(1954) and Fudenberg *et al.* (1961) have shown that in patients with very large spleens this ratio can well exceed unity. Ideally, direct plasma-volume estimations should have been made, but any gross error seems unlikely. If it is assumed that the true whole-blood volume in the first two tests was inaccurate and really as expected from his weight, then the body/venous haematocrit ratio would have to be between 1.2 and 1.8, which seem to be impossibly high figures. Fudenberg *et al.* (1961) noted a reduction in the whole-body/venous haematocrit ratio when there was oedema associated with adrenocorticosteroid therapy, but my patient never showed any clinical oedema, so the ratio is unlikely ever to have been below 0.91.

Summary

A case of Felty's syndrome is described in which the patient made a dramatic improvement with adrenocorticosteroid therapy. Red-cell volume and survival studies have shown that the improvement in the anaemia could be explained by a reduction in a grossly excessive plasma volume. It is suggested that inhibition of the reticuloendothelial hyperplasia by the steroid therapy has given the patient a "medical splenectomy," the benefits of this having been due to reduction in the size of the organ, with a reduction in the size of the body's vascular compartment, the same changes having sometimes been observed after splenectomy in patients who had had enlarged spleen.

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Preliminary Communications

Chromatography and Microbiological Assay of Vitamin B_{12} in Smokers

Brit. med. J., 1966, 2, 988–990

The hypothesis that one of the forms of vitamin B_{12}—hydroxocobalamin—is concerned in the detoxication of exogenous cyanide (Boxer and Rickards, 1952; Wokes and Picard, 1955; Wokes, 1958; Smith, 1961a, 1961b) has aroused much interest and speculation, and it has been suggested that inactivation of hydroxocobalamin by conversion to the cyano–form might be concerned in the pathogenesis of certain demyelinating disorders (Smith, 1961a). For some years it has been known that when serum B_{12} is assayed microbiologically with *Lactobacillus leichmannii* the values obtained after extraction in the presence of added cyanide are usually much higher than those obtained when cyanide is absent (Boger *et al.*, 1955; Spray, 1955; Girdwood, 1960). The values obtained with added cyanide represent total serum B_{12}, while the low values obtained in the absence of added cyanide are associated with loss of the vitamin in the protein precipitate (Matthews, 1961, 1962).

Smith (1961a) stated that the difference between the values obtained with and without cyanide was "insignificant" in heavy pipe-smokers. He attributed this to the effects of the high cyanide content of tobacco smoke (Osborne *et al.*, 1956; Johnstone and Plimmer, 1959; Surgeon-General, 1964), and maintained that the value obtained without added cyanide represented serum cyanocobalamin, while the difference between this and the value obtained with added cyanide represented serum hydroxocobalamin. Thus, according to Smith's hypothesis, the high proportion of serum B_{12} extractable without cyanide in heavy smokers meant that most of their serum hydroxocobalamin (which he regarded as the physiologically active form) had been inactivated by conversion to cyanocobalamin.

Until recently there was no means of testing this hypothesis (Matthews, 1961, 1962; Basil *et al.*, 1965; Matthews *et al.*, 1965; Wilson and Matthews, 1966), since the forms of B_{12} actually existing in the blood were unknown, but the development of suitable chromatographic and bioautographic techniques (Lindstrand and Stählberg, 1963; Lindstrand, 1964) has now made it possible to identify these components, which include methylcobalamin, hydroxocobalamin, and DNB coenzyme B_{12}. If the results of the "differential" microbiological assay (with and without added cyanide) do in fact reflect the proportions of B_{12} components in the blood stream, this simple technique may be valuable, particularly in the investigation and diagnosis of conditions in which it is suspected that functional deficiency of B_{12} may exist owing to excessive conversion to the cyano– form.

This communication reports an attempt to see whether increased amounts of cyanocobalamin could be detected in the plasma of smokers, and, if so, whether this could be related to the results of microbiological assay.

Methods

Fifty-millilitre blood samples were taken from healthy volunteers, two non-smokers, and 10 moderate or heavy smokers.

30 ml. was heparinized for plasma thiocyanate estimation and chromatography of plasma B_{12}, and the remainder allowed to clot for serum B_{12} estimation. Blood was withdrawn in semidarkness with foil-wrapped syringes, and handling of samples for chromatography was carried out with minimal exposure to light. Plasma thiocyanate was determined on deproteinized samples by the method of Aldridge (Aldridge, 1945). Serum B_{12} was estimated with *L. leichmannii* (Matthews, 1962); each sample was divided into two parts, one of which (A) was assayed after extraction of serum in the presence of cyanide (giving total B_{12}) and the other (B) after extraction in the absence of cyanide. The amount of B_{12} extractable in the absence of cyanide will be termed "non-CN-extracted B_{12}." Chromatography of plasma B_{12} was carried out by the method of Lindstrand and Ståhlberg (1963), with the following modification. Before concentration for application to the chromatogram, the final aqueous plasma extract was adjusted to *p*H 3.8–4, and passed through a column of carboxymethylcellulose in the H^+ form, to remove hydroxocobalamin. This step was necessary because "trailing" of the hydroxocobalamin normally present in plasma extracts tends to obscure the cyanocobalamin position. This process also removes coenzyme B_{12}.

Results

Results are summarized in the Table. Plasma thiocyanate concentrations tend to be high in smokers (Støa, 1957; Matthews *et al.*, 1965; Wilson and Matthews, 1966) as a result of detoxication of the cyanide in tobacco smoke, and they are probably some indication of the exogenous cyanide exposure.

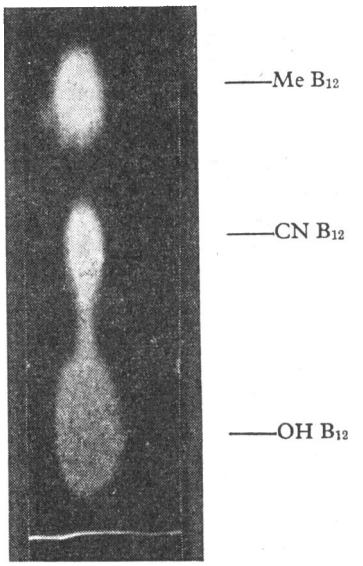
Plasma Thiocyanate Concentrations, Serum B_{12} Concentrations, and Results of Chromatography of Plasma B_{12}

Subject No.	Smoking Habits	Plasma-SCN (μmol/100 ml.)	Total Serum B_{12} (A) (μμg./ml.)	Non-CN-extracted B_{12} (B)* (μμg./ml.)	(A) minus (B)†	(B) as % of (A)	Chromatography—cyanocobalamin present (+) or not detected (0)
1	Non-smoker	8·0	420	245	175	58	0
2	„	8·0	300	160	140	53	0
3	Cigs. 20/day	6·7	400	220	180	55	0
4	„ 20/ „	14·5	440	210	230	48	0
5	„ 25/ „	8·8	450	240	210	53	0
6	„ 25/ „	8·4	740	475	265	64	+
7	„ 30/ „	8·0	260	145	115	56	0
8	„ 50/ „	12·9	620	420	200	68	+
9	Pipe 2 oz./week	12·3	460	240	220	52	0
10	„ 2½ „	11·2	785	360	425	46	0
11	„ 7 „	7·4	550	270	280	49	0
12	„ 7 „	20·6	595	325	270	55	+

* "Cyanocobalamin" according to Smith's hypothesis. † "Hydroxocobalamin" according to Smith's hypothesis.

The values for total B_{12}, non-CN-extracted B_{12}, and the values derived from these two measurements are not greatly different, in the present small series of smokers, from those in non-smokers. We have, however, shown elsewhere (Wilson and Matthews, 1966) that total B_{12} minus non-CN-extracted B_{12} tends to be slightly lower, and non-CN-extracted B_{12} as a percentage of total B_{12} tends to be slightly higher, in smokers than in non-smokers. We have not yet observed a smoker in whom the proportion of non-CN-extracted B_{12} approached 100%, as described by Smith (1961a). Values of more than 65% are uncommon, and the highest value in a series of 39 smokers (Matthews *et al.*, 1965; Wilson and Matthews, 1966) was 75%.

Chromatography and bioautography of plasma B_{12} components showed methylcobalamin in all samples. Small to moderate amounts of cyanocobalamin were found in only three of the 10 smokers (see Fig. and Table). Two of these were very heavy smokers with high plasma thiocyanate concentrations. It may be significant that in two of the samples containing cyanocobalamin the proportion of non-CN-extracted B_{12} was over 60%—though in the third sample this proportion (55%) was within the range customarily found in non-smokers. Very small quantities of cyanocobalamin (less than 5% of the total B_{12}) would pass undetected by the techniques employed.



Chromatogram of plasma B_{12} in a heavy cigarette smoker (Subject 8). Both methylcobalamin and cyanocobalamin are present. (Since steps were taken to remove plasma hydroxocobalamin, the presence of small amounts of this compound is probably the result of partial breakdown of methylcobalamin.)

Discussion

The present work does not provide evidence for the view that smokers usually have large amounts of cyanocobalamin in the blood. In fact, this compound was detected in only a minority of smokers, and, when detected, was present in relatively small amounts. Owing to the fact that only two non-smokers were studied, we cannot be sure that a similar incidence of cyanocobalamin in the blood might not occur in non-smokers also. Lindstrand and Ståhlberg (1963) found small amounts of cyanocobalamin in the blood of all their eight normal subjects, but did not record whether these were smokers or not; the apparent discrepancy in results may be due to differences in technique. The results do not support Smith's hypothesis concerning the interpretation of the "differential" microbiological assay, as it is clear that the non-CN-extracted B_{12}, which is always a very substantial proportion of the total, cannot represent cyanocobalamin, which, if present, occurs only in small amounts. At the same time, it has been shown that, on a statistical basis, the values for non-CN-extracted B_{12} tend to be slightly raised in smokers (Wilson and Matthews, 1966), and the present results certainly do not exclude the possibility that this may be due to an increase in cyanocobalamin in some of the subjects.

Though Smith's original hypothesis appears to be precluded, it is possible that the components extractable without cyanide are methylcobalamin, and cyanocobalamin if present, while those requiring cyanide to extract them are hydroxocobalamin and coenzyme B_{12}. The present results, however, are inconclusive. Even if they had been based on a larger series, with more non-smokers, difficulties in interpretation might still remain. Both an increased concentration of cyanocobalamin in blood samples and an increase in the proportion of non-CN-extracted B_{12} *could* be entirely *in vitro* phenomena resulting from a high concentration of cyanide pre-existing in the blood (Basil *et al.*, 1965; Wilson and Matthews, 1966), and need not necessarily represent alterations in the B_{12} components in the blood stream. We say this to stress the importance of avoiding premature conclusions in this field.

In spite of the reservations expressed here, a good deal of evidence has now accumulated suggesting that there is some connexion between the metabolism of cyanide/thiocyanate and vitamin B_{12}—for example, Boxer and Rickards, 1952 ; Mushett *et al.*, 1952 ; Wokes and Picard, 1955 ; Braekkan *et al.*, 1957 ; Matthews *et al.*, 1965. There appears to be an inverse relationship between total serum B_{12} concentration and cyanide concentration in healthy smokers and non-smokers (Wilson and Matthews, 1966). Hydroxocobalamin will protect against the neuropathological effects of chronic cyanide administration in animals (Smith *et al.*, 1963 ; Smith and Duckett, 1965). A patient believed to be suffering from acute cyanide encephalopathy who responded to hydroxocobalamin has been described (Smith, 1964). At least one neurological disorder—Leber's optic atrophy—appears to be the result of a disorder of cyanide metabolism (Wilson, 1963, 1965), and there are grounds for suspecting that tobacco amblyopia, the retrobulbar neuritis of pernicious anaemia, and possibly other neurological and metabolic complications of B_{12} deficiency are all associated with linked disturbances in the metabolism of cyanide and B_{12} (Heaton *et al.*, 1958 ; Smith, 1961a ; Wilson and Matthews, 1966). As yet there is no definite information about possible alterations in B_{12} components in these conditions, and though the present work has produced no evidence that the plasma B_{12} components are grossly altered in a small series of healthy smokers, we feel that further investigation in this field may well be rewarding.

SUMMARY

In view of claims that smoking may cause inactivation of vitamin B_{12} by conversion to cyanocobalamin, and that such inactivation may be concerned in the pathogenesis of certain neurological disorders, chromatography and bioautography of plasma B_{12} was carried out in a small series of smokers and non-smokers. Cyanocobalamin was detected in samples from only 3 out of 10 smokers, and occurred in relatively small amounts. The results do not support the hypothesis that the values obtained in the *Lactobacillus leichmannii* assay with and without added cyanide indicate the relative proportions of hydroxocobalamin and cyanocobalamin in the blood, though the possibility that these values may reflect certain alterations in plasma B_{12} components has not been excluded.

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Medical Memoranda

Radiological Demonstration of Renal Papillary Necrosis in Sickle-cell Anaemia

[WITH SPECIAL PLATE]

Brit. med. J., 1966, **2**, 990–991

Renal papillary necrosis is rarely demonstrated at intravenous urography. Clinicians seem to be largely unaware that papillary necrosis occurs in sickle-cell anaemia, even though the pathological changes in the kidney were long ago described by Goodwin *et al.* (1950). Middlemiss (1958), reporting on the visceral manifestations of sickle-cell anaemia, does not mention renal changes. Two of the standard textbooks do not list sickle-cell disease as a cause of papillary necrosis (Shanks and Kerley, 1958 ; Emmett, 1963). A survey of the literature in the English language reveals a total of seven cases demonstrated radiologically (Harrow *et al.*, 1963 ; Vix, 1965).

CASE REPORT

A 24-year-old secretary from British Guiana, resident in this country, a known case of homozygous haemoglobin S disease, was admitted to hospital with a 10-day history of bilateral loin pain and episodes of shivering. She had no dysuria, haematuria, or frequency. There was no history of diabetes mellitus, chronic phenacetin ingestion, or recurrent infection of the urinary tract.

On physical examination she was obviously unwell ; her temperature was 104° F. (40° C.). There was bilateral loin tenderness. The right kidney was palpable. The pulse rate was 120 and regular ; blood-pressure 120/90 mm. Hg. Clinically there was no other abnormality. Haematological examination confirmed the known homozygous haemoglobin S disease, and showed an associated iron-deficiency anaemia. The pertinent findings in the blood were: haemoglobin 5.6 g./100 ml. (38%), with marked hypochromic red cells ; white-cell count 18,600/c.mm., with 16,700 neutrophils/c.mm. ; reticulocytes 3.2% ; sickling test positive ; haemoglobin electrophoresis showed haemoglobin S only ; serum iron 80 µg./100 ml. ; erythrocyte sedimentation rate 115 mm./1 hr. ; blood urea 38 mg./100 ml. ; serum bilirubin 1.5 mg./100 ml. (0.6 mg./