Carriers

During July three apparently healthy faecal carriers of *Salm. paratyphi B* phage type 3A were admitted and treated with chloramphenicol. The dosage given was 16.5 g. in seven days (0.5 g. four-hourly for four days, and then 0.25 g. four-hourly for three days). This dosage had no effect on the carrier state.

Comment

The satisfactory evaluation of the effect of chloramphenicol is not possible in so small a series of cases, especially in the absence of a control group. The rapid improvement and clinical recovery which coincided with treatment, however, were most impressive. This was particularly so in those treated early in the disease.

The dosage of chloramphenicol adequate to produce a well-marked clinical response did not in all cases render the faeces free from the infecting organisms. A similar observation was made in the case of typhoid fever by Woodward *et al.* (1949) and by Rumball and Moore (1949).

In vitro tests showed that both *Salm. paratyphi B* phage type 3A, and *Salm. paratyphi B* phage type Taunton were sensitive to chloramphenicol. It was encouraging to note that in a case in which sensitivity tests were carried out after treatment the organism had not become resistant to chloramphenicol.

It remains to be seen whether larger doses over longer periods will prevent the development of the convalescent carrier state, and whether the drug is effective in treatment of the chronic carrier condition.

I am indebted to Dr. J. Grant, medical superintendent, for his invaluable advice in the preparation of this paper, and to Dr. R. Norton, Director of the M.R.C. Public Health Laboratory, Blakelaw, Newcastle-upon-Tyne, who carried out all bacteriological investigations, including the chloramphenicol sensitivity tests.

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DIFFRACTION METHODS OF MEASURING RED CELL DIAMETERS

THEIR USE IN CLINICAL HAEMATOLOGY*

BY

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An important feature of many anaemias is the alteration which occurs in the size of the average red cell. This alteration is important in diagnosis and in treatment, since, for example, many anaemias associated with increased red cell size have a common aetiological basis and respond to liver therapy.

The recognition of changes in red cell size can be made in various ways. Probably the oldest is direct inspection of blood films, stained or unstained, under the microscope—a method fraught with difficulty even for those accustomed to making blood examinations and with positive danger for those not so accustomed. Actual measurement of the diameters of a large number of cells (Price-Jones, 1929) converts the method into one of considerable accuracy

but of such tediousness that it is ruled out except for special research. An entirely different method, depending upon measurements of the packed cell volume and of the red cell count, gives a value for the mean corpuscular volume (Wintrobe, 1946). While the packed red cell volume can be determined with an accuracy of $\pm 1\%$, the red cell count (and consequently the mean corpuscular volume) is subject to errors of at least $\pm 5\%$.

An ingenious alternative method for measuring the mean diameter of red cells depends upon the diffraction of light around the edges of the cells in an unstained film in a manner similar to that which occurs in a diffraction grating. By suitable optical methods the diffracted light can be projected upon a ground-glass screen as a series of coloured haloes (when ordinary white light is used). The diameter of the haloes varies inversely with the average diameter of the red cells. This method was originally described by Pijper (1919), and a later article (Pijper, 1947) gives a full historical review, including references to modifications by other workers, together with a detailed account of the technique and its application. It is claimed not only that the mean cell diameter can be determined but also that the degree of anisocytosis can be estimated by the degree of spread between the inner violet and outer red rings. Brief descriptions of the method will also be found in the standard textbooks on haematology (Whitby and Britton, 1939; Todd and Sanford, 1943; Wintrobe, 1946). Some workers have cast doubt upon the accuracy of claims made for diffraction methods (e.g., Fullerton *et al.*, 1933), and the general impression derived from a study of the literature is that the method is a useful confirmatory one but not reliable enough to be of use as a single critical method of diagnosis. A modification of the method, using two lamps (Pryce, 1929), has simplicity to commend it, but has been criticized by Pijper on the ground that it does not measure the diameter of the average cell but that of the smallest cell present in any considerable number.

Objects and Methods of Present Investigation

The object of this investigation was to determine whether the simple diffraction method of measuring the mean red cell diameter was of value in addition to or in place of more elaborate studies. Several uses were considered: (1) as a "screening" device in the diagnosis of untreated cases of anaemia; (2) as a control in the treatment of cases of pernicious anaemia; and (3) in the investigation of certain other conditions, particularly liver disease.

The study covers a period of almost two years, during which time all new cases showing features of haematological interest in one medical unit were personally investigated, and many followed up at intervals. In addition to conditions usually grouped under the heading of primary blood diseases a considerable number of disorders primarily involving other systems were also dealt with. Excluding a large number of investigations in which the diffraction method was used but for various reasons a complete blood examination was not made, there remain 353 blood examinations on 205 individuals in which haemoglobin, red and white cell counts, haematocrit, absolute values, sedimentation rate, and icterus index were determined in addition to the measurement of the mean cell diameter by the diffraction method and examination of stained films. Table I shows the numbers of each type of disease investigated.

Blood in all cases was withdrawn from a vein into a small bottle containing the appropriate amount of oxalate mixture (Wintrobe, 1946). Haemoglobin was determined by the oxyhaemoglobin method (Bell *et al.*, 1945), using a

*The proofs of this article were passed by Dr. Hird shortly before his tragic death on December 11, 1949.

TABLE I.—*Diagnosis of 205 Cases Studied (In Order of Frequency)*

Diagnosis	No.	Diagnosis	No.
Pernicious anaemia ..	34	Bronchiectasis	4
Hypochromic anaemia ..	27	Steatorrhoea	
Normal ..	16	Infectious mononucleosis	
Chronic lymphatic leukaemia	8	Chronic myeloid leukaemia	
Acute hepatitis ..	9	Cardiac failure	3
Bronchial carcinoma ..	7	Lymphadenoma	
Lead-poisoning ..	6	Myelomatosis	
Purpura ..	5	Haematemesis and melaena	
Chronic renal failure ..	5	Gastric carcinoma	46
Cirrhosis of liver ..	5	Banti's syndrome	
		Myxoedema	
		Miscellaneous ..	

"Spekker" photo-electric absorptiometer; red and white cell counts were made with the same pipettes, counting-chamber, and cover-glass throughout the entire period, and packed cell volumes were measured by Wintrobe's method. Mean red cell diameters were measured on a "blood cell tester" of Pijper's (1935) design manufactured by Zeiss, the diameter of the yellow ring being used and the conversion table constructed by Pijper (1947) employed to express the value in μ .

In addition, 100 films were also measured by the two-lamp method, which was chosen on account of the extreme simplicity of the apparatus required. Little difficulty was experienced in obtaining consistent readings in films with pronounced macrocytosis, since in them the haloes were small and correspondingly bright: in some films with microcytosis, on the other hand, the colours, being spread out over a wider area, were lessened in intensity, and it was much more difficult to make measurements that could be duplicated. The Pijper apparatus can be read in an ordinarily illuminated room provided that the ground-glass screen is shielded from direct light, but the two-lamp method requires a practically darkened room and a considerable space in which to carry it out. The use of oxalated blood, the delay (never more than one hour) between the withdrawal of blood and the making of films, and the thickness of the film (provided that haloes could be elicited at all) appeared to have little effect on the reading by either method.

Results

Comparison of Pijper's Instrument with the Two-lamp Method of Merlin Pryce.—One hundred consecutive films were read by the two methods, both yellow and red rings being measured in Pijper's instrument, since the two-lamp method depends upon the position of the latter ring. The average mean cell diameter by the two-lamp method was almost exactly 1μ less than by Pijper's method using the yellow ring (6.91 compared with 7.89) and 0.5μ less when the red ring was used. There was moderately good correlation between individual readings.

Relation of Mean Cell Diameter to Mean Corpuscular Volume.—The mean cell diameters for the 353 observations were plotted against the mean corpuscular volumes obtained from the red cell count and the haematocrit (Fig. 1). As will be seen, there is a general correlation between the two values, but the degree of scatter is disappointingly wide—e.g., a mean cell diameter of 7.5μ by the diffraction method was obtained with bloods in which the mean corpuscular volume varied between 58 and 98 c. μ (with an isolated figure of 116). Part of this error is likely to be due to the error in determining the red count, but the discrepancies are greater than can be explained solely on this basis. As a method of estimating macrocytosis (in the sense of increased corpuscular volume), therefore, the mean cell diameter as determined by diffraction methods must be considered unreliable except in the extreme upper range ($>8.5 \mu$), being subject to errors of both commission

Fig. 1.—Mean cell diameters as determined by Pijper's instrument plotted against mean corpuscular volumes (353 observations).

and omission, while in assessing microcytosis it seems to be valueless, although, unfortunately, no cases showing microspherocytes were encountered in this series.

Use as a Screening Examination in the Investigation of Untreated Cases of Anaemia.—Here the principal, if not the sole, interest centres upon cases showing a high mean cell diameter. Taking the figure of 8μ as a dividing-line, there were 108 examinations in which a higher value was obtained. Fifty-five occurred in patients with pernicious anaemia or steatorrhoea (including some under treatment), 23 in liver disease, and 30 in miscellaneous conditions. No normal subject was found to have a mean cell diameter greater than 8μ . There were 11 individuals in whom the value was higher than 8.5μ , and 9 of these were patients with pernicious anaemia or steatorrhoea, untreated or in relapse. It is of interest that 18 cases of pernicious anaemia or steatorrhoea seen before treatment or in a state of relapse all showed a mean cell diameter of more than 8μ . In some of these the anaemia was not severe (in one case which presented with subacute combined degeneration of the cord the haemoglobin was 13.9 g. %). It seems improbable, therefore, that an untreated case of pernicious anaemia will be missed by diffraction methods of estimating the mean cell diameter, but the exact diagnosis and the distinction from other causes of a high cell diameter depend of course upon fuller blood examination. Fig. 2 shows the distribution of conditions showing a mean cell diameter greater than 8μ at the initial examination (excluding patients with pernicious anaemia first seen while under treatment).

Use in Control of Treatment in Pernicious Anaemia.—Fig. 3 (average of 8 cases) shows the return to normal of mean corpuscular volume and mean cell diameter under specific therapy. The fall in mean cell diameter roughly

Fig. 2.—Distribution of cases (other than pernicious anaemia under treatment), showing a mean cell diameter greater than 8μ . The variations in the scale for the mean cell diameter are due to the effect of the conversion table, which does not include certain intermediate values.

FIG. 3.—Return of mean corpuscular volume and mean cell diameter to normal in pernicious anaemia under specific therapy (average for eight cases).

rhoea, has shown a haemoglobin of around 10–12 g. % over long periods with, usually, a hypochromic picture, which has responded poorly to oral iron and not at all to increased dosage of liver or to administration of folic acid. Five patients have shown a mean cell diameter of more than 8μ on one or two occasions, while symptomless and with otherwise normal blood findings. Three have shown a mean cell diameter fairly consistently above 8μ . One was a woman of 68 years with a severe uterine prolapse for which no radical treatment was possible and which was associated with recurrent cystitis. Despite heavy dosage of liver preparations, haemoglobin averaged 10–11 g. % and macrocytosis persisted. Two other patients, both male, have enjoyed excellent health, with no neurological complications, and shown normal haemoglobin values, but have had persistent macrocytosis (confirmed by mean corpuscular volume determination) despite increases in dosage of liver. It can probably be said that most properly treated patients with pernicious anaemia will show a mean cell diameter of less than 8μ ; while a value higher than this should suggest the need for further investigation but is not by itself necessarily an indication of inadequate treatment.

Use in Other Conditions Showing a High Mean Cell Diameter.—These included a wide variety of diseases (Table II), but the most notable group was that of diseases involving the liver as shown in Table III. It is of interest that in only two of these 18 cases was the mean corpuscular volume greater than 100 c.u. One case with cirrhosis and one with Banti's syndrome showed a persistently high cell diameter despite the presence of a severe hypochromic microcytic anaemia resulting from oesophageal bleeding.

The degree of spreading of the haloes was determined by measuring the diameters of the red and violet rings in 14 films from patients with liver disease and 12 from patients

parallels the decrease in macrocytosis but takes somewhat longer to return to normal levels. Of 25 cases of pernicious anaemia or steatorrhoea followed up as out-patients and about which sufficient data have accumulated, 17 have consistently displayed a mean cell diameter of less than 8μ , and of these only one has been regarded as not being satisfactorily controlled by treatment. This patient, suffering from steator-

TABLE III.—Mean Cell Diameters in Cases of Liver Disease (Initial Findings in Each)

Condition	No. of Cases, with M.C.D.		Total
	$>8 \mu$	$<8 \mu$	
Acute hepatitis	7	2	9
Cirrhosis	4	1	5
Banti's syndrome	1	2	3
Obstructive jaundice	1	—	1
Total	13	5	18

TABLE IV.—Mean Cell Diameter and Degree of Variation in Liver Disease and Pernicious Anaemia

Condition	M.C.D. (μ)	Range (μ)
Liver disease	8.38	7.80–9.09 (–0.58 to +0.71)
Pernicious anaemia	8.56	8.00–9.33 (–0.56 to +0.77)

with pernicious anaemia in relapse, in all of which the mean diameter was greater than 8μ . The results shown in Table IV, demonstrate no appreciable difference in the degree of anisocytosis in the two conditions. This finding is not in accord with the results of inspection of the films. The red cells in liver disease showed anisocytosis little greater than normal, certainly nothing like that seen in pernicious anaemia, nor was there greater rouleaux formation than normal in the thin parts of the film which were used in the diffraction method. It seems that diffraction methods by themselves are unable to differentiate between the macrocytosis of pernicious anaemia and that of liver disease, and the claim that separation of the rings in the halo represents anisocytosis seems debatable. It is hoped to make this macrocytosis of liver disease the subject of a further study.

Conclusions

The measurement of the mean red cell diameter by diffraction methods, as an alternative to the determination of the mean corpuscular volume, cannot be recommended, since the relationship between the two is not close enough.

The finding of a mean cell diameter greater than 8μ by Pijper's instrument (using the yellow ring) will lead to detection of the great majority of cases of pernicious anaemia, either untreated or in relapse, but a number of other haematological conditions will also be included, of which the most strikingly consistent group is that associated with liver disease.

In the control of treatment of pernicious anaemia the presence of a normal mean cell diameter ($<8 \mu$) suggests that treatment is reasonably adequate, but the satisfactory response to treatment of the majority of patients in this small series makes this difficult to assess. On the other hand, the finding of a mean cell diameter greater than 8μ , even repeatedly, does not necessarily imply inadequate therapy.

Some doubt is cast upon the ability of the method to estimate the degree of anisocytosis.

Summary

The use of diffraction methods of measuring mean red cell diameters is described and their value in routine blood examination discussed. They are of value as a "screening" examination, particularly in detecting untreated cases of pernicious anaemia, but fuller blood examination is required for an exact diagnosis. In following up cases of pernicious anaemia under treatment these methods are of value, in conjunction with other blood findings, but moderately abnormal findings do not always imply an unsatisfactory clinical or haematological state. Liver disease

TABLE II.—Diagnosis in All Cases Showing a Mean Cell Diameter Greater than 8μ on One or More Occasions

Condition	No.	Condition	No.
Liver disease	13	Cardiac failure	1
Carcinoma of colon	2	Chronic lymphatic leukaemia	
Chronic renal failure		Dermatomyositis	
Lymphadenoma		Hypochromic microcytic anaemia	
Myeloma		Lead-poisoning	
Aplastic anaemia	1	Lympho-epithelioma	
Bronchial carcinoma		Refractory anaemia	

is often associated with a high mean cell diameter as shown by these methods, a result which may not be arrived at by other means.

My thanks are due to Professor J. W. McNee for advice and criticism in the preparation of this paper.

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TWO SUCCESSFUL RESECTIONS FOR CARCINOMA OF PANCREAS

BY

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Two cases of carcinoma of the pancreas in which a resection operation was carried out successfully are described below. The first patient recovered from the operation, gaining 5 lb. (2.27 kg.) in weight, but died five months later from recurrences in the abdomen. The second patient was alive and well when seen 12 months after her operation; she had put on weight, and was able to do her work as a housewife. Up to that date there was no sign of recurrence. The procedure included excision of the caecum, ascending colon, and right transverse colon.

So far no record of a colon excision with the pancreas has been traced in the literature. Hunt (1941) exhaustively reviews all cases of carcinoma of the ampulla of Vater up to that date, and includes synoptic tables showing the type of operation and the results achieved. Cattell (1948) goes more fully into technique, and Brunschwig (1942) reviews the different operative treatments but makes no mention of the colon.

Case 1

A man aged 55 was first seen in October, 1947, when he complained of jaundice of four months' duration. He gave a history of general lassitude, breathlessness, dizziness on exertion, and loss of weight for two years; he said that in the last five months he had lost 19 lb. (8.62 kg.). For 30 years he had had intermittent epigastric pain two hours after a meal, and this was relieved by taking more food and by alcohol. He had had the usual infectious diseases of childhood and also occasional attacks of influenza. In 1917, in Italy, he had had an attack of dysentery, and in 1922 appendicitis, which was treated medically. His mother had died of pulmonary tuberculosis, and his father, at 76 years of age, of heart disease.

On examination he was seen to be a thin spare man, deeply jaundiced. The liver was palpable $\frac{1}{2}$ in. (1.25 cm.) below the costal margin, and the gall-bladder was felt to extend from the ninth costal cartilage to the right iliac fossa. On October 30 he was admitted to a medical ward with a tentative diagnosis of carcinoma of the head of the pancreas.

Operation, November 4.—On opening the abdomen under local analgesia supplemented with open ether (Dr. Goldman)

the gall-bladder was seen to be distended to the caecum and there was the scar of a healed duodenal ulcer. The liver was normal in size and consistency, as were also the abdominal viscera. The pancreas was of hard consistency throughout; around the entrance of the common bile duct there was a stony-hard portion which, by inserting the blunt end of a straight needle, was proved to be composed of tissue and not a stone in the common bile duct. A diagnosis was made of carcinoma of the head of the pancreas around the entrance of the ducts into the duodenum. It was considered that a resection of it was possible.

Procedure.—The duodenum was mobilized from the posterior abdominal wall and the head of the pancreas exposed from behind, where a firm irregular mass the size of an almond was present around the opening of the ducts. The stomach was divided above the pyloric antrum. The common bile duct was ligated with stout thread and sewn over, and the duodenum was mobilized to 1 in. (2.5 cm.) beyond the duodeno-jejunal flexure. The pancreas was divided at the junction of its distal and middle thirds; its duct was found to be distended to a size rather larger than that of the ureter. The first 6 in. (15 cm.) of the jejunum was easily drawn up through the mesocolon, which was closed snugly round it. The neck of the gall-bladder was anastomosed to the distal end of the jejunum. The end of the pancreatic duct was anastomosed to the side of the jejunum about 2 in. (5 cm.) distal to the first anastomosis, where the two were easily approximated. The end of the stomach was anastomosed to the side of the jejunum, immediately below the pancreas. A soft rubber drain was passed to these anastomoses and the wound was closed.

Examination of sections of the hard mass around the common bile duct confirmed the clinical diagnosis of carcinoma of the head of the pancreas. The tumour was infiltrated by large anaplastic epithelial cells with pronounced hyperchromatosis and mitosis. Differentiation to glandular structure was seen in parts of the growth. Extensive lymphoid-cell infiltration was also present.

On January 16, 1948, the patient weighed 9 st. 3 lb. (58.51 kg.)—an increase of 5 lb. (2.27 kg.). By February 13 he was again jaundiced, and a recurrence was palpable in the scar of the abdominal incision. He died in April of asthenia.

Comment.—In this case the common bile duct was so large that its anastomosis to the jejunum would have been practicable, and I think this would have been a sounder operation. The patient had been jaundiced for four months when he was first seen, and I make a plea that in cases in which painless jaundice has been present for a month the possibility of carcinoma of the pancreas should be considered and a surgical opinion sought. The mortality of laparotomy is probably 1 to 2%; that following observation of carcinoma of the bile ducts or pancreas is 100%. Some 80% of the patients will recover after resection of the pancreas.

Case 2

A married woman aged 43 was first seen in September, 1948, when she complained of a symptomless lump in the right side of the abdomen, which she had first noted two and a half years before but which had enlarged in the last seven weeks. She had some flatulence and nausea, but no pain or vomiting. Recently her appetite had been poor. She weighed 7 st. 6 lb. (47.17 kg.), but her weight had never been more than 8 st. (50.8 kg.).

On examination a tense cystic, smooth globular swelling the size of a child's head was found on the right side of the abdomen (Fig. 1a). The tumour was movable from left to right but not up and down; on pressure it flattened into the loin, but reappeared on coughing; it did not move on respiration. The liver, gall-bladder, kidneys, and spleen could not be felt. On rectal examination the pelvis was found to be clear. Provisionally the diagnosis rested between (1) mobile retroperitoneal sarcoma in the area of the pancreas, duodenum, and transverse mesocolon; (2) an atypical carcinoma of the pancreas; and (3) an atypical pelvic tumour rising into the abdomen.