and that increasing refinement of chemical and other investigations may in time render biopsy obsolete also in many cases of neurolipidosis.

Pneumoencephalography was of no value in selecting cases for rectal biopsy, since it showed cerebral atrophy with equal frequency in patients whose biopsies later did and did not show diagnostic changes.

No diagnostic abnormalities were found on rectal biopsy in patients without dementia, a positive family history of relevant disease or other features pointing strongly towards a specific neurological condition.

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Effect of Total Dose Infusion of Iron-dextran on Iron, Folate, and Vitamin B₁₂ Nutrition in Postpartum Anaemia

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The efficacy of total dose infusion of iron-dextran (T.D.I. Imferon) in the treatment of antepartum and postpartum iron-deficiency anaemia is well established (Varde, 1964; Basu, 1965; Bonnar, 1965). Detailed studies on the effect of T.D.I. Imferon therapy on the iron, folate, and vitamin B₁₂ nutritional status of iron-deficient women have, however, not yet been reported. Such information is necessary in view of the poor response to T.D.I. Imferon noted in folate-deficient women (Basu, 1965; Bonnar, 1965), and the suggestion that reactions to T.D.I. Imferon may be related to folate deficiency (Clay *et al.*, 1965; Lane and Scott, 1965). In the present study comprehensive tests of iron, folate, and vitamin B₁₂ nutrition were carried out before and after administration of T.D.I. Imferon therapy to postpartum anaemic subjects. This therapy generally resulted in restoration of normal nutrition, but the postpartum improvement in folate nutrition, shown in untreated control subjects, was retarded.

Material and Methods

All subjects were Bantu admitted to Baragwanath Hospital, Johannesburg (altitude 5,760 ft.; 1,756 m.). Consecutive patients with haemoglobin levels of less than 10 g./100 ml. and decreased marrow iron stores on the third postpartum day were included in the study. A total of 81 patients received T.D.I. Imferon, and 61 of them returned for follow-up six weeks later; 12 of these 61 received folic acid as well as Imferon. Control subjects consisted of 18 patients who received no therapy. The control group is much smaller than the group receiving therapy, for during the course of the study pronounced differences between the groups became apparent, and it was not felt warranted to leave more patients untreated.

Procedure

Before therapy on the third postpartum day venous blood was drawn for measurement of haemoglobin, haematocrit, serum iron, unsaturated iron-binding capacity (U.I.B.C.), serum

folate activity, serum vitamin-B₁₂ concentration, and for the preparation of films. Blood for serum iron and U.I.B.C. estimation was allowed to drip direct from the needle into iron-free containers. Films were prepared from bone marrow aspirated from the sternum.

Imferon was administered as follows. The dose for each patient was calculated as described by the manufacturers. The Imferon was diluted in normal saline, not more than 20 ml. being added to a litre of saline. The infusion was started at 10–15 drops a minute until 100 ml. had been administered, and thereafter at 45 drops a minute. Before infusion basal readings of the pulse and blood pressure were made, and these were repeated at hourly intervals during the infusion. Any untoward symptoms occurring during the infusion were recorded. Forty-eight hours after completion of the infusion the site was examined for pain, tenderness, swelling, and redness, and the lower limbs were examined for evidence of venous thrombosis. The 14 patients who were to receive folic acid were then given folic acid tablets (5 mg.) and instructed to take at least one tablet daily for a period of 40 days.

The patients were studied again six weeks later. All previous investigations, including bone-marrow-puncture, were repeated. Patients were re-examined for symptoms or signs of venous thrombosis at the site of infusion and in the legs. After withdrawal of the blood and marrow samples the investigation of the patients was complete; those who had received only Imferon therapy were supplied with folic acid tablets, and those who had received no therapy since delivery were treated with iron and folic acid by mouth.

Laboratory Methods

Haemoglobin was estimated as cyanmethaemoglobin, a commercially obtained standard being used. The haematocrit was

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measured in a microhaematocrit centrifuge, and the mean corpuscular haemoglobin concentration (M.C.H.C.) calculated in the usual way. Serum iron and unsaturated iron-binding capacity were measured by the methods of Bothwell and Mallett (1955) and Charlton *et al.* (1965) respectively. Serum vitamin B₁₂ was assayed with *Lactobacillus leichmannii* (Brandt and Metz, 1961) and serum folate activity with *L. casei* (Herbert, 1961; Waters and Mollin, 1961). Blood films were stained with Wright's stain and examined for hypochromia, and for hypersegmented neutrophils. When more than 3% of the neutrophils showed five or more lobes in the nucleus, a "shift to the right" was regarded as being present. Wright's stained bone-marrow films were examined for the presence of megaloblasts and giant myeloid cells. In addition, marrow films were stained for haemosiderin by the Prussian-blue reaction, and iron stores in the particles graded from 0 to +6. Grades +3 and +4 were regarded as normal, grades +5 and +6 as excessive, and grades 0 to +2 as diminished iron stores. In grade 0 no stainable iron was observed; in grade +1 stainable iron was just visible in one or two particles, and in grade +2 stainable iron was just visible in several particles. Only subjects with marrow iron stores in grades 0 to +2 were included in the study.

Results

In comparing the results obtained at the first and final assessments of the patients studied, only paired observations were used. Thus if a particular index was not available in a patient at the first assessment—for technical reasons, for example—it was excluded from the second assessment, and vice versa.

The mean values for haemoglobin, haematocrit, M.C.H.C., serum iron, U.I.B.C., serum folate, and vitamin B₁₂ before and after therapy in patients receiving Imferon without added folic acid are shown in Table I. The iron stores are shown in Fig. 1, and the incidence of right shift, megaloblasts, and giant myeloid cells in Fig. 2. There was a marked rise in the mean haemoglobin, haematocrit, serum iron, and percentage saturation and a fall in U.I.B.C. values; the differences in the mean values were all statistically significant (Table II). In no patient after treatment was the haemoglobin value less than 11 g./100 ml. There was a rise of 0.41% in the mean value for the M.C.H.C., but this difference is not statistically significant. There was also little change in the percentage of subjects showing hypochromia (40% at delivery, 36% six weeks later), but the degree of hypochromia was invariably less at six weeks. There was an increase in iron stores in all patients after Imferon therapy.

The mean serum folate activity after Imferon showed little change to the pretherapy level, the difference in the mean values not being statistically significant (Table II). That folate nutri-

FIG. 1.—Marrow iron stores at delivery and six weeks later. X = Patients who received folic acid as well as Imferon.

FIG. 2.—Morphological evidence of folate deficiency at delivery and six weeks later.

TABLE I.—Results at Delivery and Six Weeks Later. Results are Expressed as the Mean Value $\pm$ Standard Error

		Haemoglobin (g./100 ml.)	Haematocrit (%)	M.C.H.C. (%)	Serum Iron (μ g./100 ml.)	U.I.B.C. (μ g./100 ml.)	Percentage Saturation	Serum Folate (μ g./ml.)	Serum Vitamin B ₁₂ (μ g./ml.)
T.D.I. Imferon .. {	Delivery	8.73 $\pm$ 0.17	28.2 $\pm$ 0.53	31.2 $\pm$ 0.24	52.0 $\pm$ 7.01	371.2 $\pm$ 14.13	12.5 $\pm$ 0.72	3.42 $\pm$ 0.21	414.1 $\pm$ 27.8
	6 weeks	12.88 $\pm$ 0.18	40.6 $\pm$ 0.46	31.6 $\pm$ 0.24	82.6 $\pm$ 11.23	198.7 $\pm$ 8.86	30.4 $\pm$ 1.52	3.59 $\pm$ 0.26	556.2 $\pm$ 46.5
T.D.I. Imferon + folic acid .. {	Delivery	8.60 $\pm$ 0.34	27.3 $\pm$ 1.02	31.2 $\pm$ 0.58	51.4 $\pm$ 5.32	323.1 $\pm$ 26.75	14.4 $\pm$ 1.17	2.93 $\pm$ 0.32	505.4 $\pm$ 66.6
	6 weeks	13.33 $\pm$ 0.32	41.46 $\pm$ 0.86	32.2 $\pm$ 0.51	80.4 $\pm$ 5.77	233.8 $\pm$ 19.69	26.9 $\pm$ 3.47	23.10 $\pm$ 5.32	561.1 $\pm$ 56.5
Controls .. {	Delivery	8.78 $\pm$ 0.65	28.3 $\pm$ 0.67	31.0 $\pm$ 0.34	50.2 $\pm$ 4.70	273.3 $\pm$ 21.55	16.8 $\pm$ 2.00	3.40 $\pm$ 0.32	539.0 $\pm$ 72.0
	6 weeks	11.92 $\pm$ 0.27	37.9 $\pm$ 0.77	31.4 $\pm$ 0.44	68.3 $\pm$ 6.17	349.7 $\pm$ 10.83	16.3 $\pm$ 1.28	6.17 $\pm$ 0.57	484.0 $\pm$ 61.8

TABLE II.—Statistical Significance of the Differences in the Mean Values Compared with Six Weeks Later

		Haemoglobin	Haematocrit	M.C.H.C.	Serum Iron	U.I.B.C.	% Saturation	Serum Folate	Serum Vitamin B ₁₂
T.D.I. Imferon .. {	n	98	92	92	100	96	96	104	104
	t	8.68	26.68	1.21	4.47	7.09	8.80	0.60	0.25
	P	<0.001	<0.001	>0.1	<0.001	<0.001	<0.001	>0.1	>0.1
Imferon + folic acid .. {	n	28	26	26	28	26	26	26	28
	t	4.73	4.60	1.38	3.03	2.38	2.85	3.05	0.63
	P	<0.001	<0.001	>0.1	<0.01	<0.05	<0.01	<0.01	>0.1
Controls .. {	n	42	40	40	42	42	42	42	40
	t	5.30	5.25	0.63	2.19	2.85	0.20	3.80	0.58
	P	<0.001	<0.001	>0.1	<0.05	<0.01	>0.1	<0.001	>0.1

tion was not obviously improved after Imferon was further reflected in the constant number of subjects who showed megaloblastic change in the marrow before and after therapy. There was, however, a moderate fall in the number of subjects who showed a right shift in the neutrophils and giant myeloid cells in the marrow. The mean serum vitamin-B₁₂ concentration after Imferon was higher than the pretreatment level, but the difference is not statistically significant.

The mean values of the various haematological indices and the indices of iron, folate, and vitamin B₁₂ nutrition at the first and final assessment in the group who received no therapy are shown in Tables I and II and Figs. 1 and 2. Six weeks after delivery these subjects also showed a marked rise in haemoglobin and haematocrit, the differences in the mean values being statistically significant. In four of the 21 subjects (19%), however, the haemoglobin value was less than 11 g./100 ml. and in two of these it was less than 10 g./100 ml. The mean serum iron level also rose, but in contrast to the patients who received Imferon the U.I.B.C. rose markedly, so that the mean percentage saturation of transferrin showed little change after six weeks. The striking difference in percentage saturation of transferrin between the control and Imferon-treated group was further reflected in the iron stores. In patients who had no stainable iron in the marrow after delivery the particles remained free of demonstrable iron six weeks later. In all but two of the patients with some stainable iron after delivery no iron could be found six weeks later. In these two patients some iron remained in the particles, but a smaller amount than at delivery. There was also a striking increase both in the incidence (10% at delivery and 66% six weeks later) and the degree of hypochromia in this group after six weeks.

In contrast to the group who received Imferon (without folic acid), the control group showed a statistically highly significant rise in mean serum folate levels after six weeks. This improvement in folate nutrition was reflected in the virtual disappearance of megaloblastic change in the marrow. The incidence of giant myeloid cells in the marrow and right shift in the neutrophils also fell, but in many patients these changes were still present six weeks post partum. The mean serum vitamin-B₁₂ concentration was slightly lower after six weeks, but the difference is not statistically significant.

The mean values of the various haematological indices and tests of iron, folate, and vitamin B₁₂ nutrition in the subjects who received Imferon and folic acid are shown in Tables I and II and Figs. 1 and 2. After treatment with Imferon and folic acid these subjects showed highly significant rises in haemoglobin, haematocrit, serum iron, and percentage saturation, and a fall in U.I.B.C. as observed in the group who received Imferon alone. The mean rise in haemoglobin levels was greater than in the group who received Imferon alone. Iron stores increased in all subjects. The mean serum folate levels showed a marked rise, and morphological evidence of folate deficiency was much less apparent than in the subjects who received only Imferon. The mean serum vitamin-B₁₂ concentration was higher after six weeks, but the difference is not statistically significant.

Reactions in Those Having Total Dose Imferon Infusions

Of the 81 patients who received Imferon, five (6.2%) developed thrombophlebitis at the site of the infusion within 48 hours. Two patients also complained of headache during the infusion, but the infusions were completed without further ill-effect. Two patients complained of pain in the arm at the site of the infusion during the administration of the Imferon, but did not develop any evidence of thrombophlebitis. One patient, not included in the series, had an attack of shivering soon after the start of the infusion, but there was no fall in blood pressure or rise in pulse rate. This infusion was imme-

diately discontinued. Major anaphylactic reactions were not encountered.

Compared with the rest of the group there was no obvious difference in the folate nutrition, as determined by serum folate levels and blood and bone-marrow changes, in the patients who showed any form of reaction.

Discussion

The value of intravenous infusion of Imferon has been well substantiated as a rapid means of correcting anaemia in late pregnancy. In a recent study Bonnar (1965) has also demonstrated its effectiveness in correcting anaemia after abortion and in postnatal blood-loss anaemia. In the present study of iron-deficient postpartum anaemia, a mean rise in haemoglobin of 4.15 g./100 ml. was obtained in six weeks in the group receiving Imferon alone. This was greater than the rise obtained in the control group (3.14 g./100 ml.) but not as great as that (4.73 g./100 ml.) in the group receiving folic acid in addition to T.D.I. Imferon. These changes were reflected in the haematocrit values after six weeks. It is thus apparent that iron-deficient postpartum patients can spontaneously correct their haemoglobin levels almost to those found in the subjects given iron therapy. However, the rise in haemoglobin levels in the group of untreated women often failed to reach normal values, and was invariably associated with deterioration in their already precarious iron nutrition. What little iron was present in the marrow particles usually disappeared, hypochromia became more marked, and the percentage saturation of transferrin failed to rise. By way of contrast, in patients who received T.D.I. Imferon therapy iron stores increased markedly, hypochromia decreased, and the percentage saturation of transferrin more than doubled.

The effect of parenteral iron therapy on folate nutrition in pregnancy has been referred to by previous workers. Bonnar (1965) noted that in antenatal patients who received intravenous Imferon, 20% developed signs of an unsuspected folate deficiency which resulted in an impaired response to the iron therapy. Similar findings have been reported by Scott (1963) in a comparable group treated with intramuscular iron. She suggested that the earlier diagnosis of folate deficiency rather than any specific effect of the parenteral iron accounted for this high incidence. However, a recent study by Combrink *et al.* (1966) indicated that parenteral iron therapy itself in antenatal anaemic women may lead to signs of folate deficiency. The administration of folic acid with T.D.I. Imferon therapy will prevent this complication (Varde, 1964; Goldthorp *et al.*, 1965).

The results of the present study provide further evidence for a direct effect of parenteral iron therapy on folate nutrition in anaemias associated with pregnancy. In untreated subjects there was marked improvement in the indices of folate nutrition in the six-week period after confinement. This improvement was negligible in patients who received Imferon, the serum folate levels and the incidence of bone marrow megaloblastic change being similar before and after treatment. Addition of folic acid to Imferon therapy resulted in high serum folate levels and disappearance of megaloblasts from the marrow. The mechanism whereby parenteral iron retards improvement in folate nutrition is unknown. It is probably related to increased utilization of haemopoietic factors following on intense stimulation of erythropoiesis due to rapid replenishment of iron stores in the iron-deficient patient. These findings also substantiate the recommendation to administer folic acid routinely with Imferon infusion (Varde, 1964; Goldthorp *et al.*, 1965).

The observed changes in serum vitamin-B₁₂ concentrations were relatively small. Compared with delivery, the mean values at six weeks were higher in patients who received T.D.I. Imferon and lower in the control group. There was, however,

considerable variation in individual levels within the groups, and the observed differences in the mean values are not statistically significant. The results of the present study do not suggest any consistent effect of T.D.I. Imferon therapy on vitamin B₁₂ nutrition.

Though most of the subjects in the present study showed evidence of folate deficiency, no major reaction to T.D.I. Imferon therapy was encountered in a total of 81 infusions. This finding does not substantiate the suggestion that major reactions may be related to folate deficiency (Lane and Scott, 1965). Thrombophlebitis at the site of infusion occurred in five patients; it was not related to the use of 5% dextrose water as diluent for the Imferon, as only normal saline was used. The slow rate of infusion may possibly have been a factor in producing thrombophlebitis.

Summary

Iron-dextran by the total dose infusion technique was administered to 81 postpartum anaemic subjects with diminished marrow iron stores. Tests of iron, folate, and vitamin B₁₂ nutrition were carried out before therapy and repeated six weeks later.

Iron-dextran therapy was regarded as satisfactory in that anaemia was corrected and tests of iron nutrition returned to normal. In untreated control subjects anaemia was often spontaneously corrected, but this was accompanied by deterioration in iron nutrition.

Iron-dextran therapy retarded the improvement in tests of folate nutrition after confinement which occurred in untreated subjects. This substantiates the recommendation that folic acid be administered routinely to patients receiving Imferon therapy for anaemia associated with pregnancy.

No consistent effect of iron-dextran therapy on vitamin B₁₂ nutrition could be shown.

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Influenza in the West of Scotland, 1966

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The west of Scotland experienced concurrent epidemics of influenza type A and type B in the first quarter of 1966. The present study describes virological and clinical findings in 119 cases diagnosed as influenza by the Regional Virus Laboratory, Ruchill Hospital. Four cases of encephalitis associated with one particular age group are also reported.

Materials and Methods

During the epidemics tests for influenza-A and for influenza-B infections were carried out on routine specimens received by the laboratory from patients either in hospital or at home. The patients investigated comprised not only those with influenzal symptoms but also those with infections of the upper and lower respiratory tracts or other pyrexial illnesses for which no other cause could be found. From most of the children only specimens for virus isolation were obtained because of the difficulty of obtaining sera. In contrast, from most of the adults sera only were collected because of limited laboratory resources for isolation of respiratory viruses.

Virus Isolation.—Specimens for virus isolation comprised swabs of throat or throat and nose. Each swab (cotton-wool on wooden shaft) at collection was broken into 2 ml. of transport medium (Grist *et al.*, 1966). Of each extract 0.2 ml. was inoculated into one tube of secondary rhesus monkey kidney tissue culture, which was examined and maintained as described by Grist *et al.* (1966). Growth of either influenza-A (three strains) or influenza-B (23 strains) produced haemadsorp-

tion and generally also cytopathogenic effect in three to seven days. The type of influenza was determined by complement-fixation tests against both influenza-A and influenza-B human convalescent sera. Influenza complement-fixing antigen was usually detected at the fifth to eighth day in the primary culture; the remaining strains all fixed complement in the first passage, antigen titres ranging from 2 to 32 for the type B strains and being 32 for all three type A strains. Titres of influenza-B haemagglutinin varied from <4 to 128 in the primary culture and from 8 to 256 in the first pass; titres of influenza-A haemagglutinin were lower, being <4 in the primary culture and not above 8 after two passes. Representative type A and type B strains from children and adults were sent to Dr. H. G. Pereira, W.H.O. Influenza Centre, London, who reported that they were antigenically similar to recently isolated type A and B strains.

Serology.—Paired sera were examined for complement-fixing antibodies against influenza-A and influenza-B soluble antigens and against other relevant viral antigens as described by Grist *et al.* (1966). As the optimal concentrations of both influenza antigens were generally twofold to eightfold greater for young children than for older children or adults, two doses of each antigen were used: the higher concentration for testing sera from children under 6 years of age, and a four times lower

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