

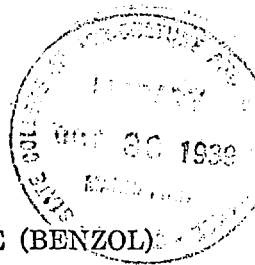
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THE HEMATOLOGICAL EFFECTS OF BENZENE (BENZOL) POISONING*

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A SUFFICIENT number of cases of refractory anemia following exposure to benzene have been reported to leave no doubt concerning the existence of the disorder as a clinical entity. In many instances, however, the clinical, pathological, and toxicological data are insufficient to allow a clear understanding of the nature of the disturbed hematopoiesis. This communication presents an account of 9 individuals (6 rotogravure printers, 1 chemist, 1 beauty parlor worker, and 1 person who used large quantities of a cement containing benzene) who were exposed to benzene fumes and who developed concomitant serious disorders of the blood. Sufficiently detailed studies were made of these cases to substantiate certain conclusions for which adequate evidence has not been available hitherto.

Selling (1), in a comprehensive review, stated that the erythropoietic tissue of the bone marrow was injured in chronic benzene poisoning, although the circulating erythrocytes were relatively unaffected. Reznikoff and Fullerton (2) advanced a similar opinion and stated, as did Selling, that benzene exerted a leucolytic effect. Greenburg (3), after citing many references, felt that in addition to the leucolysis there was an increased erythrolysis during

the early stages of poisoning. Selling (1) and later Brandino (4) studied experimental benzene intoxication in rabbits and dogs. They reported that the earliest hematological change was a hyperplasia of the marrow and that subsequently aplasia was found. Hamilton (5), Browning (6), and Greenburg (3) reviewed the reports of the histological alterations of the hematopoietic tissue of human beings dead of benzene poisoning. The marrow may be aplastic (Selling, 1), normal (Cremiru, 7) hyperplastic (Cabot, 8) (Anderson, 9), or even leucemic (Weil, 10). Penati and Vigliani (11) described the following results of chronic benzene poisoning: (a) Aplastic anemia with aplastic marrow, (b) refractory (aplastic) anemia with hyperplastic marrow, (c) refractory (aplastic) anemia with myeloid metaplasia of the marrow, (d) acute or chronic leucemia. Lignac (12) reported the production of leucemia in mice poisoned with benzene.

There are in the literature many indications that circulating erythrocytes may be destroyed at an abnormally rapid rate in patients poisoned with benzene. Many authors (Paul, Friedlander and McCord, 13), (Mitsnik, 14) (Pulford, 15), (Cabot, 8) have recorded increased numbers of circulating reticulocytes in the blood of

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human beings exposed to benzene, a finding which was assumed to indicate abnormally active hematopoiesis. Other workers have found elevated icterus indices in similar cases (Cabot, 8), (Schneider, 16), (Hunter and Hanflig, 17), (Martland, 18), (Anderson, Boyd and Jackson, 19). Schneider, (16), Oettinger (20), and Ronchetti (21) have reported an increased excretion of urobilinogen in the urine, and the last author expressed the opinion that the increase was due to a rapid destruction of erythrocytes. Hemosiderosis of the liver, spleen, kidneys, and bone marrow is a frequent pathological alteration both in human beings and in experimental animals dead of benzene poisoning. Mallory (8) stated that the spleen in Cabot's case 13321, contained deposits of pigment similar to those found in spleens of individuals dead of unequivocal hemolytic anemia. The suggestion, derived from clinical study, that a hemolytic process is abnormally active is supported by experimental evidence. Ponder (22) has shown that benzene in concentrations of 10 millimols per liter accelerates the hemolytic action of saponin and sodium taurocholate, agents known to destroy human erythrocytes. Selling and Osgood (1) have shown that benzene alone is not lytic for erythrocytes *in vitro* until concentrations 0.1 to 0.5% are reached.

METHODS

Sternal Bone Marrow Biopsy. Biopsies of sternal bone marrow were made and the tissue was prepared for histological examination as described by Rhoads and Castle (23). Smears to be stained supravitaly and by

Wright's method were made at the time of operation.

Examination of the Blood With the exception of the differential counts of supravitaly stained preparations, all of the examinations were made of oxalated blood. Four cc. of venous blood were withdrawn and placed in small rubber-stoppered glass bottles containing 0.02 cc. of a 20% solution of potassium oxalate as an anti-coagulant. Standardized pipettes and chambers were employed for cell counts. The hemoglobin was determined by the Sahli method. Enumeration of reticulocytes, determinations of mean corpuscular volume, icterus index, and fragility of red blood cells, were all made by the accepted methods in common use.

Liver Function Tests. (a) The bilirubin excretion test described by Harrop and Barron (24) was made by injecting intravenously 1 mg. of crystalline bilirubin per kilogram of body weight. A retention after 4 hours of more than 5% of the injected pigment was regarded as evidence of hepatic dysfunction.

(b) The sodium benzoate conversion test described by Quick (25) was made by administering orally 5.9 gm. of sodium benzoate. An excretion in the urine in 4 hours of less than 3 gm. of hippuric acid in the presence of normal renal function was regarded as evidence of hepatic dysfunction.

Renal Function Tests. The urea clearance test (Peters and Van Slyke, 26) was used. The expected clearance is between 70 and 100% of a normal standard.

Excretion of Urobilinogen. The content of urobilinogen in the feces and in the urine was determined quanti-

tatively by the Watson-Terwen (27) method, and the total amount excreted was considered to be an index of the rate of destruction of erythrocytes. Watson states that the normal excretion of urobilinogen in the feces may reach 250 mgm. a day in males, with 100% hemoglobin in the circulating blood. However, in 26 normal individuals with hemoglobin levels between 80 and 100%, studied in this laboratory, the amount of urobilinogen excreted in the stools varied from 75 to 150 mgm. daily, while the amounts excreted in the urine were less than 2.0 mgm. daily.

CASE REPORTS

Case 1. C. DuV., male, aged 38. Hospital No. 9717. Admitted January 4, 1936, complaining of weakness, dyspnea, occipital headaches and bleeding gums.

History: In 1918 the patient and his brother worked in a studio in which benzene was used. The brother developed anemia and epistaxis, and died the same year. The patient changed his occupation and was not exposed to benzene again until 17 years later, when in January, 1935 he began to use a rubber cement which contained benzene. He was exposed to the fumes constantly for 14 hours a day; anorexia appeared in February and by July his weakness was intolerable. A blood count revealed erythrocytes, 2,440,000, hemoglobin 44%, leucocytes 35,400. Differential count: polymorphonuclears 47%, lymphocytes 25%, atypical cells 28%. Fragility of erythrocytes, normal. Icterus index 8. A fasting specimen of gastric juice contained 29.5% of free HCl. The patient was treated with transfusions, x-ray therapy, iron, and liver extract before admission.

Physical Examination: A well nourished and well developed male showing a noticeable pallor with a slightly yellowish tint. The gums were hypertrophic and spongy with multiple bleeding areas. There was no enlargement of the liver or the peripheral lymph nodes. The tip of the spleen

could just be felt. There were no other pertinent findings.

Laboratory Findings on Admission: Erythrocytes 2,930,000, hemoglobin 57%, leucocytes 5,750, platelets 84,000, reticulocytes 5.2%. Differential count: polymorphonuclears 68%, eosinophils 2%, basophils 1%, myelocytes C 2%, lymphocytes 22%, monocytes 5%. Icterus index 15. Sodium benzoate test: 3.15 gm. benzoic acid excreted in 4 hours. Fasting gastric juice contained 37% of free HCl. Fragility, normal. Daily excretion of urobilinogen in feces 556 mg.; in urine 1.5 mg. A sternal bone marrow biopsy was done and the marrow found to be hyperplastic. Supravital differential count of the living marrow cells: * primitives 5%, neutrophilic myelocytes 27%, myeloblast 1%, polymorphonuclears (from stab nucleus to 5-lobed nucleus) 36%, normoblasts 24%, erythroblasts 6% megaloblasts 1%. Bleeding time 12 min.; coagulation time 30 min. Clot retraction, normal. The determination of the stool urobilinogen was repeated 1 month after admission and the output was found to be 1000 mg. daily, nearly 10 times the normal. Wassermann negative.

Course: The patient was given raw liver orally and ascorbic acid intravenously without effect. After 5 weeks he left and 2 months later died in another city. The picture of leucemia gradually appeared. The white blood cells increased in number to 137,000 just before death, and the majority were myelocytes. The spleen became very large as did all of the peripheral lymph nodes. A post mortem examination revealed findings typical of myeloid leucemia, a diffuse infiltration of the organs with immature myeloid cells.

Case 2. M. L., male, aged 23. Hospital No. 9875. Admitted July 10, 1936 complaining of weakness, sore gums, fever and pallor.

History: For the 18 months preceding admission the patient had been employed as a chemist using benzene as a solvent for benzoic acid. Two months before admission he noticed pallor, anorexia and swollen

* Sabin classifies the small lymphoid cells with a thin rim of cytoplasm as primitive.

gums, and the condition was diagnosed elsewhere as *Icucemia*.

Physical Examination: The patient was a well developed, well nourished young man with pallor of the skin and a slight icteric tinge to the sclera. The gums were red, edematous and swollen. The spleen could be felt on deep inspiration and the peripheral nodes were palpable. There were no other pertinent findings.

Laboratory Findings on Admission: Erythrocytes 2,380,000, hemoglobin 54%, leucocytes 3,500, platelets 120,000, reticulocytes 14%. Differential count: polymorphonuclears 7%, eosinophils 1%, small lymphocytes 51%, intermediate lymphocytes 31%, large lymphocytes 8%, monocytes 2%. Icterus index 15. Fragility, normal. Free hydrochloric acid present in fasting gastric juice. Daily excretion of urobilinogen in feces was 570 mg., in the urine 1.54 mg. A sternal bone marrow biopsy was done and the marrow was found to be hyperplastic. A supravital differential count of the living marrow cells: primitives 4%, neutrophilic myelocytes 35%, myeloblasts 6%, polymorphonuclears 18%, normoblasts 29%, erythroblasts 5%, megaloblasts 3%. Coagulation 15 min. Wassermann and heterophile antibody reactions were negative.

Course: The patient was given liver extract and ascorbic acid orally, and discharged after 2 weeks. With the exception of an ischio-rectal abscess 6 weeks after discharge, he improved steadily. In 3 months the erythrocyte count was 3,940,000, hemoglobin 72%, leucocytes 6,550. Differential count: polymorphonuclears 79%, eosinophils 2%, lymphocytes 18%, monocytes 1%. Two years later he was in perfect health.

Case 3. A. S., female, aged 35. Hospital No. 9174. Admitted June 23, 1931 complaining of dyspnea, weakness, purpura, ecchymoses and bleeding from gums, nose, genital tract and gastro-intestinal tract of 6 weeks duration. Five transfusions had been given.

History: Fourteen years previously the patient was supposed to have had a positive Wassermann test. Anti-luetic therapy had been given once yearly for 10 years in spite

of persistently negative serological reactions. The last course was completed 9 months before admission. The patient was a beauty parlor operator and 7 months before admission she began to use at least once a day as a dry shampoo a preparation containing 30% benzene. On 2 occasions she washed her own hair in this preparation and on the second she fainted. Three months after the initial exposure she complained of fatigue and anorexia. Six months later she became nauseated and dyspneic. Ecchymoses appeared on the extremities and trunk, and finally bleeding from the nose, gums and vagina.

Physical Examination: A well developed, slightly obese, adult female with a pale yellowish skin marked by purpuric spots. The gums were red and edematous without bleeding. No enlargement of the peripheral lymph nodes or spleen.

Laboratory Findings: Erythrocytes 2,080,000, hemoglobin 49%, leucocytes 2,700, platelets 22,000, reticulocytes 8.0%. Differential count: polymorphonuclears 49%, eosinophils 2%, lymphocytes 46%, monocytes 3%. Fragility, normal. Histamine induced 46° of free hydrochloric acid in the gastric juice. A sternal bone marrow biopsy was done and the marrow found to be hypoplastic. A supravital differential count of the living marrow cells: primitives 34%, neutrophilic myelocytes 15%, polymorphonuclears 44%, normoblasts 6%, erythroblasts 1%. Bleeding time 15 minutes. Coagulation time 41 minutes. Wassermann negative.

Course: The patient was given daily for 6 weeks by mouth 200 gm. of raw liver and 20 gm. of ventriculin. A small ulcer on the right tonsil appeared but healed. There was a gradual improvement up to the time of discharge, after which there was no recurrence in 4 years.

The next 6 patients to be discussed were rotogravure printers who were exposed to atmospheres containing benzene, the concentration of which varied from 24 to 1060 p.p.m. parts of air as reported by the Division of Industrial Hygiene, New York State Department of Labor. These cases were referred to us by this Division in the course of an investigation made by them of ben-

zene poisoning in the rotogravure printing industry.

Case 4. J. Z., male, aged 26. Hospital No. 10557. Admitted April 21, 1935, complaining of weakness, epistaxis, ecchymoses and salivation.

History: The patient had been employed in a rotogravure printing company for 3 years before his admission. For 2 months before admission he noted gradually increasing weakness, anorexia, salivation, somnolence and dizziness. One week before a severe epistaxis occurred and ecchymoses appeared.

Physical Examination (before therapy): Erythrocytes 2,620,000, hemoglobin 62%, leucocytes 5,650, reticulocytes 5.2%. Differential count: polymorphonuclears 88%, lymphocytes 8%, monocytes 4%. Fragility normal. Fasting gastric contents contained free hydrochloric acid. Urea clearance 110-120% of normal. A sternal bone marrow biopsy was done, and the marrow was found to be hyperplastic, although the megakaryocytes were reduced in number. Increased amounts of pigment were present in the reticulo-endothelial cells. Supravital differential count of the living marrow cells showed: primitives 10%, neutrophilic myelocytes 24%, polymorphonuclears 25%, normoblasts 24%, erythroblasts 12%, megaloblasts 5%. Wassermann negative. Serum bilirubin level was 1.0 mg.%. Fecal urobilinogen: 81, 90, 110 mg. daily, during 3 different periods each of 3 days. Urinary urobilinogen: 2.9 mg. and 2.4 mg. excreted daily during 3-day test periods. Bilirubin excretion: retention 10%. Sodium benzoate test: 3.9 gm. of benzoic acid excreted in 4 hours.

Course: Uneventful. In the hospital the patient received liver extract and thiamin intramuscularly for a period of 4 weeks, and in the out-patient department for 4 months. One year after admission the patient was perfectly well.

Laboratory Findings (5 months after admission and after therapy): Erythrocytes 4,470,000, hemoglobin 100%, leucocytes 9,300, platelets 152,000. Differential count: polymorphonuclears 54%, eosinophils 8%, lymphocytes 24%, monocytes 14%. Serum bilirubin 0.7 mg.%. Urobilinogen excretion:

feces, 86 mg. and 74 mg. daily in 2 periods; urine 2.7 mg. daily. Bilirubin excretion test: retention 10%. Sodium benzoate test: 3.5 gm. benzoic acid excreted.

Case 6. W. R., male, aged 47. Hospital No. 10481. Admitted May 19, 1938, complaining of weakness and ecchymoses.

History: The patient had worked for a rotogravure printing company for 3 years, and only during the month preceding admission had he noticed increasing weakness and fatigability. He had used solvents containing benzene and was constantly exposed to the fumes. Ten days before admission, subcutaneous ecchymoses appeared on the extremities.

Physical Examination: The patient was a well developed and well nourished male, showing no evidence of recent ecchymoses or purpura. The oral mucous membranes appeared normal and the liver extended 2 finger-breadths below the right costal margin. There were no other pertinent findings.

Laboratory Findings: Erythrocytes 2,700,000, hemoglobin 72%, leucocytes 2,450, platelets 50,000, reticulocytes 6.2%. Differential count: polymorphonuclears 70%, eosinophils 2%, lymphocytes 20%, monocytes 8%. Fragility, normal. Icterus index 5. Fasting gastric contents contained free hydrochloric acid. Urea clearance 135-120% of normal. Urobilinogen excretion: fecal, 410 mg. and 336 mg. excreted daily during 2 periods; urine 3.8 mg. and 1.1 mg. daily for 2 periods. A sternal bone marrow puncture was done and the marrow was found to be normal. Supravital differential count of the living marrow cells: neutrophilic myelocytes 22%, myeloblasts 2%, polymorphonuclears 21%, normoblasts 45%, erythroblasts 10%. Wassermann negative.

Course: Uneventful. The patient received liver extract and thiamin intramuscularly for 3 weeks in the hospital and 3 months in the out-patient department.

Laboratory Findings (4 months after admission): Erythrocytes 3,830,000, hemoglobin 95%, leucocytes 7,700, platelets 250,000, reticulocytes 4.8%. Differential count: polymorphonuclears 52%, eosinophils 4%, lymphocytes 38% and monocytes

6%. Serum bilirubin 0.85 mg.%. Urobilinogen excretion: fecal, 191 mg. and 192 mg. excreted daily during 2 periods; urine, 0.8 mg. and 0.6 mg. daily during 2 periods. The patient recovered fully and was followed over a period of 1 year with no recurrence of symptoms.

Case 6. J. L., male, 75. Hospital No. 10454. Admitted April 20, 1939, complaining of weakness, ecchymoses, and nausea.

History: The patient had been employed for 2 years as a rotogravure printer using a benzene solvent. For 6 months prior to admission he had noticed increasing weakness, dyspnea, nausea and ecchymoses of the extremities.

Physical Examination: The patient was an elderly, well developed and well nourished male with numerous ecchymoses and pale mucous membranes without ulcerations. The physical examination otherwise was negative.

Laboratory Findings: Erythrocytes 4,130,000, hemoglobin 80%, leucocytes 6,500, platelets 160,000, reticulocytes 3.8%. Differential count: polymorphonuclears 68%, eosinophils 16%, lymphocytes 8%, and monocytes 8%. Fragility, normal. Icterus index 5. Free hydrochloric acid present in the fasting gastric contents. Urea clearance: 70% and 72% of normal. Serum bilirubin 0.75%. Urobilinogen excretion: fecal, 83 mg. daily; urine, 0.92 mg. daily. The bilirubin excretion test: 7.2% retention. Sodium benzoate test: 3.6 gm. of benzoic acid in 4 hours. Wasserman negative.

Course: Uneventful. The patient received liver extract and thiamin for 4 weeks in the out-patient department, and was followed without recurrence for a year.

Case 7. M. S., male, aged 33. Hospital No. 10467. Admitted May 3, 1938, complaining of weakness and ecchymoses.

History: The patient had been a rotogravure printer for 18 years but only during the 2 years preceding admission was he exposed to solvents containing benzene. He noticed weakness and ecchymoses 1 month before admission.

Physical Examination: The patient was a well developed, well nourished, pale man, whose skin had a peculiar yellowish color

and was studded with purpuric spots. No other pertinent findings.

Laboratory Findings: Erythrocytes 2,070,000, hemoglobin 63%, leucocytes 1,750, platelets 42,000, reticulocytes 3.7%. Differential count: polymorphonuclears 72%, lymphocytes 16%, monocytes 12%. Icterus index 8. Fragility 0.50-0.34 (normal 0.44-0.32). Free hydrochloric acid as present in the gastric contents after histamine. Urea clearance: 155-1007, normal, Serum bilirubin 1.3 ms.%. Urobilinogen excretion: fecal, 225 mg. and 212 mg. daily during 2 periods; urine 6.4 mg., 5.4 mg. and 3.8 mg. daily during 3 periods. Bilirubin excretion test: 17.6% retention. Sodium benzoate test: 1.8 gm. of benzoic acid in 4 hours. A sternal bone marrow biopsy was done and the marrow found to be hypoplastic with a shift to the left of the white cell elements. Supravital differential count of the living marrow cells: primitives 10%, neutrophilic myelocytes 28%, myeloblasts 8%, polymorphonuclears 14%, normoblasts 29%, erythroblasts 6%, megakaryoblasts 5%. Wassermann negative.

Course: Uneventful. The patient received liver extract and thiamin intramuscularly for 2 weeks in the hospital and 4 months in the out-patient department; he was observed for over a year without recurrence.

Laboratory Findings (5 months after admission and after therapy): Erythrocytes 3,800,000, hemoglobin 101%, platelets 124,000, reticulocytes 1.2%. Differential count: polymorphonuclears 74%, lymphocytes 10%, monocytes 16%. Serum bilirubin 0.7 mg.%. Urobilinogen excretion: fecal, 72 mg. and 75 mg. excreted daily during 2 test periods; urine, 1.5 mg. and 1.8 mg. excreted daily during 2 periods. Bilirubin excretion test: 16.7% retention. Sodium benzoate test: 3.9 gm. of benzoic acid excreted in 4 hours.

Case 8. L. P., male, aged 29. Hospital No. 10500. Admitted June 14, 1938, complaining of anorexia, fatigue and muscular cramps.

History: The patient had been working in a rotogravure printing establishment for 4 years and for 1 year had applied with bare hands a benzene preparation. Anorexia

had been present for a year, fatigue and weakness for 6 months, and pallor for 1 month.

Physical Examination: The patient was a small, well developed male. The skin was pale but without purpura. No other pertinent findings.

Laboratory Findings: Erythrocytes 1,850,000, hemoglobin 47%, leucocytes 1,900, platelets 18,000, reticulocytes 4.5%. Differential count: polymorphonuclears 64%, eosinophils 4%, basophils 4%, lymphocytes 12%, monocytes 16%. Fragility, normal. Free hydrochloric acid present in the fasting gastric contents. A trace of occult blood in the stool. Urea clearance: 93 and 98% of normal. Serum bilirubin level 1.12 mg.%. Urobilinogen excretion: feces, 150 mg. and 112 mg. daily during 2 periods; urine, 10, 12, and 17.4 mg. excreted daily during 3 periods. Bilirubin excretion test: no retention. Sodium benzoate test: 3.77 gm. of benzoic acid excreted in 4 hours. A sternal bone marrow biopsy was done and the marrow found to be hypoplastic with a shift to the left of the white cell elements. A supravital differential count of the living marrow cells: primitives 10%, neutrophilic myelocytes 23%, polymorphonuclears 19%, normoblasts 39%, erythroblasts 7%, megaloblasts 2%. Wassermann negative.

Course: Uneventful. The patient was given liver extract and thiamin intramuscularly for 2 weeks in the hospital and for 3 months in the out-patient department. The patient was followed 1 year without recurrence.

Laboratory Findings (after 3 months of therapy): Erythrocytes 3,930,000, hemoglobin 96%, leucocytes 6,700, platelets 82,000, reticulocytes 1.8%. Differential count: polymorphonuclears 66%, eosinophils 4%, basophils 2%, lymphocytes 24%, monocytes 4%. Serum bilirubin level: 0.85 mg.%. Urobilinogen excretion: fecal 76 mg. daily; urine 4.1 mg. daily. Bilirubin excretion test 10.4% retention. Sodium benzoate test: 3.73 gm. of benzoic acid excreted daily during 2 periods.

Case 9. F. M., male, aged 20. Hospital No. 10503. Admitted June 16, 1938, complaining of fatigue, dyspnea, and irritability.

History: The patient had worked for 3 years as a rotogravure printer exposed constantly to solvents containing benzene. Pallor and dyspnea for 4 months.

Physical Examination: The patient was a well developed, well nourished pale male. No other pertinent findings.

Laboratory Findings: Erythrocytes 3,220,000, hemoglobin 81%, leucocytes 2,100, platelets 82,000, reticulocytes 4.1%. Differential count: polymorphonuclears 68%, eosinophils 2%, lymphocytes 16%, monocytes 14%. Icterus index 6. Fragility, normal. Urea clearance 160—130% of normal. Serum bilirubin 1.0 mg.%. Urobilinogen excretion: feces, 126 mg. and 115 mg. daily during 2 periods; urine 1.4, 1.6 and 2.9 mg. daily during 3 periods. Bilirubin excretion test: no retention. Sodium benzoate test 3.9 gm. excreted in 4 hours. A sternal bone marrow biopsy was done and the marrow was found to be moderately hyperplastic with a slight shift to the left. Supravital differential count of the living marrow cells was: primitives 6%, neutrophilic myelocytes 34%, polymorphonuclears 22%, normoblasts 19%, erythroblasts 17%, megaloblasts 2%. Wassermann negative.

Course: Uneventful. Liver extract and thiamin were given intramuscularly for 2 weeks in the hospital and for 4 months in the out-patient department. The patient was followed for a year without recurrence.

Laboratory Findings (after 4 months of therapy): Erythrocytes 5,010,000, hemoglobin 94%, leucocytes 5,850, platelets 152,000, reticulocytes 1.6%. Differential count: polymorphonuclears 62%, lymphocytes 32%, monocytes 6%. Serum bilirubin 0.45 mg.%. Urobilinogen excretion: feces, 84 mg. daily; urine, 0.9 mg. daily. Bilirubin excretion test: 11.8% retention. Sodium benzoate test: 3.9 gm. excreted in 4 hours.

RESULTS

The 9 patients herein reported were exposed to varying concentrations of benzene fumes. The 6 rotogravure printers worked in atmospheres con-

taining between 24 and 1060 p.p.m. *in all, of the patients. The color and often washed their hands in the chemical. Of the remaining 3 patients, one was a hairdresser who worked in a small cubicle, one was a chemist who worked in an adequately ventilated room, and one patient worked in a poorly ventilated cellar room.

No correlation between the severity of disease and the intensity of exposure can be made. Symptoms appeared after 6 months to 3 years of exposure, and had been present from 1 to 6 months before medical aid was sought. The most frequent complaints were weakness, fatigue, headache, anorexia, epistaxis, bleeding gums and ecchymoses.

The hematological findings varied (table 1); anemia (hemoglobin values from 47% to 81% and erythrocyte counts from 1,850,000 to 4,130,000), leucopenia (leucocyte counts between 1,750 and 6,500), thrombocytopenia (platelet counts between 18,000 and 160,000), and elevated reticulocyte levels (from 14% to 3.8%) were present in all of the cases. The results of the differential counts were markedly dissimilar; monocytosis was present in 3 cases, eosinophilia in one, and lymphocytosis in one. The fragility of the erythrocytes in hypotonic saline was within normal limits except in one case. Varying degrees of anisocytosis and poikilocytosis were present

indices were elevated (1.1 to 1.5) in 7 of the 9 patients, and the mean corpuscular volumes were increased (94 to 111) in all but two.

A study of the tissue removed by biopsy from the sternal bone marrow of 8 patients revealed histological changes which varied from a hypoplasia with a left shift of the cellular elements to a hyperplasia with normal maturation. The erythroid-myeloid ratio of the marrow varied from 1:3 to 1:1 except in 1 case. The myelocyte-polymorphonuclear ratio varied from 3:1 to 1:3.

The Wassermann reaction was negative in all the patients although 1 had received several courses of neoarsphenamine following a single positive test 14 years previously. The last course was finished 9 months prior to admission but only 2 months prior to exposure to benzene. Free hydrochloric acid was found in the gastric juice of all the patients though two were achlorhydric until histamine was injected. Roentgenological examinations of the gastro-intestinal tract of the 6 roto-gravure printers revealed no abnormalities. The results of the urea clearance tests were normal in these 6 individuals also. The icterus indices were determined in 5 patients, and were elevated in four. The serum bilirubin levels were 1 mg. % or above in 5 of the 6 patients tested, whereas a normal level was present in one who had very little anemia. Vaughn and Haslewood (28) have shown that the normal serum bilirubin is between 0.2 and 0.8 mg. %.

As treatment, the patients were, of course, removed from their exposure to benzene and supplementary therapy

*The National Safety Council considers a history of long exposure to benzene and the presence of a leucopenia of less than 5,000 white blood cells per cubic millimeter to be reasonable evidence for chronic benzene poisoning. The Council also advises that a person working 8 hrs. a day should not be permitted to work in an atmosphere containing more than 100 p.p.m. (WINSLOW, C.-E. A.: Summary of National Safety Council Study of benzol poisoning. THIS J., 9, 61 (1927).)

was given, the value of which is unknown. Of the 9 patients, 3 received orally 100-300 gm. of raw minced liver with 20-30 gm. of ventriculin daily for 2 to 4 weeks; 2 received in addition, daily intravenous injections of 1 gm. of ascorbic acid for 1 week, while the 6 printers received, intramuscularly, 5 cc. of liver extract, with 20 mg. of thiamin twice a week for from 2-5 months.

After 2 to 5 months of treatment, 8 of the 9 patients were clinically improved. The hemoglobin values rose to between 78% and 101%, the leucocyte counts to between 3,200 and 9,300, the platelet counts to between 82,000 and 256,000, and the reticulocyte counts fell to between 1.2% and 4.1%. The mean corpuscular volume increased in 3 patients, remained the same in 1, decreased in 3, and fell to within normal limits in 2. The color index increased in 2, remained the same in 2, and decreased in 5. Of the last group, in only 1 case did the color index fall below 1. The serum bilirubin levels fell to between 0.42 and 0.85 mg.%. The marrow eventually became leucemic in 1 case, whereas it returned to the normal state in the other 8, as indicated by the complete clinical recovery of the patients.

Studies of the rates of excretion of urobilinogen showed that on admission to the hospital 5 of the 8 patients studied had elevated levels in the feces (150 to 1000 mg. daily). The test was repeated in the 6 printers after their recovery. The quantity excreted was normal in 5 but still elevated in 1 (192 mg. daily).

On admission 4 of the 8 patients had increased rates of excretion of urobilinogen in the urine (2.7 to 17.4

mg. daily). After their recovery, 3 of the 4 excreted normal quantities, while the remaining one excreted less than he had previous to treatment. Although the quantitation of urobilinogen in the urine is a measure of one of the hepatic functions, two other tests (bilirubin excretion test and sodium benzoate conversion test) were made on the 6 printers before and after recovery. On admission 3 of the 6 printers, on whom the excretion test was made, had no retention of bilirubin, but after clinical recovery each had a retention (9.1, 10.4, and 11.6%). The other three on admission had abnormal retention (7.2, 17.6, and 10%), but after clinical recovery the first had no retention, the second had less retention (17.6-16.7%) and the third showed no change. No explanation for these findings is at hand. The results of the sodium benzoate conversion test were normal in 5 of the 6 patients both before and after recovery. The 1 case that had a decreased excretion of hippuric acid before recovery was able to excrete a normal amount after recovery. Of the 3 hepatic function tests, the first was most reliable in this series of cases since the amount of urobilinogen excreted in the urine was quite closely correlated with the clinical condition of the patient.

Eight of the 9 patients recovered, as judged by clinical and laboratory evidence, within less than 6 months after admission; whereas 1 died of acute myeloid leucemia within the same period of time. The 6 printers returned to work 6 months after admission, since the use of benzene was discontinued in the printing plants. None had shown any return of their

	CASE 1 (DuV)		CASE 2 (M. L.)		CASE 3 (A. S.)		CASE 4 (J. Z.)		CASE 5 (W. R.)	
	Before therapy (2/7/36)	After therapy (4/8/36)	Before therapy (7/10/36)	After therapy (12/14/36)	Before therapy (6/23/34)	After therapy (8/8/34)	Before therapy (3/20/38)	After therapy (10/12/38)	Before therapy (3/19/35)	After therapy (10/2/35)
1. Hospital number.....	9717		9875		9174		10457		10451	
2. Sex.....	male		male		female		male		male	
3. Age.....	38		23		35		26		47	
4. Chief complaints.....	a. Weakness b. Headache c. Bleeding gums		a. Weakness b. Fever c. Sore gum		a. Weakness b. Bleeding c. Hema- temesis d. Purpura		a. Weakness b. Dizziness c. Ecchymoses d. Epistaxis		Weakness Ecchymoses	
5. Duration of chief complaints.....	6 months		2 months		2½ months		2 month		1 month	
6. Duration of exposure to benzene.....	7 months		9 months		6 months		3 years		1 year	
7. Peripheral blood:										
Hemoglobin.....	57%	51%	84%	78%	49%	94%	62%	103%	77%	95
Erythrocytes.....	2,930,000	2,700,000	2,350,000	3,740,000	2,080,000	3,980,000	2,620,000	3,560,000	1,700,000	3,830
Leucocytes.....	5,750	137,000	6,400	6,550	2,103	6,540	5,850	9,300	2,450	25
Platelets.....	84,000		120,000		22,000	158,000	28,000	156,000	50,000	4
Reticulocytes.....	5.2%		14.0%	4.1%	8.0%	2.0%	5.2%		1.2%	
Differential:										
Polymorpho- nuclears.....	68%	22%	7%	79%	49%		88%		70%	62
Eosinophils.....	2%	3%	1%	2%	2%				1%	4
Basophils.....	1%	2%								
Myelocytes.....	2%	63%								
Lymphocytes.....	22%	8%	90%	18%	46%		8%		10%	38
Monocytes.....	5%	2%	2%	1%	3%		4%		1%	6
Hematocrit.....	950/275	60/190	950/210	970/390	940/105	950/380	950/235	950/235	950/235	930
Mean corpuscular volume.....	99	74	100	108	100	100	94	107	99	
Color index.....	0.90	0.90	1.17	1.05	1.22	1.20	1.1	1.4	1.33	1
Plasma bilirubin level (mgm. %)	1.00						1.00	0.7	1.2	
Icterus index.....	15		18							
Fragility of erythro- cytes.....	0.44-0.34		0.44-0.34		0.42-0.30		0.44-0.34		0.44-0.38	
Wassermann.....	Negative		Negative		Negative		Negative		Negative	
8. Bone marrow.....	Hyperplastic		Hyperplastic (left shift)		Hypoplastic		Hyperplastic		Normal	
Differential										
1. Primitive cells...	5%		4%		32%		10%			
2. Myelocytes and myeloblasts...	28%		41%		15%		24%		24%	
3. Segmented my- eloid cells.....	36%		18%		44%		25%		21%	
4. Erythroid cells*	31%		37%		7%		41%		45%	
9. Free hydrochloric acid in fasting gastric contents.....	Present		Present		Present (his- tamine)		Present		Present	
10. Urobilinogen (mgm. daily):										
a. Urine.....	1.54		1.40				2.9 and 2.4	2.1	1.5	
b. Stool.....	556 and 1,000		570				90.0 and 111.0	74.0	60 and 236	
11. Liver function tests:										
a. Bilirubin retained in plasma.....							10%	10%	8.9%	
b. Hippuric acid ex- creted in urine...	3.15 gm.						3.9 gm.	3.8 gm.	4.3 gm.	
12. Kidney function test:										
a. Urea clearance (% normal).....	80%						120	110	135	
13. Therapy.....	Removal from expo- sure Raw liver Gastric mucosal preparations Ascorbic acid Doath—myeloid leu- cemia		Removal from expo- sure Raw liver Ascorbic acid		Removal from expo- sure Raw liver Gastric mucosal preparation		Removal from expo- sure Intramuscular liver ex- tract Intramuscular thiamin		Removal from expo- sure Intramuscular extract Intramuscular	
14. Result.....			Recovery		Recovery		Recovery		Recovery	
15. Occupation.....	Used cement contain- ing benzene		Chemist		Beauty parlor opera- tor		Rotogravure printer			

* Including normoblasts, erythroblasts and megaloblasts.

CASE 5 (W. R.)		CASE 6 (J. L.)		CASE 7 (M. S.)		CASE 8 (L. P.)		CASE 9 (F. M.)	
Before therapy (3/3)	After therapy (10/2/33)	Before therapy (4/20/33)	After therapy (6/10/33)	Before therapy (5/3/33)	After therapy (10/12/33)	Before therapy (6/14/33)	After therapy (15/33)	Before therapy (6/16/33)	After therapy (11/33)
10454 male 76		10454 male 76		10467 male 33		10500 male 29		10503 male 20	
Weakness Ecchymoses Nausea		Weakness Ecchymoses Nausea		a. Weakness b. Ecchymoses		a. Weakness b. Anorexia c. Muscular cramps in arms and legs		a. Fatigue b. Dyspnea c. Irritability	
6 months		6 months		1 month		6 months		5 months	
2 years		2 years		3 years		3 years		3 years	
95%	80%	89%	63%	101%	47%	96%	81%	94%	
3,830,000	4,130,000	4,060,000	2,070,000	3,870,000	1,850,000	3,930,000	3,220,000	5,010,000	
2,450	7,710	6,500	1,750	3,200	1,900	6,700	2,100	5,850	
3,690	256.01	160,000	118,000	42,000	124,000	18,000	80,000	152,000	
2%	4.8%	3.8%	1.8%	3.7%	1.2%	4.5%	1.8%	4.1%	1.6%
52%	68%	67%	72%	74%	64%	66%	68%	62%	
4%	16%				4%	4%	2%		
38%	8%	19%	16%	10%	12%	24%	16%	2%	
6%	8%	14%	12%	16%	16%	4%	16%	5%	
935/28	970/330	970/360	940/210	970/390	940/180	960/355	970/280	10/385	
90	80	92	111	105	106	102	90	80	
1.23	0.9	0.42	1.5	1.3	1.30	1.20	1.26	0.94	
0.65	0.75		13	0.7	1.12	0.65	1.00	0.45	
	5						8		
0.42-0.32	0.42-0.32	0.50-0.34	0.44-0.32	0.44-0.32	0.44-0.32	0.44-0.32	0.44-0.34	0.44-0.34	
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	
		Hypoplastic	Hypoplastic	Hypoplastic	Hypoplastic	Hypoplastic	Hypoplastic	Hypoplastic	
		(left shift)	(left shift)	(left shift)	(left shift)	(left shift)	(left shift)	(left shift)	
		10%	10%	10%	10%	10%	10%	10%	
		35%	23%	34%	23%	34%	22%	38%	
		14%	19%	22%	19%	22%	38%	38%	
		41%	48%	38%	48%	38%	38%	38%	
Present	Present	Present (histamine)	Present	Present	Present	Present	Present	Present	
1.8	0.9	0.92	0.76	6.4 and 5.4	1.5 and 1.8	0, 12 and 17	4.1	14	0.9
and 335	191	83.00	63.00	225 and 212	72 and 75	150 and 118	76	126 and 110	64
0.0%	9.1	7.2%	0.0%	17.6%	16.7%	0.0%	10.4%	0.0%	11.6%
4.0 gm.	4.0 gm.	3.65 gm.	3.60 gm.	1.8 gm.	3.9 gm.	3.77 gm.	1.73 gm.	3.9 gm.	3.8 gm.
115	72	100	98%	130%					
Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	Removal from exposure	
Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	Intramuscular liver extract	
Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	Intramuscular thiamin	
Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	
Same	Same	Same	Same	Same	Same	Same	Same	Same	

former symptoms after 1 year of employment and no treatment was given during that interval.

DISCUSSION

With the increasing use of benzene as a solvent of gums, resins, fats, rubber and alkaloids, and as a fuel, increasing numbers of human intoxications are reported. Chronic exposure may cause varied hematological and clinical pictures as proved by the evidence here presented. Anemia, leucopenia, thrombocytopenia, reticulocytosis, high color index, increased fragility of erythrocytes, urobilinuria and prolonged coagulation time may or may not be present. The bone marrow may be aplastic, normal, hyperplastic, or leucemic, and the changes are reflected by the peripheral blood. The symptoms may vary as much as do the hematological findings, but headache, fatigue, dizziness, nausea, anorexia, weakness, nervousness and hemorrhagic manifestations, as bleeding gums, epistaxis, and menorrhagia predominate. The extreme variability of the manifestations is due possibly to the different concentration of benzene fumes to which the individual is exposed, the duration of exposure, and the individual susceptibility to the chemical, as suggested by Hamilton.

A dietary factor may be involved in individual susceptibility. Rhoads, Barker and Miller (29) have shown that dogs fed a deficient diet are more susceptible to the hemolytic effects of indol, a naturally occurring cyclic compound, than are dogs taking normal diets. This increased susceptibility was prevented by the administration of liver extract. The

experiments suggest that a dietary factor is concerned with the tolerance to certain cyclic chemicals.

The cause of the anemia and leucopenia of benzene poisoning is not well understood, although a subnormal rate of production is widely accepted (Castle and Minot, 30). The existence of a subnormal rate is supported by the histological findings in the vast majority of cases with late stages of poisoning. As an early effect, however, the marrow is hyperplastic, and this may explain the presence of myelocytes and irritated forms of leucocytes in the peripheral blood. As the marrow becomes aplastic the first reflection is a decrease in the number of circulating leucocytes, owing presumably to the brief life span of those cells. Thrombocytopenia appears next, and varies in intensity with the susceptibility of the megakaryocytes to the absorbed benzene. Anemia is the last manifestation since erythrocytes are the cells of longest life (Dekker, 31).

Although the improper formation of blood cells is a prominent feature in benzene poisoning, and possibly the result of an inhibition of some unknown maturation process in the marrow, there is clinical and laboratory evidence that the chemical may accelerate the normal mechanism for the destruction of erythrocytes. In the cases herein reported there was much evidence for the presence of increased rates of hemolysis. Elevated levels of serum bilirubin, poikilocytosis, elevated reticulocyte counts and increased outputs of urobilinogen were all found. Watson (27), Eppiriger and Charnas (32), Wilbur and Addis (34), Sparkman (35)

and others have indicated that the estimations of the urobilinogen of the stool constitute the best single measure of the rate of destruction of red blood cells. All evidence of increased hemolysis disappeared in 8 of the 9 patients when they were removed from exposure to benzene. The anemia, leucopenia, and thrombocytopenia vanished and the serum bilirubin levels returned to normal in all the patients, as did the reticulocyte counts and the fecal excretion of urobilinogen. Clinical improvement was therefore quite likely attended by the cessation of a destructive process as well as a restoration of normal hematopoiesis. Moreover, laboratory evidence exists that benzene accelerates the action of agents lytic to erythrocytes *in vitro* (Ponder, 22) and that it causes a destruction of circulating erythrocytes in experimental animals (Climenko and Abels, 36).

Watson showed that the output of urobilinogen in the urine is one of the most sensitive measures of liver function; any increase indicates an inability of the liver cells to excrete normally the pigment into the gastrointestinal tract. In this series of patients the rate of excretion of urobilinogen in the urine could be correlated with the clinical condition of the patient. This was not true of 2 other tests, which measure different liver functions (the sodium benzoate conversion test and the bilirubin excretion test).

The degree of macrocytosis in benzene intoxication varies greatly (Browning and Hamilton), and in certain instances may simulate that of pernicious anemia (Stodtmeister, 37). Dimmel (38) has shown that the

elevated color indices in benzene poisoning, like those of pernicious anemia, fall to normal values on recovery. This was true of the cases herein reported.

The secretion of hydrochloric acid in the gastric juice varies in patients suffering from benzene intoxication. Weil (39) reported that 3 of 9 cases of benzene poisoning that he studied had achlorhydria. In the 9 cases described in this communication free hydrochloric acid was present under fasting conditions in seven, and histamine was required in two.

Unfortunately, studies of the excretion of conjugated metabolic products of benzene were not possible in this series of patients. Of the absorbed benzene, over nine-tenths is excreted by the lungs (Feil, cited by Browning) and a portion of the remainder is excreted by the kidneys either as ethereal sulfates (Yant and coworkers, 40) (Jephcott and Bulmer, 41), glucuronides (Jost, cited by Browning), or as mercapturic acids (Stekol, 42).

It is of interest to note that one of the patients developed leucemia, a possible sequel to benzene poisoning which has been mentioned in the literature. Selling and Osgood (1) cite 6 cases that developed evidence of leucemia following exposure to benzene. (Lymphoid, 1 case—Falconer; myeloid, 5 cases—Weil, Hunter and Hanflig, Cabot, Delore and Bergomano, Schneider.) Hamilton (5) reported a case of leucemia, observed by Martland, following benzene exposure. Perrin, Kissel, and Pierquin (43) have described recently a case of "acute benzene leucosis."

The patient (Case 1) herein re-

ported, developed leucemia while being studied to ascertain the cause of an unexplained anemia following 6 months' exposure to benzene. The symptoms, the physical findings, and the hematological findings of leucemia developed insidiously. During the last 4 months of his life the patient became weaker, the peripheral lymph nodes and spleen gradually enlarged, and the number of circulating leucocytes increased as did the percentage of immature cells. The patient died of leucocytic, myeloid leukemia,

and the post mortem findings were typical of that disease.

SUMMARY

1. Nine patients with benzene poisoning are reported and the varied hematological findings in these cases are discussed. Eight of the patients recovered and one died of myeloid leukemia.

2. Evidence is presented that an increased rate of destruction of erythrocytes may be one cause of the anemia of benzene poisoning.

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