sure within the common hepatic duct of the rat with a strain-gauge manometer is described. Measurements of pressure in the common hepatic duct were made on a series of fasting rats with normal livers, cirrhosis or passive venous congestion of the liver, and these were analyzed statistically. The higher mean secretory pressure in the rat with cirrhosis over that in the rat with normal liver and with passive venous congestion is statistically significant. 2) Secretory pressure in rats after inhalation of carbon tetrachloride vapor fell slightly but significantly. 3) The duodenal-end pressure in the common hepatic duct was related to findings on simultaneous recordings of biliary pressure and gastric and duodenal motility. This pressure was significantly higher in rats with chronic venous congestion of the liver than in normal or cirrhotic animals. The lower end of the common hepatic duct probably has some sphincteric action in the rat. 4) Intravenous injection of chlorpromazine hydrochloride (thorazine) produced no effects on pressure in the common hepatic duct in normal rats.

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Studies on Erythropoietin. II. Production of High-Titer Plasma in Sheep.* (25361)

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Most investigators used rabbits for production of erythropoietin-containing plasma, although the literature suggests that ability to respond to various erythropoietic stimuli is widespread among mammals. At start of our program aiming toward isolation and clinical testing of the pure factor, it became clear that an animal considerably larger than the rabbit would be desirable for economical production of amounts of material needed. After careful consideration of the price and availability of various domestic animals, we selected cull breeder sheep as most suitable for our needs.

Methods and materials. Cull breeder sheep, mostly ewes, in age range of 3 to 7 years, and weighing over 100 lb. (45.5 kg) were used. Hay constituted the bulk of diet during experiments, and water was supplied *ad lib.* Anemia was induced by subcutaneous injection of phenylhydrazine† above shoulder, alternating sides, on days 1, 3, and 5. Blood was harvested by exsanguination on day 7, using the following procedure: the animal was shackled by hind feet and lowered into a plastic bag containing an atmosphere of CO_2 supplied by dry ice. When struggling ceased (approximately 20 seconds), an incision through the skin was made with sharp knife starting from chin and extending to breast

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† For injection, neutralized phenylhydrazine hydrochloride (Fisher Certified Reagent) was used at a concentration of 24 mg/ml.

TABLE I. Blood Values and Potency of Plasma of 16 Cull Breeder Sheep Given 48 mg Phenylhydrazine/kg Body Weight.

Wt (kg)	Hematocrits: Initial	Day 3	On drawn blood	Blood vol (l)	Potency of plasma (u/ml)
67	35	33	25	2.0	.34
71	33	14	7.0	1.9	1.6
79	39	31	13.5	1.7	1.8
41	40	35	33	1.7	.10
74	36	26	15.5	1.3	.86
52	42	30	21	1.7	.32
47	35	31	22	1.6	.61
72	34	26	12.5	1.8	.88
61	53	37	23	1.1	.44
72	41	30	8.0	1.5	7.4
38	48	32	17	1.3	.52
77	37	13	9.0	1.6	3.5
88	35	18	10.5	2.2	3.6
92	42	31	23	3.1	.41
59	32	21	6.5	1.9	5.9
97	37	22	13.5	2.1	1.2

bone. Retractors were inserted to spread the wound. By a stainless steel knife welded into a stainless steel funnel, both aorta and jugular were severed by a sharp upward thrust into thoracic area. The funnel directed the flow of blood through a plastic tube into a plastic vessel containing heparin. The bleeding process required one to two minutes. The cells were removed from blood by centrifugation in refrigerated Sharples laboratory super-centrifuge. During the process, temperature of plasma was reduced to 5-10°C. Plasma samples were stored frozen and assayed by procedure described previously(1).

Results. In preliminary experiments, the dose of phenylhydrazine was based solely on body weight of animals. In accordance with the work of Jacobsen, *et al.*(2), on rabbits, the sheep were given a total of 48 mg phenylhydrazine hydrochloride/kg body weight in 3 equal doses on days 1, 3, and 5. All animals were bled on day 7. Table I shows the results. Animals varied considerably in their initial hematocrit levels and in their reaction to standard dose of phenylhydrazine. More importantly, the potency of plasma varied greatly, ranging from 0.1 to 7.4 units/ml. The very high potencies obtained in plasma from animals with low hematocrit values suggested a logarithmic relationship and, indeed, the best straight-line fit was obtained by plotting on semi-log paper, as shown in Fig. 1. This relationship showed the need for obtaining low hematocrit values in the sheep before harvesting blood. Accordingly, all practical measures were taken to attain hematocrit levels in the range of 10-15% at time of blood harvest (7th day). This was done by the following procedure.

The first and second doses (on days 1 and 3) were equal and based on body weight, with a downward adjustment for subnormal initial hematocrit values. For animals with hematocrits of 40 or over, the first 2 injections were each 16 mg phenylhydrazine hydrochloride/kg body weight. Doses for animals of lower hematocrits were scaled downward proportionately until a level of 10 mg/kg was reached at hematocrit level of 30. On day 5, another hematocrit value was taken and size of third injection was then based on progress of animal's blood level toward the target value of 10-15% hematocrit on day 7. The status on day 5 could be judged quickly by making a rough plot of hematocrit readings against time and then comparing the slope of the straight line connecting initial and 5th day values with the slope obtained by drawing a

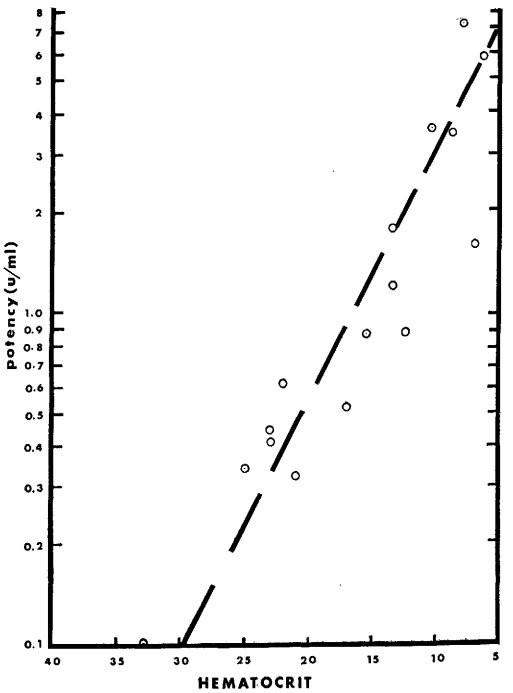

FIG. 1. Potencies of sheep plasmas plotted against final hematocrit values.

TABLE II. Blood Values of 24 Sheep Given Adjusted Phenylhydrazine Dose.

Wt (kg)	Initial het	Het on day 5	Factor for 3rd dose (day 5)	Final het
41	32	21	1.0	11
55	38	33	2.0	19
52	32	25	1.0	9
91	43	28	1.25	19
55	39	died		
77	44	25	1.0	9
64	39	29	1.5	16
64	38	died		
71	39	22	1.0	13
82	47	24	.75	15
51	36	24	1.25	18
113	45	27	.75	11
50	43	21	.75	8
62	40	27	1.5	22
66	39	17	.15	12
66	38	24	1.25	9
82	41	20	.75	died
57	37	16	.15	13
50	42	20	.4	9
71	34	24	1.25	13
114	41	18	.1	13
75	29	28	2.0	17
109	41	27	1.25	died
91	35	31	2.0	10

line from initial hematocrit value to 12.5 at day 7.

The results of a representative experiment are shown in Table II. A total of 31 liters of plasma was obtained from 20 animals which survived until blood harvest. The potency of the pool was 1.25 units/ml, giving a total of almost 40,000 units. Similar pools from 50 animals have varied in potency from 0.6 to 2.5 u/ml, with an average of 1.3.

Discussion. The logarithmic relationship suggested by Fig. 1 indicates that very effort should be made to induce low hematocrit levels. In a practical way, however, the benefits are balanced by losses incurred by deaths, as dose of phenylhydrazine is increased. We found that increasing the dose even 20% beyond that given above, results in a marked increase in mortality and the resulting decrease in plasma volume is not compensated by the increase in potency.

Another variable which we have considered is rate at which anemia is induced. Previous investigators, including Jacobsen, *et al.*(2), used approximately one week. From an economic standpoint, it would be desirable to decrease this period. However, it has been our experience that plasma from animals brought to low hematocrit levels in 4 days by injection of larger doses of phenylhydrazine has been much less potent than that from animals brought to comparable blood values in the usual 7 day period.

Goldwasser, *et al.*(3) have shown that injection of cobalt into rats markedly increases the erythropoietin titer of plasma. We obtained a similar effect in sheep. However, blood from cobalt-treated sheep contains such a large volume of cells that only about 40% of the volume is recoverable as plasma, while blood from phenylhydrazine-treated sheep yields 80% as plasma. Since cobalt plasma was not more potent than phenylhydrazine plasma, the use of cobalt was eliminated on an economic basis.

It has been estimated(1) that normal sheep plasma contains only about 0.01 u/ml of erythropoietic activity. Thus increases produced during the phenylhydrazine process are of the order of several hundred times. Since no storage organ for erythropoietin has as yet been discovered, it appears that it is all produced during the 7 day period with the greater part being made in the last 24-48 hours. At least one disease condition in humans gives rise to plasma concentrations comparable to the highest levels produced in our sheep experiments. We have recorded values as high as 8 u/ml in hypoplastic anemia patients. Others(4) have reported similar high levels.

Summary. Plasma of high erythropoietic titer has been produced in cull breeder sheep by the use of phenylhydrazine. The potency of plasma appears to have an inverse logarithmic relationship to the final hematocrit levels of the sheep blood. By making adjustments of the phenylhydrazine dose according to the condition of the individual sheep, the potency of the plasma pools from 25-50 animals has been raised to an average of 1.3 u/ml. This value is about 130 times the potency of normal sheep plasma.

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