Vitamin B₁₂ absorption in some neurological and neuroendocrine disorders

D. DONALDSON¹ AND P. T. LASCELLES

From the Department of Chemical Pathology, The Institute of Neurology, The National Hospitals for Nervous Diseases, Queen Square, London

SYNOPSIS An assessment of vitamin B₁₂ absorption in neurological patients has been made by both serum and urine counting of ⁵⁷Co cyanocobalamin during the conventional Schilling test. Although patients with pernicious anaemia, some with subacute combined degeneration of the cord, have been studied, emphasis in the discussion is placed on the significantly increased excretion of the radioactive vitamin in a group of patients with pituitary insufficiency. Results are also given for epileptic patients who have developed folate deficiency (as assessed by serum folate levels) coincidental with anticonvulsant therapy, as well as for some patients who have had neurological symptoms or signs following partial gastrectomy operations.

The Schilling test has been used for establishing the diagnosis of pernicious anaemia since 1953. It has also been a valuable laboratory investigation in patients with malabsorption syndromes. The parameters of normality have been accurately established both for urine and serum (Donaldson, Blight, and Lascelles, 1970), and the present communication describes applications of this technique to the investigation of certain neurological and neuroendocrine diseases.

Methods

COUNTING TECHNIQUE

This is described on page 559.

SELECTION OF PATIENTS

The following groups of patients were selected from an in-patient hospital population over a two-year period:

1 Patients with pernicious anaemia

Parameters used for studying this group included the following: full blood count; serum vitamin B₁₂ level (*Lactobacillus leichmanii*); serum folate level (*Lactobacillus casei*); augmented histamine test meal (in most patients); sternal marrow examination; and antibodies to gastric parietal cells and intrinsic factor (not examined in all patients).

These were all assessed before the Schilling test was performed. Table I shows the haemoglobin values at the time of diagnosis and indicates that many of the patients were only slightly anaemic. One patient (J.S., Table I) had coincidental hypopituitarism.

2 Patients with pituitary insufficiency

This state was assessed by clinical and biochemical criteria. Part I Schilling tests were done on five patients in this group. Four of the patients had pituitary tumours removed (two before and two after the Schilling test), and were examined histologically. Three of these were reported to be chromophobe adenomas, and one was stated to be an adenoma. In the remaining patient the cause of the hypopituitarism had not been established. In addition there was one patient, not included in Table II, who had coincidental

Received for publication 3 March 1970.

¹ Present address; Chemical Pathology Department, Redhill General Hospital, Earlswood Common, Redhill, Surrey. Requests for reprints should be addressed to D.D. at this address.

Patient	Sex	Haemoglobin Level (g/100 ml)	Serum Vitamin B ₁₂ Level (μg/ml)	Schilling Test			
				Part 1		Part 2	
				24-Hour Urine Excretion (% of dose administered)	Eight-hour Serum Level (% of dose/l)	24-Hour Urine Excretion (% of dose)	Eight-hour Serum Level (% of dose/l)
M.C.	F	12.2	65	2.4	—	25.0	1.65
D.T.	F	5.8	95	0.8	0.00	—	0.20
B.F.	M	14.1	130	1.3	0.11	12.2	1.08
A.F.	M	10.5	67	2.1	0.00	17.5	0.74
B.M.	F	10.0	80	1.1	0.00	12.6	1.22
G.G.	F	11.9	81	1.7	0.20	11.6	1.58
T.L.	M	12.2	—	2.4	0.18	21.3	0.84
J.S.	F	13.0	150	2.7	0.26	11.3	0.99
E.T.	F	12.6	30	0.6	0.01	20.8	0.95
E.N.	F	7.9	56	0.5	0.04	11.7	0.88
M.E.	F	11.4	130	2.2	0.18	21.6	1.41
W.C.	F	12.8	—	0.4	0.00	14.0	1.22
Mean				1.5	0.09	16.4	1.07
SD				0.8	0.09	5.2	0.39
Normal mean				18.1	1.05	—	—
SD				3.8	0.25		

Table I Results of Schilling tests on patients with pernicious anaemia

Patient	Sex	24-Hour Urine Excretion (% of dose administered)	Eight-hour Serum Level (% of dose administered/l)
W.M.	M	31.5	1.03
J.C.	M	21.2	2.67
V.T.	F	23.9	1.70
S.S.	M	29.6	1.07
P.C.	M	20.5	0.81
Mean		25.3	1.46
SD		5.0	0.76

Table II Results of part 1 Schilling tests on patients with hypopituitarism

Patient	Sex	Hb Level (g/100 ml)	Serum Folate Level (μg/ml)	24-Hour Urine Excretion (% of dose)	Eight-hour Serum Level (% of dose/l)
D.T.	M	14.9	1.8	32.2	0.84
A.R.	M	13.2	2.9	19.6	0.93
S.P.	F	13.2	1.4	27.2	1.80
P.K.	M	14.4	3.0	5.3	0.47
F.R.	F	13.2	1.8	12.0	0.53
J.S.	F	5.8	3.0	10.0	0.60
J.W.	M	13.0	1.9	33.0	1.58
J.M.	M	14.1	2.0	12.9	1.43
J.U.	F	12.6	3.6	28.9	—
M.B.	F	7.4	1.8	14.6	2.91
Mean				19.0	1.18
SD				9.7	0.72

Table III Results of part 1 Schilling tests on folate-deficient epileptic patients on anticonvulsant drugs

pernicious anaemia, and her result is therefore included under that heading. Three of the five patients investigated were receiving no replacement therapy at the time of the Schilling test. One patient (V.T., Table II) was receiving both thyroxine and cortisone acetate, and one (P.C., Table II) was only receiving cortisone acetate replacement.

3 Epileptic patients

All had been treated with anticonvulsant drugs, and except for one had folate levels of 3.0 μg/ml or less. Red cell folate levels were not arranged as a parameter for investigation in this group.

4 Patients after partial gastrectomy

The patients had had partial gastrectomy operations between one and 14 years previously. All the patients in this group had neurological symptoms and signs following the partial gastrectomy operation. In most the neurological involvement was of a type suggestive of vitamin B₁₂ deficiency, but the vitamin B₁₂ levels were in the normal range in a number of patients, possibly due to short-term vitamin B₁₂ administration before admission.

Five out of the seven patients had proved duodenal ulcers before operation. In two patients (R.S. and A.B., Table IV) the only information available is that they had peptic ulcers.

Serum vitamin B₁₂ was estimated by the microbiological method using *Lactobacillus leichmanii*, and serum folate was measured by the *Lactobacillus casei* method.

Results

PATIENTS WITH PERNICIOUS ANAEMIA

Table I shows the distribution of our data for urine and serum in patients with pernicious anaemia (for part 1 and for part 2 with intrinsic factor). In part 1 of the test the mean values for the urine excretion of ⁵⁷Co vitamin B₁₂ were significantly lower than normal, and similarly for the serum levels, usually rising to normal levels when the test was repeated with the addi-

Patient	Age (yr)	Sex	Hb Level (g/100 ml)	Serum Vitamin B ₁₂ (µg/ml)	Time since Gastrectomy (yr)	Diagnosis	Schilling Test			
							Part 1		Part 2	
							24-Hour Urine Excretion (% of dose)	Eight-hour Serum Level (% of dose/l)	24-Hour Urine Excretion (% of dose)	Eight-hour Serum Level (% of dose/l)
V.R.	60	M	12.6	160	6	DU	6.0	0.68	9.6	1.06
R.S.	54	M	12.6	190	14	PU	7.5	0.66	16.2	1.00
W.C.	67	M	14.5	517	2	DU	16.1	0.83	10.5	0.66
A.W.	62	M	13.0	—	14	DU	17.0	1.00	10.0	0.58
J.McS.	38	M	12.3	650	10	DU	14.2	1.39	10.4	0.58
A.B.	69	F	12.1	544	1	PU	3.6	0.50	10.5	1.22
R.D.	46	M	11.8	330	1	DU	11.2	0.70	9.2	0.85
Mean							10.8	0.83	11.1	0.89
SD							5.2	0.30	2.6	0.24

Table IV Results of Schilling tests on patients following partial gastrectomy

DU = duodenal ulcer

PU = peptic ulcer

Group	Number of Patients	Urine Values	Number of Patients	Serum Values
Ten normal controls and part 2 Schilling tests on patients with pernicious anaemia	11	0.50 >P> 0.40	12	0.95 >P> 0.90
Ten normal controls and part 1 Schilling tests on patients with hypopituitarism	5	0.01 >P> 0.001	5	0.20 >P> 0.10
Ten normal controls and part 1 Schilling tests on epileptic patients with folate deficiency on anti-convulsant drugs	12	0.70 >P> 0.60	11	0.60 >P> 0.50
Ten normal controls and part 1 Schilling tests on gastrectomy patients	7	0.01 >P> 0.001	7	0.20 >P> 0.10

Table V Significance between results in different groups

tion of intrinsic factor. It should be noted that neither for urine nor serum values was there an overlap with the normal range. As seen from Table I there was one patient who showed no significant change for urine or serum values when the test was repeated with intrinsic factor.

STUDIES ON PATIENTS WITH HYPOPITUITARISM

Table II shows the data for this group of patients. The mean for urine and serum is seen to be higher than for normal controls. Table V shows that there is a significant difference between the two groups for the urine values. For serum values the difference is seen to be just not significant; but the number of patients was small.

EPILEPTIC PATIENTS TREATED WITH ANTICONVULSANT DRUGS INDUCING FOLATE DEFICIENCY

Table III shows the urine and serum levels of ⁵⁷Co vitamin B₁₂ for these patients. Table V illustrates that there are no significant differences between these values and the part 1 Schilling test results on normal controls. All the patients in this group had near normal haemoglobin levels

except for two (J.S. and M.B., Table III) who had levels of 5.8 g/100 ml and 7.4 g/100 ml respectively.

STUDIES ON PATIENTS AFTER PARTIAL GASTRECTOMY

The data for this group of patients are given in Table IV. Parts 1 and 2 of the Schilling test were performed in each case except for the last patient in the list (R.D., Table IV).

Table V shows that there are no significant differences between the first and second parts of the test, either for urine or serum if the results are taken together, but individually it is seen that both R.S. and A.B. (Table IV) showed a significant rise. However, patients W.C. and A.W. both showed a fall in the second part of the test.

Discussion

Table I shows that there was clear demarcation between the part 1 Schilling test results (urine and serum levels) for patients with pernicious anaemia (including those with no evidence of anaemia) and those of normal controls, and that there was no overlap between the two groups, confirming the observations of many previous workers.

One patient (D.T., Table I) failed to show a rise in the urinary excretion of ⁵⁷Co vitamin B₁₂ when intrinsic factor was given. This may be in keeping with the observations of several investigators that there is sometimes a malabsorption for vitamin B₁₂ in patients who have suffered deficiency of the vitamin for a considerable time (Haurani, Sherwood, and Goldstein, 1964; Brody, Estren, and Herbert, 1966; Carmel and Herbert, 1967). These authors have demonstrated that this specific malabsorption is reversible when patients are treated with vitamin B₁₂, and they

showed that the Schilling test part 2 became normal after periods ranging from one to 20 months. Some of the patients had only been re-investigated after an elapse of several months or more, and it is therefore possible that the time of response may have been considerably shorter. Goldberg, Bickel, and Fudenberg (1969) similarly described a patient who showed increased absorption of vitamin B₁₂ after six months of treatment; in addition these authors draw attention to the possibility that the presence of gastric antibody to intrinsic factor may be the cause of a low absorption of vitamin B₁₂ in the second part of the Schilling test.

It is of interest that the patient (D.T.) had the lowest haemoglobin in the group (5.8 g/100 ml) and therefore may have been deficient in vitamin B₁₂ for a long period. Unfortunately there was no opportunity to investigate this patient further. There was no clinical evidence of generalized malabsorption. Sauli, Astaldi, and Malossini (1963) studied the histopathology of the intestinal mucosa in patients with pernicious anaemia, before and after vitamin B₁₂ therapy. They showed that there were appreciable changes toward normality after one month of treatment with 500 gamma per day. Foroozan and Trier (1967) demonstrated that after two or more months of vitamin B₁₂ administration the small intestines of all their eight patients were histologically indistinguishable from those of the control subjects.

Apart from this one patient there was no difference between the mean value for part 2 of the Schilling test on patients with pernicious anaemia and the part 1 tests on normal controls (see Table V).

The five patients in the hypopituitary group all showed a urinary excretion of ⁵⁷Co vitamin B₁₂ greater than 20.0%. Two of the patients had excretions greater than the upper limit of the normal range (using 95% confidence limits). In addition, two of the three remaining patients had serum levels of ⁵⁷Co vitamin B₁₂ in excess of the normal range. Hence, four of the five patients would have been separable from the normal controls taking either the urine or serum values alone as significant.

From Table II it is noted that the highest two urinary excretions belonged to patients who were not receiving replacement therapy at the time of the test (W.M. and S.S., Table II). However, the excretion in the third patient not receiving therapy was lower than these, although the eight-hour serum level was the highest of the group.

It is possible that the physiological mechanism for this increased excretion of vitamin B₁₂ in hypopituitarism is in some way related to deficiency of certain hormonal factors, either the trophic hormones produced by the pituitary, or deficient production of hormones by the target organs for these trophic hormones, namely, the thyroid, adrenals, and the gonads.

Also to be considered is the possibility that the adenomatous tissue of the pituitary tumours themselves may be producing a substance which has an effect on vitamin B₁₂ metabolism. It is hoped that further work will establish whether or not pituitary adenomas are the necessary factor or whether hypopituitarism *per se*, whatever its cause, will produce the same effect.

It is established in humans that the thyroid gland influences xylose metabolism. Broitman, Bondy, Yachnin, Hoskins, Ingbar, and Zamcheck (1964) showed that although the absorption of xylose was normal in states of thyrotoxicosis and myxoedema, the urinary excretion was low in myxoedema and increased in hyperthyroidism. Furthermore, they showed that the excretory pattern was similar when the xylose was given intravenously. They calculated that there was a decreased rate of renal clearance and an apparently normal rate of extrarenal disposal in patients with myxoedema, and that there was a decreased rate of extrarenal disposal with a concomitant increase in renal excretion in patients with hyperthyroidism.

Also of importance in this connexion are experiments involving the use of rats in which there was shown to be increased absorption of vitamin B₁₂ when the animals were fed on large doses of desiccated thyroid together with a casein-containing diet, and a decreased absorption of the vitamin when the animals were thyroidectomized (Okuda, Steelman, and Chow 1956).

It would appear, however, that these animal experiments were in conflict with our findings in hypopituitary patients if the thyroid were the factor responsible for the difference from the normal controls. One might thus expect a lowered excretion of vitamin B₁₂ if there was a degree of hypothyroidism secondary to hypopituitarism instead of the increase observed. On the other hand, the aetiology of the hypothyroidism is different in the two examples, and this fact may be of importance in the interpretation of the results. The hypothyroidism of pituitary insufficiency is mediated through the pituitary whereas that of the hypothyroid rats was induced by excision of the thyroid gland, in which case the pituitary gland in each animal of the series was intact. Experiments on vitamin B₁₂ absorption in patients with thyrotoxicosis and myxoedema are in progress.

The question arises whether the thyroid could have an effect on overall vitamin B₁₂ metabolism, or simply on transport across the intestinal mucosa. Thus it is established that the intracellular accumulation *in vitro* of the amino acids cycloleucine, histidine, and valine, and the sugar α -methyl-D-glucopyranoside, were increased in the intestinal segments of rats made hypothyroid by thyroidectomy and radioactive ¹³¹I (London and Segal, 1967). When the hypothyroid animals were treated with triiodo-

thyronine the accumulation of substrate fell toward the control level. The authors suggested that as this phenomenon was seen with an unutilizable amino acid and sugar, it strongly suggested an effect on transport rather than on metabolism.

So far we have considered only the hypothyroid element of the hypopituitary state. Another possible explanation to be considered is that the replacement therapy itself may be responsible for the increased vitamin B₁₂ absorption in the hypopituitary group. That this is not tenable is shown by the fact that three of the five patients were, at the time of the Schilling test, not on any form of therapy.

There is known to be an increased incidence of pernicious anaemia in myxoedema (Leithold, David, and Best, 1958; Forssell and Halunen, 1958; Ardeman, Chanarin, Krafchik, and Singer, 1966; and Strickland, 1969), and also of low serum vitamin B₁₂ levels in hypothyroidism without pernicious anaemia (Tudhope and Wilson, 1962). Again this observation does not account for our hypopituitary observations. Ziffer, Gutman, Pasher, Sobotka, and Baker (1957), however, demonstrated that the whole-blood vitamin B₁₂ values were lower in a group of hyperthyroid patients than in a normal control group and a myxoedematous group. The mean urinary excretion after the vitamin B₁₂ dose was considerably lower in hyperthyroid patients than in the normal group, and the myxoedematous group showed an increased excretion. These authors suggested that vitamin B₁₂ turnover and demand is greater in hyperthyroid patients and lower in myxoedema.

There have been reports of pernicious anaemia in Addison's disease (Berlin, 1952; Strickland, 1969), but no literature is available regarding vitamin B₁₂ levels. Because of the increased absorption of vitamin B₁₂ in Schilling tests in which the dose is given to fasting patients, it does not necessarily follow that there is an increased absorption of vitamin B₁₂ from the food under physiological conditions in these patients.

In order to define the mechanism for the increased excretion of vitamin B₁₂ in the Schilling test we are currently investigating a further group of such patients, some of whom are on replacement therapy. We are also establishing the effect of ACTH, prednisone, and dexamethasone on the absorption of vitamin B₁₂ in a control group of patients, as well as studying the relationship of the thyroid function of the hypopituitary group to the problem. In addition, patients with non-pituitary cerebral tumours and raised intracranial pressure are being similarly investigated.

It is well established that patients who are treated with anticonvulsant drugs, such as phenobarbitone, phenytoin, and primidone, for epilepsy are liable to develop folate deficiency (Reynolds, Chanarin, Milner, and Matthews, 1966). It is also known that the metabolism of folate and

vitamin B₁₂ is closely interdependent (Vilter, 1964). This can be seen by the changes in serum levels of the one in response to changes in serum levels of the other: for example, the tendency to high serum folate levels in pernicious anaemia (Waters and Mollin, 1961; Waters and Mollin, 1962; Waters and Mollin, 1963), and the reduction in the serum vitamin B₁₂ in epileptic patients who are treated with folic acid for folate deficiency induced by anticonvulsant therapy (Hunter, Barnes, and Matthews, 1969).

It was with these facts in mind that Schilling tests were done on a series of folate-deficient epileptic patients who had been treated with various combinations of the drugs mentioned above. Lees (1961) reported that the Schilling test indicated a lower absorption of vitamin B₁₂ in patients who had developed megaloblastic anaemia while on anticonvulsant drugs, and that in six of eight patients a considerable increase occurred when folic acid therapy was given.

In our series, however, there was no significant difference (see Table V) between the series of normal controls and the epileptic patients who were folate deficient. Only two of our series of 10 patients had a significant anaemia, and the explanation of the disparity between our results and those of Lees may lie here.

As shown in Tables III and V, there is no significant difference for the urine and serum levels of ⁵⁷Co vitamin B₁₂ in the Schilling test between the folate-deficient epileptic patients and the series of normal controls.

Table IV illustrates the importance of studying individual patients separately, as certain changes between part 1 and 2 tests do not show up when the group is studied as a whole.

It should be noted that none of these patients was given a vitamin B₁₂-free meal at the time the radioactive vitamin B₁₂ was given in the Schilling test. Deller, Germar, and Witts (1961) noted that when a vitamin B₁₂-free meal was given with the labelled vitamin B₁₂ in the Schilling test absorption was significantly enhanced in patients who had undergone partial gastrectomy. Turnbull (1967) observed similar results in a series of patients with anaemia after Polya partial gastrectomy, and suggested that the improved absorption of vitamin B₁₂ with food was likely to be due to stimulation of a small residual secretion of intrinsic factor.

These initial findings by the more precisely standardized Schilling test counting both urine and serum radioactivity confirm and extend the observations of others. The explanations for the mechanism of increased excretion of radioactive vitamin B₁₂ in patients with pituitary tumours and the fall in excretion in part 2 in some post-gastrectomy patients with neurological complications are not at present apparent and clearly need further investigation.

We wish to thank the physicians and surgeons

of the National Hospitals, Queen Square and Maida Vale, for permission to investigate patients under their care.

We are also grateful to Mrs Carol Pye at the Hospital for Sick Children, Great Ormond Street, for the serum folate assays.

References

- Ardeman, S., Chanarin, I., Krafchik, B., and Singer, W. (1966). Addisonian pernicious anaemia and intrinsic factor antibodies in thyroid disorders. *Quart. J. Med.*, **35**, 421-431.
- Berlin, R. (1952). Addison's disease: familial incidence and occurrence in association with pernicious anaemia. *Acta med. scand.*, **144**, 1-6.
- Brody, E. A., Estren, S., and Herbert, V. (1966). Coexistent pernicious anaemia and malabsorption in four patients (including one whose malabsorption disappeared with vitamin B₁₂ therapy). *Ann. intern. Med.*, **64**, 1246-1251.
- Broitman, S. A., Bondy, D. C., Yachnin, I., Hoskins, L. C., Ingbar, S., and Zamcheck, N. (1964). Absorption and disposition of D-xylose in thyrotoxicosis and myxoedema. *New Engl. J. Med.*, **270**, 333-337.
- Carmel, R., and Herbert, V. (1967). Correctable intestinal defect of vitamin B₁₂ absorption in pernicious anaemia. *Ann. intern. Med.*, **67**, 1201-1207.
- Diller, D. J., Germar, H., and Witts, L. J. (1961). Effect of food on absorption of radioactive vitamin B₁₂. *Lancet*, **1**, 574-577.
- Donaldson, D., Blight, B. J. N., and Lascelles, P. T. (1970). An assessment of serum ⁵⁷Co cyanocobalamin as an index of vitamin B₁₂ absorption. *J. clin. Path.*, **23**, 588-562.
- Foroozan, P., and Trier, J. S. (1967). Mucosa of the small intestine in pernicious anaemia. *New Engl. J. Med.*, **277**, 553-559.
- Forsell, J., and Halunen, P. I. (1958). Thyroid function and pernicious anaemia. Comments on some typical cases. *Acta med. scand.*, **162**, 61-68.
- Goldberg, L. S., Bickel, Y. B., and Fudenberg, H. H. (1969.) Immunologic approaches to malabsorption of vitamin B₁₂. *Arch. intern. Med.*, **123**, 397-400.
- Haurani, F. I., Sherwood, W., and Goldstein, F. (1964). Intestinal malabsorption of vitamin B₁₂ in pernicious anaemia. *Metabolism*, **13**, 1342-1348.
- Hunter, R., Barnes, J., and Matthews, D. M. (1969). Effect of folic acid supplement on serum-vitamin-B₁₂ levels in patients on anticonvulsants. *Lancet*, **2**, 666-667.
- Lees, F. (1961). Radioactive vitamin B₁₂ absorption in the megaloblastic anaemia caused by anticonvulsant drugs. *Quart. J. Med.*, **30**, 231-248.
- Leithold, S. L., David, D., and Best, W. R. (1958). Hypothyroidism with anaemia demonstrating abnormal vitamin B₁₂ absorption. *Amer. J. Med.*, **24**, 535-548.
- London, D. R., and Segal, S. (1967). *In vitro* studies on the intestinal transport of amino acids and a sugar in hypothyroid rats. *Endocrinology*, **80**, 623-628.
- Okuda, K., Steelman, S., and Chow, B. F. (1956). Absorption of vitamin B₁₂ in hyper- and hypothyroid rat. *Fed. Proc.*, **15**, 567.
- Reynolds, E. H., Chanarin, I., Milner, G., and Matthews, D. (1966). Anticonvulsant therapy, folic acid and vitamin B₁₂ metabolism and mental symptoms. *Epilepsia*, **7**, 261-270.
- Sauli, S., Aastaldi, G., and Malossini, L. (1963). Histopathology of intestinal mucosa obtained by Crosby's biopsy in pernicious anemia before and after vitamin B₁₂ treatment. *Acta vitamin. (Milano)*, **17**, 143-152.
- Strickland, R. G. (1969). Pernicious anemia and polyendocrine deficiency. *Ann. intern. Med.*, **70**, 1001-1005.
- Tudhope, G. R., and Wilson, G. M. (1962). Deficiency of vitamin B₁₂ in hypothyroidism. *Lancet*, **1**, 703-706.
- Turnbull, A. L. (1967). Absorption of vitamin B₁₂ in patients with anaemia after Polya partial gastrectomy. *Brit. J. Haematol.*, **13**, 752-763.
- Vilter, R. W. (1964). Inter-relationships between folic acid, vitamin B₁₂ and ascorbic acid in the megaloblastic anaemia. *Medicine (Baltimore)*, **43**, 727-730.
- Waters, A. H., and Mollin, D. L. (1961). The folic acid activity of serum in normal subjects and patients with megaloblastic anaemia. In *Proceedings of the 8th Congress of the European Society of Haematology*, Vienna (Paper 330), 1961, vol. 2, Karger, Basle and New York.
- Waters, A. H., and Mollin, D. L. (1962). Studies on the folic acid activity of human serum. *J. clin. Path.*, **14**, 335-344.
- Waters, A. H., and Mollin, D. L. (1963). Observations on the metabolism of folic acid in pernicious anaemia. *Brit. J. Haematol.*, **9**, 319-327.
- Ziffer, H., Gutman, A., Pasher, I., Sobotka, H., and Baker, E. (1957). Vitamin B₁₂ in thyrotoxicosis and myxoedema. *Proc. Soc. exp. Biol. (N.Y.)*, **96**, 229-230.