strains in continuous culture for periods of up to 6 months. Compared to Waymouth's medium (with 10% serum), growth in the tris-buffered modification was less predictable. Confluent monolayers were regularly obtained but they were seldom as thick as those fed with Waymouth's medium. With some batches of tris-buffered media, greatly improved growth was obtained by increasing the concentrations of magnesium and calcium. Tris-HCl buffers have also proven useful for controlling the pH of trypsinization media and of aged, unfed confluent monolayers; the latter could be maintained intact for at least 5 weeks when the pH was not permitted to fall below 7.0.

The author is indebted to Dr. John Paul for suggesting the use of tris-citrate buffer, which he has successfully employed in the culture of certain cell lines. The encouragement and advice of Prof. G. Pontecorvo are gratefully acknowledged. Expert technical assistance was provided by Mrs. Mary Ann Derr.

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Response of Monkeys to Erythropoietin of Rabbit, Sheep, and Human Origin.* (29192)

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Although it has been shown that erythropoietin prepared from the serum of monkeys will stimulate erythropoiesis in monkeys(1) and that erythropoietin from human urine will stimulate erythropoiesis in monkeys(2) and man(3), there has been no demonstration of the effectiveness of erythropoietin from non-primate sources in primates. Since some species differences for erythropoietin have been demonstrated(4), it was thought worthwhile to determine the effectiveness in monkeys of erythropoietin prepared from the plasma of rabbits and sheep as well as from human urine.

Materials and methods. Three young cynomolgous monkeys (3 kg each) in which the 3 preparations of erythropoietin were tested were first made polycythemic by being kept in a decompression chamber at a simulated altitude of between 15,000 and 18,000 feet for 100 days (15,000 feet for the first 14 days, increased to 18,000 feet for the remainder of exposure). The monkeys were then returned to sea level and the assay begun when erythropoiesis had become almost completely suppressed, as judged by the near absence of

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reticulocytes in the peripheral blood. The same monkeys served as their own controls by being observed without erythropoietin administration after a second 100 day exposure to hypoxia done one year following the initial study. To insure adequate iron for hemoglobin synthesis, 50 mg of iron as an iron-dextran complex (Imferon) were given prior to exposure to hypoxia.

Rabbit erythropoietin was provided by Dr. Geoffrey Keighley, California Institute of Technology, and was prepared from the plasma of phenylhydrazine treated rabbits by the method of Lowy *et al*(5). Erythropoietin from the plasma of phenylhydrazine treated sheep, prepared by the method of White *et al* (6), step 4, lot #K147137, A10566, was provided by the Hematology Study Section of the USPHS. Human erythropoietin was prepared by collodion adsorption(2) from the urine of a patient with aplastic anemia(7).

When the reticulocyte counts of the monkeys had fallen to near zero, 8 days after removal from the altitude chamber, one was given rabbit erythropoietin, one was given sheep erythropoietin, and one was given human urinary erythropoietin. When the effect of this treatment had completely subsided as judged by the return of the reticulocyte count to a low level, the monkey which had first been given rabbit erythropoietin was then given human erythropoietin and one monkey which had first been given human erythropoietin was then given sheep erythropoietin. The third, which had been given human erythropoietin was given a second and third treatment with human erythropoietin. In each instance the treatment was 70 Standard A units/kg/day for 4 days.

Microhematocrit determinations, reticulocyte counts, total leucocyte and leucocyte differential counts and platelet counts were done at frequent intervals throughout the study. Platelet counts were done with phase-contrast microscopy.

Results and discussion. At the time of removal from the decompression chamber the hematocrits were markedly elevated (81 to 88%) and the reticulocytes were 1 to 3%. The reticulocyte count fell rapidly to near zero (0 to .3%) by the 8th day post hypoxia where, when no erythropoietin was given, it remained for 80 days. Fig. 1 shows the hematocrit and reticulocyte counts of one of the monkeys during the control post hypoxic period when no treatment was given.

For evaluation of the effectiveness of erythropoietin from the 3 sources, treatment was begun on the 8th day. From Fig. 2, 3, and 4 it can be seen that each 4-day period of administration of erythropoietin was followed by a wave of reticulocytosis which was maximum 6-8 days after start of treatment. The magnitude and duration of the responses were somewhat different for the 7 treatment periods. The greatest and least responses were obtained with identical dosage of the same preparation of human urinary erythropoietin in 2 different monkeys (Fig. 2 and 3), suggesting that individual variation rather than species specificity was the responsible factor. In 2 monkeys, Fig. 2 and 3, the second period of treatment with a different preparation resulted in a lesser response, whereas in a third, Fig. 4, subsequent treatment periods with the same preparation resulted in an equal or slightly greater response, indicating no impairment of response as a result of prior exposure to the preparation. Keighley *et al* have demonstrated continued responsiveness of mice to rabbit erythropoietin up to 200 days(8).

From Fig. 1 and 4 it can be seen that the hematocrit returned to a normal value by 70 days post hypoxia. Spontaneous reticulocytosis began on about the 80th day post hypoxia.

An increase in platelet count in normal human beings given human urinary erythropoietin from the same source has been reported previously(3) and more recently an increase in platelet count in a normal human being acutely exposed to a simulated altitude of 17,000 feet has been observed(9). No consistent change in platelet or leucocyte count or leucocyte differential was related to the periods of erythropoietin administration in this study.

To rule out the possibility that the reticulocytosis observed following erythropoietin administration was a non-specific response to foreign material, one post hypoxic monkey

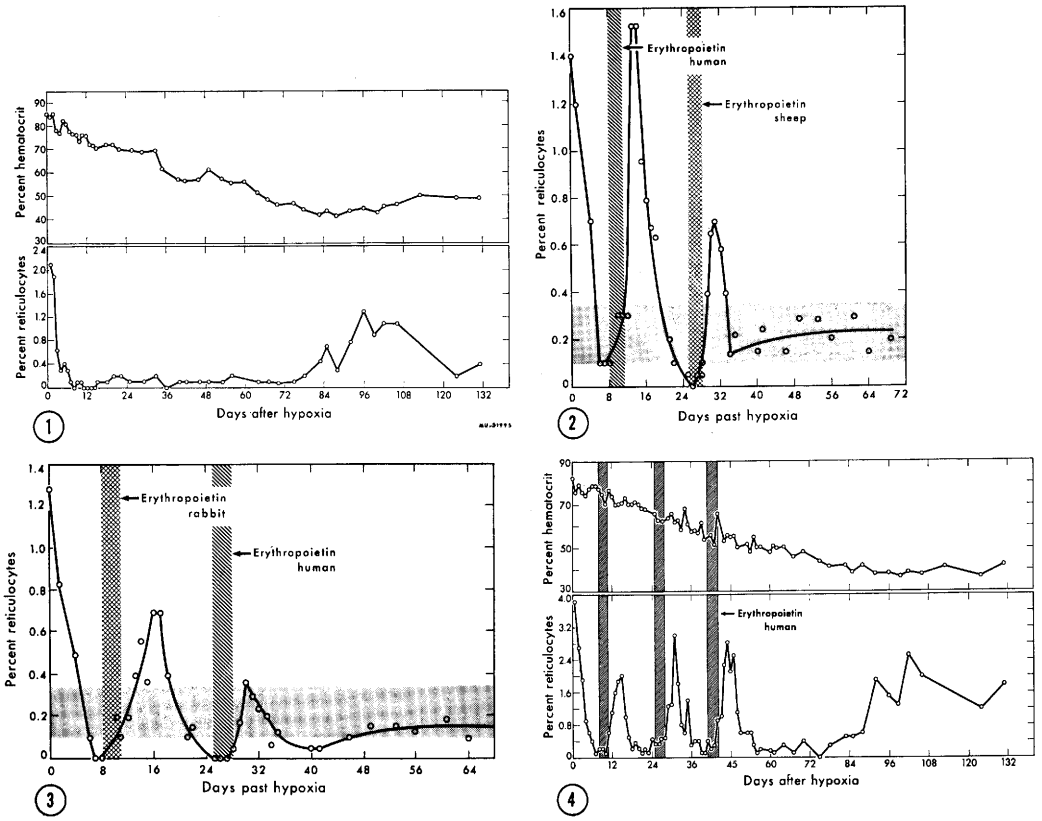

FIG. 1. Hematocrit and reticulocyte count of one monkey after return to sea level after 100 days exposure to a simulated altitude of 18,000 feet. Monkey received no treatment.

FIG. 2. Reticulocyte response of post hypoxic monkey to human and sheep erythropoietin. Horizontal shaded area indicates range of reticulocyte count in normal monkeys of same strain.

FIG. 3. Reticulocyte response of post hypoxic monkey to rabbit and human erythropoietin. Horizontal shaded area indicates range of reticulocyte count in normal monkeys of same strain.

FIG. 4. Reticulocyte response and hematocrit of post hypoxic monkey receiving human erythropoietin during 3 treatment periods.

was given an equal amount of erythropoietically inactive material prepared in the same way from the urine of a normal human being, following the same experimental design. The material was given for 4 days starting on the 8th day post hypoxia. No reticulocytosis was associated with the treatment, the results being the same as for the untreated control (Fig. 1).

Conclusions. Monkeys in whom erythropoiesis was suppressed (post hypoxic) showed a significant reticulocyte response to administration of erythropoietin of rabbit, sheep or human origin. These results indicate that erythropoietin of primate origin is not required for stimulation of erythropoiesis in primates.

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Potentiating Effect of Fractions of Eastern Equine Encephalomyelitis Virus on Interferon Production.*† (29193)

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Interferon is a soluble protein which has the interesting property of inhibiting intracellular virus replication. It is produced by cells exposed to any of a large group of viruses, both in infectious and inactivated forms(1,2). Earlier, it was found that heat inactivated Sindbis virus failed to mediate interferon production in chick cell cultures, but it increased such production when these cultures were subsequently infected with active virus(3). This potentiating effect of the non-infective virus we call "priming" of interferon production.

Attention is here directed to EEE virus, which is a pathogenic member of the same arbovirus group as Sindbis virus. In our hands, infective EEE virus is inconsistent in stimulating interferon production in chick cell cultures without priming(4). The present study was undertaken to elucidate the conditions under which priming takes place, and to define by density gradient centrifugation the components of EEE virus responsible for the priming activity.

Materials and methods. Stock suspensions of EEE virus (New Jersey) previously described(3) were purified by single plaque isolations 3 times and grown in chick embryo cell cultures (CECC). To prepare these cultures, cells obtained from 11-day-old embryos were suspended in 3 oz. bottles and nourished in 0.5% lactalbumin hydrolysate dissolved in Hanks' balanced salt solution (BSS) and 4% inactivated calf serum (LAH medium)(3). To prepare serum free virus suspensions, cells were overlaid with Eagle's basal amino acid and vitamin mixture in Hanks' BSS (E-H medium). Pellets of EEE virus were obtained by centrifuging harvests of stock virus titering about 10^8 p.f.u. per 0.1 ml at 78,000 $\times$ *g* for 3 hours at 4°C. The pellet material was suspended in E-H medium to a volume representing 2- to 100-fold of initial concentration.

For CsCl density gradient centrifugations: to 3.5 ml 25% (w/w) CsCl aqueous solution, 1.0 ml virus pellet suspension was added. The mixture was centrifuged at 100,000 $\times$ *g* for 24 hours. To collect fractions, the bottom of the tubes was pierced with a #24 needle, and successive drops were collected in a series of Wassermann tubes and diluted in E-H medium containing 5% calf serum.

For preparation of "primer" from serum-free virus suspensions or virus fractions in CsCl density gradients, 3.5 ml of such material in a 100 mm petri dish were first irradiated for one minute from a distance of 2 inches using ultraviolet light (UV) emitted from a 28.75 watt lamp with maximum emission at 2537 Å. Then the irradiated material was incubated at 37°C for 12 hours to completely eliminate viral infectivity. Excessive irradiation or heating was found to eliminate the priming effect.

Virus was titrated by plaque formation and interferon by plaque inhibition in bottle cultures. Interferon titers were expressed in percent reduction of plaque formation of 100-150 plaque forming units (p.f.u.) of EEE virus. From previous experience, an inhibition of less than 20% was not considered significant(3). In testing the interferon effect

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