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Congenital Haemolytic Anaemia Associated with Haemoglobin Anomaly and Mesobilifuscinuria

By

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Spherocytosis, thalassaemia and sickle-cell anaemia have long been known as strictly defined forms of congenital anaemia, whereas recently several pathologically independent molecular diseases have been observed. They belong to the group of non-spherocytic anaemias such as some enzyme deficiencies of the red corpuscles, certain forms of haemoglobinopathy [6, 7] and the anaemia associated with anomalous haemoglobin and mesobilifuscinuria.

The last-named disease was described by SCHMID *et al.* [15] and then by LANGE and AKEROYD [10]. After several essential problems connected with abnormal haemoglobin formation had been clarified [2, 4], about 25 cases have been reported [5]. In the present case our investigations into pyrrole metabolism might contribute new data to the pathology of the disease.

REPORT OF A CASE

Z. J., a male patient of 7 years, was admitted on March 5, 1967. He is an only child; the parents are healthy and were not examined by us. The skin had been yellowish and the mucous membranes pale since early childhood. This is why, the patient at the age of 4 years had been referred to us with the diagnosis of haemolytic anaemia. He had been admitted to our department eight times between December, 1964, and January, 1967, with the diagnosis of congenital non-spherocytic haemolytic anaemia. The more important routine examinations had yielded the following results.

RBC had varied between 2.6 and 3.1 million; haemoglobin, from 8.5 to 10.0 g per 100 ml; reticulocytes, between 150 and 350 per 1000 ml. For the erythrocytes, the following mean values had been revealed.

Volume,	M. C. V.	120 pl
Diameter,	M. C. D.	8.5 μ
Hb-content	M. C. H.	32 pg
Hb-concentration,	M. C. H. C.	27%
Osmotic resistance,		0.60—0.20% NaCl
Autohaemolysis in 24 hours,		3.5%
Haemolysis in 0.87% NaCl without glucose:		in 24 hrs. 4.5%
		in 48 hrs. 17.2%
with glucose:		in 24 hrs. 3.5%
		in 48 hrs. 10.0%

It is clear from these figures that we were dealing with a case of macrocytosis accompanied by a somewhat diminished concentration of haemoglobin, slightly decreased erythrocyte resistance, inreased autohaemolysis, as also by pathologically

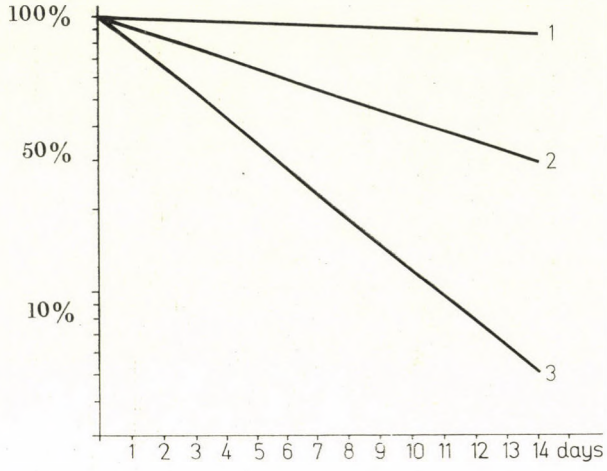

FIG. 1. 1 = Normal erythrocytes in the patient. 2 = Erythrocytes of the patient in his own circulation. 3 = Erythrocytes of the patient in two healthy recipients

increased haemolysis which responded to, but still persisted after, the administration of glucose.

The bone marrow was rich in cells. L/E was 0.42/1. Leucopoiesis and thrombopoiesis were normal. Repeated direct Coombs' tests were normal. Serum bilirubin: indirect, between 1.6 and 2.7 mg per 100 ml. Liver function tests were invariably negative.

The urine had a brownish tinge; it became dark brown during haemolytic crises. It contained neither haemoglobin nor bilirubin. Ehrlich's reaction was always normal. Repeated mesobiliviolin and pentdiopent reactions gave negative results.

The behaviour of ^{51}Cr-labelled erythrocytes is shown in Fig. 1.

The red corpuscles of the patient disappeared in two healthy recipients with a half time of 3—3½ days. The half time of the patient's labelled erythrocytes in his own circulation amounted to 8 days, while the red cells of compatible donors disappeared from the patient's blood with a half time of 33 days. The spleen: liver uptake ratio amounted to 2.1 on the first two days and to 1.9 after 10—14 days. The results of the isotope examinations, in combination with the fact that the anaemia was non-spherocytic, did not justify splenectomy.

The boy developed in a normal manner despite the haemolytic anaemia. His stature and weight were in excess of what would have corresponded to his age. X-rays of the skull and the long bones were normal. Mentally, the patient was normal. Bilateral cryptorchidism was observed. The spleen was slightly enlarged: it extended a fingerbreadth beyond the costal arch. Serious haemolytic crises occurred six times during the said period in connection with febrile diseases and after episodes of gastro-enteritis. At the time of paroxysms, RBC fell in one instance to 1.3 million, the haemoglobin to 4.4 g per 100 ml, the spleen grew in size quite considerably, and the serum bilirubin level showed a notable increase. The crises were most frequent at the age of 5 and 6 years, but subsided spontaneously so that blood transfusion had to be performed in one instance only. No crisis occurred from the spring 1967 to the end of 1968.

After congenital non-spherocytic haemolytic anaemia had been diagnosed in 1964, it was attempted to ascertain the real nature of the disease by further examinations. The result of glucose-6-p-dehydro-

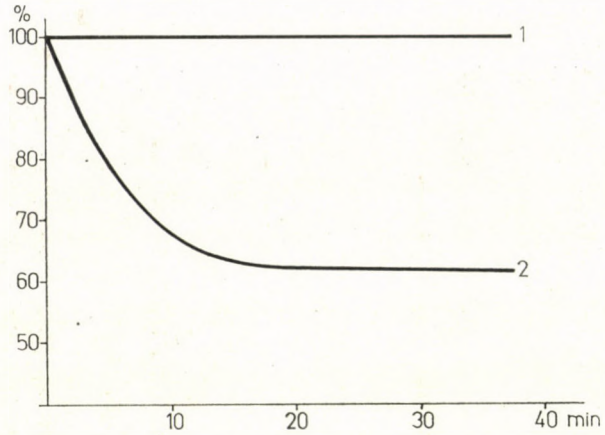

FIG. 2. Incubation of haemoglobin solutions (cyanhaemoglobin) in N/10, pH 6.8 phosphate buffer. 1 = normal control. 2 = haemolysate of patient

genase examinations, performed in 1964, was 32.3 μM/10^{11} erythrocytes, a somewhat high value that was in accordance with the high reticulocyte count. No Heinz bodies were observed in fresh smears, but after a 24-hour incubation at 37 °C about 80% of the erythrocytes displayed inclusions which stained well with brillantcresyl blue and were clearly distinguishable from the granulofilamentous substance, a phenomenon pointing to a possible glutathione reductase deficiency. The values of glutathione reductase (both the enzymes depending on TPNH and those depending on DPNH) showed, however, a slight increase in 1965, which eliminated the possibility of the known enzymopathies of the hexose-monophosphate shunt. Although haemolytic anaemias due to enzymatic disorders of haemolysis are not accompanied by the formation of inclusion bodies, we examined the ATP content of the red corpuscles. The result was 0.99 μMol/ml, a somewhat elevated value. The possibility of a glycolytic enzyme defect was, thus, likewise eliminated [11].

It was after such antecedents that the patient had been readmitted from March 5 to 19, 1967, when new diagnostic examinations were instituted with the following results.

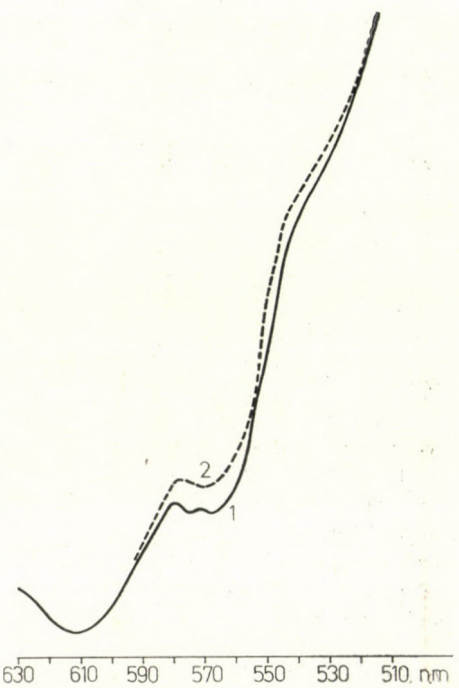

FIG. 3. Spectroscopic curves of haemolysates of the patient and of normal individuals

A. Haemoglobin

(1) The haemolysate was opalescent in diluted NaCl. Part of the haemoglobins precipitated abnormally.

TABLE I

Urinary output of pyrrole derivatives compared with that of normal individuals of corresponding age

	Values in the reported case	Normal
Ehrlich reactors, mg	0.9	0 — 3
Bilirubin	0	0
δ- ALA, mg	4.3	0.7— 1.3
PBG, mg	0.2	0.4— 0.7
Uroporphyrin, μg	15	0 —30
Coproporphyrin, μg	210	30 —70
Mesobilifuscin, mg	21	0 — 4

(2) HbF contents (Kleihaur—Betke's method): 2.5%.

(3) Starch-block electrophoresis (pH 8.6 veronal-sodium buffer) showed a large indistinct fraction next to the HbF.

(4) Using N/10, pH 6.8 phosphate buffer, we prepared a 135 mg per 100 ml cyanhaemoglobin solution. After having incubated the solution in a water bath of 66 °C, abnormal heat precipitation occurred as shown in Fig. 2.

It is clear from the foregoing that about 38% of the patient's haemoglobin was pathologically thermolabile.

(5) The erythrocyte haemolysate in N/10, pH 6.8 phosphate buffer yielded the abnormal methaemoglobin spectroscopic curve shown in Fig. 3.

It can be seen that the methaemoglobin spectrum of the haemolysate was somewhat different from the normal curve between the wavelengths 580—560 and 550—520 μm, a characteristic feature of thermolabile haemoglobins [9].

B. Pyrrole derivatives in urine

These examinations were carried out in order to observe conditions regarding the excretion of haemoglobin precursors and metabolites, and to establish the presence of increased mesobilifuscinuria. Table I shows the results in comparison with those of normal subjects of corresponding age. The patient excreted an excessive amount of mesobilifuscin. Urinary output of δ- ALA and coproporphyrin was also increased, whereas porphobilinogen excretion was normal or somewhat subnormal. Uroporphyrin excretion was normal.

DISCUSSION

Although the patient had been referred to our department for splenectomy on account of haemolytic anaemia, we decided not to perform the operation because the anaemia was of the non-spherocytic variety. ^{51}Cr examinations, too, showed the splenic involvement to be secondary. After the definite diagnosis had been established, the problem of splenectomy has again arisen. A survey of the literature revealed, however, that splenectomy improved the condition in three cases only [5, 13], while the degree of anaemia remained unchanged or decreased but for a short time in 12 cases. In one instance, the condition of the patient deteriorated after

the operation. Considering that the reticulocyte count remained excessive in all cases, the therapeutic value of splenectomy had to be considered questionable.

The appearance of Heinz bodies in cases of haemoglobin anomaly is a characteristic postoperative phenomenon of splenectomy. SCHMID *et al.* [15, 16], then LANGE and AKEROYD [10], the first authors to describe the disease, diagnosed it after preceding splenectomy, and called it, accordingly, "congenital haemolytic anaemia associated with the formation of Heinz bodies, pathologic haemoglobins and mesobilifuscinuria". Intact patients also develop inclusions, but the incidence of spontaneously formed Heinz bodies does not exceed 1% in them.

Provocation was necessary also in the present case to induce the formation of Heinz bodies *in vitro*. It is possible that the inclusion bodies contain the abnormal haemoglobins, as is the case in haemolytic anaemia caused by Hb-Zürich [1].

DACIE *et al.* [2, 4] demonstrated the thermolabile nature of anomalous haemoglobins. Studying the nature of such haemoglobins, SHIBATA *et al.* [14] found that the SH group of the cysteine at locus 93 of the beta chain was blocked, a feature distinguishing them from Hb-Zürich in which the corresponding defect occurs at locus 63 of the beta chain [1]. The structure of Hb-Köln which gives rise to a similar condition [7] is still obscure.

Mesobilifuscin is a mixture of two dipyrroles, namely mesobilifuscin I and II [18]. It is excreted with faeces and urine also under physiological conditions. It was demonstrated by STICH and STÄRK [17] that urochrome B, to which the urine owes 50% of its pigmentation, is identical with mesobilifuscin. Whether this chromogen is normally a byproduct of porphyrin synthesis or a breakdown product of haemoglobin has still to be decided. In the present case it was certainly the latter. If the erythrocytes of a patient suffering from the disease at issue are introduced into the blood of a healthy individual, a considerable quantity of mesobilifuscin will appear in the urine of the recipient [3].

The results of our investigations concerning haem synthesis are similar to those obtained in cases of lead poisoning. Increased excretion of delta ALA and coproporphyrin is accompanied by normal or slightly decreased excretion of porphobilinogen. There is further a conspicuous similarity in respect of the disposition to develop Heinz bodies. It is quite possible that congenital enzymatic disorders, characteristic of the disease in question, are somewhat similar to enzymatic disturbances caused by lead poisoning. One should remember that the disturbance of globin synthesis may combine with that of pyrrole synthesis. Looking at the structural formula of the haemoglobin molecule one cannot help to notice that the tetrapyrrole ring is attached to the histidine at loci 63 and 92 of the normal beta chain. The defect in the case of Hb-Zürich is at locus 63; in the given

disease, the anomaly appears in the SH group of cysteine at locus 93.

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Summary

The case is reported of a 7-year old patient suffering from congenital macrocytic anaemia. Osmotic resistance of the erythrocytes was diminished, autohaemolysis and haemolysis on incubation were increased. Erythrocyte enzymatic activity was normal or slightly increased. Thirty-eight per cent of the haemoglobin was abnormally thermolabile, precipitating at 66 °C. Urinary mesobilifuscin output was excessive. About 1% of the red corpuscles contained Heinz bodies. Excretion of pyrrole derivatives and the tendency to form inclusion bodies were features somewhat similar to those seen in cases of lead poisoning.

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