TREATMENT OF PERNICIOUS ANAEMIA WITH VERY SMALL QUANTITIES OF PYLORIC MUCOSA AND VITAMIN B₁₂

BY

E. MEULENGRACHT, M.D., LL.D.

(From the Medical Department B, Bispebjerg Hospital, Copenhagen, and the Biological Chemistry Laboratories of the Medicinalco)

Recently I described investigations into the localization of the anti-anaemic factor ("intrinsic factor") in the pig's stomach (Meulengracht, 1952a, 1952b). It was shown that even minute amounts of the pyloric mucosa of the pig's stomach can be therapeutically active when combined with one or other source of the "extrinsic factor" under suitable conditions regarding preparation and blending.

In the present work this advance, which arose from a fortuitous discovery, has been pursued further, and it has been proved that an apparently full therapeutic effect can be obtained with doses of the intrinsic factor far smaller than had hitherto been imagined, when these are suitably combined with vitamin B₁₂.

Technique

The source of the intrinsic factor used was the dried mucosa of the pyloric region of the pig's stomach prepared as previously described (Meulengracht, 1952a, 1952b). The vitamin B₁₂ used was an ordinary commercial product, controlled spectrographically.

Patients suffering from untreated pernicious anaemia were used in the investigation. The reticulocytes were counted daily in wet preparations. The reticulocyte maxima for the different forms of dosage were plotted against the number of erythrocytes at the beginning of treatment, for comparison with Bethell and Goldhamer's (1933) standard for the expected reticulocyte maxima after adequate intravenous and oral treatment with liver extract.

The erythrocytes were counted at weekly intervals. The rises during the first three weeks for the different forms of dosage were plotted against the number of erythrocytes at the beginning of treatment, for comparison with Schiødt's (1939) standard for the expected erythrocyte rises after adequate oral treatment with liver extract, "ventriculin," and (in accordance with my own experiences) "pylorin." Schiødt's standard was recalculated for 21 days.

Experiments With Very Small Doses of Pyloric Mucosa Administered Alone

Some orientating experiments were first made with very small amounts of pyloric mucosa given alone in the form of weighed powder. Preliminary tests showed that such doses were not entirely inactive, at any rate when given in close association with meals, which, in fact, on the ordinary mixed diet, must contain requisite amounts of the food factor.

Twelve experiments were made. In nine the dose of pyloric mucosa was 0.1 g. of dried substance thrice daily, and in three it was 0.2 g. thrice daily. In some of the experiments the administration was at meal-times—that is, at 9 a.m., 1 p.m., and 6 p.m.—in other experiments it was between meals, at 8 a.m., 11 a.m., and 9 p.m.

The following results were obtained. In one isolated case there was no demonstrable effect. In the majority there was some response, but it was not complete, since only a weak and protracted reticulocyte reaction occurred without a full rise in the haemoglobin and red cells, and with subsequent secondary reactions after adequate injection treatment with liver extract or vitamin B₁₂. Lastly, in two or three experiments there was a more pronounced effect.

The experiments showed practically no difference between the two dosages. Nor is it possible to decide definitely whether administration at meal-times or between meals played any part, although the effect seemed to be more pronounced when the pyloric mucosa was given at meal-times. It is possible that a rather more suitable planning of the experiments might produce clearer evidence on this point; thus it might be arranged that, in the case of the dosage between meals, the stomach and the upper part of the intestinal canal should be empty at the time of the administration—for example, by resorting to nightly administration. The importance of the times of oral intake of the intrinsic factor in relation to meals has never been

TABLE I.—Reticulocyte Maximum and Erythrocyte Rise in 12 Patients After Oral Administration of Small Amounts of Pyloric Mucosa Alone

No.*	Pyloric Mucosa Orally	Reticulocyte Maximum	Three Weeks' Erythrocyte Rise
180	0.1 g. × 3	4.6%	0.65
181	0.1 g. × 3	5.4%	—0.10 (14 days)
184	0.2 g. × 3	7.8%	0.60
186	0.1 g. × 3	10.8%	0.80
192	0.1 g. × 3	13.3%	0.40 (14 ")
194	0.1 g. × 3	7.8%	0.35 (14 ")
196	0.1 g. × 3	5.2%	—0.30
197	0.1 g. × 3	4.6%	0.80
200	0.1 g. × 3	1.8%	—0.10* (14 ")
1	0.2 g. × 3	16.8%	1.15*
3	0.2 g. × 3	21.0%	0.60
5	0.1 g. × 3	10.6%	0.20 (14 ")

*Blood transfusion.

properly grasped. An attempt was therefore made to elucidate this point, but, as intimated, the technique of the experiment was scarcely satisfactory for this purpose.

The results of these orientating experiments with very small doses of pyloric mucosa given by itself are shown in Table I and Fig. 1. Both the reticulocyte maxima and the three-weeks erythrocyte rises after adequate oral administration are far below the expected increases in each case.

Experiments With Very Small Doses of Pyloric Mucosa and Vitamin B₁₂

In this series of experiments the same very small amounts of pyloric mucosa were combined with vitamin B₁₂ in the proportion of 0.1 g. of dried pyloric mucosa and 5 µg. of vitamin B₁₂ in weighed powder.

There were 10 experiments in all. In the first three the mixture was given three times daily, the daily dose being 0.3 g. of dried pyloric mucosa and 15 µg. of vitamin B₁₂. In the following experiment the combination was given twice daily, the dose then amounting to 0.2 g. and 10 µg. respectively. In the rest of the experiments the compound was given only once a day, so the daily dose was narrowed down to 0.10 g. and 5 µg. respectively. It will be seen from Table II and Fig. 2 that the reticulocyte maxima and the three-weeks erythrocyte rises are now greater and approach the standards for adequate oral treatment in spite of the dosage in most of the cases being restricted to 0.1 g. of dried pyloric mucosa and 5 µg. of vitamin B₁₂.

In two of the experiments the erythrocyte rise is clearly unsatisfactory. Both of these experiments fall into the group in which the dose was reduced most drastically.

Preliminary Experiments with a Commercially Prepared Substance, "Cycoplex Mco"

Our knowledge was now so far advanced that it was decided to adopt the method in the routine treatment of pernicious anaemia.

FIG. 1.—Three weeks' erythrocyte rises in 12 patients after oral administration of small amounts of pyloric mucosa alone in comparison with Schiødt's standard.

When it is remembered that at the time the treatment with dried stomach was first introduced large amounts were administered, it must seem surprising, indeed almost sensational, that such small doses as those used in the above experiments can be effective.

For the earlier preparations the following scheme of dosage was usually recommended in the treatment of fresh cases :

Ventriculin	..	..	..	..	20 g. thrice daily
Pylorin	..	..	..	..	10 g. " "
Exo-pylorin	..	..	..	..	5 g. " "

In view of the experiments described above, it would seem that we ought to be able to come right down to centigram doses, provided that the material is taken from a site in the pig's stomach where the intrinsic factor occurs in a concentrated form—that is, the pyloric mucosa (Meulengracht, 1934; Meulengracht and Søborg-Ohlsen, 1934).

As has so often happened on previous occasions, it became apparent that, when one proceeded to the commercial production of a preparation various obstacles of a technical nature had to be overcome if uniformity and stability were to be guaranteed.

In the commercial preparation the mixture of dried pyloric mucosa and vitamin B₁₂ was put into small gelatin capsules. Each capsule contains 0.05 g. of dried pyloric mucosa and 5 µg. of vitamin B₁₂. The vitamin B₁₂ content is thus greater than that in the above-described orientating experiments. The preparation has received the name "cycloplex mco."*

Therapeutic experiments with this commercial preparation have been carried out in my hospital department and, at the manufacturer's request, in the medical departments of a number of other hospitals in this country. The dosage has been three capsules daily, corresponding to a daily dose of 0.10 g. of dried pyloric mucosa and 15 µg. of vitamin B₁₂. The four curves from my department are reproduced in Figs. 3, 4, 5, and 6, in which the reticulocyte reaction and the rises in Hb and red cells are recorded. The total number of experiments—both my own and those from the various other

*Cycobemin (*Pharmacopoeia danica*) = vitamin B₁₂.

medical departments—are recorded, with the permission of the manufacturers, by graphic presentation of three-weeks erythrocyte rises (Fig. 7).

In my four experiments subjective recovery, rapidly ensuing reticulocyte reaction with a high narrow form of curve, and a big rise in the number of erythrocytes were observed. When one is used to judging the reactions in different kinds of treatment, the rapidity and abruptness with which they set in become striking. The three-weeks erythrocyte rises for all the cases, which are plotted in Fig. 7 in comparison with Schiødt's standard, display an even distribution around the latter.

Commentary

Combined preparations—that is, preparations in which an attempt has been made to combine the intrinsic and extrinsic factors in a suitable manner—are nothing new in the treatment of pernicious anaemia. The most commonly used preparation in Denmark, just such a combination: each 5-g. carton contains 3.5 g. of dried and pulverized pig's total pylorus and 1.5 g. of "liver remains" as the sources of the intrinsic and extrinsic factors respectively (Meulengracht, 1941).

TABLE II.—Reticulocyte Maximum and Erythrocyte Rise in 10 Patients After Oral Administration of Small Amounts of Pyloric Mucosa + Vitamin B₁₂

No.	Pyloric Mucosa + Vitamin B ₁₂ Orally	Reticulocyte Maximum	Three Weeks' Erythrocyte Rise (Millions)
8	0.1 g. + 5 µg. × 3	15.2%	1.25
10	0.1 g. + 5 µg. × 3	24.6%	1.20
12	0.1 g. + 5 µg. × 3	15.4%	1.15
38†	0.1 g. + 5 µg. × 2	10.0%	1.80* (17 days)
14‡	0.1 g. + 5 µg. × 1	42.4%	1.50
16	0.1 g. + 5 µg. × 1	19.0%	1.35
18	0.1 g. + 5 µg. × 1	12.6%	0.75
19	0.1 g. + 5 µg. × 1	9.2%	0.05 (16 days)
28	0.1 g. + 5 µg. × 1	10.4%	1.25
30‡	0.1 g. + 5 µg. × 1	18.0%	0.50

* Blood transfusion.

† Complicated by fracture of the neck of the femur.

‡ Complicated by fever (pyuria, phlebitis).

FIG. 2.—Three weeks' erythrocyte rises in 10 patients after oral administration of small amounts of pyloric mucosa and vitamin B₁₂ in comparison with Schiødt's standard calculated for 21 days.

The bringing to light or, indeed, identification of the two factors which has gradually taken place, particularly the generally accepted identification of the extrinsic factor with vitamin B₁₂, has led to the production of combined preparations from concentrated sources of the two factors. In practice this means a concentrate of the intrinsic factor from the pyloric portion of the pig's stomach, where this factor, according to earlier experiments, is principally localized (Meulengracht, 1934; Meulengracht and Søeborg-Ohlsen, 1934), and the pure crystalline vitamin B₁₂ which is now easily procurable commercially.

FIG. 3.—E. V. L., a woman aged 64. Treated with cycloplex mco three capsules daily. Bispebjerg Hospital, Dept. B.

FIG. 4.—M. K. B., a woman aged 74. Treated with cycloplex mco three capsules daily.

FIG. 5.—M. B., a woman aged 53. Treated with cycloplex mco three capsules daily.

FIG. 6.—H. N. J., a woman aged 53. Treated with cycloplex mco three capsules daily.

FIG. 7.—Three weeks' erythrocyte rises in 16 patients after oral administration of cycloplex mco three capsules daily in comparison with Schiødt's standard.

Such combined preparations suggest themselves, and indeed they have recently been put on the market in different ways in a series of preparations in some countries, including Denmark (Hoff-Jørgensen *et al.*, 1952). As the source of the intrinsic factor in these preparations extracts of stomach have been used, by which means a reduction in weight can be arrived at, although rather complicated technical procedures and the probability of waste are involved. Much more simply, however, with regard to both preparation technique and accuracy, can one resort to very small—in fact, in relation to previous dosage, minimal—amounts of pyloric mucosa for the source of the intrinsic factor, as demonstrated in the investigations above. One is thereby spared an elaborate and incalculable technical process, and can be content with a statement of the weight of the substance of the organ dried at a low temperature; while, as regards the testing of the activity of the preparation and the determination of the dose, one can keep to the approved methods, using fresh cases of pernicious anaemia.

It seems as if extremely small doses are usually adequate. Profiting by earlier painful experiences (Meulengracht, 1933)—there an attempt was made to cut down the dose and to individualize it—it was felt to be more rational that the dose should be fixed in excess of what is absolutely needed, so that each patient might be covered and protected against unpleasant surprises in the form of incomplete compensation of the anaemia or a myelopathy attack.

Since the economic factor is less important than hitherto, I would recommend, as a result of the experiences here recorded, that the initial dose in fresh cases be fixed at two capsules three times daily, but the maintenance dose has yet to be fixed by further experiments.

With regard to the plan of dosage, I have endeavoured to give these between meals in order not to mix up the small quantities involved with a large meal; but whether this is reasonable and appropriate I honestly do not know, for there is no evidence in favour of this procedure, and I cannot conclude anything definite from my own experiments.

Summary

It has been shown that even very small doses of dried pyloric mucosa from the pig's stomach given alone exert a definite but, in comparison with Bethell and Goldhamer's and Schiødt's standards, slight anti-anaemic effect.

It has also been demonstrated that the same very small amounts of pyloric mucosa, when combined with vitamin B₁₂ in quantities which in themselves are inactive when given orally, exert a strong anti-anaemic effect which is practically the same as the standards referred to.

A commercial preparation, cycoplex mco, in daily dosage of 0.15 g. of dried pyloric mucosa and 15 µg. of vitamin B₁₂, exerts a strong anti-anaemic effect which corresponds to the standards cited. In the routine treatment of pernicious anaemia patients, in order to be well on the safe side, a dosage is recommended which corresponds to 0.3 g. of dried pyloric mucosa and 30 µg. of vitamin B₁₂ for the initial doses. The maintenance dose has yet to be fixed.

REFERENCES

- Bethell, F. H., and Goldhamer, S. M. (1933). *Amer. J. med. Sci.*, **186**, 480.
 Hoff-Jørgensen, E., Skouby, A. P., and Gad Andresen, J. (1952). *Nord. Med.*, **48**, 1754.
 Meulengracht, E. (1933). *Ugeskr. Læg.*, **95**, 819. Also in *Klin. Wschr.*, **1933**, **12**, 1163.
 — (1934). *Ibid.*, **96**, 179. Also in *Acta med. scand.*, **1934**, **82**, 352.
 — (1941). *Nord. Med.*, **9**, 629.
 — (1952a). *Ugeskr. Læg.*, **114**, 1. Also in *Acta med. scand.*, **1952**, **143**, 207.
 — (1952b). *Ibid.*, **114**, 1411. Also in *Acta med. scand.*, **1953**, **144**, 290.
 — and Søborg-Ohlsen, A. (1934). *Ibid.*, **96**, 190. Also in *Acta med. scand.*, **1934**, **82**, 384.
 Schiødt, E. (1939). *Acta med. scand.*, **98**, 449.

The "Odlum Fund," recently set up by the National Association for Mental Health through the generosity of Dr. Doris Odlum and others, is to be used as a permanent fund for the production of pamphlets by the association. The fund, which amounts to about £1,000, will enable the association to consider the possibility of publications without reference to its general finances. In its annual report for 1952-3 the association states that its financial position, extremely serious at the beginning of the year, has now greatly improved. The report gives a short account of considerable and varied activities. An inquiry into the necessity for a change in the law for dealing with aggressive psychopaths has reached the stage where discussions with the Home Office are imminent. In the case-work department the number of advisory cases referred during the year was 1,650.

DOMICILIARY OBSTETRICS*

A SURVEY AND COMMENTARY

BY

IVOR COOKSON, M.B., D.Obst.R.C.O.G.
D.C.H., M.M.S.A.

General Practitioner, Clinical Assistant, Gloucester
Maternity Hospital

Domiciliary midwifery is gradually diminishing in spite of the ample evidence that good results are compatible with a high proportion of domiciliary deliveries. Delivery in hospital is no panacea, and is expensive. It may be that for the same expenditure better results can be obtained by improving the domiciliary services than by expanding maternity hospitals, for the former make greater use of the patient's own resources; but it is difficult to say how improvements could be made, or how cases should be selected for hospital delivery, for the literature relating to domiciliary work is very small.

This survey is a superficial study of 4,441 births, with the primary object of collecting information on which can be based the selection of cases for hospital delivery so that the remainder will consist of those who can be delivered at home in reasonable, if not absolute, safety. It is also intended to indicate the type of obstetrical problem likely to be met in general practice, and to show whether maternity cases may safely be supervised by a practitioner who has consultant support but no post-graduate experience.

Material

The patients of a general practitioner with a list of 2,500 (rather more than average) will produce only about 40 babies a year. Approximately half will be born in hospital, and some of the remainder may be attended entirely by midwives. It is evident that a general practitioner cannot study domiciliary midwifery effectively in his own practice alone, for on an average he would take ten years to accumulate records of 150 cases. Therefore, in addition to a small personal series, two much larger groups have been studied, the survey con-

TABLE I.—Number of Primiparae and Multiparae in Each Group, and Place of Delivery

	Domiciliary			Hospital			Nursing-home		
	Prim.	Mult.	Total	Prim.	Mult.	Total	Prim.	Mult.	Total
Personal	15	85	100	16	28	44	19	5	24
City	365	1,119	1,484	892	897	1,789			
County	176	824	1,000						
Total	556	2,028	2,584	908	925	1,833	19	5	24

sisting of (1) 168 confinements of patients of a general practitioner between 1946 and 1952; (2) 3,273 confinements of residents in a city in 1949, 1950, and 1951, either at home or in hospital; and (3) 1,000 domiciliary deliveries in the surrounding district but outside the city boundary in the early months of 1952.

These three groups are called "personal," "city," and "county" cases respectively. The inclusion of hospital and nursing-home cases (Table I) makes it possible to study domiciliary cases in relation to the group from which they were selected, and to consider all the cases for which a general practitioner could assume responsibility.

*A revised version of the Sir Charles Hastings Clinical Prize essay for 1953.