cases where no swab was taken, for sepsis thought on clinical grounds to be due to staphylococci.

In all, 24 babies (7.7%) suffered staphylococcal sepsis. In 12 of these the diagnosis was made by isolation of the staphylococcus; in 12 the diagnosis was presumptive. Sepsis was distributed almost equally between the three groups of babies—7 cases among the 110 babies treated with triple dye only, 11 cases among the 97 babies treated with triple dye in conjunction with air disinfection, and 6 cases in the 105 babies treated with triple dye and hexachlorophane.

Sepsis in Relation to Nasal Carriage of Staph. aureus.—It has been shown that adult patients in this hospital are more likely to develop staphylococcal sepsis if they are nasal carriers of *Staph. aureus* than if they are not (Williams *et al.*, 1959). It was therefore of interest to see if the same trend was present in the babies. Of the 312 babies, 191 carried staphylococci by the 12th day. In this group there were 20 babies (10.5%) with staphylococcal sepsis during their stay in hospital. The remaining 121 babies were not found to be carriers by the 12th day of their life in hospital. Of these, only 4 (3.3%) developed staphylococcal sepsis. In 16 babies who were carriers and developed sepsis, staphylococci were isolated from the nose before the onset of sepsis. In a further two babies the isolation was made from the nose and septic lesion at the same time.

Discussion

In our previous work in these nurseries we found two measures that appreciably affected the nasal colonization rate of newborn babies. These were the application of triple dye to the umbilical stump, and the use of individual gowns for each baby. Combination of these two methods was no more successful than either method alone. This should be borne in mind when assessing the present results. The failure of air disinfection by ultra-violet light to alter the nasal rate despite an undoubted effect on the number of bacteria in the air clearly shows that a reduction in the number of bacteria in the environment is not necessarily followed by a reduced rate of staphylococcal spread. If, however, the ultra-violet light had been used uncombined with triple dye, a result due to it alone might have been seen. The failure may have been observed because the aerial transfer of staphylococci was not important in the circumstances, though other explanations are possible.

Hexachlorophane is used in many North American nurseries. Baldwin *et al.* (1957), for instance, found that it reduced the rate of infants' nasal colonization below that reported in other investigations. Our results support these findings in that, though the difference was not statistically significant, the nasal colonization rate was lowest with hexachlorophane bathing in conjunction with triple dye to the umbilical stump.

But there is still a long way to go. Even with our best regimen more than half the babies became nasal carriers before leaving hospital. That this phenomenon was of clinical and not only academic importance was strongly suggested by the analysis of sepsis. Staphylococcal sepsis occurred three times as often in babies who became carriers of *Staph. aureus* as it did in those who remained negative, and in most cases carriage preceded sepsis. This ratio is very close to that reported for adults. If it means, as it apparently does, that carriage of staphylococci conduces to sepsis, it provides

a further incentive and justification for attempts to prevent the colonization of newborn babies by *Staph. aureus*.

Summary

An attempt has been made in a hospital nursery to prevent the colonization of newborn babies' noses by *Staph. aureus*. When triple dye was applied to the umbilical stump 35% of babies were still not carriers by the 12th day of life. Air disinfection of the nursery with ultra-violet light combined with triple dye produced almost identical results. With triple dye and hexachlorophane 46% of babies reached the 12th day free from nasal staphylococci.

Staphylococcal sepsis occurred three times as often in babies found to be carriers as in those who were not.

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REFERENCES

- Baldwin, J. N., Rheins, M. S., Sylvester, R. F., and Shaffer, T. E. (1957). *Amer. J. Dis. Child.*, **94**, 107.
Cook, Josephine, Parrish, J. A., and Shooter, R. A. (1958). *Brit. med. J.*, **1**, 74.
Williams, R. E. O., Jevons, M. Patricia, Shooter, R. A., Hunter, C. J. W., Girling, J. A., Griffiths, J. D., and Taylor, G. W. (1959). *Ibid.*, **2**, 658.

MEGALOBlastic ANAEMIA DUE TO PHENOBARBITONE THE CONVULSANT ACTION OF THERAPEUTIC DOSES OF FOLIC ACID

BY

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Megaloblastic anaemia may develop in patients treated with the anticonvulsant drugs phenytoin sodium and primidone (Badenoch, 1954; Hawkins and Meynell, 1954; Fuld and Moorhouse, 1956) and in patients receiving large doses of barbiturates (Hobson, Selwyn, and Mollin, 1956; Calvert, Hurworth, and MacBean, 1958). Though Hawkins and Meynell (1958) observed mild macrocytosis in about a third of the patients receiving phenobarbitone, megaloblastic anaemia due to this very widely used barbiturate has not been recorded.

The purpose of this paper is to describe a patient who presented with a megaloblastic anaemia on two occasions while taking large doses of phenobarbitone, and to report observations on the pathogenesis of the anaemia and the unusual response to treatment with folic acid.

Case Report

The patient, a known epileptic aged 43, was first seen in 1955 with rheumatoid arthritis. He was uncooperative, refused to attend for treatment, and developed permanent contractures of both knees and the left arm.

Since the age of 16 he had had grand-mal attacks, which were well controlled by 1.5 gr. (96 mg.) of phenobarbitone daily, and he had been free of attacks from 1954 up to the time of his admission to hospital in 1958. However, an electroencephalogram (E.E.G.) in 1955 (Fig. 1A) showed

FIG. 1.—Electroencephalograms. A, Normal background activity followed by paroxysm typical of epilepsy. B, Severe generalized abnormality while taking large doses of phenobarbitone. C, Severe generalized abnormality leading to paroxysmal activity after treatment with large doses of folic acid. D, Background activity shows only mild generalized abnormality after withdrawal of both phenobarbitone and folic acid.

one short paroxysm consisting of regular, high-amplitude, bilaterally synchronous polyspikes and slow waves. The background activity was normal. The record was diagnostic of epilepsy.

After leaving hospital in 1955 he began to control his own dose of phenobarbitone, taking from 3 to 6 gr. (195 to 390 mg.) daily, most of the dose being taken at night.

For the previous year he had been bedridden owing to the contractures and was cared for by his mother. His diet during that year was said to have consisted of bread-and-butter, tea with milk, occasionally supplemented by a little meat or fish.

During the six weeks before admission he complained of anorexia, lassitude, and intermittent vomiting, and became increasingly pale. He was admitted to Hammersmith Hospital under the care of Dr. M. D. Milne in March, 1958.

On examination he was sallow, with gross pallor of the mucous membranes. No purpura or haemorrhages were present, and the gums were normal. There was bilateral ankle oedema. He was confused, uncooperative, and of low intelligence. Blood-pressure was 180/80, and a fourth heart sound was audible at the cardiac apex. Both the liver and spleen were palpable 2 cm. below the costal margin. There were marked muscle-wasting and flexion contracture of the left arm, and the lower limbs were fixed in extension at the knees. Ankle-jerks could not be elicited, plantar responses were flexor, and there was a variable sensory loss in the lower limbs.

Investigations

A blood count showed red cells, 1,200,000/c.mm.; Hb, 5.5 g./100 ml.; P.C.V., 17%; reticulocytes, 0.1%; M.C.V., 141 cubic microns; M.C.H.C., 32%; leucocytes, 4,200/c.mm. (neutrophils 74%, lymphocytes 16%, monocytes 6%, eosinophils 4%); platelets, 125,000/c.mm. The stained film showed macrocytosis, poikilocytosis, anisocytosis, hypersegmentation of the neutrophils, and occasional myelocytes. Aspiration of sternal marrow revealed severe

megaloblastic haemopoiesis. The serum vitamin-B₁₂ level measured by microbiological assay with *Euglena gracilis* (Ross, 1952) was within the normal range, being 230 μg./ml.

A fractional test meal showed the presence of free acid in the gastric juice; the absorption of radioactive vitamin B₁₂ measured by the hepatic uptake method of Glass (1954) was within normal range, an estimated 0.4 μg. being absorbed from a 1-μg. dose of ⁵⁸Co-vitamin B₁₂. Fat absorption using ¹³¹I-labelled triolein was normal. The absorption of folic acid (Chanarin, Anderson, and Mollin, 1958) was normal, the peak serum concentration being 56 μmg./ml. after an oral dose of 2.2 mg. of the vitamin. An ascorbic-acid-saturation test revealed a marked deficiency of the vitamin, the patient requiring 16 daily oral doses of 700 mg. to achieve saturation.

Eleven serial E.E.G.s were recorded in the period before the start of treatment with therapeutic doses of folic acid (Days 1-18, Fig. 2). None of the records showed the epileptic activity that had been seen three years before. However, the background activity now showed a moderate to severe non-specific generalized abnormality (Fig. 1B). There was marked polyrhythmia, a considerable excess of moderate-high-amplitude theta (4 to 7 cycles per second) and delta (under 4 cycles per second) waves, and poor reduction of this rhythmic activity when the eyes were opened.

Response to Treatment with Folic Acid

The haematological and clinical response to treatment is summarized in Fig. 2. An intravenous injection of 0.7 mg. of folic acid was given on the second and ninth days after admission in order to study its rate of clearance from the plasma (Chanarin, Mollin, and Anderson, 1958). Though this was followed by a small rise in the percentage of reticulocytes the marrow remained megaloblastic. The ascorbic acid was given during the same period.

Intramuscular injection of folic acid (30 mg. daily) was started on the nineteenth day. The haematological response was excellent. There was a reticulocyte response with a peak of 22% on the fifth day after the start of folic-acid therapy. The red-cell count rose rapidly and reached 4,000,000/c.mm. 30 days after the start of treatment. Aspiration of sternal marrow repeated 12 days after the start of folic-acid treatment showed that haemopoiesis was entirely normoblastic.

The clinical response was much less satisfactory. Two days after starting treatment with folic acid he began to have frequent minor epileptic attacks. These attacks consisted of jaw movements, upward deviation of the eyes, and plucking of the bedclothes. He became very vague and failed to respond to simple remarks. Each attack lasted for half an hour, and the attacks became increasingly more frequent during the next three days. He became incontinent of urine and faeces and extremely difficult to manage. The attacks were not influenced by increasing the dose of phenobarbitone to 390 mg. daily.

Nine E.E.G.s were recorded during the 10 days following the start of treatment with intramuscular folic acid. Each record now showed the changes diagnostic of epilepsy—namely, paroxysms of high amplitude, bilaterally symmetrical and synchronous polyspikes (Fig. 1C). There was no change in the moderate-to-severe background abnormality previously noted.

Subsequent Progress.—As the precipitation of epileptic attacks appeared to coincide with the administration of folic acid, treatment with this vitamin was stopped after seven days. Six days later his mental state showed considerable improvement, and the minor epileptic attacks again became infrequent and finally ceased. Four E.E.G.s recorded from the sixth to the eleventh day after stopping folic acid showed the disappearance of the paroxysmal polyspike/slow-wave activity, though the severe generalized abnormality was still present. There was also great

improvement in both his mental and his physical state, and he was discharged on 260 mg. of phenobarbitone daily 11 weeks after admission. His blood count at this time was: red cells, 4,900,000/c.mm.; Hb, 15.6 g./100 ml.; P.C.V., 48.5%. There were no abnormalities in the stained film.

Second Admission

The patient remained well during the next 16 weeks. However, a blood count at the end of this period showed the reappearance of macrocytes and also hypersegmentation of the neutrophils, and, as this finding suggested the return of megaloblastic haemopoiesis, he was readmitted for further study.

A blood count at this time showed: red cells, 4,300,000/c.mm.; Hb, 14.3 g./100 ml.; P.C.V., 46%; M.C.V., 107 cubic microns. Aspiration of sternal marrow showed the presence of intermediate megaloblasts and giant metamyelocytes. The serum vitamin B₁₂ concentration was within the normal range, being 190 μ g./ml. He was therefore again suffering from a megaloblastic anaemia which required treatment with folic acid. Though our previous experience with folic acid in this patient had suggested that the drug might have precipitated attacks of epilepsy there was no definite evidence for a causal relationship. He was therefore given an intravenous injection of 0.7 mg. of folic acid to study its rate of clearance from the plasma, and this was followed the same day by an intramuscular injection of 15 mg. Twenty hours later he was again having repeated epileptic attacks, he had become stuporous, and he was incontinent of urine and faeces. An E.E.G. record taken before treatment with folic acid showed the generalized abnormality previously described but no evidence of epileptic activity. Twenty-four hours after readministration of folic acid the E.E.G. again showed the changes characteristic of epilepsy.

Folic acid was therefore withdrawn, the patient having received 60 mg. over a period of 48 hours. Phenobarbitone was also stopped and acetazolamide, 250 mg. twice daily, was substituted. On this regime his fits were completely controlled, and his E.E.G. showed not only no paroxysmal activity but a very marked improvement in the generalized background abnormality (Fig. 1D). A marrow aspiration repeated before discharge showed a return of normoblastic erythropoiesis.

His blood count five weeks after admission was: red cells, 5,200,000/c.mm.; Hb, 15.6 g./100 ml.; P.C.V., 49%. There were no abnormalities in the stained film.

Folic-acid Studies

As already mentioned, the rate of clearance of folic acid from the plasma after an intravenous injection of 15 μ g./kg. body-weight (0.7 mg.) was studied before treatment on each admission. The results are shown in Fig. 3.

On his first admission, when the red-cell count was 1,200,000/c.mm., two tests were carried out. In both the rate of clearance was far more rapid than in normal subjects. The serum folic-acid levels three minutes after the injection in these two tests were 23 and 33 μ g./ml. respectively. In both tests the injected dose of folic acid was completely removed from the plasma by the time the 15-minute blood sample was taken.

On his second admission, when the red-cell count was 4,300,000/c.mm., the rate of clearance of injected folic acid was normal, the serum folic-acid levels 3, 15, and 30 minutes after the injection being 91, 25, and 14 μ g./ml. respectively.

FIG. 3.—Results of folic-acid-clearance test during two admissions with megaloblastic anaemia.

FIG. 2.—Response to treatment with folic acid. Folic-acid-clearance tests were carried out at times indicated by 1, 2, and 3.

Discussion

This patient presented with a megaloblastic anaemia which responded completely to treatment with folic acid. There was no evidence of vitamin-B₁₂ deficiency and intestinal function appeared normal. Though he retained excessive amounts of ascorbic acid in a saturation test this failed to influence erythropoiesis. It therefore seemed likely that the megaloblastic anaemia was the result of the large doses of phenobarbitone he had been taking.

The administration of therapeutic doses of folic acid (30 mg. daily), however, though it produced an optimal haematological response, precipitated frequent epileptic attacks, and this occurred while he was still taking large doses of phenobarbitone. This effect is all the more remarkable because treatment of the megaloblastic anaemia which develops in patients receiving anti-convulsants is said to reduce the number of attacks (Hawkins and Meynell, 1958).

Pathogenesis of the Anaemia

There is evidence that megaloblastic anaemia in patients taking primidone may arise in the absence of both deficiency of vitamin B₁₂ and folic acid (Chanarin, Elmes, and Mollin, 1958). Such an anaemia must presumably be due to an interference with folic-acid function. Phenobarbitone appears to act in a similar manner, for on the patient's second admission both the serum vitamin-B₁₂ concentration and the rate of plasma clearance of injected folic acid were normal (Fig. 3). However, when the anaemia was severe (first admission), deficiency of folic acid was present, for the clearance of the injected vitamin from the plasma to the tissues was very rapid (Fig. 3). This suggests that the ineffective folic-acid metabolism induced by the presence of the barbiturate will, if continued long enough, result in folic-acid depletion of the tissues. The development of folic-acid deficiency after prolonged administration of an antagonist has been demonstrated by Broquist (1956) and by Hiatt, Goldstein, and Tabor (1958) in patients with leukaemia treated with the folic-acid antagonist methotrexate (amethopterin). Using the excretion of formimino-glutamic acid as an index of folic-acid deficiency (Silverman, Gardiner, and Bakerman, 1952), they showed that the haematological effect of the antagonist usually preceded evidence of folic-acid deficiency by many weeks.

Convulsant Action of Folic Acid

It is difficult to understand why therapeutic doses of folic acid precipitated epileptic attacks in this patient. The mode of action of phenobarbitone as an anti-convulsant is unknown. The fact that a megaloblastic anaemia requiring treatment with folic acid may occur in patients taking barbiturates suggests that these drugs interfere with folic-acid metabolism. However, one would hardly be justified in arguing therefrom that phenobarbitone exerts its anticonvulsant action by interfering with folic-acid metabolism, and that this anticonvulsant action is negated by the administration of large doses of folic acid. Though this view cannot be excluded it would be a major assumption regarding the mode of action of a drug on the basis of a single case. If, on the other hand, phenobarbitone produces its anticonvulsant effect through another metabolic pathway the convulsant effect of folic acid is best explained as a by-product of its general effect on cellular metabolism. It is conceivable that when there

is interference with folic-acid metabolism all cell function, including that of the cerebral cells, is depressed. Under these circumstances of depressed cell function the cerebral cells may be incapable of the massive simultaneous discharge which constitutes the epileptic attack, and this may only become possible when this interference has been overcome by adequate doses of folic acid.

Whatever the explanation, it is likely that the convulsant effect of folic acid is only possible under particular circumstances, for this action has not previously been demonstrated in patients with megaloblastic anaemia associated with anticonvulsant drugs. These special circumstances may be related to the severe generalized background abnormality in the E.E.G. This abnormality in the E.E.G. is non-specific and is found in a wide variety of conditions, which might be termed "toxi-metabolic" (Laidlaw, 1959). General electroencephalographic changes of the same type are usual in cases of pernicious anaemia with or without subacute combined degeneration of the cord (Walton, Kiloh, Osselton, and Farrall, 1954). In this patient the electroencephalographic changes were not due to the anaemia, since they persisted after the anaemia had been treated by adequate doses of folic acid. Furthermore, the abnormality was present only when he was taking large doses of phenobarbitone, and was much reduced when acetazolamide was substituted for phenobarbitone. Since phenobarbitone does not usually produce such a generalized abnormality, it would seem possible that this patient showed a cerebral sensitivity to the drug. This unusual sensitivity to the action of phenobarbitone in an epileptic may have produced the special circumstances under which administration of folic acid had a convulsive effect.

Summary

The case is described of a patient who presented on two occasions with megaloblastic anaemia while taking large doses of phenobarbitone.

Treatment with folic acid (30 mg. daily) produced an optimal haematological response, but on each occasion was associated with the precipitation of frequent minor epileptic attacks.

The pathogenesis of the anaemia and the possible relationship between treatment with folic acid and the precipitation of epileptic activity are discussed.

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REFERENCES

- Badenoch, J. (1954). *Proc. roy. Soc. Med.*, **47**, 426.
- Broquist, H. P. (1956). *J. Amer. chem. Soc.*, **78**, 6205.
- Calvert, R. J., Hurworth, E., and MacBean, A. L. (1958). *Blood*, **13**, 894.
- Chanarin, I., Anderson, B. B., and Mollin, D. L. (1958). *Brit. J. Haemat.*, **4**, 156.
- Elmes, P. C., and Mollin, D. L. (1958). *Brit. med. J.*, **2**, 80.
- Mollin, D. L., and Anderson, B. B. (1958). *Brit. J. Haemat.*, **4**, 435.
- Fuld, H., and Moorhouse, E. H. (1956). *Brit. med. J.*, **1**, 1021.
- Glass, G. B. J. (1954). *Clin. Res. Proc.*, **2**, 32.
- Hawkins, C. F., and Meynell, M. J. (1954). *Lancet*, **2**, 737.
- (1958). *Quart. J. Med.*, **27**, 45.
- Hiatt, H. H., Goldstein, M., and Tabor, H. (1958). *J. clin. Invest.*, **37**, 829.
- Hobson, Q. J. G., Selwyn, J. G., and Mollin, D. L. (1956). *Lancet*, **2**, 1079.
- Laidlaw, J. (1959). *J. Neurol. Neurosurg. Psychiat.*, **22**, 69.
- Ross, G. I. M. (1952). *J. clin. Path.*, **5**, 250.
- Silverman, M., Gardiner, R. C., and Bakerman, H. A. (1952). *J. biol. Chem.*, **194**, 815.
- Walton, J. N., Kiloh, L. G., Osselton, J. W., and Farrall, J. (1954). *Electroenceph. clin. Neurophysiol.*, **6**, 45.