Observations

ON

INTRAMUSCULAR LIVER EXTRACT
THERAPY IN THE TREATMENT OF
PERNICIOUS ANAEMIA

BY

L. S. P. DAVIDSON, M.D., F.R.C.P.ED.

REGIUS PROFESSOR OF MEDICINE, UNIVERSITY OF ABERDEEN

Although it is only a little over five years since Minot and Murphy¹ demonstrated the efficacy of liver diet in the treatment of pernicious anaemia, great advances have been made since then in the purification and isolation of the active principle contained in liver. In 1927 Cohn, Minot, and their associates² produced a liver extract by a process of chemical fractioning, which was found to be effective, and which enabled treatment to be satisfactorily carried out in cases of pernicious anaemia in the severe relapse stage when nausea and vomiting prohibited the oral administration of sufficient quantities of whole liver. Animal experiments revealed the fact that such extracts were unsuitable for parenteral injection, since the small amount of contained protein was apt to produce dangerous allergic phenomena. In addition, the presence of a blood-pressure-reducing substance was detected, which appeared to be an additional contraindication to this form of treatment.

CHOICE OF PREPARATION

The obvious value of an active extract which could be given parenterally to pernicious anaemia patients who were critically ill, or in the so-called liver-resistant cases, stimulated further researches into this problem, and in 1930 Cohn, McMeekin, and Minot,³ and West and Howe,⁴ had sufficiently purified the active principle in liver to enable intravenous and intramuscular treatment to be given with entirely satisfactory results. The purification and isolation of the active principle, free from its blood-pressure-reducing properties, presented a problem of the greatest difficulty. According to Castle and Taylor,⁵ Cohn, McMeekin, and Minot used as much as 25,000 grams of liver for the production of the active principle required for the treatment of a single case. Castle and Taylor found that if aqueous solutions of fraction G (Cohn) were injected slowly—that is, at the rate of not more than 2 c.cm. per minute—the depressor effects were never serious and disappeared with rapidity. Any slight allergic manifestations which appeared could easily be controlled by the injection of 0.5 c.cm. adrenaline hydrochloride, 1 in 1,000 solution. The extract was easily made, and was of astonishing activity, since a single intravenous injection of the active principle derived from 100 grams of liver gave marked reticulocyte responses, and a gain of one million red cells within ten days. The purest preparation of the active principle so far produced has been made by Dakin, West, and Howe,⁶ who claim that maximal reticulocyte responses result from the injection of 0.1 gram. They suggest that the active principle is probably a dipeptide of β -hydroxyglutamic acid and γ -hydroxyproline. In their latest publication, of September, 1931, West and Howe⁷ state: "It must, however, be emphasized that, although clinical activity has been present after two and three recrystallizations of these quinine salts, the possibility of the presence of highly potent adsorbed material can be wholly excluded only when these products have been synthesized." In this country Wilkinson⁸ has recently reported excellent results from the use of a highly purified and extremely potent preparation from liver extract, given intravenously or intramuscularly.

The various preparations mentioned above have scarcely left the experimental stage, and the majority are not yet available to the general practitioner, so far as I am aware. Credit for having first shown the remarkable difference between the effectiveness of the active principle when administered orally and parenterally belongs to Professor Gänsslen⁹ of Tübingen. Gänsslen's extract has been used extensively on the Continent during the past year, and its potency in over one hundred cases of pernicious anaemia has been vouched for by leading physicians, such as Schilling, Naegeli, etc. Impressed by the reports appearing in the German medical journals, I asked Professor Gänsslen if he could send me sufficient material for the treatment of a case of pernicious anaemia. He did so, and the results were wholly satisfactory. Since then Gänsslen's extract has been placed on the market by Bayer Products Ltd. under the name of "Campolon." I have treated a series of cases of pernicious anaemia in the Royal Infirmary, Aberdeen, with this commercial product. The object of this communication is to bring to the notice of the practitioners of this country that there is on sale to-day at least one liver extract for parenteral injection which, after prolonged clinical trial, can be said to be highly potent, and at the same time free from danger.

ACCOUNT OF THREE CASES

CASE I

Mrs. J. B., aged 74, was admitted to Ward V, Royal Infirmary, Aberdeen, on October 30th, 1931, complaining of weakness and breathlessness on exertion.

Since May, 1931, the patient had become progressively weaker and more easily tired. She felt breathless on the least exertion, her appetite gradually failed, and for several weeks before admission to hospital she had lost all desire for food. Recently nausea and vomiting had been present. There was no history of sore tongue. Flatulence and eructations were marked. Pain was absent, and the condition of bowels satisfactory.

On examination the patient was found to be extremely emaciated—I have never seen a case of pernicious anaemia so thin and wasted as she was. Prior to the blood examination my first diagnosis was in favour of cancer of the stomach, a view held by the family doctor. The skin was dry and extremely pale, but a definite lemon-yellow tint was present, which was best seen in the conjunctivae. The tongue was pale and atrophic, but not ulcerated. The radial artery was markedly thickened. Systolic blood pressure was 150 mm. Hg; pulse rate, 88. Haemic murmurs, systolic in time, were present at all areas. Alimentary, respiratory, and renal systems were satisfactory. Tingling and numbness of the hands and feet had been present for six weeks, but no evidence of definite cord lesions was found from examination of the central nervous system.

Laboratory data.—Urine: negative for bile; urobilin positive. Van den Bergh reaction: immediate direct, negative; indirect, positive. Icteric index: 40. Test meal: complete absence of hydrochloric acid after histamine injection. Blood count: red blood cells 940,000, haemoglobin 22, colour index 1.2, white blood corpuscles 2,560, reticulocytes 1 per cent. Blood picture: marked anisocytosis and megalocytosis; megaloblasts present; platelets very scanty. Price-Jones curve showed marked broadening of the base and a shift to the right.

Diagnosis.—A case of pernicious anaemia in the severe relapse stage.

For three days the patient received no treatment, and the blood count fell to: red blood cells 730,000; haemoglobin 18 per cent. One ampoule of campolon was then injected intramuscularly on seven consecutive days, and treatment was then stopped. After the fifth injection the reticulocyte count began to rise, reaching 20 per cent. on the eighth day. By the twelfth day both the red cell count and haemoglobin had doubled. During the next week the count rose to 1,620,000 red blood cells, haemoglobin 40 per cent., and subsequently, the blood level remaining stationary, the patient was used for assaying the potency of another preparation of liver extract. A second series of injections of campolon was

recently given, and on January 20th, 1932, the red cell count was over 4 millions. The fact that no satisfactory animal test exists for estimating the activity of liver preparations, and the difficulty in securing sufficient numbers of cases of pernicious anaemia at a suitable blood level, on which to make potency tests, have recently been discussed by the writer.¹¹

That a patient of 74 years of age, with marked arteriosclerosis and emaciation, who had been severely ill for months, responded to seven injections of campolon by doubling the red cell count and haemoglobin level in twelve days, is clear evidence of the potency of the extract used.

CASE II

Mrs. W. B., aged 49, was admitted to Ward V, Royal Infirmary, Aberdeen, on October 26th, 1931, complaining of weakness and giddiness.

For the past six months the patient had been getting progressively weaker and thinner. On rising from her bed she felt giddy, and had occasionally fainted. Her ankles were slightly swollen at night. No pain was complained of in any situation. Appetite was very poor. Nausea and vomiting occurred occasionally, and the tongue was sometimes sore.

The patient was an extremely pale, anaemic woman, with a slight icteric tint in the skin and conjunctivae. The tongue was pale and atrophic. Apart from haemic murmurs, systolic in time, heard at all areas of the heart, nothing of importance was found in any system.

Laboratory Data.—Urine: negative for bile. Urobilin positive. Van den Bergh reaction: immediate direct, negative; indirect, positive. Icteric index: 20. Test meal: no free hydrochloric acid was found after histamine injection. Blood count: red blood cells 1,180,000, haemoglobin 26 per cent., colour index 1.1; white blood cells 3,100, reticulocytes 1 per cent. Blood picture: marked anisocytosis and megalocytosis; megaloblasts found. Price-Jones curve showed a pronounced shift to the right.

Diagnosis.—A case of pernicious anaemia in the severe relapse stage.

After a control period of ten days with no treatment the count had fallen slightly to: red blood cells 1,000,000, haemoglobin 22 per cent. On October 9th, 1931, an intramuscular injection of 6 c.cm. campolon was given. Within twenty-four hours the reticulocyte count started to rise, reaching 10 per cent. on the second day after injection, and 14 per cent. on the fourth day, returning to normal in about ten days. In eight days the red cell count was doubled. This patient was also used subsequently for the assaying of the potency of another liver preparation.

The potency of campolon is adequately proved by an increase of the red cell count by one million in a period of eight days following the injection of 6 c.cm. of the preparation.

CASE III

W. T., aged 70, was admitted to Ward V, Royal Infirmary, Aberdeen, on September 9th, 1931, complaining of weakness and pain over the heart.

The patient had been getting weaker during the past three years. His relations had noticed that he had become much paler, and that on occasion he had a distinctly yellow tint. Numbness and tingling of the hands had been present for the past few months. Recently his tongue had been sore, and he had noticed several ulcers at the tip and on the sides.

On examination, nutrition was good, the skin pale, with slight icteric tint best seen in the conjunctivae. Tongue: pale and glazed. Blood pressure: 118/70. Pulse: 78. Apex beat in the fifth left interspace $4\frac{1}{2}$ inches from the mid-sternal line. Faint haemic murmurs, systolic in time, at all areas. Alimentary, respiratory, renal, and nervous systems: satisfactory.

Laboratory Data.—Urine: bile and urobilin absent. Van den Bergh reaction: immediate direct, negative; indirect, positive. Icteric index: 10. Test meal: no free hydrochloric acid after histamine injection. Blood count: red blood cells 1,800,000, haemoglobin 43, colour index 1.19; white blood cells 3,400, reticulocytes 1 per cent. Blood picture: marked anisocytosis and megalocytosis. Price-Jones curve showed a shift to the right.

Diagnosis.—A moderately severe case of pernicious anaemia.

After a preliminary control period of ten days, in which the blood count remained stationary, campolon, one ampoule of 2 c.cm., was injected intramuscularly for seventeen consecutive days. On the fifth day the reticulocyte count had risen to 5 per cent., reaching a peak of 12 per cent. on the seventh day, and remaining above normal for sixteen days. In three weeks the blood count rose to: red blood cells 3,090,000, haemoglobin 62 per cent.; and a week later to, red blood cells 3,400,000, haemoglobin 75 per cent. The patient left hospital, no further treatment having been given, and reported at the Blood Clinic, Royal Infirmary, Aberdeen, at weekly intervals during the month of December. The blood count was always in the neighbourhood of four to four and a quarter millions, haemoglobin between 70 and 80 per cent. The patient appeared to be in perfect health, and complained of no symptoms. In view of the well-recognized danger of subacute combined degeneration starting in patients whose blood level is subnormal, the patient is now receiving a second course of injections of campolon.

That seventeen daily injections of campolon could raise the blood level from 1,800,000 to 4,250,000 is again adequate proof of the potency of this preparation.

DOSAGE AND POTENCY OF PREPARATION

The claims made by Gänsslen regarding the potency of his liver extract are clearly substantiated by the cases reported above. Moreover, the results are particularly satisfactory when it is remembered that minimal amounts of the material were administered. This was deliberately done with the object of estimating the potency of the extract, and also, as already stated, conserving the cases for further therapeutic trials of other preparations. In addition to a rapid increase of the red cell level by approximately 100,000 red blood corpuscles per day, remarkable changes took place within fourteen days in the red cell diameter, the shape of the Price-Jones curve, the iron content of the blood, the cell-volume index, and the plasma-red-cell ratio. These points, however, are being dealt with in a separate paper concerning a large series of cases of both primary and secondary anaemias.

Gänsslen, in his latest communication,¹⁰ states that one ampoule should be injected daily for five to eight weeks, in order to bring the blood level back to normal. In some obstinate cases daily treatment for three months may be necessary. Subsequently the number of injections may be reduced to one or two weekly, and finally, a maintenance dose of two to three ampoules in one injection every fourteen days will keep the patient in perfect health. That no danger exists from injecting larger amounts is indicated by the fact that in two cases I have given from 10 to 20 c.cm. in twenty-four hours, with no ill effects, either local or general. Many of Gänsslen's cases remained in perfect health in the remission stage for many months, without any further injections after the completion of the course. In fifty consecutive cases he has had no failures, and patients who commenced treatment with campolon two years ago are to-day in perfect health. Gänsslen is of opinion that one ampoule a day is sufficient, and that no additional benefit is derived from larger daily doses, the additional material being presumably excreted or destroyed. This is contrary to our knowledge of other forms of liver extract therapy in the acute stage of pernicious anaemia in which the body stores of the active principle are exhausted. I have repeatedly pointed out that the response is a quantitative one; therefore it is quicker and just as economical in the relapse stage to give large doses of liver extract over a short period as it is to give smaller doses over a longer period. This is also borne out in Case II, which showed a rise in the reticulocyte count within twenty-four hours of the injection of 6 c.cm. of campolon, while Cases I and III, on daily injections of 2 c.cm., both failed to show a change until the fifth day.

It is necessary, however, in assessing the potency of a remedy in cases of pernicious anaemia, to make allowance for the fact that in elderly patients, particularly if they have been ill for a long period, and in patients with arterio-sclerosis or chronic sepsis, the response is slower and less marked—a fact well illustrated in a series of cases recently published by Beebe and Lewis.¹² Nevertheless, it appears to me that in cases critically ill the dose of campolon should be about 6 c.cm. daily for the first forty-eight hours, thereafter returning to a single dose of 2 c.cm. daily until a normal blood level is reached. One ampoule of 2 c.cm. of campolon is stated to contain the active principle derived from five grams of raw liver. As judged by the cases reported above, it is as potent as the quantity of liver extract derived from 500 grams of raw liver given by the mouth. Intramuscular injection of the active principle would appear to be approximately one hundred times as effective as oral administration. It is obvious, therefore, that when liver or liver extract is given by the mouth, either a large portion is destroyed in the alimentary tract by bacterial or enzymic agencies, or assimilation of the active principle is extremely poor.

INDICATIONS FOR CAMPOLON THERAPY

There appear to be three definite indications for the intramuscular injection of liver extract:

1. In cases of pernicious anaemia, critically ill, with a blood count of one million red cells or less, and with marked nausea and vomiting, the patients may die during the period of five to seven days which must elapse before oral administration can be effective. At present blood transfusion is the treatment which is adopted to bridge this gap. It is my experience that this is far from being the safe and simple procedure which a study of the literature leads one to believe. In patients with blood counts between 500,000 and 1,000,000 red cells, marked reactions with rigors and high temperatures may occur, no matter how carefully the blood is matched or how slowly it is injected. In fact, a marked reaction may follow the intravenous injection of normal saline. I have seen three fatal terminations in this type of case during the past eighteen months. Presumably the intensely degenerated myocardium is unable to withstand the additional strain of the reaction. The explanation of these reactions is far from clear. Haemolysis did not occur in the three cases mentioned above, as judged by the absence of haemoglobinuria. It is believed that when the blood count is well below one million, auto-agglutination is very apt to take place. The knowledge that the intramuscular injection of liver extract can alter the blood picture within twenty-four hours will reduce the need for blood transfusion in severe cases of pernicious anaemia to a minimum.

2. It is recognized that the average maintenance dose of liver is in the neighbourhood of two pounds weekly. There are few of us who can contemplate a life sentence of this kind with equanimity. It is not surprising, therefore, that we are constantly seeing pernicious anaemia patients with blood counts around three and a half to four million red cells who, probably because of the haemoglobin level of about 80 per cent., are able to continue their daily occupations fairly comfortably. Inadequate intake of liver is the explanation of these subnormal counts, which can be brought back to normal by increasing the consumption of liver. Evidence is steadily increasing that one of the most important factors in allowing subacute combined degeneration of the cord to develop (or, when present, to accelerate its progress) is a subnormal blood level. For this reason we must use every means at our disposal to keep the blood level up to standard. An injection of two or three ampoules of

campolon at fortnightly intervals, provided a high protein diet containing a daily helping of meat, liver, or kidney is taken, will accomplish this object, without producing the disgust which continuous eating of large quantities of liver engenders in many patients after a period of time.

3. Intramuscular liver extract therapy is of value in the so-called liver-resistant cases of pernicious anaemia. Minot and Murphy stated several years ago that in their opinion no genuine case of pernicious anaemia ever failed to respond to liver therapy, provided a sufficient quantity of the potent material was taken. Gänsslen,¹⁰ also, agrees with this dictum in his latest paper. The so-called liver-resistant cases are frequently not examples of pernicious anaemia at all, although the blood picture may simulate that disease closely. During the past three years I have seen ten patients with red cell counts around a million, colour indices over unity, and megaloblasts in the blood, who were sent to me as cases of pernicious anaemia which had failed to respond to liver therapy. Vital staining methods clearly showed that these cases should be classified as haemolytic anaemia, and not as pernicious anaemia, since reticulocyte percentage counts of from 10 to 50 were present over long periods, with no subsequent rise in the blood level. Four of these patients, at post-mortem examination, were found to be cases of lymphadenoma, three others have died with no definite pathological lesions other than evidence of anaemia and haemolysis, while three are still alive and going downhill steadily. No family history of haemolytic anaemia could be discovered, and no fragility of the red cells to hypotonic saline was present.

POSSIBLE MISDIAGNOSIS

Other conditions which may lead to faulty diagnosis are aleukaemic leukaemia, certain obscure cases of liver disease associated with cirrhosis and hepatitis, and malignant disease of the gastro-intestinal tract, especially of the stomach. In the past eighteen months I have seen two cases of severe anaemia, diagnosed as pernicious anaemia, which reacted to liver therapy excellently. After some months the red cell count began to fall, in spite of intensive treatment. In one case numerous intramuscular injections of campolon failed to improve the blood picture, which by this time was definitely of the secondary type. Cancer of the stomach was found in both cases at post-mortem examination. It is worth remembering that, while failure to respond to adequate liver therapy is strongly against the diagnosis of pernicious anaemia, a response to such treatment is not proof that a primary anaemia is present, since several other diseases with severe anaemia may also react in a similar way. This is particularly true of diseases of the gastro-intestinal tract, where the lesion interferes with the production of the active principle from protein, or its absorption from the bowel. Thus, cases of malignant disease, sprue, dysentery, etc., may react to liver treatment with a reticulocyte response and a subsequent rise in the blood level. After making due allowance for faulty diagnosis as the principal cause of failure to respond to liver therapy, there remain a limited number of cases of genuine pernicious anaemia which fail to react to the usual dose of liver or liver extract by the mouth. Defective assimilation and absorption is presumed to be the explanation of this failure. Many of the cases are elderly persons with marked arterio-sclerosis and long-continued chronic focal sepsis. By intensive feeding of a daily dose of extract equal to 1,000 grams of raw liver, and in some patients by the additional use of large amounts of iron, the blood level can be restored to normal in the majority of cases of the so-called liver-resistant type. It is more economical and rapid, and obviously far more pleasant for the patient,

to obtain such a result by the intramuscular introduction of the active principle. Gänsslen states that he has never failed by this method in any liver-resistant case in which the diagnosis of pernicious anaemia was undoubtedly correct.

CONCLUSIONS

1. Gänsslen's liver extract for intramuscular injection is shown to be a highly potent preparation. Its effectiveness is about one hundred times that of raw liver given by the mouth. No undesirable local or general manifestations occurred as a result of its injection.

2. (a) Intramuscular liver extract therapy is of particular value in cases of pernicious anaemia which are critically ill, with marked nausea and vomiting. The necessity for blood transfusion is reduced to a minimum if adequate quantities of liver extract are given intramuscularly. (b) The so-called liver-resistant cases of pernicious anaemia should be treated by intramuscular injection of liver extract.

3. In patients in the remission stage the fortnightly injection of liver extract enables the maintenance dose of

liver to be reduced to limits which can be easily tolerated even by persons who have an active dislike for the consumption of this organ. The importance of maintaining a normal blood level in such patients, for the prevention and treatment of subacute combined degeneration of the cord, is discussed.

REFERENCES

- Minot, G. R., and Murphy, W. P.: *Journ. Amer. Med. Assoc.*, 1926, lxxxvii, 470.
- Cohn, E. J., Minot, G. R., and others: *Journ. Biol. Chem.*, 1928, lxxvii, 325.
- Cohn, E. J., McMeekin, T. L., and Minot, G. R.: *Proc. Soc. Biol. Chem.*, 1930, vii, 49.
- West, R., and Howe, M.: *Journ. Biol. Chem.*, 1930, lxxxviii, 427.
- Castle, W. B., and Taylor, F. H. L.: *Journ. Amer. Med. Assoc.*, 1931, xcvi, 1198.
- Dakin, H. D., West, R., and Howe, M.: *Proc. Soc. Exper. Biol. and Med.*, 1930, xxxviii, 2.
- West, R., and Howe, M.: *Journ. Amer. Med. Assoc.*, 1931, xcvi, 685.
- Wilkinson, J. F.: *Lancet*, 1931, ii, 791.
- Gänsslen, M.: *Klin. Woch.*, 1930, ix, 2099.
- Idem: *Deut. med. Woch.*, 1931, xli, 1926.
- Davidson, L. S. P.: *Lancet*, 1931, ii, 1395.
- Beebe, R. T., and Lewis, G. E.: *Amer. Journ. Med. Sci.*, June, 1931, clxxxi, No. 6, 796.

RESISTANT SPRUE ANAEMIA YIELDING TO INTRAVENOUS LIVER EXTRACT

BY

SIR LEONARD ROGERS, K.C.S.I., M.D., F.R.C.P.,
F.R.S., I.M.S.(RET.)

PHYSICIAN TO THE LONDON HOSPITAL FOR TROPICAL DISEASES
AND

W. E. COOKE, F.R.C.S.I., D.P.H.
RESIDENT MEDICAL SUPERINTENDENT TO THE HOSPITAL

The patient, a European male aged 47, has been under the care of one of us (L. R.) at the London Hospital for Tropical Diseases, at intervals since 1926, for relapsing sprue contracted originally in Burma in October, 1923, and complicated by severe anaemia. At first he responded well to a milk diet and returned to Burma, only to come home again in 1925 with a recurrence of his sprue, with sore tongue, fatty stools, and anaemia. In 1926 he was again treated with milk diet (and was also given small doses of novarsenobillon, as a positive Wassermann reaction had been obtained), with good effect on the anaemia. In addition, he had injections of an autogenous oral streptococcal vaccine, which resulted in the clearing up of the soreness of his tongue. In December, 1928, he was readmitted with severe anaemia; red blood cells 1,470,000, haemoglobin 50 per cent., and colour index 1. A transfusion of 500 c.cm. citrated blood was given (causing rather a severe reaction), together with liquor arsenicalis orally, and a milk diet. Once more good results occurred, and the blood was restored to about normal. In July, 1929, he was readmitted with sprue symptoms and moderate anaemia, and was treated with 1 lb. of liver extract and Fairley's high protein diet. By January, 1929, he had gained a stone in weight, and his blood was practically normal again, but he relapsed

once more in July, 1930, to pick up yet again on liver extract and high protein diet. He continued taking one to three tubes of liver extract daily after leaving hospital, but had to be readmitted in February, 1931, when the liver treatment failed to have as good an effect as previously.

On July 29th, 1931, he was again admitted to the hospital with severe anaemia, but freedom from the former sprue symptoms of diarrhoea and sore tongue. Red blood cells were 1,940,000, haemoglobin 40 per cent., and colour index 1, and there were signs of megalocytic anaemia, including the presence of scanty normoblasts and of megalocytes. On admission, in view of the good effects of the previous blood transfusion, 600 c.cm. of citrated blood were injected intravenously. This was followed by

Therapeutic failure of blood transfusion with subsequent adequate haematological response to hepatex P.A.F. intravenously and full dosage of liver extract and iron per os. (July 29th to October 22nd, 1931.)

a temperature of 104° F. on the next day, accompanied by vomiting of bile-stained fluid, and the passage of dark urine. There was drowsiness for three days and slight jaundice, while at the same time the liver became somewhat enlarged and tender, the reaction being thus a severe one. Unfortunately, instead of the hoped-for improvement in the condition of his blood, it steadily deteriorated, until on September 1st it showed red blood cells 1,000,000 and haemoglobin 20 per cent. His condition caused grave anxiety, for in the meantime both Parke, Davis and Co.'s ventriculin orally, and the same company's liver extract subcutaneously, had failed to check the downward course of the blood changes. Dr. Donald Hunter was now asked to see the patient with us, and he suggested iron orally in the form of Bland's pills, and the trial, if available, of intravenous injections of the new form of liver extract recently used by Professor Minot and his colleagues in Baltimore. A cable to Professor Minot informed us that this could be supplied by Evans Sons Lescher and Webb, in the form of hepatex P.A.F., so we decided to try it.