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Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene

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Aksoy, M., Dinçol, K., Erdem, Ş., Akgün, T., and Dinçol, G. (1972). *Brit. J. industr. Med.*, 29, 56-64. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. A study was performed on 32 pancytopenic patients who had had long-term exposure to benzene. They had been subjected to high concentrations of benzene varying from 150 to 650 p.p.m. for from 4 months to 15 years. Apart from four, in whom the platelet count was normal, all had pancytopenia. As the bone marrow punctures of the pancytopenic patients showed a great variation from acellularity to hypercellularity, the patients were classified and studied according to the bone marrow findings.

Anaemia was macrocytic in 14, in three of whom a megaloblastic erythropoiesis was detected. The findings in some patients, such as mild reticulocytosis, hyperbilirubinaemia, erythroblastaemia, an increase in quantitative osmotic fragility and in faecal urobilinogen excretion as well as elevated serum LDH levels, suggested that these might be attributed to either increased haemolysis or the presence of ineffective erythropoiesis.

The HbF content in 20 out of 24 pancytopenic patients was above normal, ranging between 3·2% and 19·5%, with a mean of 6·1%. Mean values of HbF in groups with a hypoplastic, hyperplastic, and normoplastic bone marrow were essentially the same. The absolute amounts of HbF exceeded 400 mg/100 ml in only 8 out of 24 patients, all of whom survived.

The HbA_2 level was within normal limits in 21 out of 24 pancytopenic patients. It was definitely decreased in one and slightly so in three. These findings may suggest that HbA_2 occasionally shows a tendency to decrease in some patients with chronic benzene poisoning. The maturation arrest in both the myeloid and erythroid elements was the most frequently encountered finding.

In the bone marrow examinations, giant erythroid precursors varying from 9% to 72% were detected in two patients. In one of them, who also had hepatosplenomegaly, the development of preleukaemia was accepted. Varying mortality rates were estimated in the above-mentioned groups. The results obtained from treatment with steroids, androgens, phyto-haemagglutinin, and oxymetholone are also described.

Several haematological disorders, such as aplastic anaemia, leukaemia, leucopenia, thrombocytopenia, pancytopenia, acquired pseudo-Pelger-Huet anomaly, etc., have been attributed to chronic benzene poisoning.

In the last decade, in Istanbul and in oth cities in Turkey, numerous cases of chronic be zene poisoning have been encountered amo workers using benzene-containing adhesives in t manufacture of shoes and slippers. The unhygie

conditions of their workplaces have been described elsewhere (Aksoy *et al.*, 1971a).

Case material and methods

The series comprised 32 patients with pancytopenia associated with long-term exposure to benzene. Of these, 25 patients were initially admitted to the haematology department of the 2nd Internal Clinic of Istanbul Medical School and, after discharge, were followed up by us for periods ranging from one to seven years. Five patients were followed up by us only in the outpatient department. One patient was seen in another hospital and another patient was followed up at our laboratory after discharge from a clinic in Istanbul.

The haematological methods were all standard. Platelet counts were performed with a phase-contrast microscope using the Brecher-Cronkite (1950) technique. Haemoglobin F was determined by the method of Singer, Chernoff, and Singer (1951). Haemoglobin A_2 was estimated quantitatively by DEAE-cellulose (DEAE-Sephadex A, Medium, Anion exchanger, Pharmacia Uppsala, Sweden) chromatography and by starch gel electrophoresis, according to the methods of Huisman and Dozy (1961) and Aksoy and Erdem (1965). With these methods normal values for haemoglobin A_2 range between 2 and 3% for the former and between 1·5 and 4% for the latter. G-6PD activity was determined either quantitatively or qualitatively by the methods of Gadsden and Cannon (1964) and Tönz and Betke (1962). The quantitative osmotic fragility tests before and after 24 hours' incubation at 37°C were performed by Dacie's (1960) method. Serum lactate dehydrogenase values were determined by the method of Wroblewski and LaDue (1955). Faecal urobilinogen was estimated by the method of Young, Davis, and Hogestyn (1949) using a comparator block.[1] In this method, values for faecal urobilinogen are 50 to 300 Ehrlich units for 100 g of faeces. Concentrations of benzene vapour in the air of working environments of the patients were measured by a Draeger Multigas Detector.[2]

[1] Manufactured by W. A. Taylor Co., Baltimore, Md, U.S.A.
[2] Draeger Gasspurgerat/Multi Gas Detector Mod.21/31 Lübeck, Germany.

Results

Though the clinical features and haematological data for these 32 patients were generally similar, their bone marrow findings were different. Thus, the patients have been classified into four groups as follows:

Group 1 Pancytopenia with a hypoplastic or acellular bone marrow (cases 1 to 12 inclusive)
Group 2 Pancytopenia with a hyperplastic bone marrow (cases 13 to 19 inclusive)
Group 3 Pancytopenia with a normoplastic bone marrow (cases 20 to 31 inclusive)
Group 4 Pancytopenia with giant erythroid precursors, possibly as a finding of preleukaemia (case 32).

Clinical and occupational findings

The series consisted of 30 males and 2 females, ranging in age from 15 to 57 years, with an average of 32 years (Table 1).

Twenty-four patients worked in small workshops manufacturing shoes. Two worked in a box factory, another two manufactured objects made of leather, one made glues containing benzene, one was a car painter, one a radio repairman, and another a furniture maker. All were using benzene-containing materials as adhesives. The working conditions of all the patients were hardly good as their working places were unhygienic and poorly ventilated. The concentrations of benzene in their working environment ranged between 15 and 30 p.p.m. outside working hours and were recorded to reach 210 or,

TABLE 1

COMPARISON OF SOME CLINICAL DATA IN FOUR GROUPS

	Group 1 (n = 12)		Group 2 (n = 7)		Group 3 (n = 12)		Group 4 (n = 1)
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
Age (yr)	35·2	±9·17	34·7	±12·9	26	±9·19	39
Duration of exposure (yr)	6·1	±3·91	6·7	± 1·6	6·5	±4·05	10
Duration of symptoms (mth)	2·7	±1·64	2·4	± 1·0	2	±1·64	1
Duration of follow-up till CR or death (mth)	6·3 (11 patients)	±3·79	6	± 1·24	7·5	±4·21	8
Age of fatal cases in all groups (yr)	(Mean 38·8; S.D. ±9·16; N = 8)						
Age of CR cases in all groups (yr)	(Mean 27; S.D. ±9·27; N = 19)						
% and no. of severe cases at 1st examination	66·6%	8	42·8%	3	33.3%	4	100%
% and no. of fatal cases	41·6%	5	28·5%	2	0%	0[1]	100%

[1] One of the patients in this group was critically ill and refractory to all treatment when he was discharged. It is quite probable that he died.
CR = complete recovery

rarely, 640 p.p.m. when benzene-containing adhesives were being used. The period of exposure ranged from 4 months to 15 years. In two patients the period of exposure was less than one year, in 10 between one and five years, in 17 between 5 and 10, and in three more than 10 years. There was no significant difference in the duration of exposure between all four groups, the mean duration of exposure being 6·1, 6·7, 6·5, and 10 years respectively. On the other hand, although when first seen a moderate or severe pancytopenia was detected in most of the patients, the duration of symptom attributable to pancytopenia before the first examination or admission was surprisingly very short, ranging mostly between one and three months. This may be explained by the insidious development of the disease. Five of the patients were symptomless and were discovered during a survey of workers using benzene-containing materials (Aksoy *et al.*, 1971a).

Details of the clinical findings are shown in Table 2. They included signs and symptoms attributable to pancytopenia, such as lassitude, palpitations and dyspnoea on exertion, pallor, purpura, ecchymoses, epistaxis, and bleeding from the oral mucosae and from the gastrointestinal tract, particularly in association with secondary infections. In two patients severe and rare complications of secondary thrombocytopenia were detected, a fatal haemothorax and a haemorrhagic pleurisy. On the other hand, in two patients (cases 1 and 32) a marked or moderate hepatosplenomegaly developed during the course of the illness. Unfortunately, we were not able to perform a splenic puncture or necropsy in these two fatal cases. In one patient, a marked hepatosplenomegaly causing severe abdominal pains had developed nearly two weeks before death and the cause of the hepatosplenomegaly remained obscure. In the other case, group 4, the cause of the hepatosplenomegaly was possibly preleukemia, as will be explained below.

Haematological and laboratory data

Table 3 gives some important haematological findings for each group of patients.

Red blood cells Generally, the severity of anaemia was similar in all four groups, but some pancytopenic patients with a normoplastic bone marrow had higher values than those of the pancytopenic patients with hypoplastic or hyperplastic bone marrow. In the patients with hyperplastic or hypoplastic bone marrows, the mean reticulocyte counts were slightly increased.

Erythroblastaemia In six patients nucleated red cells were present in the peripheral blood, ranging between 1 and 8 per 100 WBC.

Quantitative osmotic fragility A quantitative test was performed in 20 out of 32 patients before and after 24 hours' incubation at 37°C. It was normal in seven of them. A mild or moderate increase either before or after incubation was detected in the remaining 13 patients.

Fetal haemoglobin This was determined in 24 out of 32 patients. In the remaining eight patients, fetal

TABLE 2

NUMBER OF PATIENTS EXHIBITING THE DESCRIBED SIGNS AND SYMPTOMS AMONG THE 32 PANCYTOPENIC PATIENTS TOGETHER WITH OUTCOME AND CAUSE OF DEATH

	Group 1 (n = 12)	*Group 2* (n = 7)	*Group 3* (n = 12)	*Group 4* (n = 1)
Fever	5	2	a	p
Purpura and ecchymoses	7	4	3	p
Epistaxis	6	3	3	p
GI bleeding	5	3	a	p
Haemothorax	1	1	a	a
Splenomegaly	1	a	a	p
Hepatomegaly	2	a	a	p
Outcome	Fatal in 5, CR in 4, UT in 2, lost to follow-up in 1	Fatal in 2. CR in 5	No improvement in 1, improvement in 1, CR in 10	Fatal
Cause of death	Haemothorax and sepsis in 1. infection and bleeding in 4	Haemothorax and bleeding in 1, infection and bleeding in 1		Infection and bleeding in 1

CR = complete recovery; UT = under treatment: p = present; a = absent

TABLE 3

SOME HAEMATOLOGICAL AND LABORATORY DATA IN THE 32 PATIENTS

	Group 1			Group 2			Group 3			Group 4	
	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	n
RBC (10^6/mm^3)	2·03	± 0.5597	12	1.97	± 0.402	7	2.22	± 1·035	12	1·60	1
Hb (g %)	5·4	± 1·215	12	5·6	± 0·755	7	5.9	± 2·66	12	4.5	1
WBC/mm^3	2250	±714	12	2310	±103	7	2310	±788	12	400	1
Reticulocytes (%)	1·7	± 1.3	12	2.6	± 1.67	7	2.9	± 1.42	12	0	1
Platelets (10^3/mm^3)	80.2	± 71.6	12	59	± 31·54	7	95·5	± 51.71	12	10	1
Haematocrit (%)	18·5	± 4·5	12	20	± 4.6	7	20·8	± 10·04	12	19	1
MCV (μm^3)	91	± 5·38	12	98.2	± 8·1	7	93	± 6·32	12	118	1
MCHC (%)	306	± 4	12	29	± 6.24	7	29·2	± 5·38	12	24	1
Lymphocytes (%)	43·5	± 14.17	12	36·6	± 12·59	7	33.8	± 17	12	64	1
NRC/100 WBC	0	0	12	1.7	—	7	0·08	—	12	2	1
Total bilirubin (mg %)	1·05	± 0.32	11	1.09	± 0·51	7	0.85	± 0.72	9	1·5	1
Serum iron (μg %)	126	± 53.35	7	140	± 70	6	170	± 56·84	7	190	1
Fetal Hb (%)	5·4	± 2.09	7	5·8	± 4.7	6	6.9	± 4.4	11	x	0
Total fetal Hb (mg/100 ml)	365	±175·2	7	308·2	±193·6	6	696	±619·8	11	x	0
Haemoglobin A, (Scphadex Chromatography)	2.26	± 1·03	7	2.15	± 0·55	2	21	± 0·55	3	x	0
Haemoglobin A_2 (Starch-gel electrophoresis)	0		0	2.4	± 0.52	4	2·5	± 0.53	3	x	0
Faecal urobilinogen (Ehrlich U/100 g)	329	±159·5	6	510	±364·4	4	428	±176·9	7	x	0
Serum LDH (Wroblewsky U)	484	±225·7	7	0	—	0	609	5287.7	3	840	1
Quantitative osmotic fragility											
before incubation	Slightly or moderately increased in 3 out of 6			Slightly increased in 4 out of 6			Slightly increased in 2 out of 7			Normal	1
after incubation				Moderately increased in 1 out of 6			Slightly increased in 3 out of 7			Normal	1
G-6PD screening test	Positive in 9, negative in 1			Positive in all			Positive in all			Positive	1

S.D. = standard deviation; n = number; x = not performed

haemoglobin estimations were not performed because of large numbers of blood transfusions. In 20 patients HbF was increased (mean 6·14%; range 3·2 to 19·5%) and was within normal limits in the remaining four. However, the absolute amount of fetal haemoglobin exceeded 400 mg/100 ml in only 8 of the 24.

Haemoglobin A, In 21 out of 24 patients, HbA_2 values were found within normal limits. The HbA_2 was definitely decreased in one patient to 1·5% and slightly in two patients to 1·9 and 1.95%. In addition, in one patient of group 3 HbA_2 like HbF was within normal ranges on admission (2·2%) but three months later, following a considerable haematological improvement, it had decreased to 1·95%.

White blood cells All the patients investigated had pancytopenia. Eight patients had a relative lymphocytosis, ranging from 50 to 78%. In the remainder, there was an absolute lymphopenia. A mild increase in monocytes, ranging from 9 to 13%, was detected in four patients. One patient in group 1 showed a marked Pelger-Huet anomaly following complete recovery (53% of the leucocytes had one or two lobes). This abnormal feature persisted for two and a half years after clinical and haematological recovery. Neither his mother nor one of his sisters showed a similar leucocyte anomaly.

Platelets A severe or moderate thrombocytopenia was found in all except four patients. One of the four patients with an abnormal platelet level had a severe anaemia and leucopenia which were refractory to all treatment, and his platelet levels, determined several times during a one-year period, were always found to be within normal limits. In the blood smears of the patients with low levels of platelets, giant or morphologically abnormal platelets were detected.

Bilirubinaemia A mild hyperbilirubinaemia was found in 8 out of 28 patients studied.

Serum lactate dehydrogenase activity In 6 out of 11 pancytopenic patients the serum lactate dehydro-

genase activity was significantly increased, ranging between 600 and 930 Wroblewski units. In one patient, the LDH level returned to normal following the improvement of the haematological state. In four patients with an elevated LDH level there were findings of increased haemolysis, so the increase in LDH activity may be attributed to the presence of a haemolytic component. On the other hand, in a patient in group 4 the increased level of activity of this enzyme may be explained by a state of preleukaemia.

Life span of erythrocytes The life span of the red blood cells was measured by the ^{51}Cr method in one patient only and was moderately shortened—21 to 28 days.

Faecal urobilinogen A mild or moderate increase in the excretion of faecal urobiiinogen was observed in 9 out of 17 patients.

G-6PD activity Only one out of 22 patients studied showed G-6PD deficiency.

Serum electrophoresis and liver function tests
Serum electrophoresis and liver flocculation tests such as thymol turbidity and alkaline phosphatase levels were determined in all the patients. The results were within normal limits. Only in one patient, in group 3, did the bromsulphalein test show a 23% retention. In this patient the results of pyruvic and glutamic oxalacetic transaminase activities. thymol turbidity, and prothrombin times were within normal range. Following complete recovery the results of the bromsulphalein test remained unchanged.

Bone marrow
Table 4 gives the results of bone marrow punctures in each group. In three patients in group 1, repeated. bone marrow punctures were performed at different sites and no material was obtained. The bone marrows of these patients were considered to be acellular. In 12 patients, bone marrows were normocellular, in 9 hypocellular, and in the remaining 7 hypercellular. The bone marrow finding of a patient in group 4 were strongly suggestive of preleukaemia. A maturation arrest in the myeloid series was observed in 15 patients and in the erythroid series in 9 patients. A mild or moderate megaloblastic erythropoiesis was observed in three patients from groups 1, 2, and 4 in whom megaloblasts ranged between 4 and 22%.

In the one patient in group 4, giant erythroid precursors comprising 38 to 72% of the nucleated cells were found. In this patient these erythroid precursors appeared during the course of the disease. lasting eight months, and persisted till death. Of the four bone marrow punctures, numerous giant erythroid precursors as well as those of normal size were detected in the last three. The cytoplasms ot these erythroid precursors were deeply basophilic, some resembling plasma cells. They sometimes showed bizarre forms and were usually in clusters. The chromatin network of the nuclei of these cell: was fine and a number appeared to have two lobes They often had one to four large nucleoli and the:

TABLE 4

BONE MARROW FINDINGS IN THE 32 PATIENTS

	Group 1 (n = 9)[1]		Group 2 (n = 7)		Group 3 (n = 12)		Group 4 (n = 1)[3]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Myeloid/erythroid ratio	1:0·7		1:2		1:1·05		1:8·3	
Myeloid series (%)	397	±16·06	34.7	±14·45	46·6	±16·24	19·7	±29·51
Erythroid series (%)	26·2	±11·09	46	± 7·21	38	±13.03	16·5	± 8.42
Lymphocytes (%)	29·7	±19·46	8·2	± 6·40	12·3	± 6·48	a	± 3·31
Megaloblasts (%)	0·44	n.p.	3·1[2]	n.p.	0		8	n.p.
Giant erythroid precursors (%)	0	n.p.	0	n.p.	0		44·5	n.p.
Maturation arrest in myeloid series	Present in 2		Present in 5		Present in 8		Absent	
Maturation arrest in erythroid series	Present in 4		Present in 3		Present in 2		Present	
Megakaryocytes	Present in 7		Present in 4		Present in 8		Absent	
Vacuolation	Present in I		Present in 4		Present in 4		Present	

[1] 3 patients with acellular bone marrow are not included in the calculation of the values
[2] A megaloblastic erythropoiesis developed in one patient during the course of the disease
[3] In this patient four bone marrow punctures were performed and the mean values were calculated accordingly
n.p. = not performed

showed numerous mitotic forms. Unstained areas were also found in the cytoplasm of these giant erythroid precursors which sometimes strongly resembled promegaloblasts. As will be explained below, ue arc strongly inclined to consider these cells as a finding of preleukaemia. In this patient with numerous giant erythroid precursors, a megaloblastic erythropoiesis was present during the course of rhe illness. megaloblasts comprising 12 to 20% of the nucleated cells.

In rhe majority of the patients, a vacuolation in the myeloid and/or erythroid elements was observed.

Outcome

Eight of the patients died of complications of pancytopenia such as bleeding and/or secondary nfection (Table 2). Of the 24 surviving patients, four were pancytopenic when last observed. One of these, n group 3, required periodic blood transfusions.

Treatment

Androgens Twelve patients, of whom five had hypoplastic, two had hyperplastic, and five had normoplastic bone marrows, received only androgens. Five of them were in a serious clinical and haematological condition. Of these patients, two with a hypoplastic and one with a hyperplastic bone marrow died.

Steroid Steroids with androgens were used in seven patients, four with a hyperplastic and three with a normoplastic bone marrow. Only one patient with a hyperplastic bone marrow died during the treatment due to the complications of his severe condition.

Phytohaemagglutinin[1] Six patients, of whom three had hypoplastic bone marrow, two had hyperplastic bone marrow, and one was in a preleukaemic stage, received androgens, steroids, and PHA. In addition to this, in three patients, two with hypoplastic and one with normoplastic bone marrows, PHA was used parenterally, 50 mg per day for 20 to 21 days. Two patients with a hypoplastic and one with a preleukaemic bone marrow died.

Oxymetholone One patient with a hypoplastic and two with a normoplastic bone marrow received oxymetholone. One patient with an acellular bone marrow died despite treatment. In another, in group 3, neither PHA and androgen nor a trial with oxymetholone led to any haematological improvement. Complete recovery was observed in a patient in group 3, following a trial with oxymetholone.

[1] PHA, Burroughs Wellcome, England, 50-mg vials; Phyto PHA, Egic Laboratories, Loiret, France, 50-mg vials.

Discussion

As can be seen from Tables 2, 3, and 4, there were great variations in both the clinical and haematological findings as well as in the state of the bone marrow in this series. Thus, the initial clinical conditions of the patients ranged from asymptomatic or mild forms to 'very severe', with a corresponding variation in the haematological picture. Yet the common basis in all our cases was two main features: (1) the presence of advanced and persistent pancytopenia, and (2) benzene as a causative agent–The mean age was lowest in group 3, which comprised patients with normoplastic bone marrows. In this group, the illness was less severe and the outcome more favourable than in the patients of the other groups. As there was no significant difference in the duration of exposure in all groups, we have the impression that older patients are more severely affected by chronic benzene exposure.

The clinical manifestations in our 32 pancytopenic patients were not different from those encountered in chronic benzene poisoning (Browning, 1965). As can be seen from Table 2, the great majority of clinical symptoms, such as bleeding and fever, etc., were mostly encountered in the patients with either hypoplastic or hyperplastic bone marrows. On the other hand, in the patients with a normoplastic bone marrow, similar clinical findings were not present. One interesting clinical manifestation was the haematological pleurisy or haemothorax due to thrombocytopenia in two patients. These rare complications of thrombocytopenia were the cause of death in one patient.

Splenomegaly

This was observed during the course of illness in two of the patients who died. In one of them, a splenic puncture was not done and a necropsy was refused, so the cause was not discovered. The possibilities of a leukaemic involvement or myeloid metaplasia cannot be excluded. The other patient, from group 4, exhibited a different picture because the hepatosplenomegaly, which developed five months before death, in association with the presence of numerous giant erythroid precursors in the bone marrow, was suggestive of preleukaemia.

RBC and *signs* of increased haemolysis

Anaemia was normocytic in 18 and macrocytic in 14 patients. The majority of patients with macrocytic anaemia were in group 2, with hyperplastic bone marrow. In two patients (one each in groups 2 and 4) the mean corpuscular volume was more than 114 μm^3. As expected, the bone marrow of both these patients also showed megaloblastic erythropoiesis.

A mild reticulocytosis was found in 16 and a mild hyperbilirubinaemia in 8 out of 28 patients.

In addition, a marked increase in the excretion of faecal urobilinogen was observed in 9 out of 17 patients and a mild or moderate increase in osmotic fragility, before or after incubation, in 13 out of the 20 patients in whom this was studied. These four laboratory findings may be explained either by the presence of a haemolytic component (Goidwater, 1941; Scott, Cartwright, and Wintrobe, 1959; Erf and Rhoads, 1939; Aksoy *et al.*, 1966) or by ineffective erythropoiesis which may be present in refractory anaemias (Barrett, Cline, and Berlin, 1966; Heller and Fried, 1967; Wintrobe, 1967).

Similarly, the mild erythroblastaemia, ranging between 1 and 8 per 100 WBC, which was mostly observed in group 2, may also be attributed to increased haemolysis or to the presence of ineffective erythropoiesis. In one of these six patients with erythroblastaemia in group 2, this finding may also be explained by folic acid deficiency. In that patient, in whom there were 8 to 30 nucleated red cells per 100 WBC in peripheral blood, adequate doses of folic acid caused a gradual disappearance of erythroblastaemia.

Platelets

In four patients, three with a hypoplastic and one with a normoplastic bone marrow, the platelet levels were within normal limits. Yet, in one of them, the platelet counts were always normal during a one-year period of follow-up. Thus. considering the results obtained from these four patients and the results of a survey on 217 workers with long-term exposure to benzene (Aksoy et *al.*, 1971a), we would suggest that megakaryocytes are more resistant to benzene than the precursors of granulocytic elements. Individual susceptibility should also be considered.

Fetal haemoglobin

According to Bloom and Diamond (1968), when the total fetal haemoglobin is less than 400 mg/100 ml, the prognosis of aplastic anaemia is unfavourable. There were 16 patients with total fetal haemoglobin less than 400 mg/100 ml, three in group 1, five in group 2, and eight in group 3. They all recovered. It is noteworthy that in 12 out of these 16 patients. the initial haematological state was severe. Thus, we suggest that in pancytopenic patients exposed to benzene, the total fetal haemoglobin value does not have the same prognostic value, as suggested by Bloom and Diamond (1968), in aplastic anaemia. This point has also been emphasized by Aksoy, Dinçol, Erdem, and Akgün (1969) and by Storti, Perugini, Massini, and Manzini (1969) in patients with aplastic anaemia due to various causes.

Shahidi, Gerald, and Diamond (1962) and Aksoy and Seçer (1964) have pointed out that the production of fetal haemoglobin to excess is 'switched on' in aplastic anaemia and overproduction continues well beyond recovery. In several patients, elevate levels of fetal haemoglobin persisted for many year after recovery. Sometimes it rapidly returns t normal. This happened in one patient in group 3, i whom, after complete recovery, the fetal haemc globin content rapidly dropped from 19·5 to 2% On the other hand, occasionally the mechanism 'switched on' a little late. In one patient, in group 2 the fetal haemoglobin was within normal limi when she was first seen. After the end of exposur three months after recovery, the fetal haemoglobi was found to be 8·7% and peripheral blood coun were within normal limits.

Pelger-Huet anomaly

In one patient, this leucocyte anomaly was detect following complete recovery. An incomplete fami study did not confirm this inherited leucocy anomaly, but the persistence of this finding two ar a half years following his complete recovery strongly in favour of a true Pelger-Huet anomal Interestingly, this pancytopenic patient with Pelger-Huet anomaly showed a rapid clinical a haematological recovery. This prompted us accept the possibility that such a congenital anoma does not aggravate the haematological effects chronic benzene poisoning.

Bone marrow

A maturation arrest in the granulocytic and erythrc series and an erythroid hyperplasia were the m commonly encountered findings in our pancy penic patients with long-term exposure to benze Megaloblastic erythropoiesis in refractory anaer with a hyperplastic bone marrow is considered to due to a block in nucleoprotein formation (Vil et *al.*, 1960). Furthermore, folic acid deficiency m also play a role in the development of megaloblas erythropoiesis observed in some pancytooe patients exposed to benzene. In the patient in gro 4, the low level of serum folic acid[1] confirmed possibility. However, an unfavourable response folic acid showed that folic acid deficiency was the only factor in the development of a megalobla erythropoiesis in that patient. Furthermore, another patient. in group 2, with megalobla erythropoiesis, the disappearance of a mar. erythroblastaemia after folic acid therapy may considered as confirmation of a folic acid deficie although the folic acid level was not determin Thus, in the development of megaloblastic erytt poiesis in the patients exposed to benzene, at l two factors may play a role: (*a*) a block in nucleoprotein formation, and (*b*) folic acid ficiency.

[1]Kindly performed by Dr. I. Chanarin. Experime Haematology Research Unit, London.

Giant erythroid precursors

In two patients (one in group 2 and one in group 4), giant erythroid precursors which were found to vary between 9 and 72% of nucleated cells were similar to those described by Mallory, Gall, and Brickley (1939) and erythrogones observed by Moeschlin and Speck (1967) in animals experimentally poisoned by the injections of benzene.

Leukaemia may appear *in* patients with aplastic anaemia due *to* chronic benzene exposure (Vigliani and Saita, 1964; Browning, 1965; Hernberg, Savilahti, Ahlman, and Asp, 1966). Four such cases uere detected in our study among benzene workers (Aksoy, Dinçol, Erdem. and Dinçol, 1971b). It thus seems probable that the presence of giant erythroid precursors, which are usually very primitive cells with bizarre shapes, may be a feature of pre-leukaemia. This is a possibility in our one case in group 4.

Outcome

The overall mortality in our series was 25%. On the other hand, when rhe mortality rates of each group were considered separately, it became apparent that the rate was higher in group 1 than in groups 2 and 3, being 41%, 28·5%, and nil respectively. Funhennore, the mean age of eight patients who died is significantly higher ($P < 0{\cdot}01$) than the mean age of the 19 who recovered completely. Oider patients seem more likely *to* succumb to the deleterious effects of chronic benzene exposure.

Treatment

Because of the differences in the severity of the disease and the type of cellularity *of* the bone marrow in the patients in this series, it is difficult to evaluate the therapeutic results of this trial. However, we should stress that, in our experience, PHA may occasionally prove beneficial (Aksoy *et al.*, 1969). This was observed in four patients (two in group 1, one in group 2, and one in group 3) who had previously been treated without improvement with steroids and androgens or with androgens alone. It was only after the administration of PHA that a considerable improvement was observed.

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