HAEMOLYTIC ANAEMIA IN OSTEOPETROSIS

A REPORT OF TWO CASES

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The anaemia so frequently found in cases of osteopetrosis in children has been generally regarded as being due to poor development of the bone marrow cavity by failure of absorption and organization of primitive chondro-osteoid tissue (Warkany, 1959; Clifton and Frank, 1959; Aegerter and Kirkpatrick, 1958). In many cases, however, the severity of the anaemia has not corresponded with the radiological appearances of the medullary cavity, and alternative theories have been put forward to explain this discrepancy. Some workers have suggested that the anaemia is due to a primary bone marrow defect (McCune and Bradley, 1934), and others (Creveld and Heybroek, 1940) have postulated that the bone lesions and the anaemia are both part of a common developmental mesenchymal disturbance. Hepatosplenomegaly, a common feature of this disorder, has been taken to indicate increased extramedullary erythropoiesis, and in view of this, splenectomy in cases of osteopetrosis is considered therapeutically unsound.

Recently, however, doubt has been thrown on the above beliefs, and evidence is now accumulating that although the spleen in osteopetrosis may be a site of blood formation, blood destruction is also actively taking place in this organ. Increased destruction of red cells was demonstrated by Zetterström (1957) when he found a reduced survival time of red cells tagged with radioactive chromium in a 10-year-old patient with osteopetrosis. Engfeldt, Karlberg and Zetterström (1955) demonstrated a reduced red cell survival time of the patient's and donor's cells in a child of 6 years.

Sjölin (1959) noted a constant excess of nucleated red cells in the blood of four patients, and this, together with the presence of reticulocytosis, thrombocytopenia, and the absence of demonstrable haemolysing antibodies, led him to conclude that haemolysis and hypersplenism occurred in osteo-

petrosis. In a series of investigations on his cases, Sjölin (1959) demonstrated the presence of haemolysis. (1) Specimens of blood from the patients were labelled with radioactive chromium (Cr_{51}) and were injected into the patients and into 12 healthy adults. The apparent half life of the labelled cells in the patients was considerably shorter than in the healthy recipients. (2) Labelled blood from compatible donors was injected into the patients and into two healthy compatible recipients. Again, the survival time of the cells in the patients was much shorter than in the healthy recipients, indicating that the cause of the haemolysis was entirely extra-corporeal. Circulating antibodies were not found in the blood of these patients.

Splenectomy was performed on all four of Sjölin's cases, and post-operative red cell survival time was shown to be significantly increased in two. After splenectomy all four cases showed a rise of haemoglobin and of the platelet count, and a fall in the reticulocyte count. In one case this response was not sustained. In another, the results after splenectomy could not necessarily be accounted for by the operation alone as the patient had been given numerous blood transfusions before the procedure. In none of these cases did splenectomy seem to produce any ill effects, nor were any noted in two other instances of splenectomy cited by Sjölin (Frank, 1931; Gérard-Lefebvre, Vandendorp and Benoit, 1952).

The Present Investigation

At the time that Sjölin's paper appeared, we had under our care an infant with osteopetrosis and were thus able to confirm some of Sjölin's findings by using one of his techniques. Previously, this infant's brother had been in the same unit, also with osteopetrosis. These two patients are the subject of this presentation. They were the children of young, healthy, unrelated parents. Their mother had five pregnancies, the second pregnancy terminating after three months in an abortion. Of the

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TABLE 1
SUMMARY OF HAEMATOLOGICAL FINDINGS IN CASE 1 DURING FIRST THREE MONTHS OF LIFE

Age (days)	Haemoglobin (g./100 ml.)	R.B.C. (millions per c.mm.)	Nucleated R.B.C. per 100 W.B.C.	Reticulocytes (%)	W.B.C. per c.mm.	Differential (%)		Platelets per c.mm.	Transfusions (ml. of packed cells)
						Lymphocytes	Polymorphs		
10	4.6	1.5	5	—	6,700	96	3	'Normal'	—
11	—	—	—	—	—	—	—	—	20
21	3.7	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—	100
25-37	—	—	—	—	—	—	—	—	100
38	13.6	4	8	—	5,600	82	16	154,000	—
43	5.6	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—	130
50	4.4	—	—	—	—	—	—	—	150
90	3.3	1.1	13	7.8	9,000	65	19	9,000	—
92	—	—	—	—	—	—	—	—	180
93	11.8	3.4	—	5.5	10,500	—	—	10,500	—

four children born alive, the first and fourth had osteopetrosis, the second is a normal girl of 6 years of age, the third was born at home, weighing 3 lb. 2 oz., and died at the age of 3 days. No haematological examination or autopsy was performed on this infant.

Neither parent shows any abnormal features on radiographs taken of their long bones, spines and skulls. There is no history of osteopetrosis in other members of the family.

Case 1

Stephen was born four weeks prematurely and weighed 4 lb. 4 oz. at birth. At the age of 4 days he was jaundiced, but this gradually faded and was no longer present at the age of 11 days. At the age of 10 days, he had a slight epistaxis and was pale. At this stage a purpuric rash was present over the face, trunk and limbs.

Investigations. The haemoglobin was 4.6 g./100 ml., red blood cells numbered 1.5 million per c.mm. (Table 1). There were 6,700 white cells per c.mm., 96% of which were lymphocytes, 3% polymorphonuclears and 1% eosinophils. Five nucleated red cells were seen to every 100 white cells. Platelets were not counted, but appeared normal. The mother's blood group was A rhesus positive and that of the infant was B rhesus positive. The Coombs direct antiglobulin test on the infant's blood was negative and the Wassermann reaction of both mother and infant was negative. Bleeding time was two and a half minutes, clotting time four minutes.

Subsequent Course. The infant was given 20 ml. blood and 10 days later when the haemoglobin had fallen to 3.7 g./100 ml., a further blood transfusion of 100 ml. blood was given.

At 5½ weeks the purpura was still marked. The haemoglobin just after a blood transfusion was 13.6 g./100 ml., but during the next five days the level had fallen to 5.6 g./100 ml. The infant was given a total of five blood transfusions during the first two months of his life.

At the age of 3 months he was transferred to the

Sheffield Children's Hospital. He had a purpuric rash and his liver and spleen were both palpable. He was very pale, the haemoglobin being only 3.3 g./100 ml. The platelets numbered 9,000 per c.mm. and reticulocytes 7.8% on admission. He was given a transfusion of 180 ml. blood, but his haemoglobin continued to fall and one month later a further 180 ml. blood was given. Examination of the bone marrow showed appreciable, though low erythropoietic activity in the specimen from the scapula. The marrow of the tibia and iliac bone was strikingly hypoplastic. Radiographs of the skeleton showed generalized osteopetrosis. The infant was given intramuscular cortisone for four weeks in an attempt to retard the development of anaemia and it seemed that shortly after treatment had been started and for some six months after cortisone had been withdrawn, the level of haemoglobin remained within normal limits and no transfusions were necessary. Purpura, too, was not so marked during this time.

At the age of 4 months the child was readmitted with gastro-enteritis. This cleared up within two days, but one week later his urine showed a positive reaction to urobilin and urobilinogen and the output of urobilinogen in the stools was normal (2.2 mg. in 24 hours). The platelet count was 190,000 per c.mm.; the haemoglobin 10 g./100 ml., and a reticulocyte count of 6.5% was noted. No immune antibodies were found and the direct Coombs test was negative. The red cells showed increased resistance to complete haemolysis when suspended in serial dilutions of saline (haemolysis was 59% in a concentration of saline of 0.35% and 19% in a concentration of saline of 0.45%), but there was constant haemolysis of 10% in saline which was hypertonic (0.65%, 0.75%).

At the age of 5 months he presented with a history of jaundice for 10 days. His urine was dark and contained bilirubin, bile salts and urobilinogen and his faeces were pale. At that time it was considered likely that he had homologous serum jaundice from one of his previous blood transfusions.

During the rest of his life he required two or three monthly blood transfusions. His haemoglobin fell despite a persistent reticulocytosis of 3.5%. His liver and spleen became large and hard. He became blind

with roving nystagmus and marked optic atrophy, and also deaf. He twice fractured his tibia as a result of a trivial injury. The platelets numbered 13,000 per c.mm. at the age of 1 year and thereafter the platelet count ranged between 10,000 and 200,000 per c.mm. for the rest of his life. In all he received 32 blood transfusions before he died at the age of 6 years.

Autopsy. Naked eye examination revealed the following relevant findings:

Respiratory tract. There was mucopurulent material in the nose and nasopharynx. The lungs did not collapse on opening the chest and there were a large number of greyish areas on cut section.

The liver. This weighed 580 g. and appeared normal.

The spleen. This weighed 255 g. and was large and firm, cutting almost like fibrous tissue.

Skeletal system. All bones which were extremely hard and brittle, were surrounded by large masses, and at first sight these seemed to be large tumour deposits. On closer examination they were seen to be red plaques in the periosteum which almost covered the ribs and which were also present over the skull.

Lymph nodes. These were slightly enlarged, particularly the hilar, retroperitoneal and iliac glands, which were brownish-red in colour.

The brain. The external surface was normal.

Histological findings were as follows:

Bones. Sections of the bones showed large masses of osteoid and matrix with irregular calcification and little in the way of bone marrow production. Multiple small haemorrhages were present in the periosteum of all sections examined.

The brain and lungs. These showed evidence of old haemorrhage and increased pigment deposition.

Lymph nodes. All showed increased pigment deposition.

Spleen and liver. There was no definite evidence of erythropoiesis in either of these organs.

Iron staining. Massive quantities of iron were present in the parenchymal cells of the liver. In the lungs there was irregular distribution of iron and there was some deposition at the site of previous small haemorrhages. Small focal areas of pigment were present in the periosteum of all bones. A large quantity of iron was present in the lymph nodes and was especially marked in the cells lining the sinuses.

Case 2

Paul was born on November 26, 1959, after a normal pregnancy of 36 weeks, and weighed 4 lb. 2 oz. The haemoglobin at birth was 19.7 g./100 ml. The infant became progressively paler and at the age of 18 days his haemoglobin had dropped to 10 g./100 ml. A whole body radiograph showed a generalized increase in bone density and a lack of differentiation between cortex and medulla. The appearances were typical of osteopetrosis.

Subsequent Course. When 3 weeks old the baby was transferred to the Sheffield Children's Hospital. He

was then extremely pale, the haemoglobin being 6.6 g./100 ml. A purpuric rash was present over the face and trunk and there were scattered petechiae over the hard palate. The liver and spleen were both palpable and firm. The optic discs appeared normal.

A peripheral blood smear showed a leucoerythroblastic reaction with nucleated red cells and immature white cells. Blood culture was sterile. At the age of 24 days the serum indirect bilirubin was 4.5 mg./100 ml. The urine contained a slight excess of urobilin and urobilinogen, but no bilirubin was present. Faecal stercobilin in a single specimen was 77 mg./100 ml.

At the age of 23 days a transfusion of 120 ml. of packed cells was given. Two weeks after this transfusion the haemoglobin had fallen to 3.7 g./100 ml. and the baby received a further transfusion of 150 ml. packed cells. After these transfusions the spleen was more easily palpable for some days, but it became smaller and more difficult to feel again as the baby became more anaemic. The direct Coombs test was negative when performed on a fresh warm blood sample, but more detailed tests for haemolysis and haemagglutinins were difficult to interpret because the baby seemed to be producing very few erythrocytes and most of the red cells in his peripheral blood were those of his blood donors. Tests for haemoglobin binding showed that haemoglobin was bound to β globulin and albumin. No haptoglobin was present.

The rapid development of anaemia after each transfusion, the constant reticulocytosis (see Table 2) and the raised serum bilirubin, all suggested that haemolysis was taking place.

When the infant was 7 weeks old, tests were done using his own red cells tagged with radioactive chromium (Cr_{51}) which were injected into the infant and his father, to see whether the cells were being haemolysed more rapidly in the infant. A full description of this investigation is given. After this procedure another transfusion of 130 ml. of packed cells was given.

When he was 8 weeks old the baby's general condition deteriorated suddenly. Muscle tone in the limbs was increased and he began to have frequent generalized convulsions. The fontanelle was tense and the infant became extremely pale. He was thought to have had a cerebral haemorrhage resulting from the thrombocytopenia. Prednisolone in a dose of 10 mg. daily was given in an attempt to influence the platelet count. Phenobarbitone gr. $\frac{1}{4}$ twice a day was also given, but the fits continued, and during one such fit, two days after their appearance, the baby died.

Radioactive Chromium Studies. Red cells taken from the infant were tagged with approximately 12 microcuries of Cr_{51} and were resuspended in 21 ml. of saline: 4 ml. of the suspension of tagged cells were injected into the baby and 14 ml. into the baby's father. Blood samples were taken at regular intervals from the infant and his father; in the former for five days after injection of tagged cells, and in the latter for seven days.

Agglutination tests on the original sample from the baby showed that the blood that had been tagged was

TABLE 2
SUMMARY OF HAEMATOLOGICAL FINDINGS IN CASE 2

Age (days)	Haemoglobin (g./100 ml.)	R.B.C. (millions per c.mm.)	Nucleated R.B.C. per 100 W.B.C.	Reticulo-cytes (%)	W.B.C. per c.mm.	Differential (%)		Platelets (per c.mm.)	Transfusions (ml. of packed cells)
						Lymphocytes	Polymorphs		
1	19.7	—	—	—	—	—	—	—	—
18	10	—	—	—	—	—	—	—	—
20	9.7	3.3	—	—	7,200	70	27	'Normal'	—
23	6.6	—	—	—	—	—	—	—	120
24	13.9	4.25	'some nucleated red cells'	—	4,500	86	11	94,000	—
26	12.6	—	—	—	—	—	—	—	—
27	11	—	—	—	—	—	—	—	—
32	5.8	2.1	—	—	6,500	—	—	50,000	—
34	5.5	2.0	—	2	7,000	85	15	49,000	—
37	3.7	1.3	—	2.5	5,500	—	—	41,000	150
39	15.2	4.3	2	2.1	4,000	79	17	108,000	—
40	15.1	4.6	—	2.1	5,000	—	—	83,000	—
42	11.4	3.4	? some nucleated red cells	2.4	10,000	87	10	72,000	—
46	8.1	2.5	some nucleated red cells	2.5	7,000	87	10	40,000	—
48	8.6	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	130
51	14.7	—	—	—	—	—	—	—	—
53	10	—	—	—	—	—	—	—	—
55	6.7	—	—	—	—	—	—	—	—

not the infant's own blood, but consisted entirely of donor cells from the transfusions he had been given.

The results were expressed as the ratio of activity in the saponized whole blood sample to the activity in the counting standard. The latter was made up of a dilution of one part in 100 from the saponized suspension of tagged cells. Loss of activity in the counting solution was corrected by using as the standard count the mean count observed over the first five days before loss of activity was apparent.

The results are plotted on the attached graph (Fig. 1), in which the rapid decline of activity in the patient's blood as compared to that in the father can readily be seen.

The time to half activity was deduced from the best straight lines drawn through the points plotted logarithmically and was estimated as 2.7 days in the infant and 21 days in the father.

Autopsy. The body was that of a small pale infant with numerous petechiae in the skin, particularly of the scalp. The main naked eye changes were found in the brain and skeletal system. The brain was grossly oedematous. A large area of superficial thrombosis with an underlying haemorrhage was found over the right hemisphere.

The bones were of a deep red colour and were thickened. The ribs were acutely angulated at the costovertebral joints. Large dark red areas were present under the periosteum of the inner table of the skull.

The gross appearance of the liver and spleen was normal. The liver weighed 116 g. and the spleen 7 g.

A few petechiae were seen in both lungs.

Histological studies were carried out.

Brain. Slightly increased subependymal gliosis was present with haemorrhages around the cerebellum and

small haemorrhages in the deeper parts of the brain. The meninges were unusually thick and contained a large number of lymph and plasma cells.

Bone. A rib, skull bone, vertebra and phalanges of a toe were examined, and all showed that the bone consisted almost entirely of osteoid. Maturation seemed

FIG. 1.—Results of serial blood tests for radioactivity after injection of Cr_{51} into Case 2 and into his father. Results are expressed as a ratio of activity in the saponized whole blood sample to the activity in the counting standard. For details, see text.

to be normal until the stage of disappearance of the matrix when further maturation seemed to stop, and the entire shaft was filled with osteoid bone. The cavities of the osteoid were filled with a normal amount of haemopoietic tissue. The subperiosteal red areas over the inner table of the skull, which had been seen at autopsy, proved to be haemorrhages within the periosteum.

Spleen and liver. There was no evidence of increased erythropoiesis.

Iron staining. This was increased and was most marked in lymph nodes, testis, in the central involuting area of the adrenal and in the parenchymal cells of the liver. Iron staining was also present to a lesser extent in the brain and lungs.

Tissue samples were analysed for the presence of radioactivity. This was expressed as a percentage of the administered dose of radioactive chromium per gramme of tissue (Table 3).

The value for the spleen is clearly the highest.

TABLE 3
ANALYSIS OF TISSUE SAMPLES FOR SIGNS OF
RADIOACTIVITY

Specimen	Fraction of Total Dose per Gramme
Mediastinal lymph node	0.85×10^{-3}
Cervical lymph node	1.07×10^{-3}
Spleen	3.33×10^{-3}
Right lobe of liver	0.52×10^{-3}
Left lobe of liver	0.59×10^{-3}
Vertebra	0.42×10^{-3}
Rib	1.01×10^{-3}
Iliac crest	0.65×10^{-2}
Spine	1.43×10^{-3}

Discussion

Both cases presented the classical features of haemolytic anaemia. Reticulocytosis and nucleated red cells were noted in both. Haemolysis was also strongly suggested by the rapid development of anaemia after each blood transfusion. In both cases there was an excess of urobilin in the urine and in Case 2 the level of stercobilin was high, and the indirect bilirubin level of the blood was raised. Unfortunately, the development of homologous serum jaundice at the age of 5 months makes it difficult to evaluate the full significance of the bile pigment investigations in Case 1. Further evidence of haemolysis was provided by the test of haemoglobin binding which was described by Neale, Aber and Northam (1958). They found that in states of intravascular haemolysis, haptoglobulin, to which haemoglobin is normally bound for transport from the blood after lysis, was absent, and that alternative binding to β globulin took place. In Case 2, no haptoglobulin was demonstrated, and the haemoglobin was found to bind with β globulin

and albumin. The short life span of the red cells labelled with radioactive chromium is additional evidence for increased blood destruction in Case 2; and the accumulation of radioactivity in the spleen is suggestive, although by no means definite evidence, that most of the blood destruction was taking place in this organ.

The signs of haemolytic anaemia were only present from the age of 4 months in Case 1 and developed shortly after birth in Case 2 and it is surprising when one considers the severity of the anaemia in the latter that the infant was not anaemic at birth. This suggests some humoral factor present in the maternal blood which has kept the development of anaemia in abeyance *in utero*. In Case 1, it seems possible that the marrow could only respond to developing anaemia when the child was 3 months of age.

Although reduced in amount, haemopoiesis was shown to be present in marrow samples from Case 1 and the post-mortem appearances of the bone in Case 2 showed that there were large areas of functioning marrow in the medulla. It is unlikely that the anaemia in these cases could have been caused solely by defective haemopoiesis. Also, although haemorrhages were present in both cases in the form of purpura with slight epistaxis and there were multiple small haemorrhages in all tissues examined histologically, these could hardly be held responsible for the severe anaemia developing so rapidly after each blood transfusion. It is interesting to note that purpura was present in Case 1, even when the platelet count was normal and it is noteworthy that Aherne (1960) mentioned that in his case the same situation obtained. He described the platelets of his case as being morphologically abnormal, but this feature was not found in our patients. The disappearance of the purpura and the maintenance of a normal haemoglobin level after cortisone therapy in Case 1 is difficult to explain, and it is tempting to ascribe this response to the therapy. Because of the apparent temporary success of this treatment in this case and because of the known efficacy of steroid therapy in haemolytic anaemia, the same treatment was considered in Case 2, but he died before an adequate course could be given.

Although in Sjölin's cases, erythropoiesis in the spleen was described as being lively, in our patients and in the case described by Aherne (1960) no evidence of increased haemopoiesis was found. It is therefore not invariably the case that the spleen is an important organ of haemopoiesis in osteopetrosis. It seems likely that thrombocytopenia played at least some part in the production of the

haemorrhages found in both patients, particularly in Case 2. Sjölin found that splenectomy in his cases resulted in correction of the platelet count. It seems that splenectomy is strongly indicated in cases of malignant osteopetrosis, especially in the presence of haemorrhagic manifestations and anaemia.

Summary

Two cases of osteopetrosis in siblings are presented. They both had the malignant form of the disease, with radiological changes and severe anaemia in the neonatal period.

Reticulocytosis and a high proportion of nucleated cells were found in both patients, and this, together with a raised bilirubin level in the blood of Case 2 and the rapid development of anaemia following transfusion in both children, suggested that haemolysis was taking place.

The rapid disappearance of radioactivity in the blood of Case 2 after injection of radioactive chromium (Cr_{51}), and changes in haemoglobin protein binding are presented as further evidence for the presence of haemolysis.

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