dose and in increase in incorporation of iron per unit of stimulating material. However, in certain instances, the shorter time between injection of the isotope and sacrifice of the animals in the 8-hour splenic assay may be advantageous.

Uptake of iron^{59} into peripheral RBC's in the starved rat is directly proportional to the injected dose of ESF in the range between 2.4 and 48 cobalt units.

The minimal amount of ESF needed to increase significantly Fe^{59} incorporation above saline controls in this assay is 80 times that needed in the 48-hour blood assay using the polycythemic mouse. The greater sensitivity of the 48-hour mouse blood assay is also seen in the greater coefficient of regression of the dose-response curve. This heightened sensitivity is of most importance when the test material is limited in activity or amount. The upper limit of the linear portion of the dose-response curve in the 48-hour mouse blood assay is 2.4 cobalt units which coincides with the minimal detectable dose of the starved rat assay. These 2 assays are thus complementary and the rat asay, which does not require laborious preparation of the assay animal, may be used more conveniently when very active material is to be assayed.

Summary. Incorporation of Fe^{59} with blood and spleen of transfused polycythemic mice at various intervals has been used to find sensitive assay systems for erythropoietin. Transfusion polycythemia markedly reduces erythropoiesis as judged by low levels of Fe^{59} uptake into the spleen and into the blood after 16 hours. In polycythemic mice receiving injections of erythropoietin, incorporation of Fe^{59} is maximal in the spleen at 8 hours and in the blood at 48 hours after injection of the isotope. Incorporation of Fe^{59} into the blood of the polycythemic mouse 48 hours after giving the isotope was found to be the most sensitive assay when compared to the starved rat assay and other polycythemic mouse assays.

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Effect of Cortisol on Liver Phosphorylase Activity.* (27352)

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Phosphorylase activity in several organs and tissues is altered by various hormones. Epinephrine and glucagon cause a rapid increase in liver phosphorylase activity under *in vivo* and *in vitro* conditions(1,2,3). ACTH specifically increases adrenal phosphorylase but has no effect on enzyme activity in other tissues(4). Skeletal and cardiac muscle phosphorylases are subject to hormonal influence, epinephrine producing a rise in activity of both enzymes, whereas glucagon has greater effect on cardiac muscle phosphorylase(5,6). Adrenal cortical hormones are known to stimulate liver phosphorylase(7,8) although effects of these hormones on the enzyme have not been thoroughly investigated. The time required for phosphorylase activity to rise after corticosteroid administration and the duration of increased enzyme activity have not been established. Furthermore, it is not known whether continued hormone administration will have greater effect on liver

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phosphorylase than does a single injection. Since phosphorylase serves a major role in glucose formation and adrenocortical hormones have important effects on carbohydrate metabolism, it was of interest to investigate further the effects of cortisol on liver phosphorylase activity. The results demonstrate that cortisol causes an increase in phosphorylase activity which is apparent within 12 hours, continues to rise for 48 hours and becomes maximal after several injections of the hormone.

Procedure. Male, Holtzman rats were housed in individual cages and fed Purina laboratory chow. In the first experiment, one group of animals was given a single subcutaneous injection of cortisol (free alcohol) suspended in vehicle, a mixture of 0.5% carboxymethylcellulose, 0.4% Tween 80 and 1.5% benzyl alcohol in isotonic saline. Cortisol was administered in a dose of 5 mg/100 g body weight. Control rats were injected with the vehicle only. Animals were selected at random from both cortisol-treated and control groups and sacrificed by decapitation at intervals of 2,4,6,12 and 24 hours after injection. All rats were fasted for 24 hours before being decapitated. The liver was quickly removed, weighed and homogenized in cold 0.1 M sodium fluoride which contained 0.001 M EDTA.† Phosphorylase activity of homogenate was determined by the method of Sutherland(9), however, the incubation period was increased from 10 to 15 minutes. Protein content of liver was assayed using Lowry's technic(10) and glycogen concentration determined by the method of Walaas and Walaas(11). Blood glucose levels were measured in some rats at time of sacrifice (12).

In the second experiment, one group of rats was given 5 mg of cortisol subcutaneously while control animals were injected with vehicle. At 24, 36 and 48 hours after injection, rats from each group were randomly selected and sacrificed by decapitation. All animals were starved for 24 hours prior to decapitation. The liver of each animal was removed, weighed and homogenized

† ethylenediaminetetraacetic acid.

TABLE I. Liver Phosphorylase Activity during First 24 Hours after a Single Injection of Cortisol (Mean Values ± Stand. Error of Mean).

	Body wt (g)	Liver wt (g)	Blood glucose (mg/100 ml)	Liver glycogen (mg/liver)	Liver phosphorylase (u/g liver)	Liver phosphorylase (u/g liver protein)	Liver phosphorylase (u/total liver)
Control rats (11)	116.3 ± 1.6	4.4 ± .1	69.0 ± 4.9 (6)	21.8 ± 3.8 (8)	10.0 ± .2	53.5 ± 1.6	43.8 ± 1.2
Cortisol treated rats 2 hr after inj. (5)	112.4 ± 2.1	4.5 ± .1	77.6 ± 4.8 (5)	35.6 ± 4.8*(5)	9.3 ± .4	52.2 ± 1.1	41.7 ± 1.0
6 hr after cortisol (8)	112.3 ± 1.3	4.9 ± .1*	92.6 ± 5.4*(5)	121.0 ± 14.7†(5)	9.1 ± .3*	52.1 ± 1.6	43.8 ± 1.6
12 " " (8)	117.1 ± 1.5	5.2 ± .1†	88.4 ± 7.0*(5)	177.7 ± 12.5†(5)	10.9 ± .3*	62.4 ± 1.6†	57.0 ± 1.4†
24 " " (8)	118.6 ± 2.2	5.8 ± .2†	—	240.5 ± 34.8†(5)	12.3 ± .2†	69.5 ± 1.5†	69.7 ± 2.3†

No. of rats indicated by figures in parentheses.

* Significantly different from control value, P = <.05.

† Significantly different from control value, P = <.01.

TABLE II. Liver Phosphorylase Activity during Second 24 Hours after a Single Injection of Cortisol (Mean Values ± Stand. Error of Mean).

	Body wt (g)	Liver wt (g)	Blood glucose (mg/100 ml)	Liver phosphorylase (u/g liver)	(u/g liver protein)	(u/total liver)
Control rats (9)	128.4 ± 2.1	4.9 ± .3	66.0 ± 5.9	10.4 ± .4	50.1 ± 1.2	50.5 ± 1.9
Cortisol treated rats						
24 hr after cortisol (5)	119.5 ± 2.1	5.1 ± .2	95.8 ± 4.6*	11.9 ± .2†	59.1 ± 2.5†	60.9 ± 1.9*
36 hr " " (5)	122.1 ± 4.3	5.9 ± .2*	91.8 ± 6.1*	12.5 ± .4†	66.6 ± 1.6†	73.4 ± 3.4†
48 hr " " (5)	121.7 ± 2.2	5.9 ± .2*	87.0 ± 8.1*	13.6 ± .4†	68.9 ± 1.9†	80.5 ± 4.0†

No. of animals in parentheses.
* Significantly different from control value, P = <.05.
† *Idem* P = <.01.

as in the previous experiment. Phosphorylase activity of liver homogenates as well as hepatic protein concentrations were determined. Blood glucose levels were measured in all animals at the time they were sacrificed.

In Exp. 3, rats were given cortisol subcutaneously daily for 1, 3 or 7 days while control animals received carboxymethylcellulose vehicle. Rats that received 1 or 3 cortisol injections were given 5 mg each day. Animals injected with 7 doses of hormone received 5 mg daily for 4 days but, because of severe weight loss, the dose was reduced to 3 mg per day for the last 3 days. Food was withheld from all rats for 24 hours before sacrifice. Phosphorylase activity and protein content of liver were assayed as in the first experiment.

Results. In Exp. I, blood glucose and liver glycogen were elevated within 2 hours after cortisol injection (Table I). Blood sugar concentration reached its maximum at 6 hours; however, the greatest liver glycogen content was attained 24 hours after hormone administration (Table I). Hepatic phosphorylase activity was unchanged for 6 hours after cortisol injection (Table I). Within 12 hours, however, a rise in enzyme activity became evident and at 24 hours the increase in liver phosphorylase was even greater (Table I).

In Exp. II, blood glucose level was increased 24 hours after cortisol and remained elevated 36 and 48 hours following hormone administration (Table II). Liver phosphorylase activity 24 hours after cortisol was approximately 20% greater than that of controls; however, the rise was less than that which occurred in the first experiment. The level of enzyme activity became progressively higher 36 and 48 hours after hormone injection (Table II).

The third experiment again demonstrates increased liver phosphorylase activity 24 hours after a single injection of cortisol but also shows that activity of the enzyme is more markedly increased following several doses of hormone (Table III). The level of hepatic phosphorylase activity was high in rats given 7 injections of cortisol even though these animals demonstrated severe loss of body weight (Table III).

Administration of cortisol in all experiments was associated with increased liver weight, as has been previously noted(13).

Discussion. Phosphorylase activity is increased in various tissues following administration of several hormones including glucagon(1), epinephrine(2), ACTH(4), growth hormone and chorionic gonadotrophin(14). The rapid effect which epinephrine, glucagon and ACTH exert on phosphorylase activity is dependent on formation of cyclic adenosine monophosphate (AMP) by a particulate fraction of the cell(15). Growth hormone and chorionic gonadotrophin might also affect phosphorylase activity of corpus luteum by stimulating production of cyclic AMP since these hormones have a prompt effect on phosphorylase and are active *in vitro*. Appearance of 3′, 5′ AMP in corpus luteum after growth hormone and chorionic gonadotrophin has not been demonstrated, however. The action of cortisol and other adrenal steroids in stimulating liver phosphorylase activity is apparently different from that of other hor-

TABLE III. Effect of Multiple Injections of Cortisol on Liver Phosphorylase Activity (Mean Values ± Stand. Error of Mean).

	Body wt (g)	Liver wt (g)	Liver phosphorylase (u/g liver)	(u/g liver protein)	(u/total liver)
Control rats (5)	138.2 ± 3.0	5.1 ± .2	10.9 ± .3	57.2 ± 1.4	55.5 ± 2.0
Cortisol treated rats					
1 inj. (dosage) (5)	128.6 ± 3.2	5.8 ± .4	12.5 ± .6†	71.5 ± 1.1 †	72.3 ± 5.2*
3 daily inj. (5)	119.0 ± 1.8	6.7 ± .5†	14.1 ± .5†	89.6 ± 4.5 †	94.4 ± 7.6†
7 daily inj. (5)	86.4 ± 3.2	4.4 ± .1	16.1 ± .7†	87.7 ± 4.01†	71.7 ± 4.3*

No. of animals indicated by figures in parentheses.
* Significantly different from control value, P = <.05.
† *Idem* P = <.01.

mones since there is an appreciable delay before a rise in enzyme activity is evident. Furthermore, phosphorylase activity continues to increase for at least 48 hours after hormone injection and maximal activity is obtained only when multiple injections of steroid are given. These observations suggest that cortisol does not cause increased phosphorylase activity by stimulating formation of cyclic AMP but through some other mechanism.

Cortisol and certain other adrenal steroids have striking effects on carbohydrate metabolism in the experimental animal. Administration of these hormones is associated with elevation of blood sugar, liver and muscle glycogen and there is a concomitant increase in urinary nitrogen excretion(16). These observations support the concept that adrenal cortical hormones stimulate gluconeogenesis although there is other evidence which indicates that peripheral utilization of carbohydrate is impaired as well(16). The fact that cortisol stimulates liver phosphorylase activity suggests that this may contribute to the rise in blood sugar which occurs in response to the hormone since phosphorylase is concerned with glycogenolysis. However, in these experiments it was observed that blood sugar and liver glycogen rose within 2 hours after cortisol, whereas, phosphorylase activity was not elevated until 12 hours after hormone injection. These findings indicate that increased liver phosphorylase activity is not a primary effect of adrenal steroid treatment but is, instead, an adaptive change induced by elevated body carbohydrate stores which are produced by the hormones.

Summary. The effects of single and multiple doses of cortisol on liver phosphorylase activity have been determined and correlated with alterations in blood glucose and liver glycogen. Phosphorylase activity was increased 12 hours after a single injection of cortisol and continued to rise for 48 hours. Multiple injections of the hormone produced a much greater increase in enzyme activity than did a single dose. Elevation of blood sugar and liver glycogen which followed cortisol occurred several hours before a change in phosphorylase activity could be demonstrated.

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Folinic Acid Activity in Leucocytes.* (27353)

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Leukemic leucocytes contain a higher concentration of folinic acid active material than mature leucocytes(1,2). In these investigations it was noted that the increased levels of citrovorum factor activity (CF) correlated with degree of immaturity of the leukemic cell type, the highest elevations being present in acute leukemic cells. Furthermore, although some free CF activity was present, most of the activity in leucocytes was in a bound form. Ellison and Hutchinson(2) noted that CF activity of leucocytes was maximal only when the sample was protected from oxidation by including ascorbate in the assay procedure. Thus, the indications are that several folate forms exist in leucocytes which may be designated as free, bound, oxygen-stable and oxygen-labile CF. Other workers have demonstrated the occurrence of oxygen-labile bound CF in liver(3), red cells(4), and blood plasma(5). However, the relative amounts of each CF form in leucocytes have not been previously reported. Hence the present study was undertaken to establish values for free, bound, oxygen-stable and oxygen-labile CF in normal leucocytes. Preliminary observations in leukemic leucocytes are also included.

Method. Six healthy, well nourished medical students were studied to establish normal values. The leukemic patients (Table II) are classified according to cell morphology of peripheral blood and bone marrow. These patients had received no therapy for leukemia. All subjects were on unrestricted diets with no vitamin supplements, except for normal subject E.M. who was receiving 100 μg folic acid daily.

Venous blood was obtained from individuals in a fasting state for leucocyte separation. Approximately 300 cc of blood was necessary to obtain sufficient numbers of leucocytes (0.5-1.0 g wet weight of cells) for assay in subjects with leucocyte counts in the normal range. Leucocytes were separated by the phytohemagglutinin method(6). Microscopic examination of leucocyte morphology showed no alteration after separation, and erythrocyte contamination was negligible. However, platelets were included in the leucocyte extracts. When the buffy coat layer was aspirated directly in patients with high leucocyte counts CF assay results were not essentially different from the activity of those samples obtained by the agglutination method. The dry weight of the residue was determined by heating at 100°C for 30 minutes to correct for variations of water content in the samples. The results are expressed as mμg of CF activity/mg of leucoctyes-dry weight. The samples were assayed immediately or stored at –20° until use.

The leucocytes from each subject were divided into fractions and assayed after treatment (Table I). In Table I the column entitled *Incubation* indicates that the fraction was incubated at 37°C in 0.25 M phosphate buffer at pH 6.6-6.8** for 18-20

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** Although it has been stated that optimal pH for release of folate conjugates is 4.5(1), we have found that yields are much greater at pH 6.8, similar to Chang's findings(3).