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Erythropoietin in Children I. Findings in Hodgkin's Disease

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Introduction

Hodgkin's Disease, a somewhat rare type of lymphoma, afflicting adults mostly, has been one of the most worrisome human ailments ever encountered. To our knowledge, studies investigating the predisposing factors leading to this disease and the excessive production of the hormone called erythropoietin in this disease, have been somewhat scarce. Our present study is carried out with aim of establishing a possible relationship between erythropoietin and the anemia seen in cases with Hodgkin's Disease.

As early as the beginning of this century the scientists pointed out to the probability of the effect of a certain stimulative element in the production of red cells. In 1906 Carnot and Deflandre[1] and, in the following years several other investigators[2 3] attempted to establish the presence of a humoral factor which was later named erythropoietin.

The studies carried out on this humoral factor since the 1900's up today describe erythropoietin to a stimulative hormone acting on the bone marrow with a result of an increase in the production of red cells. The activity of this hormone which has no effect on white cells or platelets one way or the other, has been observed in the plasma and urine of many of the mammalian species as well as in birds and fish.

The hormone of erythropoietin which we based our study on has not yet been satisfactorily isolated and the time is early to make a thorough discussion of its components.[4] Some scientists, however, indicate approximate figures as to its molecular weight. According to Goldwasser,[5] erythropoietin is a glycoprotein with a molecular weight of 60,000 to 70,000.

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The main organ which has a controlling function on the production of this hormone seems to be the kidney. There are some researchers, however, who report that various external sources also produce erythropoietin.[6-8] Okçuoğlu,[9] Shalet et al[10] and Thurman et al,[11] for instance, point out to the presence of an erythropoietic factor in plasma in different types of malignant tumors in childhood. Mirand et al[12] report that minute amounts of erythropoietin can be found in individuals who are subjected to bilateral nephrectomy. Babuna,[13] in one of his studies, questioned the possibility of an increase in erythropoietin in a case with uterine leiomyoma. Krantz[14] includes cerebellar hemangioblastoma, hepatom, uterine leiomyoma, various tumors and hyperplasia of endocrine glands as possible sources of erythropoietin production.

The activity of erythropoietin in the plasma of human beings is usually determined with the usage of certain bioassay methods and, without the help of such techniques, the presence of this hormone is impossible to detect. Various methods devised for this purpose, however, disclose somewhat different results with the same organisms, making the uniformity in the experimental conditions a very important factor.

The experiments show that the level of this hormone is either increased or decreased with the presence of certain conditions. Hypoxia, for instance, usually results in an increase of the production of erythropoietin while hyperoxia causes a decrease in it. Hypoxia, as is well known, may either be due to a change in atmospheric conditions or could be caused by various types of anemia. Hyperoxia, on the other hand, occurs as a result of a decrease in altitude or plethora due to hypertransfusion.

An increase of certain hormones such as the thyroid and the pituitary result in an increase of erythropoietin production while a decrease in one of these hormones or starvation causes a decrease in the production of erythropoietin. To sum up, we can say that changes in tissue oxygenation compensatively affect the production of erythropoietin.

The present bioassay methods are developed upon the above mentioned basic relationship, between the conditions which are either internal or external to the organism and erythropoiesis. Experiments carried out with such animals as rats, mice, etc. reveal this significant relationship, in almost every instance. The most recent bioassay methods comprise either hypoxic chambers or hypertransfusion. The method

which we have employed in this study, e.g. starvation, has also been used effectively to produce a change in the production of erythropoietin.

Materials and Methods

Seventeen patients with a previously established diagnosis of Hodgkin's Disease whose age range differed between 3.5 and 15 years were included in our study. 10 cc. of anticoagulated blood was taken from each child prior to any specific therapy. The plasma of the blood specimens was separated and frozen until the time the tests were carried out. In addition routine hematological studies, Coombs and the serum iron tests[15] were performed on the patients. We then classified the cases with a hemoglobin level of 10 - 12 gm / 100 ml. as mildly anemic, those with 7-9 gm / 100 ml as moderately anemic and the ones below 7 gm / 100 ml as severely anemic.

In our experiment for each assay we have used eight to ten Swiss albino rats weighing 100-200 grams. The rats were starved for 24 hours receiving water ad lib and then, using the modified version of the Fried bioassay method previously employed by Okçuoğlu[9] they were injected 0.5 ml. of patient's plasma subcutaneously. At the end of 48 hours these rats were given the same amount of plasma while their starvation continued. After the completion of 72 hours of fasting the rats were injected one microcurie of Fe^{59} in the form of ferrous citrate through the foot veins in the hind leg. Afterwards the rats were again left in fasting until 90 hours of starvation was completed. The control group consisting of 4 or 5 rats in each case were subjected to starvation in the same manner, but were given saline injections instead of plasma. Our decision was based upon the reports of other researchers indicating no significant differences between the responses of the experimental animals to saline and normal plasma. At the end of this period, 1 ml. of blood was drawn from the rats in both groups by cardiac puncture under ether anesthesia. The radioactivity of the blood specimens was measured by employing the Tri–carb Liquid Scintillation Spectrometer – Packard for a period of five minutes. The Fe^{59} uptake was estimated, which, in turn, provided for us the level of erythropoietic activity of the patients.

Results

Table I summarize the hemoglobin and hematocrit levels, reticulocytes, changes in the bone marrow and the erythropoietic activity observed in the patients and those in the control group. Significant differences were encountered between the erythropoietic acti-

TABLE I

THE EFFECT OF PLASMA FROM PATIENTS WITH HODGKIN'S DISEASE ON THE Fe^{59} UPTEKE IN STARVED RATS

No. Initials		Age. Sex	Hb. gm./ 100 ml.	Hct. (%)	Ret. (%)	Bone Marrow	Fe^{59} Uptake % ± S.D	Mean Control
1	LC	5yr. F	14.10	40	0.5	Normocellular	0.6±0.3	1.5±1.1
2	AB	6yr. M	10.00	32	1.0	"	1.8±1.6	"
3	MS	12yr. M	11.25	34	1.0	"	1.8±0.3	"
4	EG	9yr. F	10.60	32	1.0	"	29.6±11.8(d)	"
5	DA	15yr. F	7.70	24	1.5	Normoblastic Hyperplasia	10.5±7.6(d)	"
6	BP	9yr. M	5.80	18	1.2	Not obtained	1.1±0.3	"
7	AID	8yr. M	4.00	14	7.6	Normoblastic Hyperplasia	0.7±0.2	"
8	AO	3yr. M	7.70	24	1.2	Normoblastic Hyperplasia	0.7±0.2	"
9	HS	8yr. M	12.30	–	0.5	Normoblastic Hyperplasia	0.3.4±3.4(d)	"
10	KE	11yr. F	11.50	35	1.5	Normocellular	3.2±1.0(d)	1.5±1.1I
11	BE	15yr. F	8.00	25	–	"	0.7±0.4	"
12	HHE	11yr. M	9.80	–	1.0	"	0.2±0.7	"
13	MB	3.5yr. M	8.45	27	2.0	Not obtained	0.2±0.7	"
14	ID	6yr. M	8.30	26	0.8	No change	0.5±0.1	"
15	MD	8yr. M	10.69	33	1.2	Normoblastic Hyperplasia	5.4±3.4(d)	"
16	CE	8yr. M	10.15	32	0.2	Normocellular	3.9±2.6(d)	"
17	HK	9yr. M	6.20	20	2.4	Normocellular	0.4±0.1	"

(d)Fe^{59} uptake is not significantly different from control.P> .05

vity of the seventeen patients with Hodgkin's Disease and that of the control group. The levels of six cases (Numbered 4,5,10,15,16) are pointed out in the table with an additional letter (d). In these six patients the rate of increase in erythropoietin was markedly high in two and slight in others. Excluding one patient who had a value of 7.7 gm/100 ml, all of the children had hemoglobin levels above 10 gm / 100 ml.

Discussion

Anemia is seen more often in cases with Hodgkin's Disease than in those afflicted with other types of lymphoma.[15] In one third or half of cases with this disease, anemia develops in fairly early stages.[15] The anemia observed during these stages is usually mild or moderate and normocytic in type.

The available literature does not confirm much research on the relationship between anemia caused by Hodgkin's Disease and the production of erythropoietin. Some studies, however, point distinctly to a shortened lifespan and a decrease in the production rate of red cells.[2 16] Some researchers indicate the cancer in adults to be one of the causes of iron deficiency anemia, despite the lack of studies investigating the incidence of the latter.[2 17] Others claim that erythropoietic activity is measurable in cases with iron deficiency anemia when the hemoglobin level falls under 4-5 gm/100 ml. As can be observed from the classification mentioned in a previous section of this article, none of our patients with Hodgkin's Disease showed a hemoglobin level below these figures. The fact that we were able to determine high erythropoietic levels in these cases, only points to a probability of the existence of some other factors contributing to a raise in this activity. Since the kidney is an important organ in the production of erythropoietin, we suggest further studies in renal pathology that are secondary to Hodgkin's Disease. As of today, no adequate knowledge is available on whether the lymphoma such as Hodgkin's Disease is the sole source for elevated erythropoietic activity.

We feel that our study aiming at a probable relationship between Hodgkin's Disease and the production of erythropoietin, does not, by itself, provide a completely satisfactory explanation for the anemia observed in such cases. To be able to draw a more exact conclusion, other possible factors should be evaluated.

Summary

Seventeen children with Hodgkin's Disease were subjected to erythropoietin assays to establish a relationship between this disease

and the erythropoietic activity. Six of the seventeen cases were observed to have an increased erythropoietic activity.

We were not able to determine a positive relationship between the production of erythropoietin and the level of anemia. The presence of other possible factors contributing to the increased activity of other possible factors contributing to the increased activity of erythropoietin have been suggested.

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