Polycythemia Vera in Childhood. Studies of Iron Kinetics with Fe\textsuperscript{59} and Blood Clotting Factors

By P. M. AGGELER, M. POLLYCOWE, S. HOAG, W. G. DONALD AND J. H. LAWRENCE

POLYCYTHEMIA vera has been reported in very few children. Thus, only six examples of true polycythemia were found below the age of puberty.\textsuperscript{1,4} Other reports appear to be examples of secondary polycythemia.\textsuperscript{5} Benign familial polycythemia has been reported in children.\textsuperscript{6}

We have observed a case of polycythemia vera in a girl aged nine years and nine months at the time of diagnosis. Our youngest previous case was aged 21. The average of 264 patients was 52 years.\textsuperscript{7}

REPORT OF CASE

Our nine-year-old patient had no significant past illnesses. The results of previous physical examinations and blood counts were normal, except that at the age of four the patient had a grade one systolic murmur, maximal over the second left interspace at the sternal border. In the three years prior to referral the patient had blue lips and nail beds after exposure to cold. She had a few moderate nosebleeds each year.

Initial examination showed a healthy-appearing girl of above average height and weight. There was a suggestion of ruddy cyanosis of the lips and nail beds and erythema of the mucous membranes. Her heart was of normal size, the sounds of normal intensity; P\textsuperscript{2} was split and there was a grade one systolic murmur, maximal in the second and third intercostal space to the left of the sternum; the pulse was 80 and regular. Blood pressure in the arms was 110/60, on the left leg, 130/70. The spleen was palpable 5 cm. below the left costal margin in the mid-clavicular line and was smooth and non-tender. The liver edge was not felt. There were no other masses and no lymph node enlargement. Genitalia, extremities and reflexes were normal. There was clubbing of the fingers or toes.

Laboratory studies in January, 1957, showed: Hemoglobin, 22.2 Gm. per cent; RBC, 10,500,000/mm\textsuperscript{3}; PCV 72 vol. per cent; WBC, 9000 mm\textsuperscript{3} with a normal differential. Serum iron was 58 micrograms per cent. Blood oxygen saturation before oxygen administration was 26.1 vol. per cent or 98 per cent saturation; after oxygen it was 27.7 vol. per cent or 104 per cent saturation; after exercise it was 25.7 vol. per cent or 97 per cent saturation. Blood uric acid was 6.0 mg. per cent. Bone marrow aspiration showed normal cellular elements with active hematopoiesis. Extensive bleeding and clotting tests revealed specific, mild deficiencies of proaccelerin, proconvertin, Stuart factor and prothrombin (table 1).

In February, 1957, iron kinetic studies using Fe\textsuperscript{59} were done. The concentration of iron in the plasma was 35 micrograms/100 ml. (normal 70–170 micrograms/ml). The latent iron binding capacity of the plasma was 398 micrograms/ml, resulting in a total plasma iron binding capacity of 433 micrograms/100 ml. (normal 300–425 micrograms/ml/hr.). The plasma iron turnover was normal with respect to blood volume, 0.35 mg./hr./liter (normal 0.22–0.40), but increased with respect to weight, 0.83 mg./hr./day (normal 0.35–0.65). Radioiron was incorporated into circulating erythrocytes at a slightly increased rate and attained a maximum net incorporation of 90 per cent (85 per cent–100 per cent).

Analysis of plasma radioiron over a period of 11 days showed the synthesis of 7.0 Gm. of hemoglobin per day, representing the daily formation of 1.18 per cent of 595 Gm. total hemoglobin. The absence of splenic sequestration and destruction of erythrocytes suggested that the total red cell volume might be slowly increasing.\textsuperscript{7,8} It was not possible
Table 1.—Coagulation Studies

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<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (Gm./100 mL)</td>
<td>22.2</td>
<td>15.3</td>
<td></td>
<td></td>
<td>300,000 ± 100,000</td>
</tr>
<tr>
<td>RBC (10^6/mm³)</td>
<td>10.6</td>
<td>8.0</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>72</td>
<td>57</td>
<td>48</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Platelets/mm³</td>
<td>764,500</td>
<td>480,000</td>
<td>283,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding time in min.</td>
<td>3.5</td>
<td>3.0</td>
<td>1-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clotting time in min.</td>
<td>7.5</td>
<td>10.6</td>
<td>7.8</td>
<td>7.6</td>
<td>6-12.3</td>
</tr>
<tr>
<td>Cap. fragility</td>
<td>neg.</td>
<td>neg.</td>
<td>neg.</td>
<td>0-10</td>
<td></td>
</tr>
<tr>
<td>Clot retraction % fluid vol. of clot shed RBC's per 5 ml sample of blood</td>
<td>poor clot</td>
<td>13.7</td>
<td>19.0</td>
<td>19.0</td>
<td>0-23</td>
</tr>
<tr>
<td>Quick prothrombin complex %</td>
<td>54</td>
<td>50</td>
<td>52</td>
<td>66</td>
<td>70-100+ %</td>
</tr>
<tr>
<td>Proaccelerin %</td>
<td>61</td>
<td>30</td>
<td>48</td>
<td>70-100+ %</td>
<td></td>
</tr>
<tr>
<td>Proconvertin %</td>
<td>66</td>
<td>46</td>
<td>46</td>
<td>56</td>
<td>75-125 %</td>
</tr>
<tr>
<td>Stuart Factor %</td>
<td>40</td>
<td>58</td>
<td></td>
<td>70-100+ %</td>
<td></td>
</tr>
<tr>
<td>Plasma Prothrombin + Stuart Factor %</td>
<td>54</td>
<td>51</td>
<td>63</td>
<td>70-100+ %</td>
<td></td>
</tr>
<tr>
<td>Serum Prothrombin + Stuart Factor (in % of self)</td>
<td>8</td>
<td>11.2</td>
<td>14.2</td>
<td>10-25%</td>
<td></td>
</tr>
<tr>
<td>Anti-hemophilic Factor (AHF) %</td>
<td>62</td>
<td></td>
<td></td>
<td>50-140%</td>
<td></td>
</tr>
<tr>
<td>Plasma Thromboplastin Component (PTC) %</td>
<td>66</td>
<td></td>
<td></td>
<td>70-125%</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen mg.%</td>
<td>221.7</td>
<td>224.9</td>
<td></td>
<td>300 ± 100 mg%</td>
<td></td>
</tr>
</tbody>
</table>

*See: Coagulation Methods in this paper.

to measure this increase by repeated red cell volume determinations, because of the frequent removal of large samples of blood. External scanning technics demonstrated rapid initial uptake of radioiron in the sacral marrow without initial accumulation of radioiron elsewhere. During the next 11 days there was a rapid complete release of radioiron from the marrow without secondary accumulation in the liver or spleen. This iron kinetic study showed increased hemoglobin formation within normal iron pathways which is characteristic of polycythemia vera.75

Blood volume determinations on February 11, 1957, using P32-labeled red cells, showed an increased total blood volume and an increased total red cell mass with a slightly low plasma volume (table 2).

Blood and urinary studies of 17-ketosteroids and 11-oxysteroids were normal before and after the administration of corticotropin. X-ray examination of the abdomen showed a possible slight enlargement of the liver and moderate enlargement of the spleen. X-ray of the chest showed no cardiac enlargement and the contour of the heart was entirely normal. Vascular shadows were normal and there was no evidence of pulmonary disease. An electrocardiogram was normal. Intravenous urograms in March, 1958, showed normal kidney outlines and drainage structures. "Erythropoietin" could not be demonstrated in her plasma or urine.

The patient has done very well. In August, 1958, the total blood volume was still increased, with increases in both the red cell mass and plasma volume (table 2). Examina-

Table 2

<table>
<thead>
<tr>
<th>Date</th>
<th>Total BV cc</th>
<th>Total BV cc./Kg</th>
<th>Red Cell Volume cc</th>
<th>Red Cell Volume cc./Kg</th>
<th>Plasma Volume cc</th>
<th>Plasma Volume cc./Kg</th>
<th>Micro- hematocrit vol.%</th>
</tr>
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<tbody>
<tr>
<td>Feb., 1957</td>
<td>2730</td>
<td>89.7</td>
<td>1802</td>
<td>59.2</td>
<td>928</td>
<td>30.5</td>
<td>30.5</td>
</tr>
<tr>
<td>Aug., 1958</td>
<td>3555</td>
<td>92.1</td>
<td>1927</td>
<td>50.2</td>
<td>1608</td>
<td>41.9</td>
<td>55.5</td>
</tr>
<tr>
<td>Normal values</td>
<td>62-70</td>
<td>30-35</td>
<td></td>
<td></td>
<td>32-35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
tion in December, 1959, was normal except for the palpable spleen. Her height was 64¼ inches and her weight 107 pounds, an increase of 9 inches and gain of 38 pounds in three years. She was treated with occasional venesections to keep the total red cell count and hematocrit near normal levels (fig. 1). Treatment was withheld in the hope that the hematologic values could be maintained at normal levels for some time by venesections alone.

Coagulation studies were performed at varying hematocrit levels before and after phlebotomies (table 1). On all occasions there was a considerable degree of shedding of erythrocytes from the clot, varying with the degree of elevation of the hematocrit. The Quick prothrombin complex activity was between 30 per cent and 66 per cent. Specific assays showed moderate reductions in proaccelerin, proconvertin, Stuart factor and prothrombin. The thromboplastin generation test showed borderline results when patient's adsorbed plasma and serum were used in the generating mixture (table 3). Improvement was noted with the substitution of either normal plasma or normal serum. The patient's platelets and normal platelets at levels of 125,000 mm$^3$, 175,000 mm$^3$, and 250,000 mm$^3$ behaved in an identical manner in the thromboplastin generation test. Despite these defects the patient had only one minor bleeding episode following a dental extraction.

**COMMENT**

The pathogenesis of the coagulation abnormalities is not clear. The moderate reduction in proaccelerin and Stuart factor could be responsible for these findings, but a mild plasma thromboplastin antecedent (PTA) deficiency could not be excluded with certainty. Hemorrhagic manifestations of varying degree are part of the clinical picture of polycythemia vera. As noted by Calabresi and Meyer$^5$ the incidence of this complication in different series has varied considerably, depending upon whether both major and minor bleeding
episodes were included. They estimated that 30 per cent of patients with polycythemia vera develop one or another bleeding disorder, not including easy bruising. Various abnormalities of the hemostatic mechanism consisting of poor clot retraction, thrombocytopenia, prolonged Quick prothrombin time, fibrinogenopenia and fibrinolysis have been noted by previous observers.\textsuperscript{7,10,11} In one case a specific deficiency of proconvertin was found.\textsuperscript{12} Spaet has demonstrated two factors in platelets which, in high concentration, are capable of inhibiting coagulation.\textsuperscript{13} He suggests that these factors may be partially responsible for the hemorrhagic tendency in some cases of thrombocythemia. The same mild reduction in the specific clotting factors was present in our patient at both elevated and normal platelet levels.

We believe that his case represents an example of true polycythemia vera occurring in a child of nine years. Although a systolic murmur at the "pulmonic" area suggests the possibility of an intra-auricular septal defect, this seems of no significance in the production of the polycythemia. The oxygen saturation values before and after exercise were normal; there was no evidence of cardiac enlargement or "strain" by x-ray and electrocardiograms, and venesection of amounts up to 500 cc. did not impair exercise tolerance. Other reported causes of "secondary" polycythemia have been excluded as far as possible, including uterine fibroids\textsuperscript{14} and renal lesions.\textsuperscript{15}

**SUMMARY**

A case of polycythemia vera, first diagnosed in a nine year old girl, is presented. The increase in red cell count, hematocrit and hemoglobin values was accompanied by an increase in total blood volume and true red cell volume. No other condition reported to be accompanied by polycythemia was found. Specific, mild deficiencies in certain coagulation factors were found. Iron kinetic study with Fe\textsuperscript{59} showed increased hemoglobin formation within normal iron pathways which is characteristic of polycythemia vera.
POLYCYTHEMIA VERA IN CHILDHOOD

SUMMARIO IN INTERLINGUA

Es presentate un caso de polycythemia vera, primo diagnosticate in un puera de novem annos de etate. Le augmento in le numeration erythrocytic, in le hematocrite, e in le valores de hemoglobina esseva accompaniate de un augmento del volumine de sanguine total e del volumine ver de erythrocytos. Nulle altere condition reportate in le litteratura como occurrente in concomitancia con polycythemia esseva trovate. Leve deficientias specific in certe factores de coagulation esseva constatate. Studios del cinetica del ferro con le utilisation de Fe59 revelava un augmento del formation de hemoglobin in normal circuitos de ferro. Isto es characteristic de polycythemia vera.

COAGULATION STUDIES

1. Platelets: Indirect, venous blood.
3. Clotting time: Modified Lee-White. Five tubes (12 mm. in diameter) containing 1 ml. at 37 C. tipped simultaneously at 1 min. intervals.

REFERENCES

Although considerable evidence has been presented to indicate the synthesis of δ-aminolevulinic acid from glycine by condensation with an asymmetric derivative of succinic acid to form α-amino β-ketoaspartic acid with decarboxylation to produce δ-aminolevulinic acid with subsequent polymerizations to produce porphobilinogen and then porphyrins, the evidence is indirect and based primarily on isotopic dilution techniques to show that δ-aminolevulinic acid in vitro is a more active precursor of heme than glycine. Moreover, in a variety of in vitro conditions δ-aminolevulinic acid is converted to porphobilinogen and porphyrins. The purpose of the study was to investigate further the net synthesis and the enzymatic mechanisms involved in the formation of δ-aminolevulinic acid by using particulate matter, nuclei and cell debris from chicken erythrocytes which synthesize the acid but prevent further condensation into porphobilinogen and porphyrins. Anemia was produced in leghorn chickens by intramuscular injection of equal amounts of phenylhydrazine and acetyl phenylhydrazine. Red cells were collected and hemolysates prepared; then by differential centrifugation an upper fraction of particles collected. By rather elaborate chemical procedures δ-aminolevulinic acid was isolated as a pyrrole derivative. Synthesis of δ-ALA requires the addition of glycine, succinate or α-oxoglutarate and the presence of oxygen. High concentration of succinate and α-oxoglutarate are inhibitory, but not glycine. The omission of coenzyme A, pyridoxal phosphate or magnesium chloride causes a decrease in synthesis. Cyanide, p-chloromercuribenzoate and iodoacetamide are strongly inhibitory; DPN, TPN, glucose, glucose-6-phosphate, fructose 1:6 diphosphate, D-3-phosphoglycerate and A.T.P. produce very slight and variable results. L-penicillamine, but not D-penicillamine are strongly inhibitory, probably through an interaction with pyridoxal phosphate. No δ-ALA was formed when succinate or α-oxoglutarate was replaced by synthetic succinyl-coenzyme A.—G. W. J., III.
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