Rouge, Louisiana. The fish were maintained on natural photoperiods indoors for 2 weeks in storage tanks containing 10 percent synthetic seawater (Instant Ocean). They were fed an overabundance of dried fish feed (Tetramin) supplemented by freeze-dried brine shrimp several times before and during the experimental period. The water was filtered continually and was maintained at 24° ± 2°C. From 26 October to 2 November 1971, the fish were weighed, measured, and hypophysectomized by a technique described by G. E. Pickford [*Bull. Bingham Oceanogr. Collect.* **14**, 5 (1953)]. On 2 November, the hypophysectomized fish were divided into two groups coupled with two groups of intact fish. In experimental series 1, intact and hypophysectomized fish were kept in six aquariums on a 12-hour photoperiod, with light beginning at 0800. In series 2, intact and hypophysectomized fish were placed on a 12-hour photoperiod with light beginning at 2000. The light was supplied by "daylight" fluorescence (250 lumen/m^2 at the water surface). After 4 days, some of the hypophysectomized fish showed evidence of osmotic failure; therefore, the concentration of the water was increased to full strength "seawater" for all the fish. On 17 November, each fish was anesthetized with tricaine methane sulfonate (MS-222), and the blood was collected from the caudal artery in heparinized microhematocrit tubes, was centrifuged, and was then frozen. Care was taken to disturb the fish as little as possible before removing the blood. L. E. Garcia (unpublished data) has found that our procedure has only minimal effect in raising plasma concentrations of cortisol in intact *F. grandis*, probably by less than 0.5 μg/100 ml during the time it takes to obtain the blood sample. The carcasses were used for measurement of body and testes weights.

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Fetal Liver Erythropoiesis and Yolk Sac Cells

Marks and Rifkind, in their thought-provoking article on control of erythrocyte protein synthesis during fetal and postfetal development (*1*) referred (their reference 8) to a paper by Moore and Metcalf (*2*) as supporting the following statement: "Neither in vivo nor in vitro is there substantive evidence that yolk sac cells seed fetal liver erythropoiesis (*8–10*)." This is opposite to the view that Moore and Metcalf in fact took, stating (in their summary): "Organ cultures of . . . embryos or yolk sacs after separation have shown . . . the dependence of intra-embryonic haemopoiesis, particularly in embryonic liver, on colonization by yolk sac haemopoietic cells" (*2*).

They were led to this view by a series of elegant transplantation and culture experiments, including 2-day organ cultures after which 7-day yolk sacs and embryos or yolk sacs alone each contained 55 to 81 in vitro colony-forming cells. Embryos alone contained none. Two lethally irradiated mice which survived for 30 days after injections of 8×10^6 chromosomally marked yolk sac cells showed 89 to 100 percent donor-type mitoses in bone marrow, spleen, thymus, and mesenteric lymph nodes (*2*).

The question of whether or not immature, primitive (yolk sac) cells are the precursors of the fetal liver and adult erythroid cell line is of fundamental importance in understanding the process of determination involved (*3*) (when the hemoglobin changes from fetal to adult type), and much current research is directed to this area (*3*). Even Marks and Rifkind admitted that the possibility has not been excluded "that the yolk sac contains cells which are direct precursors of fetal liver erythropoiesis" (*1*). Their contribution to one side of this question should not have misrepresented the contribution of Moore and Metcalf to the other.

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Although the work of Moore and Metcalf (*1*) cited by Harrison and Russell indeed claims to demonstrate ". . . the dependence of intra-embryonic haemopoiesis, particularly in embryonic liver, on colonization by yolk sac haemopoietic cells," in fact these studies do not document any contribution of yolk sac cells to later fetal erythropoiesis. The data cited concerning in vitro colony-forming cells (CFC) are irrelevant to this question since such cells have thus far been only shown to be granulocytic leukocyte precursors incapable of erythrocytic differentiation (*2*). The "elegant transplantation" studies cited do not provide any evidence of fetal hepatic erythropoiesis in explanted 7-day fetuses, whether or not the yolk sac is left attached, after 2 days of culture. These studies quite simply show that the yolk sac contains in vitro CFC, which may circulate into the embryo when the vasculature is developed. Furthermore, in vitro CFC are first detected in the intact fetal liver on day 10. Since the 7-day explants disintegrate after 2 days in culture, it is not possible to conclude that yolk sac in vitro CFC have colonized the liver or any other fetal organ.

Harrison and Russell further adduce experiments with chromosomally marked donor yolk sac cells injected into lethally irradiated adult recipients as evidence in support of the contribution of yolk sac in vivo CFC to later embryonic hemopoiesis. The donor cells in these experiments were from 11-day embryos. By the 11th gestational day the fetal liver is already contributing hemopoietic precursors to the embryonic circulation (*3*). There is no reason to assume, therefore, that the observed colony-forming activity in these experiments is of yolk sac origin. The simplest explanation of the data presented by Moore and Metcalf (*1*, figure 6) concerning in vivo CFC is that such hemopoietic precursors may be found in all hemopoietically active tissues and that the question of colonization remains as yet unsupported by substantive experimental data, as stated in our article (*4*).

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