Serum vitamin B₁₂ in renal failure

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SYNOPSIS The serum vitamin B₁₂ level was abnormally high in 14 out of 32 cases of renal failure. This was probably due to impaired excretion of the vitamin, but the results of measurements of the rate of excretion of radioactive vitamin B₁₂ did not provide unequivocal evidence on this point; other possible explanations are discussed. Renal failure must be added to the causes of high serum B₁₂ levels.

During a study of the metabolism of vitamin B₁₂ in diabetes mellitus (Matthews and Beckett, 1962), abnormally high serum B₁₂ concentrations were found in some patients with renal failure due to diabetic glomerulosclerosis. Consequently it seemed important to investigate serum B₁₂ concentrations in non-diabetic patients with renal failure, and the results are described here.

METHODS

Serum B₁₂ concentrations were estimated in 32 patients with renal failure (Table I) and in 60 hospital control patients not suffering from renal disease or a condition known to affect the serum B₁₂ concentration. Renal failure was considered to be present when, in the presence of primary or secondary renal disease, including urinary tract obstruction, the blood urea level was consistently raised above 45 mg./100 ml.

Serum B₁₂ was estimated microbiologically with *Lactobacillus leichmannii* (Matthews, 1962); cyanide was used in the extraction of the vitamin from serum.

The rate of excretion of radioactive vitamin B₁₂ was measured in some patients shortly after the blood sample had been taken for estimation of serum B₁₂. ⁵⁸Co-vitamin B₁₂ 0.3 microcurie, in a total of 50 µg., was injected intramuscularly and the urine collected for an eight-hour period followed by a 16-hour period. Aliquots of the urine were counted in an annular scintillation counter, and the percentage excretion of the labelled vitamin calculated.

RESULTS

The mean serum B₁₂ concentration in 60 hospital control patients was 461 µµg. per ml. (S.E. 25.3,

FIG. 1. Serum B₁₂ concentrations in hospital controls and in patients with renal failure.

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TABLE I

SERUM VITAMIN B₁₂ IN RENAL FAILURE

No.	Patient	Serum B ₁₂ (μg./ml.)	Blood Urea (mg./100 ml.)	Duration of Renal Failure (years)	Diagnosis
1	M.B.	1,560	50	About 1	Chronic pyelonephritis
2	A.B.	310	180	> 5	Chronic pyelonephritis
3	W.B.	1,410	135	> 5	Chronic pyelonephritis
4	L.B.	585	75	> 10	Chronic pyelonephritis
5	A.C.	335	50	< 1	Chronic renal failure secondary to arteriosclerosis
6	J.G.	2,270	290	> 1	Chronic glomerulonephritis
7	M.F.	525	160	< 1	Type II nephritis
8	P.K.	940	390	> 1	Chronic glomerulonephritis
9	E.M.	1,290	120	> 2	Chronic pyelonephritis
10	S.P.	625	170	< 1	Malignant hypertension
11	A.P.	810	220	> 10	Polycystic kidneys
12	A.R.	1,050	90	> 1	Nephrocalcinosis
13	J.S.	1,440	170	> 1	Chronic pyelonephritis
14	F.T.	540	200	> 8	Chronic pyelonephritis
15	E.T.	3,500	375	> 1	Chronic pyelonephritis
16	E.Y.	350	150	< 1	Carcinoma cervix; obstructive renal failure
17	A.C.	270	460	< 1	Chronic glomerulonephritis
18	A.T.	910	950	< 1	Carcinoma cervix; obstructive renal failure
19	M.B.	700	340	> 1	Chronic nephritis ? type
20	W.B.	220	65	< 1	Carcinoma prostate; obstructive renal failure
21	F.P.	1,290	100	> 1	Malignant hypertension
22	E.L.	1,475	520	> 1	Chronic renal failure due to gout
23	A.H.	985	145	> 1	Chronic pyelonephritis
24	J.S.	1,180	370	> 1	Polycystic kidneys
25	A.N.	1,020	240	About 1	Chronic renal failure; essential hypertension
26	R.H.	635	185	> 2	Chronic renal failure; cause unknown
27	S.S.	215	165	> 5	Chronic glomerulonephritis
28	E.K.	480	50	< 1	Type II nephritis causing malignant hypertension
29	B.M.	730	70	< 1	Malignant hypertension
30	A.C.	580	150	> 1	Chronic renal failure secondary to arteriosclerosis
31	E.L.	1,425	150	'	Chronic renal failure; prostatic obstruction; urinary infection
32	J.G.	730	310	< 1	Renal failure following appendicectomy ? cause

total range 115 to 950 μg. per ml., 95% range 130 to 930 μg. per ml.). In the 32 patients with renal failure, the mean serum B₁₂ concentration was 949 μg. per ml. (S.E. 115, total range 215 to 3,500 μg. per ml.). The difference between the mean values (calculated by the t test) is highly significant ($P < 0.001$).

The results in the individual patients with renal failure and the controls are compared in Fig. 1; those of the former group are given in Table I together with the diagnosis, the approximate duration of renal failure, and the blood urea level. Fourteen of those with renal failure had a serum B₁₂ concentration falling outside the upper limit of the 95% range for controls and 13 a value above the extreme limit of the control range.

The values of the patients with renal failure (Table I) show no clear relation to the height of the blood urea concentrations nor are they closely correlated with the duration of renal failure, though it is noticeable that no high values occur where the blood urea has been raised for less than a year.

The results of the excretion tests are illustrated in Fig. 2, and may be summarized as follows. In 13 control subjects the mean urinary excretion of labelled vitamin B₁₂ in 24 hours was $8.3 \pm 1.5\%$ of

the dose of which $7.7 \pm 1.6\%$ was excreted in the first eight hours and $0.6 \pm 0.2\%$ in the next 16 hours. In seven patients with renal failure, $8.9 \pm 2.8\%$ was excreted in 24 hours, $5.7 \pm 2.6\%$ in the first eight hours and $3.2 \pm 0.9\%$ in the next 16 hours. Thus in renal failure the percentage of the labelled dose excreted in 24 hours is normal, but excretion is considerably delayed, and the patients excrete more of the dose in the latter part of the collection period than do control subjects. No correlation was observed between the results of the excretion test and the serum B₁₂ concentration in individual cases.

DISCUSSION

Several authors have shown that the urinary excretion of large doses of vitamin B₁₂, such as are used in the Schilling test, is delayed in renal failure (Rath, McCurdy, and Duffy, 1957; Dunn, Walsh, and Holthaus, 1958; Kuhlback and Gräsbeck, 1958; Kuhlback, Nyberg, and Gräsbeck, 1960) but the fact that serum B₁₂ concentrations may be considerably raised has not, as far as we know, been reported. The simplest hypothesis to explain the raised values is that they are the result of failure of renal excretion of the vitamin. Although the results

¹Standard error of the mean.

FIG. 2. Urinary excretion of $^{58}\text{Co-B}_{12}$ in controls and in patients with renal failure.

of the excretion tests which we have carried out in renal failure show that a normal percentage of the dose of labelled vitamin B_{12} is excreted in 24 hours, this does not necessarily mean that there is no serious impairment of excretion of the small amounts of vitamin B_{12} appearing in the urine under physiological conditions. The physiological relevance of tests in which very large doses of vitamin B_{12} are given, enough to raise the concentration of unbound vitamin B_{12} in the serum to a high level, is uncertain, since nothing is known of the normal mechanism of excretion of the vitamin. Since this work was carried out evidence has been presented suggesting that the excretion of large doses of vitamin B_{12} is mainly dependent on the renal filtration rate (Adams, 1961; Watkin, Barrows, Chow, and Shock (1961). The fact that no high serum B_{12} values have been observed in renal failure of short duration is in favour of the hypothesis of failure of excretion, but the occurrence of normal values in patients who have had high blood urea concentrations for over 10 years suggests that this is not the whole explanation.

Failure of excretion of vitamin B_{12} , though the most obvious explanation, is not the only possible cause of raised serum B_{12} values in renal failure. The

kidney contains relatively large amounts of vitamin B_{12} (Halsted, Carroll, and Rubert, 1959), and release of the vitamin from necrosing renal tissue might raise the serum B_{12} by a similar mechanism to that operating in liver damage (Rachmilewitz, Aronovitch, and Grossowicz, 1956). Such increases would be expected to be greatest in acute renal damage such as extensive infarction or tubular necrosis; however, there are no cases of this acute type in the present series. Another possibility is that the serum B_{12} concentrations might be raised in association with an increase in serum B_{12} binding capacity, as they are in chronic myeloid leukaemia (Mollin and Ross, 1955; Miller, 1958). Such an increase in binding capacity might accompany the rise in α -globulins that is frequent in chronic renal disease (Owen, 1958). Failure of utilization of the vitamin by a bone marrow depressed by uraemia might play a part in raising the serum B_{12} concentration in the presence of inadequate excretion; however, in the present series, there was no relation between serum B_{12} and haemoglobin values. Finally it is conceivable, though unlikely, that some unknown substance that is retained in the bloodstream in renal failure might stimulate the growth of the assay organism, so giving falsely high results. These possibilities remain to be investigated. Whatever the explanation of our results, it is evident that renal failure must be added to liver damage and certain myeloproliferative disorders as a possible cause of high serum B_{12} concentrations.

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REFERENCES

- Adams, J. F. (1961). *J. clin. Path.*, **14**, 351.
- Dunn, A. L., Walsh, J. R., and Holthaus, J. M. (1958). *A.M.A. Arch. intern. Med.*, **101**, 927.
- Halsted, J. A., Carroll, J., and Rubert, S. (1959). *New Engl. J. Med.*, **260**, 575.
- Kuhlback, B., and Gräsbeck, R. (1958). *Scand. J. clin. Lab. Invest.*, **10**, 231.
- , Nyberg, R., and Gräsbeck, R. (1960). *Ibid.*, **12**, 140.
- Matthews, D. M. (1962). *Clin. Sci.*, **22**, 101.
- , and Beckett, A. G. (1962). *Ibid.*, **23**, 361.
- Miller, A. (1958). *J. clin. Invest.*, **37**, 556.
- Mollin, D. L., and Ross, G. I. M. (1955). *Brit. J. Haemat.*, **1**, 155.
- Owen, J. A. (1958). In *Advances in Clinical Chemistry*, Vol. 1, edited by H. Sobotka and C. P. Stewart, p. 251. Academic Press, New York.
- Rachmilewitz, M., Aronovitch, J., and Grossowicz, N. (1956). *J. Lab. clin. Med.*, **48**, 339.
- Rath, C. E., McCurdy, P. R., and Duffy, B. J. (1957). *New Engl. J. Med.*, **256**, 111.
- Watkin, D. M., Barrows, C. H. Jr., Chow, B. F., and Shock, N. W. (1961). *Proc. Soc. exp. Biol. (N.Y.)*, **107**, 219.