treatment differs from post-sanatorium care, but both should be of interest to the regional hospital board and the local health authority. Without the mutual co-operation of these two bodies there is little hope of the development of efficient domiciliary care and treatment. In fact, there is urgent need for a complete revision of our tuberculosis administration and the creation of a special service which the peculiar characteristics of the disease demands.

REFERENCES

Cuthbert, J. (1950). *Tubercle*, **31**, 131.
Heller, R. (1949). Ibid., **30**, 204.
Maclean, A., and Gemmill, J. S. (1948). *Lancet*, **1**, 173.
Wynn-Williams, N., Shaw, J. Brian, and Mashiter, W. E. (1950). Ibid., **1**, 221.

THE CONTENT OF HAEMOPOIETIC FACTORS IN LIVER EXTRACTS

RELATIONSHIP TO CLINICAL RESPONSE

BY

RONALD H. GIRDWOOD, M.B., Ch.B., F.R.C.P.Ed.
Lecturer in Medicine, University of Edinburgh

AND

KATHLEEN M. CARMICHAEL, M.B., Ch.B.
Assistant, Department of Medicine, University of Edinburgh

With Statistics by B. WOOLF, M.A., Ph.D., F.R.S.E.
Lecturer in Medical Statistics, Department of Public Health and Social Medicine, University of Edinburgh

In recent years there has been considerable discussion about the potency or otherwise of liver extracts available in this country for the treatment of the megaloblastic anaemias. This has been referred to by Mollin (1950), who has given clinical evidence of a decline in the potency of commercial liver extracts during the period from 1943 to 1948.

Since vitamin B_{12} was isolated from liver almost simultaneously in Great Britain (Lester Smith, 1948; Lester Smith and Parker, 1948) and in the United States (Rickes *et al.*, 1948), it has become clear that this substance is the main therapeutically active material present in refined liver extracts used for the treatment of pernicious anaemia, and vitamin B_{12} is therefore generally considered to be what was formerly known as the "specific anti-anaemic factor." Ungley (1949) has reported on the therapeutic activity of vitamin B_{12} in a series of 53 patients with pernicious anaemia, and Cuthbertson *et al.* (1949) and Shaw (1949a) have assayed certain liver extracts for vitamin B_{12} content and discussed the relationship of this to their clinical activity. A detailed review of the clinical and experimental uses of vitamin B_{12} and related factors has been made by Girdwood (1950).

The object of the present investigation was to estimate the vitamin B_{12} and pteroylglutamic acid (folic acid) content of various liver extracts that had been prepared commercially between the years 1945 and 1950, and to compare the vitamin B_{12} activity as assayed by microbiological methods with the clinical response on the basis of the results of Ungley (1949), who used vitamin B_{12} itself as the therapeutic agent in cases of pernicious anaemia and put forward a formula to indicate the rise in red cells that might be expected to occur with various doses. In addition, by the kind co-operation of certain manufacturers of liver extracts it was found possible to compare a few of our vitamin B_{12} estimations with the results obtained by these commercial houses. Estimation of vitamin B_{12} was carried out in all instances by measuring the turbidity produced by the growth of organisms in a vitamin-B_{12}-deficient medium to which had been added various dilutions of the liver extracts to be tested. These were compared with a standard obtained by measuring the growth sustained by adding known amounts of vitamin B_{12} to the same culture medium.

Methods

The patients were typical cases of Addisonian pernicious anaemia with histamine-fast achlorhydria and a megaloblastic bone marrow. There was no evidence of steatorrhoea or of any abnormality of dietetic habits. A control period without treatment preceded the injections of liver extract, and during this period there was no evidence of spontaneous remission. All the haematological investigations were carried out by one of two experienced research technicians whose results were in close agreement.

The liver extracts were kept in a refrigerator from the time of issue for clinical testing except where otherwise noted.

The microbiological assays for vitamin B_{12} were carried out by an unpublished modification of the method of Hoffmann *et al.* (1949), using *Lactobacillus leichmannii* as the test organism. Hoffmann and his co-workers in one of their methods added thioglycollic acid to the culture medium to prevent loss of vitamin B_{12} activity as a result of autoclaving, but we preferred to omit the thioglycollic acid and to add the samples aseptically after the medium had been autoclaved It has been shown (Cuthbertson and Lester Smith, 1949; Shaw, 1949b; Winsten and Eigen, 1949) that liver extracts contain substances other than vitamin B_{12} capable of sustaining the growth of *L. leichmannii*. These can be separated by methods which include partition chromatography and by destroying the vitamin B_{12} by alkaline hydrolysis and then re-estimating the ability of the extract to support growth of the test organism. We tested our liver extracts by the latter method, and showed that substances other than vitamin B_{12} were responsible for only a negligible fraction of the growth-supporting activity of the samples tested, except in the case of the extract produced by Manufacturer C, 1948 (Table III), where 25% of this activity was due to substances other than vitamin B_{12}.

In each instance our vitamin B_{12} assay figure is the mean of three readings, and, as will be seen from Table I, the readings were in close agreement. The discrepancies within a group of triplicates are approximately proportional to the absolute value of the mean. The logarithms of the readings were therefore taken for statistical treatment.

TABLE I.—*Results of Vitamin B_{12} Assays on 16 Liver Extracts, Using a Tube Method with L. leichmanii as Test Organism*

Extract No.	Results of Three Assays (μg./ml.)			Mean
1	7·5	7·7	7·6	7·6
2	5·4	5·5	5·4	5·4
3	4·1	5·5	4·7	4·8
4	4·4	3·4	4·9	4·2
5	4·3	4·0	3·7	4·0
6	3·95	3·3	3·75	3·7
7	2·85	2·3	2·9	2·7
8	1·9	2·0	2·3	2·1
9	2·05	2·2	2·0	2·1
10	2·0	2·2	1 8	2·0
11	1·3	1·6	1·3	1·4
12	1·3	1·2	1·25	1·25
13	0·92	0·99	0·82	0·91
14	0·47	0·45	0·45	0·46
15	0·36	0·34	0·35	0·35
16	0·24	0·27	0·24	0·25

The logarithmic standard error of the mean is 0.02302, and the 5% fiducial limits are ±0.04698. This implies that the standard limit of the mean is about ±5.3% of the assay result, and it can safely be taken to be within the range 90 to 111% of the true mean in 19 cases out of 20.

In addition to our own results, we were fortunate in having assays performed for us on a few extracts by three commercial firms, and the results of these tests are shown in Table II.

TABLE II.—*Comparison of Vitamin B_{12} Assay Results With Those of Three Manufacturing Firms (μg/ml.)*

Extract	Manufacturing Firm	*L. leichmannii*, Tube Method			*L. lactis* Dorner, Plate Method
		Own Assay	Firm X	Firm Y	Firm Z
1 ..	Z	3·7	3·1	3·8	5·56
2 ..	X	0·46	0·27	—	—
3 ..	X	7·6	14·4	7·2	10·2
4 ..	X	4·0	1·4	—	2·1

The pteroylglutamic acid estimations were carried out by the method of Teply and Elvehjem (1945), using *Streptococcus faecalis* as the test organism, and measuring the growth of the organisms by turbidity estimations in a Spekker photoelectric absorptiometer.

In the paper by Ungley (1949), Campbell gives a formula to express the expected red cell rise when a dosage of 5, 10, 20, 40, or 80 μg. of vitamin B_{12} is given. Campbell's formula was calculated from the actual rise of red cells over a period of 15 days in 63 cases receiving vitamin B_{12}. In our calculations we have the slight inaccuracy that in most instances the red cell count had been done on the 14th rather than the 15th day (the day of injection being calculated as Day 0). The difference in red cell level between 14th and 15th days was extremely small in the few instances where a count was done on both days.

Statistical examination reveals that Campbell's formula is not wholly satisfactory. In the first place, for reasons given in the original paper, he considered it advisable to omit all data derived from the administration of very low doses of vitamin B_{12}, and to reject a discrepant observation within the range of doses he included (a patient who showed an exceptionally large red cell rise with 5 μg. of vitamin B_{12}). In the second place, none of the three regression coefficients in the formula he actually uses is statistically significant. In fact, he gives two formulae—one linear with respect to initial erythrocyte count and log. dose, and the other containing also a product term. The simpler formula, which he rejects, actually has the higher statistical credentials. To be on the safe side we have calculated our own results in terms of both formulae. As given by Campbell, these formulae are not convenient for the calculation of the expected responses at doses other than those he used. We have therefore used algebraically equivalent forms, in which the dose is expressed in ordinary logarithms to base 10. The two formulae are:

(a) $I_{15} = 0.684 - 0.131\ E + 0.814\ P - 0.056\ PE$
(b) $I_{15} = 0.913 - 0.199\ E + 0.641\ P$

where

I_{15} = Expected increase in red blood cell count (millions per c.mm.) on the 15th day

E = Initial red blood cell count (millions per c.mm.) on day of injection

P = Logarithm of number of μg. of vitamin B_{12} injected.

Ungley has pointed out that the formula used in his paper is unsatisfactory for doses of 2.5 μg. or less of vitamin B_{12}. For this reason we have divided our findings into two groups according to whether the dosage of vitamin B_{12} given was greater or less than 4 μg.

Results

The results of our assays for vitamin B_{12} and pteroylglutamic acid on 15 extracts that were tested clinically on 20 patients are given in Table III. All but one of these had been marketed as a high potency extract. In Table IV

TABLE III.—*Vitamin B_{12} and Pteroylglutamic Acid Content of Liver Extracts*

Manufacturer	Date of Manufacture	Vitamin B_{12} Content (μg./ml.)	P.G.A. Content (μg./ml.)
A	1945	5·45	0·150
B	1945	2·7	0·594
A	1946	1·25	0·062
B	1946	1·4	0·538
B	1947	2·1	0·253
B	1947	2·1	0·525
B	1947	0·9	0·272
A	1948	4·0	0·041
C	1948	0·25	0·335
A	1948*	0·46	0·378
B	1948	2·0	0·173
A	1949	7·6	0·067
D	1949	0·35	0·906
B	1950	4·8	0·494
B	1950	3·7	0·149

* Extract was not marketed as a high-potency extract.

TABLE IV.—*Vitamin B_{12} Content of Liver Extracts and B_{12} Preparations Purchased Commercially in 1950*

Preparation	Country of Origin	Manufacturer's Statement of Vitamin B_{12} Content or Potency	Content According to Our Assay
Vitamin B_{12} ..	U.K.	20 μg /ml.	16·5 μg./ml.
,, ,, ..	U.K.	10 μg /ml.	10·0 μg /ml.
Liver extract ..	U.K.	20 μg /ml.	3·75 μg /ml.
,, ,, ..	U.S.A.	15 U.S.P. units	1·5 μg /ml.

are given the results for four preparations of stated potency purchased in 1950. The American extract was provided by a patient who visited the United States.

In Tables V and VI are given the actual red cell rises when these extracts were used clinically, the expected cell rises according to each of the above formulae, and the differences between the observed and the calculated rises. Table V refers to the 12 cases receiving 4 μg. or more of vitamin B_{12}, and Table VI to the eight patients who had less than this amount. It will be seen that the expected values on the two formulae are closely similar. Table VII summarizes the results of the comparison of observed and expected values. In the 12 cases in the first group the mean red blood cell rise was 1,068,000. The mean discrepancy on each of the two formulae was very small. The spread of values about the mean, conveniently measured by the standard deviation, was 0.508. Use of either formula reduces the standard deviation to about 0.45. This means that the formula is of some value for prediction but the reduction of standard deviation in these few cases is not statistically significant. On the other hand, in the second (low-dose) group, to which Campbell's formula is not supposed to apply, the predictive value of the formula is considerable and statistically significant.

We therefore combined the two groups, and the predictive value of the formulae on all the cases is shown at the bottom of Table VII. The standard deviation is reduced by about 22%, although, at 0.47, it is still

TABLE V.—*Red Cell Response to Injections of Liver Extract of Known Vitamin B_{12} Content (Dose More Than 4 μg.)*

Case No.	Sex	Age	Initial Red Cell Level (mills./c.mm.)	Vitamin B_{12} Given in Liver Extract (μg.)	Retic. Peak and Day	Actual Red Cell Rise (mills./c.mm.) and Day	Formula (a)		Formula (b)		Volume of Dose (ml.)
							Expected Red Cell Rise (mills./c.mm.) at 15 Days	Difference (Actual—Expected)	Expected Red Cell Rise (mills./c.mm.)	Difference (Actual—Expected)	
1	F	46	1·48	15·2	19·4 (5)	0·92 (14)	1·35	−0·43	1·38	−0·46	2
2	F	50	2·08	10·9	22·4 (5)	1·86 (15)	1·13	+0·73	1·16	+0·70	2
3	M	68	1·44	10·9	23·4 (4)	1·41 (14)	1·26	+0·15	1·29	+0·12	2
4	F	67	2·32	10·9	20·6 (5)	0·90 (15)	1·09	−0·19	1·12	−0·22	2
5	F	49	1·61	8·0	22·4 (5)	1·10 (14)	1·13	−0·03	1·17	−0·07	2
6	F	66	1·65	8·0	3·4 (6)	1·50 (14)	1·12	+0·38	1·16	+0·34	2
7	M	67	1·89	7·4	5·2 (6)	0·37 (15)	1·05	−0·68	1·09	−0·72	2
8	M	73	1·35	5·4	9·8 (6)	0·73 (14)	1·05	−0·32	1·11	−0·38	2
9	F	40	0·95	4·2	22·0 (6)	1·71 (15)	1·03	+0·68	1·12	+0·59	2
10	F	52	1·51	4·2	6·2 (6)	0·30 (15)	0·94	−0·64	1·01	−0·71	2
11	F	75	1·30	4·2	18·0 (5)	1·07 (14)	0·98	+0·09	1·05	+0·02	2
12	M	73	2·20	4·0	8·4 (6)	0·94 (15)	0·81	+0·13	0·86	+0·08	2

TABLE VI.—*Red Cell Response to Injections of Liver Extract of Known Vitamin B_{12} Content (Dose Less Than 4 μg.)*

Case No.	Sex	Age	Initial Red Cell Level (mills./c.mm.)	Vitamin B_{12} Given in Liver Extract (μg.)	Retic. Peak and Day	Actual Red Cell Rise (mills./c.mm.) and Day	Formula (a)		Formula (b)		Volume of Dose (ml.)
							Expected Red Cell Rise (mills./c.mm.) at 15 Days	Difference (Actual—Expected)	Expected Red Cell Rise (mills./c.mm.)	Difference (Actual—Expected)	
13	F	34	1·21	2·8	17·4 (5)	1·51 (14)	0·86	+0·65	0·96	+0·55	2
14	F	63	1·33	2·5	22·4 (4)	1·81 (15)	0·80	+1·01	0·90	+0·91	2
15	F	59	1·97	1·8	10·4 (5)	0·91 (14)	0·61	+0·30	0·68	+0·23	4
16	F	55	2·48	1·8	4·0 (7)	0·14 (15)	0·53	−0·39	0·58	−0·44	4
17	F	42	1·61	1·8	9·0 (5)	0·40 (15)	0·66	−0·26	0·76	−0·36	2
18	F	67	1·73	1·4	12·0 (6)	1·10 (14)	0·56	+0·54	0·66	+0·44	4
19	F	59	2·35	0·5	1·2 (5)	−0·06 (14)	0·17	−0·23	0·25	−0·31	2
20	F	62	1·74	0·5	1·9 (4)	−0·16 (14)	0·24	−0·40	0·37	−0·53	2

TABLE VII.—*Comparison of Observed and Expected Values for Red Cell Rise*

	Observed Red Cell Rise	Observed Minus Expected Red Cell Rise	
		Using Formula (a)	Using Formula (b)
Dosage of vitamin B_{12} 4 μg. or more (12 cases):			
Mean (mills./c.mm.)	1·068	−0·011	−0·059
Standard deviation ..	0·508	0·466	0·448
Dosage of vitamin B_{12} less than 4 μg. (8 cases):			
Mean (mills./c.mm.)	0·706	+0·153	+0·061
Standard deviation ..	0·738	0·531	0·510
Both groups (20 cases):			
Mean (mills./c.mm.)	0·923	+0·055	−0·011
Standard deviation ..	0·609	0·482	0·473

considerably higher than the value of 0.33 claimed by Ungley and Campbell. Formula (b) seems slightly better than formula (a).

It seemed worth while to obtain from our data the best-fitting formula, for comparison with that put forward by Ungley. The analogue of his formula (b) worked out as :

$$I_{15} = 1.474 - 0.542\ E + O.665\ P.$$

It is remarkably similar to that of Ungley, differing mainly in the increased importance given to the influence of the patients' initial counts on the final result. We believe that, by omitting his cases receiving a low dosage of vitamin B_{12}, Ungley artificially minimized the importance of this factor.

It is important to stress that, as is suggested by Table II, different results might have been obtained and different conclusions reached had the vitamin B_{12} assays been carried out by other workers using various modifications of the assay methods.

As has been found by previous workers (Stokstad and Jukes, 1946), the pteroylglutamic acid content of the extracts was in all instances therapeutically negligible.

In order to see how small a dose of vitamin B_{12} might be effective, we gave one patient, suffering from classical Addisonian pernicious anaemia, a single injection of 0.4 μg. of vitamin B_{12} itself after a control period. It will be seen from the Graph that we seem to have selected by chance a patient who was able to show some response even to this small dosage, for the red cell count rose by 670,000 per c.mm. in 13 days, and there was a partial

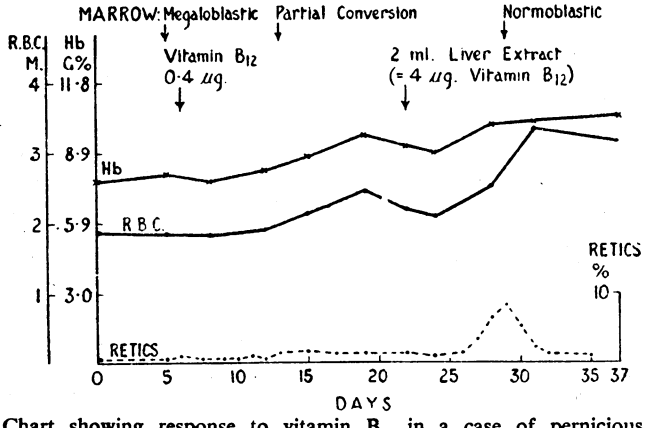

Chart showing response to vitamin B_{12} in a case of pernicious anaemia (man aged 73).

conversion of the bone marrow towards the normoblastic state, as evidenced by condensation of the nuclei of the megaloblasts.

Discussion

The results of this investigation indicate that the vitamin B_{12} content of all 15 batches of liver extracts manufactured between the years 1945 and 1950 and tested both microbiologically and clinically was low or very low. For comparison we have the figure of 25 μg./ml. for an American liver extract given by Mollin (1950). On the other hand, we have not included in the series a British liver extract, marketed in 1948 but not tested clinically by us, which we found to have a vitamin B_{12} content of 14 μg./ml. It is of interest that we obtained similar results

on assaying two samples of the same batch of one extract manufactured two years previously, one ampoule of which had been kept in a refrigerator and the other sent back to us from the Fiji Islands.

It has been suggested by Rickes *et al.* (1948) and by many workers since then that 1 U.S.P. unit of activity is approximately equal to 1 μg. of vitamin B_{12}, and the aim of manufacturers has been to market high-potency extracts containing 15 U.S.P. units of activity. How far the extracts considered in the present paper fall short of this target is evident. It should be realized that it was not the fault of British manufacturers that the vitamin B_{12} content of their products was low at a time when clinical testing was the only method of assay available to them, and individual variation to the same dose was so great that it would no doubt be possible to show the presence of "15 U.S.P. units of activity" in many of the above extracts if those testing them persevered long enough to find a sufficiently sensitive patient. Nor should it be thought that all American extracts are superior to British ones. Personal experience by one of us of the clinical results obtained in the United States by various liver extracts has convinced us that low-potency extracts were not uncommon across the Atlantic, too, when microbiological assay methods were not available, and from Table IV we see that a "high-potency" extract marketed in 1950 by a reputable American firm contained only 1.5 μg./ml.

The relationship between the actual red cell rise and the expected rise when liver extracts are used clinically, and calculating from Ungley's results with vitamin B_{12} itself, is obviously of importance. One should not, however, attach too much importance to formulae, especially those based on the relatively meagre data here considered, even if Ungley's findings and those of the present series are considered together. The random errors are so high, and the other relevant factors not taken into account are probably so important, that the formulae can do little more than indicate a qualitative relationship. All one can say with confidence at the moment is that the data indicate that the red blood cell rise is greater the lower the initial state of the patient and increases with increase of dose, and give some idea of the order of magnitude of the influence of these factors. We must add, however, that a statistical analysis of all available data may give one a fair idea of the quantitative importance of these influences. A study of this nature is planned.

In view of this, our conclusions must be guarded, but it is true to say that we have produced no evidence to support the view that the results in these cases cannot be explained on the assumption that the haemopoietic responses are due to the vitamin B_{12} content of the extracts. In order to suggest that the activity of a liver extract was not due solely to its vitamin B_{12} content it would be necessary to show a consistent and definite difference in response to the injection of that particular extract given to a relatively large number of patients. When the actual response is much greater than the expected response the chief possibilities are: (*a*) that this is a case that, by reason of some individual variation as yet not understood, responds unusually well to the dosage given; (*b*) that a spontaneous remission has coincided with the test; (*c*) that there is some substance present in the liver extract that has a haemopoietic effect and is not a growth factor for *L. leichmannii* or any other test organism used; (*d*) that the liver extract contains, in addition to vitamin B_{12}, inhibitors for the growth of *L. leichmannii*; or (*e*) that some of the vitamin B_{12} is present in a conjugate form that does not support the growth of *L. leichmannii*.

The significance of these factors is not yet understood. An artificially produced hydrogenation product of vitamin B_{12} has been named vitamin B_{12a} (Kaczka *et al.*, 1949), and it is possibly the same as vitamin B_{12b} isolated in crystalline form by Pierce *et al.* (1949) (Brockman *et al.*, 1950). More recently, vitamin B_{12c} has been isolated (Buchanan *et al.*, 1950), but so far there is no published evidence of the presence in liver of forms active clinically and not microbiologically, although reference to the possible existence of such forms has been made by Lester Smith (1950).

The problem of whether liver extract is active haemopoietically only by virtue of its vitamin B_{12} content may also be approached by comparison of the results of maintenance therapy. Recently Meacham *et al.* (1950) have reported that a vitamin B_{12} concentrate in a dosage equivalent to 1 μg. daily, but given every three or four weeks, did not maintain optimal blood levels in pernicious anaemia patients. They attribute this to inadequate dosage of vitamin B_{12}, but further work of this nature is indicated and other possible explanations require investigation, including the possibility that vitamin B_{12} is absorbed and excreted less rapidly if injected in the form of a liver extract.

It is of some interest to know how many patients would be required to assay a liver extract or vitamin B_{12} preparation clinically to any desired degree of precision. An answer to this question may emerge from our further statistical study. On the basis of the formulae used in this paper, for what they are worth, one can make the following rough estimates. If the true potency of a preparation be put at 100, then, using 4 patients, one would expect, in 19 times out of 20, to get a result only within the range 30 to 330; 16 patients would give an accuracy, in 95% of cases, between about 60 and 160; 64 patients between 80 and 125; while to get within 10% of the true result 250 or more subjects would be required. The same order of magnitude of error emerges from the results of the present series using liver extracts and from those of Ungley (1949) using vitamin B_{12}.

When microbiological assay is carried out, however, it is important that the method should be standardized, since, as we have seen in Tables II and IV, quite marked discrepancies may occur. At present the best practicable method of testing liver extracts for haemopoietic activity would appear to be by the combination of clinical tests on a few patients and microbiological assay by an agreed standard technique

Summary

The vitamin B_{12} content of 15 batches of British liver extracts prepared for commercial use between 1945 and 1950 and tested microbiologically by a tube method using *L. leichmannii* as the test organism was low, varying from 0.25 μg./ml. to 7.6 μg./ml. The pteroylglutamic acid content was negligible.

The actual rise of red cells in 20 pernicious anaemia patients treated with these liver extracts was compared with the calculated response according to the vitamin B_{12} content. Too much importance should not be attached to formulae, especially in view of the relatively meagre data available, but the results give no evidence to suggest that the haemopoietic responses cannot be attributed to the vitamin B_{12} content of the liver extracts.

Clinical testing alone of liver extracts or vitamin B_{12} concentrates may give very inaccurate information, but, although it is possible to obtain consistent results with a single microbiological method of vitamin B_{12} assay, there may be considerable variation between the results obtained by workers using different microbiological methods. Hence it is important that a standard method be agreed upon.

We are very grateful to Professor L. S. P. Davidson, Dr. W. A. Alexander, and Dr. T. R. R. Todd for access to clinical material in their wards, and to Professor L. S. P. Davidson and Professor F. A. E. Crew for their interest in this work. We wish to thank Dr. Augustus Gibson, of Messrs. Merck, Inc., Rahway, New Jersey, for supplying us with crystalline vitamin B_{12} for use as a standard. One of us (R. H. G.) was in receipt of a grant from the John Risk Bequest, and grateful acknowledgment is made for the assistance given in defraying the expenses of the investigation.

REFERENCES

Brockman, J. A., Pierce, J. V., Stokstad, E. L. R., Broquist, H. P., and Jukes, T. H. (1950). *J. Amer. chem. Soc.*, **72**, 1042.
Buchanan, J. G., Johnson, A. W., Mills, J. A., and Todd, A. R. (1950). *Chem. & Ind.*, p. 426.
Cuthbertson, W. F. J., Lloyd, J. F., Emery, W. B., and Lees, K. A. (1949). *J. Pharm. Pharmacol.*, **1**, 705.
—— and Smith, E. Lester (1949). *Biochem. J.*, **44**, v.
Girdwood, R. H. (1950). *Edinb. med. J.*, **57**, 72.
Hoffmann, C. E., Stokstad, E. L. R., Hutchings, B. L., Dornbush, A. C., and Jukes, T. H. (1949). *J. biol. Chem.*, **181**, 635.
Kaczka, E., Wolf, D. E., and Folkers, K. (1949). *J. Amer. chem. Soc.*, **71**, 1514.
Meacham, G. C., Vignos, P. J., Heinle, R. W., Weisberger, A. S., and Epstein, M. (1950). *J. Lab. clin. Med.*, **35**, 713.
Mollin, D. L. (1950). *Lancet*, **1**, 1064.
Pierce, J. V., Page, A. C., jun., Stokstad, E. L. R., and Jukes, T. H. (1949). *J. Amer. chem. Soc.*, **71**, 2952.
Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K. (1948). *Science*, **107**, 396.
Shaw, G. E. (1949a). *J. Pharm. Pharmacol.*, **1**, 701.
—— (1949b). Ibid., **1**, 695.
Smith, E. Lester (1948). *Nature, Lond.*, **161**, 638.
—— (1950). *Proc. R. Soc. Med.*, **43**, 535.
—— and Parker, L. F. J. (1948). *Biochem. J.*, **43**, viii.
Stokstad, E. L. R., and Jukes, T. H. (1946). *Proc. Soc. exp. Biol., N.Y.*, **62**, 112.
Teply, L. J., and Elvehjem, C. A. (1945). *J. biol. Chem.*, **157**, 303.
Ungley, C. C. (1949). *British Medical Journal*, **2**, 1370.
Winsten, W. A., and Eigen, E. (1949). *J. biol. Chem.*, **177**, 989.

THE MANUBRIO-STERNAL JOINT IN RHEUMATOID ARTHRITIS

BY

ANDREW BOGDAN, M.D.

AND

JUSTIN CLARK, M.D., M.R.C.P.

(*From the Rheumatism Unit, Westminster Hospital*)

[WITH SPECIAL PLATE]

Examination of the manubrio-sternal joint in a series of patients has shown that rheumatoid involvement of this joint is not uncommon. Five examples are outlined here to indicate the clinical and radiological features.

Case Reports

Case 1.—A married woman aged 55 gave a five-year history of rheumatoid arthritis, still active, and showing typical clinical and radiological involvement of feet, wrists, fingers, and right elbow. The manubrio-sternal joint became painful two years after the onset of the disease, and the patient noticed that there was swelling and tenderness over the joint. The pain came in bouts lasting two to three days; between the bouts there was a residual ache. During the bouts the pain was aggravated by coughing and sneezing, and inhibited deep respiratory movement. Examination of the joint confirmed the presence of soft-tissue swelling and tenderness (Fig. A). Radiographs showed irregular destruction of the articular margins of the joint (Plate, Fig. 1). The sedimentation rate was 31 mm. (Wintrobe corrected) at the end of one hour.

Case 2.—A married woman aged 52, who gave a twenty-two year history of rheumatoid arthritis, starting with pain, swelling, and tenderness over the manubrio-sternal joint, showed clinical and radiological evidence of active disease in almost all the peripheral joints. The manubrio-sternal pain had lasted for one month only, and had not recurred. Examination showed no clinical abnormality of the joint, but a radiograph revealed gross narrowing and irregularity of the joint space, with marked irregular expansion of the articulating ends of the bones (Fig. 2). The sedimentation rate was 30 mm. (Wintrobe corrected) at the end of one hour.

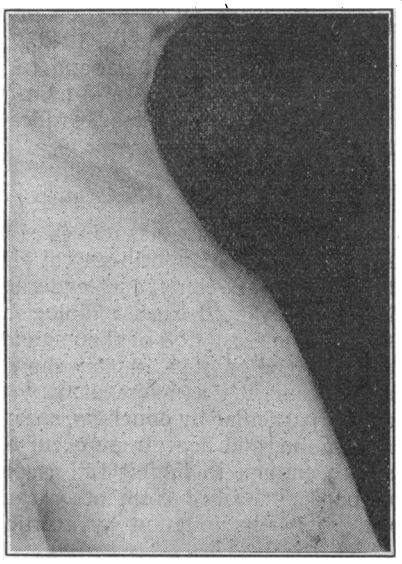

FIG. A.—Swelling over the manubrio-sternal joint (Case 1).

Case 3.—A girl aged 16 gave a one-year history of swelling, aching, and stiffness of the proximal interphalangeal joints of the right index and middle fingers and of the left index finger. Examination of these joints showed soft-tissue swelling and tenderness, with tenderness of some of the other proximal interphalangeal, metacarpo-phalangeal, and wrist joints. Radiographs of these showed only soft-tissue changes. For three weeks prior to attending the clinic she had experienced a constant tight sensation over the front of the chest, in the region of the manubrio-sternal joint, aggravated by deep inspiration and yawning. She had no cough or other chest symptoms. There was definite tenderness over the manubrio-sternal joint, which persisted for one month. A radiograph of the manubrio-sternal joint showed no abnormality.

Case 4.—A married woman aged 52 gave a nine-year history of rheumatoid arthritis affecting all the peripheral joints and the cervical spine. She was severely crippled. For the past two years she had had intermittent pain in the region of the manubrio-sternal joint aggravated by deep breathing, coughing, and yawning. Examination showed swelling and tenderness of the manubrio-sternal joint. Radiographs showed irregularity of the joint space, erosion of the joint margins, and irregular expansion of the articulating ends of the bones (Fig. 3).

Case 5.—A married woman aged 53 gave a twelve-year history of rheumatoid arthritis with involvement of the knees, shoulders, elbows, wrists, fingers, and ankles, with typical radiological changes. Pain in the region of the manubrio-sternal joint was first noticed six months before attending the clinic, and became her chief symptom. The pain occurred in attacks lasting up to twenty-four hours, and was aggravated by yawning and coughing. A striking feature of the pain was its tendency to occur on exertion, especially on walking uphill, after which it often lasted several hours. When severe, she found difficulty in taking a deep breath. This pain, which had the same quality as her arthritic pains elsewhere, was relieved by aspirin. There was marked tenderness over the manubrio-sternal joint. A radiograph of the joint showed no abnormality. Examination of the cardiovascular system, cardioscopy, and the electrocardiogram were normal. Blood Wassermann and Kahn reactions were negative. The sedimentation rate was 31 mm. (Wintrobe corrected) at the end of one hour.

Anatomy

The manubrio-sternal joint is of the cartilaginous type, the articular surfaces being covered by a layer of hyaline cartilage and connected by a disk of fibro-cartilage. A limited amount of movement is permitted, and occurs with each thoracic respiratory movement. It is stated that in rather more than 30% of people conversion into a synovial