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INDUCTION OF ENEMIA IN SHEEP AND RABBITS BY PHENYLHYDRAZINE

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ABSTRACT : Five sheep and twenty rabbits were subjected to phenylhydrazine intraperitoneal injections. An injection dose of approximately 3.0 mg. per Kg. body weight was used. The 2.5 % phenylhydrazine sterile soiution was administered every day for sheep and every two-day for rabbits. Hemoglobin concentration and hematocrit of the experimental animals were determined. It was observed that phenylhydrazine caused a 67 % and 59 % decrease after administrations for 28 days and 18 days in sheep and rabbits respectively. Erythropoietic activity in the anemic plasma filtrate of the experimental animals assayed by Fe^{59} incorporation method was in the range of 0.07-0.09 Cobalt Unit per mg actual weight.

INTRODUCTION : Anemia is still an important problem in Thailand. Conditions of anemia can be investigated in animal models. Anemia in animals can be artificially induced by bleeding, administration of some chemical agents, or drug, e. g. trinitrotoluene (1, 2), phenacetin (3, 4), primaquine (5) and phenylhydrazine has been commonly used on this purpose for a long time. Anemic plasma of the experimental animals becomes a good souce for preparation of erythropoietin. In this paper, the hematologic effect of phenylhydrazia in sheep and rabbits will be reported and erthropoietic activity in their anemic plasma filfrate will be assayed.

MATERIALS AND METHODS

A. **Animals :** Five male sheep weighing from 30 to 40 Kg, 3-5 years of age and 20 rabbits in both sexes weighing from 2-5 Kg, 1-2 years of age were used in the experiment. They were locally bought in Chiang Mai, Thailand.

B. **Chemicals :** Phenylhydrazine hydrochloride was obtained from Matheson Coleman and Bell, Norwood, Cincinati, U.S.A. Sodium chloride was taken from

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City Chemical Corporation, New York, U.S.A. Cobalt chloride as $CoCl_2 \cdot 6H_2O$ crystals was obtained from May and Baker Ltd., Dugenham England. Fe^{59} as ferric chloride was directly purchased from the Radiochemical Center, Amersham, U.S.A.

C. **Preparation of Phenylhydrazine-Anemic Plasma:** The sheep and rabbits were intraperitoneally injected with 2.5% sterile solution of phenylhydrazine hydrochloride using each injection dose of approximate 3.0 mg. per Kg body weight. The injections were performed every day for sheep and every two-day for rabbits. The animal blood was frequently drawn for determinations of their hemoglobin concentration and hemotocrit compared with the normal values. It took 28 injections for sheep and 9 injections for rabbits. After hemoglobin concentration and hematocrit decreased below 10 gm% and 15 vol% respectively, the sheep were venously bled and the rabbits were bled by cardiac puncture. The whole blood of each animal was pooled using ACD solution as an anticoagulant and the plasma was collected. The anemic plasma would be used for an eryhropoietin study.

D. **Preparation of Anemic Plasma Filtrate:** The preparation of anemic plasma filtrate (APF) was done by the method of Rambach et. al. (g). The pH of the anemic plasma, 3.5 litres from sheep and 500 ml. from rabbits, was then adjusted to 5.5 with 1N HCl., boiled for 10 minutes and finally filtered. The anemic plasma filtrate was dialyzed against distilled water at 4°C for 24 hours, and lyophilized.

E. **Determination of Hemoglobin Concentration:** Exactly 0.02 ml of the animal blood was pipetted by a micro-pipette. Five ml of cyanomethemoglobin solution of Drabkin's solution were added and thoroughly mixed. The optical density was measured at 540 millimicron compared with standard value, using a Bausch and Lomb Spectronic 20.

F. **Determination of Hematocrit:** The animal blood was drawn and placed in balanced oxalate tube. Fer the micro-hematocrit determination method, the blood was filled into the sealed capillary tubes approximate three-fouths of total volume of tubes. The tubes were centrifuged by a Sorvall angle centrifuge with speed of 3,000 rpm. for 5 minutes. The hematocrit in volume % was determined by a Micro-capillary Reader, model C.R.

G. **Biological Assay of Erythropoietic Activity**

The erythropoietic activity assay was modified from the method of Graham (10, 11), using technique of radioisotope iron incorporation into red cells. The method required at least 4 albino rats per group

for testing materials and with two remaining group for a control NSS solution[1] and a cobalt chloride standard solution (8). The erythropoietic activity was expressed in Cobalt Unit[2] per mg actual weight.

EXPERIMENTAL RESULTS

The time response of hemoglobin concentration and hemetocrit in sheep and rabbits to phenylhydrazine hydrochloride is shown in Figure I and II respectively. From Figure I, it was found that after administrations ef phenylhydrazine into sheep the hemoglobin concentration and hematocrit obviously and rapidly decreased from the starting day to day 8, and then gradually changed to a plateau level. The hemoglobin concentration dropped from 15.5 gm% to 5.0 gm%; and the hematocrit from 35 vol% to 13 vol%. The reduction was about 67 %. Similary, in Figure II, the hemoglobin concentration and hematocrit in rabbits were 59 % decreased after total administrations. Erythropoietic activity in the anemic plasma filtrate of both sheep and rabbits assayed was 0.07 and 0.09 Cobalt Unit per mg. actual weight, respectively, as shown in Table I.

When all phenylhydrazine injections were done, some anemic signs in the experimental animals. eg., pale eyes, less activity, weakness, and loss of appetite were also observed.

DISCUSSION

In this experiment, it has been shown that phenylhydrazine effectively induced an anemia in sheep and rabbits. The hemoglobin concentration and hematocrit continously decreased during injections. The values became nearly constant on day 18 for sheep and on day 12 for rabbits. The plots between hemoglobin concentration and hematocrit against the injection time showed a good correlation in both kinds of animals. The difference in the figures between the sheep and the rabbits might depend upon the blood composition, such as plasma proteins, red cell number, and total blood volume.

The plasma filtrate of both sheep and rabbits showed the presence of an erythropoietically active substance. As reported by other investigators (12), phenyldrazine was shown to secondarily affect the erythropoietin production by inhibiting the respiratory mechanism. Anemia or

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[1] *NSS solution = Normat saline solution; 0.85% NaCl solution.*

[2] *One Cobalt Unit is equal to the erythropoietic activity by which 5 micro moles of* $CoCl_2 . H_2O$ *as a total dose (8, 12), is injected into starved rate. In this investigation one Cobalt Unit is equivalent to 7.76 percent of* Fe^{59} *incorporation.*

hypoxia due to phenylhydrazine injection increases the erythropoietin synthesis. This was confirmed by our experiments.

The injection dose of phenylhydrazine investigaled in this experiment is quite suitable. If higher dose was used, it caused the animals sudden death. It was also observed that the withdrawn blood was easily hemolyzed, therefore, it ought to be immediately centrifuged after withdrawing to avoid the hemoglobin contamination and to maintain high biological activity of erythropoietin in the sample.

This preliminary report on induction of anemia in animals by using phenylhydrazine may be a technical guide for those who want to study anemia in animal models. Other kinds of animals such as dogs, goats, and monkeys which are available in our country could be employed. The anemic plasma obtained is useful for erythropoietin studies.

	Anemic Plasma Filtrate (APF)	
	Sheep	Rabbit
Erythopoietic Activity (Cobalt Units per mg. actual weight)	0.07	0.09

Table I. Erthropoietic Acitivity in Anemic Plasma Filtrate (APF) Prepared from Plasmas of Sheep and Rabbit Treated with Phenylhydrazine.

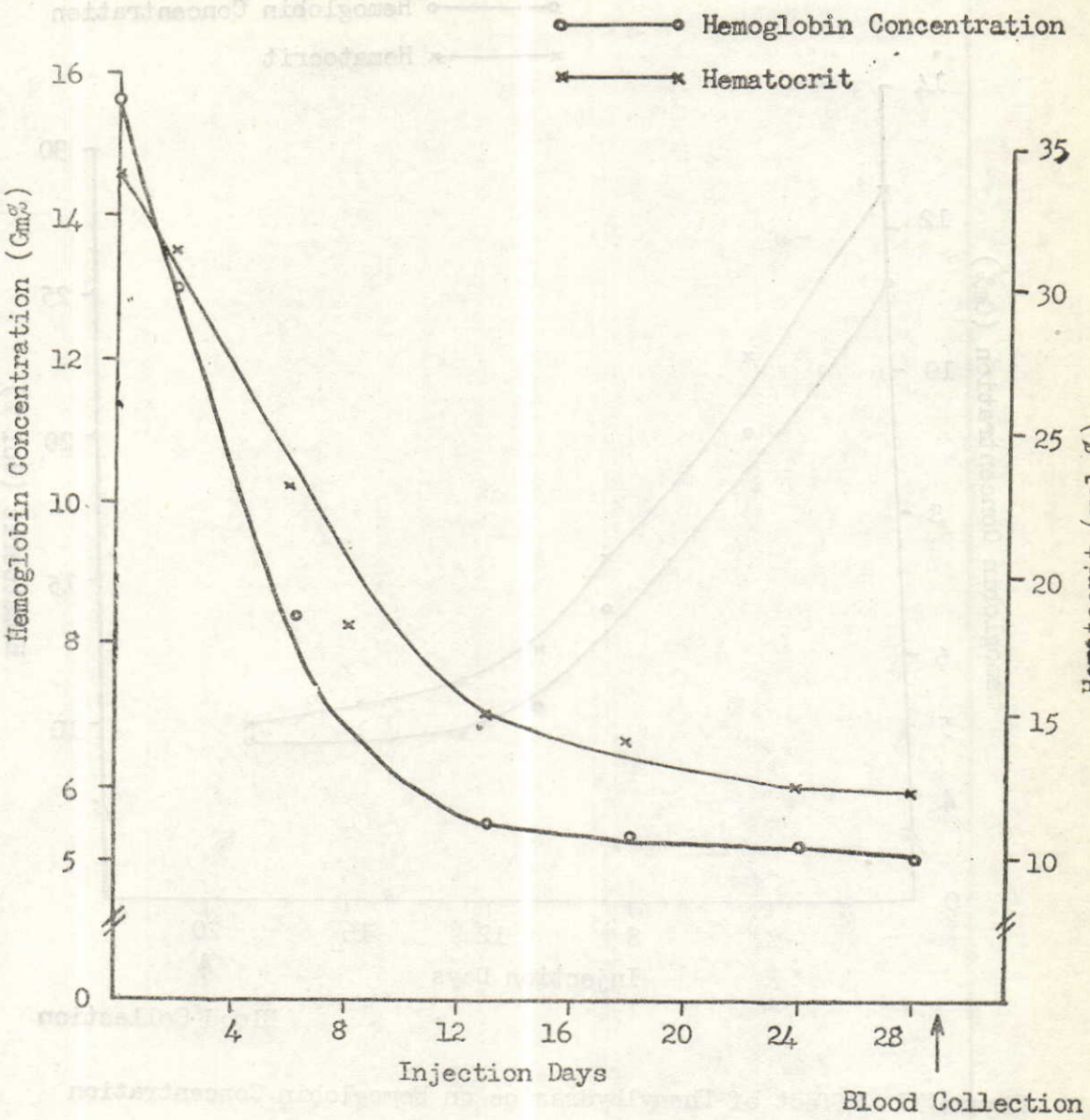

Figure I Effect of Phenylhydrazine on Hemoglobin Concentration and Hematocrit in a Sheep. The experiments were performed as described in Materials and Methods.

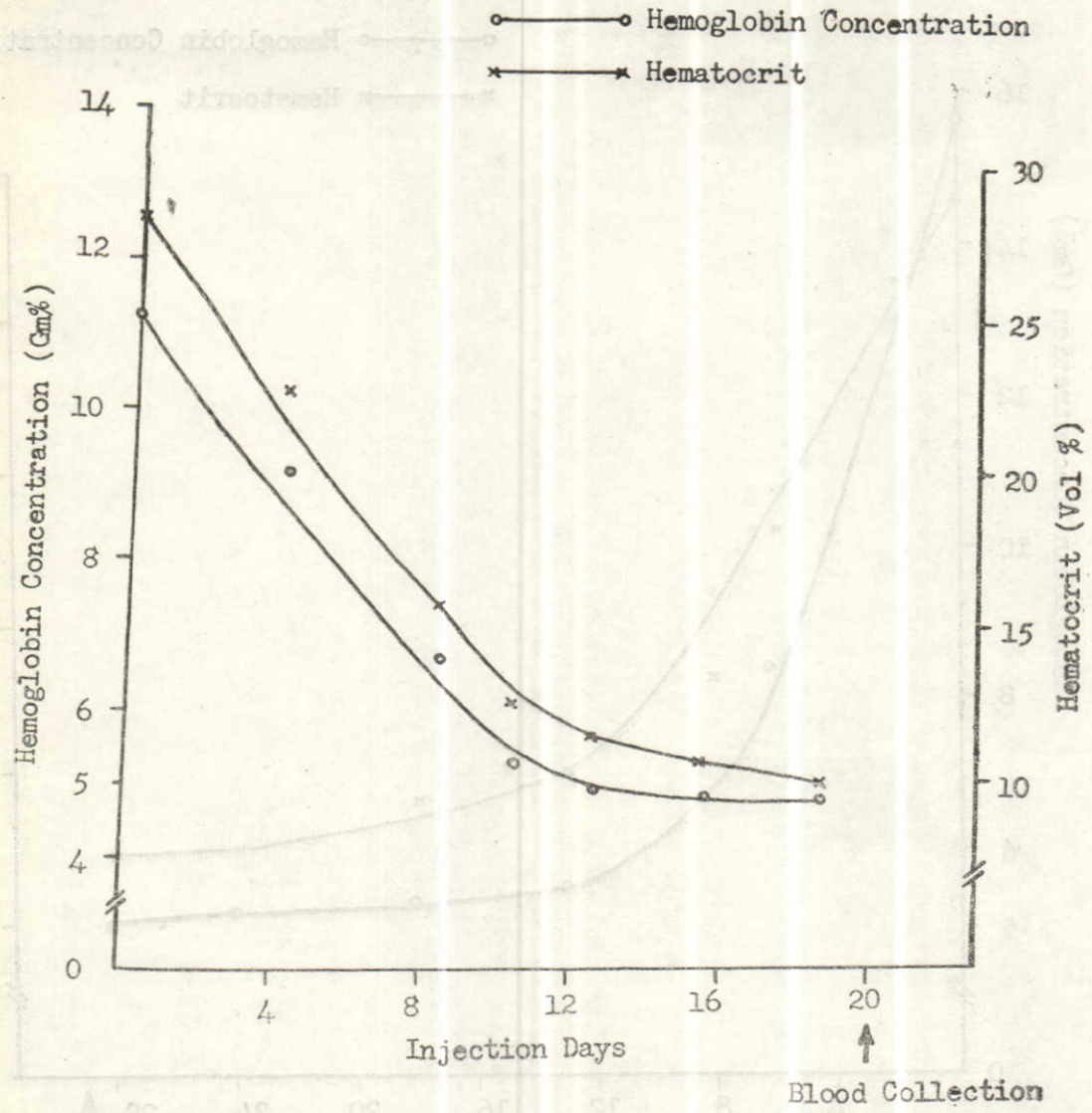

Figure II Effect of Phenylhydrazine on Hemoglobin Concentration and Hematocrit in a Rabbit. The experiments were performed as described in Materials and Methods.

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