Folate Status in the Elderly

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It is only within recent years that a satisfactory method for estimating the serum folate level has been available, and since this test was introduced a number of investigators have reported the occurrence of nutritional megaloblastic anaemia due to folic acid deficiency in the United Kingdom, particularly in elderly persons (Gough, Read, McCarthy, and Waters, 1963; Varadi and Elwis, 1964; Forshaw, Moorhouse, and Harwood, 1964, 1965). However, as has been previously reported, nutritional megaloblastic anaemia has not been seen in the South-east of Scotland (Girdwood, 1960a, 1964); hence it was decided to make use of a study of elderly persons, which was being undertaken for other purposes, to assess the serum folate level of a number of geriatric subjects in the region.

In 1965 Read, Gough, Pardoe, and Nicholas reported studies of the nutritional status of 51 consecutive subjects on arrival at an old people's home in Bristol. Only 10 of these had serum folate levels within their accepted normal range of 6-21 $\mu\text{g./ml.}$, and in 10 the reading was less than 3 $\mu\text{g./ml.}$ The excretion of formiminoglutamic acid after a histidine load test (Kohn, Mollin, and Rosenbach, 1961) was excessive in 62% of the subjects, and this was thought to give further evidence of deficiency. Low serum vitamin-B₁₂ levels were not a feature. More recently Hurdle and Williams (1966) reported low levels of serum folate in 28 out of 72 persons over the age of 70 admitted to a geriatric hospital in South London.

Subjects Studied

Three groups of patients were included in the survey:

Group 1. Geriatric Survey Group.—This consisted of persons of either sex over the age of 65 who were on the N.H.S. list of practitioners in a working-class area of the city. They were either apparently well, many not having consulted their doctor for a number of years, or were attending on account of minimal illnesses. Seventy-two subjects were studied, 40 being women. Twenty-five subjects lived alone. The mean age of the women patients was 75.1 years (range 65-90), and that of the men was 77.4 years (range 65-88).

Group 2. Geriatric Patients in Hospital.—This group consisted of 39 patients over the age of 65. All were in hospital and had been there for more than three months. Their appetites were good and investigations had shown that they did not suffer from gastrointestinal, malignant, endocrine, or collagen diseases. The blood urea levels were normal and no patient was receiving antibiotics. Nineteen were women, their mean age being 79.1 years (range 65-92), and 20 were men, with a mean age of 78.8 years (range 65-90).

Group 3. Younger Controls.—This group consisted of 62 healthy subjects, all of whom were taking a good diet. None was receiving drugs. The group consisted of volunteer medical students, members of the departmental staff, and blood donors. There were 25 women, with a mean age of 35.4 years (range 21-48), and 37 men, with a mean age of 37.6 years (range 20-60).

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All the patients in group 1 were visited by a social worker as part of the larger survey, and their financial, social, and nutritional state was assessed. They were then seen at a hospital outpatient department, where a complete physical and full psychiatric examination was carried out. The haemoglobin level, packed cell volume, and red blood cell count were estimated.

A trained dietitian visited the homes of six subjects studied in group 1, including three whose serum folate levels were among the lowest in the group, and made a detailed analysis of their food intake. The patients lived in a working-class area of the City of Edinburgh. The dietitian also assessed in detail the adequacy of the diet of eight of the geriatric patients in group 2, including four of those with the lowest serum folate levels. In all instances the total calorie and protein content was satisfactory, as was the intake of vitamin B₁₂. There are no satisfactory tables of folate content of foodstuffs and no agreement on how this should be measured, but it is possible for a dietitian to state whether the diet is grossly deficient in the content of folate. There was no evidence of any such deficiency in the diet of the subjects studied, or, in general, in the two geriatric hospitals involved in the present investigation.

Records were kept of drugs being taken by each of the subjects.

Methods

Serum Folate Level.—This was measured microbiologically with *Lactobacillus casei* as the test organism, using Waters and Mollin's (1961) modification of the method of Baker *et al.* (1959). Difco Laboratories' *L. casei* folate medium was used, but higher concentrations of ascorbic acid were added to the serum (10 mg./ml.) and buffer (200 mg./100 ml.) than in the published method. Ascorbic acid was not added to the standard.

Folic acid clearance tests were carried out in a few instances (Girdwood and Delamore, 1961) by the method of Chanarin, Mollin, and Anderson (1958), with *Streptococcus faecalis* as the test organism.

Serum vitamin-B₁₂ levels were estimated with *Euglena gracilis* z strain as the test organism (Girdwood, 1960b).

Results

Serum Folate Levels.—In the 62 healthy control subjects the serum folate levels ranged from 2.2 to 13.6 $\mu\text{g./ml.}$ (mean 6.1; S.D. 2.5 $\mu\text{g./ml.}$). These results are shown, together with the findings in the other groups, in the Chart; it will be seen that in four men and one woman the level was less than 3 $\mu\text{g./ml.}$ These five were completely healthy, were not taking drugs or other medicaments, and were eating a normal diet. In the case of the 72 persons in group 1, all of whom were geriatric subjects living at home, the serum folate levels ranged from 2 to 25 $\mu\text{g./ml.}$ (mean 6.4; S.D. 3.5 $\mu\text{g./ml.}$). In five instances the level was less than 3 $\mu\text{g./ml.}$ When the 39 geriatric patients in hospital were investigated it was found that the levels ranged from 1.6 to 9.1 $\mu\text{g./ml.}$ (mean 4.8; S.D. 2 $\mu\text{g./ml.}$). In seven instances the level was less than 3 $\mu\text{g./ml.}$ There was no significant difference between the results in

the younger control subjects and those of the geriatric subjects living at home, and an analysis of variance performed on the results of the subjects in both these groups, considered in decades, showed no significant difference between the decades. When the results of the geriatric subjects living at home were compared with those in hospital the application of the *d*-test showed the latter to be significantly lower at the 1% level.*

Serum Vitamin-B₁₂ Levels.—In 44 controls aged 20 to 60 the range was 140 to 640 $\mu\text{g./ml.}$ (mean 360; S.D. 135 $\mu\text{g./ml.}$). The serum vitamin-B₁₂ level was estimated in 64 subjects in group 1 and ranged from 120 to 756 $\mu\text{g./ml.}$ (mean 346.2 $\mu\text{g./ml.}$) except in four patients in whom the readings were 80, 97, 104, and 108 $\mu\text{g./ml.}$ These four were studied further and were found to be suffering from pernicious anaemia. It was discovered that one had previously been diagnosed in another city, but treatment had not been continued. In three other patients, included above, the level was less than 140 $\mu\text{g./ml.}$, but they were not suffering from pernicious anaemia. Their serum folate levels exceeded 4 $\mu\text{g./ml.}$ In 36 subjects studied in group 2 the levels ranged from 128 to 717 $\mu\text{g./ml.}$ (mean 350 $\mu\text{g./ml.}$). In three instances the levels were below 140 $\mu\text{g./ml.}$, while the serum folate levels exceeded 4 $\mu\text{g./ml.}$ No patient suffered from pernicious anaemia.

Haemoglobin Levels.—The haemoglobin levels of 68 patients in group 1, not including those with pernicious anaemia, ranged from 8.8 to 17.6 g./100 ml. (mean 14.1 g./100 ml.). Eleven women had iron-deficiency anaemia (haemoglobin level less than 12.4 g./100 ml.), but in all instances it was mild. One man had a haemoglobin level of 8.8 g./100 ml. associated with iron deficiency, and for this no cause was found. In group 2 the range in 39 subjects was from 11 to 17.4 g./100 ml. (mean 14.3 g./100 ml.). Two women and one man had very mild iron deficiency.

Discussion

The results of this survey show no difference between the serum folate levels in old persons living in a working-class area of the City of Edinburgh and those of younger healthy control subjects, many of whom were medical students. There was, however, a lower mean value in a group of geriatric subjects who had been in hospital for more than three months. The reason for this is not clear, since the diet was in every way satisfactory in the two hospitals concerned, though it is possible that the aged are less inclined to eat when in hospital than when at home. Those in hospital were less active and a number were confined to bed. The difference was not one of age or

of sex, since the two groups were matched. An analysis of drugs administered in hospital did not suggest that any of those in use caused an apparent lowering of the serum folate level. A few folic acid clearance tests were done, but the results fell within the limits of those in control subjects, so were not helpful.

In making a survey of this type one must accept that estimation of the serum folate level is a relatively crude method for investigating folate deficiency. However, if the level is normal and vitamin-B₁₂ deficiency is not a feature, then it is reasonable to believe that the patient does not suffer from folate deficiency. It has been our experience that in untreated patients with gluten enteropathy, a condition in which folate depletion is common, the serum folate level is usually lower than 3 $\mu\text{g./ml.}$, though even here the readings may appear to be lower than their true value because of inhibitors in the serum (Cowan, Hoffbrand, and Mollin, 1966).

A problem in the present survey is to determine what level of serum folate should be taken to indicate depletion. That the low results in some controls were not due merely to technical error is evidenced by the fact that in 17 subjects, including the two young controls with the lowest results, duplicate estimations were done independently on other blood samples in another laboratory of the Department of Therapeutics by a second worker, who obtained almost identical results without being aware of the original findings. It does appear, however, that there is some overlap between results in patients with folic acid depletion and in normal subjects, so that with the present technique a level even as low as 2.2 $\mu\text{g./ml.}$ does not necessarily indicate folate depletion. It is inconceivable that our healthy young controls, who were eating a normal diet, could be suffering from folate deficiency.

Altogether it seems reasonable to claim that there is no evidence from this survey that folate deficiency was a serious problem in geriatric subjects living at home or in hospital, and vitamin-B₁₂ depletion of nutritional origin did not constitute a problem. Iron deficiency of a mild degree was not uncommon in elderly women at home.

The reason why folate deficiency is less of a problem in this region than in certain others already reported is no doubt because there are differences in dietetic habits, and this is a matter for further study. It is possible that this is also the reason why megaloblastic anaemia of pregnancy is seldom seen in the South-east of Scotland.

Summary

Serum folate estimations were carried out on 72 subjects over the age of 65 living at home in a working-class area within the City of Edinburgh, on 39 patients in the same age group in geriatric hospitals in the city, and on 62 healthy younger controls. No significant difference was found between the levels in the geriatric subjects and the younger controls, but the results were lower in the patients in the geriatric hospitals. There was little evidence of folate deficiency.

There was no evidence of vitamin-B₁₂ deficiency due to malnutrition.

Of the geriatric subjects living at home, 11 women had mild iron deficiency and one man was anaemic. In the group of geriatric hospital patients, two women and one man had mild iron-deficiency anaemia.

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* The *d*-test is a generalization of the more commonly employed *t*-test. It does not assume the variances of the two samples to be equal.

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Simultaneous Administration of Live Measles Virus Vaccine and Smallpox Vaccine

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Smallpox has been virtually eradicated from Europe and America by means of efficient control measures practised over many years. The disease is, however, still prevalent in large areas of Africa and Asia, and the possibility of global eradication was discussed by an expert committee of the World Health Organization (W.H.O., 1964).

Measles is also a serious disease in developing countries (Gans *et al.*, 1961; Morley *et al.*, 1963; Hendrickse and Sherman, 1965), having many clinical similarities to the illness as seen in Europe in the early part of the present century (Morley, 1964).

During the past few years methods of active immunization against measles have been developed (W.H.O., 1963). Much emphasis has been placed on the use of live vaccines, and there have been numerous reports on the use of the attenuated types of measles vaccines (Collard *et al.*, 1961; Hendrickse *et al.*, 1964; Krugman, 1965; Smorodintsev *et al.*, 1965; Rey *et al.*, 1965b), as well as on the comparison between *attenuated* and *further attenuated* vaccine strains with regard to clinical reactions and serological response (Du Pan, 1965; Hendrickse *et al.*, 1964, 1965, 1966a, 1966b).

Satisfactory results have been reported from Senegal and Nigeria in trials of further attenuated measles vaccines administered by needleless injectors (Rey *et al.*, 1965a; Hendrickse *et al.*, 1966a). It is clear that this method of injection would be required in any large-scale vaccination programme.

Plans for the eradication of smallpox and the control of measles in the near future, in 19 West African countries, "sponsored by the U.S. Government in co-operation with the World Health Organization," announced recently in the Nigerian press (*New Nigeria*, 1966; *Daily Times*, 1966), bring the problem of the simultaneous use of measles and smallpox vaccines to the forefront.

Meyer *et al.* (1964) reported on the response of 545 children in the Upper Volta who were given combined measles, smallpox, and yellow fever vaccines, which were physically mixed before being used in a single injection. They gave little detailed

information about the clinical responses to the procedure apart from that concerning febrile responses and rashes. Budd *et al.* (1965) evaluated measles and smallpox vaccines administered simultaneously to 258 children in Togoland; information, however, is not available at present whether or not the vaccines were physically mixed. The attenuated Enders B strain of measles vaccine was used in both trials.

The results of the trials indicated that antibody responses were good, but were accompanied by a high rate of severe febrile reactions. Similar trials have not been carried out in other West African territories, nor has there been a direct comparison between the responses to attenuated and further attenuated measles vaccine when given simultaneously with smallpox vaccine.

If developing countries are to embark on mass smallpox/measles vaccination campaigns information is urgently needed regarding the nature of reactions that are likely to follow the procedure. We need also to know which of the live measles vaccines currently available are most suitable for use in such a campaign in developing African countries.

In an attempt to answer these questions a clinical trial in children was undertaken in a town in Western Nigeria. In this trial one group was given *attenuated* measles vaccine plus smallpox vaccine; a second group was given *further attenuated* measles vaccine plus smallpox vaccine; and a third group, which acted as controls, was given smallpox vaccine only. Our aim was to determine the pattern of reaction to the simultaneous vaccination procedure, including antibody responses, and to discover whether there were any significant differences between reactions provoked by the attenuated or further attenuated measles vaccines when given with smallpox vaccine. We report here the results of the trial.

Material and Methods

The study was undertaken at Ipetu Ijesha, a town of some 43,000 to 47,000 persons, situated about 97 miles (156 km.) from Ibadan, in the Western Provinces of Nigeria.

Healthy children between the ages of 6 months and 3 years who had had neither measles nor smallpox vaccination were

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