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Effect of Acute Liver Damage Plus Hypoxia on Plasma Erythropoietin Content. (23176)

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The factors which regulate erythropoiesis are gradually being elucidated. That a circulating humoral factor in the plasma is important in stimulating red cell production under many circumstances is gaining increasing acceptance(1-6). This material has been demonstrated in the plasma from experimental animals and humans with anemias of several different etiologies(7,8), and from several animal species(9-11). A consistent finding has been that the plasma from animals made anemic with phenylhydrazine was more active in stimulating erythropoiesis than the plasma from bled animals(12-14). Some have maintained that degree of anemia was the important factor here, a more severe anemia being produced by phenylhydrazine administration than could be produced by bleeding, with survival of the animal. Recently the observation has been made that this enhanced erythropoietic activity of plasma from phenylhydrazine-anemic animals is in some way related to liver damage(15).

The present study is a further effort to clarify the status of the liver as a controlling influence on the presence and titer of erythropoietic factor in the plasma. During the past year and one-half efforts have been made to demonstrate EPF* in the plasma of normal rats placed in chambers and subjected to atmospheres of 8-12% oxygen for varying periods of time. These results were uniformly negative. At no time were we able to demonstrate any ability of the plasma from these rats kept in this low oxygen atmosphere to stimulate increased Fe^{59} uptake in recipient hypophysectomized or normal rats. It was postulated that perhaps if acute liver damage were induced prior to placing the animals in the chamber an erythropoietic factor might then be demonstrable in their plasma. This proved to be the case and the results are here reported.

Materials and methods. The chambers were constructed of plexiglass. Into each chamber was directed a mixture of nitrogen and oxygen, the proportions of which, along with ambient pressure, determined the simulated altitude. The flow of nitrogen and oxygen was regulated by a rotameter type of flowmeter† and remained quite constant over the experimental period. The chamber O_2 concentration was checked by reading of the oxygen and nitrogen flowmeters and also by a portable Beckman Oximeter. Random air samples were also taken from the chamber in a Douglous bag and checked with a mass spectrograph for O_2, N_2 and CO_2. Good agreement in results was obtained. A flow of approximately 5 liters/minute was maintained through the chamber and soda lime and sulfuric acid were placed to minimize CO_2 and humidity levels.

Ten to 15 normal rats were placed in each chamber and kept there for periods ranging from 48 hours to 5 days. Food (Purina Laboratory Chow) and water were replaced as necessary. At the end of the experimental period the rats were removed, bled from the dorsal aorta and the plasma frozen and stored. A final hematocrit was done on each animal and no animal with

* Refers to plasma erythropoietic factor.

† Chicago Anesthesia Equipment Co.

TABLE I. Normal and Hypophysectomized Rats that Received Plasma from Hypoxic Animals on 2 Consecutive Days, 1 μc Fe^{59} on Day 3, and 24-Hour Fe^{59} Uptake on Day 4.

*	No. rats	Recipient rats	cc plasma per day	24-hr uptake (%) + 5.0
NP	5	Hypox	2	12.0 ± 4.62
CP	5		2	16.6 ± 9.54
NP	6		2	6.0 ± 4.53
CP	3		2	3.4 ± 1.99
NP	5		2	7.0 ± 7.4
CP	4		2	11.4 ± 10.98
NP	6		2	2.4 ± 2.35
CP	5		2	2.6 ± 1.80
NP	6	Normal	6	53.0 ± 8.40
CP	6		6	50.6 ± 4.98
NP†	10		4	62.6 ± 14.52
CP	9		4	64.6 ± 10.07
NP†	4		6	48.0 ± 10.14
CP	6		6	52.0 ± 3.88

* NP = Normal plasma; CP = Chamber plasma.
† Fe^{59} given on same day as last inj. of plasma.
The "t" test of significance for differences between means was applied to all exp. and control groups and no significant differences existed.

hematocrit below 40 was used as a source of plasma. Acute liver damage was produced in rats by injecting .25 cc CCl_4 subcutaneously just once. These rats were placed immediately in the chamber and left there for 48 hours in an atmosphere of approximately 10% oxygen. They were then withdrawn, exsanguinated from the dorsal aorta and the plasma frozen and stored until used. A final hematocrit was done on each animal and no animal with hematocrit below 40 was used as a source of plasma.

Recipient animals were Sprague-Dawley rats hypophysectomized between the ages of 2 and 3 months and used as test animals approximately 40 days after hypophysectomy. They received 2 cc of plasma I.V. via the jugular vein on 2 successive days. On day 3 approximately 1 μc Fe^{59} was given I.V. via femoral vein. Both the experimental and control groups were then split in half. Half of each group received Fe^{59} 3 hours after the last plasma injection and an 18-hour uptake was done the following day (day 3). The other half received Fe^{59} 24 hours after their last plasma injection, and a 24-hour uptake was done 24 hours thereafter (day 4).

Results. When normal rats were placed in low oxygen atmosphere (8 to 12%) for periods of either 48 hours or 5 days, we could demonstrate no significant stimulating effect of their plasma in either normal or hypophysectomized recipient animals (Table I). However, when acute liver damage‡ was induced with CCl_4 and the animal then placed at the same altitude as the normals for 48 hours, their plasma possessed distinct stimulating properties (Tables II and III). It is apparent by comparison of Table II with Table III that more clearcut differences in hypophysectomized recipients were obtained when Fe^{59} was given at a later period after the last plasma injection and a 24-hour uptake then carried out.

Discussion. The present results suggest that the normal liver inhibits the ability of the normal rat in a hypoxic atmosphere to deliver to or elaborate in its plasma a factor capable of stimulating an increased Fe^{59} uptake in another animal. However, when the liver is damaged acutely with CCl_4 this inhibiting effect is decreased and marked increments of such a factor then appear in the plasma of the hypoxic animal.

Jacobsen *et al.*(15) have demonstrated the importance of liver damage in phenylhydrazine-anemia as a prerequisite to the outpouring of EPF into the plasma. Our findings tend to support these observations and demonstrate that production of significant amounts of EPF can be stimulated in an animal with no anemia provided hypoxic hypoxia and liver damage are present in combination.

The method of demonstrating an erythropoietic response in recipient test animals is a problem in itself. What constitutes reliable evidence of significant erythropoietic stimulation and what does not? A measurement of total red cell volume in each animal with labeled red cells before and after the experiment to detect a significant increase is per-

‡ Sections of livers show varying degrees of liver damage. This appears as vacuolization of the cells of part or all lobules and necrosis in the centrilobular region. Mitotic figures are present indicating regeneration. The rats subjected to low oxygen tension show a more severe degree of damage than those in normal atmosphere.

TABLE II. Hypophysectomized Rats Given 2 cc of Plasma I.V. on 2 Consecutive Days, 1 μc Fe^{59} I.V. 3 Hr after Last Plasma Injection on Day 2, and 18-Hr Uptake Done on Day 3.

	No. of animals	Avg wt (g) & range	Avg Hct (%) & range	Avg Fe^{59} uptake (%)
Group I—Receiving plasma from rats given .25 cc CCl_4 and kept at 10% oxygen 48 hr	6	198 (172-206)	47 (45-48)	9.9 ± 8.45*
Group II—Receiving plasma from rats given .25 cc CCl_4 and kept at normal oxygen tension 48 hr	7	199 (193-205)	46 (40-48)	1.5 ± .03
Group III—Receiving normal plasma	7	198 (193-208)	45 (43-47)	3.2 ± 1.24
Group IV—Untreated	6	205 (199-221)	50 (46-52)	3.7 ± 3.5

* ± S.E.

The "t" test of significance for differences between the means was applied to group I *vs* groups II, III and IV. Values were significant at 1% level comparing groups I and II. Significance was between 1% and 2% comparing group I *vs* III and IV.

TABLE III. Hypophysectomized Rats Given 2 cc of Plasma I.V. on 2 Consecutive Days, 1 μc Fe^{59} I.V. on Day 3, and 24-Hr Uptake Determined on Day 4.

	No. of animals	Avg wt (g) & range	Avg Hct (%) & range	Avg Fe^{59} uptake (%)
Group I—Receiving plasma from rats given .25 cc CCl_4 and kept at 10% oxygen 48 hr	7	194 (185-205)	44 (43-47)	37.5 ± 3.8 *
Group II—Receiving plasma from rats given .25 cc CCl_4 and kept at normal oxygen tension 48 hr	6	193 (186-217)	47 (45-50)	10.7 ± 2.88
Group III—Receiving normal rat plasma	7	209 (186-218)	45 (43-47)	7.0 ± 4.86
Group IV—Untreated	6	191 (180-203)	48 (45-50)	3.1 ± 1.8

* ± S.E.

The "t" test of significance for differences between means was applied to group I *vs* groups II, III and IV. The difference was significant at the level of less than 1% in all.

haps the most direct assessment of response. Other methods such as 1) response of Hb, Ht, RBC and retic. count 2) marrow cellularity and differential, possess quantitative errors largely due to fluctuations in plasma volume and faulty sampling. The 24-hour Fe^{59} uptake is simple and widely used to demonstrate response or lack of it. Its reliability as a criterion of red cell production under varied circumstances needs further elucidation. Assuming reliability, it has marked advantages in that it shows an earlier response and greater magnitude of response than the other criteria available. Comparative studies of the various methods are in progress. The fact that one group using one method(15) finds plasma from bled-anemic animals totally inactive and others using different methods(5) find it sufficiently positive, illustrates the possible effect on conclusions that methodology may produce.

Stohlman *et al.*§¶ have reported erythropoietic stimulating effects of plasma from normal animals placed at various simulated altitudes, when given to sublethally irradiated recipients. 24-hour Fe^{59} uptake was the criterion used for presence or absence of response. We are unable to explain the discrepancy between their results and ours. The only significant differences in method were 1) their test animal was the sublethally irradiated rat whereas ours was either the normal or hypophysectomized rat. 2) Their altitude levels were simulated in decompression chambers whereas ours were accomplished using various mixtures of nitrogen and oxygen at ambient pressure. The degree of hypoxia was comparable. 3) Their animals were kept hypoxic for 24 hours whereas

§ Personal communication.

¶ Reported at Sixth International Congress of Hematology.

ours were kept so for 48 hours or longer.‖

Summary. Normal rats placed in a low oxygen atmosphere did not elaborate in their plasma a factor capable of stimulating increased incorporation of Fe^{59} into red cells of normal or hypophysectomized recipients. However, when their liver was acutely damaged with carbon tetrachloride and they were then placed in low oxygen atmosphere, significant amounts of such a factor appeared in their plasma. This is interpreted as further evidence that the liver, under ordinary circumstances, has a significant part in the mechanisms that control red cell production.

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‖ Subsequent studies have shown 1) there is variation in susceptibility of groups of rats to the hepatotoxic effect of carbon tetrachloride. Only those with more severe damage show increased amounts of erythropoietic factor in their plasma at 48 hours. 2) increased levels of plasma erythropoietic factor have been found regularly in the plasma of hypoxic rats without liver damage at 24 hours. This has disappeared in every instance at the 48 hour interval.

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Uterotrophic Effect of Digoxin Administration in the Rat.* (23177)

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Le Winn(1) has described gynecomastia in men receiving prolonged digitalis medication for management of congestive heart failure. He suggested that digitalis steroids have an estrogen-like effect. A search of the literature has failed to reveal any description of estrogen-like stimulation in digitalized human or animal females. During the course of investigating the relationship between cardiotonic glycosides and protein metabolism, we observed that the uteri of mature ovariectomized female rats were trophically stimulated by the administration of digoxin. As a result, experiments were designed to further investigate this observation.

Material and method. Nineteen 68-day-old female Sprague-Dawley rats were bilaterally ovariectomized and divided into 4 groups following a 21-day post-operative period. The groups were treated as follows for

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