I am of opinion that the toxic action of salvarsan compounds on the liver is not sufficiently borne in mind in the very extensive use of this drug at the present day. Professor Maclean has suggested that liver damage is less likely to follow salvarsan administration if the liver cells have stored in them a certain amount of glycogen, and he has recommended that danger may be avoided by taking glucose shortly before the drug is given. This precaution is undoubtedly of value, and it has been emphasized by the use of a drug having like effects on the liver—namely, chloroform in its production of delayed chloroform poisoning after anaesthesia.

I wish to express my sincere thanks to Mr. J. Webster and Dr. Roche Lynch for valuable and much appreciated help in the work connected with this paper.

THE BLOOD SERUM IN PERNICIOUS ANAEMIA.

WITH NOTES ON THE NATURE OF THE DISEASE AND ITS TREATMENT.

A Preliminary Note.

BY

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Two years and a half ago, when making some observations on the phosphorus content of the corpuscles, clot, serum, and plasma of pernicious and other anaemias, I noticed that the colour of the plasma or serum of pernicious anaemia differed from that seen in the plasma or serum of any other anaemia or of normal blood. Since making the observation I have used it regularly in the diagnosis of pernicious anaemia and have found it a most useful and reliable, if not a pathognomonic, test of this anaemia.

By pernicious anaemia I mean a severe anaemia of unknown cause with high colour index, almost always above normal; urobilinuria; a tendency to megaloblast formation, and leucopenia; absence or great scarcity of blood plates (as seen on smears); and, judging from observations which are recorded in this paper, serum and plasma with special characteristics.

The serum in pernicious anaemia is always of a definite yellow colour, varying in tint from that of a cowslip to the deeper buttercup yellow of Canada balsam, whilst the serum of all other anaemias is much paler, and resembles straw-coloured water. The yellow colour is so distinct that it can be recognized in the serum or plasma if only two or three drops of blood are drawn into, and half fill, a small bulb made of thin glass with inlet and outlet of a narrow bore which can be closed hermetically by sealing-wax. Care must be taken not to heat the glass bulb and blood with the wax, for this would tend to haemolyse the blood; it is for this reason that sealing the ends of the bulb with heat is risky. For the past two years I have regularly carried these glass bulbs about with my blood slides, and used one in each case of anaemia examined. The blood runs freely into the small bulb from a needle puncture (when it is held lower than the blood drop on the finger), and when the ends of the bulb are sealed I stand it upright, or if taken at a patient's house I carry it upright in a small box in a waistcoat pocket until serum and clot separate. The colour of the serum is then seen, and, judging from a score or more of cases of pernicious anaemia and twice the number of other anaemias—Banti's disease, the leukaemias, secondary anaemia from any cause, purpura haemorrhagica—a diagnosis of pernicious anaemia from any other anaemia can be made. I have not applied the test to the anaemia of intestinal parasites which simulates pernicious anaemia, as I have never had any case of the condition under my care. A very important point is that serum from cases of severe secondary anaemia is pale straw colour, not yellow, in all the cases I have tried, whether due to septic infection, haemorrhage, or concealed malignant disease, which all have a high colour index (though not above 0.9) and a microscopic red blood cell appearance simulating pernicious anaemia but with leucocytosis. The blood in these severe secondary anaemias is specially referred to in the diagnosis of pernicious anaemia by Gulland and Goodall.¹

a. Glass bulb;
b. with clot and serum.

This serum bulb test is very simple and has been a most useful aid to me many times in the diagnosis of the type of anaemia under investigation. In its average appearance I can tell from the serum alone, and without the estimation of the colour index or an examination of the blood film, whether I am dealing with a case of pernicious anaemia; but, of course, I always make and examine a blood smear, and wherever possible estimate the colour index as well. On some occasions I have had my clinical opinion of a case confirmed or altered on my way home in a train by the colour of the serum which has separated from the clot.

There is no question of any pink colour in the deeper coloured yellow serum—nothing whatever to suggest the presence of any trace of haemoglobin or haemolysis; nor is it of a bile pigment tint.

In taking larger amounts of blood from a vein haemolysis is liable to occur in health or disease if there is any trace of alcohol or distilled water in the syringe or needle. This is avoided by drawing 0.5 c.cm. of sterile 5 per cent. sodium citrate solution through the sterilized needle into the sterilized syringe, and withdrawing the piston to its full extent, thus rinsing the barrel of the syringe with the citrate solution, which is then expelled from the syringe. The needle is then put into the vein, and blood withdrawn. Finally, the blood is expelled gently into a sterile test tube, which is handled very gently and laid down in a sloping position to facilitate separation of clot and serum. The serum is pipetted away from clot without disturbing red corpuscles and then well spun in a centrifuge.

Every precaution is taken to avoid haemolysis. Some of my specimens were taken with dry Wassermann outfit needles direct into a dry sterile test tube without any syringe; some were taken with an oiled syringe by Dr. Loveday, who has been director of our clinical laboratory for many years, and is thoroughly experienced in the dangers of haemolysis and the methods of avoiding it.

At first I could find no reference to the colour of the serum in pernicious anaemia in textbooks of medicine and pathology or monographs on blood diseases. There is no mention of it in Hunter's monograph, in French's full article on pernicious anaemia in Allbutt's *System of Medicine*, or in Gulland and Goodall's book on the blood. In the last named work a suggestion that the increased haemoglobin index may be accounted for by the presence of haemoglobin in the serum is mentioned, but no evidence is advanced in support of it.

In the section by Lazarus (p. 20) on the clinical methods of blood investigation in *Die Anaemie* (Ehrlich, 1909) there is no reference to the colour of the serum. The only mention of it that I have been able to find is in Pantou's *Clinical Pathology*, where it is said:

"The bile colour of some serums is more closely simulated by the greenish-yellow pigment present in the serum of nearly all cases of pernicious anaemia. A certain percentage of these cases are, in addition, actually jaundiced."

In none of my cases of pernicious anaemia has the serum had a greenish tint.

Cause of the Colour of the Serum.

A larger amount of serum than that obtained in the bulbs is necessary to make any observations on the cause of the colour; for this purpose 5 c.cm. of blood or more are withdrawn from a vein in the arm with the precautions already referred to. The colour of the blood serum and plasma in pernicious anaemia calls to mind the striking yellow colour of the fat in the body, and the question of any possible connexion between the two and of the colouring matter being a lipochrome naturally arises. There is also the possibility of the presence of some bile pigment in the serum even in cases in which there was no real jaundice. I therefore examined the serum with the spectroscope—using a simple pocket or straight vision instrument—and immediately saw two very distinct absorption bands in the position of those of oxyhaemoglobin, and a much less distinct narrow third band farther to the right in the green in some specimens. These absorption bands appeared in all the specimens of pernicious anaemia serum or plasma examined except the very pale ones. This difference may be due possibly to the small quantity used, but possibly, as I believe, to there being more than one form of the disease. In the deeper coloured serum the bands are very distinct when the depth of fluid is only one-third of an inch and the shadows are about the same intensity as those of oxyhaemoglobin in 0.01 per cent. solution as depicted in Starling's *Textbook of Physiology*. I never saw any bands to the left of the D line in the red. In normal serum and

plasma, with their paler colour, I found the same absorption bands, though generally less distinct.

No modern work of reference that I know of has any mention of the presence of oxyhaemoglobin in normal serum. MacMunn and Thudichum refer to it and attribute the third band in the green to "lutein," which is probably a lipochrome.

Spectroscopic Observations.

With the help of Mr. Ernest Jones, M.Sc., M.B., my house-physician in 1920, the observations with the pocket spectroscope were checked with a laboratory instrument with which we could measure the wave-lengths of the definite absorption bands. In all the cases examined these were identical with the absorption bands of oxyhaemoglobin—namely:

The band near	...	...	D 575 to 585
The band near	...	...	E 530 to 550
D line - 590	...	...	E line 530.

There was not the slightest indication of any absorption band in the red.

No absorption bands were seen in any other form of anaemia—Banti's disease; purpura haemorrhagica; septic anaemia; secondary anaemia from haemorrhage, malignant disease, or any other cause; the leukaemias—and, especially, I repeat, as a very useful point of differential diagnosis, that they were not present in those forms of anaemia, previously referred to, which simulate by their high colour index (for a secondary anaemia) and by their microscopic blood picture pernicious anaemia. The yellowness of the serum, which contained enough oxyhaemoglobin to give its characteristic absorption bands, was striking: there was no trace of pink tint in it.

Haemoglobin, in the small quantity seen microscopically in a solitary blood corpuscle, is yellowish. Many such yellow corpuscles together give the normal pink blood colour, but I have been unable to find any statement in textbooks of physiology or works on the blood that haemoglobin is ever yellowish in solution. I have diluted normal blood with water so much that I could see no colour of any kind in the fluid, but only in the meniscus at the top of a small test tube, and this was pink. The question as to whether haemoglobin in the serum of pernicious anaemia which was yellow existed in some new combination or solution then arose, but I came to the conclusion that the deeper yellow shades of the serum of pernicious anaemia are produced by a weak pink solution of haemoglobin strengthening a lighter yellow pigment, possibly that which colours all the tissues of the body, especially the fat. This is seen by examining a specimen of the serum with the naked eye in the dark by artificial light. The dominant yellow colour is then less visible, whilst the haemoglobin is seen faintly pink, like weak laked blood, and not in the least brown.

The Nature of the Yellow Pigment.

It is difficult to carry out accurate chemical investigations with the small amount of serum which can be obtained from a case of pernicious anaemia, but Mr. Jones applied standard tests to some cases. He found that the yellow colouring matter of the serum was not extracted by ether. It remained after hydrolysis with alcoholic potash and subsequent precipitation of fatty acids by hydrochloric acid.

These two results suggest that the colouring matter is not a lipochrome. It is precipitated by reagents which precipitate proteins. This observation of Mr. Jones is interesting in view of the fact that a protein ricin when injected into the bone marrow produces blood appearances like those of pernicious anaemia. The identification of the pigment which colours the fat and other tissues might give a useful clue to the causation of the disease.

I have been unable to find any reference in recent English literature or textbooks to the spectroscopic examination of the blood serum of pernicious anaemia, and consequently of the absorption bands found therein. The classical work on the *Spectroscope in Medicine*, by MacMunn (1880), and *A Manual of Chemical Physiology*, by Thudichum (1872), though valuable and interesting, are out of date, especially in the nomenclature of the haemoglobin derivatives. I accordingly wrote to Sir Archibald Garrod, with whom I had previously had some correspondence about the spectroscopic appearance of the blood in enterogenous cyanosis. He kindly directed my attention to some work by Schumm in the *Zeitschrift für physiologische Chemie*, 1916, vol. 97. This observer recorded in this and previous volumes of the same journal his observations on haematin as a pathological constituent of the blood in certain diseases,

including pernicious anaemia. He gives references to similar observations by Schottmüller in the *Münchener medizinische Wochenschrift*, 1914, No. 5, and by Hijmans van den Bergh and Snapper in the *Berliner klinische Wochenschrift*, 1915, No. 42. Schumm worked with a grating spectroscope and took photographic records of the spectra.

All these observers pay a great deal of attention to haematin as the chief spectroscopic feature of the serum of the various diseases, including pernicious anaemia, investigated by them, and say very little about the presence of haemoglobin, and, as far as I have seen, nothing about the colour of the serum. Schumm does refer to the brownish colour of any solution of haematin. After getting as far as this I was introduced by letter to Professor A. A. Hijmans van den Bergh of Utrecht by Sir Archibald Garrod, and he kindly offered to examine a specimen of the serum which I obtained from my patients. I sent him one, and received the following comments in reply, which I have his permission to print:

"I believe that the colour of the serum which you kindly sent me is due to a small increase of the bilirubin content and to the (oxy)haemoglobin dissolved in the serum. There was no haematin to be discovered in this specimen.

"We generally find a moderate increase of the bilirubin in the serum of patients with pernicious anaemia, especially during the periods of intensive blood destruction. Whereas the normal rate is found at about 0.2 to 0.3 or 0.5 of our units, in pernicious anaemia we find 1 to 1.5 or even 2 units. In your specimen the bilirubin value amounted to 1 unit.

"Haematin is not always present in the serum of pernicious anaemia, not even during the period of going downhill. In this disease it seldom is present in such a quantity that the colour becomes brownish, but in exceptional cases this condition is met with. (In other diseases the quantity of haematin may be so large as to give a brownish tinge to the serum—as, for instance, in some cases of anaerobic sepsis.) The quantity of haematin being only very small in pernicious anaemia, you will very seldom find a band in the red region of the spectrum. We demonstrate the haematin by adding ammonium sulphide, whereupon the very dark bands of 'haemochromogen' appear. (As they disappear rapidly, one has to look immediately after having added the sulphide or whilst an assistant adds it before the spectroscope.) But, as I have mentioned already, in your specimen there were no traces to be found, even in this way.

"Now as to the presence of the haemoglobin in the serum. This point is a very difficult and important one. We should be very careful, of course, in drawing conclusions from it. For it is always possible that the haemoglobin has escaped from the corpuscles because of the manipulations during and after collecting the blood. Therefore we always take the most careful precautions that we can for preventing artificial haemolysis (dry and warmed tubes, no shaking of the tubes, careful centrifugalizing, etc.).

"Might it not be possible that the presence of haemoglobin in the serum in your cases of pernicious anaemia is due to artificial haemolysis? For in our cases I do not remember to have found more haemoglobin than can be met with in every normal serum. Perhaps we did not look at it with sufficient attention. I must confess that the supposition of an artificial haemolysis as a cause of the presence of haemoglobin in your specimens of pernicious anaemia cannot explain the curious fact that you never find it in other anaemias.

"Theoretically, of course, it is quite intelligible that a slight intravascular haemolysis should occur in patients with pernicious anaemia. I think it would be of the greatest interest to repeat your experiments with the most careful precautions. I hope you will succeed in bringing this problem into clearness. I shall pay attention to it and let you know the results of our observations."

After receiving the letter I took blood from a patient with a sterilized and perfectly dry Wassermann outfit needle and rubber tubing, letting the blood flow into a paraffined test tube. This was carefully placed in an inclined position and the serum separated well; it was perfectly clear and of yellow colour. It was withdrawn by pipette introduced well away from the clot and well centrifuged. The oxyhaemoglobin bands were there as before. I could not get the haemochromogen bands by adding ammonium sulphide. This suggests that my previous methods were equally careful. I have purposely avoided the immediate treatment of the blood in a centrifuge, fearing that some traumatic haemolysis might be caused.

When the yellow serum of pernicious anaemia is kept in sealed tubes it begins to lose its colour in a few days, and by the end of a week the absorption bands of oxyhaemoglobin become much less clear and eventually indistinguishable. In one case it turned to a light cherry colour after being kept in a sealed tube a couple of months, and gave a spectrum of haemochromogen. I could not understand what had happened when the serum lost its colour and the bands of oxyhaemoglobin until I heard from Professor Hijmans van den Bergh of his methods of identifying haematin by reducing it with ammonium sulphide and production of haemochromogen. It

then became quite clear, especially when it is remembered that the spectrum of haemochromogen will appear in dilution of 1 in 25,000, and that its formation is even a more delicate test for blood than the spectrum of oxyhaemoglobin itself.² The explanation of the loss of colour in the sealed tubes seems to be that the oxyhaemoglobin changes to haematin in time, and this in weak solution does not deepen the colour of the serum to the same extent as the haemoglobin does. This explanation I put to the test by unsealing an ampoule of serum which I had kept for a couple of months, and which, from being quite yellow and giving good oxyhaemoglobin bands when fresh, had become pale and showed no spectrum. On adding to this some ammonium sulphide I obtained a very clear absorption spectrum of haemochromogen, clearer than that of oxyhaemoglobin had originally been. The change to the cherry red in the ampoules is probably due to the oxyhaemoglobin being changed to haematin, and this body being further changed, probably by decomposition, to haemochromogen, which has a cherry-red colour in solution, haematin being in the same strength nearly colourless.

Professor Hijmans van den Bergh says that he has not paid much attention to the oxyhaemoglobin in pernicious anaemia serum, being chiefly interested in the haematin. It is possible, however, that the haemoglobin is the forerunner of the haematin and its presence in the plasma in the blood vessels the essential feature of pernicious anaemia. Some haemoglobin compound most probably does circulate in the plasma ready to be deposited as haemosiderin in the liver. An explanation of the difference in my results and those of other Continental observers may be that there is more than one variety of pernicious anaemia, and clinical appearances of the disease, in my opinion, support this view.

In two or three of my original cases in which the serum was rather orange-coloured I found the oxyhaemoglobin bands more distinct than usual, but only in one, which I met with quite recently, have I seen a band in the red. I did not try Professor Hijmans van den Bergh's method of getting the haematin bands until the last month because I did not know of it earlier. I failed to get haemochromogen by adding ammonium sulphide in the ordinary way.

The fact that oxyhaemoglobin occurs in the serum of cases of pernicious anaemia obtained from the vein of a living person and separated from the clot in a test tube is, of course, no proof that the oxyhaemoglobin is present in the plasma of the circulating blood. It is quite possible that in spite of every precaution taken to prevent it some haemoglobin escapes from the red corpuscles during the process of withdrawal of blood from a vein; but even if artificial haemolysis does occur this does not affect the fact that oxyhaemoglobin appears in the serum of pernicious anaemia, but not in that obtained by identical methods and with identical precautions from any other form of anaemia. There is, however, some reason to think that oxyhaemoglobin does exist in the plasma of the circulating blood. It is an unquestioned fact that the red corpuscles in pernicious anaemia carry, on the average, more than the normal amount of oxyhaemoglobin, sometimes as much as 1.5 times this amount; and it is quite possible that more oxyhaemoglobin is manufactured in pernicious anaemia than there are red corpuscles to carry it, and that some circulates free in the plasma. In pernicious anaemia there is not such a deficiency of oxyhaemoglobin as there is in an anaemia of low colour index and low blood count.

Is Pernicious Anaemia a Malignant Disease of the Blood?

Pernicious anaemia may be a form of malignant disease of the blood—that is, a reversion to embryonic type of blood and blood formation. The scarcity of blood plates with such evidence of red-marrow activity always strikes me as peculiar. At most, the blood plates number very few, as compared with normal blood, and are insignificant as compared with the enormous numbers found in some cases of secondary anaemia, such as that due to moderate haemorrhage, chronic nephritis, and cancer. In some cases of pernicious anaemia scarcely any can be seen. As blood plates contain nuclein and resist peptic digestion, they are probably the remains of the nucleus of the bone-marrow erythroblast. Their presence, and that of nucleated red cells, is, in my opinion, an indication of an attempt by the bone marrow to regenerate red blood corpuscles, and when they occur the prospects of improvement in pernicious anaemia, temporarily at any rate, are good. But their small number conveys the impression that with all its apparent activity, judged from its appearance, the marrow in pernicious anaemia is not pro-

ducing red cells well, and this raises the question, Are they being produced anywhere else? Some justification for this question is to be found in the resemblance which the megalo-cytes, microcytes, and poikilocytes of the disease have to similar forms met with in embryonic mammalian blood, as described by Emmel.³ His observations are striking, and in my opinion support the view that in pernicious anaemia there is a reversion to embryonic type of blood and blood formation. I hope at a later date to go more fully into this question and discuss the bearing which the scantiness of blood plates has on the nature of the disease.

Treatment.

In conclusion, I may refer briefly to the very remarkable improvement which generally takes place for a longer or shorter time when pernicious anaemia is treated by liquor arsenicalis in graduated doses. I always begin with 4 minims, three times a day after food, and increase the dose by one minim every forty-eight hours until 8 minims for each dose are being taken. Then by a similarly graduated decrease in dose the original 4-minim dose is reached. Forty-eight hours later the arsenic should be stopped altogether for a week and then the same graduated course of treatment carried out again. The patient should be kept in bed and in the open air as much as possible and have well-ventilated rooms. Arsenic should be given in this way on and off for several months. When there is any cardiac muscle failure, with dilatation of the heart and oedema, as there often is in pernicious anaemia, digitalis in doses of 1/240 grain digitoxin (Nativelle's granule) daily in addition to the arsenic soon brings about a wonderful improvement; and I have seen patients with both feet in the grave recover in six weeks comparative health and activity. They all tend to relapse, however. Very few patients suffering from pernicious anaemia cannot take liquor arsenicalis. Occasionally I have given it even when vomiting and diarrhoea were present when the case first came under observation, with improvement in the symptoms.

Conclusions.

1. The serum of pernicious anaemia is always definitely yellow and that of all other anaemias nearly colourless. The colour may be seen in two or three drops of serum in a small glass bulb.
2. Oxyhaemoglobin absorption bands appear in the serum of the great majority of cases of pernicious anaemia, and can easily be seen by a pocket spectroscope. They are not seen in the serum of other anaemias.
3. Continental observers have found haematin in the serum of some cases of pernicious anaemia, and they believe that bilirubin gives rise to the yellow colour.
4. There may be some free oxyhaemoglobin in the plasma of the circulating blood of the disease.
5. It is possible, judging from the serum, that there are at least two varieties of the disease.
6. Blood plates are generally very scanty or absent in the disease, but when present are a sign of potential regeneration of red cells by the bone marrow and indicate a good temporary prognosis.
7. The abnormal conditions of the red corpuscles suggest the possibility of pernicious anaemia being a malignant disease of the blood.

REFERENCES.

- ¹ *Diseases of the Blood*, p. 138. ² Starling's *Principles of Physiology*, p. 827. ³ *Amer. Journ. of Anatomy*, 1914, vol. 16, p. 127, on "The Origin of Non-nucleated Erythrocytes by a Process of Cytoplasmic Constriction."

HIGH PERCENTAGE OF ABNORMAL OBSTETRIC CASES IN GENERAL PRACTICE.

BY

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FROM January 1st to June 15th, 1922, the percentage of abnormal obstetric cases met with in my practice has been unusually high. Out of a total of 42 cases attended, 15 (45.2 per cent.) have presented some definite abnormality. These cases occurred in the ordinary course of practice, only one having been seen on the requisition of a midwife. The abnormal cases were as undernoted.

1. Primipara, aged 34. Great oedema of feet and legs for one week before confinement; very slight albuminuria. On commencement of labour great headache and flashes before the eyes. One