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Received July 21, 1965. P.S.E.B.M., 1965, v120.

## Role of a Plasma Factor in Porphyrin Biosynthesis.* (30587)

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The presence of a circulating erythropoietic-stimulating factor ("erythropoietin") in the plasma of anemic animals has been demonstrated by a number of investigators(1,2). Evidence for its presence is derived from studies with animals made anemic by bleeding, or by hemolytic agents such as phenylhydrazine(3,4,5), or by other methods. Linman *et al*(6) have suggested that there are probably 2 factors in plasma affecting erythropoiesis, one a thermostable factor which stimulates the division of erythroblasts and another relatively thermostable factor which enhances hemoglobin synthesis. Thomas(7) showed that normal rabbit serum markedly enhanced the rate of incorporation of $C^{14}$ labeled glycine into heme by rabbit bone marrow. There is, however, no general agreement as to the presence of a heme-stimulating factor in the plasma. For example, Goldberg *et al*(8) could not find any difference in the uptake of radioiron into heme between deproteinized plasmas of normal chickens and of chickens made anemic by either bleeding or by phenylhydrazine administration. These workers found that the uptake of radioiron into heme was much greater when saline was substituted for any of these plasma extracts. Other workers(9,10) have shown that polycythemia causes the appearance in the plasma of an active substance which is antagonistic to erythropoietin. This inhibitory factor "erythropoiesis inhibitor"(10) is thermostable. More recently, Bruns and London (11) have shown that hemin is inhibitory to the biosynthesis of the heme moiety of hemoglobin but stimulates the formation of the globin moiety. Because of the foregoing conflicting evidence for erythropoietic stimulating and inhibitory factors in blood plasma or serum, the present investigation was undertaken to study further the effects of different plasmas and plasma preparations on the biosynthesis of porphyrins, as an index of erythropoietic activity.

*Materials and methods.* The effects of several different plasmas and plasma or serum preparations on porphyrinogenesis were determined by adding these substances to porphyrin-synthesizing systems, chicken erythrocytes or beef liver acetone powder, under controlled conditions. The chicken erythrocyte preparations were made as previously described(12). Normal mongrel dogs were made anemic by the administration (on 2 successive days) of 40 mg phenylhydrazine per kg body weight to obtain the anemic plasma. The blood was taken when the hematocrit reached 20% or less. Beef liver acetone powder was obtained from General Biochemicals Co., Chagrin Falls, Ohio. The incubation procedures, extraction, and determination of porphyrins were essentially the same as described previously(12).

*Results and discussion.* In preliminary experiments, the effect of several types of incubation media on the biosynthesis of porphyr-

* This work was supported by grants from the Nutrition Foundation, Inc., and Michigan State Board of Alcoholism.

TABLE I. Effect of Plasma or Serum on Porphyrin Biosynthesis.

| Medium | Porphyrins formed Copro | Proto |
|---|---|---|
| (a) *With chicken erythrocytes* (2 ml packed cells) | | |
| KRP | 7.1 | 102. |
| KRP (calcium omitted) | 8.4 | 138.8 |
| Eagle's (10% dialyzed serum albumin) | 5.1 | 110.4 |
| Eagle's (20% dialyzed serum albumin) | 5.4 | 83.7 |
| Plasma | 2.3 | 24. |
| (b) *With beef liver acetone powder* (250 mg) | | |
| KRP | 11. | 58.2 |
| KRP (calcium omitted) | 13.2 | 66.4 |
| Eagle's (10% dialyzed serum albumin) | 1.2 | 7.7 |
| Eagle's (20% dialyzed serum albumin) | 1.4 | 4.9 |
| Plasma | 5.4 | 16.4 |

Values are averages of 6 determinations from 3 experiments each run in duplicate. Values from different experiments agreed within ± 10%. Amounts are expressed in μg of porphyrinogen-porphyrins formed per incubation flask, corrected for the preformed porphyrins found in controls incubated without precursors.

ins by nucleated (chicken) erythrocytes was studied. Krebs - Ringer - Phosphate buffer (KRP) was compared with KRP without calcium chloride, with Eagle's medium(13) containing either 10% or 20% dialyzed dog serum and with dog plasma alone. KRP, (without calcium) was found to be the best medium in terms of the amount of protoporphyrin formed in the incubation mixture (Table I). In the presence of calcium, porphyrin formation was decreased. Eagle's medium, containing either 10% or 20% dialyzed serum, as well as plasma decreased the formation of porphyrins, particularly the latter. These experiments were repeated using beef liver acetone powder as the enzyme system for porphyrin biosynthesis instead of chicken erythrocytes. As can be seen from Table I, KRP without calcium again was the best medium. Here, also, both Eagle's media and plasma inhibited the formation of porphyrins.

Eagle's medium with dialyzed serum contains all of the factors known to be necessary for cellular growth. It appeared from the foregoing results, therefore, that some plasma or serum factor tended to decrease porphyrin synthesis under the conditions employed. To investigate this possibility further, the following series of experiments using beef liver acetone powder as the porphyrin-synthesizing system was performed:

1. The plasma was first boiled for 20 minutes and then added as a suspension to the incubation mixture. 2. The plasma was brought to pH 5.5 by the addition of 1 N HCl and then boiled for 10 minutes and filtered. The filtrate was added to the incubation mixture. 3. Plasma was exhaustively extracted with ether and the ether extract, after removal of ether, was incorporated into the incubation mixture. 4. Plasma was dialyzed against saline and the dialyzed plasma was added to the incubation mixture.

From the data given in Table II, it is evident that boiled plasma did not inhibit porphyrin formation. However, the filtrate of plasma adjusted to a pH 5.5 and then boiled inhibited particularly protoporphyrin formation. The inhibitory factor does not seem to be ether-soluble since the ether-soluble fraction did not influence porphyrin formation. The factor is not dialyzable since dialyzed plasma decreased the synthesis of por-

TABLE II. Effect of Plasma Preparations on Biosynthesis of Porphyrins.*

| Addition | Porphyrins formed Coprot | Protot |
|---|---|---|
| Saline | 14.0 | 70. |
| Normal plasma | 2.6 | 34. |
| " " (boiled 20 min) | 12.8 | 72. |
| Plasma "filtrate" (filtrate obtained at pH 5.5) | 12.0 | 10. |
| Ether soluble fraction of plasma | 14.5 | 66. |
| Dialyzed plasma | 4.1 | 40. |
| Albumin | 15. | 68. |
| Alpha 1-globulin | 14.8 | 72. |
| Alpha 2-globulin | 13.6 | 68.8 |
| Beta globulin | 15. | 65.8 |
| Anemic plasma | 16.6 | 81. |

* Five ml of saline or plasma or extract from 5 ml of plasma added to incubation flask containing 250 mg beef liver acetone powder and the necessary incubation mixture and KRP (without calcium). Fifteen mg each of purified rat serum albumin, alpha 1-globulin, alpha 2-globulin, and beta globulin were used.

† Amounts are expressed in μg of porphyrin formed per incubation flask corrected for the preformed porphyrins found in the contents incubated without precursors. Values are averages of 6 determinations from 3 separate experiments each run in duplicate. The values agreed within ± 10%.

phyrins to a similar extent as the normal plasma.

The results thus point to the presence of a non-dialyzable inhibitory factor in normal plasma which is possibly a plasma protein or related substance but one apparently not heat-coagulable at a pH 5.5. As can be seen from Table II, however, the possibility of the inhibitory factor being one of the major serum proteins was ruled out by adding electrophoretically pure serum proteins (rat) to the incubation mixture. The serum protein fractions in the concentrations used did not decrease the formation of porphyrins. Anemic plasma did not decrease porphyrin formation under similar conditions. The inhibitory factor, therefore, seems to disappear or to be destroyed or inhibited when the animal is made anemic as in phenylhydrazine-treated dogs.

Since the inhibitory factor is non-dialyzable, ether insoluble, and thermolabile at pH 7.4, it could be some type of plasma protein or a related substance possibly attached to a protein as an association complex. In this connection, Karibian and London(14) have recently presented evidence that hemin itself may have a regulatory effect on porphyrin biosynthesis by a "feedback mechanism." It appears possible that the inhibitory plasma factor observed in the present investigation may indeed be related to the factor described by London and his co-workers, perhaps bound as a protein complex. This inhibitor may play an important physiological role in regulating porphyrin formation to supply normal demands. Thus, when anemic plasma was used, less of the inhibitory factor was present or was less active and more porphyrin formation occurred than when normal plasma was used.

*Summary.* Evidence is presented which indicates that there is present in the plasma of normal dogs a factor, possibly some type of plasma protein or a related substance, which inhibits *in vitro* biosynthesis of porphyrins from ALA. The amount of activity of this factor is decreased in the plasma of anemic phenylhydrazine-treated dogs. It is suggested that this plasma inhibitory factor may play a physiological role in regulating porphyrinogenesis.

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Received July 22, 1965. P.S.E.B.M., 1965, v120.

## Serum Vitamin E Determined by Thin-Layer Chromatography. (30588)

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Values in the literature for human serum tocopherol levels have been obtained with analytical methods which yield results expressed as "total tocopherols" (see *e.g.*, 1,2). It would be desirable to know what proportions of the total serum tocopherols are contributed by the various individual tocopherols. This paper describes a rapid, thin-layer chro-