clizine. Thus it was apparent that enzyme induction occurred in both the adult and weanling mice, regardless of dietary intake. Since it is believed that the induction of increased microsomal enzyme activity involves the synthesis of new protein (3), it was interesting to note that such protein synthesis appeared to be as efficient in the starved adults and weanlings fed the low protein diet as it was in the adult and weanling control mice stimulated by these potent inducers.

It was also apparent from the studies with the weaker inducers that the degree of enzyme induction by these inducers was always greater in the starved adult and weanling mice than it was in the control adult mice. The greater activity of these weaker inducers may result from the fact that the hepatic enzymes are at a subadult level in these animals and there is therefore a greater inherent stimulus for enzyme synthesis.

The observation that 3,4-benzpyrene caused a decrease in hexobarbital sleeping time in both weanling groups, but not in the adult mice again points out the susceptibility of the weanling mouse to enzyme induction. It also suggests that the specificity of action of this compound in inducing only certain drug-metabolizing enzymes may be dependent on the presence of a more stabilized state of protein synthesis that exists in the adult mouse.

Summary. Potent inducers of drug-metabolizing enzymes such as phenobarbital and chlorcyclizine are as effective in fed and in starved adult mice as they are in weanling mice maintained on 8 or 27% casein diets. Weaker enzyme inducers such as hexobarbital, antipyrine, or metronidazole are more effective agents in starved adults and weanling mice than they are in the well-fed adult mice. Compounds such as 3,4-benzpyrene which do not induce increased hexobarbital-metabolizing activity in adult mice are capable of inducing such activity in weanling mice.

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A Difference in Erythropoietin Production between Anemic and Hypoxic Mice* (32961)

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Genetic variations in various species of mice have shown a number of interesting abnormalities including hematological disturbances. The WWv mouse is anemic with an elevated plasma level of erythropoietin and a markedly diminished response to exogenous erythropoietin (1). However, the strain is capable of adequate, if not excessive, erythropoietin production in response to hypoxia. That the defect is one of erythropoietic cells seems confirmed by improvement following transplantation with normal coisogenic cells (2). In like manner, the Steel mouse is characterized by a macrocytic anemia with adequate erythropoietin production. The pluripotential cells of this strain have adequate transplant potential in irradiated normal coisogenic and WWv mice. However, the irradiated SL/SL mice do not adequately support colony formation even with parabiotic normals (3). This implies a tissue rather than an erythropoietic cellular defect.

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In our laboratory the CF_1 mouse regularly responds to altitude hypoxia with the development of marked polycythemia. However, the CAF_1 mouse shows only minimal response to altitude. The failure of the CAF_1 mouse to respond to hypoxia was thought to be secondary to failure either to produce or respond to erythropoietin. Accordingly, both strains of mice were assessed for their response to exogenous erythropoietin and for their ability to produce erythropoietin in response to anemic and hypoxic stimuli.

Materials and Methods. Eight- to 14-week-old CF_1S (Carworth)[1] and CAF_1/J (Jackson)[2] mice were used throughout. The effect of long-term hypoxia on erythropoiesis in these 2 strains of mice was evaluated after 3 weeks of exposure to 23,000 feet of simulated altitude for 16 hours a day. The effect of exogenous erythropoietin was determined in groups of mice which received daily injections of 1 unit of erythropoietin subcutaneously for 14 days. The erythropoietin used was an alcoholic extract of human urine (4). Animals were sacrificed at 3, 7, and 14 days after the initiation of treatment. In both the hypoxic-stimulated and erythropoietin-treated animals the red cell mass was estimated by isotope dilution using ^{51}Cr-labeled isogenic red cells; the chromium-labeled cells were injected intravenously 20–30 min prior to sacrifice. Eighteen-hour iron incorporations were measured by technics previously described (4).

The capacity to produce erythropoietin was evaluated in animals after exposure to hypoxia, bleeding, and in mice in which phenylhydrazine hemolytic anemia had been induced. Those animals stimulated by hypoxia were exposed to 23,000 feet of simulated altitude for periods of 12–24 hours. Immediately upon return to ambient pressure the animals were bled by cardiac puncture and the plasma was separated. Posthemorrhagic anemia was accomplished by bleeding the animals by cardiac puncture an amount of blood equivalent to 2.5% of the body weight. Twenty hours later they were sacrificed and the plasma was assayed for erythropoietin. Hemolytic anemia was induced by the subcutaneous injection of 5 mg/100 gm of body weight of phenylhydrazine hydrochloride on days 0, 1, and 3. Twenty-four hours after the last injection of phenylhydrazine the plasma was harvested.

Erythropoietin assays were carried out in plethoric animals. The CF_1 mice were exposed to altitude for a period of 23 days and returned to ambient pressure. On the fourth and fifth days after return to ambient pressure, 0.4–0.6 ml of pooled test plasma was administered subcutaneously. On the sixth day ^{59}Fe-labeled mouse serum was injected intravenously and the animals were sacrificed 18 hours later. In other assays plethora was induced by transfusion of 0.7 ml of washed red cells given intraperitoneally on days −1 and 0. On days 4 and 5 the plasma to be assayed was given subcutaneously. On day 6 ^{59}Fe-labeled serum was given and the animals were sacrificed 18 hours later. For both groups of assay animals a blood volume of 8% of the total body weight was assumed; this was based on previous measurements. Assay animals with an hematocrit of less than 55 were excluded. Originally the iron incorporation values in assay animals were estimated on unwashed samples of blood. It was noted, however, that these gave control values significantly higher than those observed when the red cells had been washed 3 times. Accordingly, in later experiments washed red cells were used. It was also noted that in those assay animals in which plethora had been induced by hypoxia with subsequent return to ambient pressure, the iron incorporation was significantly greater than in the hypertransfused animals. Accordingly, absolute iron incorporations cannot be compared between experiments. The oxygen dissociation curve was measured in the 2 strains using a spectrophotometric technique with an IL[3] co-oximeter and PO_2 and pH determination on an IL[3] electrode model 113. Tonometry was performed with an IL[3] tonometer apparatus with controlled gas humidification.

Results. The effect of prolonged exposure to altitude is shown in Table I. The measurements were made 1 week after descent to

[1] Carworth Farms, New City, Rockland County, New York.

[2] Jackson Laboratories, Bar Harbor, Maine.

[3] Instrumentation Laboratories, Watertown, Massachusetts.

TABLE I. The Effect of 3-Weeks Exposure to 23,000 Feet Simulated Altitude.[a]

Strain	Treatment	No. of animals	Hematocrit (%)	RCM (ml)	Increase in RCM (%)
CF_1 female	A	15	47.2 ± 0.52	0.78 ± 0.03	
CF_1 female	H	14	63.4 ± 0.98	1.41 ± 0.05	80.8
CAF_1 female	A	6	44.3 ± 1.31	0.66 ± 0.03	
CAF_1 female	H	6	50.1 ± 1.62	0.79 ± 0.05	19.7
CAF_1 male	A	5	46.7 ± 0.73	0.78 ± 0.03	
CAF_1 male	H	7	52.9 ± 1.08	0.89 ± 0.03	14.1

[a] Values are the mean ± 1 SE for the indicated number of animals; measurements were made 7 days after discontinuation of hypoxia; A, ambient pressure; H, simulated altitude of 23,000 feet; RCM, red cell mass.

avoid the problem of hemoconcentration secondary to dehydration. The degree of polycythemia developed by the CF_1 mouse was substantially greater than that of the CAF_1; there appeared to be no sex difference in the response to hypoxia.

Studies on the effectiveness of erythropoietin in inducing polycythemia are shown in Table II. Comparable increases in hematocrit, hemoglobin, reticulocyte counts, and iron incorporation were noted in both strains of mice. Significant increases in these parameters were noted after 7 and 14 days of treatment in both strains. In these age-matched animals the CF_1 mouse consistently had higher red cell masses than the CAF_1 mouse, both in control and treated groups. In part this is explained by the smaller size of the CAF_1 mouse, but a difference exists even when the red cell mass is expressed as percentage of body weight; the values were 2.9–3.2% in control CAF_1 mice as contrasted with 3.2–3.8% of the body weight in the CF_1 mouse. The extent of increase in the red cell mass after treatment, however, was not different between the 2 strains.

The results of assay for erythropoietic activity in the plasma of mice exposed to 23,000 feet for periods of 12, 18, and 24 hours is shown in Table III. There was a marked increase in the erythropoietic activity in the plasma of the CF_1 mouse, but only minimal activity in that of the CAF_1 mouse. After exposure at various altitudes from 15,000–25,000 feet for a period of 18 hours, a dose–response relationship could be demonstrated in the CF_1 mouse, but the increase in erythropoietic activity in the CAF_1 mouse was inadequate to construct a dose–response curve.

The effect of anemia on erythropoietin production is shown in Tables IV and V. In those animals in which a hemolytic anemia had been induced by phenylhydrazine the hematocrits at the time of sacrifice on day 4 ranged

TABLE II. The Effect of Erythropoietin in CF_1 and CAF_1 mice.[a]

		CF_1			CAF_1		
Days	Treatment	HCT (%)	RCM (ml)	Increase in RCM (%)	HCT (%)	RCM (ml)	Increase in RCM (%)
3	C	47.2 ± 0.96	0.70 ± .01		47.4 ± 0.29	.51 ± .04	
3	EP	50.3 ± 0.42	0.74 ± .02	6.0	50.5 ± 0.66	.59 ± .03	16.6
7	C	45.9 ± 0.93	0.77 ± .05		49.6 ± 0.49	.58 ± .03	
7	EP	52.9 ± 2.36	1.10 ± .06	43.1	55.7 ± 0.37	.76 ± .05	32.8
14	C	48.4 ± 0.51	0.86 ± .07		46.5 ± 1.47	.62 ± .04	
14	EP	59.2 ± 1.53	1.26 ± .12	46.3	56.2 ± 1.16	.85 ± .05	37.6

[a] Values are mean of 5 animals/group ± 1 SE; RCM, red cell mass expressed in ml; EP, erythropoietin treated; C, control. Animals were sacrificed 24 hours after last injection of EP.

TABLE III. The Erythropoietic Activity of Plasma After Indicated Duration of Hypoxia.[a]

Treatment	^{59}Fe uptake (%)	
	CF_1	CAF_1
12 hour HP	14.6 ± 0.48	2.8 ± .27
18 hour HP	16.0 ± 0.89	2.4 ± .58
24 hour HP	16.4 ± 1.38	2.4 ± .15
CP	1.8 ± 0.13	1.6 ± .12
Control	1.8 ± 0.16	

[a] Values represent the mean of 6 assay animals ± 1 SE; plasma was pooled from 30–50 donor mice. HP, hypoxic plasma; CP, plasma from normal animals at ambient pressure. Recipients with plethora induced by hypoxia were used. Red cells were unwashed.

from 20–33% with a mean of 27% in both groups. The erythropoietic activity in the plasma of both CF_1 and CAF_1 phenylhydrazine-treated animals was similar. There were no differences between the hematocrits of control and treated assay animals, excluding the possibility of residual phenylhydrazine in the plasma affecting the assay. In other animals treated with phenylhydrazine using the above schedule hematocrit and reticulocyte values were measured on days 5, 6, and 7. The increase in hematocrit was comparable in both strains of mice reaching levels of 38–44% by the seventh day, at which time the reticulocyte values were in the range of 45–65%.

In the animals with posthemorrhagic anemia the hematocrits at the time of sacrifice 20 hours after the original bleeding ranged from 21–34% with a mean of 28% for the CF_1 and 29% for the CAF_1 animals. The response to doses of 0.6 ml of plasma on 2 consecutive days in hypertransfused mice is shown in Table V. Again, there was substantial erythropoietic activity in the plasma of both strains.

TABLE IV. The Erythropoietic Activity of Plasma from Phenylhydrazine Treated Animals.[a]

Treatment	Source of plasma	
	CF_1	CAF_1
Phenylhydrazine plasma	10.2 ± 2.3	12.0 ± 3.4
Control plasma	0.02 ± 0.01	0.04 ± 0.01
Controls	0.01 ± 0.003	

[a] Values represent mean 18-hour ^{59}Fe uptake of 6 animals/group ± 1 SE. Hypertransfused CF_1 recipients were used for assay and red cells were washed prior to counting. Plasma pools were from 30–50 donors of the CAF_1 or CF_1 strains, respectively.

Oxygen dissociation curves for both strains of mice were determined by tonometry of the whole blood against gas mixtures containing 2, 4, 6, and 8% oxygen. Figure 1 (corrected to pH 7.4, temperature 37°C) shows no appreciable difference between the two curves.

Discussion. From these results it is apparent that the failure of the CAF_1 mouse to respond to prolonged altitude exposure is due to a markedly decreased ability to produce erythropoietin in response to hypoxia. By contrast the CF_1 mouse responds well both to acute and chronic hypoxia. The adequate response of the CAF_1 strain to a moderate dose of erythropoietin excludes a stem cell or tissue defect such as has been shown in the WWv or Steel mouse. Of interest is the comparable production of erythropoietin in both strains after the production of a posthemorrhagic or hemolytic anemia. This indicates that the CAF_1 mouse has intact "receptors" for the sensing of "anemic hypoxia" as well as the apparatus for the generation of and response to erythropoietin.

TABLE V. The Erythropoietic Activity of Plasma from Animals with Posthemorrhagic Anemia.[a]

Source	^{59}Fe uptake (%)
CF_1 plasma	4.7 ± 1.4
CAF_1 plasma	5.6 ± 1.0
Untreated controls	0.004 ± 0.001

[a] Values represent mean 18-hour ^{59}Fe incorporation of 6 animals ± 1 SE. Hypertransfused CF_1 recipients were used and the red cells washed prior to counting. Pools were from 30–50 donors.

The possibility was considered that the failure of the CAF_1 mouse to respond to hypoxia reflected a difference in the hemoglobin molecule and its affinity for oxygen, similar to that which has been described in man with hemoglobins Chesapeake (5) and Yakima (6,7). However, polycythemia has been a primary finding in such high oxygen

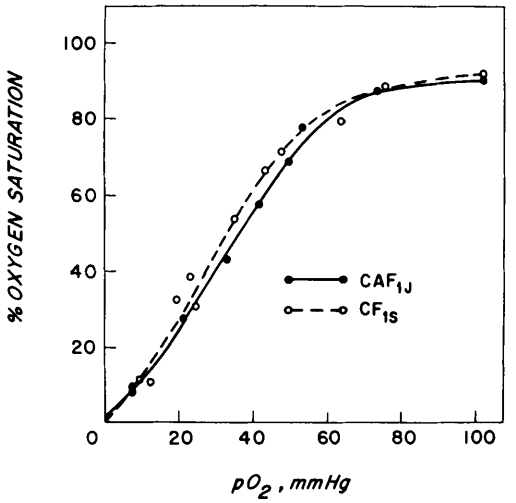

FIG. 1. Oxygen dissociation curves for the hemoglobin from CAF_1 and CF_1 mice (values corrected to pH 7.4, temperature 37°C).

affinity hemoglobinopathies; this is thought to be due to impaired release of oxygen at the tissue level. Against such an explanation was the obvious lack of polycythemia in the CAF_1 mouse with a red cell mass lower than that of the responsive CF_1 strain. Direct measurement of the oxygen affinities of these hemoglobins, as was shown in Fig. 1, confirmed the impression that no basic difference existed in the capacity of the red cells to transport or release oxygen. In view of this similarity in oxygen dissociation curves for the two strains, the difference in response to hypoxia and anemia would suggest that there is a basic difference in the two mechanisms responsible for erythropoietin production. The nature of this difference is not clear, but further exploration of this area may provide important insight into the regulatory mechanism of erythropoiesis.

It is of interest to note that other workers have observed that some strains of mice failed to develop a substantial polycythemia after prolonged exposure to hypoxia (8–10). For example, McDonald *et al.* (9) reported that the BALB/cJ mouse had a relatively mild increase in hematocrit, from 50–56%, after 3 weeks of hypoxia. The BALB/cJ is a parent strain of the CAF_1 mouse used in the present experiments. It would appear, therefore, that the reduced capability of responding to hypoxia is genetic in origin. We would emphasize, then, the importance of considering possible strain differences in interpreting results of physiologic studies on hematopoiesis.

Summary. A strain difference in the response to hypoxia of the CAF_1 and CF_1 mice is described. The CF_1 strain developed significant erythropoietin production in response to hypoxia, and after 3 weeks marked polycythemia. In the CAF_1 mouse both erythropoietin production and polycythemia were minimal. Both strains produced comparable amounts of erythropoietin in response to posthemorrhagic or hemolytic anemia. The implications of these differences are discussed.

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Addendum. Since submitting the manuscript it has come to our attention that the BALB/c mouse also has a reduced capacity to produce erythropoietin in response to hypoxia (M.L. Nohr, Am. J. Physiol. **213**, 1285, 1967).

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