This group of 8 samples, 6 treated and 2 controls, was incubated for 21 days at 4°C, then subjected to electrophoresis. It was found that the presence of the various chemicals under the stated incubation conditions caused no significant changes in the electrophoretic pattern of the human plasma.

Discussion. The results of these experiments indicate that glyoxal and Kethoxal may have value as virucidal agents. Both chemicals were more potent in destroying the infectivity of influenza A, Newcastle disease and mouse hepatitis viruses than was an equimolar amount of BPL. Glyoxal appeared to be less lytic than BPL or Kethoxal, and none of the chemicals caused significant changes in human plasma proteins at concentrations which were effective in sterilizing artificially-infected blood. These results suggest that glyoxal and Kethoxal hold some promise as blood-sterilizing agents. Glyoxal gave results as good or better than Kethoxal. Since thorough studies have not been made on the toxicity of glyoxal for mammalian hosts(7,8), further work is required before it can be applied to the large-scale sterilization of blood and plasma. The marked superiority of glyoxal over formaldehyde as a virus inactivating agent suggests also the possible application of glyoxal in the preparation of "killed" vaccines or as a general viral disinfectant.

Summary. Both glyoxal and β-ethoxy-α-ketobutyraldehyde (Kethoxal) were more potent virucidal agents than β-propiolactone (BPL). Glyoxal was less lytic for human erythrocytes than was Kethoxal or BPL. None of the chemicals showed significant toxicity for plasma proteins as measured by electrophoresis. These experiments indicate that glyoxal may be of potential value in the sterilization of human blood and plasma.

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Increased Erythropoietic Stimulant in Plasma of Pregnant Rats.* (22777)

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The existence of an erythropoietic substance in the blood stream was first suggested by Carnot and Deflandre(1) who showed that plasma from rabbits which had been bled repeatedly when injected into other rabbits resulted in hyperplastic bone marrow and increased reticulocyte and erythrocyte counts. Several investigators have since(2-19) confirmed the presence of erythropoietic activity in plasma of animals which had been bled. Plasma from animals exposed to low oxygen tensions has similarly been found to stimulate erythropoiesis in recipients (6b,9,20-22, 24b). Human umbilical cord blood, also fetal sheep and dog blood, have been found to increase red blood cell count in recipient animals(22-26). As these reports of an erythropoietic substance in blood were based either

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on reticulocyte counts, red blood cell counts, or hemoglobin determinations, and as these methods are subject to error introduced by changes in plasma volume, it is not surprising that other investigators have been unable to confirm the presence of an erythropoietic substance in blood(27-29).

It was the object of this study to reinvestigate the existence of an erythropoietic stimulant in the blood stream of the rat, using a more satisfactory method for determination of increased numbers of red cells in the circulation, namely, the total circulating red cell volume. Changes which may exist under differing physiological conditions, such as pregnancy and fetal life, would then be on a more sound basis. This paper compares the levels of erythropoietic factor in plasma of pregnant and normal female rats.

Material and methods. The test animals for measuring red cell volume were female rats of the Long-Evans strain, hypophysectomized on the 26th to the 28th day of life and maintained for 45 to 50 days to allow stabilization of the hematological values at the low level characteristic of hypophysectomized rats. The anemia which develops within this period in such animals is severe, the red cell volume being 50% below normal. This anemia is known to be promptly repaired after administration of erythropoietic stimulants such as cobalt, plasma factor and pituitary extracts(15,30-32). The plasma from pregnant rats was newly prepared for each day's injection, as follows: Between the 15th and 18th days of pregnancy the donors were exsanguinated by withdrawal of blood from the aorta into a heparinized syringe. The blood was transferred at once to centrifuge tubes containing a small amount of mineral oil to exclude air, and was centrifuged for 12 minutes at 2,000 rpm. The plasma was drawn into a syringe and immediately injected intraperitoneally. This procedure was repeated daily for 14 days. Groups consisting of 6 to 8 hypophysectomized rats received daily doses of 2 or 4.8 cc. Other groups received 2 or 4.8 cc of plasma similarly prepared from normal adult female rats of the same age as the pregnant donors. At the termination of the ex-

TABLE I. Increased Hematological Values of Hypophysectomized ($\overline{H}$) Rats Injected with Plasma from Normal (N) or Pregnant Rats.

Type of plasma	Daily dose 14 d, ml	No. rats	Body wt, g*	Hemoglobin, g		Blood vol per 100 g body wt, ml	Plasma vol per 100 g body wt, ml	Hematocrit, %	Red cell vol, ml	
				Per 100 ml	Per 100 g body wt				Total	Per 100 g body wt, ml
Pregnant	2	26	82 ± 1	9.3 ± .2	.51 ± .01	5.52 ± .10	3.85 ± .20	30.4 ± .2	1.38 ± .12	1.68 ± .05
	4.8	7	84 ± 1	10.3 ± .1	.67 ± .02	6.44 ± .15	4.45 ± .10	30.8 ± .1	1.67 ± .09	1.99 ± .03
Normal	2	22	85 ± 1	9.2 ± .2	.47 ± .02	5.18 ± .18	3.73 ± .08	28.5 ± .1	1.23 ± .11	1.45 ± .02
	4.8	6	81 ± 1	10.0 ± .2	.58 ± .01	5.82 ± .15	4.04 ± .10	30.5 ± .1	1.44 ± .11	1.78 ± .02
Controls										
$\overline{H}$		19	76 ± 1	9.3 ± .2	.45 ± .01	4.80 ± .07	3.41 ± .09	28.9 ± .1	1.06 ± .07	1.39 ± .04
N		29	198 ± 3	13.0 ± .2	.67 ± .02	5.13 ± .08	2.88 ± .08	43.9 ± .5	4.46 ± .09	2.25 ± .03

* Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

periment control hematological values were determined in uninjected hypophysectomized and normal rats of the same age as the experimental groups. Red cell volumes of all rats were determined by the Fe^{59} tagged cell method(31). The result obtained from injection of the lower dose level (2 cc of plasma) was confirmed 3 times. All hypophysectomized rats were fed a standard laboratory diet (Diet XIV) consisting of: wheat 68.5%, casein 5%, fish meal 10%, alfalfa leaf meal 10%, fish oil 5%, NaCl 1.5%, KI added (analysis 1 μg iodine per g diet). Each afternoon a dish of liquid diet was given consisting of Diet I (modified McCollum Diet I): wheat 67.5%, casein 15%, skim milk powder 7.5%, fish oil 1%, NaCl 0.75%, $CaCO_3$ 1.5%, KI added (analysis 1 μg iodine per g diet). Lettuce was fed twice weekly. Pregnant and lactating rats were maintained on Diet I.

Results. The results are presented in Table I. The hypophysectomized rats injected with the daily dose of 2 cc of plasma from pregnant rats showed a significant increase in total circulating red cell volume (21%), also a significant increase in red cell volume per 100 g of body weight ($P = < .001$). The increase in circulating red cell volume was accompanied by an increase in total circulating hemoglobin of 14%, and of 13% in hemoglobin per 100 g of body weight ($P = <0.01$). Injection of the larger dose of plasma from pregnant rats (4.8 cc) increased total circulating hemoglobin by 51%, the hemoglobin per 100 g of body weight by 40%, circulating red cell volume and red cell volume per 100 g body weight by 43% ($P = <.001$). No increase in hematological values occurred in hypophysectomized rats injected with the 2 cc dose of plasma from normal rats. At the higher daily dose of normal plasma (4.8 cc) which was equivalent to, or greater than, the recipient's total blood volume, an increase of 23% in red cell volume per 100 g body weight ($P = <.001$), and of 21% in hemoglobin per 100 g body weight resulted ($P = <.001$). The stimulation to erythropoiesis, when normal plasma was used, was definitely less than that observed from injection of plasma from pregnant rats ($P = <.001$).

The hematocrit and hemoglobin/100 ml were not increased in any injected hypophysectomized rats as there was a concomitant increase in plasma volume and in total blood volume. Increase in plasma volume was more pronounced in those injected with plasma from pregnant rats, ranging from 13% ($P = <.001$) at the lower dose level to 34% ($P = <.001$) at the higher level. The increase in plasma volume in rats injected with normal serum was only 8% ($P = <.001$) at the lower dose level, increasing to 19% ($P = <.001$) at the higher level. The increased plasma volume probably is attributable to the growth promoting substances (or growth hormone) which has been shown to be present when this amount of plasma from normal or pregnant rats was given. Pituitary growth hormone has been shown to increase plasma volume(34).

Increase in red cell volume and hemoglobin in hypophysectomized rats injected with plasma from normal rats is further evidence that a circulating erythropoietic factor is normally present in the blood stream, and the higher values in rats injected with plasma from pregnant rats indicates that increased amounts of erythropoietic substance circulate during pregnancy. Increased production during pregnancy of this factor, or factors, may be responsible for the adult level of the red cell volume which characterizes the rat at birth; withdrawal of this stimulus at birth may account for the physiological anemia developing in the neonatal period(33). If this is true, then plasma from pregnant rats should prevent the neonatal anemia.

To test this concept newborn rats were injected during the postnatal period with plasma from pregnant rats and the results were compared with those from injection of normal rat plasma. Experimental groups were so constituted that each group consisted of 6 male rats, each from a different litter. Two of the 6 rats received normal rat plasma, 2 received pregnant rat plasma, and 2 were uninjected controls. (The newborn rats were identified by clipping toes, or by their color and coat pattern.) Care was taken to maintain the mothers on a diet satisfactory for lactation. The injections were started the 4th

TABLE II. Prevention of Neonatal Anemia by Injections of Pregnant Rat Plasma.

Type of plasma inj.	No. rats	Body wt, g*	Hemoglobin, g		Blood vol, ml	Plasma vol, ml	Hematocrit, %	Red cell vol, ml	
			Per 100 ml	Total	Per 100 g body wt	Per 100 g body wt		Total	Per 100 g body wt
Pregnant	40	45.0 ± .5	6.8 ± .1	.21 ± .01	.46 ± .01	6.78 ± .10	4.78 ± .08	29.5 ± .3	.90 ± .03	2.00 ± .03
Normal	29	45.2 ± 1.0	6.3 ± .1	.19 ± .01	.41 ± .01	6.52 ± .09	4.89 ± .10	27.6 ± .7	.83 ± .01	1.83 ± .04
No inj.	45	44.1 ± .5	6.7 ± .2	.18 ± .01	.41 ± .01	6.25 ± .10	4.60 ± .07	26.4 ± .3	.73 ± .01	1.66 ± .02

* Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

day of life and were continued daily for 14 consecutive days or until the rats were 18 days of age at which time the neonatal anemia is known to be most severe(33). The suckling rats were removed from cages only for the short periods required for injection. After injection each group of 6 suckling rats was placed with a different lactating female in order to equalize the milk supply. With proper care the young were not rejected because of this procedure.

The plasma was prepared by the procedure described previously. The dose given was 1 cc per day for the first 5 days, and 2 cc per day for the remainder of the experimental period.

The combined results of 4 repetitions of this experiment are presented in Table II. A statistically significant ($P = <.001$) increase (22%) occurred in the red cell volume of the newborn rats injected with plasma from pregnant rats. A smaller increase, 10% ($P = <.05$), occurred when the newborn rats received plasma from normal female rats. A significant increase also occurred in other hematological values. The anemia of the newborn rat was ameliorated, though not completely prevented, by injection of plasma from pregnant rats; the values were 10% below normal adult levels but were 22% above typical postnatal levels. From the results obtained in newborn rats, as well as in hypophysectomized rats, it may be concluded that the plasma from pregnant rats has an erythropoietic potency greater than that of the plasma from normal adult female rats. These results therefore support the assumption that an erythropoietic principle in pregnant rat serum plays a role in fetal red cell production. The site of the production of this erythropoietic factor is under investigation.

Summary. 1. Plasma of normal adult female rats contains a factor capable of stimulating erythropoiesis in hypophysectomized and newborn rats, as judged by increased circulating red cell volume and hemoglobin. 2. This humoral erythropoietic factor is markedly increased during pregnancy. 3. Injections of plasma from non-pregnant and pregnant rats resulted in an increase in plasma

and blood volumes of hypophysectomized and newborn recipients.

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