Effect of Alcohol on Haemopoiesis

A. H. WATERS,† M.B., B.S., PH.D., M.C.PATH., M.C.P.A.; A. A. MORLEY,* M.D., M.R.A.C.P.
J. G. RANKIN,* M.B., B.S., M.R.A.C.P.

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Disturbance of haemopoiesis is often found in patients who drink large quantities of alcohol (ethanol). Anaemia is common in alcoholic cirrhosis, and many factors may be involved, such as gastrointestinal blood loss, deficiency of iron and folic acid, increased haemolysis, and depressed erythropoiesis (Jarrold and Vilter, 1949; Movitt, 1950; Jandl, 1955; Jandl and Lear, 1956; Krasnow *et al.*, 1957; Zieve, 1958; Herbert *et al.*, 1963; Kimber *et al.*, 1965; Klipstein and Lindenbaum, 1965; Leevy *et al.*, 1965). That alcohol may have a direct toxic effect on the bone marrow is suggested by the presence of vacuoles in the primitive erythroid and myeloid cells in alcoholic subjects (McCurdy *et al.*, 1962). Furthermore, Sullivan and Herbert (1964) have shown that the administration of alcohol may depress the haematological response to physiological doses of folic acid in patients with folate-deficient megaloblastic anaemia.

The present study was undertaken to determine the effect of alcohol on haemopoiesis in a group of 16 alcoholic patients who were not suffering from cirrhosis. Ferrokinetic studies were used in in attempt to quantitate this effect based on a similar study by Rubin *et al.* (1960) to detect early drug-induced depression of erythropoiesis. The incidence of vitamin B_{12}, folate, and iron deficiency in these patients was also studied.

Material and Methods

Sixteen male patients aged 25 to 64 were studied. They drank heavily, of the order of 20–40 glasses (6–8 litres) of beer a day (3½% alcohol content), often with wine or spirits in addition. At the time of admission to hospital many were intoxicated or in early delirium tremens. Patients were excluded from the study if they had stopped drinking for more than 36 hours before admission, if they suffered from a complicating factor (e.g., blood loss, infection), or if they were found to have clinical, biochemical, or histological evidence of cirrhosis. An accurate dietary history was usually difficult to obtain, but the diet was regarded as poor in all except one patient.

On the day of admission blood was taken for routine haematological studies (Dacie and Lewis, 1963) and for measurement of the serum levels of iron (Wootton, 1964), vitamin B_{12} (Hutner *et al.*, 1956), and folate (Waters and Mollin, 1961). Blood examinations (including reticulocyte and platelet counts) were performed three times weekly thereafter.

A bone-marrow aspiration was carried out on all patients on admission. The sternum opposite the second intercostal space was the site of aspiration in 14 patients and the posterior iliac crest in two. In 10 patients bone-marrow aspiration was repeated after one week at the same site as the first aspiration.

All subjects had biochemical liver-function tests and seven had percutaneous liver biopsies.

Ferrokinetic studies with ^{59}Fe were carried out as described by Dacie and Lewis (1963). Six patients were studied both on the day of admission and one week later. Measurements were made of the plasma iron half-life, plasma iron turnover rate, and the accumulation of radioactivity over the heart, spleen, liver, and sacrum by surface scintillation counting. A sacrum-to-liver ratio was calculated from the surface counts at these sites two hours after the injection of ^{59}Fe. The percentage of iron incorporated into red cells was not measured because ^{59}Fe injected during the first study would contribute to results obtained during the second, and direct comparison of the two studies would not be possible. The six patients in this study were kept on a diet calculated to contain approximately 8 mg. of iron and less than 5 μg. of folic acid (Herbert, 1963), whereas the remaining patients received the normal hospital diet. The only drugs administered were chlorpromazine or chlordiazepoxide, which were necessary in many patients to relieve agitation. In view of the possible suppressive effect of chlorpromazine on the growth of *Euglena gracilis* (Herbert *et al.*, 1965), these drugs were not given until blood had been taken for serum vitamin-B_{12} assay.

* University of Melbourne Department of Medicine, St. Vincent's Hospital, Melbourne.
† Haematologist, St. Vincent's Hospital, Melbourne. Present address: Department of Haematology, St. Bartholomew's Hospital, London.
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Results

Haematological Data

The blood counts of the 16 patients were within normal limits on admission: haemoglobin 14.3–18.9 g./100 ml., P.C.V. 40–54%, M.C.H.C. 32–35%, white cell count 5,000–11,000/cu. mm., platelet count 110,000–288,000/cu. mm., and reticulocyte count 0.3–2.0%. The blood counts did not change significantly during the week after admission. There was no evidence of macrocytosis or neutrophil hypersegmentation in the peripheral blood. Occasional nucleated red cells were seen in the peripheral blood of two patients on admission, but there was no associated reticulocytosis which might suggest blood loss or haemolysis.

Bone Marrow

It was not uncommon to experience difficulty with bone-marrow aspiration, and then to obtain only a few fragments. Cellularity of the marrow was at the lower limit of normal. The myeloid:erythroid ratio ranged from 1.5:1 to 5:1. Occasional intermediate megaloblasts were present in the bone marrow of one patient who had a subnormal serum folate level of 3.2 mμg./ml. and a normal serum vitamin-B_{12} level of 840 μμg./ml. Defective haemoglobinization of the normoblasts was not seen in any patients.

In 14 of the 16 patients vacuoles were present in the cytoplasm and occasionally in the nucleus of primitive erythroid and myeloid cells (Fig. 1). The vacuoles did not stain with periodic-acid Schiff or Sudan red. In view of the presence of similar vacuoles in phenylalanine deficiency (Cockburn *et al.*, 1965), serum phenylalanine levels were measured in five patients with vacuoles in the marrow. With the method described by McCaman and Robins (1962), the levels ranged from 1.7 to 2.8 mg./100 ml., which is within or above the range for normal subjects found by these workers. Marrow aspiration was repeated one week after admission in 10 of the 16 patients.

Vacuoles had disappeared in nine of the ten patients and had become less frequent in one. However, the cellularity and myeloid : erythroid ratio did not show any significant change.

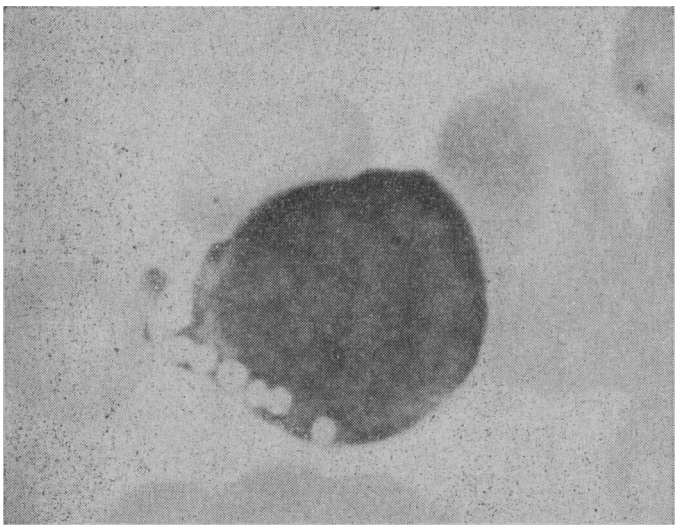

FIG. 1.—Bone-marrow smear during acute alcohol intoxication showing cytoplasmic vacuolation of an early normoblast (May-Grünwald-Giesma stain).

Serum Vitamin-B_{12} and Folate Levels

Serum vitamin-B_{12} levels measured by the *E. gracilis* assay were normal in all patients, ranging from 230 to 840 $\mu\mu$g./ml., with a mean of 490 $\mu\mu$g. Serum folate (*Lactobacillus casei*) levels were subnormal (<6 mμg./ml.) in seven of the 16 patients, and in two of these the level was less than 4 mμg./ml. (i.e., in the range found in folate-deficient megaloblastic anaemia). One of the latter two patients had early megaloblastic changes in the bone marrow. Three patients had abnormally high levels of 18, 24, and 28 mμg./ml. Bacterial contamination was excluded as a cause of false high levels in these cases (Waters and Mollin, 1963).

Serum and Bone-marrow Iron

On admission the serum iron level was low in five patients (i.e., <70 μg./100 ml.), normal in ten (i.e., 70–160 μg./100 ml.), and in one patient it was abnormally high, being 267 μg./100 ml. The serum iron estimation was repeated in 12 patients one week after admission. The level had decreased in eight and increased in four, but the change in the mean (a decrease of 41 μg./100 ml.) was not statistically significant ($0.1>P>0.05$).

Stainable iron in the bone marrow was absent in two patients, reduced in five, and normal in nine. Increased siderotic granulation was present in one of the latter patients, who also showed the most marked vacuolation of the early myeloid and erythroid cells. However, abnormal "ring" sideroblasts were not found in any of the patients in the present study. In the ten patients in whom bone-marrow aspiration was repeated there was no significant change in the iron stores in the second specimen.

Ferrokinetic Studies

The results in six patients who were studied on admission and again one week later are summarized in the Table.

The initial plasma iron clearance (^{59}Fe $T\frac{1}{2}$) was within normal limits (70–140 minutes) in four patients and decreased in two, one of whom was iron-deficient. One week later it had decreased in all patients to below the lower limit of normal, the change being highly significant ($P<0.0025$). A representative clearance pattern is shown in Fig. 2.

Ferrokinetic Studies on Six Alcoholic Patients

Patient	Serum Iron (μg./100 ml.)		Plasma Clearance (^{59}Fe $T\frac{1}{2}$ min.)		Plasma Iron Turnover (mg./kg./day)		Sacrum: Liver Ratio	
	1*	2	1	2	1	2	1	2
1	104	49	68	28	0·71	0·94	0·56	1·06
2	139	107	106	58	0·58	0·90	0·95	1·24
3	102	85	100	45	0·72	1·01	0·71	1·47
4	104	95	129	56	0·36	0·77	1·00	1·15
5	267	126	92	45	1·13	0·79	0·91	2·86
6	48	36	31	27	0·69	0·79	1·36	1·77

* 1 = On admission. 2 = One week after withdrawal of alcohol.

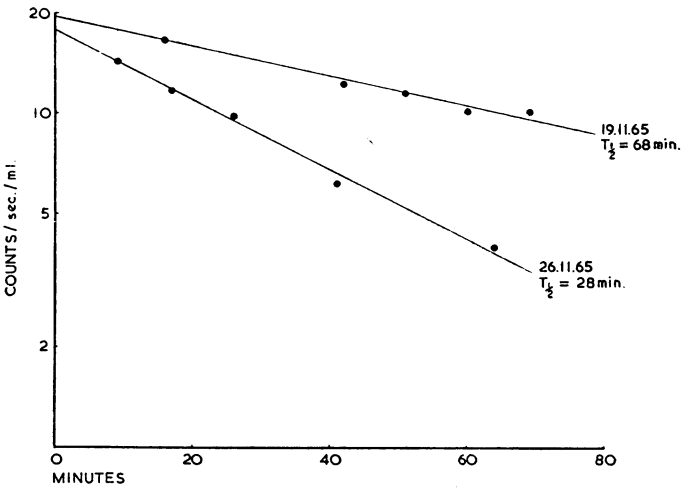

FIG. 2.—Plasma ^{59}Fe clearance of Case 1 (Table) on admission to hospital (19 November) and one week after withdrawal of alcohol (26 November). The ^{59}Fe $T\frac{1}{2}$ was 68 minutes on admission and 28 minutes one week later.

The plasma iron turnover rate was initially within normal limits or slightly increased in five patients, and in all of them it had risen to well above the normal range after one week in hospital. In one patient (Case 5), who had an initial plasma iron of 267 μg./100 ml., the plasma iron turnover rate on admission was markedly raised, being 1.13 mg./kg./day, and after one week it was still raised, though lower than previously. However, the mean change in the plasma iron turnover rate, an increase of 0.17 mg./kg./day, was not statistically significant ($0.1>P>0.05$).

A representative surface pattern obtained in one patient (Case 1) by counting over the heart, spleen, liver, and sacrum

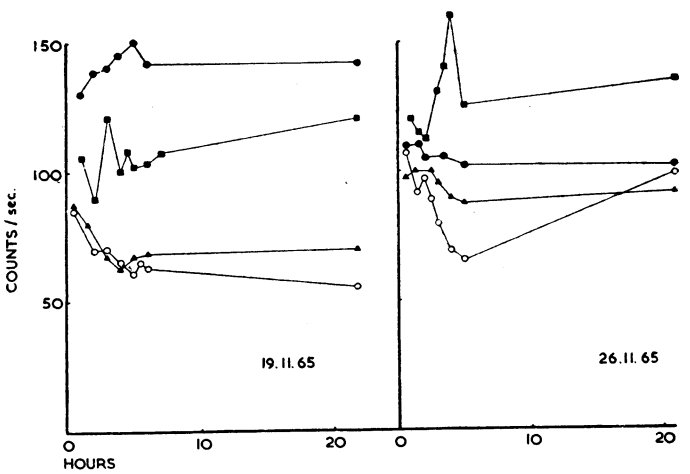

FIG. 3.—^{59}Fe surface counting pattern of Case 1. Left, on admission (19 November). Right, one week after withdrawal of alcohol (26 November). Heart counts O——O, spleen ▲——▲, liver ●——●, and sacrum ■——■.

is shown in Fig. 3. The initial surface pattern showed reduced or approximately equal uptake by the sacrum relative to the liver, indicating that uptake by the marrow was depressed. One week later uptake by the sacrum had increased, with a significant rise in the sacrum-to-liver ratio ($P<0.01$) (see Table).

Discussion

The present study of a group of 16 alcoholic patients without cirrhosis showed that disturbance of haemopoiesis was common even in the absence of anaemia.

Nutritional folate deficiency is common in patients with alcoholic cirrhosis, and in some there may also be increased utilization of the vitamin due to marrow hyperactivity after blood loss or haemolysis (Herbert *et al.*, 1963 ; Knowles *et al.*, 1963 ; Kimber *et al.*, 1965 ; Klipstein and Lindenbaum, 1965). All of the 16 patients in the present study had normal serum vitamin-B_{12} levels, but seven had subnormal serum folate levels (i.e., <6 mμg./ml.). Two of these latter patients had serum folate levels less than 4 mμg./ml. (i.e., in the range found in folate-deficient megaloblastic anaemia), and in fact one showed megaloblastic changes in the bone marrow. Since none of the patients studied had evidence of haemolysis or loss of blood on admission, and since all but one seemed to take a poor diet, the folate deficiency in these patients was attributed mainly to inadequate intake. Abnormally high serum folate levels of 18, 24, and 28 mμg./ml. were found in three patients. These high levels may have been due to the release of folate from liver cells damaged by alcohol, for in two of these patients there was direct evidence of liver-cell necrosis, as shown by raised serum glutamic-oxaloacetic-transaminase levels of 160 and 150 units. None of these patients had radiological evidence of intestinal blind loops or diverticula which may be associated with abnormally high serum folate levels due to bacterial contamination of the small intestine (Hoffbrand *et al.*, 1966). In this respect the ingestion of large quantities of alcohol may predispose to bacterial contamination of the small intestine in some patients.

Low serum iron levels were found in five patients and decreased amounts of bone-marrow iron in seven, but none of the patients was anaemic or showed evidence of hypochromasia of the red cells. The cause of the mild iron deficiency was uncertain, but several patients gave a past history of haematemesis, and all but one seemed to take an inadequate diet. This high incidence of iron deficiency in the present study is of interest in view of the suggested aetiological association between chronic alcoholism and haemochromatosis (MacDonald and Mallory, 1960). However, the patients studied by MacDonald and Mallory probably differed from those in the present study in that they drank large quantities of wine, which has a high iron content (McCance and Widdowson, 1960 ; MacDonald, 1963), and, furthermore, they were found to have a high incidence of cirrhosis and chronic pancreatitis, both of which may cause increased absorption of iron (Conrad *et al.*, 1962 ; Davis and Badenoch, 1962 ; Callender and Malpas, 1963).

Vacuoles were seen in the bone marrow of 14 of our patients at the time of admission to hospital. They were present in the primitive cells of both erythroid and myeloid series, usually in the cytoplasm, but occasionally in the nucleus also. They did not stain for glycogen or lipid. This phenomenon was first described by McCurdy *et al.* (1962), and similar vacuolation has been found in association with chloramphenicol administration (Rosenbach *et al.*, 1960 ; Saidi *et al.*, 1961), riboflavine deficiency (Lane and Alfrey, 1965), and phenylalanine deficiency (Cockburn *et al.*, 1965). There was no evidence of this latter deficiency in five patients in this series in whom it was sought. In most patients the vacuoles were not present one week after withdrawal of alcohol, and it seems likely that this reversible vacuolation was due to a direct toxic effect of alcohol on the marrow rather than to deficiency of some unrecognized factor.

As mentioned previously, the studies of Sullivan and Herbert (1964) suggest that alcohol may have a direct depressing effect on the marrow, perhaps by interference with folate metabolism, and a report by Bertino *et al.* (1965) suggests that this may be due, at least in part, to reversible inhibition of the enzyme tetrahydrofolate formylase. In the present investigation ferrokinetic studies with ^{59}Fe were used to assess the effect of alcohol on erythropoiesis. Six patients were studied on admission and again after one week in hospital, each being thus used as his own control. All six showed a decrease in the plasma iron clearance (^{59}Fe $T\frac{1}{2}$), five showed an increase in the plasma iron turnover rate, and in all patients the initially low sacrum/liver ratio had returned towards normal.

These results suggest that a reversible depression of erythropoiesis had been corrected by the withdrawal of alcohol. However, certain objections might be raised to this explanation. Since three of the six patients were folate-deficient and one was iron-deficient, it could be argued that our findings might be due to response to dietary folate or iron. This possibility seems unlikely, for the folate and iron contents of the diet were purposely kept low and the serum levels of these substances did not change significantly during the week of study. Furthermore, the plasma-iron turnover is faster than normal in megaloblastic anaemia and iron-deficiency anaemia (Bothwell *et al.*, 1957 ; Sheehy *et al.*, 1960), and studies in patients with pernicious anaemia show that this decreases or remains unchanged after treatment with vitamin B_{12} (Finch *et al.*, 1956). It is also possible that there may have been a release of iron into the plasma from necrotic liver cells in acute alcohol intoxication, as has been reported in acute hepatocellular necrosis associated with virus hepatitis or toxic liver damage (Peterson, 1952 ; Reissmann and Dietrich, 1956). If this had occurred in our patients one might expect to find an initially high plasma iron turnover, as has been reported in virus hepatitis (Peterson, 1953), and a low hepatic uptake of ^{59}Fe, both returning to normal after withdrawal of alcohol. Since the reverse was found, this possibility also seems unlikely.

The most probable interpretation of the present results is that a real improvement in marrow function occurred after one week in hospital. This could have been due to removal of some toxic factor in beer or correction of some unrecognized deficiency. However, the most likely reason for the improvement in marrow function would seem to be the withdrawal of alcohol itself.

Summary

The present study of a group of 16 alcoholic patients without cirrhosis showed that disturbance of haemopoiesis was common even in the absence of anaemia. Radioactive ferrokinetic studies in six subjects showed depressed iron utilization by the bone marrow ; this improved after withdrawal of alcohol. Bone-marrow aspiration showed cytoplasmic or nuclear vacuolation of early myeloid and erythroid precursors in 14 of the 16 patients, and when repeated in 10 patients one week later it showed that the vacuolation had disappeared in nine and was much less obvious in one. These observations suggest a direct toxic effect of alcohol on the bone marrow.

There was also a high incidence of both folic acid (approximately 50%) and iron (approximately 30%) deficiency in the present study. None of the patients was vitamin-B_{12}-deficient.

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Investigation of Folic Acid Requirements in Pregnancy

M. L. N. WILLOUGHBY,* M.A., M.D., M.C.PATH. ; F. J. JEWELL,* F.I.M.L.T.

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The minimum daily oral requirements of pteroylglutamic acid in the non-pregnant adult are probably of the order of 50 μg./day (Herbert, 1962), and there is little doubt that requirements are increased in pregnancy (Chanarin *et al.*, 1959 ; Ball and Giles, 1964 ; Hibbard, 1964), but the magnitude of increase is not known (*Brit. med. J.*, 1964).

There is evidence that oral supplementation of 500 μg. daily is adequate (Lowenstein *et al.*, 1964), and of 20 to 30 μg. is inadequate (Chanarin *et al.*, 1965) to meet the folic acid requirements of pregnancy in urban communities. Since 1964 we have been investigating the effects of oral supplements at levels of 100, 300, and 450 μg./day—namely, intermediate between the the level employed by Lowenstein and that by Chanarin—in an attempt to define the minimum level more closely.

As part of this investigation the fasting serum folate levels were measured between the second and fourth day postpartum in a consecutive series of 350 patients from five different randomly allocated supplementation groups as described below. These results are shown in the Chart and Table II, where they are compared with similar results from cases of megaloblastic anaemia of pregnancy and also with a group of patients not allocated to a supplementation group.

Clinical Material

All patients attending the antenatal clinic were randomly allocated, according to their hospital number, to one of five trial groups at their first visit, which was usually at about three months' gestation. Patients with initial haemoglobin levels below 10 g./100 ml. were excluded from the trial. Thereafter all patients had haemoglobin estimations performed at every visit to the clinic. If the haemoglobin level fell below 10 g. they were removed from the trial and treated appropriately according to the haematological findings. This surveillance continued after delivery until the postnatal visit.

Group 1 patients received no haematinics. The remaining groups were given three bottles labelled "breakfast," "lunch," and "tea" containing chelated iron aminoates with or without various amounts of folic acid, so that the daily dosage of three tablets supplied the amounts shown in Table I. All patients were also given a multivitamin preparation (Vitavel) free of folic acid.

TABLE I.—*Details of Antenatal Oral Supplements*

Group:	1	2	3	4	5
Iron (mg./day)	0	105	105	105	105
Folic acid (μg./day)	0	0	100	300	450
" " " actual* ..	0	0	124	355	530

* These figures are derived from microbiological assays on batches of tablets at different periods of storage arranged by Riker Laboratories.

Every endeavour was made to encourage the patients to take the tablets regularly by the nursing staff, dietitians, and obstetricians, and by enlisting the help of the patients' general practitioners. Where it was clearly established in the case notes that a patient had not been taking the tablets their results were excluded from analysis.

Between the second and fourth days postpartum a venous sample for serum folate estimation was collected at approximately 11 a.m., more than two hours after the last meal.

Methods

Screening haemoglobin estimations were performed from finger-prick blood samples with an oxyhaemoglobin method and an EEL haemoglobinometer. In patients with a haemoglobin level in the region of 10 g./100 ml. this was confirmed on a venous sample of blood by means of a cyanomethaemo-

* Haematology Department, Queen Mother's Hospital, Glasgow.