1960

EPH - and the kidney - and the liver : a review and discussion of the literature

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A Review and Discussion of the Literature

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

March 31, 1960

Omaha, Nebraska

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I. Introduction:

Erythropoietin, plasma erythropoietic stimulating factor, Carnot serum, hematopoietine, erythropoietic stimulant (1). What a variety of names for the elusive agent which seems to control red blood cell production. It appears that all the above titles, formidable or otherwise, refer to the same substance. What, then, is this substance?

For the sake of convenience, we will refer to this substance as EPH (erythropoietic hormone). It is a humoral agent which apparently controls the number of circulating red blood cells in the body. How it does this is not known. Most authors agree that its site of action is the bone marrow. It seems to have no effect on the red cells once they are in circulation. It has been postulated that its function is to regulate the release of mature red cells from the marrow (1). Others have said it stimulates red cell development from the stem cell. Still others feel that it controls the speed of maturation of the cells from existent erythroid precursors (1). Some feel that it may mobilize factors essential for RBC production, i.e. - iron, copper, nucleic acids, etc.

Another question is - What controls the level of the circulating EPH? It appears that the major factor is the oxygen carrying capacity of the blood, or more correctly, the ratio of blood oxygen supply to tissue oxygen demand. If this is reduced,

as in anemia of hemorrhage or hemolysis, or in hypoxemia of pulmonary or cardiac disease, or the anemia of marrow depression, then the level of EPH is increased. This increase is real, and can be assayed in various laboratory test animals. It is necessary, however, that the other needed factors for red cell production be present in adequate amounts for a full response to occur.

The above paragraph poses another question - What cells of the body, in response to a diminished oxygen supply, react by producing EPH? This is the principal question with which I wish to deal in this paper. Before delving into this, let's briefly review some of the research which has led to our present knowledge of EPH.

II. History:

Carnot and DeFlandre (1), in 1906, coined the word Hemopoietine to designate an erythropoietically active substance in the plasma of rabbits rendered anemic by bleeding. Five decades later, Reissmann (1) substantiated their theory by showing that if one member of a pair of parabiotic rats is exposed to reduced barometric pressure, while its mate breathes room air, both develop the same degree of polycythemia and erythroid hyperplasia. Further support was added by Grant (1), who showed that rats, suckled by mothers who had been intermittently exposed to low oxygen tension, showed higher red cell counts, hematocrits and blood oxygen levels than littermates nursed by unexposed mothers. Much other work has also been done lately.

The utilization of similar methods of bio-assay in the last 5 to 10 years has made research of EPH more fruitful, and has made comparison between results of different sets of workers more valid. The accepted test donor animal for erythropoietically active serum is one, such as a mouse, rat, rabbit, or dog, which has been rendered hypoxic through hemorrhage, phenylhydrazine treatment, or exposure to low oxygen tensions. The serum is obtained usually through rapid exsanguination via cardiac puncture. Serum is also used from severely anemic patients, such as those suffering from hypo- or aplastic anemias, pernicious anemia or refractive anemia. Such serum is then pooled or kept

separate, frozen, boiled, set on the shelf or mixed with antibiotics, as each group sees fit.

The test recipient animal is like those mentioned above, which is normal and fed, normal and starved, hypophysectomized and fed or starved. The serum is usually given in multiple injections, either sub-Q or intravenously. Control animals are treated similarly, except that saline or normal serum is used for the injections. It is important that the procedures used in a test be similar for each group of animals, so as to restrict the unknown elements to the injected serum.

The indices used for measuring the erythropoietic activity of the test serum are many. After a set period of time following the injections, the recipient animals' hemograms are examined. This may include reticulocyte counts, hematocrit determinations, red cell counts, hemoglobin levels and bone marrow studies. A new aid in this type of study is the use of radioactive isotopes. The one most commonly used is Fe^{59}. A known quantity of this substance is injected into the recipient animal intravenously after the test serum has been given. At a set time later, an aliquot of the recipient's blood is withdrawn and from it is determined the percentage of the given dose of Fe^{59} which has been incorporated into the red cells. All of the above procedures, and specifically the Fe^{59} studies and the reticulocyte

determinations (9), have been deemed accurate guides to the erythropoietic response of the recipient animals.

I will briefly refer to the chemical nature of EPH. "Discrepancies exist in the area of the chemistry of ESF" (1). In short, it is confusing at the present time.

III. The Kidney:

The basic theory I wish to discuss is that the kidney is the site of production of EPH. It is reasonable to assume that the kidney, that bean shaped mass of functionally phenomenal cells, may have a cellular system which is instrumental in keeping our circulating red cell mass at an optimum level. Let's examine some of the literature which deals with this subject.

There are numerous articles, and I will mention only a few, which cite cases of polycythemia coexistant with renal lesions, predominantly hypernephromas. DeWeerd and Hagedorn (5) present 7 cases of such coexistant entities. Each of the patients was treated surgically for the kidney tumor, and in 5 cases there was a remission of the polycythemia. The article makes no mention of cardiac or pulmonary diseases which might account for the polycythemia on a secondary basis, but the remissions following surgery suggest that there was no other cause. Frey (6) cites a case similar to the ones above, and excludes the other diseases which commonly might account for a secondary polycythemia. Berger and Sinkoff (7) reveiwed 273 cases of hypernephroma, and found that 1.8% of the patients had polycythemia, and that 5.5% had hemoglobin values of 15.0 grams per cent or better. They did not include a calculation of the expected rate of polycythemia in this number of patients. Lawrence and Donald (12) point out that Forsell, in 1946, strongly felt that

there was a relationship between polycythemia and hypernephroma. These authors presented statistics on 325 cases of polycythemia, (251 of which were primary or vera), and found an actual incidence of associated hypernephroma of 0.93%. The expected occurrence, calculated from national statistics, is 0.003%.

While this is not ironclad evidence, it suggests that the kidney is related to EPH production. It is easy to visualize how the kidney tumor could account for the increased red cells, if it were the site of origin of EPH. The tumor, which contains hyperplastic renal tissue assumed to be functional, could produce EPH autonomously, without the stimulation of hypoxia, and thus prod the marrow to produce excessive red cells. Another possibility is that the tumor tissue, in response to a moderate hypoxic stimulation, would hyperfunction and more EPH would be released than needed, and a polycythemia result.

In order to put a firmer base under this idea, it would be necessary to show that all cases of hypernephroma are associated with a polycythemia, or at least with hemoglobin values above, or at, the upper limit of normal. It is also important to discover the relationship of other kidney tumors - Wilm's, clear-cell, etc. with polycythemia. This brings up another consideration, which is, that if only hypernephromas are associated with an increased red cell mass, what cellular elements, which could

produce EPH, are present in hypernephromas, but are not found in the other renal tumors?

Substantiation for this line of reasoning is not to be had at the present, however, and we have a weakness in our theory. We will go on to stronger points in this argument and find better evidence.

Two most interesting articles by Dr. Sverre Osnes, of Norway, support the theory that the kidney is the site of EPH production, or as he puts it, an erythropoietic principle. His papers reflect a great deal of work, and the complexity of some of his procedures is impressive.

In his first report (2), he studied the response of mice to bleeding, after various alterations in their kidneys. In the first group, mice, who had been subjected to bilateral nephrectomies, failed to respond to acute hemorrhage. Failure of response was reflected by a lack of reticulocyte increase over 3 to 4 days. A group of mice whose ureters had been ligated did respond with a moderate reticulocyte increase, while mice having only partial nephrectomies showed a good reticulocyte rise following the bleeding.

In his second report (3), his investigation went even further. He began by subjecting 3 groups of mice to renal destructive procedures. All 3 groups had 8,000 r. delivered to the left kidney. In group A, the right kidney was removed, in

group B, the right ureter was ligated, and in group C, the right ureter was given a peritoneal outlet. The uremia which developed after seven weeks was similar in all 3 groups (BUN - 156,152, and 160 mgs.% respectively). The interesting development was that the hemoglobin values at the end of the seven weeks were quite different. In group A - 46.5%; group B - 66.3%; group C - 99.0%. Each group had shown a steady decline from the first week, but group C had leveled off at 85% at 3 weeks, and then slowly rose again.

Only group C mice had much functioning tissue in their right kidneys, as the right kidney of group B mice became hydronephrotic with loss of tissue substance. This points up two items of interest. First, that functioning kidney tissue is essential for a response to hemorrhage, and second, that uremia, per se, does not inhibit red cell production.

He supported this further by work on three more groups of mice, all of which were bled 0.5 ml. Group A showed a reticulocyte increase in 28 hours, (2% to 4%), group B, a steady decline, (2% to 1%) while group C showed an increase the same as group A. This again shows that the absence of kidney tissue prevents a response to an acute hemorrhage, but also shows that the absence of kidneys does not prevent a response to erythropoietically active serum. This suggests that the kidneys play an important role in the production of EPH.

Osnes now comes to the main point of his paper, which is, that there are two factors involved in the control of red cell production. The first he calls Erythropoietin, and states that it is probably produced outside of the kidney. The second he calls the Erythropoietic Principle produced by the kidney. He supports this postulate by some additional experiments.

He first showed that both normal and nephritic mice (made so by treating both kidneys with 4,000 r.) respond to active serum from normal bled mice with a good reticulocyte increase, over a 6-day period. He then used two groups of mice, the first normal and the second group nephritic, to each of which he gave a single injection of serum from nephritic mice whose hemoglobins ranged from 30-68%. The normal mice showed a good response over 4 days, while the nephritic mice showed a steady decrease in reticulocytes. A third experiment along this line used the same recipient mice (normal and nephritic), but the donor serum was obtained from nephrectomized mice, kept alive by peritoneal lavage, which had been bled. Once again, the normal mice showed a reticulocyte increase over 4 days, while the nephritic mice failed to show any such increase.

These studies show that: (1) The active serum from bled normal mice is capable of stimulating both normal and nephritic mice; (2) The active serum from bled nephritic mice is capable of producing red cell production in normal mice, but not in

nephritic mice; and (3) The factor necessary for a reticulocyte response in nephritic mice is missing in active serum from nephritic mice. These experiments support his idea that there are two substances capable of stimulating erythropoiesis.

The first, erythropoietin, produced outside the kidney, is present in the serum from both normal and nephritic bled mice. The second, the kidney factor, while present in active serum from normal mice, is missing in serum from nephritic mice. This explains why normal mice, with intact kidneys to produce the factor, can respond to active serum from nephritic mice, which contains increased erythropoietin only. It also explains why nephritic mice, whose kidneys cannot produce their own factor, do not respond to serum of nephritic mice, which also lacks the kidney factor. These experiments also support Osnes' theory that both of the factors are needed for erythropoiesis, and that lack of the kidney factor is responsible for the anemia which develops in nephritic mice.

Additional support is given this theory by Osnes' work using serum from anemic human patients, one with a hemorrhagic anemia and one with an iron-deficiency anemia. The injection of these serums produced a reticulocyte increase in both the normal and the nephritic mice. Also tested were the serums from two other anemic patients, both with chronic nephritis. In these tests, the normal mice showed a reticulocyte response, while the nephritic mice showed none.

Gordon, in his recent review of this subject (1), states that if the site of production of EPH is postulated, a sensitive test for ascertaining the correctness of the postulate is to determine an animal's response with that organ system removed or its function decreased. I think it fair to say that Osnes has gone a long way in fulfilling this requirement.

The work of Jacobsen, et al (13) tends to confirm these results. They showed that rats, having had their pituitary, adrenals, thyroid, gonads, stomach, intestines or 7/8 of their liver removed, could still respond to anoxia with an increased EPH level. Rats subjected to bilateral nephrectomies, however, failed to so respond. This assay method was to give serum from the above animals, after bleeding, to normal rats, and measure the Fe^{59} uptake. They also showed that rats with both ureters ligated could respond to the stimulus, although to a lesser degree. While these results are less convincing, they are indicative that the kidney is important in erythropoiesis.

The results of the work of Erslev (10) are also compatible with Osnes' experiments. He used normal rabbits for recipient animals and measured their response by reticulocyte and hemoglobin determinations. He showed that: (1) Rabbits subjected to bilateral nephrectomies do not respond to hemorrhage, and that their hemoglobin levels drop slowly over 6-8 days. (2) Rabbits, with a unilateral nephrectomy and the opposite ureter ligated,

showed a slight increase in hemoglobin and reticulocyte count following hemorrhage. In another experiment he found that: (3) Serum from bilaterally nephrectomized rabbits, which were kept anemic for 20 hours, produced as good a response in reci-pient animals as did serum from normal rabbits similarly anemic.

The results in (1) and (2) agree with Osnes' work, that the lack of kidney tissue prevents a response to bleeding, but that even a portion of functioning renal tissue will help to offset the anemia. The results in (3) are in accord with Dr. Osnes' work on the normal animal's response to anemic neph-ritic serum.

As is to be expected, there are authors who do not share the opinions of Osnes and Jacobsen as to the role of the kidney in EPH production. It is interesting, however, that their results are compatible with the results of the other authors. Prentice and Mirand (4), in a similar project, used hypophysec-tomized rats as recipient animals and Fe^{59} uptake as a measure-ment of donor serum activity. They first placed a series of normal rats in an atmosphere of reduced oxygen tension, for varying periods of time. The serum from such rats, after 4 hours exposure, produced a 15.6% uptake in the recipient rats; after 8 hours exposure, 32% uptake, and after 24 hours, 34.5%. Control values for this procedure averaged 2.7%. A second and third group of rats, group A having had unilateral hephrectomies

and group B, bilateral nephrectomies, were subjected to similar reduced oxygen levels. The results:

Group A -	4 hr. exp. - 13.9%	Group B -	4 hr. exp. 17.9%
	8 hr. exp. 31.2%		8 hr. exp. - 13.2%
	24 hr. exp. - 40.0%		24 hr. exp. - 37.9%

The authors felt that the EPH response in these animals suggested that the kidney was not the site of EPH production. Again, however, these results are compatible with the previous work showing the normal animal's response to anemic nephritic serum.

An interesting sidelight here is the theory, put forth by Dr. Osnes in his first article (2), that the juxtaglomerular bodies are related in some way to the production of the kidney factor. By doing sections on the kidneys of the mice used for his studies, he showed changes in the granules of the juxtaglomerular cells, which he said were significant. These included: (1) Under normal conditions, the granules were present in moderate numbers, and stained well with crystal violet and light green dyes. (2) If the animal was anemic from bleeding, the number of granules was decreased. (3) In kidneys which have been irradiated, both the j.g. cells and the granules are normal to increased in number. Unfortunately, he does not delve into the full significance of such changes, but said that more will be published on this at a later time.

I also want to mention an article by P. M. Hartroft (8), discussing changes in the j.g. granules associated with renal disease. She points out that: (1) In hypertension, the granules are decreased so long as the blood supply is adequate. (2) If the cells become ischemic, the granules increase, but a rising blood pressure will cause them to decrease again. Again, unfortunately, the significance of these findings is not clear, but it does make me anxious to see Dr. Osnes' discussion of this matter.

will next present some information concerning the second theme of this paper, namely, the role of the liver in the production of EPH.

IV. The Liver:

Dr. Osnes, in his experiments with anemic human serum (3), obtained some from two patients suffering from chronic hepatitis. This serum produced no response in normal mice, but a good response was seen in the nephritic mice. This result is explained if we assume the liver to be the source of erythropoietin.

The hepatitis patient, anemic due to lack of erythropoietin, still has a high titer of kidney factor. Such serum will then produce no response in normal mice (no increased erythropoietin level), but will in the nephritic mice (high kidney factor in the donor serum; high erythropoietin titer in the nephritic recipient).

Jacobsen, et al (9) showed that, in rabbits made anemic by phenylhydrazine treatment, those rabbits showing the greatest liver damage (at necropsy) were the ones whose serum was the most active in the recipient animals. He felt that this may represent a decrease in the animals ability to destroy or inhibit EPH, and that the liver may destroy, or produce an inhibitor of EPH.

Prentice & Mirand (11), in another project, studied the effects of liver damage on rats ability to respond to anoxia. The liver damage was produced by subcutaneous injections of CCl_4, and the anoxia produced by exposure to 10% oxygen atmosphere. He measured the serum activity in hypophysectomized rats.

The results showed that the liver damaged rats' serum gave a good response in the recipient animals, and that as the degree of liver damage increased, so did the recipient's response. This, too, suggests the liver as the site of inhibition rather than the site of production of EPH.

V. Discussion:

In evaluating the research done to determine the site of EPH production, it is important that each group's work be used as a test of any theory advanced. I shall discuss two theories brought out in this paper, and test each against the results which have been presented.

The first theory is a combination of the ideas of Dr. Osnes and those of Jacobsen, et al, and Prentice and Mirand.

Theory A. The kidney produces the factor essential for stimulating erythropoiesis, and the liver acts to inhibit or destroy it, as needed, to keep the red cell mass at an optimum level.

Experimentor and support provided:

1. Jacobsen, et al - YES - A decrease in the amount of functioning liver tissue produces increased EPH levels in response to anemia. (No direct support for the kidney as the site of EPH production)

2. Prentice and Mirand - YES - Same as above.

3. Osnes - YES - The normal mice did not respond to the hepatitic serum because they were not anemic, and thus their liver inhibited the increased EPH in the donor serum. The nephritic mice, being anemic, responded to the increased EPH in the hepatitic serum as their liver did not inhibit it.

4. Other authors - YES to ? - Polycythemia develops in only a few patients with hypernephroma because the liver, if intact, destroys the excess EPH produced by the tumor tissue. In those few, there may be too much EPH for the liver to handle, or else there is some degree of liver damage which prevents complete inhibition of the EPH.

5. Medical finding - YES to ? - Many nephritic patients are anemic.

6. Osnes - NO - Why is the hepatitis patient anemic? Why is serum from nephritic mice capable of producing a reticulocyte response in normal mice?

7. Mirand and Prentice - NO - Why does serum from the bilaterally nephrectomized mice produce a response in the normal recipient animals?

Theory B. The kidney and the liver each produce a factor, and that both are needed for erythropoiesis.

1. Osnes - YES - His work described in this paper.

2. Osnes - ? - Why do normal mice respond to anemic nephritic serum, which means that they must make increased kidney factor, while normal mice do not respond to anemic hepatitic serum, meaning that they are not making increased liver factor?

3. Jacobsen, et al - ? - It is possible that the increased liver damage in the phenylhydrazine-treated animals could account for a high level of liver factor, assuming that the factor was

released as the cell membranes were disrupted (similar to the transaminase release in myocardial infarction). If this were true, why then don't the normal animals respond to it, as it should be high in both kidney and liver factor?

4. Prentice and Mirand - ? - As in 3 above.

5. Other authors - ? - Here again we would have to assume some undiagnosed liver disease, in those hypernephroma patients with polycythemia, which would account for the necessary increase in liver factor.

6. Mirand and Prentice - ? - What prompts the normal recipients of anemic nephritic serum to produce the increased kidney factor which is needed to give an increased Fe^{59} uptake?

It is evident that neither theory can be conclusively supported at the present time. am impressed with the work that Dr. Osnes has done, and feel that his results give good support to his theories. I feel that his ideas are sound, but accept them with reservations, inasmuch as there are flaws in his reasoning, as pointed out above. I look forward with interest to his next publication.

Some experiments which would be of value in testing Osnes' work would involve anemic serum from animals with both kidneys and liver functionally kaput. Aside from being an interesting technical exercise just to keep such an animal alive, the response of normal animals to such serum would shed more light

on the two-factor idea. In theory, such a serum should demonstrate no EPH activity in the recipient animal.

Another study needed is to check for any history of liver disease in those hypernephroma patients with polycythemia. Still another experiment would be to study the effect of serum, from an anemic human patient, on a patient with chronic nephritis who is anemic. Such serum could come from a patient with an aplastic or refractory anemia, and which had shown a high EPH activity in normal test animals.

Even now, someone may have found the link which will join together this chain of facts and theories, and provide us with the answer. We may know soon.

VI. Conclusion:

EPH is the term used to designate the humoral agent which controls the production of red blood cells. It has many other titles. Its existence was proposed 50-odd years ago, and experiments since that time, especially in the last decade, have substantiated its presence.

The works of different experimentors, principally Dr. Sverre Osnes of Norway, demonstrate the significance of both the kidney and the liver in the production of this agent. It is postulated that there are two factors, one from the kidney and one from the liver, both of which are necessary for an erythropoietic response.

In analyzing this theory in light of data from different sources, flaws appear which cannot be reconciled at the present time. We must await the results of additional research before making final resolution of the problem.

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