cell preparations as "suspensions" rather than "cultures."

Summary. Multiplication of measles virus has been demonstrated in suspensions of human and simian blood leucocytes. Under the experimental conditions employed the virus failed to multiply in canine, rabbit, guinea pig, mouse and chicken blood leucocytes. Evidence is presented which indicates that multiplication takes place in mononuclear cells.

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Response of Rats of Various Ages to Erythropoietin.* (26410)

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New-born rats develop an anemia soon after birth and it becomes most severe when the animals are 15-20 days of age(1,2). In the rat this is the period of maximum growth rate of the body as a whole as well as the period of maximum growth rate of total circulating red cell volume. During the time that the anemia is developing, iron kinetics studies reveal that erythropoiesis is proceeding more rapidly than at any other time in the life span of the animal(3). From these observations it has been suggested that the anemia in the rat results from the disproportionate growth of the body in relation to the ability of the marrow to supply erythrocytes. This implies that the marrow is functioning normally and at maximum capacity during the anemic period. It has been shown that rats do not show an erythropoietic response to hypoxia during the neonatal period(4).

It was considered of interest to determine whether erythropoietin, which is active in adult rats, would stimulate erythropoiesis during the period of neonatal anemia.

Materials and methods. Both total circulating red cell volume measurements and Fe^{59} red cell incorporation studies were used to judge the erythropoietic response to erythropoietin. Groups of normal male rats of the Long-Evans strain of 12, 23, 87, 230, and 470 days of age were used to determine the effect of erythropoietin on total circulating red cell volume. Control and experimental groups, equal in number and of the same average body weight at the beginning of experiment, were studied at each age period. Half

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of each litter served as the controls in the youngest group studied. No litter exceeded 6 in number. Erythropoietin was injected daily for 10 days. Twenty-four hours after last injection, total red cell volumes were measured by the labeled red cell dilution technic(2). Hematocrits and hemoglobin concentrations were also determined. In the young animal total red cell volume is normally increasing due to growth. Total red cell volumes and total circulating hemoglobins of the animals receiving erythropoietin were thus compared with those of their respective controls, to determine whether there was any gain in total red cell volume in excess of that due to growth and only as a result of injection of erythropoietin. Since the anemia, which normally occurs in young rats, is most severe at 15-20 days after birth, 12-day-old rats were chosen for the youngest group so that the 10-day injection period would fall within the anemic period.

The effectiveness of erythropoietin was also measured by the red cell uptake of Fe^{59} (5) both in normal adult and in 14-day-old rats. Erythropoietin was administered for 2 days, and on the third day Fe^{59} was given intraperitoneally. Eighteen hours later a sample of blood from the aorta was counted. The results were expressed as per cent of administered Fe^{59} appearing in peripheral blood, using a value for the blood volume appropriate to the age of the animal. Measurements of blood volume gave values of 5.0 and 6.2% of body weight respectively for the adult and 14-day-old rat. Because of the possibility that young rats are producing red cells at a maximum rate, it was decided to test the effect of erythropoietin on such animals after depressing their red cell production by hypertransfusion. The same erythropoietin and Fe^{59} injection schedules were used except that prior to injection of erythropoietin the rats were injected intraperitoneally for 2 days with 1 ml of whole blood from an adult donor. Only animals with an hematocrit that exceeded that of normal adult rats (45%) were included. A value for blood volume of 7.2 ml per 100 g of body weight was used in the hypertransfused rat to express the results as per cent of administered Fe^{59}. This value had been measured previously in rats of the same age and hypertransfused in the same way.

The erythropoietin was obtained by ultrafiltration of urine through a collodion membrane from 2 anemic patients. The method of extraction has been described(6,7). The erythropoietin used in the red cell volume study was obtained by ultrafiltration of urine from an hypoplastic anemic patient, a summary of whose history has been published (6). The erythropoietin used in the Fe^{59} red cell uptake assay was obtained by ultrafiltration of urine from a patient with chronic bleeding from congenital telangiectases. A summary of the case history is to be published elsewhere. The erythropoietin was dissolved in saline and administered subcutaneously at a dose of 2 mg (0.2 ml) daily for 10 days for the blood volume assay, or 1 mg (0.2 ml) daily for 2 days for the Fe^{59} red cell incorporation assay. All controls received an equivalent volume of saline.

All rats were fed a complete laboratory diet.†

Results and discussion. The animals in all of the 5 groups responded to the erythropoietin with significant increases in total red cell volume and total circulating hemoglobin with the exception of the rats which were 12-days-of-age at beginning of experiment. Results of the red cell volume study are summarized in Table I. Since estimations of hemoglobin concentrations as well as hematocrit determinations were made, the value for total circulating hemoglobin as well as total red cell volume was calculated. In the 230- and 470-day-old rats, the percentage increase in hemoglobin is less than the percentage increase in red cell volume, indicating a degree of hypochromia in the cells produced under the influence of erythropoietin. This difference is even greater in the 23- and 87-day-old rats. Such a difference has been observed in the erythropoietic response

† The diet was obtained from Simonsen Laboratories, Gilroy, Calif. It consisted of 59.0% wheat, 11.7% skim milk, 11.2% casein, 11.2% rice bran, 3.3% vegetable oil, 1.3% $CaCO_3$, 0.7% NaCl, and vitamin and mineral mixtures to make up 100%.

TABLE I. Response of Male Rats of Various Ages to Treatment for 10 Days with 2.0 mg Daily of Human Urinary Erythropoietin.

Age, days	Treatment	No. rats	Wt, g (final)	Hct, %	Hgb, g/100 ml	Total red cell vol ml	% change	p*	Total circulating hemoglobin g	% change	p
12–22	2 mg "E"	12	49	28.0	7.1	.88	4.8	.5	.220	.7	.9
"	Control	12	52	25.4	6.7	.84			.219		
23–33	2 mg "E"	10	93	44.6	12.6	2.72	18.8	<.001	.769	11.1	<.02
"	Control	10	99	37.1	11.2	2.29			.692		
87–97	2 mg "E"	10	320	54.8	15.7	9.19	21.1	<.01	2.62	9.2	<.05
"	Control	10	336	46.7	14.8	7.59			2.40		
230–240	2 mg "E"	10	400	55.2	16.7	10.70	22.1	<.001	3.24	17.8	<.001
"	Control	10	407	47.4	14.9	8.76			2.75		
470–480	2 mg "E"	7	511	57.5	16.9	13.43	24.7	<.001	3.95	18.3	<.01
"	Control	7	519	48.6	15.1	10.77			3.34		

* Fisher's t test.

to hypoxia(4). Since the same total dose of erythropoietin was injected into the rats in the various age groups, the dose per unit body weight increased from the oldest to the youngest animals studied. Of the 4 groups which responded however, the absolute and relative increase is greatest in the oldest animals studied, *i.e.*, the ones that received the lowest dose per unit body weight. It would appear that the older the animal, the more responsive it is to erythropoietin.

It is interesting to note that the erythropoietic response to hypoxia is similar to that resulting from erythropoietin administration in at least 2 respects. Both stimuli fail to increase erythropoiesis in very young rats, and the response seen in older rats to either hypoxia or erythropoietin is characterized by production of slightly hypochromic cells.

Table II presents the results of Fe^{59} red cell uptake studies. One mg of erythropoietin was sufficient to elicit a significant increase in the red cell incorporation of Fe^{59} in normal adult male rats. When this dose of erythropoietin was injected into normal 14-day-old rats, no increase in incorporation of Fe^{59} into red cells was seen. When such young animals were hypertransfused, incorporation of Fe^{59} into red cells was reduced from the normal value of 37.7 to 24.6%. Injection of erythropoietin into such animals resulted in a significant increase, giving a value of 45.8%.

These data support the concept that erythropoiesis is very active in young rats during the period of neonatal anemia. Such animals cannot be stimulated to any greater extent with erythropoietin. Rats of this age do not respond erythropoietically to hypoxia. That young rats are capable of responding to erythropoietin, however, is shown by the fact that when erythropoiesis is depressed by hypertransfusion, they respond to injection of erythropoietin by an increased incorporation of radioiron into red cells.

Conclusions. Erythropoietin of human

TABLE II. Red Cell Incorporation of Radioiron in Normal and Hypertransfused Rats Following Injection of Erythropoietin.

	Age, days	No. rats	Hematocrit, %	Fe^{59} uptake in red cells, %	p*
Normal control	57	10		14.4	<.001
" 1 mg erythropoietin	57	10		31.0	
" control	14	7	22.0	37.7	
" 1 mg erythropoietin	14	6	24.5	38.6	
Hypertransfused control†	14	4	51.0	24.6	<.001
" 1 mg erythropoietin	14	5	52.7	45.8	

* Fisher's t test. † 1 ml whole blood I.P. for 2 days prior to inj. of erythropoietin.

urinary origin was injected into groups of rats of various ages, after which both measurements of total circulating red cell volume and Fe^{59} red cell incorporation were made. Total red cell volume of young rats throughout the period of neonatal anemia did not increase in response to administration of erythropoietin, whereas it did increase in all older groups. According to this assay, the rat most sensitive to erythropoietin was the oldest rat studied. Using the Fe^{59} red cell incorporation assay, no increase in erythropoiesis was observed in normal young rats (14 days of age) following erythropoietin administration. However, when erythropoiesis was depressed in young rats by hypertransfusion, red cell uptake of Fe^{59} was greatly reduced. When such hypertransfused rats were injected with erythropoietin, red cell incorporation of Fe^{59} was significantly increased.

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Organic Acid Excretion, Enhanced Calcium Absorption and Body Fat of Rats Fed Incompletely Digested Carbohydrates.* (26411)

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A relatively low content of body fat has been found in rats fed diets containing the incompletely digested carbohydrates, lactose, sorbitol, cellobiose or raw potato starch(1). The mechanisms involved are not known. Other characteristic effects associated with feeding of poorly digested carbohydrates include an initial transient diarrhea, enlargement of the cecum, establishment of an aciduric bacterial flora in the intestine, and enhanced gastrointestinal absorption of calcium and other alkaline earth elements(2). Recently Fournier and Digaud(3) have reported an increased urinary excretion of α-ketoglutaric, citric, aconitic, malic and succinic acids but not of pyruvic or hippuric acid. The present study investigated the possibility that the increased excretion of acids of the Krebs cycle is a consequence of metabolic alterations that result in a decreased deposition of body fat.

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Methods. Male weanling Sprague-Dawley rats, individually caged, were fed diets whose basal composition consisted of casein 24, carbohydrate 52, fat mixture 20, salts 4 and adequate amounts of vitamins(1). Twenty-four hour urine samples were collected under toluene. Body fat content was determined by whole carcass analysis(1) or estimated from the weight of the epididymal fat pads. The weight of the cleaned cecum was determined as an index of the digestibility of the dietary carbohydrate. Total organic acids in the urine were determined by the method of Van Slyke and Palmer(4). As this method is not specific, determination of keto acids by the simple and rapid method of Friedemann and Haugen(5) was routinely used to follow the effect of dietary carbohydrate on organic acid excretion. The organic acids were identified by silica gel chromatography. A 10-fold concentration of lyophilized urine in 2 N H_2SO_4 was mixed with an appropriate amount of dry silic acid and placed on top of a column prepared, and then eluted, by the