.4°C), was observed. Two days later (day 14) fever of 104.0°F (40°C) was recorded. By day 16, the parasite count in volunteer B.R. was approximately 37,000 per cubic millimeter of blood, and fever of 105.0°F (40.6°C) was recorded.

The fact that this transmission was possible is not only interesting, but indeed unexpected on the basis of previous reports. Whereas mosquitoes fed on owl monkeys infected with *P. vivax* became infected (*1*), gametocytes in chimpanzees or gibbons infected with *P. falciparum* are immature and cannot undergo development in mosquito vectors (*4*, *6*). Geiman and Meagher (*3*) stated that immature and eventually mature gametocytes appear in owl monkeys infected with *P. falciparum.* However, no reports of the ability of such gametocytes to infect anopheline mosquitoes are available.

It remains to be seen if *P. falciparum* can be transmitted back to the monkey from man through the bites of infected mosquitoes. If such transmission can be achieved experimentally, the implications of these cycles of transmission, insofar as they relate to malaria eradication and control, are obvious.

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