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ELECTROPHORESIS OF HUMAN GASTRIC JUICE IN RELATION TO CASTLE'S INTRINSIC FACTOR

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In the period 1929–37 Castle and his colleagues showed that normal gastric juice (intrinsic factor) administered orally to patients with pernicious anaemia would potentiate the haemopoietic effect of beef muscle (extrinsic factor). Later they showed that the extrinsic factor in beef muscle was vitamin B_{12} or a related compound (Gardner *et al.*, 1949), and that vitamin B_{12} itself behaved like extrinsic factor when small doses were administered orally (Berk *et al.*, 1948). Meanwhile there were many unsuccessful attempts to isolate Castle's intrinsic factor, which is heat-labile and readily inactivated by chemical procedures (see Ungley, 1951–2; Welch and Heinle, 1951; Welch and Nichol, 1952).

The methods employed in the present study were mainly physico-chemical: ultrafiltration and freeze-drying followed by preparative electrophoresis on filter paper. The techniques used were devised by one of us (A. L. L.). The method of electrophoresis has already been demonstrated (Latner *et al.*, 1952).

METHODS

These are the methods finally adopted. Their development from earlier ones is described below.

Acid gastric juice was collected from students and patients after stimulation with histamine or aminoethyl pyrazole dihydrochloride. The juice was neutralized and stored at approximately –20° C. Ultrafiltration was carried out at 0–5° C. Then the ultrafiltrate residuum was freeze-dried and reconstituted with buffer so that the gastric juice had been concentrated 150–200 times.

The filter paper used for electrophoresis was Whatman No. 31 extra thick. Each of the six strips was subdivided into 25 segments 1 cm. wide. The ultrafiltrate residuum from 500 to 800 ml. of gastric juice was applied with a paintbrush to these six strips so that each contained an approximately equal amount. After electrophoresis, separation of liquid from the segments by centrifuging gave 25 fractions labelled from 1 to 25, starting at the cathode end. A barbitone buffer at *p*H 8.6, used initially, was later replaced by a buffer at *p*H 6.35.

To test their vitamin B_{12}-binding power as a basis for grouping fractions for clinical trial, samples of 0.1 ml. from each fraction were added to 1 ml. of a solution containing 200 μg. vitamin B_{12}. Any vitamin left unbound was then estimated by plate assay using *Bact. coli* (Bessell, Harrison, and Lees, 1950). Fig. 3 shows vitamin B_{12}-binding peaks demonstrated in this way. (Figs. 1 and 2 relate to some preliminary observations in which vitamin B_{12} was added before electrophoresis.) Later, a method used for staining protein was found equally effective for the grouping of fractions (see Experiment E).

Clinical Trials

Tests were conducted in 15 cases of pernicious anaemia. Methods are described elsewhere (Ungley and Campbell, 1949; Ungley, 1950), but certain points need re-emphasis. The diet was restricted to reduce the intake of extraneous haemopoietic factors, particularly vitamin B_{12}. Food was withheld for at least six hours before and after the administration of test material. Test material was given orally as a single dose with 50 μg. vitamin B_{12}. The administration of vitamin B_{12} with a possible source of intrinsic factor was preceded by a test in which an equal dose of vitamin B_{12} was admin-

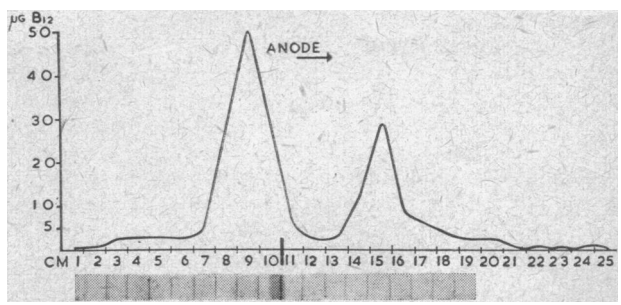

FIG. 1.—Tests on fractions obtained by electrophoresis (in barbitone buffer) of gastric juice concentrated 20 times: the curve shows two B_{12}-binding peaks. Shown below is a strip of paper stained for protein. The thick line on the abscissa represents the point of application of the material.

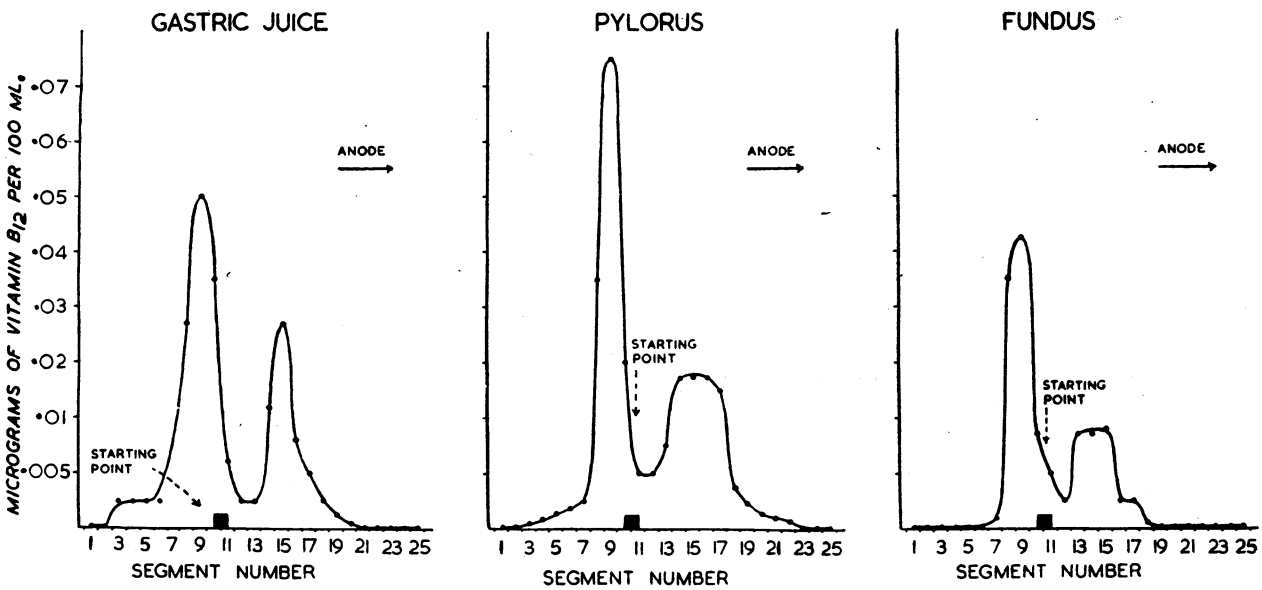

FIG. 2.—Results of simultaneous electrophoresis (in barbitone buffer) of gastric juice and extracts of pylorus and fundus mucosa of pig's stomach. Two vitamin B_{12}-binding peaks are shown in each case.

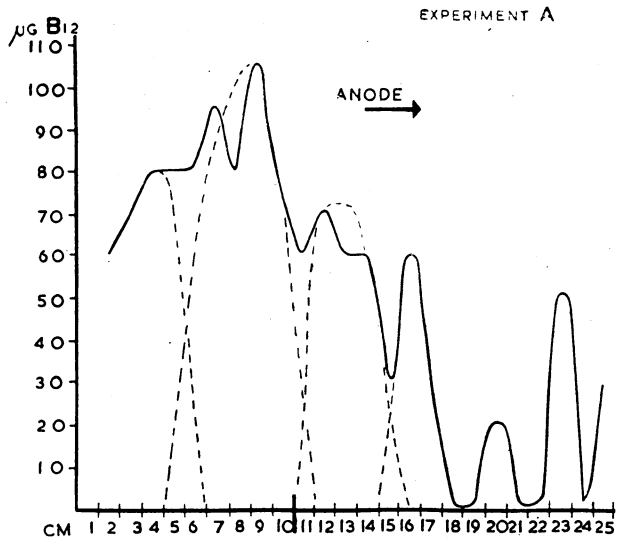

FIG. 3.—Results of electrophoresis (in buffer at *p*H 6.35) of gastric juice used in Experiment A. Vitamin B_{12} was added after electrophoresis, and the vitamin B_{12} bound by each fraction was determined. The thick line on the abscissa indicates the point of application of material.

istered alone or with an inactive fraction. A second test was not begun until any haemopoietic response initiated in the previous period had ceased: reticulocyte counts were <2%, serial estimations of red blood cells (R.B.C.), haemoglobin (Hb), and packed cell volume (P.C.V.) were level or falling, and the marrow was megaloblastic. A further point—namely, the use of one rather than several subjects for the comparison of fractions derived from one electrophoresis—is mentioned later.

Haematological Methods

Reticulocytes were counted daily by a wet method. Multiple determinations (usually two to five) of R.B.C., Hb, and P.C.V. were made every three to five days. Only mean values determined at five-day intervals are recorded here. Reticulocyte responses were observed, but were used only as an indication of the presence or absence of activity. For the quantitative evaluation of haemopoietic responses the rate of increase of R.B.C. and P.C.V. in 15 days was expressed in terms of the number of micrograms of injected vitamin B_{12} which would be expected to produce a similar response (Ungley and Campbell, 1949, and unpublished).

PRELIMINARY OBSERVATIONS

In an early study gastric juice was concentrated 20 times by ultrafiltration and vitamin B_{12} was added to the concentrate. Fig. 1 indicates the amounts of bound vitamin B_{12} in each of the 25 fractions obtained by subsequent electrophoresis.

Two peaks of vitamin B_{12}-binding can be seen—one on the cathode side near the starting-point and one on the anode side. For comparison, a strip of filter paper from the same electrophoresis was fixed and stained for protein. Protein could be demonstrated in the region of the cathode vitamin B_{12}-binding peak, but not in the region of the anode peak. This did not necessarily mean that the vitamin B_{12}-binding compound on the anode side was not a protein, for protein may have been present in amounts too small to be demonstrated. If this were the case, the vitamin B_{12}-combining power of the compound per mg. would be very high—a possibility which we thought might indicate the presence of intrinsic factor.

This possibility was strengthened by the result of experiments in which extracts of pig's gastric mucosa (Ungley and Moffett, 1936) were subjected to ultrafiltration and electrophoresis. For when the source was fundus mucosa, which is clinically inactive, the anode peak was smaller—both relatively in comparison with the corresponding cathode peak, and absolutely, in terms of vitamin B_{12} bound per mg. of protein in the extract—than when the source was pylorus mucosa, which in the pig is a rich source of intrinsic factor (see Fig. 2).

The findings could also be expressed in terms of the amounts of material needed to ensure that 50 μg. vitamin B_{12} would be bound in the anode peak—namely, 500 ml. gastric juice, extract from 400 g. pylorus mucosa, and extract from 800 g. fundus mucosa. It was of interest to compare these amounts with doses used clinically—for example, a single dose of 500 ml. of gastric juice to potentiate 50 μg. vitamin B_{12} (Ungley, 1950) and daily doses of extract from 40 g. pylorus mucosa given with yeast extract

as extrinsic factor (Ungley and Moffett, 1936). The weaker binding activity of fundus mucosa was matched by its clinical inactivity in the dosage used.

These findings encouraged us to undertake electrophoresis on a larger scale, so that the fractions could be tested clinically. Some of this work was reported at the International Congress of Biochemistry in Paris in July, 1952,* when it was stated that material with intrinsic-factor activity had been separated from other vitamin B_{12}-binding substances in gastric juice. For at this time the selection of fractions was based on the distribution of peaks of vitamin B_{12}-binding activity. Subsequently, a method used for staining and scanning protein on filter paper after electrophoresis (Latner, 1952a, 1952b) was found to be equally effective.

In these earlier experiments vitamin B_{12} was added before electrophoresis, and barbitone buffer at *p*H 8.6 was used. Fractions from the cathode and anode vitamin B_{12}-binding peaks, tested in Cases 1 and 2, gave unsatisfactory results, which will not be described. About this time one of us (A. L. L.), in the course of collaborative work with Professor A. A. Harper in which paper electrophoresis was used, found that barbitone buffer at *p*H 8.6 could destroy the physiological activity of secretin. As it was thought that barbitone buffer might destroy intrinsic factor also, the following experiments were conducted with another buffer, the *p*H of which was 6.35.

MAIN OBSERVATIONS

Experiment A

The ultrafiltrate residuum from one litre of gastric juice was applied to paper strips and separated into 25 fractions by electrophoresis. The vitamin B_{12}-binding power of these fractions is shown in Fig. 3. Two or possibly three peaks were visible on the cathode side and four on the anode side of the line of application of the concentrate. As a first step towards determining where the intrinsic-factor activity lay, the 25 liquid fractions were separated into two groups. The group from the anode side was tested in Case 3, the cathode group in Case 4.

Case 3 (a Female Aged 62). Anode

In a preliminary period 50 μg. vitamin B_{12} administered orally alone produced merely a slight reticulocytosis of 4.2% without a rise in R.B.C. or P.C.V. When the same dose of vitamin B_{12} was given with the liquid fractions from the anode side, reticulocytes rose on the third day and reached a peak of 17.6% on the sixth day (Fig. 4). Clinical improvement was evident and R.B.C., Hb, and P.C.V. increased as follows:

Days:	0	5	10	15
R.B.C. (10^6 per c.mm.) ..	2·00	2·24	2·44	2·68
Hb (g. per 100 ml.) ..	9·3	10·1	10·5	10·5
P.C.V. %	21·5	24·0	27·0	29·0

Values continued to rise up to 81 days.

The increase of R.B.C. and P.C.V. in 15 days was slightly less than that which would be expected from an injection of 5 μg. (Ungley and Campbell, 1949, and unpublished). This result falls within the lower range of responses obtained with 50 μg. vitamin B_{12} and 500 ml. of gastric juice (Ungley, 1950).

Case 4 (a Female Aged 63). Cathode

The liquid fractions from the cathode side were administered orally together with 50 μg. vitamin B_{12}. There was no haemopoietic response. Subsequently a good response to the injected vitamin was obtained, but unfortunately the response to a potent oral preparation was never observed, so that the patient may have been resistant to oral therapy. Results reported later indicate that liquid from the cathode side usually does in fact possess some intrinsic factor activity. At this stage, however, we knew only that liquid from the anode side possessed such activity, whereas liquid from the cathode side might or might not be active. For this reason in the next three experiments, B, C, and D, only material from the anode half of the papers was tested clinically. In each instance material derived from 800 ml. of gastric juice was applied to six strips of paper.

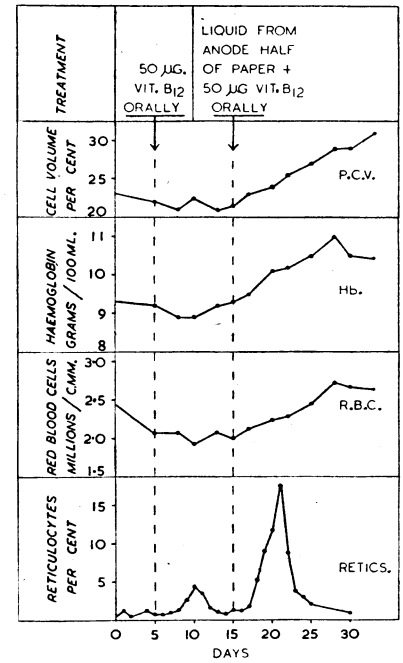

FIG. 4.—Clinical response to anode fractions in Case 3 (Experiment A).

Experiment B

Segments 1 cm. wide were drawn on the anode side of the line of application of material and numbered from 1 to 15. Liquid from segments 2, 3, and 4, which represented the B_{12}-binding peak on the anode side nearest to the starting position (see Fig. 5), was tested in Case 5.

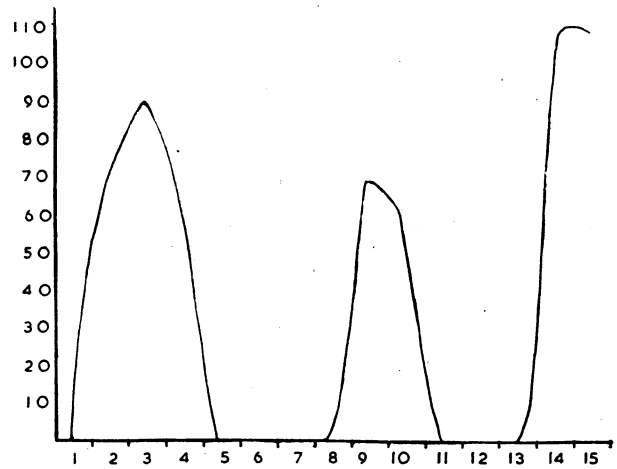

FIG. 5.—Vitamin B_{12}-binding in fractions obtained by electrophoresis of gastric juice used in Experiment B, in which only the anode side was tested.

Liquid fractions from the rest of the anode side were tested in Case 6. In this and subsequent experiments the anode and cathode peaks nearest to the line of application of concentrated gastric juice are referred to as the "first anode peak" and "first cathode peak" respectively.

Case 5 (a Female Aged 72). First Anode Peak

There was no reticulocyte response to 50 μg. vitamin B_{12} administered orally alone and the absolute values fell.

*Unfortunately, an account does not appear in the abstract, which was submitted long before the meeting.

When the same dose of vitamin B_{12} was given with the liquid from segments 2, 3, and 4, reticulocytes rose to a maximum of 10.3% on the sixth day. The R.B.C., Hb, and P.C.V. changed little :

Days:	0	5	10	15
R.B.C.	1·48	1·54	1·64	1·48
Hb	6·2	6·2	7·3	6·5
P.C.V.	15·0	16·5	19·0	17·5

There was subsequently a considerably greater response to a single oral dose of 3,000 μg. vitamin B_{12}.

Case 6 (a Male Aged 64). Rest of Anode Material

There was no response to 50 μg. vitamin B_{12} administered orally alone. An equal dose of the vitamin was then given together with the liquid from segment 1 and segments 5 to 15. Reticulocytes rose only to 2.8%, but there was some increase in absolute blood values.

Days:	0	5	10	15
R.B.C.	2·20	2·42	3·12	2·92
Hb	10·4	11·3	12·1	11·4
P.C.V.	31·0	35 0	37·0	35 0

Unfortunately, liquid from segment 1, which was erroneously included, might have had sufficient intrinsic factor activity to account for these changes. Desiccated stomach, 40 g. daily, subsequently produced a satisfactory increase in absolute values without a reticulocytosis.

Our conclusion from Experiment B was that the material corresponding to the first B_{12}-binding peak on the anode side had some intrinsic factor activity. The rest of the material from the anode side (which may have included part of the first peak) perhaps also had some intrinsic factor activity.

Experiment C

Fractions from this electrophoresis caused no response in Cases 7 and 8. Either the patients were resistant to oral therapy or any intrinsic factor present had been inactivated.

Experiment D

In this experiment a 1-cm. segment on the cathode side was included, together with the first four on the anode side, and tested in Case 9. Liquid from the rest of the anode side was tested in Case 10.

Case 9 (a Male Aged 38). First Anode Peak

There was no reticulocyte response to 50 μg. vitamin B_{12} administered orally alone, and absolute values fell slightly. When this amount of vitamin was given with the first anode peak (and one segment from the cathode side) reticulocytes rose to 8.6% on the fifth day. Absolute blood values (R.B.C., 1,200,000 ; Hb, 5 g.; P.C.V., 13.0%) showed little change. There was subsequently an excellent response to injected vitamin B_{12}.

Case 10 (a Female Aged 59). Rest of Anode Material

There was no response to 50 μg. vitamin B_{12} given orally alone. When 50 μg. was given with liquid from the rest of the anode side the response was negligible. There was no reticulocytosis, and the increase in absolute values was insignificant. The patient responded to desiccated stomach and later to injections of vitamin B_{12}.

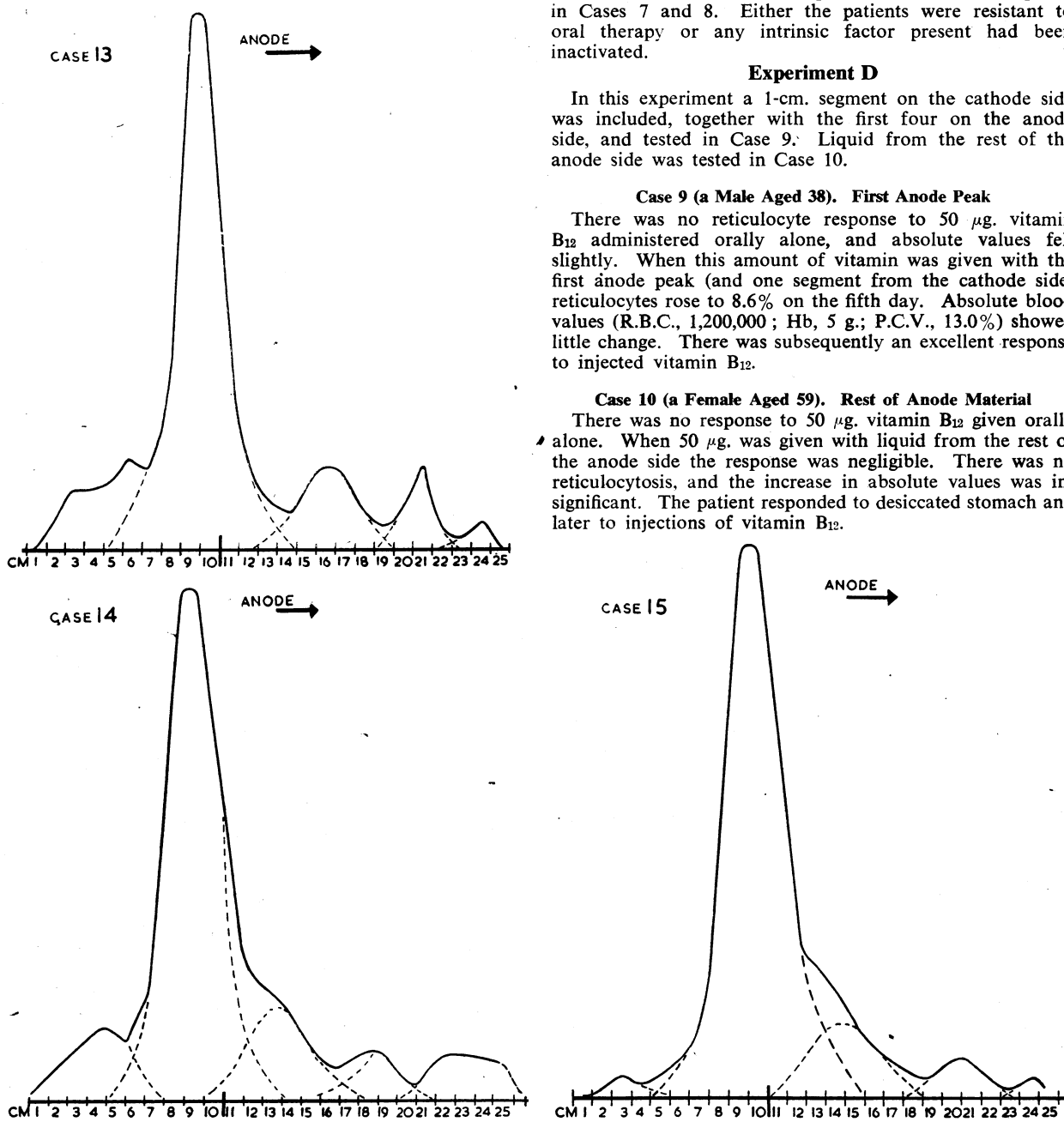

FIG. 6.—Intensity of protein staining in 25 fractions obtained by electrophoresis of aliquots of a large pool of gastric juice, and tested in Cases 13, 14, and 15 (Experiments F, G, and H).

Our conclusion from Experiment D is that any intrinsic-factor activity in the liquid from the anode side was mainly concentrated in the first anode peak, which in this case included one segment from the cathode side. There was little or no intrinsic-factor activity in the remainder of the anode side.

Experiment E

Neither of the patients (Cases 11 and 12) responded to the fractions administered. The same remarks apply as in Experiment C.

We now reached the conclusion that testing the various fractions from one electrophoresis in different patients was not completely satisfactory since individuals differ in their responses, especially to oral therapy. It was decided to test the fractions successively in the same patient, who then acted as his or her own control. We also reverted to our original procedure of testing fractions from both the anode and the cathode side of the starting position.

The three final experiments were carried out with a single pool of concentrated gastric juice. The aliquots taken for each electrophoresis corresponded to an original volume of 800 ml. The fractions after electrophoresis were tested not only for vitamin B_{12}-binding power but also for protein content. For the latter purpose 0.02-ml. portions of each fraction were placed in line on a strip of filter paper. The spots were fixed in the oven and stained by a technique already described (Latner, 1952a). The stain used, naphthalene black, is not necessarily specific for protein. The density of each stained spot was determined with a modified Spekker absorptiometer (Latner, 1952b), and plotted graphically as shown in Fig. 6. The peaks so obtained agreed fairly closely with the peaks of vitamin B_{12}-binding obtained microbiologically. Since the staining method was less arduous and gave smooth, easily reproducible curves, it was used henceforth as the guide for grouping fractions for clinical trial.

Experiment F

Several peaks were demonstrated by the staining method (Fig. 6, Case 13). The material was subdivided into three groups of fractions corresponding to the first anode peak, the first cathode peak, and the rest of the material. These were tested in successive periods in Case 13. Disappointingly, there was no response to any of them. A review of the technique suggested that there may have been undue warming during manipulation. In subsequent experiments, therefore, special care was taken to keep the material cool at all stages and in particular during centrifuging.

Experiment G

The peaks demonstrated by staining are shown in Fig. 6, Case 14. This material was also subdivided into three groups of fractions and tested in successive periods in Case 14 (see Fig. 7).

Case 14 (a Male Aged 60)

In the first period 50 μg. vitamin B_{12} was given with liquid from segments 1 to 6 and 18 to 25. There was no response. In the second period the same amount of the vitamin was given with liquid from segments 7 to 10 (the first cathode peak). Reticulocytes rose to 15% on the fifth day and absolute blood values increased.

Days:	0	5	10	15
R.B.C.	1·52	1·60	2·12	2·02
Hb	5·9	6·4	7·8	7·7
P.C.V.	15·0	18·2	24·6	24·2

In the third period, when liquid from segments 11 to 17 (first anode peak) was given with 50 μg. vitamin B_{12}, there was a further reticulocytosis of 8.6% on the seventh day. Because of the higher initial R.B.C. level, this reticulocytosis is as significant as the peak of 15% in the preceding period. For similar reasons the increases in absolute blood values were also more significant.

Days:	0	5	10	15
R.B.C.	1·94	2·00	2·92	2·96
Hb	7·4	7·8	10·1	10·4
P.C.V.	23·5	25·5	34·0	32·5

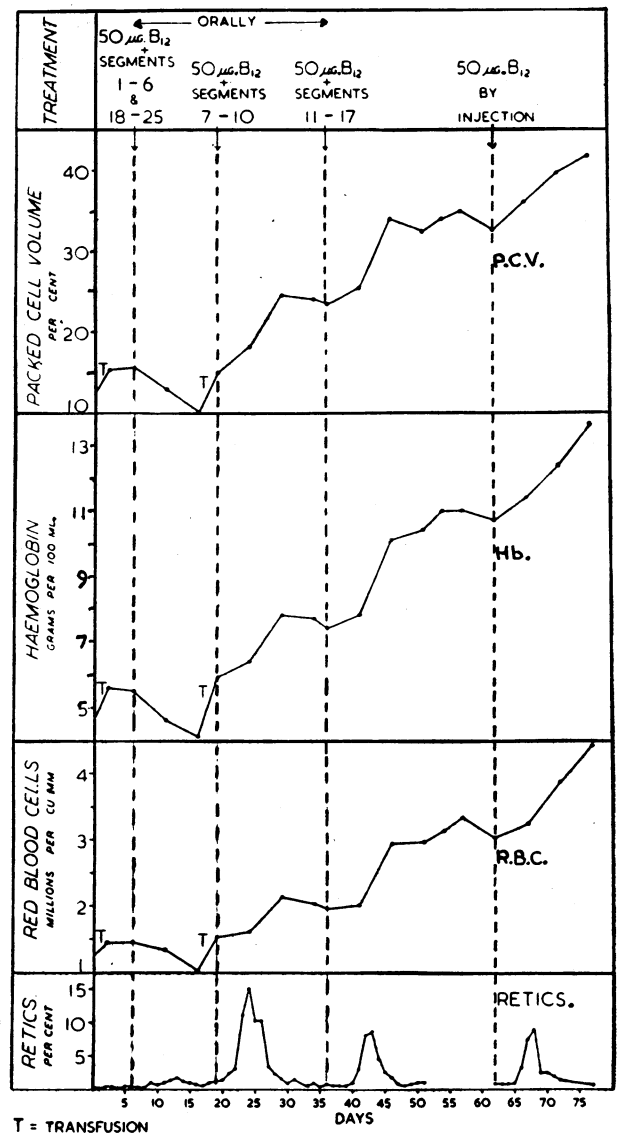

FIG. 7.—Clinical trials in Case 14; responses to first cathode peak (segments 7–10) and first anode peak (segments 11–17), but no response to rest of material (segments 1–6 and 18–25).

The increases of P.C.V. and R.B.C. in the second period were much less than would be expected from an injection of 5 μg. vitamin B_{12}. In the third period the increases were equivalent to those which would be expected from an injection of 5–10 μg.

Our conclusion from Experiment G is that the material corresponding to the first anode peak had a substantial amount of intrinsic-factor activity. It was particularly rich in terms of activity per mg. of stained material. Some intrinsic-factor activity stayed with the first cathode peak, but the activity of this region per mg. was much less than that of the first anode peak.

Experiment H

This experiment was similar to the previous one. The peaks demonstrated by staining are shown in Fig. 6, Case 15. The various fractions were tested in Case 15.

Case 15 (a Female Aged 56)

In the first period 50 μg. vitamin B_{12} was given with liquid from the outer segments of cathode and anode sides (segments 1–6, 18–25). Reticulocytes rose only to 4.4% on the sixth day, and absolute blood values remained unchanged.

In the next period 50 μg. vitamin B_{12} was given with the liquid corresponding to the first cathode peak (segments 7–10). Again reticulocytes rose to only 4.2% on the sixth day, although this time there was a very slight increase in absolute values.

Days:	0	5	10	15
R.B.C.	1·76	1·68	2·16	2·16
Hb	7·8	7·8	8·7	8·4
P.C.V.	22·0	22·0	26·0	26·0

In the third period 50 μg. B_{12} was given with the material corresponding to the first anode peak (segments 11–17). Reticulocytes rose to 7.2% on the sixth day, and the increase in absolute values was more significant.

Days:	0	5	10	15
R.B.C.	2·40	2·81	3·08	2·88
Hb	9·6	10·8	11·1	10·8
P.C.V.	25·7	31·0	31·0	29·0

The same conclusions were drawn from this experiment as from Experiment G. The liquid corresponding to the first anode peak was the richest in intrinsic factor, especially in terms of activity per mg. of stained material. Some activity stayed with the liquid corresponding to the first cathode peak.

Discussion

It has thus proved possible to fractionate intrinsic-factor activity in human gastric juice by preparative paper electrophoresis. Vitamin B_{12}-binding and later a method of protein staining were used as aids to the grouping of fractions for clinical trial.

When highly concentrated gastric juice was used, a "protein" peak could be detected in association with every vitamin B_{12}-binding peak. This suggests that our inability to demonstrate such a peak corresponding to the anode vitamin B_{12}-binding peak in an earlier experiment (see preliminary observations) was probably due to insufficient concentration of the gastric juice. It will be remembered that in this experiment the juice was concentrated only 20 times.

In experiment A, intrinsic-factor activity was clearly demonstrated in the anode fractions. But the absence of response to the cathode fractions did not necessarily mean absence of intrinsic-factor activity, for the recipient (Case 4) may have belonged to a group of patients who respond poorly to orally administered material. Of 10 patients with pernicious anaemia to whom 50–80 μg. vitamin B_{12} was administered with 500 ml. gastric juice, no fewer than three had very trivial responses, and one failed to respond (Ungley, 1950, and unpublished).

It is interesting to note that in the last two experiments, G and H, when larger quantities of gastric juice were used, tests showed the presence of some intrinsic factor in material corresponding to the first cathode peak as well as to the first anode peak. The reaction to the anode peak material seems to be the more significant, for its activity is as great as or greater than that of the cathode peak material although the former contains much less protein.

In view of our finding that intrinsic-factor activity is associated with material corresponding to two peaks, it is interesting to speculate that the substance may exist in gastric juice both in a free and in a combined state. The former might be represented by the first anode peak and the latter by the first cathode peak. We already have evidence that the material of the first anode peak contains either a mucoprotein or a mucopolysaccharide, and that heparin, a related substance, has significant vitamin B_{12}-binding power. These facts will be reported in greater detail elsewhere. We have also shown that the material of the first cathode peak, although it contains much more mucoprotein, has less intrinsic-factor activity. This may well represent the fraction isolated by Glass *et al.* (1952). It is possible, however, that the activity of the cathode peak material may have been due not to a chemical combination but to overlap of the peaks or to some physico-chemical phenomenon associated with the electrophoresis on filter paper of concentrated and highly viscous solutions.

In Experiments G and H the weight of organic material from the first anode peak was of the order of 40 mg. It would be misleading to compare this quantity with the daily dose of 0.6 mg. with which Welch and Heinle (1951) produced a response in one case. Our method has been to give a single dose orally and to evaluate the result on the rate of increase of R.B.C. and P.C.V. in 15 days (Ungley, 1950). Reticulocyte responses may provide evidence of the presence or absence of activity, but they are not a satisfactory basis for quantitative assessment (Ungley and Campbell, 1949). As determined by reticulocyte response, even 10 ml. of gastric juice daily was enough detectably to potentiate an oral dose of 5 μg. vitamin B_{12} (Schilling *et al.*, 1950; and Castle, 1951, personal communication). Until details of their case are published it cannot be assumed that the 0.6 mg. of material used by Welch and Heinle (1951) had any greater activity than 10 ml. of gastric juice.

In Experiments G and H our material produced not only a reticulocytosis but a significant increase of R.B.C. and P.C.V. within the range of responses we have obtained with a single dose of 500 ml. gastric juice and 50 μg. vitamin B_{12} (Ungley, 1950, and unpublished). The magnitude of these responses makes it reasonably certain that appreciably smaller doses, perhaps differing little from those used by Welch and Heinle in the single case referred to above, might have sufficed to produce a reticulocytosis alone.

But, whatever the basis of haematological assessment, allowance must be made for the wide scatter in the responses of different patients to equal dosage. This scatter is wide even when vitamin B_{12} is administered parenterally, especially if the dose is less than 10 μg. (Ungley and Campbell, 1949, and unpublished). The scatter is even wider when the vitamin is administered orally, for increases of R.B.C. and P.C.V. tend to be small and individual differences in absorption are great. When different observers are concerned there are certain also to be differences in strictness of control, limitation of diet, and so on. Because of all these variables, attempts to compare the activity of our material with that of others might involve errors of several hundreds per cent.

Even an approximate comparison would call for many more clinical trials—preferably trials in each of which the two materials would be tested serially in one patient, as in our Experiments G and H. Alternatively, activities could be compared by the technique of Welch, Scharf, Heinle, and Meacham (unpublished data cited by Welch and Nichol, 1952). These authors measured the radioactivity of the stools of patients with pernicious anaemia in remission after administration of radioactive vitamin B_{12}, at first alone and then together with a source of intrinsic factor.

Welch and Nichol (1952) remark that a daily dose of 3 mg. of a crude concentrate of hog's stomach will promote the absorption of 0.5 μg. radioactive vitamin B_{12}. It is possible, however, that 10 times this daily dose of their intrinsic factor concentrate might be needed to potentiate 5 μg. vitamin B_{12} administered orally to patients with pernicious anaemia in relapse.

Summary

Concentrated human gastric juice was subjected to preparative electrophoresis on filter paper. Several peaks were demonstrated by a protein-staining method. All had vitamin B_{12}-binding activity. Material from two of them—one on the cathode side and one on the anode side of the starting-point—was shown clinically to possess intrinsic-factor activity. Material correspond-

ing to the anode peak, which appeared to be the more active of the two, has been shown to contain either a mucoprotein or a mucopolysaccharide.

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THE DEFINITION AND ASSESSMENT OF RESPIRATORY FUNCTION*

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PART II

FACTORS AFFECTING THE TRANSFER OF OXYGEN IN THE LUNGS

It has already been emphasized that the main function of the lungs is the transfer of adequate quantities of oxygen to the blood to maintain a normal oxygen tension and content in the blood flowing to the body, no matter what the degree of activity or how great the oxygen utilization of the tissues. The removal of excess carbon dioxide from the blood rarely presents any difficulty in disease, except in a few special circumstances. I will therefore confine my main discussion to the problem of oxygen transfer.

Distribution of Blood and Gas to the Alveoli

The alveolus is the unit of lung anatomy and lung function. All aspects of structure and function are subservient to the proper supply of each alveolus with blood and gas in correct quantities so that an adequate oxygen tension gradient and gas transfer between them is maintained. In a theoretically perfect lung the inspired air that passes the anatomical dead space would be equally divided among all alveoli. In other words, if there were, for argument's sake, one million alveoli, then each would receive one-millionth of the effective inspired air that enters the alveoli. Similarly in such a lung each alveolus would receive one-millionth of the cardiac output. Such a state of affairs would represent perfect blood and gas distribution and each of the million alveoli would have the same ratio of ventilation and perfusion as all the other alveoli and the total lung itself. The resultant gas and blood tensions in each alveolus would be similar to all other alveoli, and each will be a miniature lung in all respects.

It is easy to imagine various disturbances of blood and gas distribution that could occur. Certain alveoli could receive less than their "share" of ventilation and yet be perfused with their normal "quota" of mixed venous blood. The blood leaving such alveoli and joining the left heart outflow would be inadequately oxygenated.

Next there could be imperfect distribution of blood; this would result in alveoli which are adequately ventilated no longer receiving their quota of mixed venous blood from the right heart. Such blood-distribution defects would not cause any deficiency of blood oxygenation but would manifest themselves in the expired air, which would contain a larger-than-normal percentage of unchanged inspired air.

We shall see later how near normal and diseased lungs come to these entirely theoretical concepts of perfection and imperfection of blood and gas distribution.

Oxygen Transfer (Diffusion) in the Lungs

The diffusion characteristics of a membrane, in regard to a particular gas such as oxygen, can be described by the quantity of oxygen that passes across it in unit time when there is a unit pressure gradient of oxygen across the membrane. This quantity of gas, or the diffusion coefficient, will depend on a number of fairly obvious factors—surface area, thickness, and physical characteristics. If we wish to consider the diffusion characteristics and coefficient of the pulmonary membrane, we must remember that we are dealing with a very complicated structure. It will include all the tissues and fluids between alveolar gas and the haemoglobin molecule in the red blood cells in the pulmonary capillary. It is also possible that the effective area of this "membrane" may vary greatly in the normal lung during various degrees of activity. Marie Krogh (1915) attempted to measure the oxygen diffusion coefficient of the lungs in normal subjects by the use of carbon monoxide. This gas was used because it is taken up by haemoglobin with such avidity that its tension in the pulmonary capillary blood (amount in simple solution) was assumed to be zero at all times. The diffusion coefficient of oxygen was easily determined from these data, as the coefficient of each gas is a simple function of its solubility and molecular weight.

Since that time little definitive work has been done on this subject, but attempts have recently been made to measure the diffusion coefficient of oxygen more directly during normal respiration (Lilienthal *et al.*, 1946). This presents great difficulty even in the normal lung, where it can be reasonably assumed that the alveolar oxygen tension is fairly constant through all alveoli. If the alveolar oxygen tension is known, then the initial pressure gradient between this and the mixed venous blood entering the alveoli can be determined by cardiac catheterization. However, the determination of the final and mean gradient between the alveolus and capillary blood presents more difficulty. These difficulties are even greater in subjects with lung disease, in whom the alveolar oxygen tension may also vary greatly. The diffusion coefficient cannot be measured unless the mean alveolar capillary oxygen tension gradient is known.

Alveolar Gas Tensions. The Concept of Ideal Alveolar Gas Tensions

The determination of the actual gas tensions in the alveoli during normal respiration presents great difficulties. Without this information the analysis of blood and gas distribution and gas transfer would seem impossible. The Haldane (1915) technique of making a subject expire deeply and collecting the sample at the end of expiration has long been used for this purpose. There is no certainty that the gas obtained in this way is an accurate, statistically repre-

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