normal population (mean 9·1, S.D. 4·8) (Eysenck and Eysenck, 1964). It is possible that underlying depression may have contributed to their initial seeking of treatment for their smoking. The fact that the depression was serious only in the two (Cases 8 and 9) who gave a history of a previous depressive episode suggests that an underlying depression or depressive tendency may be exacerbated by the treatment.

Conclusion

Electric aversion is a powerful suppressor of cigarette smoking. In most subjects it causes a rapid reduction in the number of cigarettes smoked and induces a negative attitude to smoking. More experience is needed to ensure its best use as a measure to achieve permanent abstinence from smoking. Though a few cases are less responsive, there is reason to believe that it may be effective in a fair proportion of those dependent smokers who have proved immune to other anti-smoking measures. Its use is limited to a small group of persistent smokers with strong internal motivation to break their habit.

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Suppression of Erythropoiesis by Alcohol

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Summary: Serial measurements in alcoholic subjects showed a profound fall of serum iron for three days after withdrawal of alcohol and a reversion of abnormal accumulation of erythroblastic haemosiderin to normal. These findings suggest an interference in normal haem synthesis, most probably by a direct effect.

Introduction

Anaemia and the prolonged ingestion of alcohol (ethanol) are frequently associated. The aetiology of this anaemia has been variously ascribed to gastrointestinal blood loss, dietary deficiency of iron and folic acid, increased haemolysis, and depressed haemopoiesis. The subject was reviewed by Kimber *et al.* (1965).

A direct effect of alcohol on haemopoiesis was suggested by Jandl (1955) and was supported by the finding of vacuoles in the primitive erythroid and myeloid cells in alcoholic patients (McCurdy *et al.*, 1962). Waters *et al.* (1966) supported the mechanism of a direct effect, while Sullivan and Herbert (1964) produced evidence of folate inhibition in alcoholic subjects. While this paper was in preparation additional support for an effect of alcohol on normoblast haemosiderin in folate-depleted subjects (Hines, 1969) and for a direct effect on serum iron levels in well-nourished non-anaemic volunteers (Lindenbaum and Lieber, 1969) has been presented.

The purpose of the present study was to investigate the changes in criteria of iron metabolism following cessation of prolonged alcohol intake in subjects who were well nourished and not anaemic, and had no clinical evidence of cirrhosis.

Methods and Materials

The subjects studied were alcoholic patients admitted to a private psychiatric hospital. Patients in this institution pay for their management and treatment and tend to be well nourished and non-anaemic.

Within two hours of their admission the subjects were selected on the basis of known prolonged alcohol intake (the admitted daily consumption of alcohol varied from one to three bottles of spirits and/or 10 to 30 pints (5·7 to 17 litres) of beer), adequate nutrition, and absence of anaemia. A total of 36 patients were investigated. Thirteen, however, declined to have any further investigations thereafter. The remaining 23 (22 men and 1 woman aged 28 to 72 years) had blood taken by venepuncture for a routine blood count (Dacie and Lewis, 1963), serum iron (Peters *et al.*, 1956), and serum folate (Temperley and Horner, 1966) both on admission (day 0) and on days 1, 2, 3, 7, 14, and, in a few instances, 21 days after admission. Except following admission venepuncture was per-

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formed before 11 a.m. In all 23 subjects a bone marrow puncture was performed within two hours of admission and was repeated two to seven days later in 11 patients. The remainder either refused a repeat puncture or had a normal bone marrow at the initial examination. Bone marrow smears were prepared routinely with Romanowsky stains and with potassium ferrocyanide for estimation of iron content (Dacie and Lewis, 1963). The degree of iron staining in the erythroblasts was assessed and classified as suggested by Dacie and Mollin (1966). A normal sideroblast contains two or three very fine blue granules, scattered randomly in the cytoplasm, and in normal marrow less than half of the erythroblasts contain detectable granules.

The patients were treated initially with sedative drugs, and they usually accepted a normal ward diet three to four days after admission. The dietary history showed that most had made an effort to have one adequate daily meal that included animal protein, usually in the evening.

Results

Of the 23 patients on whom serial studies were performed, the admission serum folate levels ranged from 1·0 to 9·2 ng./ml. (mean of 3·2 ng.). This compares with the normal range for our laboratory, obtained from 40 subjects, of 2·1 to 9·5 ng./ml. (mean 5·1 ng.). The serum vitamin-B_{12} levels ranged from 225 to 850 pg./ml. (mean 400 pg.), the normal range being 125 to 1,025 pg./ml. (mean 472 pg.) (Temperley and Collery, 1965). The serum iron levels varied markedly from 70 to 309 μg./100 ml. (mean 172 μg.), the normal range being 60 to 180 μg. The results of the serial serum iron estimations are shown in Fig. 1 and demonstrate a precipitous and sustained fall for 72 hours after admission, slowly rising back towards the normal range. The serum iron level fell after admission in 18 out of the 23 patients. The serial folate values are shown in Fig. 2, and again demonstrate a fall in the mean level for 48 hours after admission. The serum vitamin-B_{12} levels did not vary significantly during this period. The haemoglobin level on admission varied from 12·3 to 15·5 g./100 ml., with a mean of 14·0 g. (S.D. ± 1·14 g.).

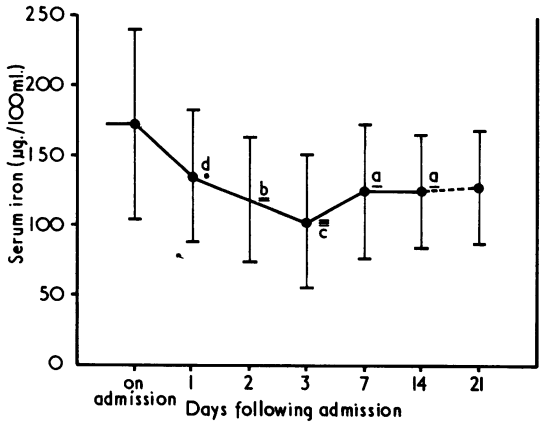

FIG. 1.—Mean serum iron values in 23 alcoholic patients plotted against time following admission to hospital. The significance of the fall in the serum iron when compared with the admission value is indicated as follows: $P < 0{\cdot}02\%$ = a; $P < 0{\cdot}01\%$ = b; $P < 0{\cdot}001\%$ = c; $P > 0{\cdot}05$ = d (mean values ± S.D.).

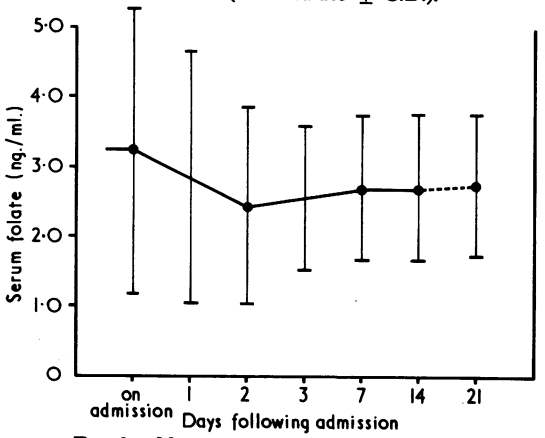

FIG. 2.—Mean serum folate values in 23 alcoholic patients plotted against time following admission to hospital. The fall of the serum folate when compared with the admission value failed to reach a significant level, $0{\cdot}1 > P > 0{\cdot}05$ (mean values ± S.D.).

Five non-alcoholic psychiatric patients who were admitted at the same time and who were given a similar sedative and dietary regimen had serial studies performed as controls. The results show no comparable fall of serum iron or serum folate levels (Fig. 3).

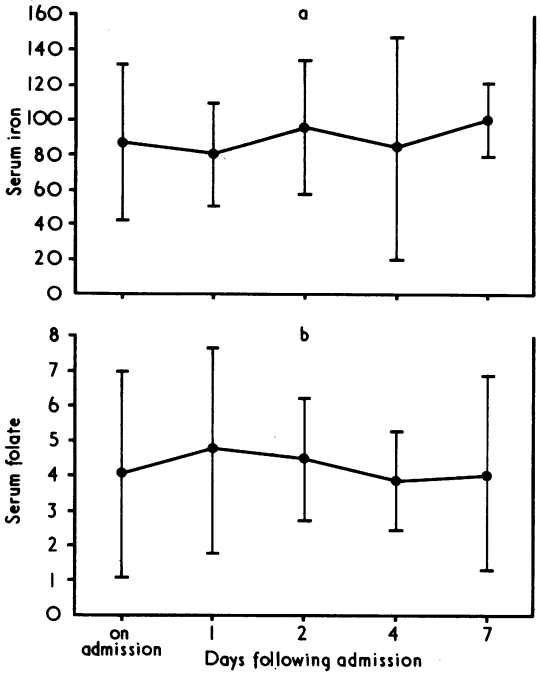

FIG. 3.—Serial serum iron values (above) and serial serum folate values (below) in five depressed subjects treated by sedation similar to that used for alcoholics (mean values ± S.D.).

Sternal marrow aspiration was performed within two hours of admission in 23 subjects, and a second aspirate was taken two to seven days later in 11 of these. Marrow fragments were readily obtained, were normocellular, and showed no megaloblasts or giant metamyelocytes. The myeloid/erythroid ratio was normal in all.

Cytoplasmic vacuoles were present in some haemocytoblasts in eight subjects, and were occasionally also found in pro-

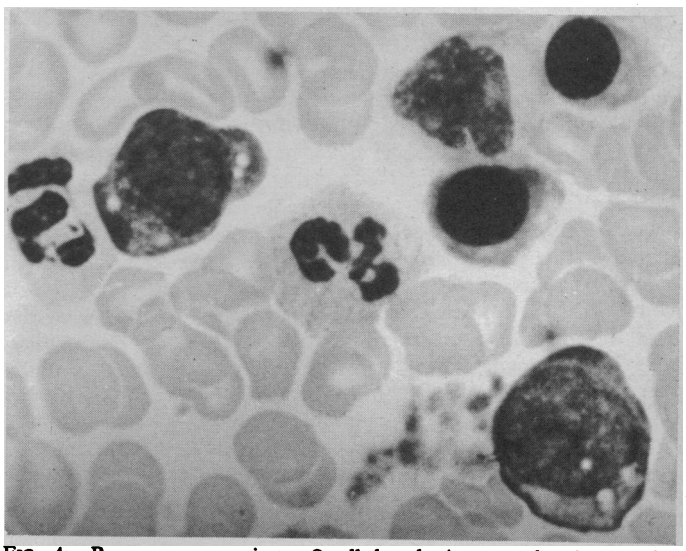

FIG. 4.—Bone-marrow aspirate. Small largely intracytoplasmic vacuoles in two primitive haemopoietic cells. (May-Grünwald-Geimsa × 1,350.)

myelocytes and early erythroblasts (Fig. 4). Megakaryocytes were detected in normal numbers.

Haemosiderin granules were examined after staining with acid ferrocyanide. The quantity of haemosiderin within marrow fragments was thought to be excessive in only two subjects, but 17 of the 23 showed abnormalities within the developing normoblasts. These 17 marrows included the eight with vacuolated cells. Over half of the normoblasts contained distinct blue granules, many having six to eight and some containing up to 13 (Fig. 5). These granules did not have the distinct perinuclear distribution of some refractory anaemias (Fig. 5), but there was a tendency to aggregate along the

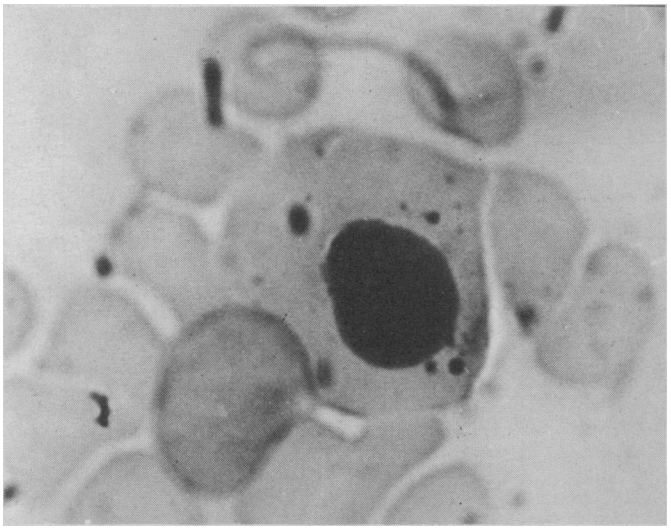

FIG. 5.—Normoblast with numerous haemosiderin granules, some of them abnormally large. (Acid ferrocyanide and Safronin × 2,250.)

membrane in some sideroblasts, even when their number or size was not excessive. A fine perinuclear blue "dust" was present in a few cells, and occasional definite "ring" sideroblasts were seen in four subjects. Abnormally large granules were present in many cells (Fig. 5).

Repeat examination two to seven days later showed a reversion to normal haemosiderin distribution within the erythroblasts in all except two patients—in these two excess granules remained and a very occasional ringed sideroblast was seen, but the appearances had improved since the initial examination.

Discussion

In this report most alcoholic patients have been shown to have an abnormality of iron metabolism which rapidly reverts to normal after admission to hospital.

The profound fall in serum iron levels after admission resembles that seen in pernicious anaemia following therapy with vitamin-B_{12} (Hawkins, 1955) and, together with the reversal of cellular distribution of haemosiderin, suggests the sudden onset of effective erythropoiesis. Similar serum changes follow cessation of chloramphenicol treatment (Rubin *et al.*, 1958), a drug which produces vacuoles in primitive marrow cells (Saidi *et al.*, 1961), resembling those found in alcoholics (McCurdy *et al.*, 1962). The low serum iron levels which may be found in alcoholics must be scrutinized in the light of these findings, as the rapid decrease after admission may falsely suggest iron depletion unless allowance is made for the changes which we describe here (Hilal and McCurdy, 1967).

Sullivan and Herbert (1964) showed that alcohol can suppress the normal erythropoietic response in anaemic folate-deficient alcoholics with florid megaloblastic change. The mechanism of suppression is not clear, but could be overcome by large (therapeutic) doses of folic acid, so that an inhibition of folate was suggested. Our subjects differ from the three described by these authors in so far as anaemia was not a feature, serum folate levels were normal, and megaloblastic erythropoiesis was not present.

Our patients were healthy "bout" alcoholics with no signs of malnutrition or neglect, and resembled the patients studied by Lindenbaum and Lieber (1969) rather than those of Hines (1969). The latter showed that ringed sideroblasts were common in folate-depleted anaemic alcoholics, and that they spontaneously disappeared on a ward diet as the anaemia also reverted after a reticulocytosis. He wa sable to induce ringed sideroblasts and megaloblasts in one subject by administration of ethanol, and the addition of pyridoxal phosphate to the regimen led to the disappearance of sideroblasts while megaloblasts remained. The results suggested that alcohol could lead to the formation of ringed sideroblasts in the presence of folate depletion (*Lancet*, 1969).

Our subjects were similar to those of Lindenbaum and Lieber (1969) in that they were well nourished and not anaemic. These authors were able to produce cytoplasmic vacuolation of marrow cells together with a rise of serum iron when well-nourished volunteer alcoholics were placed on an adequate vitamin-supplemented diet with alcohol substituted for some carbohydrate. No sideroblasts were found in the marrow during this experiment, though the serum iron levels rose slightly during ethanol administration and fell significantly on its withdrawal. In a ferrokinetic study of a similar group of patients Waters *et al.* (1966) were able to show an inhibition of marrow iron uptake which reverted to normal within seven days of admission to hospital and which was thought to be due to a direct suppression of erythropoiesis by alcohol. The distribution of marrow haemosiderin in our subjects is not that of ringed sideroblasts, but possibly represents a less severe change, as Hines (1969) described similar normoblasts during reversion from ringed sideroblasts to normal. These non-ringed sideroblasts, together with the highly significant alterations in serum iron levels and the synchronous change in serum folate levels, all support the hypothesis of a direct effect of alcohol on erythropoiesis, and may be added to the growing evidence for its existence (Waters *et al.*, 1966; Lindenbaum and Lieber, 1969). Whether the haematosuppressive effect of alcohol on folate-depleted subjects is mediated through the same or separate metabolic pathways remains obscure.

Our findings suggest an inhibition of haemoglobin synthesis which cannot have been long-standing, as the subjects were not anaemic, but which nevertheless affected the majority of subjects at the time of admission. Occasional ragged or vacuolated normoblasts supported the suggestion of dyshaemopoiesis, as did the accumulation of iron granules within normoblasts. Serial folate levels fell synchronously with serum iron after admission, so that a response to folic acid in the ward diet seems most unlikely, more especially since these patients did not accept a normal diet until after the profound drop in iron and folate had begun.

Though abnormalities of pyridoxine may result in sideroblastic marrow changes we have no data on this from our study. Enzymes concerned with pyridoxine metabolism such as delta-aminolaevulinic acid (Horrigan and Harris, 1964) or pyridoxal phosphate (Cooper *et al.*, 1963) might be affected, though we feel that a deficiency of pyridoxine is unlikely in these patients. A suppression of these or similar enzymes, however, might well account for dyshaemopoiesis, and though the classical "ringed" sideroblasts of pyridoxine deficiency were only scantily present in a few patients, the possibility of a less severe or less chronic deficiency or inhibition of pyridoxine must remain. The observation that in many cells the haemosiderin granules tended to aggregate on the nuclear membrane might be taken to support this possibility.

Our serum folate levels do not preclude a more subtle effect on folate activity, such as inhibition of the enzyme tetrahydrofolate formylase (Bertino *et al.*, 1965), and this must also be considered as a possible pathogenesis. If, however, the finding of Gitlin (1969) is confirmed, then a specific toxic effect of alcohol may have been uncovered. Gitlin described transient porphyrinuria in an alcoholic, which had disappeared seven days after cessation of alcohol. This porphyrin might be of hepatic or erythroblastic origin—if the latter, then an abnormality of haem synthesis differing from that found in folate or vitamin-B_{12} deficiency is likely, probably affecting an earlier stage in the build-up of haemoglobin.

We feel that a direct toxic effect of alcohol an haem synthesis is the most likely explanation of our findings. Evidence for a direct effect of alcohol on the liver has been brought forward in recent years (Lieber and Rubin, 1968), and a transient suppression of platelets (Lindenbaum and Hargrove, 1968; Post and Desforges, 1968) and leucocytes (McFarland and Libre, 1963) is probably also a direct effect.

Experimental ethanol feeding of dogs results in leucopenia (Beard *et al.*, 1963) and there is evidence from in-vitro experiments that ethanol suppresses some hepatic enzymes (Rubin and Lieber, 1968). Our findings may well indicate a similar action on erythropoietic cells, but acute experiments have produced no alterations in the levels of serum iron. A single three-hour bout of alcohol intake in 10 volunteer students had no effect on serum iron readings despite alcohol levels of up to 240 mg./100 ml. Our patients usually had a single three-hour bout of alcohol intake in 10 volunteer students had no effect on serum iron readings despite alcohol on erythropoiesis is first manifested.

The incidence of haemosiderosis in chronic alcoholics has been reported to be 50% of patients with alcoholic cirrhosis. The cause has been variously recorded as due to excess iron in alcoholic liquors (MacDonald, 1963) or to increased iron absorption as a consequence of pancreatic damage (Davis and Badenoch, 1962). It may be that a further factor leading to excess accumulation of iron in the liver and other tissues in chronic alcoholic patients is defective utilization of iron by the bone marrow during prolonged alcohol consumption.

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Preliminary Communications

Experimental and Clinical Studies on Rifampicin in Treatment of Leprosy

British Medical Journal, 1970, **1**, 89-92

Summary: Rifampicin showed high activity against experimental leprosy, inhibiting the multiplication of dapsone-sensitive and dapsone-resistant strains of Mycobacterium leprae in mice fed 5 mg./kg. body weight. In a formal pilot-type trial on six previously untreated patients with active lepromatous leprosy, rifampicin (600 mg. daily by mouth) was as effective as standard treatment with dapsone. Myco. leprae, however, appeared to be killed more rapidly by rifampicin than by dapsone or other antileprosy drugs so far studied. This was confirmed on a further 10 patients, including two with dapsone resistance, and from the infectivity in mice of bacilli recovered from patients during treatment with rifampicin or dapsone. These results are consistent with the bactericidal activity of rifampicin against other micro-organisms, which could be important to the chemotherapy of leprosy, since all antileprosy drugs in current use are bacteriostatic.

Introduction

Until recently all drugs for the treatment of leprosy were chosen empirically or on the basis of their efficacy against tuberculosis and then had to be submitted to clinical trials in man. No preliminary screening of potential antileprosy drugs in the laboratory was possible, because the causative organism, *Mycobacterium leprae*, could not be grown in vitro or in vivo. Since 1960, however, as a result of the successful transmission of leprosy to experimental animals (Shepard, 1960; Rees, 1964), it has been shown that antileprosy drugs effective in