THE HAEMOLYTIC COMPLICATIONS OF PROSTHETIC HEART VALVE REPLACEMENT

A thesis submitted

by

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DECLARATION

The planning and institution of all of the investigations in this thesis was entirely my own work, as was the coordination of laboratory data and the analysis of results. All clinical aspects (choice of cases, history taking and clinical examination, supervision of anticoagulant control, and specimen collections) were conducted by myself, apart from the routine evaluation of cardiac status. This was carried out by Mr W. H. Bain and his colleagues of the cardiac surgery unit from whom I obtained information regarding the prosthetic valve function in the patients.

The majority of the laboratory techniques utilized in these studies have been of routine nature, and I have received the willing cooperation and assistance of my laboratory colleagues (p 14). The extent of my personal contribution in relation to this technical assistance can be outlined as follows:-

1. Haematological Investigations

Standard blood counts, reticulocyte counts, preparation of blood films and urinary haemosiderin specimens, plasma haptoglobin and serum F.D.P. estimations, and serological tests were carried out by technical staff. The examination and reporting of all blood films and urinary haemosiderin specimens, and all counts of fragmented and distorted red cells were personally performed as were iliac crest bone marrow examinations. ^{51}Cr-labelled red cell survival studies were carried out by me with technical assistance.

2. Biochemical Investigations

With the exception of the examination of urine for urobilinogen and bilirubin, all biochemical investigations were carried out by technical staff. Special mention must be made of the measurement of urinary iron levels. This was part of a joint study of urinary iron losses in prosthetic valve patients with Dr G. S. Fell of the Department of Pathological Biochemistry, Glasgow Royal Infirmary, who was responsible

for the evaluation of an auto-analyser technique for estimating the urinary iron levels.

3. Renal Investigations

These were all carried out by technical staff, but supervised by me in collaboration with Dr R. M. Lindsay and Dr M. Rahman of the renal unit at Glasgow Royal Infirmary with whom the investigation of renal function in prosthetic valve patients was a joint study.

4. Statistical Methods

I have personally carried out all the statistical tests but I acknowledge the statistical advice of Mr A. S. McLelland of the Department of Pathological Biochemistry, Glasgow Royal Infirmary.

Drawings of abnormal red cell morphology were made by me, and I personally prepared all the photographs of abnormal red cells and urinary haemosiderin.

SUMMARY

A study of the haemolytic complications of prosthetic heart valve replacement forms the subject of this thesis. A chronic traumatic intravascular haemolysis is the principal complication and its investigation constitutes the major part of this study. The history of haemolysis with intracardiac prosthetic devices extends over 20 years and is outlined, and haemolysis in patients with valvular heart disease without prostheses is discussed. The incidence of traumatic haemolysis and haemolytic anaemia, their diagnosis, pathogenesis, complications, and treatment are reviewed in detail.

There are four basic objectives to this study.

1. To assess the relative value of different tests employed in the diagnosis of intravascular haemolysis, with particular reference to serum lactate dehydrogenase levels, the urinary haemosiderin test, and the evaluation of red cell morphology.

2. To compare and contrast the incidence and severity of traumatic haemolysis with different prosthetic valve types and to attempt to identify factors of aetiological importance.

3. To examine potential complications of chronic intravascular haemolysis, including the assessment of urinary iron losses and accompanying iron deficiency, the effect of renal haemosiderosis upon renal function, and the possible potentiating effect on the thrombo-embolic complications of the release of red cell haemolysate.

4. To investigate immune haemolytic factors, including the possibility of an autoimmune component to the traumatic haemolysis, and the frequency with which blood group specific alloantibodies develop as a consequence of the considerable blood transfusion required at valve replacement operations.

From this study I have obtained the following results and conclusions.

1. Serum lactate dehydrogenase levels, known to be a sensitive index of intravascular haemolysis, were found to correlate inversely and significantly with the half-life of ^{51}Cr-labelled autologous red cells.

Urinary haemosiderin was found to be an equally sensitive indicator of chronic intravascular haemolysis and its degree relates directly to the severity of haemolysis. It is therefore a useful and simple screening procedure in the long-term care of patients with prosthetic valves.

The degree of red cell fragmentation and distortion was also found to relate directly to the severity of haemolysis. A less subjective and more accurate counting procedure for the enumeration of these abnormal forms was devised, with which it was determined that minor yet abnormal degrees of red cell fragmentation might frequently go unrecognized on routine film inspection, and that even the observation of only a very occasional fragmented or distorted red cell should be regarded as likely evidence of haemolysis. With a normal fragment count, however, the presence of a traumatic haemolysis cannot be excluded.

The various types of abnormal red cells encountered in these patients have been classified and illustrated. Of these various types only the number of triangular-shaped cells appeared to relate to the degree of haemolysis and were extremely uncommon in normal blood films. They may, therefore, have diagnostic value. It is also proposed that many of the different appearances exhibited are the result of sequential changes in morphology following trauma.

The reticulocyte count and estimation of serum bilirubin and urinary urobilinogen levels were found to be of very limited value in the detection of intravascular haemolysis and assessment of its severity.

High serum aspartate aminotransferase levels were detected in most patients with marked haemolysis and should not be regarded as necessarily indicating another complicating disorder.

2. The traumatic haemolytic complications in 87 patients with two different types of Starr-Edwards cloth-covered, metal ball and cage prosthesis, in 18 with the earlier non cloth-covered, Silastic ball models , and in 44 with the Björk-Shiley tilting disc valve were compared. Intravascular haemolysis, as detected by the presence of haemosiderinuria, was considerably more common with the cloth-covered Starr-Edwards valves irrespective of valve site than with the other models, the overall incidence with aortic, mitral or multiple replacement being 94%, 92% and 88% respectively; the corresponding figures for the Björk-Shiley valves were 31%, 15% and 20%. It was equally relatively uncommon (20%)in patients with the non cloth-covered Starr-Edwards mitral prosthesis; there were insufficient patients available for study who had the corresponding aortic model.

The degree of haemolysis was also greater with the Starr-Edwards cloth-covered valves as evidenced by more frequent red cell fragmentation and haemolytic anaemia, lower haemoglobin levels and higher reticulocyte counts and serum lactate dehydrogenase levels. It was particularly marked with the initially developed 2300 cloth-covered aortic model, although a serious degree of anaemia was uncommon.

Haemolysis was found to be promoted by prostheses of small orifice size, and evidence is also presented for the likely aetiological importance of prosthesis cloth wear and tear. It is proposed that with the cloth-covered valves systolic factors at aortic prostheses are of equal importance in the causation of severe haemolysis as are prosthetic leaks. The evaluation of the systolic murmur commonly present with aortic prostheses may be of diagnostic importance, and an unusually long and loud murmur was found in association with significant

prosthesis cloth wear and tear. It is also proposed that in the absence of regurgitation or functional stenosis, the presence of cloth and of a metal ball may be of greater significance in the genesis of haemolysis than any factor of turbulence determined by the geometry of the valve.

3. Study of the potential complications of chronic intravascular haemolysis revealed that excessive urinary iron losses were very common with the Starr-Edwards cloth-covered prostheses irrespective of the site of valve replacement, but were particularly high with the 2300 aortic model. By contrast, they were usually normal with non cloth-covered valves and in patients with Björk-Shiley valves. The degree of haemosiderinuria was observed to correlate directly with the 24-hour urinary iron level. A complicating iron deficiency was found to be common and to frequently respond to oral iron, and it is recommended that oral iron be given prophylactically if there is marked haemosiderinuria or a daily urinary iron loss exceeding 2.0 mg. A rapid, accurate and precise method of estimating urinary iron is also presented.

No evidence of impairment of renal glomerular or tubular function was detected as a consequence of renal haemosiderosis. Nor was there any indication of a possible potentiating effect of intravascular haemolysis on the incidence of thrombo-embolism.

4. There was no evidence of an autoimmune haemolytic factor, in that all direct anti-human globulin tests were negative. Blood group specific antibodies, however, developed post-operatively in 77% of 156 patients and this high incidence of alloimmunization suggests that its development is directly related to the volume of blood transfused at operation. Rhesus anti-E was the most frequent antibody detected and the antigenic potency of the (Rh)-E antigen may have been underestimated. It is recommended that tests for alloantibodies should become part of the routine follow-up of these patients.

INTRODUCTION

The studies I have undertaken which form the subject of this thesis were carried out over the course of three years from 1970 to 1973 during the tenure of my post as Registrar in Haematology at Glasgow Royal Infirmary, and latterly as Senior Registrar in Medicine at the Southern General Hospital, Glasgow.

One of my duties as Registrar in Haematology was the responsibility for the management of patients on long-term anticoagulant therapy. Amongst the various groups of patients attending the anticoagulant clinics were those who had undergone prosthetic valve replacement for rheumatic or congenital heart disease in the cardiac surgery unit at Glasgow Royal Infirmary. Shortly after taking up these duties three patients with prosthetic valves presented at the clinics who were obviously anaemic, and investigations revealed the presence of intravascular haemolysis with red cell fragmentation. The prostheses were all of the Starr-Edwards metal ball and totally cloth-covered cage type, but a prosthetic leak was present in only one.

At that time, April, 1970, while traumatic intravascular haemolysis had become a well recognized complication of cardiac valve prostheses, there was little published information available regarding the haemolytic properties of the totally cloth-covered, metal ball and cage models. They had been introduced by Starr and Edwards in 1968 in an attempt to reduce the risk of thrombo-embolism in comparison with the bare metal cage models, and to obviate the development of ball variance, a degenerative process occasionally noted with the Silastic rubber ball used in the non cloth-covered valves. There were a few reports suggesting an increased incidence of haemolytic anaemia with the cloth-covered prostheses, although Starr's group were of the opinion that this was not a severe problem in the absence of a peri-prosthetic leak. A modification to the original

cloth-covered design had already been made for haemodynamic reasons, and as the cardiac surgery unit had begun to use this modified valve there was the opportunity to study patients with different types of cloth-covered prosthesis. Investigations were, therefore, instituted on a routine basis to identify and compare the haemolytic properties of these valves, and to contrast them with those associated with the older non cloth-covered models. The study was further extended to include patients with the entirely differently designed Björk-Shiley tilting disc valve with which operations were subsequently performed, and whose haemolytic properties had not previously been studied in any detail.

The main object of this thesis has been to compare and contrast the incidence and severity of traumatic intravascular haemolysis with different prosthetic valve types, and from the results to attempt to identify factors of aetiological importance.

The large number of investigations performed during the course of this study has also enabled an analysis to be made of the relative value of the different tests employed in the diagnosis of intravascular haemolysis. A particular study has been made of the sensitivity of the urinary haemosiderin test, and the relationship between the degree of haemosiderinuria and the severity of haemolysis. This was undertaken with a view to determining whether or not, by analogy with the management of diabetes, a simple urine test might have value as a screening procedure in the care of patients with prosthetic valves. Furthermore, it seemed likely, though this had never been demonstrated, that the degree of haemosiderinuria might also relate to the total daily urinary iron excretion. It became particularly important to investigate this relationship and to obtain a more precise estimate of daily urinary iron losses, when it was soon noted that considerable quantities of haemosiderin were frequently excreted in patients with

mitral valve replacements in whom previous limited study had suggested a usually normal urinary iron output. The measurement of 24-hour urinary iron levels was, therefore, instituted in all patients using a simple and rapid auto-analyser technique which was found to be accurate and precise.

The demonstration of persistent gross haemosiderinuria in many patients raised the question of the effect of renal haemosiderosis upon renal function. This question had been posed in the past with regard to patients with paroxysmal nocturnal haemoglobinuria. In these patients it was suggested that serious impairment of renal function was uncommon. However, probably because of the rarity of this condition, and until now of other causes of chronic or recurrent intravascular haemolysis, little detailed study of renal function had been reported, and none has been carried out in patients with prosthetic valves. An investigation of renal function was therefore undertaken.

A particular study has also been made of the abnormal red cell morphology encountered in patients with prosthetic valves. This arose from the need to attempt to rationalize the varied terminology used in descriptions of fragmented and distorted red cells in different disorders, to check on the reliability of the routine inspection of blood films for red cell fragmentation, and to assess the relationship between the extent of red cell fragmentation and other evidence of intravascular haemolysis.

The red cell appearances in severe cases of prosthetic valve haemolysis are similar to those in disorders associated with micro-angiopathic haemolytic anaemia. In this condition a vicious cycle of recurrent fibrin deposition due to the release of pro-coagulant factors from damaged red cells has been proposed, and a thrombus-promoting effect of intravascular haemolysis has been suggested in other conditions. A search was therefore made for an association between the

haemolytic and thrombo-embolic complications of prosthetic valves, a possibility not hitherto explored. This was supplemented by the estimation of fibrinogen-fibrin degradation product (F.D.P.) levels which may be elevated in association with intravascular fibrin formation.

Other potential haemolytic complications following prosthetic valve replacement have also been reviewed. These relate more to the process of extracorporeal circulation required at the time of operation than specifically to the insertion of a prosthetic valve. Amongst these is the risk of blood group specific alloantibodies developing following the considerable blood transfusion that is usually required in open-heart surgical procedures. In prosthetic valve patients, who for a variety of reasons are particularly likely to require subsequent blood transfusion, alloimmunization represents a hazard. Since reports of the frequency of alloimmunization after massive blood transfusion have been few and contradictory, an investigation of this aspect was pursued in the patients under study. The possibility of an auto-immune component to the traumatic haemolysis with prosthetic valves has also been raised because of the occurrence in a few patients of a haemolytic anaemia associated with a positive direct anti-human globulin (Coombs') test after prosthetic valve replacement. Direct anti-human globulin tests were, therefore, also carried out as a matter of routine in all patients.

These studies are presented in this thesis in the following order. The relevant literature is reviewed (Ch. 1), and details of the methods employed in the investigations and descriptions of the patients examined and of their prosthetic valves are given (Ch. 2). The documentation and discussion of results of investigations is then divided into four sections, and at the end of each section conclusions are drawn and listed. The first section (Ch. 3 & 4) is concerned with

the diagnosis of intravascular haemolysis and includes observations on the urinary haemosiderin test and on red cell morphology. The second (Ch. 5 & 6), details the incidence and outcome of haemolysis with the different prosthetic valves and also factors of possible aetiological importance. In the third section (Ch. 7 & 8) the potential complications of chronic intravascular haemolysis are examined, and include the assessment of urinary iron losses and accompanying iron deficiency, the effect of renal haemosiderosis upon renal function, and the possible pro-coagulant action of red cell haemolysis. The fourth section (Ch. 9 & 10) on immune haemolytic factors deals with the question of alloimmunization following massive transfusion at valve replacement operations, and with the possibility of an autoimmune component to prosthetic valve haemolysis. A final commentary is presented in Chapter 11.

Some of the material included in this thesis has been incorporated in the following publications.

1. Slater, S.D. & Fell, G.S. (1972) Intravascular haemolysis and urinary iron losses after replacement of heart valves by a prosthesis. Clinical Science, 42, 545-553.
2. Slater, S.D., Rahman, M. & Lindsay, R.M. (1973) Renal function in chronic intravascular haemolysis associated with prosthetic cardiac valves. Clinical Science, 44, 511-515.
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CHAPTER I

REVIEW OF THE LITERATURE

I HISTORICAL REVIEW

The history of haemolysis with prosthetic heart valves extends back over the past nearly 20 years following the advent of open heart surgery. The main subject of this thesis is the chronic haemolytic complications, particularly of traumatic intravascular aetiology. Other potential haemolytic problems associated with prosthetic valve replacement are, however, reviewed. The existence of haemolysis in patients with unoperated heart disease is also discussed.

(1) Haemolysis with Cardiac Prostheses

Rose and Hufnagel and their colleagues in 1954[1] are widely quoted as first documenting the occurrence of anaemia due to a cardiac prosthetic device. They reported on nine patients with severe aortic incompetence in whom a Hufnagel plastic ball valve[2] had previously been inserted. This prosthesis was positioned in the descending aorta just distal to the origin of the left subclavian artery, and the improvements in cardiac function achieved clinically and experimentally were regarded as due to a reduction in the absolute volume of regurgitation permitting a more effective ventricular contraction and emptying. They noted that the post-operative arterial haematocrit was 3 to 8 mm (mean 5.5 mm) lower than the pre-operative value, with in one patient a drop of 16 mm from 48% to 32%. Apart from blood volume measurements which showed no significant change, no further haematological investigations were reported and no explanations suggested for these falls in haematocrit which reached anaemic levels in eight of the patients. Since these investigations were performed only 14 to 36 days (mean 24 days) after surgery, the changes in haematocrit observed may have been due to operative blood loss. An extracorporeal circulation was not employed in these operations.

It is Sarnoff, Stohlman, Case and co-workers who, while conducting

animal experiments into the surgical relief of aortic stenosis,[3] appear to have been the first to establish that a traumatic intravascular haemolysis may follow the insertion of a cardiac prosthesis[4]. They presented preliminary results of their haematological researches in 1955[5], and to them must be attributed the suggestion that the post-operative cases of anaemia of Rose et al.[1] might be related to such a haemolytic process. They described many of the now widely recognized features of traumatic intravascular haemolysis except for the changes in erythrocyte morphology upon which they made no comment. Fifteen dogs were studied in whom experimental aortic stenosis had been induced and a by-pass anastomosis between the apex of the left ventricle and the thoracic aorta constructed by means of a Lucite (methyl methacrylate) plastic tube containing a Hufnagel ball valve. All dogs developed increased red cell destruction as evidenced by a reduction in haematocrit with frequent anaemia, a reticulocytosis, haemoglobinaemia and haemoglobinuria, and gross haemosiderinuria. The survival of ^{51}Cr-labelled normal donor red cells was found to be reduced when measured in six of the dogs, and a shortened red cell half-life also demonstrated in three dogs using their own red cells. Varying degrees of haemolysis continued during the observation periods of up to 10 months. Post-mortem examinations carried out in four dogs showed heavy deposits of haemosiderin in the kidney, particularly in the proximal convoluted tubules, although renal failure was not the cause of death. The authors considered it likely that the red cells were being traumatized between the rigid ball and rigid valve housing, and that the degree of damage would be related to the impact force of the ball, in turn directly related to the magnitude and rate of change of pressure gradients across the valve. This last suggestion seemed borne out by the finding of a milder haemolysis in three other dogs

and no haemolysis in two when a Hufnagel valve was placed in the "Hufnagel position" in the descending aorta after the animals' aortic valves had been made moderately incompetent. They considered also the possibility of chemical injury to the red cells by some constituent of the plastic valves, but deduced this was unlikely as no evidence of haemolysis was found in a further four dogs with normal valves in whom a two-inch Lucite plastic, non-valved, tube was substituted for a segment of thoracic aorta.

Following this report, it was not until 1961 that Sayed, Dacie, Handley, Lewis and Cleland[6] published the first account of a mechanical haemolytic anaemia developing after insertion of an intracardiac prosthetic device in man. This occurred in a 25 year old man who, by the eighteenth post-operative day following a Teflon patch repair of an ostium primum defect, developed a conspicuous anaemia of 6.5 g/100 ml associated with numerous irregularly shaped "crenated", "fragmented", and "burr" red cells. There was a reticulocytosis, haemoglobinaemia, methaemalbuminaemia, haemoglobinuria and haemosiderinuria, and serum haptoglobins were absent. ^{51}Cr-labelled normal donor erythrocytes were eliminated rapidly from his circulation. Other investigations for possible drug-induced or immune haemolytic factors were negative. There was no response to prednisone, and blood transfusions were repeatedly required. Six months after surgery with no improvement in the anaemia re-operation was undertaken, as it seemed likely that some mechanical intracardiac factor must be responsible for the haemolysis. The left atrium was explored and a small cul-de-sac of bare Teflon was found in the septum just above the aortic cusp of the mitral valve, while the rest of the Teflon patch was covered with endothelium. This cul-de-sac was in close relation to a partial cleft in the aortic cusp which,

together with a consequent but slight degree of mitral incompetence, had originally been noted but had not been repaired (Fig. 1).

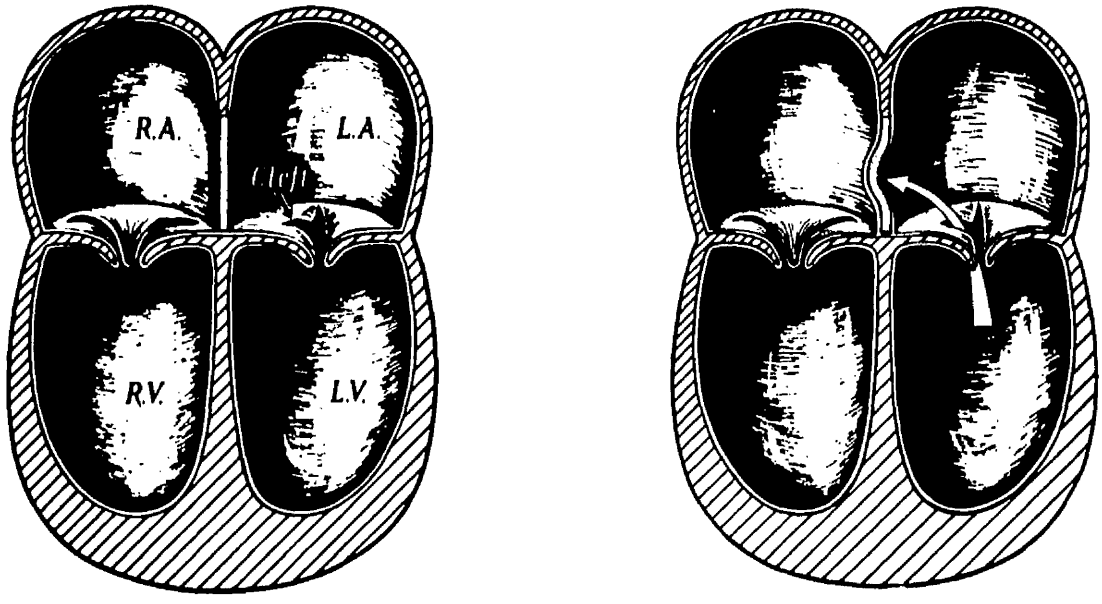


Fig. 1.

"Diagrammatic representation to show the Teflon felt patch in position, the partial cleft in the mitral valve, and the mechanism whereby the jet of blood was impinging against the patch causing the cul-de-sac and preventing endothelialization of the Teflon" (Fig. 4 of the paper by Sayed et al., reprinted by permission of the editor of Thorax)

The bare area of Teflon was covered by suturing the adjacent edges of endothelium but the mitral valve defect again left alone. By the second post-operative day the haemoglobinaemia and haemoglobinuria had disappeared, and at follow-up by four months the haemoglobin, reticulocyte count and blood film had remained normal and serum haptoglobins were normal. Haemosiderinuria, however, persisted. The authors concluded that the patient's haemolysis had resulted from mechanical conditions in the heart, and

specifically as a consequence of a mitral regurgitant jet of blood being driven against bare Teflon fibre and presumably preventing its endothelialization. Whether the Teflon fibre itself, the presence simply of a roughened non-endothelialized surface, or a turbulent blood stream was responsible was uncertain. The persisting haemosiderinuria at four months despite disappearance of other evidence of haemolysis was considered due to continued shedding of iron from the previously heavily loaded renal tubular cells.

After this important paper in 1961 other cases of mechanical intravascular haemolysis associated with prosthetic repair of endocardial cushion defects or other congenital cardiac lesions were published[7-11]. Among these the following merit particular attention because they introduced principles important in the management and investigation of patients with traumatic intravascular haemolysis.

Sigler et al. in 1963[8] suggested that turbulence might be of greater significance in the genesis of haemolysis in these patients rather than collision of red cells with some intracardiac structure, as re-operation undertaken in two of their three cases showed complete endothelialization of the Teflon inter-atrial septa but incompetence through clefts in the mitral valves which had previously been sutured. They argued too, that haemolysis might therefore even be detected pre-operatively if appropriate investigations were undertaken. They documented the iron deficiency that may occur through loss of iron as haemosiderinuria and the benefit of iron supplements. In the three cases studied there was hypochromia, a low serum iron, and increased urinary iron excretion

amounting to as much as 15 mg per day, and in one the anaemia responded well to oral iron. Of the two cases in whom re-operation was performed, repair of the mitral cleft corrected the haemolysis in one but the other died shortly after operation. In this patient post-mortem examination showed absence of stainable iron in the marrow, liver and spleen, but large accumulations of haemosiderin were present in the renal proximal tubular cells accompanied by areas of necrosis and regeneration. Attention was drawn to the similarities of the haematological features in these patients to the findings encountered in paroxysmal nocturnal haemoglobinuria.

Sanyl et al. the following year (1964)[10] confirmed these observations regarding iron deficiency, and drew attention to a deteriorating sequence of events that might develop as a consequence. Namely, that the precipitation or aggravation of anaemia by iron deficiency could result in a state of high-output cardiac failure which in turn might intensify the mechanical factors responsible for the haemolysis. They presented evidence that this cycle was operative in their patient in whom a Teflon patch repair of an atrial septal defect had been performed. Treatment of the anaemia with blood transfusion and iron appeared to break the cycle and allow the establishment of a compensated haemolytic state, thereby avoiding the dangers of re-operation.

That circumstances associated with an increase in cardiac output might increase the rate of traumatic haemolysis received experimental support from the work of Sears and Crosby in 1965[11]. They found a diurnal variation related to activity in the urinary excretion of haemoglobin in a patient with intravascular haemolytic anaemia secondary to a Teflon patch repair of an ostium primum defect. The degree of haemoglobinuria by day was constantly and appreciably greater than that excreted during the night, and short periods of increased activity during the day were associated with further

increments in haemoglobinuria. This pattern of excretion was reversed when the patient was allowed to sleep by day and be up at night. The level of total plasma haemo pigments was also higher during activity than at rest. Similar results for plasma free haemoglobin were obtained in a second patient whose anaemia followed aortic valve replacement, but the haemolysis was not sufficiently pronounced to cause haemoglobinuria so that the diurnal pattern of haemoglobin excretion could not be evaluated. The authors considered that this evidence of a direct relationship between degree of haemolysis and cardiac output, as determined by level of activity, lent credence to the view that the red cells were being mechanicallydamaged within the heart. They, too, reasoned that anaemia by increasing cardiac output might aggravate the underlying haemolytic process, whereas its correction should lead to a reduction in red cell trauma. This hypothesis was supported by the amelioration of the haemolysis that occurred after blood transfusion in their first case. Their studies also supplied objective evidence that rest might be of potential value in the management of these cases.

With the increasing use of prostheses in the surgical treatment of valvular heart disease and awareness of the potential traumatic intravascular haemolytic complications of cardiac devices, it was not long before cases of haemolytic anaemia secondary to prosthetic valve replacement were recognized, and in 1964 the findings of several workers were published.

Larson and Kirklin[12] briefly commented on the development of a chronic haemolytic anaemia, 16 months after operation in two of 48 patients in whom one or more defective aortic cusps had been replaced with a Bahnson Teflon prosthesis[13]. In both patients there was regurgitation at the aortic valve site, although other patients with prosthetic leaks did not develop anaemia. Whether or not these other patients had a compensated haemolysis was not documented.

Gehrmann and Loogen[14] described two patients in whom aortic incompetence had been treated by excision of the damaged cusps and their replacement with Hufnagel silicone-rubber impregnated Dacron aortic cusps[15]. Pre-operatively and for some time following operation haemoglobin levels and erythrocyte morphology were normal, and there was a good clinical response to the procedure. However, five months after operation in one and at ten months in the other sudden aortic incompetence developed, and the patients were found also to have an intravascular haemolytic anaemia with "schistocytes" and "burr cells" in the peripheral blood. At re-operation in one, a loose Hufnagel cusp was resutured to the aorta but a mild degree of regurgitation and of anaemia persisted. The second case was not subjected to further operation as the incompetence was not haemodynamically significant and the haemolysis was well compensated. The authors considered that leakage of blood through the suture line onto the non-endothelialized reflex edge of the cusp was responsible for the haemolysis. It is of interest that this paper appears to have been the first to document high serum lactate dehydrogenase levels in these cases.

Stevenson and Baker,[16] and Marsh,[17] each reported on two cases of haemolytic anaemia that followed the insertion of a Starr-Edwards ball and cage aortic valve prosthesis. Stevenson and Baker's cases developed the features of an intravascular haemolytic anaemia immediately after operation in one and at five months in the other. In both there was regurgitation of blood around the site of insertion of the valves. They were of the opinion that turbulence and collision of red cells with a rigid prosthesis were more likely to be responsible for haemolysis than the precise surface characteristics of the prosthesis, and that the development of this type of haemolytic anaemia indicated the presence of a haemodynamic defect. One of their

patients spontaneously improved, the other died of bacterial endocarditis. They emphasized the diagnostic importance of "pyknocytes", fragmented and distorted erythrocytes, which accounted for 17-27% of the red cells in their patients. Marsh also found marked erythrocyte distortion and fragmentation with haemoglobinaemia, methaemalbuminaemia, absent serum haptoglobins and considerably reduced ^{51}Cr-erythrocyte survival. His two cases developed severe anaemia within three to six weeks of operation. Both patients remained anaemic with short-lived responses to blood transfusion during the few months of follow-up, and investigations for other causes of haemolysis were negative. They were two in a series of seven patients with Starr-Edwards aortic prostheses, and the author found difficulty in accepting that only some factor of turbulence or direct red cell trauma had differentiated them from the unaffected patients. They both, nonetheless, subsequently developed signs of aortic incompetence. Furthermore, the other five patients appear to have been screened for haemolysis only with standard blood count, reticulocyte count and blood film examinations, so that a mild degree of haemolysis was by no means excluded.

Reed and Dunn[18] described a patient in whom severe intravascular haemolysis developed shortly after replacement of the aortic valve with a ball and cage Starr-Edwards prosthesis, and which led to his death in acute renal failure with plasma haemoglobin levels of nearly 900 mg/100 ml. Although post-operative regurgitation had developed coincidentally with the haemolysis, the haemolysis was uninfluenced by its correction at a second operation. Subsequent post-mortem examination confirmed the competence of the valve, but when viewed from above the aorta was found to so encroach upon the superior aspect of the cage of the prosthesis that, with the ball in the open position, the aorta was severely obstructed with very little clearance for blood flow around the ball. Persistence of a significant systolic gradient across

the valve had been noted at the second operation. Tests for immune haemolytic factors were negative, and the authors concluded that the haemolysis was most likely to be the result of erythrocyte trauma due to severe blood turbulence and obstruction to flow during systole. This report was of obvious surgical value, but it had significance also in its demonstration that traumatic haemolysis could occur in the absence of valvular incompetence and that systolic factors might instead be of aetiological importance.

Brodeur et al. made an assessment of the incidence of haemolysis in patients with valvular prostheses, and their findings were presented in 1964[19] and published in detail the following year[20]. The ^{51}Cr-labelled erythrocyte survival technique was used, and of 12 patients with a Starr-Edwards aortic ball and cage prosthesis eight had a reduced erythrocyte survival, three of whom were anaemic. Haemolysis was also detected in five of seven patients with aortic plus mitral ($\pm$ tricuspid) ball and cage prostheses, and three of them were anaemic. There was, however, no difference in the mean erythrocyte survival between the multiple replacement group and those with single aortic prostheses. The authors suggested that intracardiac turbulence was responsible for the haemolysis, but due to the low pressure system over the atrioventricular valves the turbulence set up by a mitral or tricuspid prosthesis appeared to be insufficient to add significantly to the rate of destruction caused by an aortic prosthesis. Extra turbulence produced by incompetence of aortic prostheses, or by mitral prosthetic leaks which would occur under high pressure in systole, would result in further shortening of red cell survival leading in some cases to anaemia. Incompetence of an aortic prosthesis was considered to have precipitated the anaemia in three of the six cases, and a mitral prosthetic leak in a fourth.

Despite the lesser turbulence expected over well functioning

mitral prostheses, Björk and Malers[21] in a study of the long-term results of mitral valve replacement with the Starr-Edwards ball and cage valve, found evidence of slight haemolysis in that serum haptoglobin levels were reduced in nine out of ten patients. Only one patient had a prosthetic leak and this was slight. No patient was anaemic.

From 1965 onwards many reports of traumatic haemolytic anaemia associated with prosthetic valve replacement, particularly of the aortic valve, have appeared in the literature. These reports include some further instances of anaemia in patients with prosthetic Teflon or Dacron aortic cusps,[22-27] but refer mainly to total prosthetic valve replacements. Different types of valve for total replacement have been constructed[28] although most are based on a ball or disc and cage arrangement, but probably none are entirely immune from haemolysis. Haemolytic anaemia has been associated, for example, with the Starr-Edwards ball and cage valve,[26,27,29-51] and indeed most reports refer to this prosthesis probably because it is the most widely used. Anaemia has also occurred with the Magovern,[40,51] Smeloff-Cutter,[52] and Surgitool[52] ball and cage valves, with the Cross-Jones caged lenticular disc prosthesis,[53] the Barnard, University of Cape-Town lenticular or disc prostheses,[54,55] and with the Gott-Daggett hinged leaflet valve[56].

The great majority of anaemic cases have developed with aortic valve replacements and usually in association with regurgitation around or through the prosthesis. In a few isolated cases obstruction to, or interference with, forward flow appears to have been held responsible.[18,41,43,44,46,48] Most of these latter cases have been due to so-called ball variance. This is the term used for the physico-chemical changes that may occur in the Silastic rubber ball component of some ball and cage prostheses, usually of aortic valves, that may lead

to swelling, fissuring, cracking or shrinking of the ball.[38,41,43,46,48,57] If swelling occurs the normal free movement of the ball may be impeded, and obstruction to flow with or without incompetence will ensue. Cracking and fissuring may distort forward flow, and shrinking of the ball is likely to lead to a leak through the valve and the ball may even escape from the cage.

While most accounts of haemolytic anaemia relate to aortic prostheses, very occasional instances have developed secondary to mitral valve replacement, and in almost every case an associated prosthetic leak has been reported.[20,29,36,37,39,42,49,53,54,58,59] Altogether these references document a total of only 15 cases of haemolytic anaemia with mitral prostheses, in 13 of whom a prosthetic leak was known to be present.

An appreciation of the incidence of this type of haemolytic anaemia as well as the frequency of a milder haemolytic state in patients with prosthetic valves also developed from evidence presented in many of the above reports and from the work of others[60-65] as studies of series of patients were undertaken. It further became apparent that a similar haemolytic process might occur in some cases of unoperated valvular heart disease and this facet of the problem requires some amplification at this point.

(2) Haemolysis in Unoperated Valvular Heart Disease

The possibility that valvular heart disease that had not been the subject of prosthetic replacement might cause a traumatic intravascular haemolysis appears to have been first raised by Damashek in 1964[66] during a case discussion at the Massachusetts' General Hospital, although it had previously been alluded to by Sigler et al.[8] The case was that of a female patient with calcific rheumatic aortic and mitral valve disease and a mild anaemia. The anaemia appeared to be haemolytic in nature and in particular was associated with

"helmet cells" in the peripheral blood suggesting red cell trauma. Subsequent post-mortem examination disclosed a striking deposition of haemosiderin in the kidneys but no stainable iron in the spleen, bone marrow or liver, a pattern typical of intravascular haemolysis. Although paroxysmal nocturnal haemoglobinuria could not be entirely ruled out, erythrocyte trauma caused by the abnormal passage of blood through diseased cardiac valves remained a possible though unproven diagnosis.

Following this report, Brodeur et al.[19,20,58] during their investigations of haemolysis in patients with prosthetic heart valves also studied patients with unoperated valve disease. They found a reduction in ^{51}Cr-labelled erythrocyte survival in 16 of 21 patients with aortic valve disease ($\pm$ mitral valve disease), and in nine of 13 with mitral valve disease alone. There was, however, no correlation between the type or severity of the valvular disease and the result of the red cell survival study. None of the patients was anaemic or showed red cell fragmentation.

In contrast to these results, Yacoub, Rogers and Taylor[67] found normal ^{51}Cr-labelled erythrocyte survival in 11 patients with severe aortic valve disease, six of whom had severe regurgitation and five had stenosis with gradients of 50 mm Hg or more. Their findings were supported by the work of Andersen, Gabrieli and Zizzi[29] who studied 18 patients with aortic or mitral valve disease pre-operatively and several months after prosthetic valve replacement. Whereas following operation all patients developed intravascular haemolysis as shown by absent or barely detectable levels of serum haptoglobin and elevated serum lactate dehydrogenase levels, pre-operatively only two of 13 tested had a

reduced haptoglobin value and none of the 18 had a high lactate dehydrogenase level.

An intriguing case of rheumatic aortic and mitral valve disease was described by Miller et al. in 1966[68]. The patient had a mild anaemia with persisting haemosiderinuria and small numbers of fragmented cells in the peripheral blood. The acidified-serum test for paroxysmal nocturnal haemoglobinuria was negative. Although ^{51}Cr-erythrocyte survival was normal when first tested this had been performed with the patient sitting or lying most of the time. When repeated with the patient undertaking more exercise the half-life of the ^{51}Cr-labelled erythrocytes fell from the initial normal value of 28 days to 17 days. There was also a decline in haemoglobin level and an increase in reticulocyte count. They studied 20 other patients with valvular heart disease but found no evidence of haemolysis in them.

Ziperovich and Paley[69] also described that year a case of haemolytic anaemia which appeared to be secondary to very severe mitral regurgitation. The anaemia and regurgitation developed concurrently after an open operation for mixed mitral stenosis and incompetence in which the stenotic element had been relieved and a 3 cm tear in the anterior leaflet of the mitral valve repaired with sutures. At the subsequent operation the recurrent regurgitant jet was estimated to be "one of the largest the surgeon had encountered in his experience both as regards volume and forcefulness". Insertion of a Starr-Edwards mitral prosthesis corrected the regurgitation and anaemia although a slight degree of haemolysis probably persisted.

Westring[35] reported a patient who, while developing a very severe haemolysis secondary to a peri-prosthetic leak after aortic valve replacement, was considered to have had a mild haemolytic anaemia

for two years pre-operatively due to her aortic valve disease. The evidence presented for this case of pre-operative haemolysis appears, however, rather unconvincing. He studied 12 other patients with unoperated aortic valve disease ($\pm$ mitral valve disease), and although three had slightly reduced ^{51}Cr-labelled erythrocyte survival, in only one did he find other corroborative laboratory signs of haemolysis.

Roberts[70] presented pathological evidence for pre-operative intravascular haemolysis. In an autopsy study on 132 patients dying of valvular heart disease he found renal haemosiderosis ("blue kidney") in four. Each of these four patients had calcific aortic stenosis and incompetence. The bone marrow in the four cases also showed severe erythroid hyperplasia, and a mild anaemia in three of them could have been due to the haemolysis although the nature of the anaemia was not precisely identified.

That traumatic intravascular haemolysis might occur due to valvular heart disease alone seemed, therefore, established notwithstanding the generally low estimates of its frequency. Further studies of the incidence of haemolysis in unoperated valve disease followed.[27,36,39,40,50,62,63,65,71-73] From these it became apparent that at laboratory level it was a not uncommon manifestation, but in only a small minority of cases did the process appear to be of clinical significance. Furthermore, erythrocyte distortion and fragmentation in non anaemic cases appeared to be rare[74]. These facts may go some way towards explaining the lack of recognition of the phenomenon prior to the advances in cardiac surgery. Indeed, Dacie[75] writing in 1967, was "not aware of a single proved case at Hammersmith Hospital within a 20-year period".

(3) <u>Other Haemolytic Complications of Prosthetic Valve Replacement</u>

Finally, the chronic traumatic intravascular haemolysis that is the main subject of this thesis must be distinguished from three other possible haemolytic complications of prosthetic heart valve

replacement.

(i) The red cell damage that occurs at operation in the extracorporeal circulation of the heart-lung by-pass circuit is well recognized and may result in a post-perfusion anaemia of several days duration.[76-83] The principle features of this complication are that the degree of cell damage is directly related to the duration of perfusion, it appears to be caused mainly by the intracardiac blood suction apparatus that keeps the operation field dry and returns blood to the main circuit, and subsequent extravascular destruction of cells damaged but not immediately lysed during extracorporeal circulation appears largely responsible for the post-perfusion anaemia. Little has been written on the effect of extracorporeal circulation upon erythrocyte morphology. One study[81] has reported the development of "burr cells", red cell fragments and spherocytes, while in another[82] no consistent morphological abnormality was seen.

(ii) Pirofsky et al. in 1965[84] also described a haemolytic anaemia that developed within one to two weeks of prosthetic valve replacement in seven patients and which in all but one case was characterized by a positive direct anti-human globulin (Coombs') test. Further investigation in two patients led them to conclude that the serological reaction was due to a true autoantibody. The authors postulated that the mechanical trauma of the prosthetic valves may have altered the surface antigens of the red cells and so precipitated an autoantibody response. The anaemia in their patients either regressed spontaneously or with corticosteroid therapy.

Hjelm et al. in 1964[85] had described one case of transient autoantibody formation six months after prosthetic valve replacement but there were no signs at that time of haemolysis. Other workers[86-88] following Pirofsky's paper reported the development of positive direct

antiglobulin reactions usually shortly after open heart surgery with cardio-pulmonary by-pass. In one study[88] a positive result was obtained in as many as 33 out of 100 patients. Four of the five prosthetic valve patients with a positive reaction shortly after surgery in one of these investigations[86] had a transient anaemia and resembled the cases reported by Pirofsky, but in neither of the other studies were Coombs' positive cases associated with "gross"[87] or "clinically significant"[88] haemolytic anaemia. The direct antiglobulin reactions in these reports were frequently weak and transient and not all patients had had prosthetic valves inserted, and the overall significance of the serological findings was considered obscure. It should be added that in none of the studies of traumatic haemolysis secondary to prosthetic valves that have been reviewed earlier in this chapter have positive direct antiglobulin tests been described. It is perhaps notable that erythrocyte fragmentation was absent in the patients studied by Pirofsky[89]. The theory of a mechanical basis for a possible autoimmune type of haemolytic anaemia with prosthetic valves remained, therefore, attractive but unproven. However, that the anaemic cases described by Pirofsky et al.[84,89] were, nonetheless, examples of a haemolytic process distinct from traumatic haemolysis is suggested from a consideration of other evidence.

Four of the seven patients described by them had in addition to their haemolytic anaemia clinical features of the so-called "post-perfusion syndrome". This is a generally benign illness lasting one to four weeks characterized by fever, splenomegaly, atypcal lymphocytes in the peripheral blood and a negative Paul-Bunnell reaction, and was originally described following usually two to four weeks after heart-lung by-pass procedures.[90-94] Cytomegalovirus infection transmitted in transfused blood has since been established as the cause of this disorder on the basis of serological studies and the

isolation of the virus from affected patients[95-97]. Haemolytic anaemia has frequently been associated with cytomegalovirus infections in children, usually accompanied by a positive direct antiglobulin test[98], but appears to be a less common complication of adult infections in which possible autoimmune factors have also been identified[97,99-101]. Haemolytic anaemia, usually of cold antibody type, is a well recognized complication of other viral infections[102]. It seems possible, therefore, that at least some of Pirofsky's cases were examples of acquired haemolytic anaemia secondary to post-transfusion cytomegalovirus infection. This condition in any event clearly requires to be considered in the differential diagnosis of haemolysis developing in the first month after valve replacement. In this regard erythrocyte fragmentation does not appear to be one of its features. It is possible, too, that it has been responsible for some of the cases of post-perfusion anaemia attributed to damage to red cells during extra-corporeal circulation.

(iii) The third potential mechanism of haemolysis in relation to prosthetic valve replacement is the development of blood group specific antibodies as a reaction to minor incompatibilities in the massive blood transfusion that is frequently required in heart-lung by-pass procedures. There have, however, been few studies of the incidence of allo(iso)-immunization after massive transfusion[39,86-88,103] and results are conflicting. In the larger series blood group specific alloantibodies have been reported to develop in as few as 1.5%[87] and as many as 24%[86] of patients exposed to large transfusions. The haemolytic potential of alloimmunization is not immediate as an immune antibody is unlikely to develop rapidly enough to react against the initiating transfusion stimulus[104]. Its establishment would be important to recognize, nonetheless, especially in patients with prosthetic valves who through traumatic haemolytic anaemia, bleeding from anticoagulant therapy, or the

need for re-operation are at risk of requiring further transfusion.

This review will continue with a consideration of traumatic intravascular haemolysis secondary to prosthetic heart valve replacement under the following headings: incidence of haemolysis and haemolytic anaemia, laboratory findings, pathogenesis, complications, and treatment. The incidence of haemolysis in unoperated patients with valvular heart disease will also be summarized. It should be added that this subject was reviewed by Dacie in 1967[75] and by Marsh and Lewis in 1969[105]. The chapter will end with a consideration of the varied terminology applied to the distorted and fragmented red cells seen in this and other conditions.

II INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA WITH PROSTHESES.

While different parameters have been employed in the diagnosis of intravascular haemolysis it has become apparent that chronic haemolysis in patients with prosthetic heart valves is very common. This is true irrespective of the site of valve replacement, although judged by the frequency of anaemia the degree of haemolysis is more severe with aortic replacements. The data from reported series of apparently unselected patients with prosthetic valves have been analysed and the results are detailed in Tables I to III. In most reports the conclusions appear to be based usually on the results of investigations done on a single occasion which has varied from weeks to years following operation. In some studies a mixture of different types or sites of valve replacement have been investigated. In these, only the results from homogeneous groups of eight or more patients are recorded in the Tables.

(1) Aortic Prostheses

Haemolysis has developed in 62.5-100% of aortic valve replacements although the incidence has generally exceeded 80%, with an overall

incidence from available data of 84%. A consequent anaemia, the definition of which has usually not been clearly specified in these reports, has occurred altogether in 8% of cases. This agrees with the figure of an 8--10% incidence of "clinically significant" haemolytic anaemia quoted by Pirofsky[61] in his experience of 317 valve replacement operations involving the aortic valve. Since an unknown number of these were probably multiple replacements and the precise valve type or types was not clearly specified this large series has not been included in Table I. It also approximates to the 5% incidence of "clinically significant" anaemia in 200 patients studied by Rodgers & Sabiston[49]. No information regarding the distribution of valve type or site was given in this report but it included cases of aortic, mitral, and multiple valve replacement.

The data from these reports do not show any definite difference between the haemolytic properties of the various valve types with one possible exception. The series studied by Hodam et al.[47] was composed of patients with the Starr-Edwards metal ball and cloth-covered cage prosthesis compared to the earlier Silastic ball and non cloth-covered cage model which has been the subject of most other reports. In this study there was an unusually high incidence of haemolytic anaemia of 31% in 39 patients. Of the 89 patients studied by Rees et al.,[51] nine had a cloth-covered Starr-Edwards valve and four of these contributed to the total of eight anaemic patients in the series. Milam et al.[52] found a substantially greater degree of haemolysis in five patients with a Dacron velour covered ball and cage aortic prosthesis compared to 15 with a non cloth-covered model, and postulated that the cloth covering might be responsible for the increased haemolysis. On the other hand, Walsh, Starr and Ritzmann[50] found no difference in the severity of haemolysis between 13 patients with cloth-covered Starr-Edwards valves and 32 with the non cloth-covered type in their apparently selected study of patients known to have haemolysis.

In studies in which information regarding the haemodynamic function of the valve has been given, a high proportion of anaemic cases have been associated with prosthetic leaks. Not all patients with leaks, however, have been anaemic nor even apparently shown evidence of haemolysis.

Prosthesis Model[a]	Total No. of Patients	No. (& % No.) with Haemolysis	Criterion of Haemolysis[b]	No. (& % No.) with Anaemia	Criterion of Anaemia	No. of Anaemic Patients with Prosthetic Leaks	No. of non-anaemic Patients with Prosthetic Leaks (& No. haemolysing)	Author(s) Reference
S-E (1)	8	8 (100%)	Hpt.<50 mg%	3 (37.5%)	PCV < 37%	N.Sp.[c]	N.Sp.	29
S-E (1)	11	11 (100%)	Hpt.reduced	5 (45%)	Calc. from available data (Hb.<13.5 g% males:<11.5 g% fem.)	N.Sp.	N.Sp.	27
S-E (1)	11	10 or 11 (91 or 100%)	Hpt.<60 mg%	0	N.Sp.	-	N.Sp.	62
S-E (1)	15	14 (93%)	Hpt.<30 mg%	0	N.Sp.	-	3 (2 or 3)	63
S-E (1)	15	12 (80%)	^{51}Cr<25 days	5 (33%)	N.Sp.	5	1 (1)	45
S-E (1)	?58	N.Sp.	-	1 (?2%)	"significant anaemia"	N.Sp.	8 or 9 (?)	64
S-E (1)	?74	N.Sp.	-	3 (4%)	N.Sp.	3	16 (?)	60
S-E (1)	80	N.Sp.	-	4 (5%)	N.Sp.	0	N.Sp.	51
S-E (1)	176	N.Sp.	-	10 (6%)	PCV < 33%	10	14 (?)	42
S-E (?)	45	37 (82%)	Hpt.<15 mg%	?	"No case of serious haemolytic anaemia seen"	N.Sp.	N.Sp.	65
S-E (2)	39	N.Sp.	-	12 (31%)	PCV < 35%	3 or more	N.Sp.	47
Magovern	8	7 (87.5%)	Hpt.<70 mg%	1 (12.5%)	?PCV<35%	N.Sp.	N.Sp.	40
Magovern	68	N.Sp.	-	1 (1.5%)	N.Sp.	0	? 6 (?)	51
Smeloff-Cutter	15	14 (93%)	Hpt.<70 mg%	1 (7%)	N.Sp.	N.Sp.	N.Sp.	52
UCT	104	N.Sp.	-	4 (4%)	N.Sp.	2	22 or more (?)	55
Gott-Daggett	24	15 (62.5%)	Hpt.reduced	3 (12.5%)	N.Sp.	3	9 (6)	56
?	54	N.Sp.	-	6 (11%)	N.Sp.	N.Sp.	N.Sp.	59
Total available from data		128/152 = 84%		59/760 = 8%				

Notes: a S-E (1) = Starr-Edwards Silastic ball & non cloth-covered cage models
S-E (?) = Starr-Edwards ball & cage models of unspecified type, probably S-E (1)
S-E (2) = Starr-Edwards metal ball & cloth-covered cage model 2300
UCT = Barnard, University of Cape-Town prostheses

b Hpt. = Serum haptoglobin levels
^{51}Cr = ^{51}Cr-labelled red cell survival study half-life

c N.Sp. = Not specifed

TABLE I REPORTED INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA WITH AORTIC VALVE PROSTHESES.

Prosthesis Model[a]	Total No. of Patients	No. (& % No.) with Haemolysis	Criterion of Haemolysis	No. (& % No.) with Anaemia	Criterion of Anaemia	No. of Anaemic Patients with Prosthetic Leaks	No. of non-anaemic Patients with Prosthetic Leaks (& % No. Haemolysing)	Author Ref.
S-E (1)	10	9 (90%)	Hpt. < 30 mg%	0	N.Sp.	-	1 (1)	21
S-E (1)	10	10 (100%)	Hpt. < 50 mg%	1 (10%)	PCV < 37%	N.Sp.	N.Sp.	29
S-E (1)	10	5 (50%)	^{51}Cr < 24 days	0	N.Sp.	-	1 (1)	58
S-E (1)	10	10 (100%)	Hpt. < 30 mg%	0	N.Sp.	-	N.Sp.	63
S-E (1)	10	10 (100%)	Heat stable LDH >100 iu/l	? 1 (?10%)	N.Sp.	N.Sp.	N.Sp.	36
S-E (1)	11	3 (27%)	^{51}Cr < 25 days	?	N.Sp.	?	0	45
S-E (1)	13	12 or 13 (92-100%)	Hpt. < 60 mg%	0	N.Sp.	-	N.Sp.	62
S-E (1)	81	N.Sp.	-	1 (1%)	PCV < 33%	1	7 (?)	42
UCT	105	N.Sp.	-	1 (1%)	N.Sp.	1	? 5 (?)	54
Gott-Daggett	8	5 (62.5%)	Hpt. reduced	0	N.Sp.	-	3 (2)	56
?	14	N.Sp.	-	1 (7%)	N.Sp.	1	N.Sp.	59
Total from available data		64/82 = 78%		4/261 = 1.5%				

Notes: a S-E (1) = Starr-Edwards Silastic ball & non cloth-covered cage models.
UCT = Barnard, University of Cape-Town prostheses

TABLE II REPORTED INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA WITH MITRAL VALVE PROSTHESES.

Prosthesis Model[a]	Total No. of Patients	No. (& % No.) with Haemolysis	Criterion of Haemolysis	No. (& % No.) with Anaemia	Criterion of Anaemia	No. of Anaemic Patients with Prosthetic Leaks	No. of non-Anaemic Patients with Prosthetic Leaks (& No. Haemolysing)	Author(s) Reference
S-E (1) (7 = A+M 3 = A+M+T)	10	8 (80%)	^{51}Cr < 24 days	3 (30%)	N.Sp.	3 (1 = aortic) 2 = mitral)	1 (1) (aortic)	20,58
S-E (1) (all = A+M)	27	N.Sp.	-	1 (4%)	PCV < 33%	1 (mitral)	2 (?) (1 = A+M) (1 = aortic)	42

Notes: a S-E (1) = Starr-Edwards Silastic ball & non cloth-covered cage models. A = aortic, M = mitral, T = tricuspid.

TABLE III REPORTED INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA WITH MULTIPLE VALVE REPLACEMENT.

(2) Mitral Prostheses

The incidence of haemolysis in mitral valve replacements has been more variable, the figures calculated from available data ranging from 27-100%, although the overall frequency of 78% approaches that with aortic prostheses. Anaemia, however, has been distinctly uncommon with an overall incidence of 1.5%. Where present it has nearly always been associated with incompetence of the prosthesis (p. 37). Again, conversely, mitral prosthetic leaks have not necessarily been associated with anaemia or even with haemolysis.

(3) Multiple Prostheses

Very few series of multiple valve replacement patients have been subjected to detailed haematological investigation. However, the results in patients with aortic plus mitral ($\pm$ tricuspid) replacements appear so far very similar to those in patients with single aortic prostheses.

III INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA IN PATIENTS WITH VALVULAR HEART DISEASE WITHOUT PROSTHESES.

The incidence of haemolysis judged by different parameters in patients with valvular heart disease who have not had valve replacement operations performed has shown a considerable variation in the reported series (Table IV). The frequency has ranged from zero to 76% with an overall incidence calculated from available data of 20%. That it is very seldom of clinical significance is indicated by the correspondingly calculated figure of 2% for the incidence of anaemia.

No consistent correlation between the type or severity of the valvular disease or the presence or absence of valve calcification and the incidence or degree of haemolysis has been observed. Brodeur et al.[20] and Roeser, Powell and O'Brien[73] in studies mainly of aortic valve disease found no significant relationship between these variables. On the other hand, Gehrmann, Bleifeld and Kaulen[71] found haemolysis to be

Valve(s) Affected	Total No. of Patients	No. (& % No.) with Haemolysis	Criterion of Haemolysis[a]	No. (& % No.) with Anaemia	Criterion of Anaemia	Author(s) Reference
Aortic	11	0	^{51}Cr < 23.5 days	0	N.Sp.[b]	67
	26	2 (8%)	Hpt. < 30 mg%	0	N.Sp.	63
	65	11 (17%)	Hpt. < 15 mg%	1 (1.5%)	Hb. < 13.5 g%	65
Mitral	13	9 (69%)	^{51}Cr < 24 days	0	N.Sp.	58
	28	0	Hpt. < 35 mg% + retics. incr.	0	N.Sp.	72
Aortic &/ or Mitral (± Tricuspid)	11	0	Hpt. < 40 mg%	0	PCV < 38%	39
	12	1 (8%)	^{51}Cr < 25 days + Hpt. reduced or Plasma Hb. incr.	1 (8%)	N.Sp.	35
	12	3 (25%)	Hpt. < 70 mg%	2 (17%)	?PCV < 35%	40
	13	2 (15%)	Hpt. < 50 mg%	N.Sp.	-	29
	13	7 (54%)	Hpt. < 60 mg%	N.Sp.	-	62
	18	1 (6%)	Heat stable LDH > 100 iu/l	0	N.Sp.	36
	20	0	N.Sp.	0	N.Sp.	68
	21	16 (76%)	^{51}Cr < 24 days	0	N.Sp.	20
	24	6 (25%)	Hpt. reduced	N.Sp.	-	27
	35	18 (51%)	^{51}Cr < 24 days	0	N.Sp.	71.
	42	7 (17%)	Hpt. < 35 mg% + retics. incr.	3 (7%)	N.Sp.	72
	132	4 (3%)	Renal haemosiderosis	?3 (?2%)	PCV < 40%	70
Unspecified	22	16 (73%)	^{51}Cr < 25 days	0	N.Sp.	73
Total from available data		103/518 = 20%		7/336 = 2%		

Notes: a ^{51}Cr = ^{51}Cr-labelled red cell survival study half-life
Hpt. = Serum haptoglobin
b N.Sp. = Not specified

TABLE IV REPORTED INCIDENCE OF HAEMOLYSIS AND HAEMOLYTIC ANAEMIA IN UNOPERATED VALVULAR HEART DISEASE.

commoner in aortic or mitral stenosis and considered this might be related to transvalvar pressure gradients. Myhre and Rasmussen[65], however, reported that haemolysis occurred twice as frequently in aortic incompetence as in aortic stenosis, although there were several patients with markedly incompetent aortic valves who were not haemolysing. The study of Yacoub et al.[67] has already been mentioned (p. 38) in which despite evidence of severe regurgitation or stenosis in 11 patients with aortic valve disease, ^{51}Cr-labelled erythrocyte survival studies were normal. In lone mitral valve disease Brodeur et al.[58] detected evidence of haemolysis just as frequently as in aortic valve disease, whereas Dupont and Wennevold[72] in their larger series concluded that mitral valve disease alone did not give rise to mechanical haemolysis. Roberts[70] in his autopsy study of patients dying from valvular heart disease could also find no evidence of intravascular haemolysis, as determined by the presence of renal haemosiderosis, in those who only had mitral valve lesions. Certainly, apart from the case reported by Ziperovich and Paley[69] discussed above (p. 39), in none of the other few reported instances of haemolytic anaemia with valvular heart disease has it occurred in patients with disease limited to the mitral valve.

This lack of a consistent relationship between the nature and especially the severity of the valve abnormality and the frequency or severity of haemolysis requires explanation.

IV LABORATORY FINDINGS

(1) Peripheral Blood, Urine, and Bone Marrow

The blood picture varies depending on the degree of haemolysis, in mild cases the haemoglobin and reticulocyte count being normal, anaemia and a reticulocytosis developing in the more severe cases. The blood film in anaemic patients characteristically shows fragmented and distorted red cells variously described as "burr cells", "schistocytes", "pyknocytes" and

"helmet cells", and these have accounted for up to 27% of the red cells[16,22,33]. A review of the varied terminology applied to these damaged cells is given at the end of this chapter. Hypochromia of the red cells may develop and in the absence of other causes of iron deficiency is presumed due to the chronic loss of iron in the urine as haemosiderin and ferritin.

The accepted mechanism of formation of haemosiderinuria and ferritinuria is outlined in figure 2.

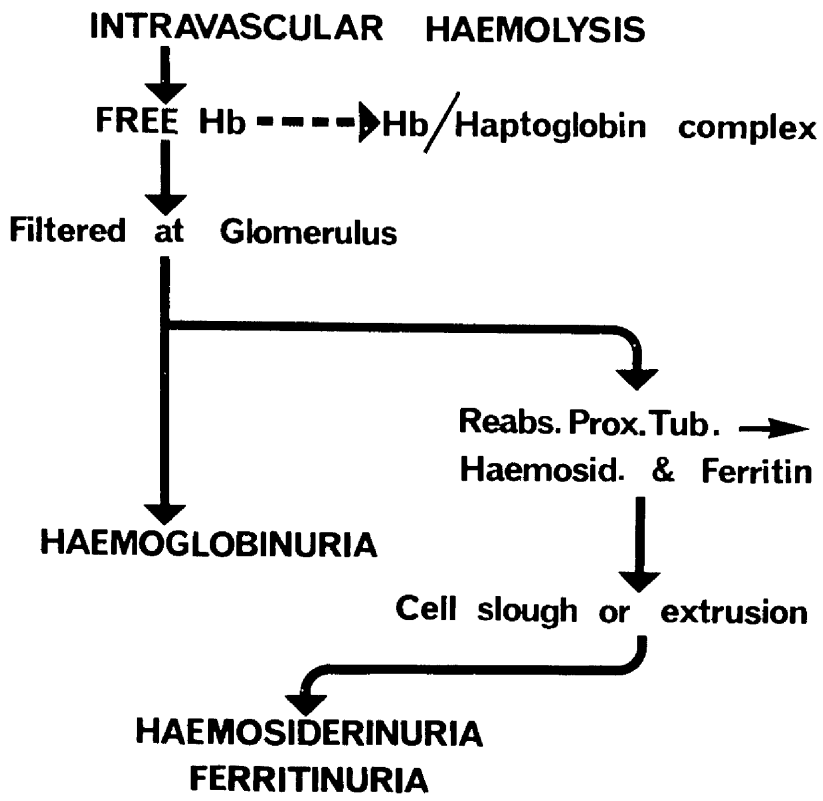


Fig. 2.
Outline of the mechanism of formation of haemosiderinuria and ferritinuria.

Intravascular haemolysis liberates haemoglobin into plasma, and once the haptoglobin binding capacity is saturated any remaining free haemoglobin that is not then complexed with the β_1 globulin, haemopexin (as haeme-

haemopexin), or with albumin (as methaemalbumin), may be filtered at the glomerulus.[106-114] The actual process of filtration of free haemoglobin molecules in enhanced probably by their reversible dissociation into sub-units of $\alpha\beta$ dimers[115]. A proportion of the filtered haemoglobin is then absorbed mainly by the proximal renal tubular cell and catabolized therein to haemosiderin and ferritin which are subsequently released into the urine with cell sloughing and possibly by extrusion from the intact tubular cell[25,116-118]. Unless the haemolysis is severe the tubular absorptive capacity may be sufficient to prevent the concurrent appearance of haemoglobinuria[29,119-121].

Extimation biochemically of the total urinary iron excretion in prosthetic valve patients has demonstrated elevated levels of up to 34 mg in 24 hours,[34,37,40,46,50,118] and most of the iron appears to be in the form of haemosiderin[118].

Bone marrow examination may show a reduction or absence of stainable iron [34,40,118] in addition to erythroid hyperplasia.

No particular alteration in white blood cells has been reported, but moderately low platelet counts appear to have been recorded in a few cases[75]. Reduced ^{51}Cr-labelled platelet survival has also been found,[50,122-124] and from the results in two of these studies[123,124] it seems likely that this is due more to platelets being consumed in thrombus formation at the valve site than to their being damaged by the prosthesis although both mechanisms could be operative.

(2) Other Investigations

No intrinsic abnormality of the red cells has been found and an extracorpuscular mechanism for the haemolysis has been confirmed by cross-circulation studies of erythrocyte survival using ^{51}Cr-labelled red cells.[16,22] Usually no excess splenic localisation of radioactivity has been detected on surface counting,[17,20,22,31,34,59] although splenic sequestration has been found in a few isolated cases[14,23,46].

Elevation of serum total lactate dehydrogenase (LDH) and of the heat stable isoenzyme, appears to be an almost constant finding in

patients with prosthetic intra-cardiac devices and a sensitive, although not specific, parameter of intravascular haemolysis[36,65]. The degree of elevation of the serum LDH has, moreover, been found to correlate well with the results of erythrocyte survival as determined by the half-life of ^{51}Cr-labelled red cells[50,125].

Serum haptoglobin levels have also been found almost invariably reduced or absent in these patients[27,29,62,63]. The data from two studies showed that these changes in haptoglobin level might occur in the presence of normal LDH values and normal ^{51}Cr-erythrocyte survival studies, and in the absence of haemosiderinuria, suggesting that haptoglobin estimation might be the most sensitive test[52,56].

While some elevation of plasma free haemoglobin is usual, methaemalbuminaemia may be detected only with the more pronounced degrees of haemolysis[29,50,52].

As is to be expected in primary haemolytic disorders serum bilirubin levels are frequently normal despite other definite evidence of intravascular haemolysis[52,56,126].

V PATHOGENESIS

All the laboratory findings implicate an extracorpuscular and intravascular basis for the haemolysis in patients with prosthetic valves. The process appears to be essentially one of physical damage to the red cells, and the morphological appearances of these cells, the greater severity of haemolysis with aortic valve prostheses, and the evidence for accentuation of haemolysis by exercise as found by several workers[11,29,40,41,46,50,53,118,127] supports this view. The precise mechanism producing the red cell trauma remains to be defined but a number of possibilities have been suggested.

Turbulence of red cells in the blood stream in association with haemodynamic abnormalities such as regurgitation through or around prostheses or obstruction to flow has generally been accepted as a causal factor. Certainly, the more marked degrees of haemolysis encountered have been related usually to prosthetic leaks. Under these circumstances

it appears from experimental data that the red cell is subjected to shearing stresses as well as to any direct buffeting action. Nevaril et al.[128] studied the effect of shearing stress on human and rabbit erythrocytes in-vitro. They identified a critical shearing stress for human erythrocytes of 3000 dynes per cm^2 above which there was a marked and steep increase in haemolysis as determined by the level of plasma haemoglobin. The authors also found, however, that lesser degrees of shearing stress produced morphological changes in the red cells similar to those in patients with prosthetic valve haemolysis. These changes began at shearing stresses of approximately 1500 dynes per cm^2 and there was a direct linear relationship between the magnitude of the stress and the percentage of abnormal cells produced. In studies of rabbit blood, ^{51}Cr-labelled red cells exposed to high shearing stresses exhibited diminished survival after re-injection into the donor animals, and splenic sequestration of the damaged cells occurred. They calculated that diastolic or systolic pressure gradients likely to be operative in patients with leaking or functionally stenotic prostheses could be sufficient to result in shearing stresses that would exceed the critical tolerance of the red cell. For example, a pressure gradient of 50 mm Hg would be associated with a shearing stress of approximately 4000 dynes per cm^2. The red cell membrane in a field of high shearing stress is stretched, and Rand and Burton[129] had previously presented evidence that, while capable of withstanding large bending strains, the red cell membrane had a more limited tolerance to tangential strains.

It has also been suggested that red cells may be traumatized by direct contact with any artificial cloth incorporated in a prosthesis. The classical case of Sayed et al.[6] described in detail previously (p.28) is frequently quoted as an example of such a mechanism. In this case the patient's severe and unremitting haemolysis ceased once a bare area of Teflon upon which a regurgitant jet of blood played was covered over,

even though the regurgitation must have continued. Damage by bare Teflon fibres would be analogous to the red cell fragmentation and distortion that is considered to be produced by intravascular fibrin strands in conditions causing microangiopathic haemolytic anaemia[130].

Simple direct collision of red cells with or between component parts of a prosthesis other than cloth has also been considered responsible for haemolysis[4,16]. However, Fok and Schubothe[131] questioned the role of collision factors in an in-vitro study of mechanical haemolysis induced by quartz beads, and suggested that indirect forces acting on the red cell might be more important. Their description of these hypothetical forces would correspond to shearing stress.

A possible unifying mechanism for haemolysis with prosthetic valves that incorporates many of the above ideas is suggested from a consideration of the work of Blackshear et al.[132]. In in-vitro studies they found that red cells become tethered to artificial surfaces and eventually tear losing a fraction of their substance when the connecting strand breaks (Fig. 3).

Fig. 3.
Diagrammatic representation of the cell-wall mechanism of haemolysis proposed by Blackshear et al.

They considered that such a cell-wall interaction was more important in the genesis of haemolysis than shearing stresses induced by increased velocity or turbulence of blood flow remote from walls. Increased turbulence, however, would promote the flow of cells to the cell-wall interface, and cells anchored to a wall were calculated to be more sensitive to any given shearing stress. The number of cells lysed would also be greater the more rough or sticky the wall, and would exceed the calculated figure of one lysed per 1000 red cells of those reaching a "benign" wall such as stainless steel or Silastic. Cells damaged in this way would be more susceptible to further trauma in the blood stream.

An estimate of the frequency with which a red cell might be subjected to the overall hostile environment of a prosthetic valve was made by Rubinson, Morrow and Gebel[26] for cases of prosthetic aortic incompetence. They derived a mathematical relationship between the severity of regurgitation and the number of times a regurgitant cell would pass the valve. For example, with relatively mild regurgitation a red cell was theoretically calculated to contact the valve on average only 1.5 times before it entered the systemic circulation, whereas with more severe regurgitation it would pass it on average 5.7 times before being finally ejected. Increased regurgitation would also have the effect of augmenting the absolute number of cells being regurgitated and traumatized because of the associated larger stroke volume.

Whatever may be the mechanism of haemolysis, it is clear from the high incidence in patients with apparently normally functioning prostheses that its development is not necessarily dependent upon valve malfunction.

VI COMPLICATIONS

There are two recognized potential complications that are peculiar to chronic intravascular haemolysis.

(1) Urinary Iron Loss

High urinary iron levels may occur in patients with chronic traumatic intravascular haemolysis associated with prosthetic heart valves as a consequence of the development principally of haemosiderinuria and ferritinuria, and the subject has already been discussed (p. 52). This may lead to iron deficiency which may precipitate anaemia in a patient whose haemolysis has been hitherto well compensated or aggravate an already established haemolytic anaemia.

Most of the measurements of urinary iron excretion have been carried out in patients with an aortic or aortic plus mitral prosthesis and generally elevated levels have been found[34,40,50,118]. Only a few patients with isolated mitral prostheses have been so studied,[37,50] and the urinary iron excretion in nearly every case has been within normal limits. This difference in urinary iron losses between patients with an aortic and those with a mitral replacement undoubtedly reflects the usually greater severity of haemolysis produced by an aortic prosthesis, and an approximate inverse correlation between urinary iron levels and the half-life of ^{51}Cr-labelled erythrocytes has been reported[50]. As haemolysis appears to occur almost as frequently with mitral prostheses, the urinary iron results to date suggest that the degree of haemolysis produced by them must usually be sufficiently slight as to cause no saturation of the plasma haemoglobin binding capacity and therefore no haemosiderinuria and ferritinuria.

(2) Renal Haemosiderosis

Chronic intravascular haemolysis may lead to heavy and selective deposition of iron in the kidneys, particularly in the cells of the proximal convoluted tubules[25,116,117,133]. The effect of renal haemosiderosis upon renal function has not been studied in detail. General experience mainly with cases of paroxysmal nocturnal haemoglobinuria suggests that it seldom, if ever, leads to serious or even

significant renal functional impairment[25,134-136]. However, formal investigations in patients with chronic intravascular haemolysis[116,117,137] have been limited in extent, often confined to isolated cases, and altogether appear inconclusive. Furthermore, in the occasional case of renal failure considered likely to be directly related to renal haemosiderosis[138-140] there have been other complicating pathological factors.

The question of possible eventual renal impairment in patients with haemolysis secondary to prosthetic heart valves has frequently been raised,[29,45,118,141] but no study of renal function in them has been reported and this whole subject clearly merits further attention.

VII TREATMENT

The only definitive treatment for the traumatic haemolytic anaemia of prosthetic valves is to re-operate and repair any associated prosthetic defect or completely replace the prosthesis[22,24,33,41-43,46-49,54,142]. This, however, appears to be necessary in only occasional cases.

There are three conservative measures that are often beneficial and certainly merit a trial in severe anaemia before proceeding to re-operation unless haemodynamic considerations are of pressing urgency. These are blood transfusion, rest and iron treatment, and the basis for the therapeutic value of each in prosthetic valve patients with traumatic haemolysis has been discussed. Several reports attest to the frequent efficacy of iron therapy which may require to be given parenterally[34,37,46,47,49,50,58]. The aggravation of haemolysis with exercise and its alleviation with rest is also documented[11,22,29,40,41,46,50,53,118,127]. Together in severe anaemia these three measures may avert the need for re-operation by allowing the haemoglobin to stabilize at a more satisfactory level, but should severe anaemia recur on resumption of activities necessary for a meaningful existence and despite adequate iron repletion then operation is probably unavoidable. In lesser degrees of anaemia

iron treatment alone may produce a satisfactory rise in haemoglobin.

Folic acid has also been given to anaemic cases on the grounds that chronic haemolysis can lead to folate deficiency[41,46,49,50]. Aside from two patients in whom low serum folate levels were recorded one of whom responded to folic acid[50], possible folate deficiency in prosthetic valve patients does not appear to have been documented. In the patients in whom folic acid was empirically prescribed, the improvements in haemoglobin level obtained in a few[46,49] are likely to have been due to the iron treatment or exercise restriction that was also instituted. Nonetheless, a secondary folate deficiency should always be excluded in anaemic cases.

A number of other therapeutic measures have been tried to alleviate the traumatic haemolysis in patients with anaemia but they have been uniformly ineffective. These have included corticosteroids[18,22,23,30,32,41,44,49] and immunosuppressive drugs.[23] Immune aetiological factors responsible for, or contributing to, the traumatic haemolysis were not evident in any of these studies. Splenectomy has been carried out in two patients, one with splenomegaly[30] and the other with excess splenic sequestration of ^{51}Cr-labelled red cells[23], but in neither did the procedure appear beneficial.

For patients with a well compensated haemolysis no treatment may be necessary although the value of prophylactic iron supplements in this setting does not appear to have been assessed. It has been suggested that they should probably be given if urinary iron losses exceed 4 mg per day[50].

VIII THE TERMINOLOGY OF RED CELL FRAGMENTATION AND DISTORTION

A multiplicity of descriptive terms are used in discussion of the red cell morphology in disorders associated with red cell fragmentation and distortion. This section is concerned with an historical review and appraisal of this varied terminology.

In 1949 Schwartz and Motto[143] introduced the term "burr cell". In the course of the routine examination of blood films they had noted a

"peculiar red cell" which they described as "a mature erythrocyte about 7.5 micra or less in diameter, which has one to several (large) spiny projections along its periphery". To this they gave the name "burr cell" because of its resemblance to the prickly envelope of a burr. They found these appearances also in wet film preparations and concluded that the burr cell was most likely to be a pre-formed poikilocyte and not a crenated cell. Crenated cells they described as having many fairly symmetrically arranged small spiny projections often occurring on the surface as well as the periphery. Furthermore, crenated cells tended to occur in groups, sometimes more in one area than another, in comparison with burr cells which they found to be fairly evenly distributed throughout a blood film. It was admitted, however, that occasionally it was difficult to differentiate the two on morphological grounds and this difficulty is illustrated in their paper. They found burr cells in a variety of conditions but most frequently in cases of uraemia, gastric carcinoma and bleeding peptic ulcer. They were absent in one hundred hospital patients without obvious haematological abnormalities used as controls.

Aherne[144] in a subsequent study of the relationship between the presence of these abnormal cells and the level of uraemia described and illustrated them as varying in size and being in the main basically triangular or crescentic in shape. The abnormal ("burr") cells in his illustrations correspond to some of the cells demonstrated by Schwartz and Motto.

Dacie[126] considered that the burr cell, although a distinct entity, was an "irregularly crenated cell" produced as an artefact as the cell dried on the slide, and that it was doubtful whether its presence denoted increased haemolysis. Certainly, in his photograph of "?burr cells" there are many crenated looking cells. He used the term "irregularly contracted" or "shrunken and distorted" for abnormal red cells observed in some haemolytic anaemias associated with drugs, acute liver necrosis,

renal failure and carcinomatosis[126,145]. He also drew attention to and originally described "triangular cells" which he considered probably always indicated increased haemolysis[126,145,146]. These have "smoother (triangular) outlines; they rarely have more than two spines and may have none; they are always microcytes and stain deeply with Romanowsky dyes". His illustrations of all these abnormal cell types show many to be of very similar if not essentially identical appearances to the burr cells of Schwartz and Motto and especially to the crescentic and triangular burr cells of Aherne.

Other authors[134,147-151] have illustrated very similar looking abnormal cells and described them as being deeply stained, irregularly distorted or contracted, fragmented, as having indentations "as if small pieces had been bitten out of them with a punch"[147], or as appearing like "burst egg shells"[148]. The term "burr cell" was not used in these reports, some of which antedate Schwartz and Motto's paper, and the abnormal cells were described in certain drug-related haemolytic anaemias[134,147-149] and in children with haemolytic anaemia associated with renal disease and thrombocytopenia[149-151].

Tuffy, Brown and Zuelzer in 1959[152] proposed the term "pyknocyte" for a "much distorted, completely irregular, densely stained erythrocyte, usually appreciably smaller than the undistorted cells, and having from several to many spiny projections". They emphasized the increased density of these cells and considered them a type of burr cell, and described them in certain infants with haemolytic anaemia although they were present also in small numbers in healthy children and adults. The illustrations in their paper demonstrate well the densely stained, contracted and spiky characteristics they described, though a number of the cells in their photomicrographs resemble the crescentic burr cells of Aherne. Others, however, would be difficult to differentiate from crenated cells and some, as they pointed out, may be indistinguishable from the "acanthrocytes" described by Bassen and Kornzweig in 1950[153] and

Singer, Fisher and Perkstein in 1952[154] in the disease now known as hereditary acanthocytosis.

There appear, therefore, to be semantic difficulties in this area. Illustrations of these various abnormal cells in the published reports referred to above show them to be undoubtedly pleomorphic, yet in the main of essentially similar appearance whether they be described as "burr cells", "pyknocytes" or "irregularly contracted cells". It is clearly important to question their significance, however, when their appearance is difficult to distinguish with certainty from crenated cells, as occurs especially when there are numerous spiny projections. Crenated cells usually, nonetheless, have a distinctive morphology and tend to occur in groups because of irregular drying of the slide, and they are not infrequently seen in blood films from normal individuals or patients without haematological problems.

A further source of potential semantic argument is apparent when the morphology of the red cell fragment is considered. "Schistocyten" (schistocytes; Gk, σχιζω -split) was the name given by Ehrlich in 1891[155] and "schizocytes" (after Ehrlich) by Rous and Robertson in 1917[156] to denote the products of red cell fragmentation. Yet the illustrations or descriptions by these authors reveal that schistocytes take a diversity of forms, and apart from recognizable cell fragments they may resemble the burr cells described above as well as appearing like microspherocytes or dumb-bells. Other illustrations of "red cell fragmentation" or "schistocytosis" show cells the majority of which are identical to the previously described triangular and crescentic burr cells or irregularly contracted cells[134,157]. Thus, the interpretation of what is meant by a schistocyte, schizocyte or fragment, in contradistinction to a burr cell, pyknocyte or irregularly contracted cell appears open to question. Small portions of red cell material may without argument be accepted as actual fragments. The triangular cell is probably a fragment,[126] and the "helmet cell" may also be considered as

such[158]. "Helmet cell" was the name given by Adelson, Heitzman and Fennessey in 1954[159] to cells of helmet shape which they found in cases of thrombotic thrombocytopenic purpura, and Damashek[66] has outlined the varying types of helmet shape that may be assumed. Nonetheless, both helmet and triangular shaped cells figure prominently in the illustrations of burr cells already quoted. On the other hand, Bell[160] after detailed microscopic observation, suggested that the following sequence of events led to the formation of a burr cell. First, a vacuole forms at the periphery of a red cell; this then ruptures leaving a crater and a deformed erythrocyte. Subsequent flattening and opening up of the crater leads to crescentic shapes and, with further distortion in the blood stream, to more bizarre forms. Burr cells could, therefore, be regarded as fragmented cells and indeed are defined as such by Harper[158].

A better understanding of the possible relevance and interrelationship of the varied morphology presented by all these abnormal cells has come from the work of Brain and his colleagues[130,161-165]. They have introduced a likely unifying pathogenetic mechanism for the distortion and fragmentation of red cells observed in many of the disorders mentioned above. That is, that the red cells are damaged as they are forced through small arterioles partially blocked by a fibrin meshwork, laid down either because of local endothelial disease, which by itself may be responsible for the red cell damage, or as a result of some generalised stimulus to intravascular coagulation. They considered that this process may operate in renal disease, especially if there was malignant hypertension, in the haemolytic-uraemic syndrome, and in disorders complicated by disseminated intravascular coagulation such as may occur, for example, with metastatic carcinoma, Gram-negative septicaemia, abruptio placentae and retained dead foetus. The term they applied to the frequently resulting haemolytic anaemia is microangiopathic haemolytic anaemia. In their experimental studies it was demonstrated by cinephotomicrography as well as by visual observation that the shape of the red cell fragments

depended upon the position and plane in which the cells were arrested by fibrin strands, the point at which the cell membrane ruptured, the distribution of haemoglobin and membrane between the resulting fragments, and on whether or not crenation of the fragments supervened[130]. Dehaemoglobinized portions of damaged cells were considered possibly to correspond to the vacuoles described by Bell,[160] and certainly the appearances on the photomicrographs are virtually identical. Fragments were also frequently noted to have irregularities or "burrs" along the line of closure of the torn membrane. Overall, the appearances of the abnormal cells in their study are indistinguishable from previously reported burr cells or schistocytes. The interaction of erythrocytes with fine fibrin strands was further elegantly demonstrated by stereoscan electron microscopy by Bull and Kuhn[157]. That a panorama of such red cell deformities may be the result of a single aetiological factor is further evidenced by the similarly distorted and fragmented cells produced in patients with cardiac valve prostheses.

It seems clear, therefore, that the degree of cell damage and distortion that occurs under certain circumstances is variable, resulting in the production of a variety of abnormal cell shapes. Some of these may be readily recognized as fragments or bits of cells, or as torn cells, others are probably best described as irregularly contracted and distorted. Crenation of a number of the abnormal forms may well take place subsequently on the slide leading to further distortion and bizarre shapes. This wide spectrum of morphological abnormalities appears to occur irrespective of the precise underlying aetiology. This is to be expected if, as Weed has stated[166], red cell fragmentation is accepted as an important final common pathway for red cell destruction in a variety of haemolytic states. It remains to be determined, though appears unlikely, that any single geometric configuration would have specific diagnostic significance.

CHAPTER 2

METHODS AND PATIENTS

I HAEMATOLOGICAL INVESTIGATIONS

(1) Standard Blood and Reticulocyte Counts

Haemoglobin estimation, packed cell volume, red cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and mean corpuscular volume were determined using a Coulter Counter, Model 'S'. The normal values are[167]:

Hb 13.5 - 18.0 g/100 ml (males), 11.5 - 16.5 g/100 ml (females); P.C.V. 40 - 54% (males), 35 - 47% (females); R.B.C. 4.5 - 6.5 million/mm^3 (males), 3.9 - 5.6 million/mm^3 (females); M.C.H. 27 - 32 pg; M.C.H.C. 30 - 35%; and M.C.V. 76 - 96 μm^3.

Reticulocyte counts were performed as described by Dacie and Lewis[167] (normal: 0.2 - 2.0% of R.B.C.)

(2) Red Cell Morphology

This was evaluated by the examination of well prepared stained blood films using a Watson-Barnet, Bactil-60 microscope (magnifications and objective sizes: low-power x 150, obj. 6mm; high-power x 600, obj. 4 mm; oil-immersion x 1350, obj. 2 mm). The stains used were May-Grünwald-Giemsa's and Wright's.

The following classification of the abnormal red cell morphology encountered in patients with prosthetic valves has been constructed. It defines those abnormalities described in this thesis under the heading "red cell fragmentation and distortion." The abnormalities are illustrated by drawings made under oil-immersion magnification and subsequently printed (Figs. 4-10), and by photomicrographs taken using a Leitz Orthoplan microscope with an Orthomat camera (Figs. 11-17).

A. Fragmented and Torn Red Cells

These forms vary in size and shape but four distinct types can be recognized

1. Cell pieces. Small portions of cellular material usually

of pale or "ghost" staining character and of varied shape (Figs. 4, 11, 15-17).

2. Triangular cells. Microcytic red cell forms of triangular shape, usually densely staining, and at one or two corners of which there may be a sharp spiny projection (Figs. 5, 12 & 17).

3. Cell segments. Larger segments of cells basically helmet, half-moon or crescent shaped, which usually have a frequently ragged tail of red cell material in the middle or at the end of the torn edge, or sometimes a sharp spiny projection at one or both ends (Figs. 6, 16 & 17).

4. Indented cells. Red cells which are intact except for a variable sized loss of material in one and very occasionally two segments as if a piece had been punched or bitten out of them. The edge of the defect or crater may be clearly defined or rather ragged and less well defined with sometimes tails or wisps of red cell material trailing out from it and lending credence to the view that the cell has been torn (Figs. 7, 13, 15 & 16).

B. Distorted and Irregularly Contracted Red Cells

These are red cell forms of pleomorphic shape with an irregular outline. They are often smaller and more densely staining than normal cells and may have occasional sharp spines protruding from their periphery (Figs. 8, 14, 16 & 17). As discussed in Chapter 1, these forms may well originate from, and probably have the same significance as, fragmented and torn cells, but cannot on visual inspection be assigned to that category.

C. Pincered Cells

These are red cells which appear "as if part of their substance had been indented and pulled outwards by a pair of pincers" (Figs. 9 & 14), and were described thus by Dacie et

al.[146] in cases of atypical congenital haemolytic anaemia. They are found also in other types of haemolytic anaemia[145] and cells of similar appearance ("dumb-bell" shape) were described by Ehrlich[155] and in normal rabbit blood by Rous and Robertson[156] as representing a stage in the process of fragmentation. They are illustrated also in the in-vitro studies of red cell fragmentation carried out by Bull et al.[130]

D. Other Abnormal Forms

1. "?Crenated cells". Cells difficult to distinguish as distorted and irregularly contracted or as "normal" crenated cells were not taken by themselves as evidence of haemolysis (Figs. 10, 15 & 16). They are characterized mainly by a serrated outline and include cells of similar appearance to some of the "irregularly crenated (?burr) cells" of Dacie[126]. Many are almost certainly otherwise distorted or fragmented cells that have undergone crenation. They were, however, relatively uncommon.

2. Vacuolated cells. Cells with a peripheral vacuole were occasionally observed in small numbers (Fig. 7).

3. Microspherocytes. Small densely stained spherocytic cells. These were only very occasionally seen in the prosthetic valve patients.

It has been found possible to identify the very great majority of abnormal erythrocytes with one or other of the above categories. However, forms intermediate in appearance between two categories were occasionally seen. For example, indented cells with an incomplete thin bar of red cell material across the mouth of the crater resembled vacuolated cells; indented cells with large opened-up craters merged with segmented cells; distorted but minimally irregularly contracted cells sometimes appeared to have a basically segmented cell type of out-

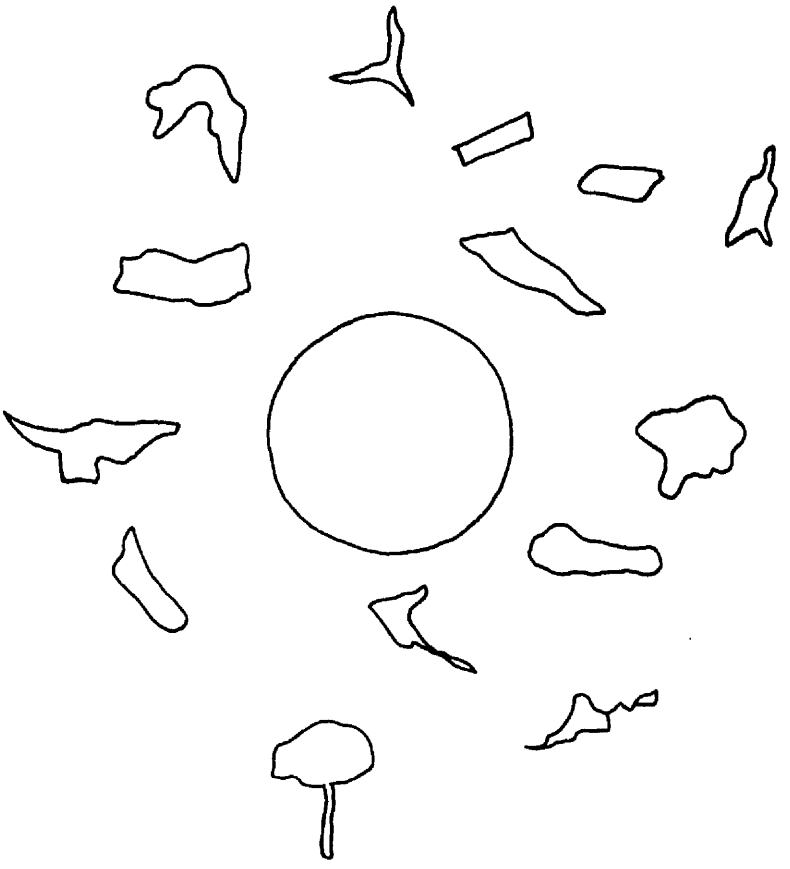


Fig. 4 Cell pieces. A normal red cell is shown in the centre for size comparison.

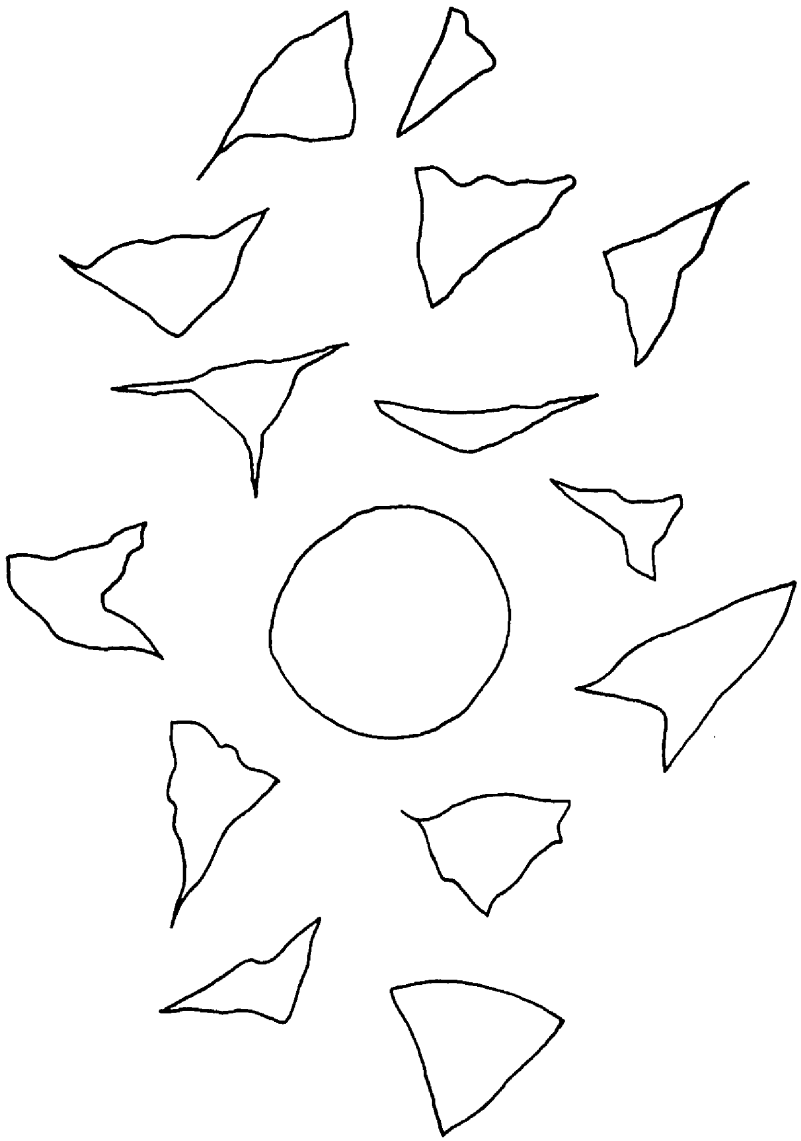


Fig. 5 Triangular cells.

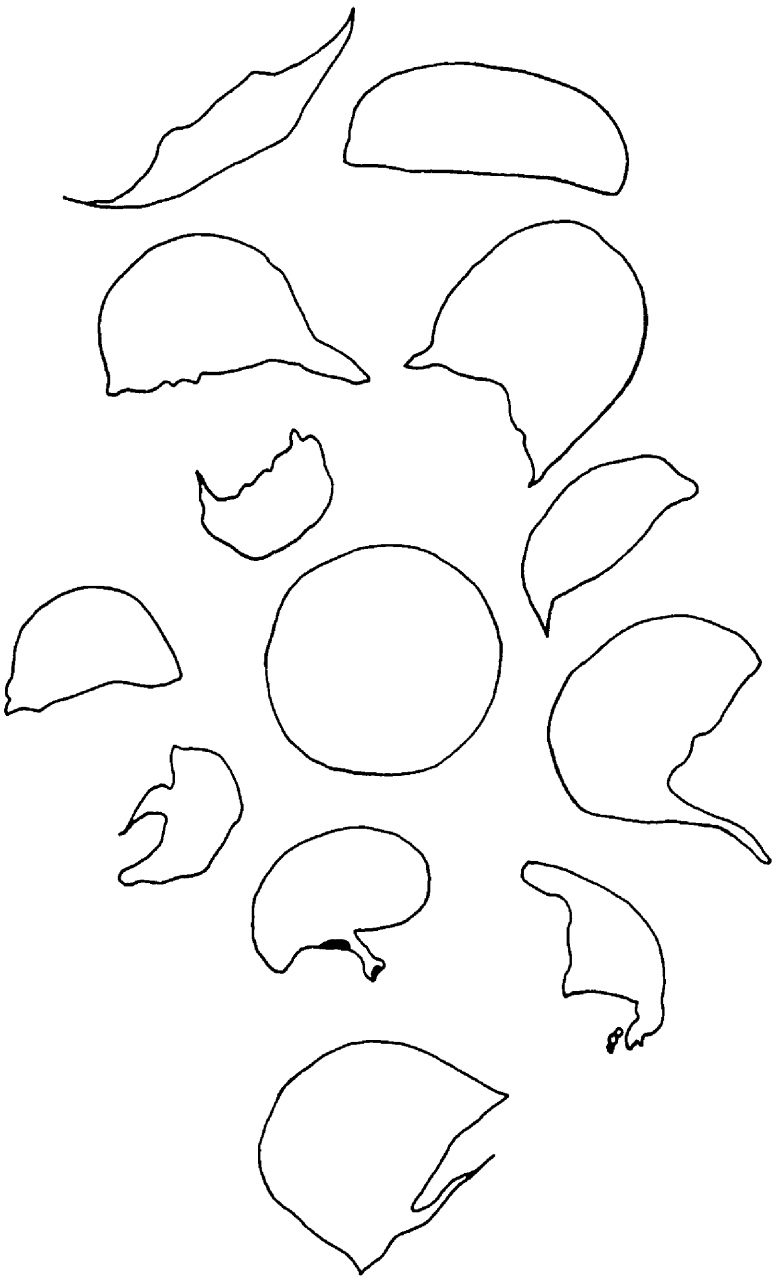


Fig. 6 Coil segments.

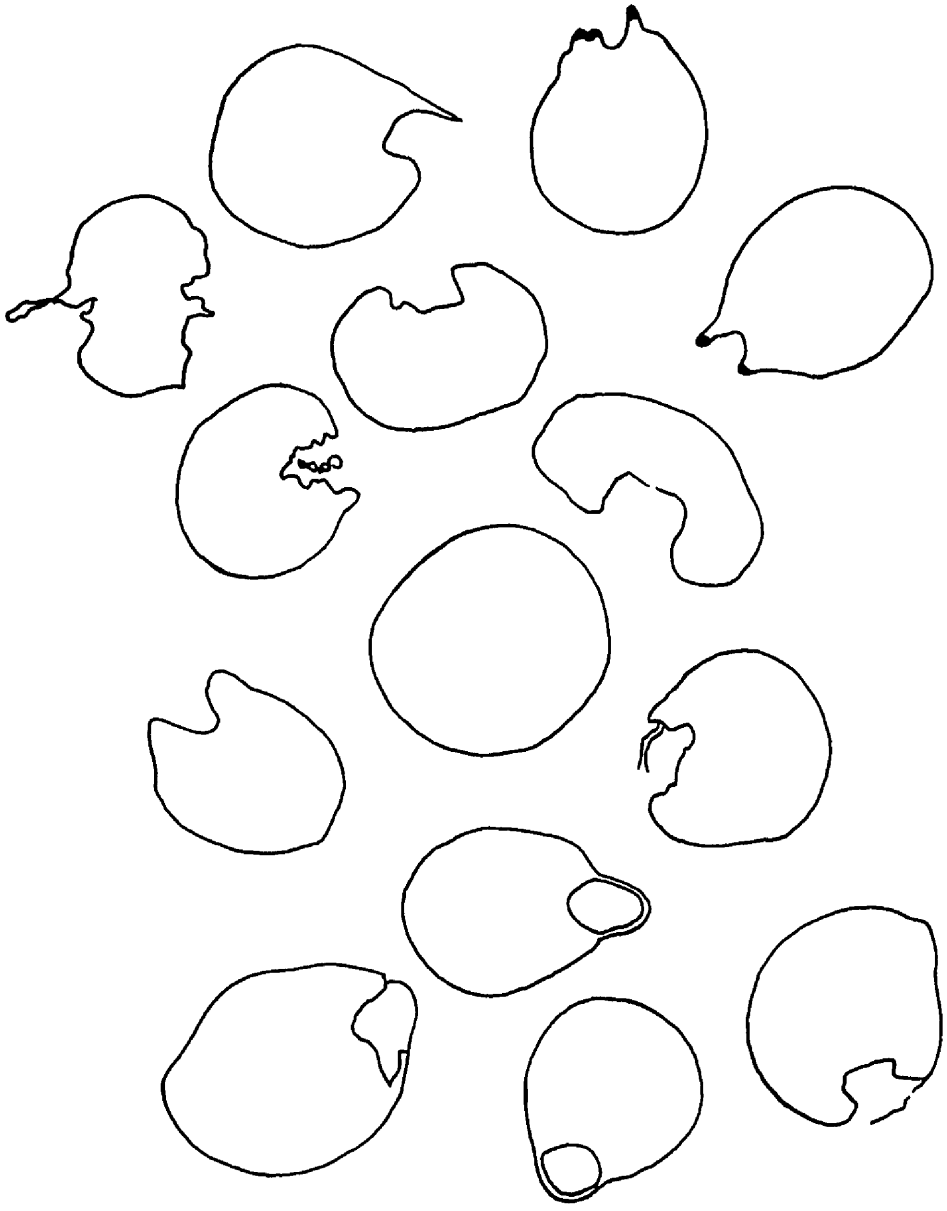


Fig. 7 Indented and vacuolated cells.

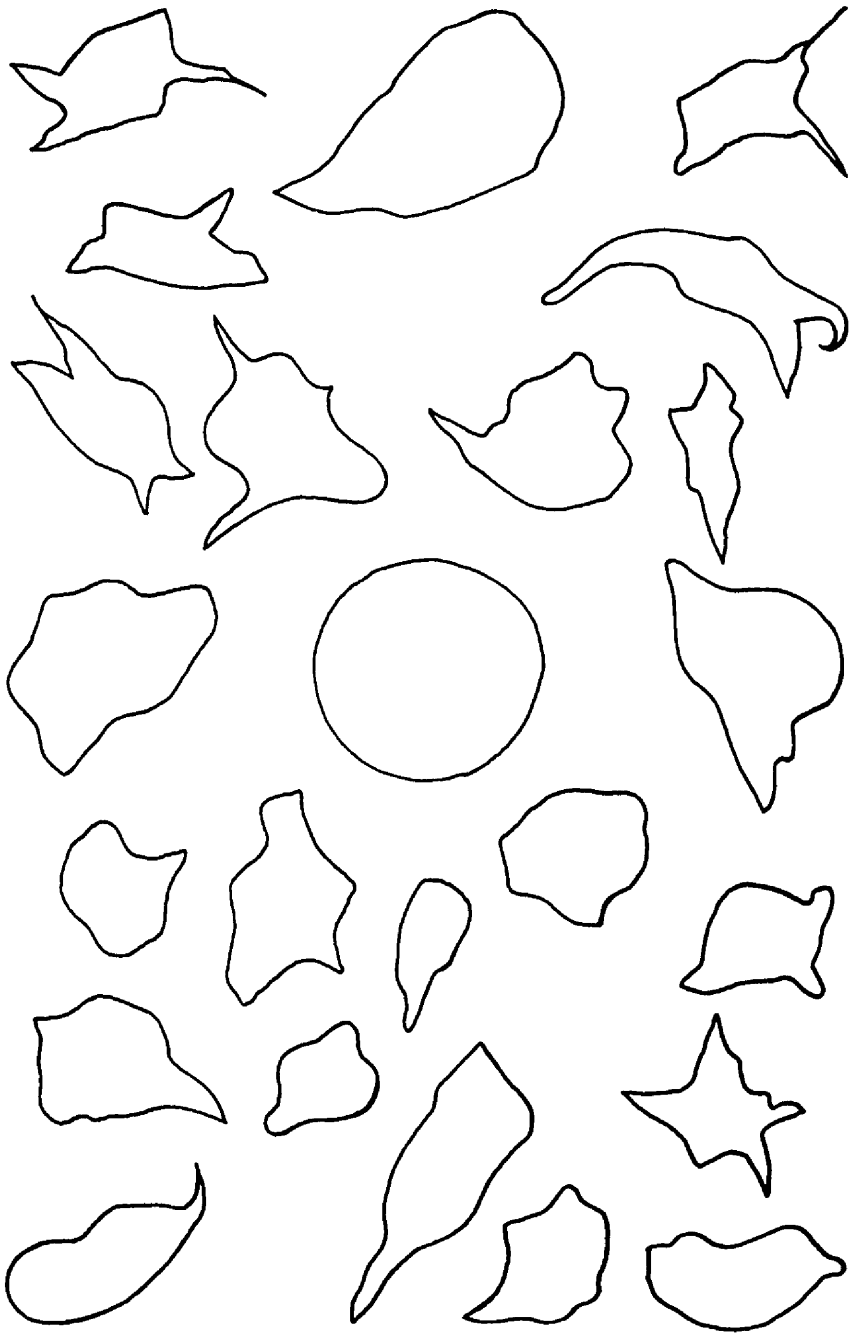


Fig. 8 Distorted and irregularly contracted red cells.

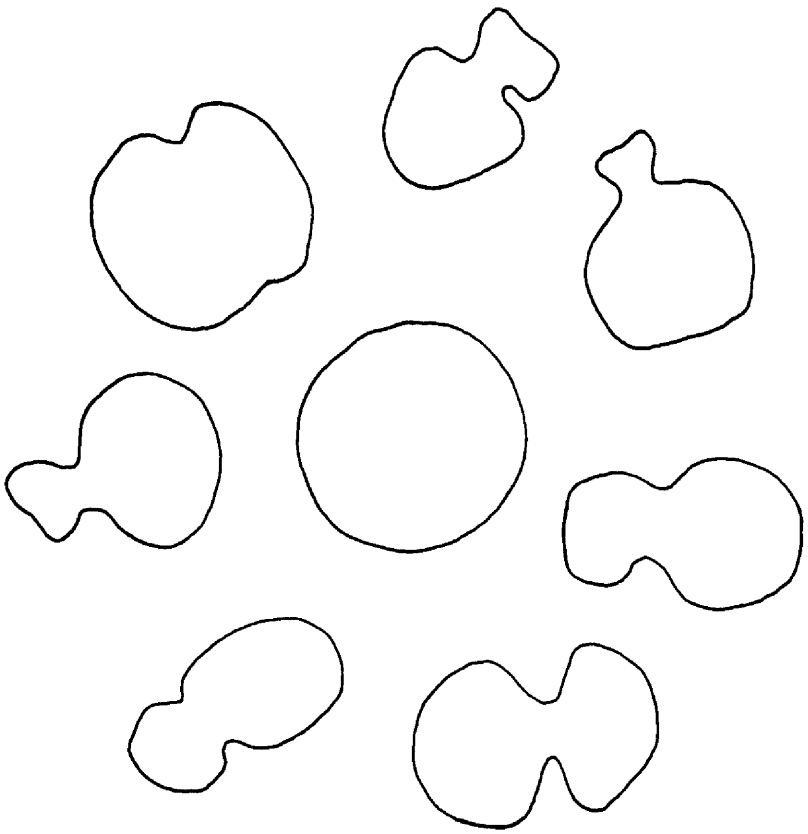


Fig. 9 Pincered cells.

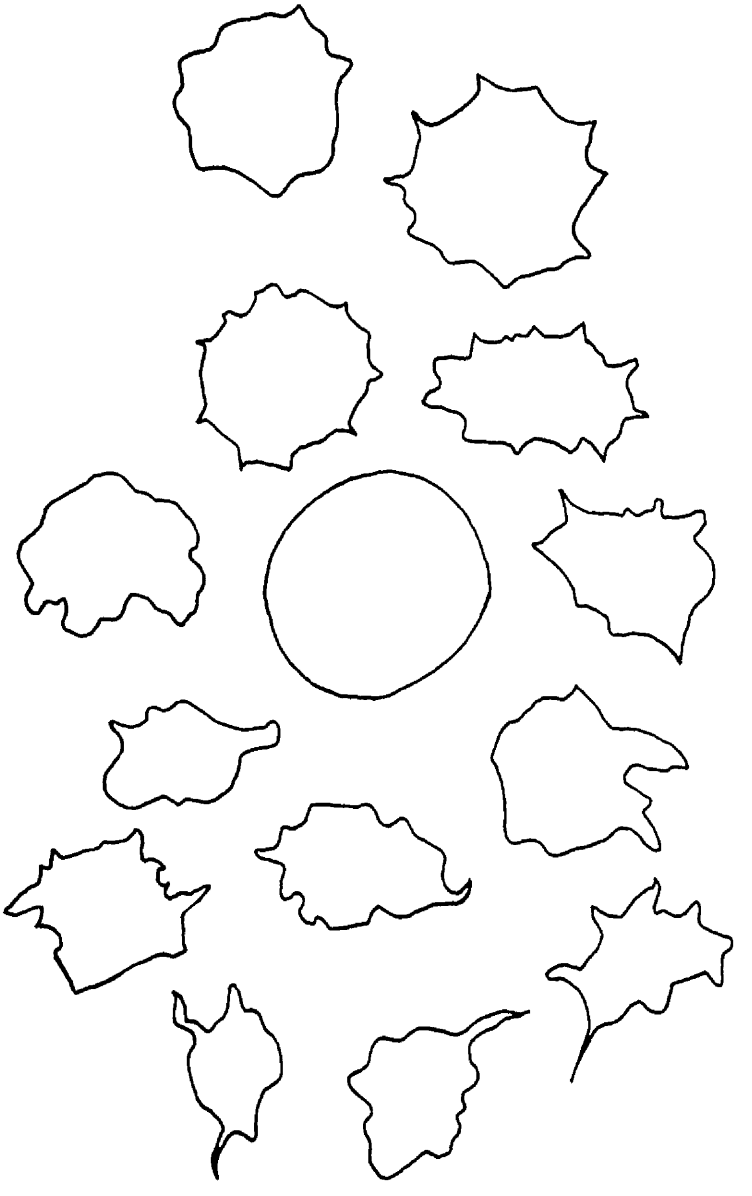


Fig. 10 "?Crenated cells". The top four cells are "normal" crenated cells shown for comparison.

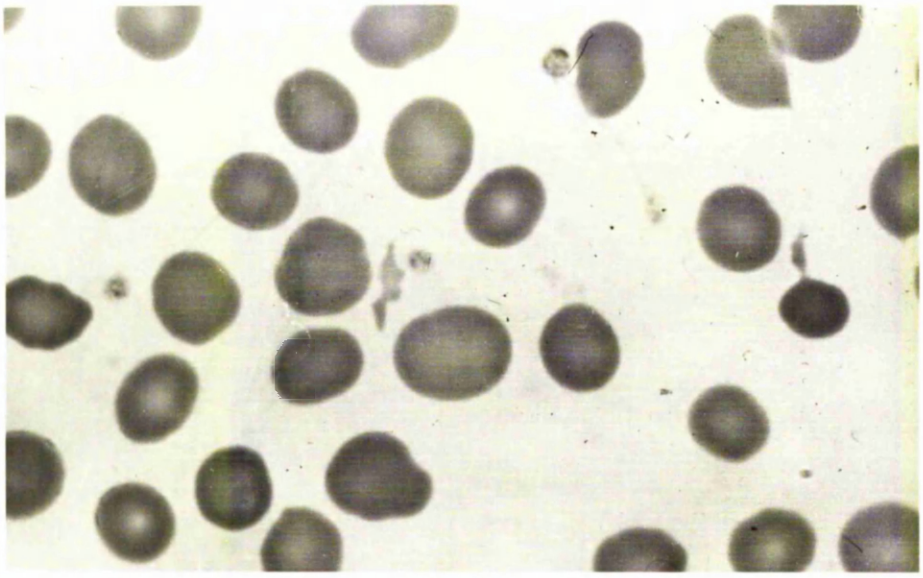


Fig. 11 Two elongated cell pieces (centre & right). Three round platelet clumps also present.

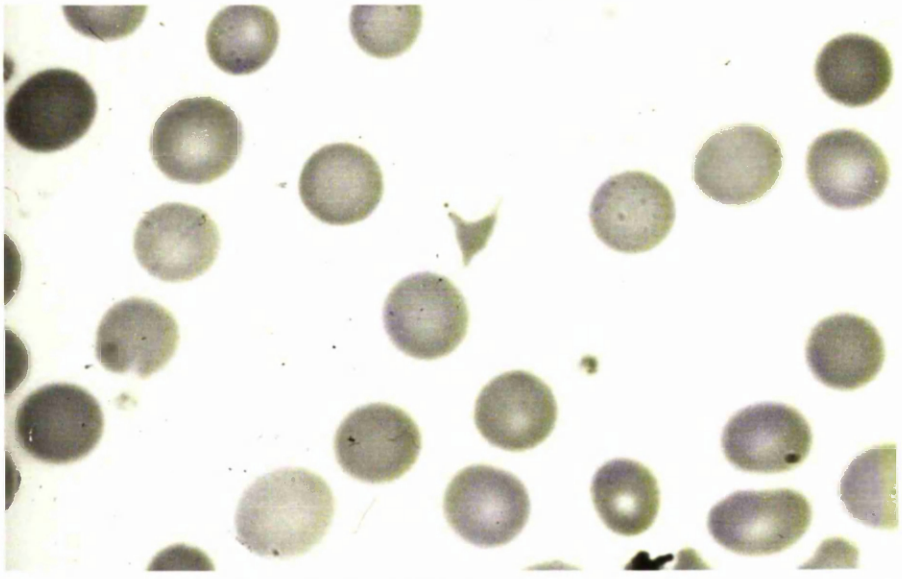


Fig. 12 A triangular cell.

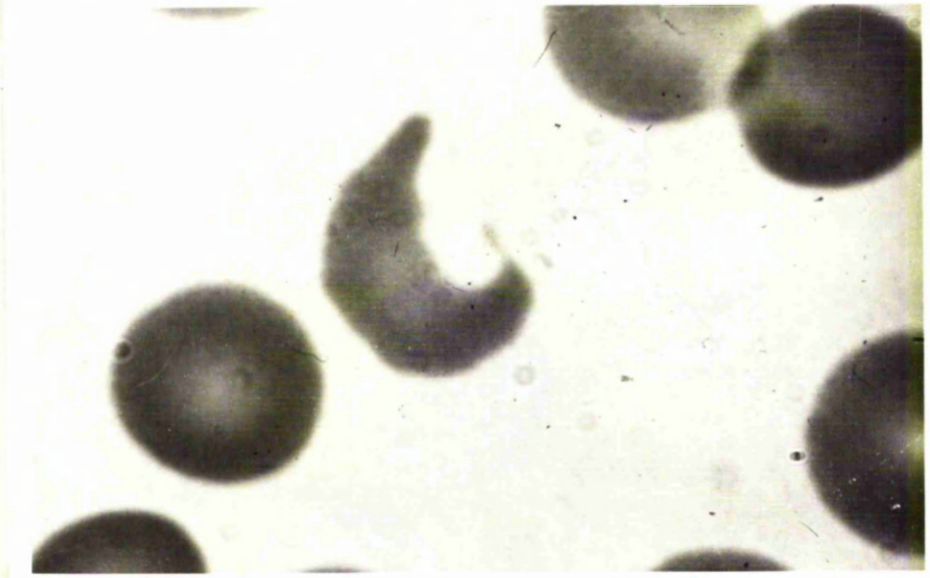


Fig. 13 An indented cell.

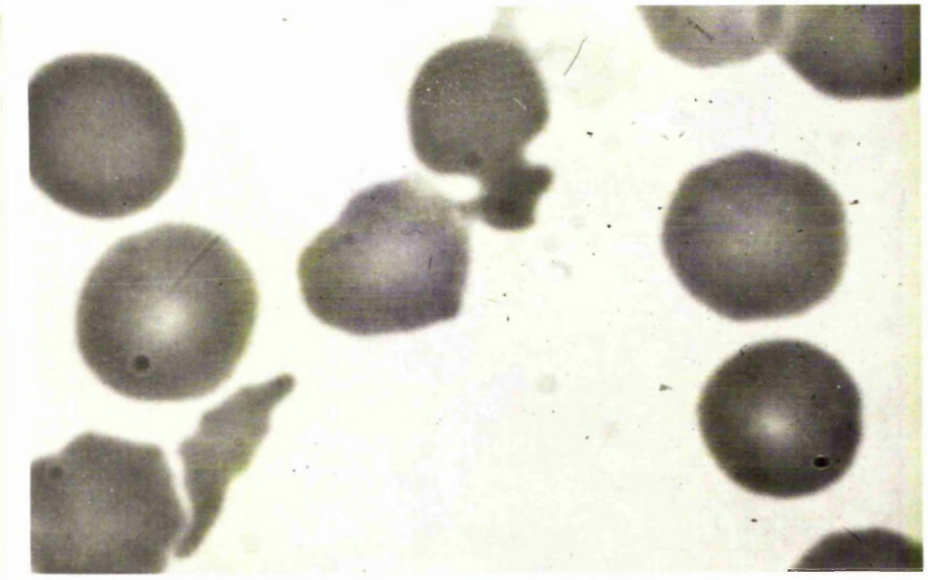


Fig. 14 A distorted and irregularly contracted cell (bottom left) and a pincered cell (top centre).

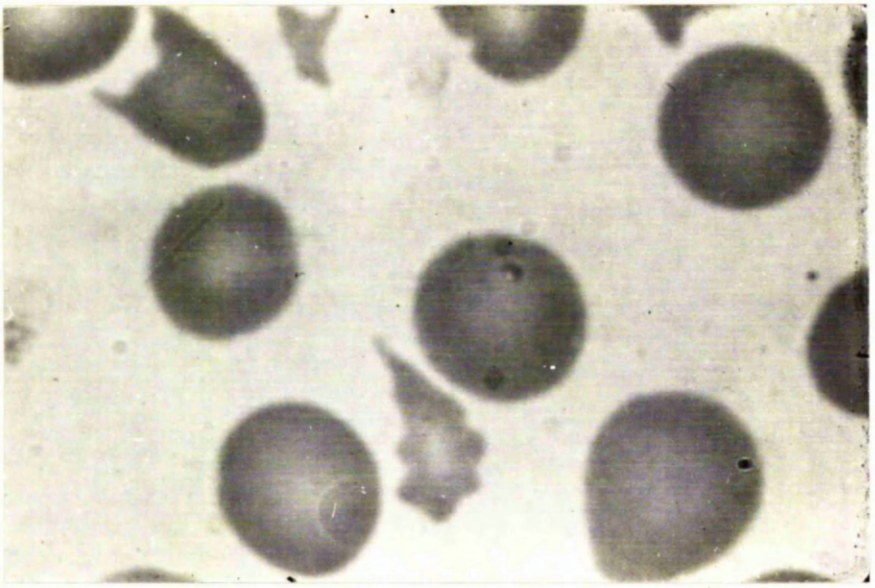


Fig. 15 A cell piece (top centre), an indented cell (top left) and a "?crenated cell" (bottom centre).

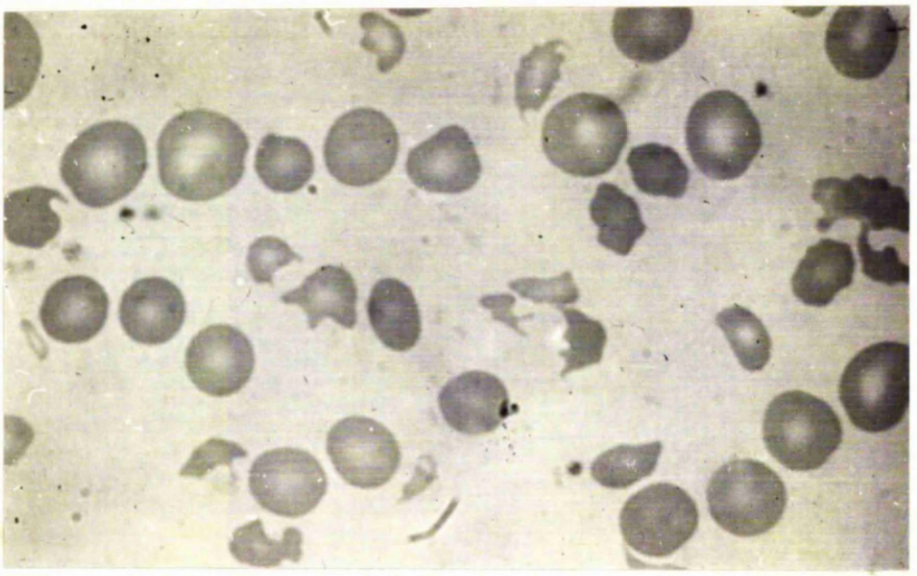


Fig. 16 Several cell segments, two cell pieces (extreme left & with a de-haemoglobinized portion, bottom centre), two indented cells (extreme left & bottom left), two distorted and irregularly contracted cells (centre), and a "?crenated cell" (top centre).

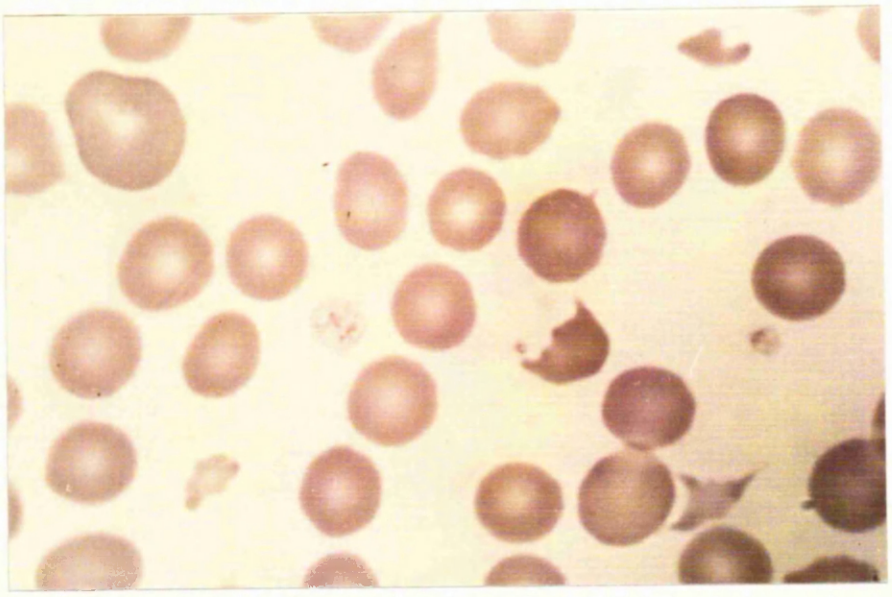


Fig. 17.
A cell segment (centre right), a distorted and irregularly contracted cell (top right), a ?cell piece or distorted and irregularly contracted cell (bottom left), and a triangular cell (bottom right). A reticulocyte is present at the top left corner.

line; and very small distorted and irregularly contracted cells especially if pale staining were difficult to distinguish from cell pieces.

The description "red cell fragmentation and distortion" has been applied only if blood films contained abnormal cells of types A and B. The degree of red cell fragmentation and distortion in each film was assessed semi-quantitatively as follows:-

Grade 0 = no evidence of red cell fragmentation and distortion

" 1 = occasional fragmented and distorted red cell approximating to one or two per 2-3 high-power-fields (minimal fragmentation)

" 2 = a few fragmented and distorted red cells in most high-power-fields (moderate fragmentation)

" 3 = numerous fragmented and distorted red cells present in nearly all high-power-fields (marked fragmentation)

(3) Abnormal Red Cell Counts

The following technique was employed for counting fragmented and distorted red cells.

Binocular Watson-Barnet adjustable squared eyepiece lenses were used, and counts were made under oil-immersion with a final magnification of x 1350. The area of the square was adjusted so that the number of red cells contained in it equalled 55 to 75 cells. Only cells whose entire outline could be seen were counted. Each film examined consisted of a monolayer of red cells. The films were not scanned for abnormalities but fields were examined at random so that there was no search for "suitable" fragment-containing fields and any such conscious or unconscious bias avoided. The method of calculating the number of abnormal cell types was based on the procedure for reticulocyte counting as recommended by Dacie and Lewis[167]. The total number of red cells in every fifth field was counted until the total cells present in 10 fields were recorded. Surveyal of successive fields was continued until 100 abnormal cell types had been enumerated or until 100 fields had been carefully inspected, each film examination taking about 15 minutes to complete. Therefore, in all but the most abnormal of films 5500 to 7500 red cells were examined, and the number of abnormal cell types was expressed as a percentage of this total number of red cells. Knowledge of the overall total red cell count allowed expression of the results also in absolute numbers per mm^3 of blood. The following are two examples of the calculations employed:-

A.

No. of red cells per 5th field	No. of additional fields surveyed	No. of abnormal red cell types observed	R.B.C. count
64	15	100	3.26 million/mm^3
70			
64			
60			
70			
78	100 abnormal cells present in (5 x 10) + 15 = 65 fields		
73			
70	677 total red cells present in 10 fields		
72	∴ 67.7 x 65 total red cells present in 65 fields = 4400 cells		
+ 56			
677			

∴ % abnormal forms = $\frac{100 \times 100}{4400}$ = 2.27% of RBC's

Absolute number of abnormal cell types = 2.27% of 3.26 million RBC/mm^3 = 74002/mm^3

B.

No. of red cells per 5th field	No. of additional fields surveyed	No. of abnormal red cell types observed	R.B.C. count
66	50	35	4.81 million/mm^3
70			
69			
67			
76			
70	35 abnormal cells present in (5 x 10) + 50 = 100 fields		
74			
75	705 total red cells present in 10 fields		
69	∴ 70.5 x 100 total red cells present in 100 fields = 7050 cells		
+ 69			
705			

∴ % abnormal forms = $\frac{35 \times 100}{7050}$ = 0.50% of RBC's

Absolute number of abnormal cell types = 0.50% of 4.81 million RBC/mm^3 = 24050/mm^3

With each examination the abnormal red cell types were also subdivided according to their particular morphological appearance. A hand counter as for differential white blood cell counts was used for recording, and cells falling into types A, B and C of the above classification, a total of six individual categories, were enumerated. The other forms (D) were relatively infrequently observed and were not recorded.

All abnormal cell counts were made without knowledge of the identity of the blood film or of the initial semi-quantitative assessment of red cell fragmentation and distortion, so that bias might be further avoided. This was arranged by giving the blood films to another member of staff once their initial routine inspection and assessment had been completed. The slides were relabelled at random according to a code of identification determined by him, and under these "blind" conditions the total and differential abnormal red cell counts were then carried out.

(4) Urinary Haemosiderin Test

A modification of the method of Gömöri[168] for demonstrating haemosiderin in tissue sections was used. A sample of urine (20 ml) was centrifuged at 2000 rev/min (700 g) for 10 minutes, and the resulting sediment and a small quantity of supernatant mixed and a loopful spread on each of two glass slides. The smears were heat-fixed and then alcohol-fixed for 10 min. They were then stained for 30 min with a mixture of equal volumes of 20% (v/v) HCl and 10% (w/v) potassium ferrocyanide that was prepared immediately before use. After this the smears were washed in water for 20 min to remove excess of acid and then counter-stained with 0.1% Neutral Red for 30-60 seconds. The preparations were finally washed briefly in water, allowed to dry, and mounted with DPX.

Haemosiderin appears as isolated or grouped blue-staining granules. Sometimes tiny amorphous dots of usually pale blue material or streaks of

blue stain were seen. These were not regarded as haemosiderin whose granular structure is usually easily identified if necessary using oil-immersion magnification. The degree of haemosiderinuria observed microscopically was assessed semi-quantitatively as follows, and is illustrated by photomicrographs taken as in section (2) above and all at the same magnification.

Grade 0 = no haemosiderin seen

" 1 = occasional very small group or isolated granule of haemosiderin approximating to one or two per several high-power-fields (Fig. 18)

" 2 = a few small groups or isolated granules of haemosiderin per 2-3 high-power-fields (Fig. 19)

" 3 = a few to many small groups and isolated granules of haemosiderin in most high-power-fields (Fig. 20)

" 4 = many groups of haemosiderin of varying size, often rather larger and denser than in grade 3, and present in nearly all high-power-fields

" 5 = gross haemosiderinuria with large dense clumps and innumerable smaller clumps in nearly all high-power-fields, often to the extent that the blue of the positive iron stain is visible on naked-eye inspection of the slide (Fig. 21)

The haemosiderin, usually only in grades 4 and 5, sometimes appeared to be incorporated in tubular epithelial cells.

This classification of haemosiderinuria can be summarized as follows:-

Grades 1 & 2 = slight haemosiderinuria, likely to be missed unless a careful search is made, especially if there is little counter-stainable material to assist in identifying the depth of focus

Grade 3 = moderate haemosiderinuria

Grades 4 & 5 = marked haemosiderinuria

The examination of blood films was always carried out before urinary haemosiderin preparations so that the semi-quantitative assessment of red cell distortion and fragmentation might not be influenced by a knowledge of the urinary haemosiderin status.

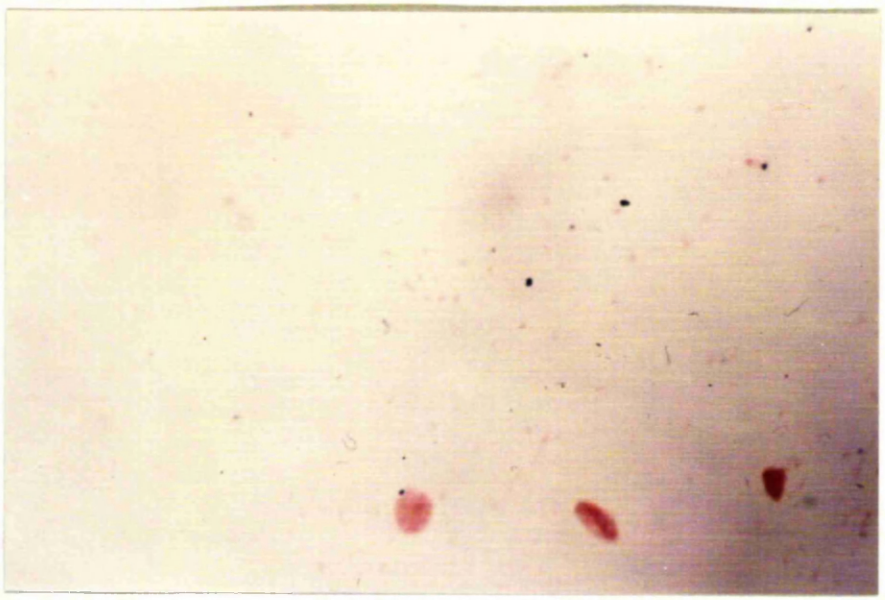


Fig. 18.
Grade 1 haemosiderinuria. Isolated blue staining granules seen in top right quadrant. Pink staining debris and nuclei of squamous epithelial cells present.

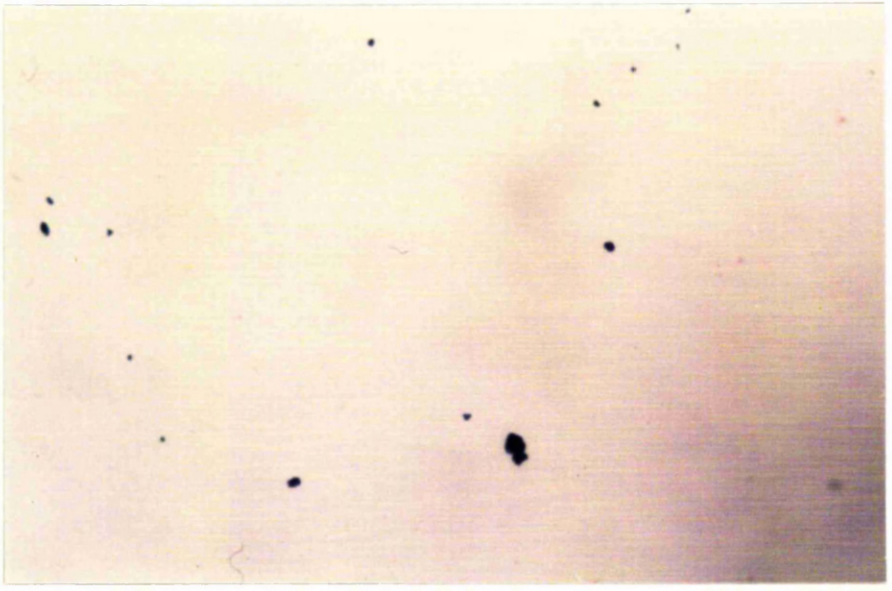


Fig. 19 Grade 2 haemosiderinuria.

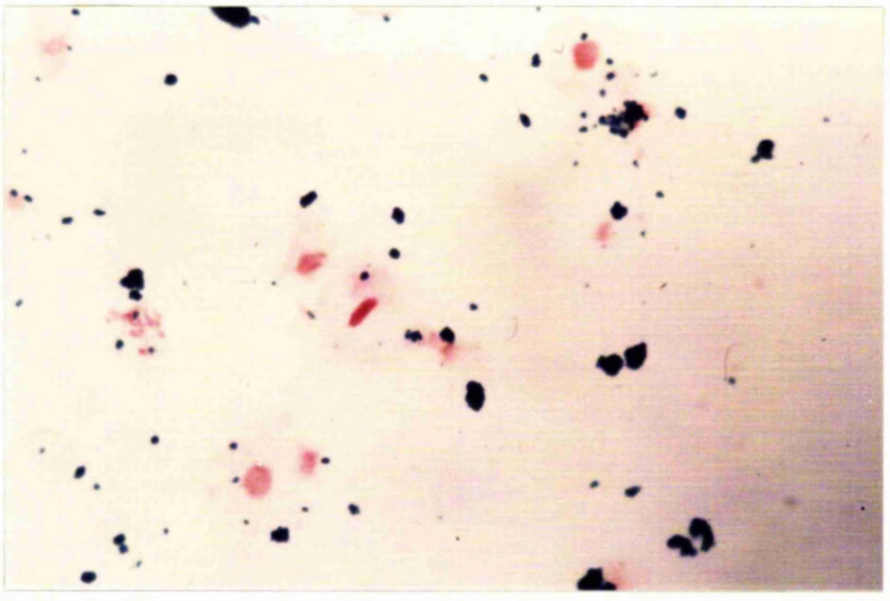


Fig. 20 Grade 3 haemosiderinuria. Squamous epithelial cells and debris present.

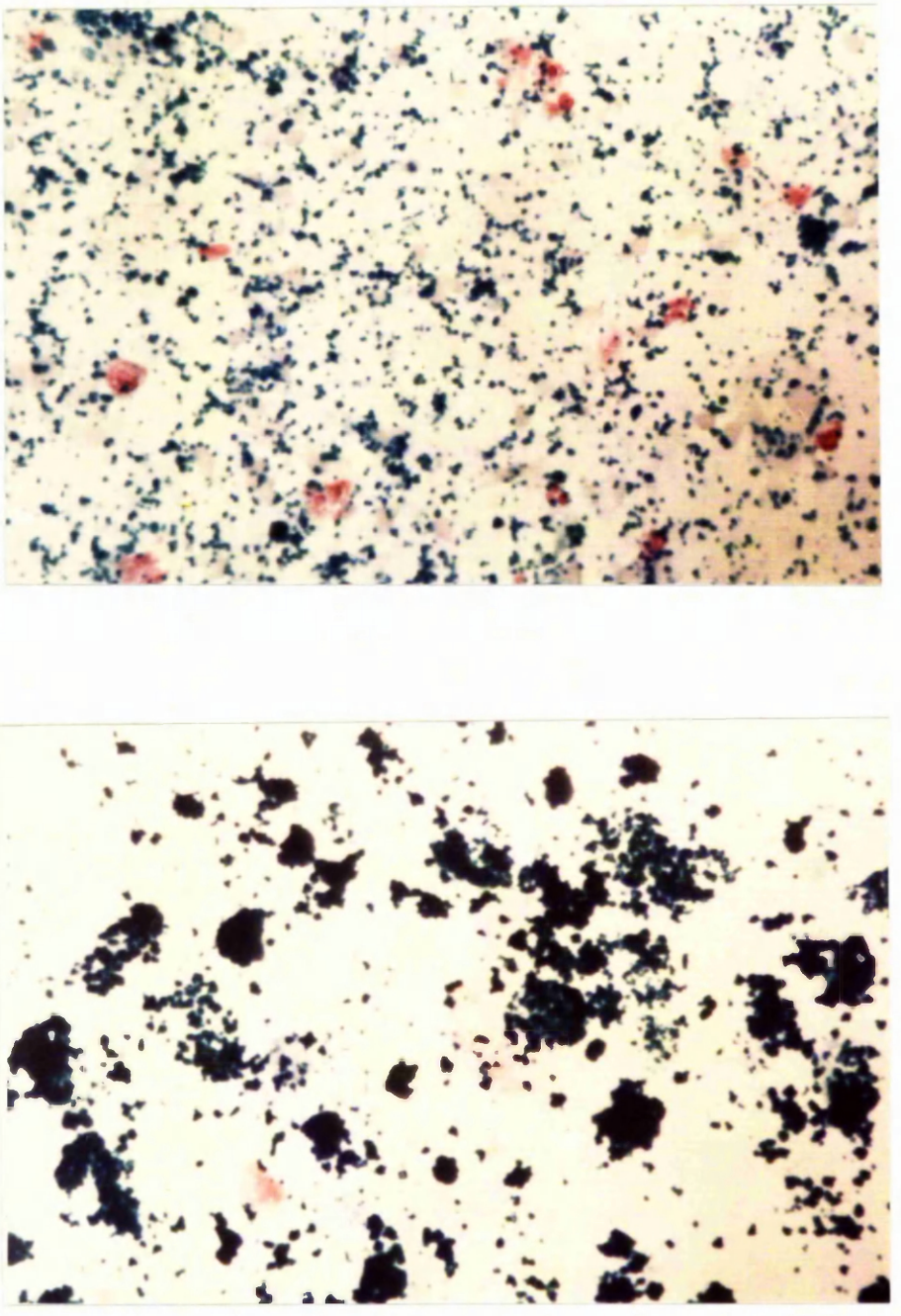


Fig. 21 Grade 5 haemosiderinuria (gross); two examples shown, and haemosiderin clump size is often larger. Pink staining debris and epithelial cell nuclei present.

(5) Other Haematological Studies

(i) ^{51}Cr-labelled Red Cell Survival

This was carried out according to the method of Dacie and Lewis[167] (normal half-life ($T_{\frac{1}{2}}$): 25-33 days).

(ii) Plasma Haptoglobin

The plasma haptoglobin level was estimated by an immuno-diffusion technique (M-Partigen plates, Behringwerke AG). The normal range established in this laboratory is 48-216 mg/100 ml.

(iii) Serum Fibrinogen-Fibrin Degradation Products (F.D.P.)

Blood samples for F.D.P. measurement were collected in 0.04% tranexamic acid and allowed to clot for four hours at room temperature before separation of the serum. F.D.P. levels were estimated by the tanned red cell haemagglutination inhibition immunoassay of Merskey, Kleiner and Johnson[169]. The upper limit of normal in this laboratory is 5 μg/ml.

(iv) Bone Marrow Examination

The aspiration and examination of bone marrow was carried out by a standard method[167]. Samples were obtained from the iliac crest as the sternal area had been involved in the previous thoracotomy procedures in the patients under study.

(6) Serological Investigations

These procedures were based on standard methods[167]. They included blood grouping with identification of Rhesus phenotype and Kell and Duffy^a blood group status, the direct anti-human globulin (Coombs') test, and the screening of serum for the presence of allo (iso)antibodies. This latter was carried out against a comprehensive panel of red cell antigens obtained from the Regional Blood Transfusion Service using the following techniques: (A) room temperature saline, (B) 37°C saline, (C) enzyme (ficin-treated test cells), and (D) anti-human globulin. The specificity of any

antibody detected was then precisely identified using a panel of cells supplied by Ortho Diagnostics.

II BIOCHEMICAL INVESTIGATIONS

(1) Serum Bilirubin, Aspartate and Alanine Aminotransferase, and Lactate Dehydrogenase

Serum total bilirubin was estimated on the Technicon Auto-analyser by the method of Gambino and Schreiber[170] (normal: 0.2 - 0.9 mg/100 ml).

Serum aspartate aminotransferase (aspartate transaminase, AsT), alanine aminotransferase (alanine transaminase, AlT), and lactate dehydrogenase (LDH) were assayed on the LKB 8600 Reaction Rate Analyser using Boehringer test combination kits at 37°C. While this study was in progress the Department of Pathological Biochemistry instituted a change in the methodology of these enzyme estimations in that the concentrations of substrate, coenzyme and phosphate buffer were altered to achieve optimization of the assay procedures at 37°C.

Initially the Boehringer Company UV-tests for AsT and AlT levels had been based on the methods of Karmen[171] and of Wróblewski and La Due[172] (normal values: AsT 9 - 35 iu/l; AlT 5 - 26 iu/l.) Since December 1972 the optimized methods for AsT and AlT estimations described by Bergmeyer and Bernt[173] have been used (normal values: AsT 13 - 42 iu/l; AlT 11 - 55 iu/l.)

Serum LDH assay was initially performed according to Wróblewski and La Due[174] (normal: 116 - 467 iu/l). Since December 1972 the concentrations of substrate, coenzyme and phosphate buffer optimal at 37°C have been employed for LDH assay as described by McQueen[175], and a normal range of 240 - 525 iu/l established[176]. This last result was obtained in a healthy volunteer group none of whom were hospital patients, while the foregoing quoted normal values for enzyme activity and bilirubin level were established using hospital in-patient

populations[177].

These earlier and later methods of estimating serum AsT, AlT and LDH are hereafter referred to as method (a) and method (b) respectively.

Care was taken during collection of blood to avoid in-vitro haemolysis. The minimum of venous compression was employed, wide bore gauge 19 needles were used when venous size and position were suitable, blood was withdrawn without excessive suction and then after removing the needle expelled from syringe into sample tube evenly and under low pressure, and specimens were transferred to the laboratory without delay.

The examination of both blood films and urinary haemosiderin preparations was always completed before the results of these serum estimations were available, so that the haematological assessment might not be prejudiced by prior knowledge of the biochemical features.

(2) Serum Iron and Total Iron Binding Capacity

The serum iron and total iron binding capacity (T.I.B.C) were determined by a routine auto-analyser method as described by Young and Hicks[178] and Babson and Kleinman[179]. The normal values in this laboratory are: serum iron 100 - 250 μg/100 ml (males), 80 - 180 μg/100 ml (females); and T.I.B.C. 250 - 430 μg/100 ml.

(3) Urinary Iron

The urinary iron output was estimated by the following method.

Urine collections (24 h) were made in iron-free plastic bottles containing 5 ml of conc. HCl as preservative and to aid in solution of the iron. Samples of urine (2 ml) were analysed directly by the auto-analyser method referred to above for the determination of serum iron in which a dialysed iron-containing sample is mixed with tripyridyl-triazine to form a coloured complex which is measured colorimetrically.

The useful working range was found to be from about 0.2 mg Fe/l to 3.0 mg Fe/l. Samples with a higher concentration of iron were reanalysed after dilution with 0.1 M-HCl. Conversely, for more precise estimates of iron at concentrations less than 0.2 mg/l a standard addition technique was used. Two additions of known amounts of standard iron solutions were made to the unknown urine, and the original iron concentration was calculated graphically from the increments in colour absorbance produced[180].

Repeated analysis of a urine sample gave a mean result of 0.62 mg Fe/l (s.d. 0.01, n = 10) with a coefficient of variation of 1.6%.

The same urine analysed by an atomic absorption spectrophotometric method after chelation and solvent extraction[181] gave a mean of 0.63 mg Fe/l (s.d. 0.04, n = 10), the coefficient of variation being 6.3%.

The quality control results from a suitable control serum gave, during the period of collection of the urine samples, a mean value of 1.88 mg Fe/l (s.d. 0.07, n = 20) with a coefficient of variation of 3.7%.

(4) Urinary Urobilinogen, Bilirubin, and Haemoglobin

The presence or absence of excess urinary urobilinogen was determined in a fresh spot sample of urine using Urobilistix reagent strips (Ames Co.) (normally < 1.0 Ehrlich unit/100 ml). The urine was concurrently tested for bilirubin by means of Bili-Labstix reagent strips (Ames Co.).

Urine was examined for haemoglobin using a Hartridge reversion spectroscope.

III RENAL INVESTIGATIONS

The normal values given are those reported by de Wardener[182] unless otherwise stated.

(1) Tests of Glomerular Function

(i) Plasma Urea

This was estimated on the Technicon Auto-analyser by the method of Marsh, Fingerhut and Miller[183]. The normal range is 15 - 35 mg/100 ml.

(ii) Serum Creatinine and Creatinine Clearance

An endogenous creatinine clearance was performed using a 24-hour urine collection with a mid-point blood sample, the serum and urine creatinines being estimated by the method of Hare[184]. The normal range for serum creatinine is 0.8 - 1.4 mg/100 ml, and a creatinine clearance of 80 ml/min/ 1.73 m^2 was taken as the lower limit of normal having regard to the age of the patients in whom this investigation was performed.

(2) Tests of Tubular Function

(i) Water Concentration Test

This was assessed by measuring the urine osmolality with an Advanced osmometer after an 8-hour period of dehydration. The kidney should normally be capable of concentrating urine to an osmolality of 800 mosmol/kg H_2O or more.

(ii) Acid Load Test

The short acid load test of Wrong and Davies[185] was carried out. Normally under the conditions of this test the urinary pH should fall to within the range 4.60 - 5.24, and the titratable acidity, ammonium excretion and total hydrogen ion excretion should rise to 24 - 51 μEq/min, 33 - 75 μEq/min and 60 - 124 μEq/min respectively.

(iii) Urinary Amino Acid Content

The amino acid content of the urine was analysed by colorimetry[186] and paper chromatography[187].

(3) Urinary Protein and Cell Excretion Rates

Proteinuria was quantitated by the biuret method of Wootton[188] on a 24-hour urine collection, and the upper limit of normal was taken as 90 mg/24 h.

Urinary red and white blood cell excretion rates were measured by the method described for white cells by Houghton and Pears[189] using a 3-hour urine collection. The normal values are: R.B.C. 0 - 200000/h; W.B.C. 0 - 400000/h.

(4) Bacteriological Examination of the Urine

Quantitative urine cultures were carried out as described by McGeachie and Kennedy[190].

IV STATISTICAL METHODS

(1) Standard Parameters

Mean ($\bar{x}$), variance, standard deviation (s, or s.d.) and standard error of the mean values were calculated on a Wang electronic calculator, Model 370, with the aid of a suitable computer pgogram.

(2) Significance Tests

The following tests of significance were used.

(i) Student's t tests for comparison of the means of two samples. For non-paired comparisons the appropriate t test and calculation of degrees of freedom (d.f.) were used according to whether or not there was a significant difference between the variances of the two samples[191].

(ii) Calculation of correlation coefficient (r) and linear regression analysis.

(iii) Chi-squared (X^2) test for the association between two systems of classification, employing Yates' correction for 2 x 2 contingency tables[191,192].

Student's t tests, calculations of correlation coefficients and

linear regression analysis were performed with the aid of Wang computor programs. The statistical tables in Documenta Geigy[193] and the Biometrika Tables for Statisticians[194] were used. The significance of all probabilities was judged by "two-tailed" tests.

V PATIENTS STUDIED

A total of 159 patients with prosthetic heart valves have been studied (64 men and 95 women; mean age 42 years, range 17 - 66 years). In nearly every case the operation had been performed in the cardiac surgery unit at the Royal Infirmary, Glasgow. A few (12 patients) had undergone prosthetic valve replacement at other cardiac centres in Britain but were domiciled in Glasgow. The investigations that are the subject of this thesis were carried out during the patients' attendances at the anticoagulant clinics of the Glasgow Royal Infirmary for control of the long-term anticoagulant therapy instituted as a prophylaxis against thrombo-embolism. A small number of patients also had investigations performed while in-patients, and special admission or out-patient arrangements were made for ^{51}Cr-labelled red cell survival studies, bone marrow examinations, and renal function tests.

Different types of prosthesis had been used to replace the damaged valves in these patients. The patients also differ in respect of the time of entry into this study relative to the date of operation as well as in the duration of post-operative follow-up.

These two factors will now be detailed.

(1) Types of Cardiac Valve Prosthesis

The great majority of the patients had received either one of the different types of Starr-Edwards ball and cage prosthesis[195] or the tilting disc prosthesis introduced by Björk and Shiley[196]. A few had Starr-Edwards non-tilting disc prostheses[195]. Descriptions of these different prostheses are given below and illustrated in figures 22-26.

(i) Starr-Edwards Ball and Cage Prostheses

In order of development:-

A. Models 1000 and 1200 (Aortic), and 6000 and 6120 (Mitral)

These have bare metal cage struts, a Silastic rubber

ball, and an outer sewing ring of cloth (Fig.22). In the 1200 and 6120 developments, which have supplanted the earlier models, the cloth of the sewing ring is extended medially on the inflow side of the valve ring to cover the metal up to the orifice rim.

B. Models 2300 (Aortic) and 6300 (Mitral)

In these models the metal cage struts are covered with Teflon cloth which also completely covers the valve ring internally as well as constituting the outer sewing ring. The ball is a hollow Stellite metal sphere (Fig. 23).

C. Models 2310 and 2320 (Aortic), and 6310 and 6320 (Mitral)

The basic design of these models with complete cloth-covering and a Stellite metal ball is as in type B. In addition, metal studs alternate with the cloth on the outflow side of the valve ring and together they form a "composite seat" for the ball (Fig. 24). The valve orifice diameter is also larger for any given external tissue diameter in comparison with type B. Further minor modifications are included in the more recent 2320 and 6320 models.

(ii) Starr-Edwards Low-Profile Disc Prostheses

These comprise a disc that moves (non-tilting) within a bare Stellite metal cage (Fig. 25). The cloth of the sewing ring, as in the 1200 and 6120 models, extends to the orifice rim on the inflow side of the ring leaving no exposed metal on the inflow face. They are used in the mitral or tricuspid position, and prostheses with plastic discs (Model 6520) have supplanted the earlier type that had a hollow metal disc (Model 6500).

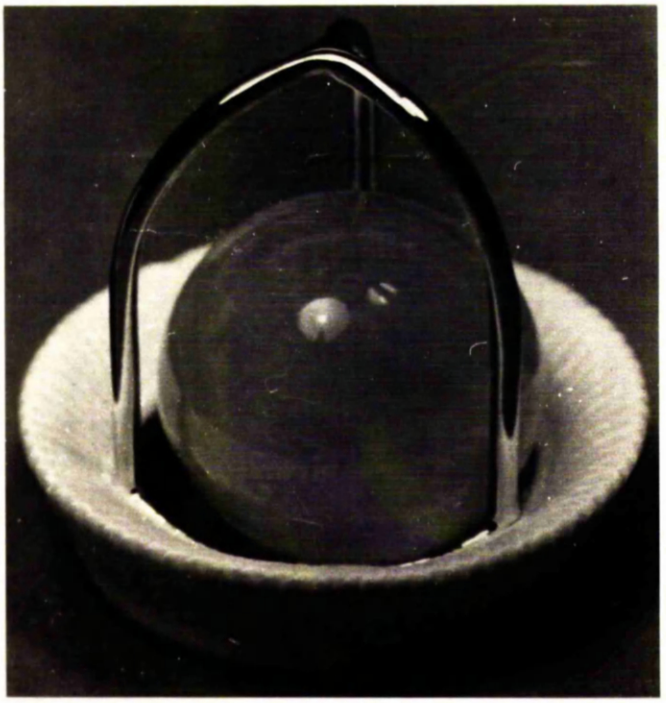


Fig. 22 Starr-Edwards Silastic rubber ball and bare metal cage prostheses; an aortic 1200 model (above) and mitral 6120 model (below) shown.

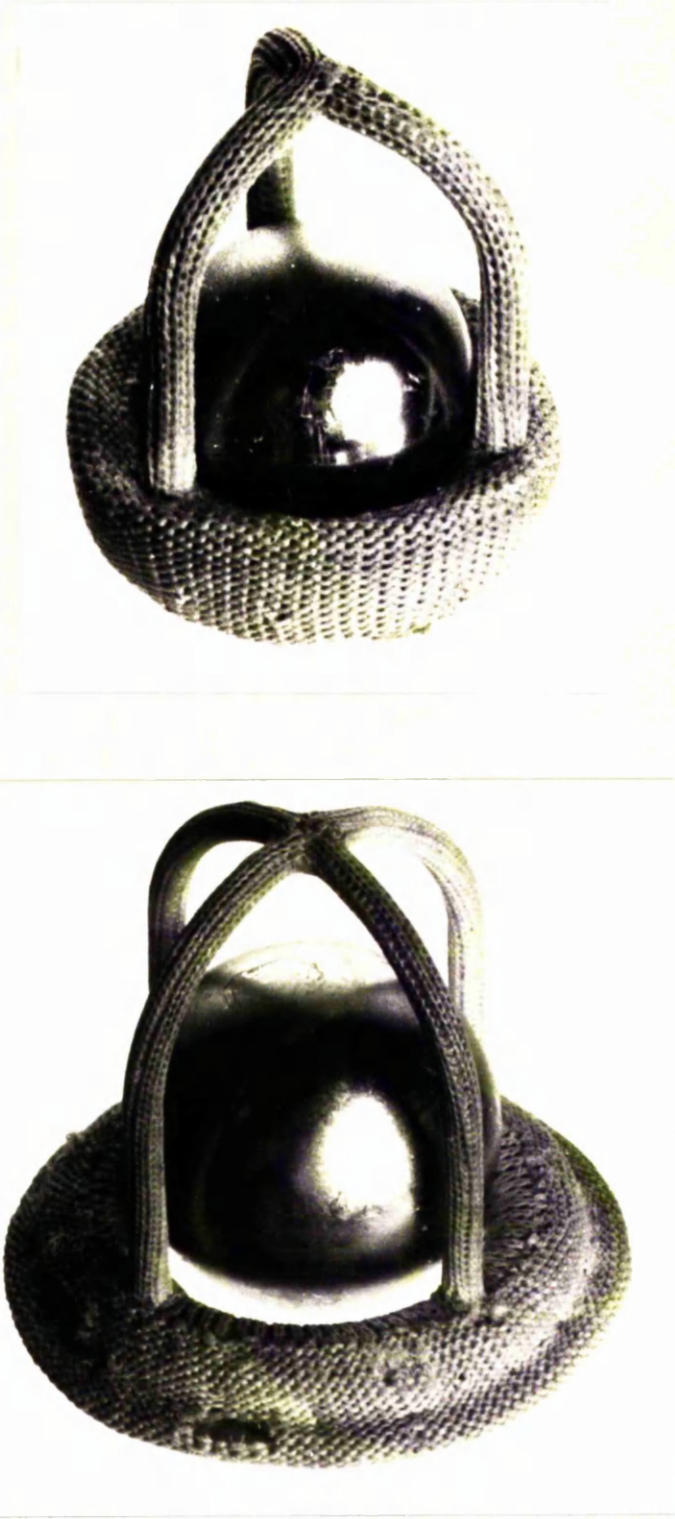


Fig. 23 Starr-Edwards Stellite metal ball and cloth-covered cage prostheses; an aortic 2300 model (above) and mitral 6300 model (below) shown. Ignore the torn cloth in the latter photograph.

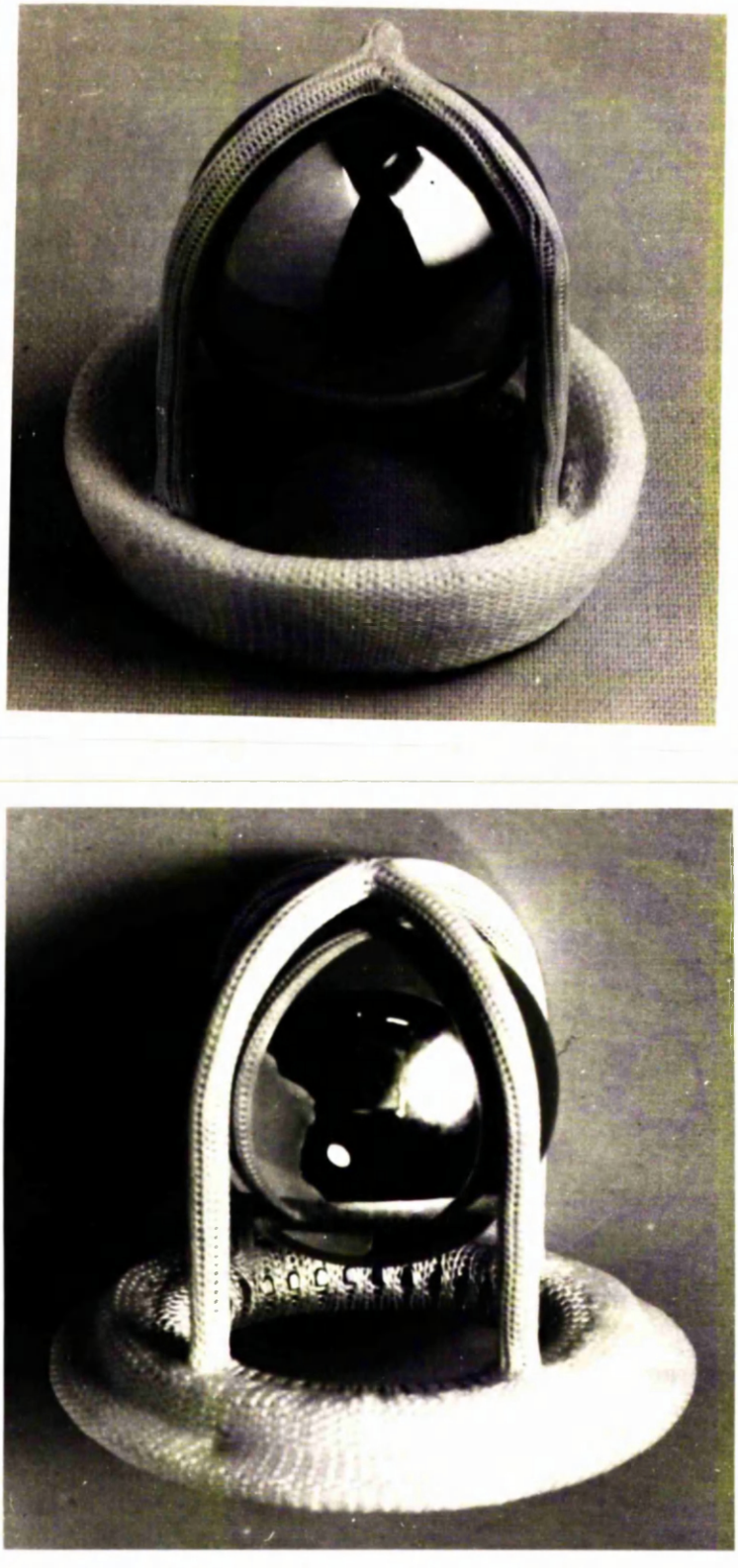


Fig. 24 Starr-Edwards Stellite metal ball and cloth-covered cage with composite seat prostheses; an aortic 2310 model (above) and mitral 6310 model (below) shown. The metallic studs alternating with cloth on the outflow side of the valve ring (the "composite seat") are clearly seen.

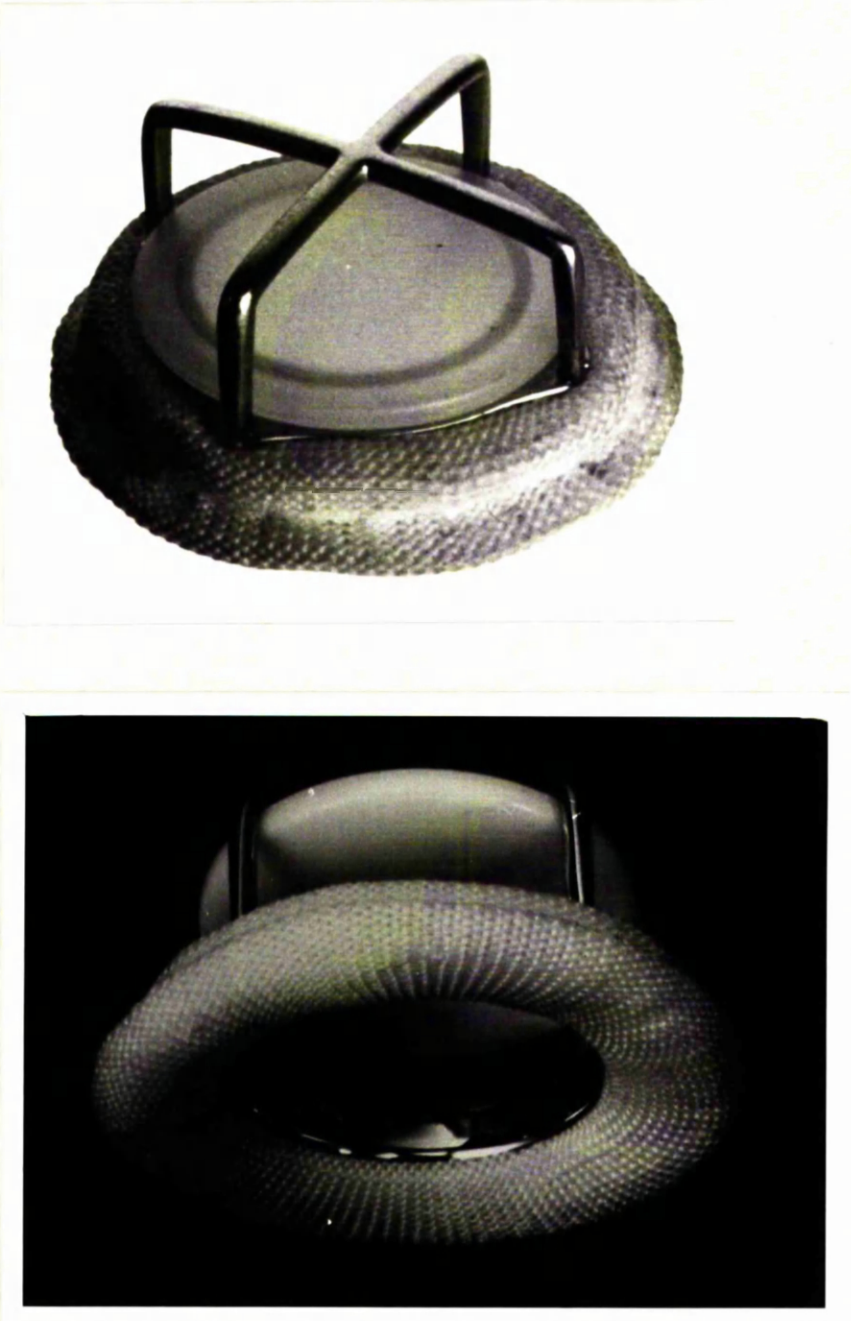


Fig. 25 Starr-Edwards low-profile caged disc prosthesis; a mitral 6520 model shown, with views from the ventricular aspect (above) and from the atrial side (below). The cloth from the sewing ring can be seen to extend inwardly to the orifice rim on the inflow atrial side of the valve ring.

(iii) Björk-Shiley Tilting Disc Prosthesis

This is an entirely different design from the foregoing types of prosthesis. It comprises a free-floating Delrin plastic (or now pyrolytic carbon) disc which is suspended and pivots between two eccentrically placed support legs attached to the Stellite metal valve ring (Fig. 26). The disc tilts open to an angle of 60° in aortic models and to 50° in mitral and tricuspid models. Apart from a sewing ring of Teflon there is no cloth incorporated in the model.

Fig. 26.
The Björk-Shiley tilting disc prosthesis. (A mitral model).

The numbers of patients with these different types of prosthesis related to the site of valve replacement are detailed in Table V. They amount to an overall total of 51 cases of aortic valve replacement, 72 of mitral valve replacement and 38 of multiple valve replacement, as two of the patients were re-operated upon during the study with replacement of their original prosthesis with a different type.

(2) Timing of Investigations

The differences between the patients in the times of beginning and completing investigations in relation to the date of operation were determined principally by the prosthesis model used. Thus, the Starr-Edwards Silastic rubber ball and non cloth-covered cage prostheses which had been initially used some years before this study commenced had been supplanted by the fully cloth-covered types. These, and specifically the "composite-seat" models, were already an ongoing surgical commitment when this investigation began, and valve replacement with the Bjork-Shiley tilting disc prosthesis was then subsequently instituted. In those cases recently operated upon, investigations were nearly always delayed until three months had elapsed since operation so that the effects of operative blood loss, extracorporeal circulation, blood transfusion and blood regeneration would not interfere with the interpretation of results. Details of the timing of investigations are given in Table VI for all patients. In 63% of cases these were begun within 12 months of operation; conversely, in 50% of cases post-operative follow-up exceeded two years. The differences in timing according to the prosthesis model used are shown in Table VII. This time factor is dealt with further in chapters 5 & 6 when the haemolytic complications of the different valve types are compared.

Valve Replaced (No. of Cases)[a]	Starr-Edwards (S-E) Ball and Cage Models 1000 (A)[b] 6000 (M)	1200 (A) 6120 (M)	2300 (A) 6300 (M)	2310 (A) 6310 (M)	2320 (A) 6320 (M)	Bjork-Shiley (B-S) Tilting Disc Models	Other Cases[c]
Aortic (51)	-	3	15	17	-	16	-
Mitral (72)	6	9	21	13	2	13	8
Aortic plus Mitral (21)			10[d]			6	5
Aortic plus Mitral plus Tricuspid (4)			1[e]			3	-
Aortic plus Tricuspid (1)			-			1	-
Mitral plus Tricuspid (12)			6[f]			6	-

Notes:
a Total cases = 161 since two of the 159 patients were re-operated upon during the study and a different valve type inserted (a 2300 replaced by a B-S (A); a 6310 replaced by a B-S (M)).
b A = aortic, M = mitral; S-E tricuspid valve replacements (T) are described in the text.
c Six patients with a 6520 (M) S-E disc model and seven who do not fit into any of the defined categories. Two of the latter have a cloth-covered ball and cage S-E (M) of uncertain type; three an A + M of mixed type (B-S (A) + 6520 (M) (2 cases); B-S (A) + 6310 (1 case)); and two an A + M of uncertain type.
d Comprises a 2300 + 6300 (5 cases); a 2300 + 6310 (1 case); a 2310 + 6300 (1 case); a 2310 + 6320 (2 cases); and a 2310 + 6520 (M) (1 case).
e Comprises a 2300 + 6300 + 6500 (T).
f Comprises a 6300 + 6500 (T) (1 case); a 6310 + 6520 (T) (4 cases); and a 6320 + B-S (T) (1 case).

TABLE V DETAILS OF PROSTHETIC VALVE TYPE AND SITE IN THE 159 PATIENTS.

Months After Operation	Investigations begun (No. & % No. of Cases)		Investigations completed (No. & % No. of Cases)	
0 - 3	5	(3%)	1	(1%)
3 - 6	65	(40%)	7	(4%)
6 - 12	32	(20%)	22	(14%)
12 - 24	35	(22%)	51	(32%)
24 - 36	12	(7%)	33	(20%)
36 - 48	3	(2%)	19	(12%)
48 - 60	3	(2%)	14	(9%)
60+	6	(4%)	14	(9%)

TABLE VI TIMING OF POSTOPERATIVE INVESTIGATIONS IN THE 161 CASES

Valve Replaced	Prosthesis Model[a] (No. of Cases)		Months Postoperative: Investigations begun Mean	(s.d.)	Investigations completed Mean	(s.d.)
Aortic	2300	(15)	14.8	(5.0)	41.4	(12.3)
	2310	(17)	5.0	(2.9)	26.1	(12.3)
	B-S	(16)	4.5	(2.1)	16.2	(7.8)
Mitral	600/6120	(15)	52.1	(16.9)	72.0	(17.3)
	6300	(21)	18.0	(6.8)	36.5	(14.2)
	6310/20	(15)	5.6	(2.1)	22.9	(10.8)
	6520	(6)	8.8	(6.0)	15.7	(10.6)
	B-S	(13)	7.7	(4.1)	16.2	(5.1)
Aortic + Mitral (± Tricuspid)	S-E	(11)	6.7	(4.5)	29.8	(14.0)
	B-S	(9)	5.0	(1.7)	12.2	(4.0)
Mitral + Tricuspid	S-E	(6)	7.5	(3.7)	12.7	(5.1)
	B-S	(6)	7.0	(4.2)	13.8	(7.4)

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards

TABLE VII TIMING OF POSTOPERATIVE INVESTIGATIONS ACCORDING TO PROSTHETIC VALVE TYPE AND SITE

SECTION I

THE DIAGNOSIS OF INTRAVASCULAR HAEMOLYSIS

CHAPTER 3

RESULTS

I SERUM LACTATE DEHYDROGENASE LEVELS AS A MEASURE OF INTRAVASCULAR HAEMOLYSIS

The correlation between serum lactate dehydrogenase (LDH) levels and red cell survival as estimated by ^{51}Cr labelling was examined in 27 patients with prosthetic heart valves. Full details of the investigations are given in Table VIII. An inverse exponential relationship was found between the LDH values and the half-life ($T\frac{1}{2}$) of the ^{51}Cr-labelled red cells, and a straight line is obtained when the observations are plotted in a semi-logarithmic scale (Fig. 27). The regression line fits the equation:-

$$\text{Log LDH} = 3.69 - 0.04 \times T\tfrac{1}{2} \text{ (days)}$$

or $$T\tfrac{1}{2} \text{ (days)} = 92.25 - 25.0 \times \log \text{LDH}$$

and the correlation coefficient is -0.82 which is highly significant ($P < 0.001$). However, there are appreciable variations in LDH levels in patients with similar red cell survival, and the individual observations are scattered around the regression line with a standard deviation of 0.175 for log LDH. Conversely, therefore, for any given serum LDH value the range of predicted red cell survival half-life is rather wide.

The LDH estimations in this study were all done by assay method (a) (p. 76).

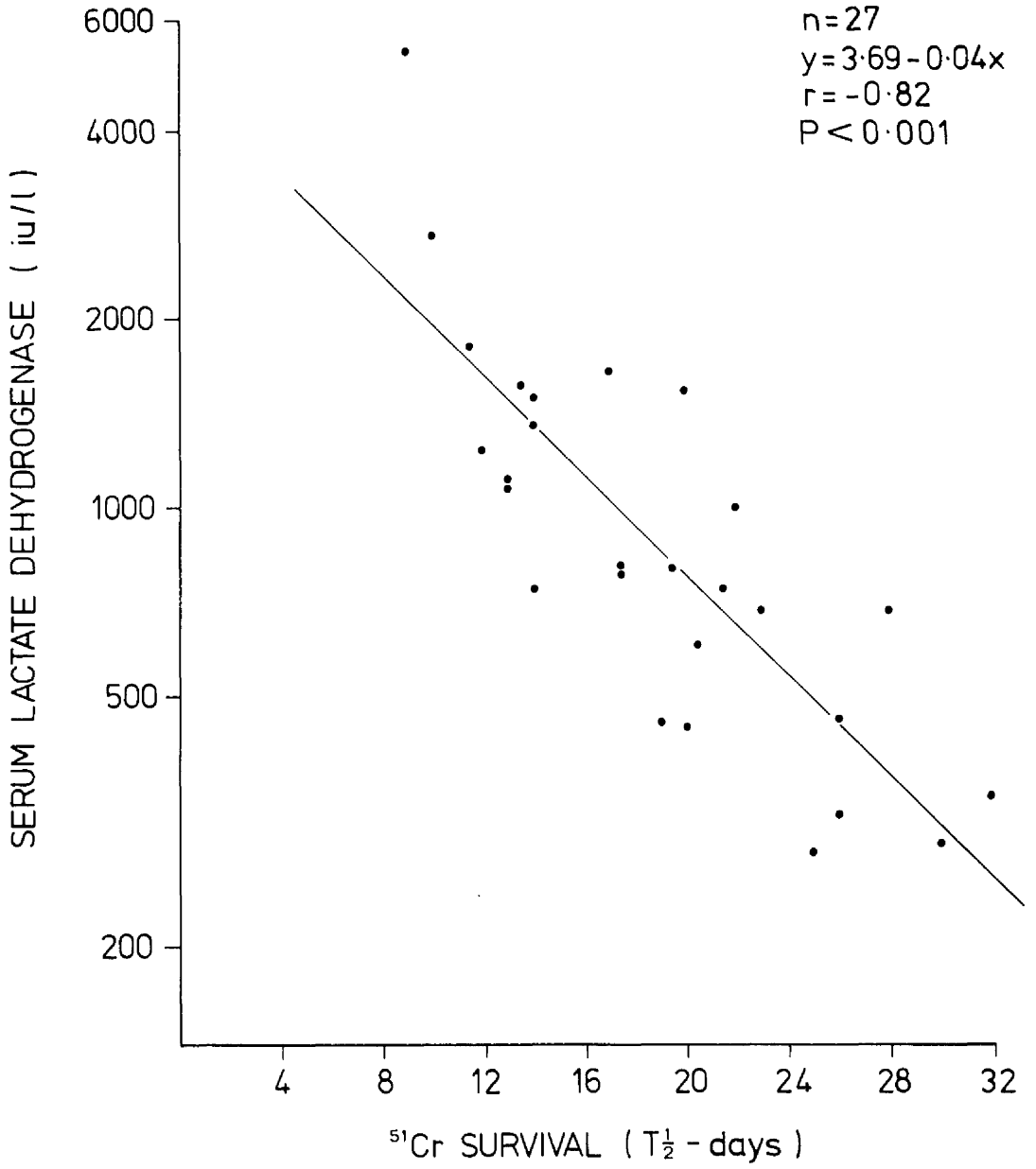


Fig. 27 Relationship between serum lactate dehydrogenase level and the half-life of ^{51}Cr-labelled red cells.

(Normal LDH 116-467 iu/l; normal $T_{\frac{1}{2}}$ 25 - 33 days).

Subject[a]	Sx	^{51}Cr Survival[b] ($T\frac{1}{2}$ - days)	Serum LDH[c] (iu/l)	Hb. (g/100 ml)	Retics. (%)	Red Cell Fragmentation (Grade)	Urinary Haemosiderin (Grade)
1*	F	32	345	14.9	1	0	0
2*	M	30	290	18.9	1	0	0
3	M	28	680	15.2	4	0	4
4	M	26	320	17.7	2	0	0
5	M	26	460	16.1	2	0	0
6*	M	25	280	16.3	2	0	0
7*	F	23	680	12.3	2	0	4
8	M	22	1000	16.3	5	0	3
9	M	21.5	740	15.6	1	1	2
10*	M	20.5	600	13.9	<1	0	3
11*	F	20	445	13.2	<1	0	0
12	M	20	1520	12.3	1	2	5
13	M	19.5	800	15.0	1	0	3
14	F	19	450	15.6	2	0	0
15*	F	17.5	780	14.6	1	0	3
16*	M	17.5	800	12.6	2	1	3
17**	F	17	1620	11.6	3	2	4
18	F	14	740	15.3	2	1	4
19	M	14	1330	10.1	9	2	5
20**	F	14	1480	10.9	8	3	5
21	F	13.5	1560	7.5	13	3	5
22*	F	13	1080	12.3	1	2	3
23**	F	13	1100	9.9	5	1	5
24	M	12	1220	11.8	4	2	5
25	M	11.5	1800	13.1	3	2	5
26	M	10	2700	7.9	9	2	3
27	F	9	5360	9.0	13	3	5

Notes: a Subjects marked * had a mitral valve prosthesis; ** an aortic plus mitral prosthesis; other cases had an aortic valve prosthesis.
b Normal $T\frac{1}{2}$ = 25 - 33 days.
c Normal LDH = 116 - 467 iu/l.

TABLE VIII CORRELATION BETWEEN SERUM LACTATE DEHYDROGENASE (LDH) LEVELS AND ^{51}Cr RED CELL SURVIVAL TIMES: DETAILS OF INVESTIGATIONS.

II HAEMOSIDERINURIA AND RED CELL FRAGMENTATION AS INDICES OF THE DEGREE OF INTRAVASCULAR HAEMOLYSIS

The value of the urinary haemosiderin test and the assessment of red cell morphology as indices of the severity of intravascular haemolysis was examined by comparing the results of these investigations in 133 patients with estimates of red cell survival as determined by serum lactate dehydrogenase (LDH) levels, and with the overall haematological status. In 37 of these patients more than one set of results was available from investigations performed at different times, so that the final number of paired and simultaneous investigations was 180.

The 180 cases were grouped as shown in Table IX according to the presence or absence of haemosiderinuria, of red cell fragmentation and distortion, and of anaemia attributable to intravascular haemolysis.

Haemolytic Group	Haemosiderinuria	Red Cell Fragmentation	Haemolytic Anaemia	No. of Patients	No. of Sets of Results
0 =	-	-	-	55	64
1 =	+	-	-	35	39
2 =	+	+	-	34	45
3 =	+	+	+	21	31

Notes: + = present, - = absent

TABLE IX CLASSIFICATION OF HAEMOLYTIC GROUPS

For example, two patients placed in Haemolytic Group 2 had a very mild anaemia but this was likely to have been precipitated by blood loss and not by their haemolysis. Of the 37 patients with more than one set of results, 12 are included in two Groups as there was a change in their urinary or blood findings during the study. Only one set of results out of the 180 would not fit into one or other Group. This was a patient in whom red cell fragmentation with anaemia and a high LDH level were present, but haemosiderinuria could not at that time be detected.

(1) Relation between Haemolytic Groups and Serum Lactate Dehydrogenase Levels

The correlation between the degree of haemolysis arbitrarily defined by Haemolytic Groups 0 to 3 and as estimated by LDH levels was examined by comparing the results between each Group of the serum LDH assays (Fig. 28). Since the method of estimating LDH altered during the course of the investigation the results are divided according to whether method (a) or (b) had been used.

While there is overlap in LDH levels between Haemolytic Groups especially with assay method (a), there is a progressive and at each step significant increase in the mean LDH level from patients in Group 0 to those in Group 3 (method (a): Group 0 v 1, $t = 5.75$, d.f. = 29, $P < 0.001$; Gp. 1 v 2, $t = 3.32$, d.f. = 46, $0.005 > P > 0.001$; Gp. 2 v 3, $t = 2.54$, d.f. = 38, $0.02 > P > 0.01$; method (b): Gp. 0 v 1, $t = 4.62$, d.f. = 14, $P < 0.001$; Gp. 1 v 2, $t = 4.26$, d.f. = 29, $P < 0.001$; Gp. 2 v 3, $t = 4.18$, d.f. = 32, $P < 0.001$). This corresponds, using the data from assay method (a) and from section I above, to a progressive approximate decrease in the ^{51}Cr red cell survival half-life from 28 days in Group 0, to 20 (Gp. 1), 16 (Gp. 2), and 13 days in Group 3.

(2) Haemosiderinuria as a Measure of Intravascular Haemolysis

A very close and significant agreement ($P < 0.0005$) was found between the presence of haemosiderinuria and an elevated LDH level, and between the absence of haemosiderinuria and a normal LDH level. The distribution of results for all 180 cases is shown in Table X.

Test		Serum Lactate Dehydrogenase Elevated	Normal
Haemosiderinuria	Present	111	4
	Absent	14	51

$X^2 = 106.53$, d.f. = 1, $P < 0.0005$

TABLE X ASSOCIATION BETWEEN HAEMOSIDERINURIA AND SERUM LACTATE DEHYDROGENASE

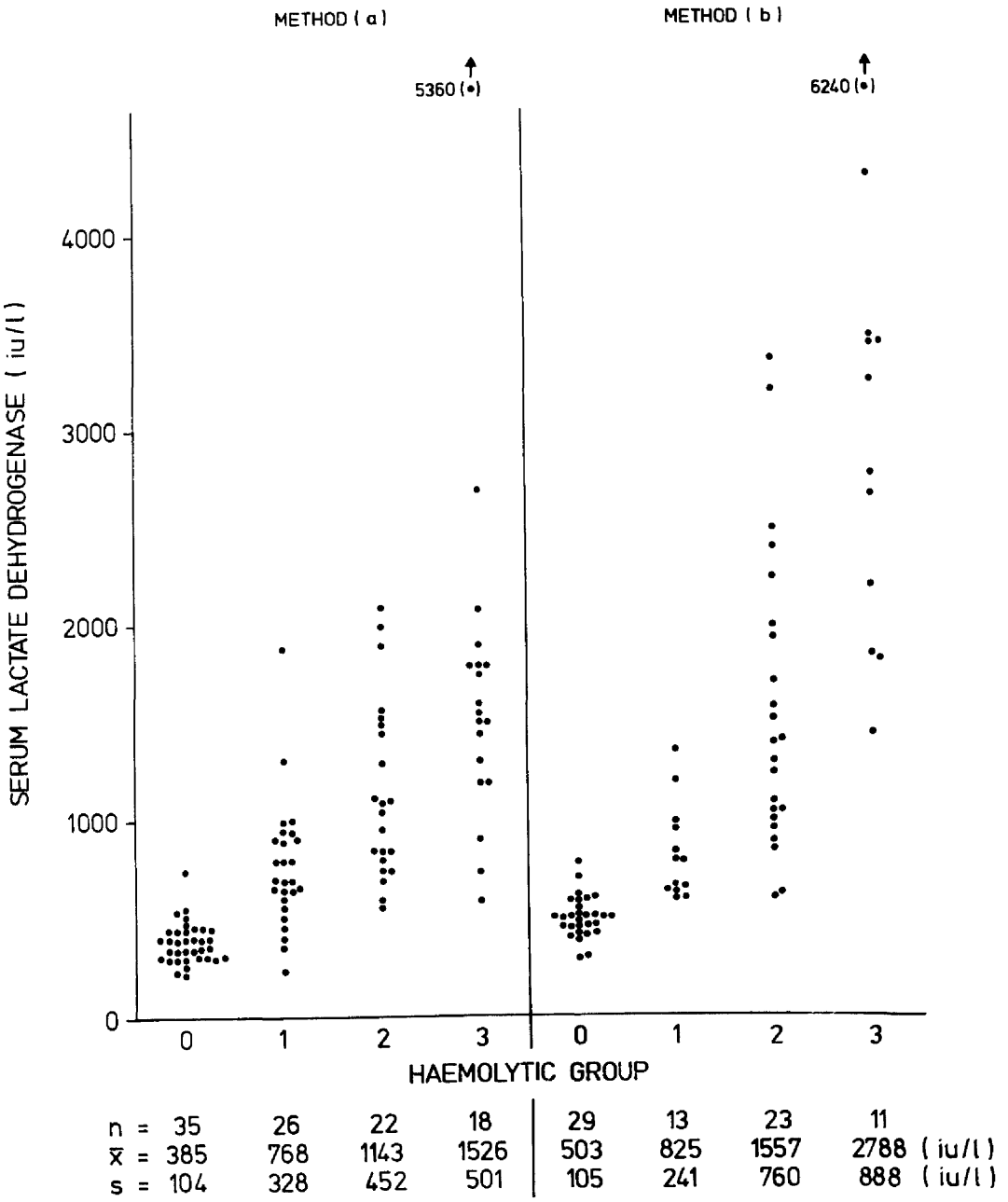


Fig. 28 **Relationship between Haemolytic Group and serum lactate dehydrogenase level.**

Results divided according to whether LDH assay method (a) (normal 116-467 iu/l) or method (b) (normal 240-525 iu/l) was used. The points in brackets are excluded in calculations of mean ($\bar{x}$) and standard deviation (s) values.

Four patients with haemosiderinuria had normal LDH levels. The amount of haemosiderin in each was very small (grade 1), and blood count and film examinations were normal. There were 14 instances in 12 patients of a negative haemosiderin test associated with high LDH levels. The increases in LDH were not marked and 10 of the 14 values fell within 100 iu/l of the upper limit of normal. Blood counts and filmswere also normal in all but one of these patients.

There is also a direct approximate correlation between the degree of haemosiderinuria and LDH levels. This is demonstrated in figures 29 (assay method (a)) and 30 (assay method (b)). LDH levels in patients without haemosiderin are included for comparison.

A highly significant association ($P < 0.0005$) was found when the degree of haemosiderinuria was compared with the extent of haemolysis as defined by Haemolytic Groups 1 to 3 (Table XI). No patient with slight haemosiderinuria (grades 1 & 2) had anaemia, whereas nearly half of the cases with marked haemosiderinuria (grades 4 & 5) were anaemic.

Haemolytic Group	Number of Cases in Haemosiderinuria Grades				
	1	2	3	4	5
1	15	5	9	6	4
2	7	6	10	10	12
3	-	-	4	6	21

$X^2 = 37.74$, d.f. = 8, $P < 0.0005$

TABLE XI ASSOCIATION BETWEEN DEGREE OF HAEMOSIDERINURIA AND HAEMOLYTIC GROUP

(3) Red Cell Fragmentation as a Measure of Intravascular Haemolysis

All patients showing red cell fragmentation and distortion ($\pm$ anaemia) had elevated LDH levels, and the transition from haemosiderinuria only to the additional finding of fragmentation was associated with an approximate fall in ^{51}Cr red cell survival half-life of from 20 to 16 days (v.s.).

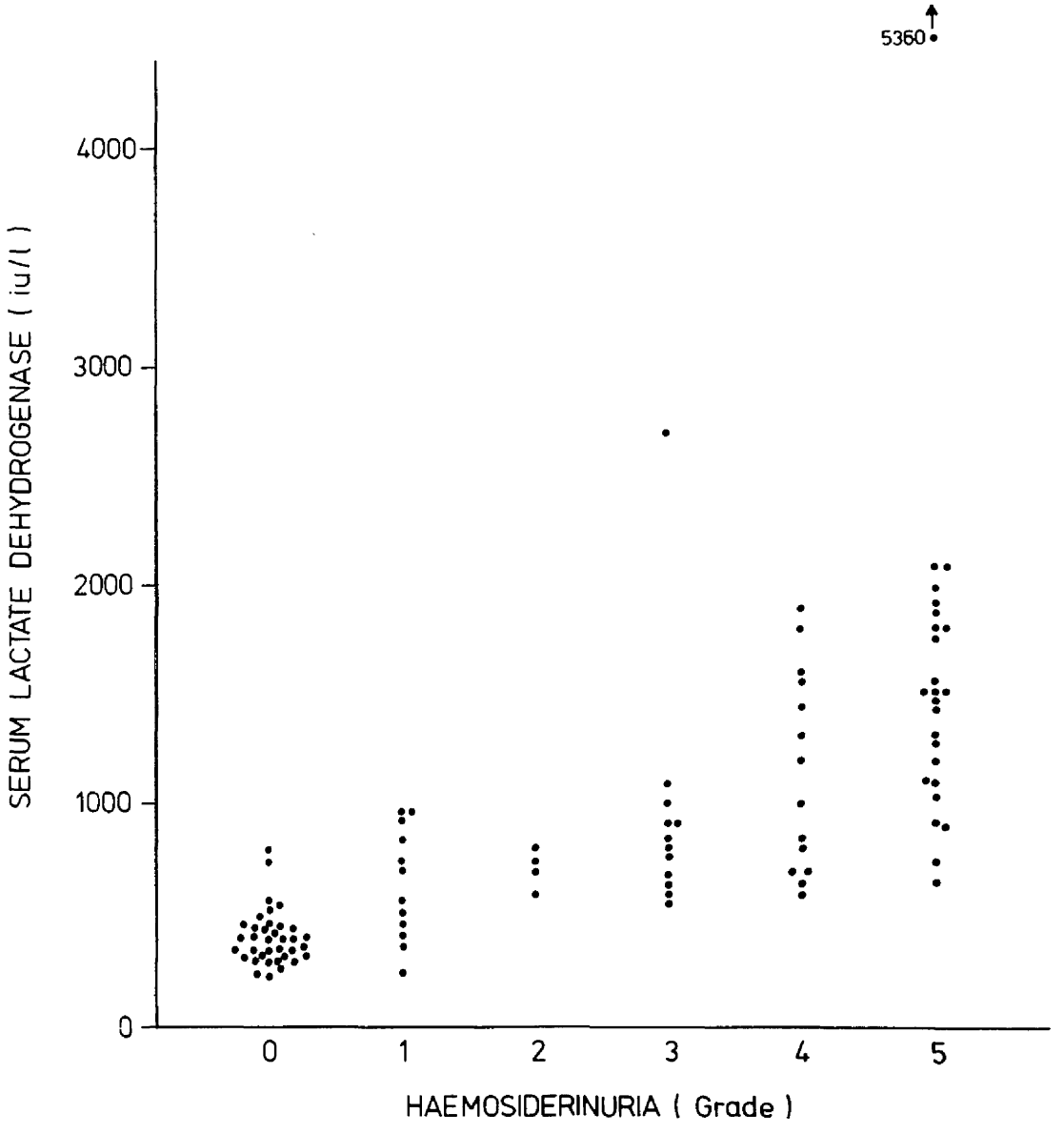


Fig. 29 Relationship between degree of haemosiderinuria and serum lactate dehydrogenase level (assay method (a)).

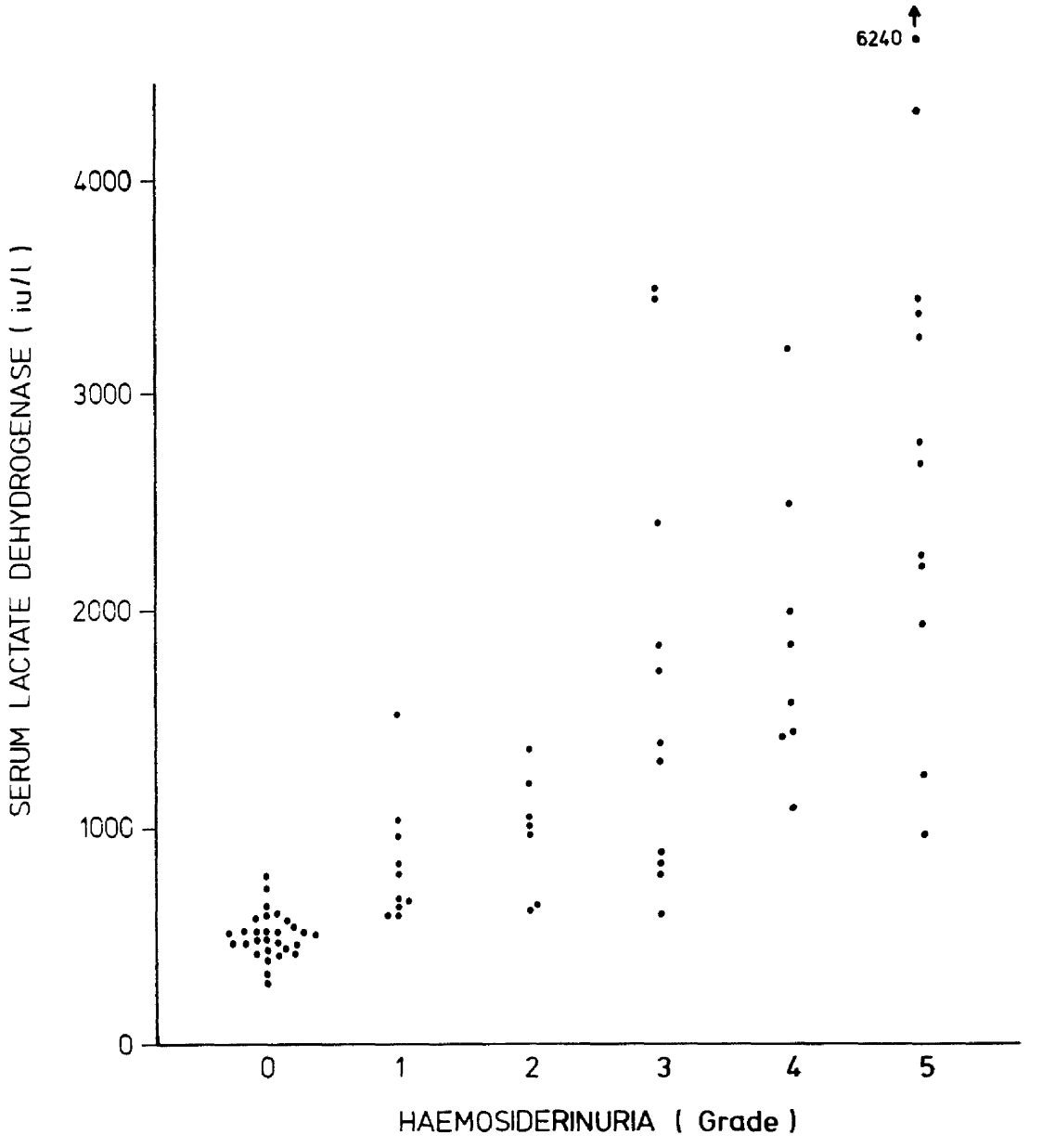


Fig. 30 Relationship between degree of haemosiderinuria and serum lactate dehydrogenase level (assay method (b)).

The quantitative relationship between the degree of red cell fragmentation and distortion and LDH levels is shown in figures 31 (assay method (a)) and 32 (assay method (b)). LDH levels in patients with haemosiderinuria but no fragmentation are included for comparison. A direct approximate correlation is present for the data with assay method (b) although is not evident for the results with assay method (a). A significant correlation was, however, found when counts of the numbers of damaged cells were compared with the LDH levels (v.i.).

There was a highly significant association ($0.005 > P > 0.001$) between the degree of red cell fragmentation and distortion and the presence or absence of haemolytic anaemia (Table XII). Only a minority of cases with minimal fragmentation had anaemia whereas most of those with marked fragmentation were anaemic.

Haemolytic Anaemia	Number of Cases in Fragmentation Grades		
	1	2	3
Absent[a]	23	15	7
Present[b]	6	11	14

$\chi^2 = 10.69$, d.f. = 2, $0.005 > P > 0.001$

a-corresponds to Haemolytic Group 2
b-corresponds to Haemolytic Group 3

TABLE XII ASSOCIATION BETWEEN DEGREE OF RED CELL FRAGMENTATION AND DISTORTION AND HAEMOLYTIC ANAEMIA

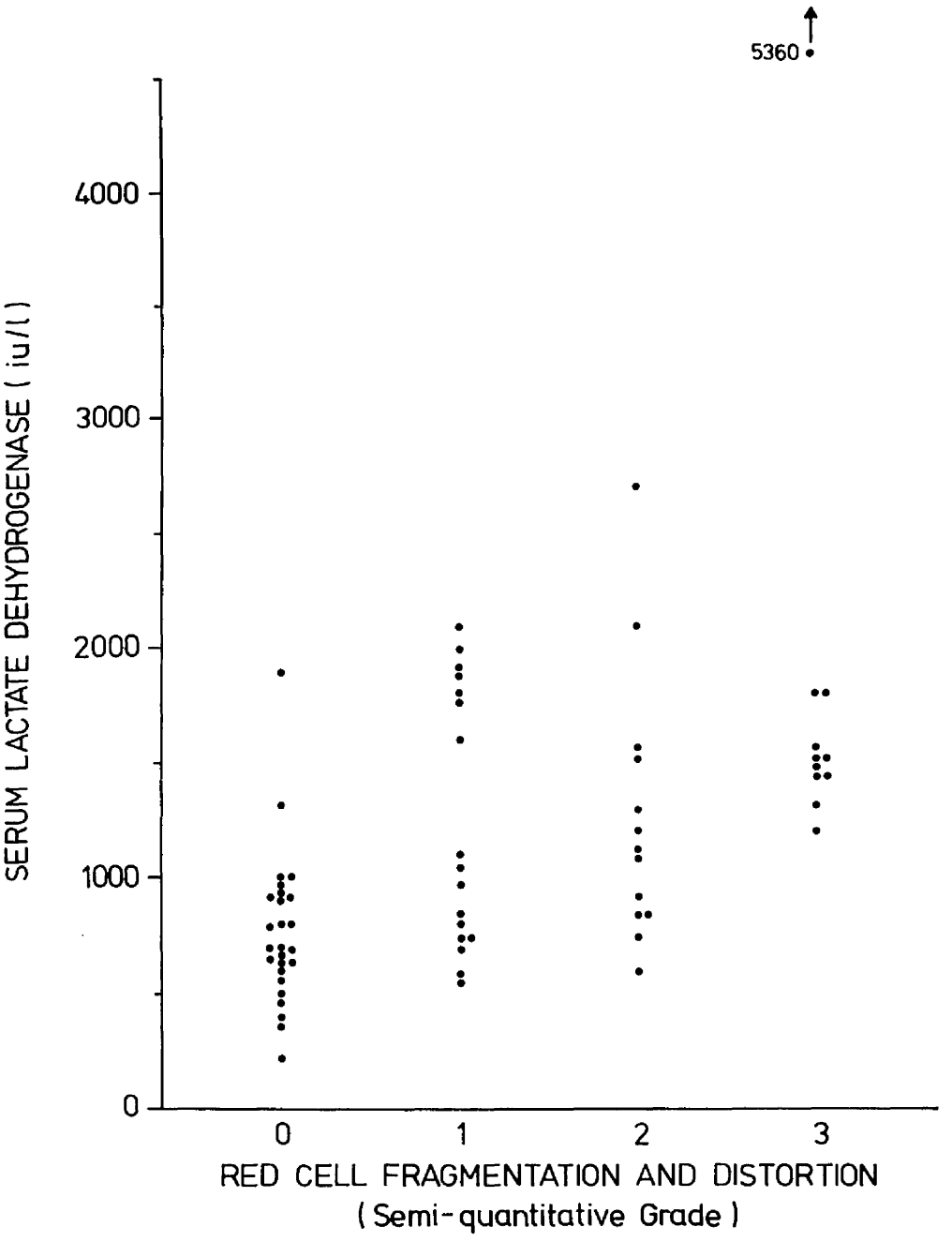


Fig. 31 Relationship between degree of red cell fragmentation and serum lactate dehydrogenase level in patients with haemolysis (assay method (a)).

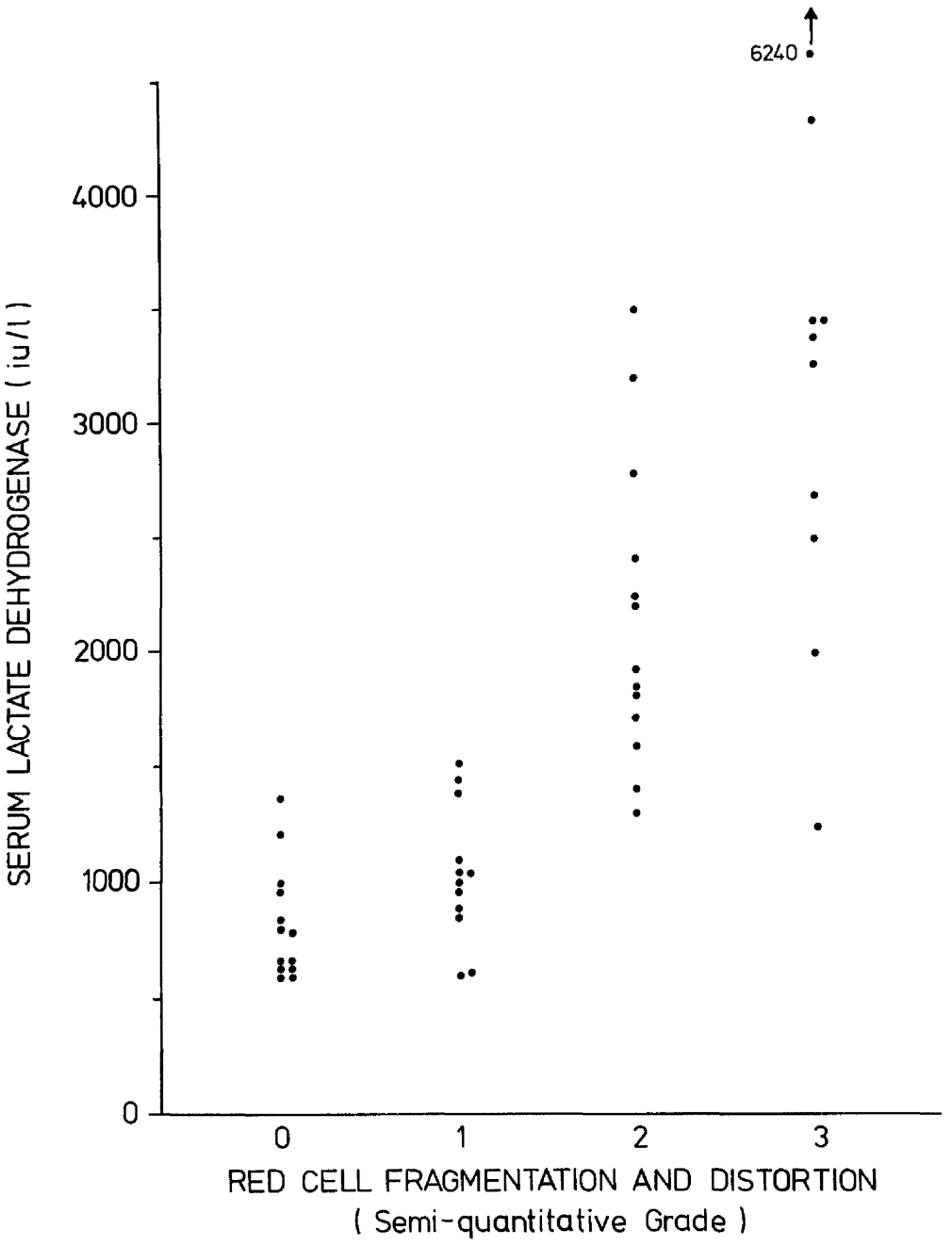


Fig. 32 Relationship between degree of red cell fragmentation and serum lactate dehydrogenase level in patients with haemolysis (assay method (b)).

III THE VALUE OF THE RETICULOCYTE COUNT, SERUM BILIRUBIN AND URINARY UROBILINOGEN ESTIMATIONS IN THE DIAGNOSIS OF INTRAVASCULAR HAEMOLYSIS

The value of the reticulocyte count, serum total bilirubin and urinary urobilinogen estimations in the diagnosis of intravascular haemolysis was examined among the 180 cases detailed in section II above, by comparing results with the degree of haemolysis as defined by Haemolytic Groups 0 to 3.

The percentage reticulocyte count plotted according to the degree of haemolysis is shown in figure 33. Despite appreciable overlap in values there is a significant association between a reticulocytosis and the presence of haemolysis when comparison is made between those with slight haemolysis (Group 1) and those considered to have no haemolysis (Group 0) ($X^2 = 8.19$, d.f. = 1, $0.005 > P > 0.001$). In most cases with more marked haemolysis (Groups 2 & 3) the reticulocyte count was elevated. The mean reticulocyte count also shows a progressive and significant increase from Groups 0 to 3 (Gp. 0 v 1, $t = 3.00$, d.f. = 101, $0.005 > P > 0.001$; Gp. 1 v 2, $t = 3.94$, d.f. = 65, $P < 0.001$; Gp. 2 v 3, $t = 3.65$, d.f. = 73, $P < 0.001$). However, altogether, 34 out of 115 cases with haemolysis had normal reticulocyte counts, an overall false negative rate of 30%, and 49% of those with slight haemolysis had a normal count. Reticulocyte counts expressed as "< 1.0%" have been regarded as 1.0% in these calculations.

Compared to reticulocyte counts, elevated bilirubin levels (Fig. 34) were significantly more frequent in comparison to cases in Group 0 only in those with more marked haemolysis (Gp. 0 v 2, $X^2 = 4.62$, d.f. = 1, $0.05 > P > 0.025$), but even in them and in cases with haemolytic anaemia (Group 3) over 60% had normal bilirubin levels, the overall false negative rate being 71%. There was also no significant difference in mean bilirubin levels between adjacent Groups.

The results of urinary urobilinogen estimations related to Haemolytic Group are shown in Table XIII. An approximate direct

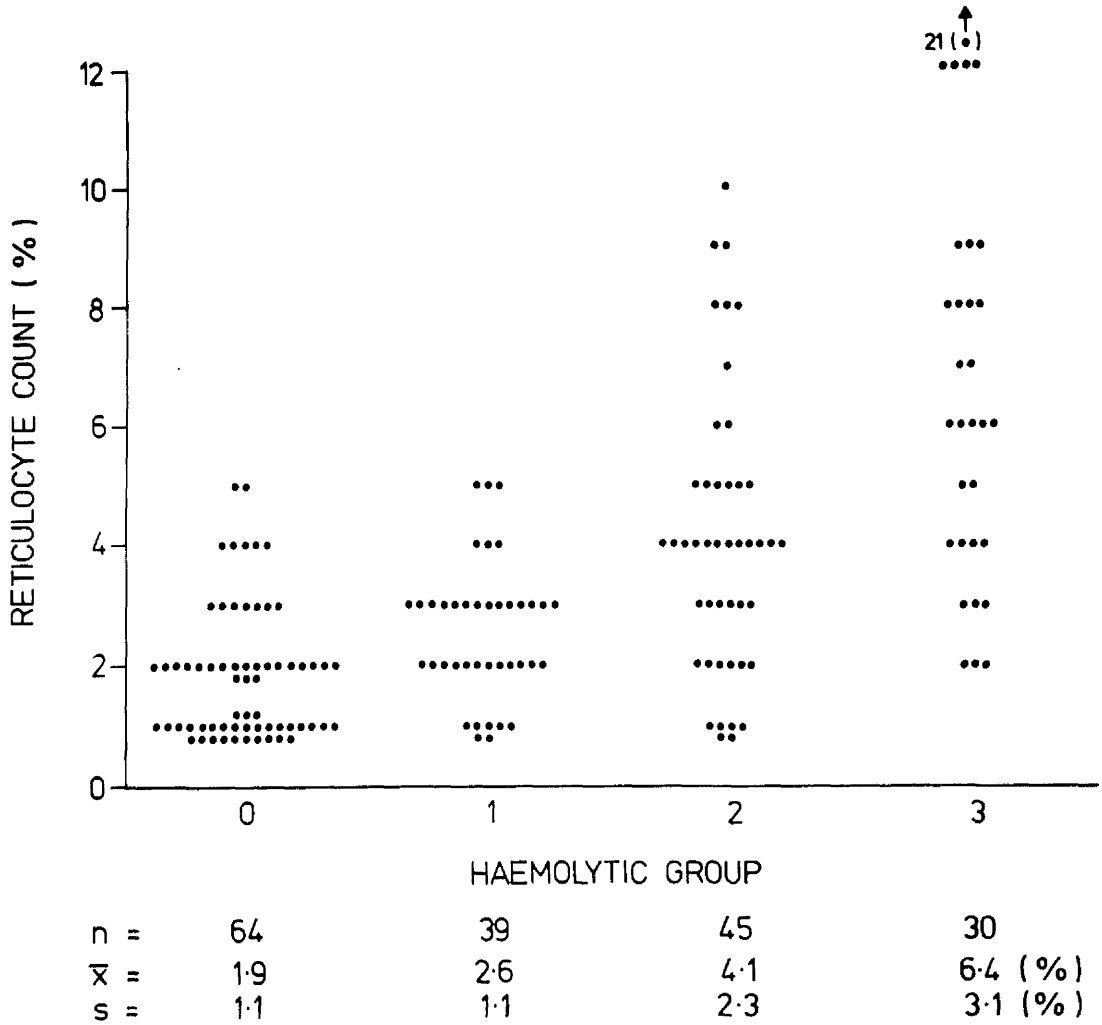


Fig. 33 Relationship between reticulocyte count and Haemolytic Group.

The point in brackets is excluded in the calculation of mean ($\bar{x}$) and standard deviation (s) values. (Normal reticulocyte count 0.2 – 2.0%).

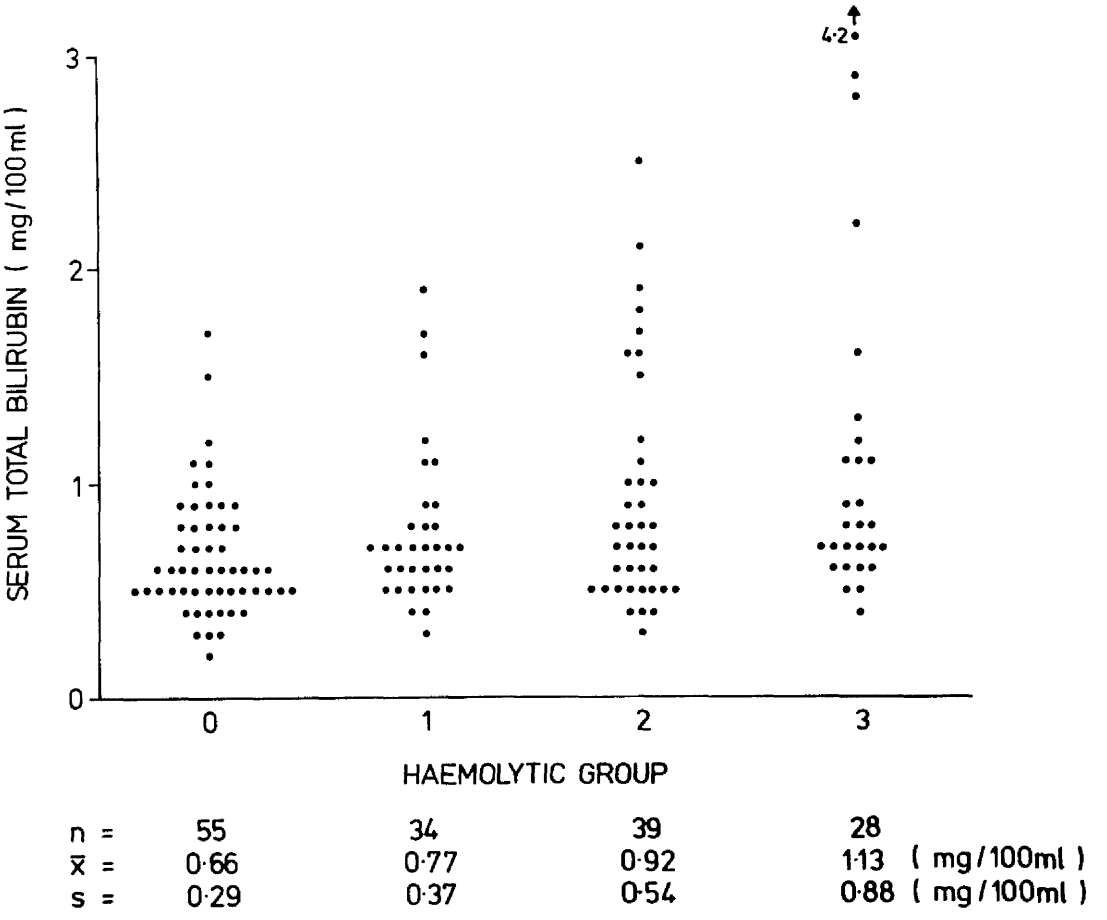


Fig. 34 Relationship between serum total bilirubin level and Haemolytic Group.

Mean ($\bar{x}$) and standard deviation (s) values given. (Normal bilirubin 0.2 - 0.9 mg/100 ml).

association between the degree of urobilinogenuria and Haemolytic Group is evident. When the distributions in Groups 2 and 3 are amalgamated to raise the theoretical frequencies so that a X^2 approximation is valid,[191,192] the association is found to be statistically significant ($0.05 > P > 0.025$). However, the maximum degree of urobilinogenuria detected even in cases with pronounced haemolysis corresponds to 1.0 Ehrlich unit/100 ml, which is only a marginally abnormal result, and the overall false negative rate was 78%. All urines examined for urobilinogen were free of bilirubin.

Urinary Urobilinogen (Ehrlich units per 100 ml - by "Urobilistix")	Number of cases in Haemolytic Groups			
	0	1	2	3
0.1	10	7	9	-
0.1 - 1.0	5	7	7	5
1.0	-	1	6	3

$X^2 = 10.09$, d.f. = 4, $0.05 > P > 0.025$ (see text)

TABLE XIII ASSOCIATION BETWEEN DEGREE OF UROBILINOGENURIA AND HAEMOLYTIC GROUP

IV INTRAVASCULAR HAEMOLYSIS AND SERUM ASPARTATE AMINOTRANSFERASE LEVELS

Serum aspartate aminotransferase (AsT) assays were also carried out among the 180 cases detailed in section II, and the results related to Haemolytic Group and divided depending on whether assay method (a) or (b) had been used (p. 76) are shown in figure 35.

The AsT levels were within the normal range in Group 0 and Group 1 cases, but were elevated in 21% of all Group 2 cases, and in 63% of Group 3 (haemolytic anaemia). These cases represent 16 individual patients who developed high AsT levels out of a total of 106 tested. There is also a progressive and at most steps statistically significant increase in mean AsT level from Groups 0 to 3 (method (a): Group 0 v 1, $t = 2.20$, d.f. = 42, $0.05 > P > 0.025$; Gp. 1 v 2, N.S.; Gp. 2 v 3, $t = 2.63$, d.f. = 28, $0.02 > P > 0.01$; method (b): Gp. 0 v 1, N.S.; Gp. 1 v 2, $t = 3.47$, d.f. = 31, $0.005 > P > 0.001$; Gp. 2 v 3, $t = 6.09$, d.f. = 32, $P < 0.001$). Alanine aminotransferase assays performed concurrently were normal in all patients.

Further observations on AsT level are described in Section V below.

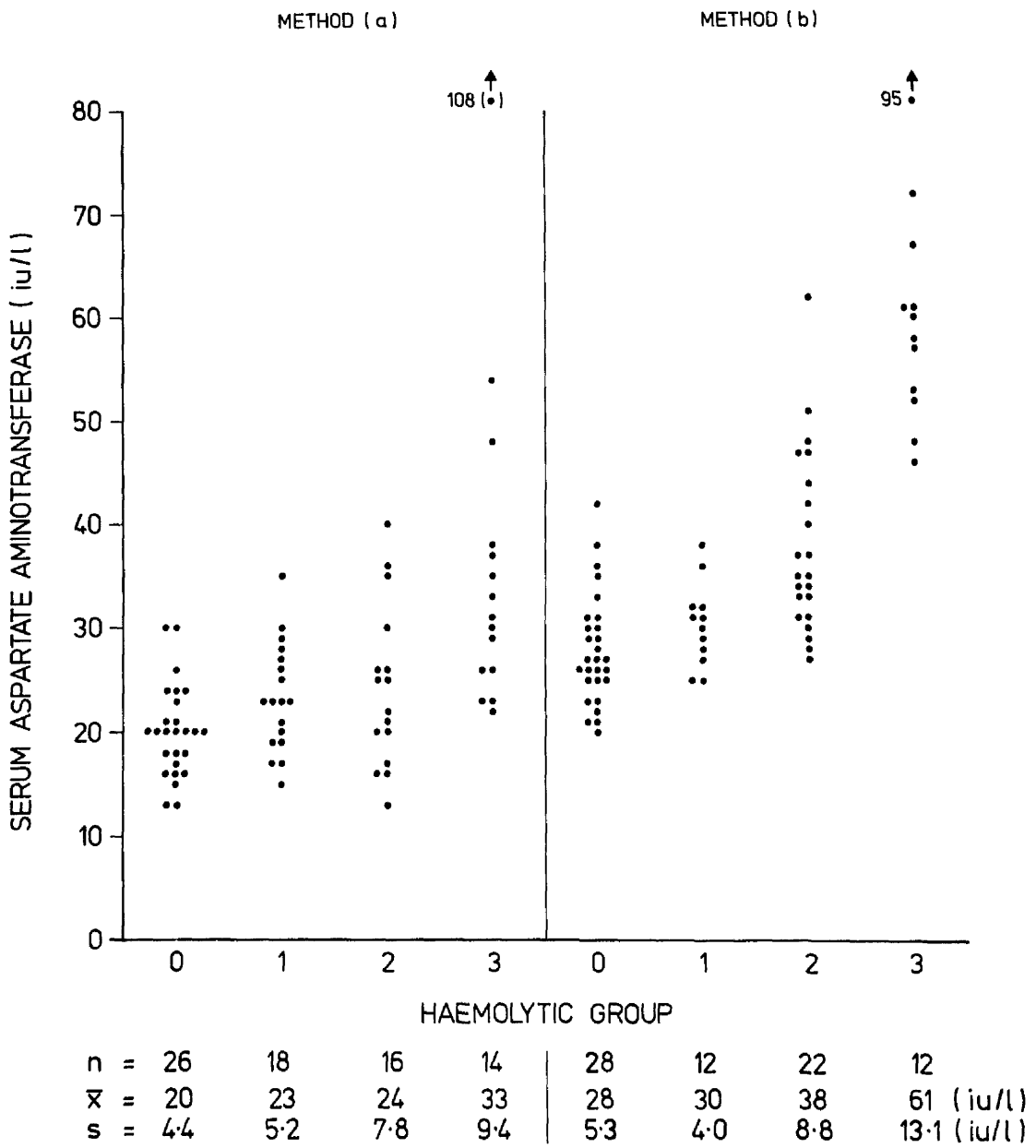


Fig. 35 Relationship between serum aspartate aminotransferase level and Haemolytic Group.

Results divided according to whether AsT assay method (a) (normal 9 – 35 iu/l) or method (b) (normal 13 – 42 iu/l) was used. The point in brackets is excluded in the calculation of mean ($\bar{x}$) and standard deviation (s) values.

V THE ABNORMAL RED CELL COUNT

Abnormal red cell counts (p . 69) were carried out in 67 blood films made at different times from 47 patients with prosthetic valves (17 men and 30 women; mean age 43 years, range 23 - 57 years). Twenty healthy hospital employees served as control subjects (15 men and 5 women; mean age 33 years, range 22 - 63 years). Standard blood and reticulocyte counts, urinary haemosiderin tests, and estimations of serum lactate dehydrogenase (LDH) and aspartate and alanine aminotransferase (AsT and AlT respectively) were obtained in parallel.

The principal aim of the study was to test the reliability of the routine inspection of blood films for the presence and degree of red cell fragmentation and distortion. In addition, from the differential abnormal count an analysis was made of the frequency distribution of the various types of deformed cells encountered. The quantitative relationship between the abnormal red cell count and the levels of LDH and AsT was also examined.

The presence and degree of red cell fragmentation and distortion was first assessed semi-quantitatively (p. 69) and the patients thereby divided into four groups: fragmentation and distortion absent (grade 0), minimal (grade 1), moderate (grade 2), and marked (grade 3). Total and differential abnormal red cell counts were then carried out in patients and controls in random order and without knowledge of the initial assessment or of the identity of the slide. When the entire series of duplicate film examinations had been completed the identity code was broken, and the results of the abnormal red cell counts were compared with the semi-quantitative assessments of the films and the other analyses carried out.

(1) Abnormal Red Cell Count in Normal Subjects

The 20 healthy controls had normal standard blood and reticulocyte counts and normal blood films on initial inspection. There was no haemosiderinuria and serum lactate dehydrogenase levels were normal.

The total abnormal red cell count obtained "blind" varied from 0 to 0.10% of the red cells with a mean ($\pm$ s.d.) of 0.05 $\pm$ 0.03%, and 0.10% was taken as the upper limit of normal. The absolute number of distorted and fragmented red cells varied from 0 to 4720/mm^3 of blood with a mean ($\pm$ s.d.) of 2553 $\pm$ 1515/mm^3.

(2) Abnormal Red Cell Count Versus Semi-quantitative Assessment of Fragmentation

(i) Quantitative Correlation

The results of the abnormal red cell counts related to the initial semi-quantitatively determined grade of red cell fragmentation and distortion in the 67 cases are shown in figure 36 along with the values obtained in the control group. Significant differences in the mean count level between consecutive grades are present (grade 0 v 1, $t = 3.94$, d.f. = 12, $0.005 > P > 0.001$; gd. 1 v 2, $t = 3.45$, d.f. = 10, $0.01 > P > 0.005$; gd. 2 v 3, $t = 3.27$, d.f. = 20, $0.005 > P > 0.001$). A highly significant difference in counts was also found, however, between cases initially judged to have no abnormalities (grade 0) and the normal controls ($t = 6.76$, d.f. = 38, $P < 0.001$), twenty-five out of 32 of these cases having an elevated count.

The semi-quantitative method of assessing the degree of red cell fragmentation and distortion has, therefore, correlated well with the abnormal red cell count. Furthermore, all but one of the 35 cases initially considered to show these abnormalities proved to have elevated abnormal cell counts. It would appear, however, that very minor yet abnormal degrees of fragmentation and distortion may frequently go unrecognized on routine blood film inspection.

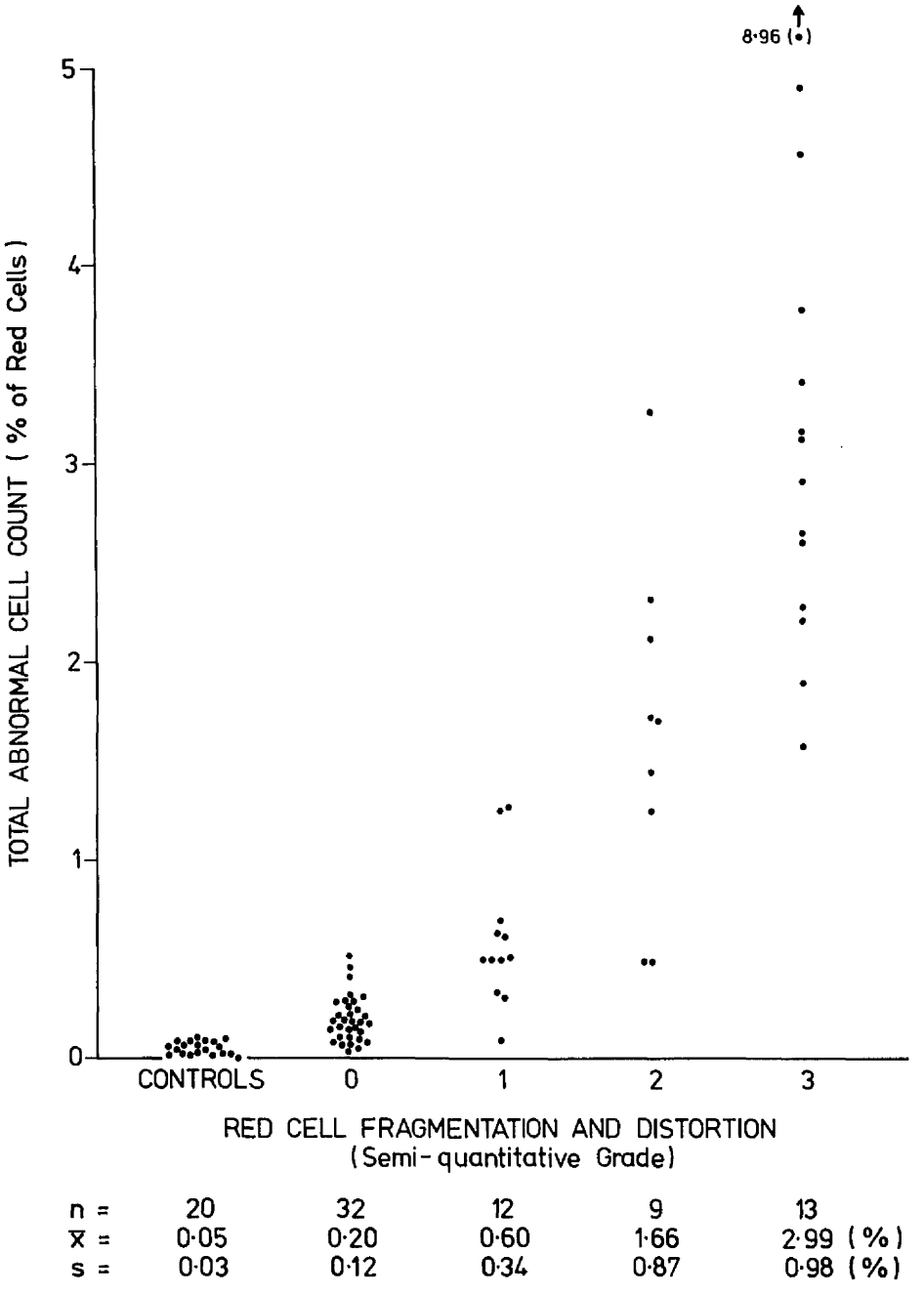


Fig. 36 Red cell fragmentation and distortion: comparison of the initial semi-quantitative grading with the subsequent abnormal red cell count in patients and controls.

The point in brackets is excluded in the calculation of mean ($\bar{x}$) and standard deviation (s) values.

(ii) Diagnostic Levels

While an abnormal cell count of 0.10% has been taken as the upper limit of normal, there were six cases with higher counts (0.11, 0.18, 0.19, 0.19, 0.28 and 0.32%) who had no haemosiderinuria or elevation of LDH. They also had a normal haemoglobin and reticulocyte count. Conversely, all other 38 cases with counts in excess of 0.30% had both haemosiderinuria and, where measured (34 cases), a high LDH level, except for one with a count of 0.42% whose only other possible evidence of haemolysis was a slightly elevated LDH value. Counts above 0.30% appear, therefore, to be virtually diagnostic of intravascular haemolysis in patients with prosthetic valves.

All 35 cases in which significant red cell abnormalities were considered to be present on the initial semi-quantitative inspection had haemosiderinuria and, where measured (31 cases), an elevated LDH level. Except for one with a count of 0.10% these were all patients whose abnormal cell counts ranged from 0.32% upwards. The level, therefore, at which the presence of red cell fragmentation and distortion has been recognized on routine examination corresponds to the level found to have positive diagnostic value when the abnormal forms are counted.

However, whether an abnormal red cell count of 0.10% or 0.30% were regarded as the upper limit of normal, half of the cases with normal counts (3/8 and 14/28 respectively) were haemolysing as evidenced by both haemosiderinuria and high LDH levels. The absence of red cell changes even on counting clearly does not exclude the presence of haemolysis. The degree of haemolysis under these circumstances was, however, slight with no case of anaemia.

(3) Distribution of Abnormal Red Cell Types

The great majority of the different abnormal red cell types fell into

one or other of the six individual categories recorded in the total abnormal cell count ($p_{.72}$). As has been noted ($p_{.68}$), intermediate forms were occasionally observed. The proportionate representation of each of these abnormal types related to the degree of abnormality as defined by the total count is given in Table XIV. Only patients in whom the total number of abnormal red cells recorded was 25 or more are included in the Table, as the allocation of each of the individual types on a percentage basis would be too inaccurate with smaller numbers. Of 35 cases finally analysed, 100 abnormal cells were surveyed in eighteen, 50 - 99 in eight, and 25 - 49 in nine. This represents all patients with counts in excess of 0.40%.

A fairly uniform pattern of abnormalities was found, and suggestive evidence of a consistent change with changing total abnormal red cell count was present only in regard to the triangular cell. Thus, an overall average of 57% comprised the various types of fragmented and torn cells, 17% being cell pieces, 5% triangular cells, 28% segmented cells and 7% indented cells. The remaining 43% of abnormalities was made up largely of irregularly contracted and distorted cells (37%) with a small proportion of pincered cells (6%). A significant increase in the overall proportion of fragmented and torn cells as well as specifically in triangular forms occurred between patients with counts of 0.40 - 1.49% and those with counts of 1.50 - 2.99% (fragmented and torn cells: $t = 2.24$, d.f. = 25, $0.05 > P > 0.025$; triangular cells: $t = 3.09$, d.f. = 25, $0.005 > P > 0.001$). There was a reciprocal significant fall in the combined proportion of irregularly contracted, distorted and pincered cells. These changes were not, however, progressive when patients with counts in excess of 3.00% were examined except in the case of the triangular cell, although the sequential change was not of statistical significance ($t = 1.25$, d.f. = 18, $0.3 > P > 0.2$).

Total Abnormal Red Cell Count (No. of cases)	Proportion of Abnormal Cell Types[a]						
	Cell Pieces	Triangular Cells	Cell Segments	Indented Cells	Total Fragmented & Torn Cells	Distorted & Irregularly Contracted Cells	Pincered Cells
0.40 - 1.49% (15)	$\bar{x}$ = 15.9	3.5	25.5	7.5	52.4	40.5	7.1
	s = 7.7	2.3	14.9	5.4	17.2	17.5	4.5
1.50 - 2.99% (12)	$\bar{x}$ = 18.2	6.3	34.5	6.0	64.9	31.0	4.1
	s = 6.6	2.4	9.3	4.4	9.8	9.5	2.5
3.00 +% (8)	$\bar{x}$ = 17.3	7.6	24.8	6.3	55.9	39.4	4.8
	s = 5.7	2.1	13.1	3.4	10.2	7.8	3.3
0.40 - 3.00+% (35)	$\bar{x}$ = 17.0	5.4	28.4	6.7	57.5	37.0	5.5
	s = 6.8	2.8	13.2	4.6	14.4	13.7	3.8

Notes: a as per cent of Total Abnormal Red Cell count; mean ($\bar{x}$) and standard deviation (s) values given.

TABLE XIV THE PROPORTIONS OF EACH OF THE DIFFERENT ABNORMAL CELL TYPES IN THE TOTAL ABNORMAL RED CELL COUNTS OF FRAGMENTED AND DISTORTED ERYTHROCYTES.

In patients with abnormal red cell counts of less than 0.40% and in the normal controls the numbers of abnormal cells recorded in each film were considered too small to attempt a similar analysis of differential morphology. However, fragmented and torn cells altogether constituted on average 67% of the abnormalities. These were usually of segmented type and the triangular cell was particularly infrequently observed, one or two examples being found in each of only eight of the 32 cases, and one example in each of two of the 20 normal controls. Seven of the eight cases with triangular cells were haemolysing compared to 10 of the 24 without. The remaining abnormalities were made up of irregularly contracted and distorted cells and very occasional pincered cells.

(4) Abnormal Red Cell Count and Serum Lactate Dehydrogenase and Aspartate Aminotransferase Levels

In 62 cases parallel results for LDH levels were obtained and in 59 for AsT levels. In view of the changes in LDH and AsT assay procedures instituted at the time of this study, correlations were sought between the abnormal red cell count and LDH level in 42 of the cases, and between the abnormal cell count and AsT level in 39, the enzyme estimations having been performed by methods (b).

Highly significant correlations were observed between the numbers of abnormal cells and the levels of both LDH and AsT ($P<0.001$). This was true whether counts were expressed as percentages of the total red cell count (Figs. 37 & 38) or in absolute values per mm^3 of blood, and whether total abnormal cell counts, fragmented and torn forms, or irregularly contracted, distorted and pincered cells were compared.

In the total group of 62 cases in which LDH estimations were performed there were a few (7/54) with high percentage total abnormal red cell counts (of up to 9.32%) and normal LDH levels, and some (4/8) with normal counts who had high LDH levels. By comparison the considerably lesser sensitivity of AsT values as a possible means of diagnosis of intravascular haemolysis was demonstrated by the fact

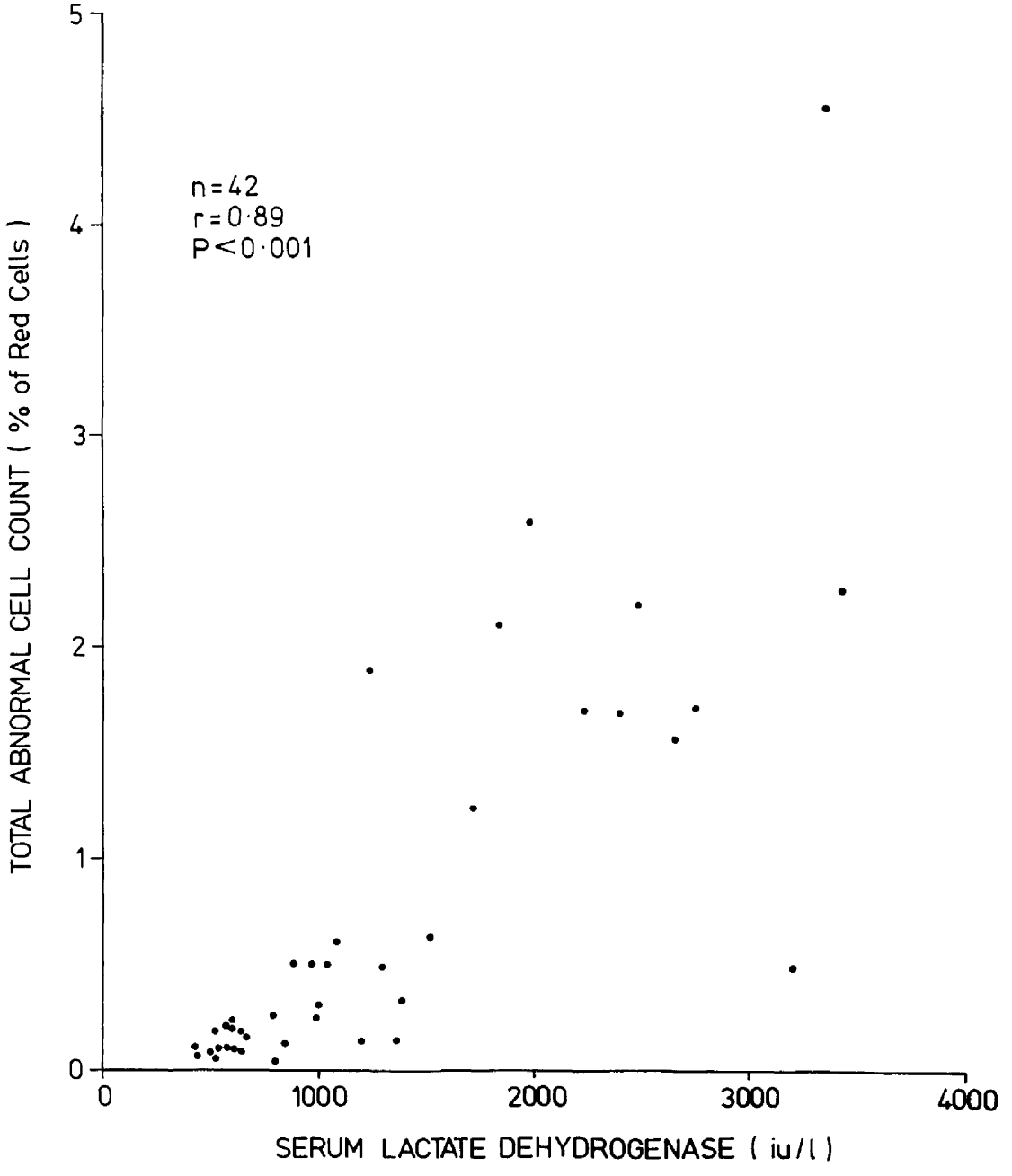


Fig. 37 Relationship between total abnormal red cell count and serum lactate dehydrogenase level.

The two points not shown in the figure but included in the correlation calculation are 3.15% - 4320 iu/l and 8.96% - 6240 iu/l. (Normal LDH 240 - 525 iu/l).

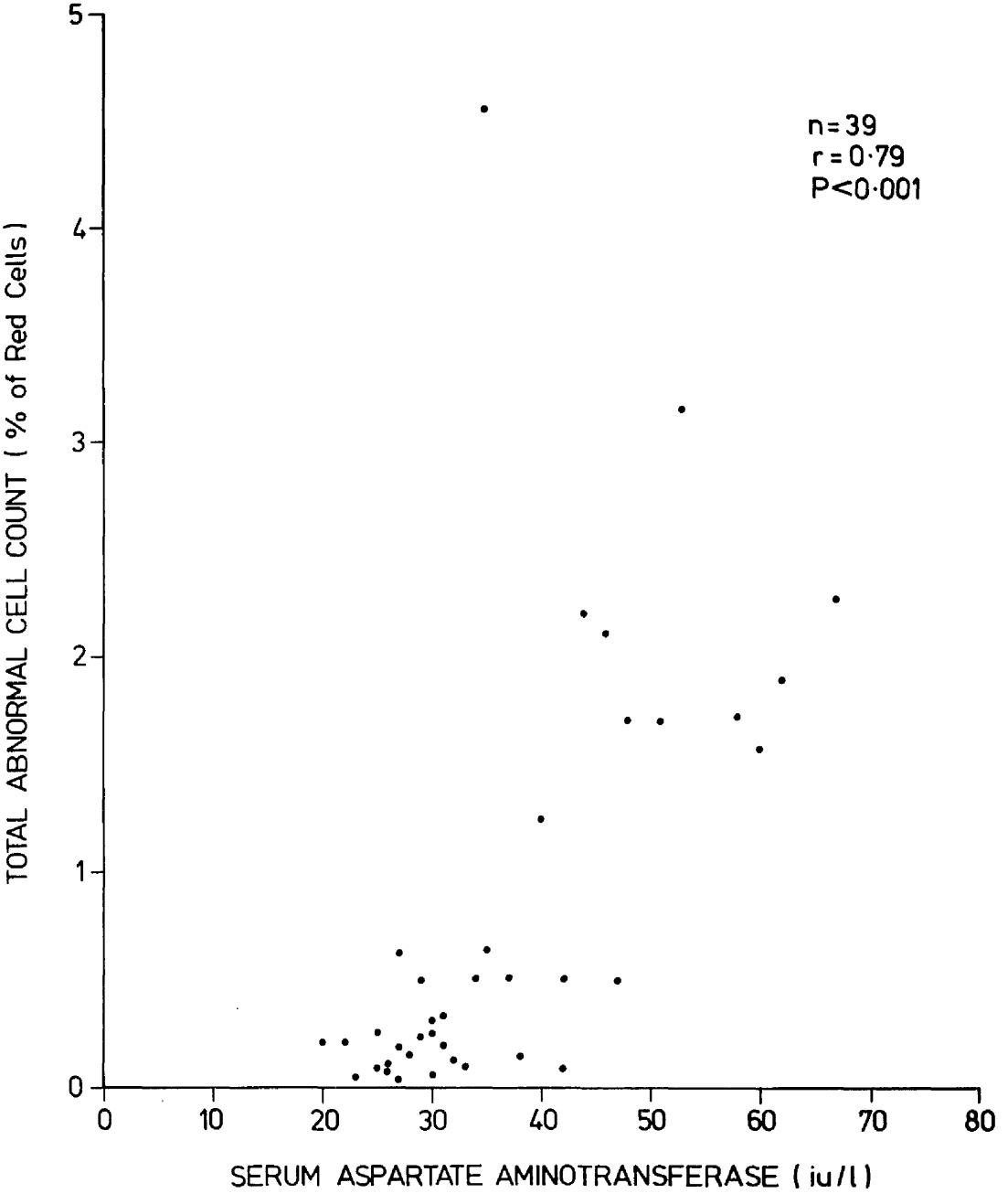


Fig. 38 Relationship between total abnormal red cell count and serum aspartate aminotransferase level.

The one point not shown in the figure but included in the correlation calculation is 8.96% - 95 iu/l. (Normal AsT 13 - 42 iu/l).

that as many as 36 of 51 cases with high counts (of up to 4.55% though usually < 1.25%) had normal AsT levels, and none of the eight cases with normal counts had an elevated level. Alanine aminotransferase assays concurrently performed were normal in all cases.

With regard to the results described in this Chapter involving analysis of serum lactate dehydrogenase or aspartate aminotransferase levels, it should be noted that any case in whom a cause other than intravascular haemolysis was likely to be responsible for, or contributing to, raised LDH and/or AsT values was excluded. Six patients fell into this category. One with a megaloblastic anaemia due to folate deficiency which was not with certainty caused by chronic haemolysis; two with cardiac failure and hepatic congestion; two with a history of excess alcohol consumption and possible cirrhosis; and one with a sub-clinical hepatitis of uncertain origin.

The other point in respect of these enzyme estimations is that the results in these studies indicate a likely difference in sensitivity between the earlier and later assay methods for both LDH and AsT. Although the patients under study are not the same, reference to figures 28-32 (LDH) and 35 (AsT) shows generally higher enzyme levels with assay methods (b) for similar degrees of haemolysis, suggesting an increase in sensitivity with the later optimized methods.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

Red cells contain a high concentration of lactate dehydrogenase (LDH), principally fractions 1 & 2, the alpha, fastest moving, anodic isoenzymes on electrophoresis[174,197,198]. Elevated serum levels have been demonstrated in patients with haemolysis, especially of intravascular nature[29,199,200]. They appear to be a sensitive although not specific parameter of haemolysis in patients with prosthetic valves[36,65] and an inverse correlation between the level of serum LDH and the half-life of ^{51}Cr-labelled red cells has been reported[50,125]. The results in this study confirm a significant relationship between serum LDH levels and red cell survival. They have, therefore, been used as a measure of the degree of haemolysis, although should not be regarded as a precise estimate of the severity of red cell destruction since, in agreement with previous experience,[125] the range of predicted ^{51}Cr red cell survival for any given LDH value was rather wide.

Persistent haemosiderinuria is a reliable sign of significant chronic intravascular haemolysis[126]. The results in this investigation suggest that the urinary haemosiderin test may be as sensitive as serum LDH assay in the detection of haemolysis, since there was a very close agreement between the presence of haemosiderinuria and an elevated LDH level, and between the absence of haemosiderinuria and a normal LDH level. It has the advantage over the estimation of LDH levels which are commonly increased in a variety of disorders,[201] in that it is highly specific for intravascular haemolysis. Indeed, haemosiderinuria appears to be almost diagnostic of this condition, which of course has a number of causes, as it has been consistently reported otherwise only in occasional cases of haemochromatosis[134,202]. It should be noted that it may take several days for haemosiderinuria to become evident after the first onset of haemolysis,[126] and conversely haemosiderinuria may be expected to persist

for some time after haemolysis has ceased[6,46].

Both the urinary haemosiderin test and LDH assay may be somewhat less sensitive investigations for the presence of very slight degrees of intravascular haemolysis than the estimation of serum haptoglobin levels[52,56] which were not routinely measured in this study. Low or absent serum haptoglobins while most commonly due to haemolysis, whether predominantly intravascular or extravascular, are not, however, diagnostic, and normal or even increased levels may occasionally be present despite haemolysis[167,203,204].

An approximate direct correlation between the amount of haemosiderin excreted in the urine and the degree of elevation of plasma haemoglobin levels was found by Crosby and Damashek[202]. Otherwise, no evaluation of the extent of haemosiderinuria as a measure of the severity of haemolysis appears to have been reported. Nor has any detailed study of haemosiderinuria in patients with prosthetic heart valves previously been carried out. In this investigation the degree of haemosiderinuria was found to bear an approximate direct relationship to increases in serum LDH level. It was also found that no patient with slight haemosiderinuria had anaemia, whereas nearly half of those with marked haemosiderinuria were anaemic.

The urinary haemosiderin test appears, therefore, to be a sensitive as well as a highly specific parameter of the presence of intravascular haemolysis, and to be of value in assessing the severity of haemolysis. It is a simple test to perform, although care is required in the detection of minor degrees of haemosiderinuria, and it has been found easy to apply on a repetitive basis to large numbers of patients.

Few studies have attempted to routinely count the numbers of red cell fragments and other distorted forms in patients with disorders characterized by red cell fragmentation, or to assess their numbers in healthy normal individuals. Rous and Robertson[156] in 1917 in their

investigations into the normal fate of erythrocytes detected about one to 20 "schizocytes" usually within a 5-minute search of thick films made from healthy rabbits' blood, and they noted that in the individual the number was remarkably constant from day to day. Schwartz and Motto[143] in their original study of "burr cells" recorded the numbers present in counts of 1000 red cells in patients with uraemia, carcinoma of the stomach, and bleeding peptic ulcer, and found them to range from 0.1 to 3.7%. None were detected in 100 control patients without obvious haematological abnormalities. Allison[149] described children with haemolytic anaemia of congenital origin, following vitamin K administration, and in association with thrombocytopenia and uraemia, in whom contracted, distorted and fragmented cells constituted up to 50% of the red cells. A Miller squared eyepiece was used and 20 fields containing 100 cells per field were examined as suggested for reticulocyte counts by Brecher and Schneiderman[205]. No control counts were made. Tuffy et al.[152] also found that up to as many as 50% of the red cells (average 16.4%) in counts of 1000 cells were distorted and contracted ("pyknocytes") in 11 infants with an unexplained haemolytic anaemia ("infantile pyknocytosis"). By contrast, in 102 full-term healthy infants pyknocytes equalled only 0.3 - 1.9% of the red cells, and in 60 premature but otherwise normal infants, 0.3 - 5.6%. Forty unselected healthy adults used as controls had no more than 0.3% abnormal cells though usually had none. Bell[160] stated that "rare burr cells and rare erythrocytes with vacuoles can be found after a meticulous examination of some normal smears".

In a few patients with haemolysis secondary to intracardiac prosthetic devices including total prosthetic valve replacements, the numbers of deformed and fragmented cells have been counted and found to represent up to 27% of the red cells, although have usually amounted to less than 10%[11,16,22,33,37,39-41,206]. In these reports the normal fragment count has been quoted as rare[37], $< 0.8\%$[41], $< 1\%$,[206] and not

> 1.0%,[39] but without reference or any further information. In a study of haemolysis in unoperated valvular heart disease[35] a control population of 32 healthy normal subjects was examined. In them fragments constituted 0 - 0.6% (mean 0.28%) of the red cells in counts of 1000 red cells, no increase beyond these values being found in the patients. In the more recent study by Eyster, Rothchild and Mychajliw[207] who investigated 54 patients with different types of aortic ball-valve prostheses, "schistocytes" ("small irregularly shaped cells or cell pieces") numbered between 0 and almost 100 per 1000 red cells (0 - 10%), the counts in most instances having been done in duplicate and results averaged. A control population of 40 consecutive hospitalized patients with normal haemoglobins and red cell indices had schistocyte counts of 0 - 5/1000 red cells (0 - 0.5%).

The counting of red cell fragments and distorted forms is an undoubted laborious procedure with its own limitations as to accuracy. The purpose of this study of abnormal red cell counts was primarily to check on the sensitivity of the routine inspection of blood films for the presence of fragments, and upon the reliability of the semi-quantitative assessment of the degree of fragmentation and distortion. The initial concern was that these abnormal forms might be over-reported rather than the reverse, since the "occasional fragmented and distorted red cell approximating to one or two per 2-3 high-power-fields", the definition of grade 1 fragmentation (p. 69), was found occasionally either to be overlooked by other observors or regarded as of no or dubious significance. An attempt was made to increase the accuracy of the abnormal red cell count by surveying and counting larger numbers of red cells, by precise definition of the abnormalities under consideration, and by the methods designed to eliminate observor bias particularly the use of the "blind" technique. Furthermore, a healthy control population was studied.

In the normal population the total abnormal red cell count ranged from 0 to 0.10% of the red cells with a mean ($\pm$ s.d.) of 0.05 ($\pm$ 0.03)%, and the upper limit of normal was taken as 0.10%. In the patients the counts varied from 0.04 to 8.96%, 88% of values being above the normal range. There were a few patients with counts in excess of 0.10%, ranging up to approximately 0.30%, who showed no other evidence of haemolysis or other haematological abnormality, whereas counts greater than 0.30% were found to be virtually diagnostic of haemolysis as indicated by haemosiderinuria and high LDH levels. The red cell abnormalities were, moreover, not recognized on the initial blood film inspections until they corresponded to abnormal cell counts of over 0.30%. The figure of 0.30% in this study appears, therefore, not only to have clear positive diagnostic value in that counts above it were almost invariably associated with haemolysis, but also represented a degree of red cell damage capable of recognition on routine blood film inspection. Put another way, the presence of fragmented and distorted red cells however few in number should be regarded as likely evidence of haemolysis. Interestingly, 0.30% was the level quoted by Tuffy et al.[152] for the upper limit of normal of "pyknocytes".

The routine examination of blood films as described in this thesis has, therefore, proved to have a sensitivity of diagnostic value, and far from over-estimating the presence of fragments very minor yet abnormal degrees of fragmentation and distortion have gone unrecognized. The overall reliability of the semi-quantitative assessment of the extent of red cell fragmentation and distortion where judged to be present has also proved very satisfactory when measured against the results obtained by counting the abnormal cells. The absence of recognizable fragments on film examination or even the finding of a normal fragment count, however, by no means excludes

haemolysis, as about 50% of patients in these categories were found to be haemolysing, although the haemolysis was slight with no case of anaemia. The transition in patients with haemolysis from the absence to the presence of red cell fragmentation on routine inspection was calculated to correspond to an approximate fall in ^{51}Cr red cell survival half-life of from 20 to 16 days. The overall broad classification of patients according to the severity of haemolysis as defined by the presence or absence of haemosiderinuria, red cell fragmentation and distortion, and anaemia (Haemolytic Groups 0 - 3) was associated with significant changes in serum LDH level and in the calculated red cell survival, and has proved useful for comparative purposes.

The association between marked red cell fragmentation and distortion and the severer degrees of haemolysis is obvious, but there have been few systematic studies of the correlation between the extent of red cell damage as witnessed on the blood film and the degree of haemolysis as defined by other parameters. Schwartz and Motto[143] found no correlation between the number of "burr cells" and the level of anaemia in their patients with uraemia, carcinoma and bleeding peptic ulcer. Brain, Dacie and Hourihane[161] in their classic study, however, demonstrated a statistically significant inverse relationship between the numbers of contracted, distorted and fragmented red cells determined semi-quantitatively and the level of haemoglobin in patients with renal failure. Tuffy et al.[152] reported a "fairly good degree of correlation" between the intensity of the "pyknocytosis" and the severity of anaemia in their cases of "infantile pyknocytosis".

In this investigation a direct quantitative relationship was observed between the degree of fragmentation and distortion and the level of serum lactate dehydrogenase, and this correlation was particularly close when the comparison was made with counts of the

abnormal cells. As increased serum LDH levels in intravascular haemolysis originate from damaged red cells such a correlation is to be expected but had not hitherto been demonstrated, although has since been reported in the more recent paper by Eyster et al.[207]. A significant association was also found between the severity of fragmentation and the presence or absence of haemolytic anaemia. Only a minority of cases with minimal fragmentation had anaemia, whereas most of those with marked fragmentation were anaemic.

Analysis of the frequency distribution of the different forms of fragmented and distorted cells showed a fairly uniform pattern at all grades of severity. Fragmented and torn cells constituted 52 - 67% of the abnormalities. There was no consistent change in the proportion of any one type of cell for every increment in the total degree of fragmentation except in respect of the triangular cells. These were almost never observed in normal blood or in patients without evidence of haemolysis, but became significantly more numerous as haemolysis advanced, although with the most severe degrees of fragmentation and haemolysis the proportion appeared to plateau out. This approximate direct association of the number of triangular cells with the presence and degree of haemolysis suggests that they may have diagnostic value, in that if observed in a film otherwise considered of doubtful significance the possibility of intravascular red cell destruction might be more seriously considered. This observation is in agreement with Dacie's suggestion that the triangular cell probably always denotes increased haemolysis[126].

Apart from the triangular cell no other specific morphologic type appeared to have any possible diagnostic significance, which would be unlikely in view of the basically single aetiological nature of the underlying pathology. An interrelationship between many of the abnormal forms was, furthermore, suggested in that appearances intermediate between two types of abnormality were occasionally seen

(p. 68). From these observations likely sequential changes in morphology can be envisaged (Fig. 39) which parallel the conclusions reached by Bell in his study of the development of "burr cells".[160] Namely, that where trauma has resulted only in vacuole formation this may subsequently rupture producing an indented cell. This in turn with opening-up and flattening of the crater may lead to a segmented cell outline which, with further distortion in the blood stream, may ultimately come to appear as an irregularly contracted and distorted cell without special features.

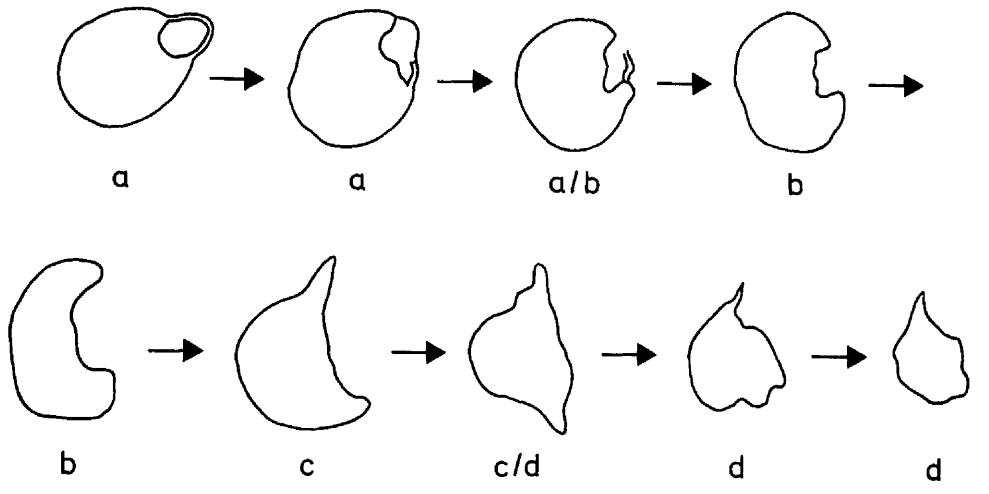


Fig. 39.

Likely sequential changes in abnormal red cell morphology. Drawings made under oil-immersion magnification: a-vacuolated cells, b-indented cells, c-cell segment, d-distorted and irregularly contracted cells, and a/b & c/d intermediate forms.

Presumably the degree and, as illustrated by Bull et al.,[130] the precise localization of the initiating trauma determines the starting point in this sequence, and also the appearance in the circulation of recognizable cell pieces, triangular cells and pincered cells. There seems no obvious reason, however, why the number of triangular cells

should be related to the overall severity of haemolysis.

The value of the classical indices of haemolysis, namely the reticulocyte count, serum bilirubin and urinary urobilinogen levels, in the detection of intravascular haemolysis and assessment of its severity was found to be limited. This was particularly so with the bilirubin and urobilinogen estimations which are well known to be unreliable measures of haemolysis[126]. The false negative rate with respect to the detection of haemolysis was 71% for serum bilirubin values, and even in patients with haemolytic anaemia the serum bilirubin level was usually normal. Urinary urobilinogen estimation as performed with Urobilistix (Ames), which has a stated reliability of 92% in the differentiation between normal and elevated urinary urobilinogen levels, had a false negative rate of 78% in the diagnosis of haemolysis. The maximum degree of urobilinogenuria detected even in cases with pronounced haemolysis was 1.0 Ehrlich unit/100 ml which is considered a marginally abnormal result. Interestingly, a significant direct association was detected between increasing degrees of urobilinogenuria within the normal range and the severity of haemolysis. Although elevated reticulocyte counts correlated with the degree of haemolysis, normal counts were found in 30% of all cases of haemolysis and in almost 50% of those with slight haemolysis.

As indicators of the presence of intravascular haemolysis in patients with prosthetic valves the serum bilirubin and urinary urobilinogen estimations and the reticulocyte count are clearly insensitive investigations, and should not be relied upon for diagnosis. Of the three, only the reticulocyte count showed value as a parameter of the degree of haemolysis.

The concentration of aspartate aminotransferase (AsT) like that of lactate dehydrogenase is much higher in red cells than in serum[208]. Zimmerman, West and Heller[199] found elevated serum levels in a minority of patients with haemolysis, though in nearly all cases there

was concomitant liver disease. Other workers have reported slight to moderate increases in serum levels in acute haemolytic crises[201] and other haemolytic states[198]. Few prosthetic valve patients have been studied in this respect. Sears and Crosby[11] found increases in AsT levels in two patients with chronic intravascular haemolysis secondary to intracardiac prostheses, and these were unexplained on the basis of cardiac or hepatic damage but correlated with the levels of total plasma haeme pigment. Walsh et al.[34] recorded elevated levels in seven of 12 patients with haemolysis due to cardiac valve prostheses but without comment. Other isolated instances of high AsT levels in patients with haemolysis associated with cardiac prostheses have been documented,[8,9 23,26,209] but there has usually either been complicating congestive cardiac failure or no comment regarding the cause of the elevation has been made. Rasmussen et al.[127] found no increase in AsT levels following exercise in 14 patients with aortic valve replacements in whom high serum LDH levels were present at rest and increased significantly on exercise. The actual AsT values at rest were unfortunately not given. The possibility that serum AsT levels may rise with intravascular haemolysis does not seem to be generally appreciated, and Myhre, Rasmussen and Andersen[125] have stated that in prosthetic valve patients causes of a high LDH level apart from intravascular haemolysis may be excluded by finding normal levels of other serum enzymes including aspartate aminotransferase.

In this study no cause other than intravascular haemolysis could be found to account for the high AsT levels found in 16 of 106 patients. The elevations in most cases were slight but levels of approximately 100 iu/l were reached in two patients. High levels did not develop until haemolysis had progressed to the stage of obvious red cell fragmentation, and a direct correlation was observed between them and the numbers of distorted and fragmented cells. On the other hand, raised values were present in most cases with haemolytic anaemia. There

were five additional patients each with elevated AsT levels, but these were due at least in part to disordered hepatic function and were therefore excluded from further consideration.

Estimation of serum AsT levels is not, therefore, a sensitive investigation for the detection of intravascular haemolysis, but high levels may be expected in the more severe degrees of haemolysis and shouldnot be regarded as necessarily due to a complicating myocardial, hepatic or other disorder.

One final point in this discussion of the results presented in the previous chapter relates to the methodology of the serum lactate dehydrogenase and aspartate aminotransferase assays. Despite very similar upper limits of normal values, the separation of the results according to the method employed revealed a very suggestive increase in sensitivity with the later optimized methods in comparison with the earlier assay procedures. The change in methodology consisted of alterations in the concentrations of substrate, coenzyme and phosphate buffer to those that were optimal for assay at 37°C. McQueen[175] estimated the LDH level by both methods in 20 serum samples and found a mean increase of activity of 50% with the optimized assay compared to the earlier method, the two assays having been carried out on the same day on the same serum sample from each of the 20 subjects.

These results underline the need for caution when comparing different enzyme studies. They do not alter the conclusions reached in this investigation although better correlations of the various parameters of the degree of haemolysis were obtained in comparison with enzyme levels estimated by the later optimized methods.

Conclusions

This section has sought to determine the value in the investigation of intravascular haemolysis in patients with prosthetic valves of serum lactate dehydrogenase assay, the examination of urine for haemosiderin and of blood films for red cell fragmentation and

distortion, reticulocyte counts and the estimation of serum bilirubin and urinary urobilinogen levels. An analysis has also been made of the frequency distribution and possible significance of the various types of deformed cells encountered, and the influence of intravascular haemolysis upon serum aspartate aminotransferase levels has been studied. The following conclusions can be drawn:

1. Serum lactate dehydrogenase levels correlate inversely and significantly with the half-life of ^{51}Cr-labelled red cells although with a limited precision. They are a useful measure of the severity of intravascular haemolysis.
2. The urinary haemosiderin test appears to be a sensitive parameter of intravascular haemolysis and the degree of haemosiderinuria bears an approximate direct relationship to the severity of haemolysis. It is also highly specific for this condition.
3. Red cell fragmentation and distortion visible on routine blood film examination is associated with an increase in haemolysis and the degree of fragmentation relates directly to the severity of the haemolysis. The absence of these red cell changes excludes neither the presence of haemolysis nor abnormal degrees of fragmentation as detected by a less subjective and more accurate counting procedure, and the finding on routine inspection of fragments and distorted forms however few in number should be regarded as likely evidence of haemolysis.
4. Counts of the numbers of red cell fragments and distorted forms show them to amount to up to nearly 10% of the red cells in patients with prosthetic valves compared to a normal range found in a healthy population of from 0 to 0.10%. A count of 0.30% has significance in that values above it are virtually diagnostic of intravascular haemolysis although even normal

counts do not exclude haemolysis. It also represents a degree of red cell fragmentation and distortion capable of recognition on routine blood film inspection.

5. Analysis of the frequency distribution of the various types of deformed cells encountered in patients with prosthetic valves shows a fairly uniform pattern, fragmented and torn cells constituting 52 - 67% of the abnormalities, irregularly contracted and distorted cells and a small proportion of pincered cells making up the remainder. Many of these different forms appear likely to be the result of sequential changes in morphology following trauma by the valve. Only the numbers of triangular-shaped cells appear to be related to the overall degree of intravascular haemolysis and they may be of diagnostic value in its detection.

6. The reticulocyte count and the estimation of serum bilirubin and urinary urobilinogen levels are of limited value in the detection of intravascular haemolysis and the assessment of its severity. This is particularly so with bilirubin and urobilinogen estimations which should not be relied upon for diagnosis.

7. High serum aspartate aminotransferase levels may develop in patients with intravascular haemolysis due to prosthetic valves and are frequently present with the more severe degrees of haemolysis although absent with slight degrees. They should not be regarded as necessarily due to a complicating myocardial, hepatic or other disorder.

SECTION II

THE INCIDENCE, OUTCOME, AND PATHOGENESIS OF PROSTHETIC VALVE HAEMOLYSIS

CHAPTER 5

RESULTS

I THE INFLUENCE OF PROSTHETIC VALVE TYPE AND SITE ON THE DEVELOPMENT OF HAEMOLYSIS

The relationship between prosthesis model and site of valve replacement and the incidence and degree of haemolysis was evaluated in the 161 cases studied. The distribution of the different prosthetic valves used in these patients according to type and site has been detailed in Table V (p. 86) and the results of investigations in them are compared. The cases are divided into a main group of patients which comprises the great majority of cases studied, and includes most of the Starr-Edwards ball and cage and Björk-Shiley prostheses, and a miscellaneous group of 17 patients with small numbers of different prostheses not included in the main group.

(1) Incidence of Haemolysis, Red Cell Fragmentation and Haemolytic Anaemia

The criterion for the diagnosis of intravascular haemolysis was the presence of haemosiderinuria. Anaemia has been defined as a haemoglobin of less than 13.5 g/100 ml together with a packed cell volume of less than 40% in the male, the corresponding values for the female being 11.5 g/100 ml and 35%[167]. Haemolytic anaemia was diagnosed when anaemia occurred in the presence of haemosiderinuria and red cell fragmentation and in the absence of any other unrelated precipitating cause.

The incidence in the 161 cases of haemolysis, red cell fragmentation and distortion, and haemolytic anaemia is detailed in Tables XV - XVII according to valve type and site.

(i) Comparison between Valve Types

The results of statistical analysis are given in Appendix A (Table XL). Since the ratio of aortic plus mitral (± tricuspid) replacements to mitral plus tricuspid replacements

Valve Replaced	Prosthesis Model[a] (No. of Cases)		Patients without Haemolysis		Patients with Haemolysis[b]					
					All Cases		Number Fragmenting		Number Anaemic	
Aortic	2300	(15)	-		15	(100%)	12	(80%)	10	(67%)
	2310	(17)	2	(12%)	15	(88%)	10	(59%)	5	(29%)
	B-S	(16)	11	(69%)	5	(31%)	3	(19%)	1	(6%)
Mitral	600/6120	(15)	12	(80%)	3	(20%)	1	(7%)	-	
	6300	(21)	3	(14%)	18	(86%)	8	(38%)	2	(10%)
	6310/20	(15)	-		15	(100%)	8	(53%)	4	(27%)
	B-S	(13)	11	(85%)	2	(15%)	1	(8%)	-	
Aortic plus Mitral (± Tricuspid)	S-E	(11)	-		11	(100%)	10	(91%)	6	(55%)
	B-S	(9)	6	(67%)	3	(33%)	2	(22%)	-	
Mitral plus Tricuspid	S-E	(6)	2	(33%)	4	(67%)	2	(33%)	1	(17%)
	B-S	(6)	6	(100%)	-		-		-	

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards.
b Patients with haemolysis: 'all cases' = all cases with haemosiderinuria; 'number fragmenting' = all cases with red cell distortion and fragmentation in addition to haemosiderinuria; 'number anaemic' = all cases with haemosiderinuria and fragmentation who have developed anaemia due to haemolysis.

TABLE XV INCIDENCE OF HAEMOLYSIS, RED CELL FRAGMENTATION, AND HAEMOLYTIC ANAEMIA ACCORDING TO VALVE TYPE AND SITE IN THE MAIN GROUP OF PATIENTS.

Valve Replaced	Prosthesis Model[a] (No. of cases)	Patients without Haemolysis	Patients with Haemolysis		
			All Cases	Number Fragmenting	Number Anaemic
Aortic	1200 (3)	2	1	1	-
Mitral	6520 (6)	2	4	1	1
	?6300/10/20 (2)	-	2	-	-
Aortic plus Mitral	B-S/S-E mixed[b] (3)	-	3	1	-
	type uncertain (2)	1	1	-	-
Aortic plus Tricuspid	B-S (1)	1	-	-	-

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards
b Comprises a B-S (aortic) + 6520 (mitral)(2 cases); and a B-S (aortic) + 6310 (mitral) (1 case).

TABLE XVI INCIDENCE OF HAEMOLYSIS, RED CELL FRAGMENTATION, AND HAEMOLYTIC ANAEMIA ACCORDING TO VALVE TYPE AND SITE IN THE MISCELLANEOUS GROUP OF PATIENTS.

is very similar in the Starr-Edwards and Björk-Shiley groups they are combined for statistical purposes under the heading "multiple valves".

The most striking differences in results lie between the Starr-Edwards cloth-covered, ball and cage valves (the models 2300/10 (aortic) and 6300/10/20 (mitral))and the Björk-Shiley tilting disc valves. While haemolysis has been almost invariably present in association with these Starr-Edwards valves, it has occurred much less frequently with Björk-Shiley prostheses at either aortic, mitral or multiple sites. Red cell fragmentation has been a correspondingly less common manifestation in patients with Björk-Shiley valves, and only one case of haemolytic anaemia has developed with them and this was transient and mild. Comparison between each of the two types of Starr-Edwards cloth-covered valve at either the aortic (2300 v 2310) or mitral (6300 v 6310/20) position shows no significant difference in the high incidence of haemolysis or red cell fragmentation. Haemolytic anaemia, however, has been particularly common with the aortic 2300 valves, although the difference in comparison with the 2310 models does not quite reach statistical significance in the numbers studied. ($X^2 = 3.07$, d.f. = 1, $0.1 > P > 0.05$).

In contrast to the results in patients with cloth-covered Starr-Edwards valves, the non cloth-covered Starr-Edwards ball and cage mitral valves, models 600/6120, have proved to have as low an incidence of haemolysis and the same freedom from haemolytic anaemia as the Björk-Shiley mitral prostheses. The number of patients available for study who have a non cloth-covered Starr-Edwards ball and cage aortic valve (model 1200) (Table XVI) is too small to permit valid comparisons; only one of the three had evidence of haemolysis.

The Starr-Edwards mitral non-tilting disc valve (model 6520) was also used in only a few patients (Table XVI) but the results suggest that it may have a somewhat lesser tendency to produce haemolysis than the cloth-covered, ball and cage type.

(ii) Comparison between Valve Sites

The results of statistical analysis are given in Appendix A (Table XLI). Patients with Starr-Edwards or Björk-Shiley mitral plus tricuspid replacements have been combined into a total group of mitral plus tricuspid valves.

There has proved to be no significant difference in the incidence of haemolysis between aortic and mitral replacements, between patients with aortic prostheses and those with aortic plus mitral ($\pm$ tricuspid) prostheses, or between mitral and mitral plus tricuspid replacements. Comparisons were made between equivalent valve types and also between the total number of cases in each category. Nonetheless, haemolysis has been more severe with the aortic 2300 cloth-covered, ball and cage valve than with the corresponding mitral 6300 prosthesis, as evidenced by a significantly higher incidence of red cell fragmentation and of haemolytic anaemia. It has been equally severe in patients with Starr-Edwards aortic plus mitral ($\pm$ tricuspid) valves. These differences between the 2300 and 6300 valves have largely determined the significantly greater severity of haemolysis when aortic valves as a whole are compared to mitral valves. The lesser degree of haemolysis with the aortic 2310 model has not differed significantly from that produced by the mitral 6310/20 valves when judged by the same paramtters, and the Björk-Shiley aortic and mitral valves have a similar low incidence of fragmentation with a virtual absence of anaemia. Comparisons between patients with mitral prostheses and those with mitral plus tricuspid prostheses has shown no obvious difference either in the incidence or degree of

Valve Replaced (No. of Cases)	Patients without Haemolysis	Patients with Haemolysis		
		All Cases	Number Fragmenting	Number Anaemic
Aortic (51)	15 (29%)	36 (71%)	26 (51%)	16 (31%)
Mitral (72)	28 (39%)	44 (61%)	19 (26%)	7 (10%)
Aortic plus Mitral (± Tricuspid) (25)	7 (28%)	18 (72%)	13 (52%)	6 (24%)
Mitral plus Tricuspid (12)	8 (67%)	4 (33%)	2 (17%)	1 (8%)

TABLE XVII INCIDENCE OF HAEMOLYSIS, RED CELL FRAGMENTATION, AND HAEMOLYTIC ANAEMIA IN ALL PATIENTS ACCORDING TO VALVE SITE.

haemolysis.

(2) Haemoglobin Level and Reticulocyte Count

Details of the haemoglobin levels and reticulocyte counts in the main group of patients are given in Table XVIII. The values recorded are the means (± s.d.) of the lowest haemoglobins and highest reticulocyte counts observed in the patients during the periods of follow-up. Allowance has to be made for sex differences between the groups in interpreting haemoglobin levels, there being more men than women with aortic prostheses and the reverse in patients with mitral and multiple replacements.

(i) Comparison between Valve Types

The results of statistical analysis are given in Appendix A (Table XLII).

The prominent haemolysis associated with the aortic cloth-covered Starr-Edwards valves (2300 and 2310), particularly with the earlier 2300 model, is again apparent from the results of haemoglobin estimations and reticulocyte counts. There are significant differences in those results between patients with either type of Starr-Edwards valve and those with Björk-Shiley aortic valves, and between Starr-Edwards and Björk-Shiley aortic plus mitral (± tricuspid) valves, the patients with Björk-Shiley valves having the higher haemoglobin values and lower reticulocyte counts. The results with the 2310 Starr-Edwards aortic model occupy an intermediate position between those with the 2300 valves and those in patients with Björk-Shiley aortic valves. The difference in haemoglobin level between the 2300 and 2310 valves does not, however, reach statistical significance in the numbers studied.

Comparison of the Starr-Edwards cloth-covered, ball and cage mitral valves (6300 and 6310/20) with Björk-Shiley models reveals small but in some instances significant differences

Valve Replaced	Prosthesis Model[a] (No. & Sex of Cases)		Haemoglobin (g/100 ml) Mean	(s.d.)	Reticulocytes % Mean	(s.d.)
Aortic	2300	(12M, 3F)	11.8	(2.9)	9.7	(8.1)
	2310	(12M, 5F)	13.1	(1.6)	3.7	(2.2)
	B-S	(12M, 4F)	14.8	(1.5)	2.1	(1.1)
Mitral	600/6120	(6M, 9F)	14.5	(1.7)	2.6	(0.6)
	6300	(4M, 17F)	13.4	(2.0)	3.3	(2.1)
	6310/20	(7M, 8F)	13.1	(1.2)	4.6	(2.3)
	B-S	(2M, 11F)	14.3	(1.4)	2.4	(1.2)
Aortic + Mitral (± Tricuspid)	S-E	(1M, 10F)	11.2	(1.8)	7.6	(5.0)
	B-S	(2M, 7F)	13.8	(0.9)	2.2	(1.1)
Mitral + Tricuspid	S-E	(1M, 5F)	12.9	(1.3)	3.0	(1.9)
	B-S	(2M, 4F)	13.9	(0.8)	2.8	(1.2)

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards

TABLE XVIII HAEMOGLOBIN AND RETICULOCYTE COUNTS ACCORDING TO VALVE TYPE AND SITE.

in haemoglobin and reticulocyte count, the Björk-Shiley mitral valves again having the higher haemoglobin levels and lower reticulocyte counts. No significant difference is present between the results in patients with either of the Starr-Edwards valves (6300 v 6310/20).

The haemoglobin levels and reticulocyte counts in patients with non cloth-covered, ball and cage Starr-Edwards mitral valves (600/6120) have been very similar to those in patients with Björk-Shiley mitral valves in keeping with their equally low incidence of haemolysis.

The values in the six (female) patients with the 6520 Starr-Edwards mitral disc prosthesis approximate to those obtained with the 6300 ball and cage valve (6520: haemoglobin 13.7 ($\pm$ 1.4) g/100 ml; reticulocytes 3.2 ($\pm$ 2.6)%).

(ii) Comparison between Valve Sites

The greater haemolysis produced by the cloth-covered Starr-Edwards 2300 aortic valve compared to the corresponding mitral 6300 model is again evident, as is the equally more marked haemolysis in patients with Starr-Edwards aortic plus mitral ($\pm$ tricuspid) prostheses, from the lower haemoglobin and higher reticulocyte values with which they are associated. Although there is a marginal absence of statistical significance in the difference in haemoglobin levels between the 2300 and 6300 valves ($t = 2.00$, d.f. = 34, $0.1 > P > 0.05$), this is probably due to the disparate sex distribution between the two groups which almost certainly also conceals a greater clinical difference than is apparent if the figures are taken at face value. The difference between the reticulocyte counts is statistically significant ($t = 2.99$, d.f. = 15, $0.01 > P > 0.005$).

Haemoglobin levels and reticulocyte counts in patients with the Starr-Edwards aortic 2310 model have not differed significantly from those in patients with the corresponding mitral 6310/20 valves. Nor has there been any significant difference in these values between patients with Björk-Shiley aortic or mitral prostheses. These findings are in keeping with the correspondingly equal incidences of haemolysis and haemolytic anaemia. Haemoglobin and reticulocyte values observed in patients with mitral plus tricuspid prostheses have been similar to those in patients with single mitral valve replacements of the same type.

(3) Serum Lactate Dehydrogenase, Aspartate Aminotransferase and Bilirubin Levels

The frequency of elevated levels of serum lactate dehydrogenase (LDH), aspartate aminotransferase (AsT) and total bilirubin are detailed in Tables XIX - XXI, and the results of statistical comparisons in Appendix A (Table XLIII). Any case in which a cause other than intravascular haemolysis was likely to be responsible for or contributing to raised levels was excluded. The results of LDH assays are also illustrated in figure 40, and include in cases where repeated estimations were performed the highest recorded levels irrespective of assay procedure.

As has been previously determined (p. 93) the presence of high LDH levels closely parallels the occurrence of haemosiderinuria. It follows that the frequency distribution of abnormal LDH levels for different valve types and sites is almost the same as for the incidence of haemolysis as determined by the presence of haemosiderinuria. Thus, LDH levels were usually normal with Björk-Shiley aortic and mitral valves compared to the almost constant presence of abnormal levels with each of the corresponding Starr-

Valve Replaced	Prosthesis Model[a] (No. of Cases)		Lactate Dehydrogenase		Aspartate Aminotransferase		Total Bilirubin	
			No. estimated	No. elevated	No. estimated	No. elevated	No. estimated	No. elevated
Aortic	2300	(15)	13	13 (100%)	12	7 (58%)	15	7 (47%)
	2310	(17)	13	11 (85%)	10	2 (20%)	17	6 (35%)
	B-S	(16)	16	6 (38%)	13	-	16	1 (6%)
Mitral	600/6120	(15)	13	4 (31%)	8	-	15	1 (7%)
	6300	(21)	16	15 (94%)	7	-	20	4 (20%)
	6310/20	(15)	14	14 (100%)	11	1 (9%)	15	4 (27%)
	B-S	(13)	12	2 (17%)	12	-	12	-
Aortic plus Mitral (± Tricuspid)	S-E	(11)	10	10 (100%)	9	5 (56%)	11	5 (45%)
	B-S	(9)	9	3 (33%)	7	-	8	1 (13%)
Mitral plus Tricuspid	S-E	(6)	4	4 (100%)	2	-	5	1 (20%)
	B-S	(6)	5	1 (20%)	5	-	5	-

Notes: a B-S = Bjork-Shiley; S-E = Starr Edwards.

TABLE XIX INCIDENCE OF ELEVATED SERUM LACTATE DEHYDROGENASE, ASPARTATE AMINOTRANSFERASE, AND TOTAL BILIRUBIN LEVELS ACCORDING TO VALVE TYPE AND SITE IN THE MAIN GROUP OF PATIENTS.

Valve Replaced	Prosthesis Model[a] (No. of cases)	Lactate Dehydrogenase		Aspartate Aminotransferase		Total Bilirubin	
		No. estimated	No. elevated	No. estimated	No. elevated	No. estimated	No. elevated
Aortic	1200 (3)	2	-	1	-	3	-
Mitral	6520 (6)	3	3	3	1	6	-
	?6300/10/20 (2)	2	-	2	-	2	-
Aortic plus Mitral	B-S/S-E mixed[b] (3)	2	2	2	-	3	2
	type uncertain (2)	2	-	2	-	2	-
Aortic plus Tricuspid	B-S (1)	1	1	1	-	1	-

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards.
b Comprises a B-S (aortic) + 6520 (mitral) (2 cases); and a B-S (aortic) + 6310 (mitral) (1 case).

TABLE XX INCIDENCE OF ELEVATED SERUM LACTATE DEHYDROGENASE, ASPARTATE AMINOTRANSFERASE, AND TOTAL BILIRUBIN LEVELS ACCORDING TO VALVE TYPE AND SITE IN THE MISCELLANEOUS GROUP OF PATIENTS.

Valve Replaced (No. of Cases)		Lactate Dehydrogenase		Aspartate Aminotransferase		Total Bilirubin	
		No. estimated	No. elevated	No. estimated	No. elevated	No. estimated	No. elevated
Aortic	(51)	44	30 (68%)	36	9 (25%)	51	14 (27%)
Mitral	(72)	60	38 (63%)	43	2 (5%)	70	9 (13%)
Aortic plus Mitral (± Tricuspid)	(25)	23	15 (65%)	20	5 (25%)	24	8 (33%)
Mitral plus Tricuspid	(12)	9	5 (56%)	7	-	10	1 (10%

TABLE XXI INCIDENCE OF ELEVATED SERUM LACTATE DEHYDROGENASE, ASPARTATE AMINOTRANSFERASE, AND TOTAL BILIRUBIN LEVELS IN ALL PATIENTS ACCORDING TO VALVE SITE.

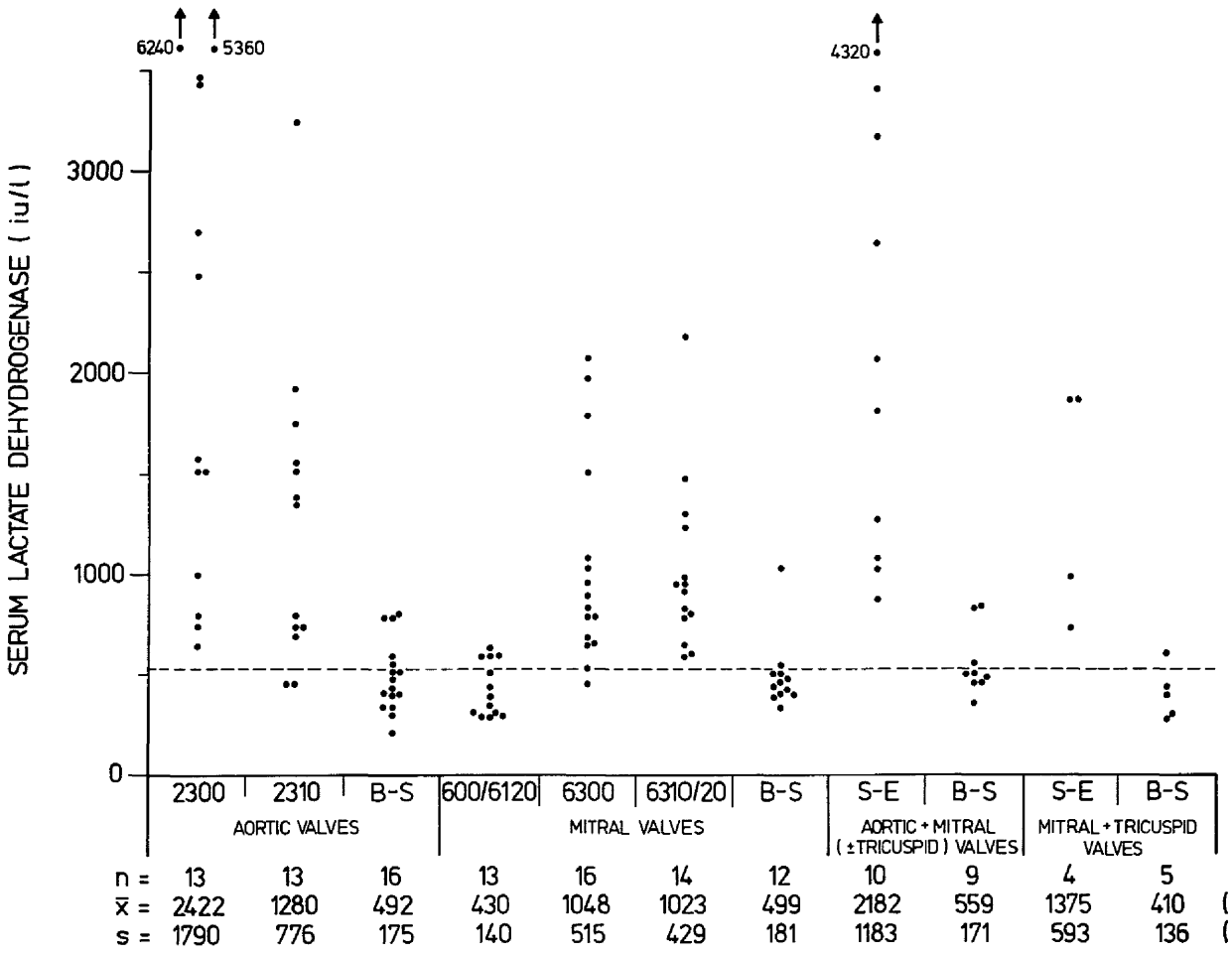


Fig. 40 Serum lactate dehydrogenase levels according to prosthetic valve type and site. Mean ($\bar{x}$) and standard deviation (s) values given. (B.S. = Bjork-Shiley; S-E = Starr-Edwards; interrupted line = upper limit ofnormal at 525 iu/l).

Edwards cloth-covered, ball and cage valves (models 2300/10 and 6300/10/20). The same contrast obtains between Björk-Shiley and Starr-Edwards multiple valve replacements. There are, however, differences in the magnitude of LDH elevations in the patients with Starr-Edwards valves. While statistical comparisons are not strictly applicable in view of the different assay methods employed, particularly high LDH levels have been found in association with the 2300 aortic valves and with the Starr-Edwards aortic plus mitral (± tricuspid) valves (Fig. 40). Patients with non cloth-covered Starr-Edwards 600/6120 mitral valves had a similar low incidence of abnormal LDH levels to those with Björk-Shiley mitral prostheses. The numbers of results in the miscellaneous group of patients (Table XX) are too few to assess.

When the frequency of high LDH levels between different valve sites is considered, no significant differences are found between aortic and mitral replacements, between patients with aortic prostheses and those with aortic plus mitral (± tricuspid) prostheses, or between mitral and mitral plus tricuspid replacements. Comparisons were made between equivalent valve types and also between the total number of cases in each category, and these results parallel the findings when the incidence of haemolysis (haemosiderinuria) between the different valve sites was assessed (v.s.)

High aspartate aminotransferase levels have occurred almost exclusively in patients with Starr-Edwards aortic or aortic plus mitral (± tricuspid) cloth-covered, ball and cage valves, particularly in those with a 2300 aortic model, 12 out of the 16 patients with elevated values having this valve type. None of these patients had high alanine aminotransferase levels nor any other obvious explanation apart from intravascular haemolysis for the high AsT levels which ranged up to 108 iu/l.

Elevated serum bilirubin levels have also developed in only a minority of patients with haemolysis, again mainly in those with Starr-

-Edwards aortic or aortic plus mitral (± tricuspid) cloth-covered valves. With one exception the values have been under 3.0 mg/100 ml.

II TEMPORAL FACTORS IN THE DIAGNOSIS OF HAEMOLYSIS AND FOLLOW-UP STUDIES

(1) The Onset of Haemolysis

The time of commencement of investigations following valve replacement varied, due mainly to the type of prosthesis under study as previously outlined (p. 85). Furthermore, in those cases recently operated upon, investigations were nearly always delayed until three months after operation so that any effects of operative blood loss, extracorporeal circulation, blood transfusion and blood regeneration would not interfere with the interpretation of results. It has not been possible, therefore, to make an accurate assessment of the earliest time of onset of haemolysis. However, where haemolysis developed, as determined by the presence of haemosiderinuria, it was demonstrable in almost all cases (96/102) at the time of the first post-operative investigation. This took place within six months of operation in 43% of patients, within 12 months in 63%, and within 24 months in 85% (Table VI, p. 87). The approximate times of first diagnosis of haemolysis are given here in Table XXII for all patients and for patients with haemolysis secondary to Starr-Edwards cloth-covered, ball and cage valves who constitute the majority of affected cases. Haemolysis was detected within six months of operation in 37%, within 12 months in 58%, and within 24 months in 89% of all the patients with haemolysis. Consideration (Table XXII) of those specifically with "composite-seat" Starr-Edwards cloth-covered prostheses (models 2310 & 6310/20) who were investigated sooner after operation than most other patients with haemolysis (Table VII, p. 88) shows that in nearly 70% of cases haemosiderinuria was detected within three to six months of operation. These results suggest that where haemolysis develops it is likely to do so within the first three to six months of operation, at least in respect of Starr-Edwards cloth-covered, ball and cage valves. Of all the patients with haemolysis there were only six in

Time of Diagnosis of Haemolysis (Months post-op.)	PATIENTS WITH HAEMOLYSIS		
	All Patients (No. & % No.)	Patients with Starr-Edwards Ball and Cage, Cloth-covered Prostheses	
		All Cases (No. & % No.)	"Composite-Seat" cases[a] (No. & % No.)
0 - 3	4 (4%)	3 (4%)	3 (8%)
3 - 6	34 (33%)	28 (35%)	23 (60%)
6 - 12	21 (21%)	17 (21%)	9 (24%)
12 - 24	32 (31%)	25 (31%)	3 (8%)
24 - 36	8 (8%)	7 (9%)	-
36 - 48	1 (1%)	-	-
48 - 60	1 (1%)	-	-
60+	1 (1%)	-	-
Total Patients =	102	= 80	= 38

Notes: a i.e. Patients with models 2310 (aortic) and/or 6310/20 (mitral)

TABLE XXII THIE OF DIAGNOSIS OF HAEMOLYSIS.

whom it was not detected when investigations were first performed, but haemosiderinuria was found and persisted at subsequent examinations.

(2) Follow-up Studies and the Outcome of Haemolysis

The duration of observation as with the time of the first post-operative investigation depended principally upon the prosthesis model and varied considerably (Fig. 41). Sixty percent of all patients were studied for periods in excess of 12 months, many of those with Starr-Edwards cloth-covered valves being under observation for the longest periods of time.

Standard blood and reticulocyte counts, blood film examinations and urinary haemosiderin tests were routinely repeated on average at 6-monthly intervals, more frequent tests being performed in patients with haemolysis. The total numbers of these repeat investigations are shown according to the main prosthetic valve types in figure 42. Altogether they were performed on 592 separate occasions, simultaneous peripheral blood and urinary haemosiderin examinations being carried out in 438, blood count and film examinations only in 138, and urinary haemosiderin tests alone in 16. They were particularly numerous in patients with Starr-Edwards cloth-covered valves because of the longer observation periods, and the greater incidence and severity of haemolysis associated with these valves.

The duration of follow-up and the number of repeat investigations was limited in 20 patients because of death in 10, transferral to another part of the country in eight, and the finding of Australia antigen or antibody in two. These accounted for eight of the 23 patients in whom repeat investigations were not performed. Of the 10 patients who died, two did so in the immediate period following replacement of malfunctioning prostheses. One other patient died of sub-acute bacterial endocarditis, one of congestive cardiac failure, and one of carcinoma of the breast. Five patients died suddenly and

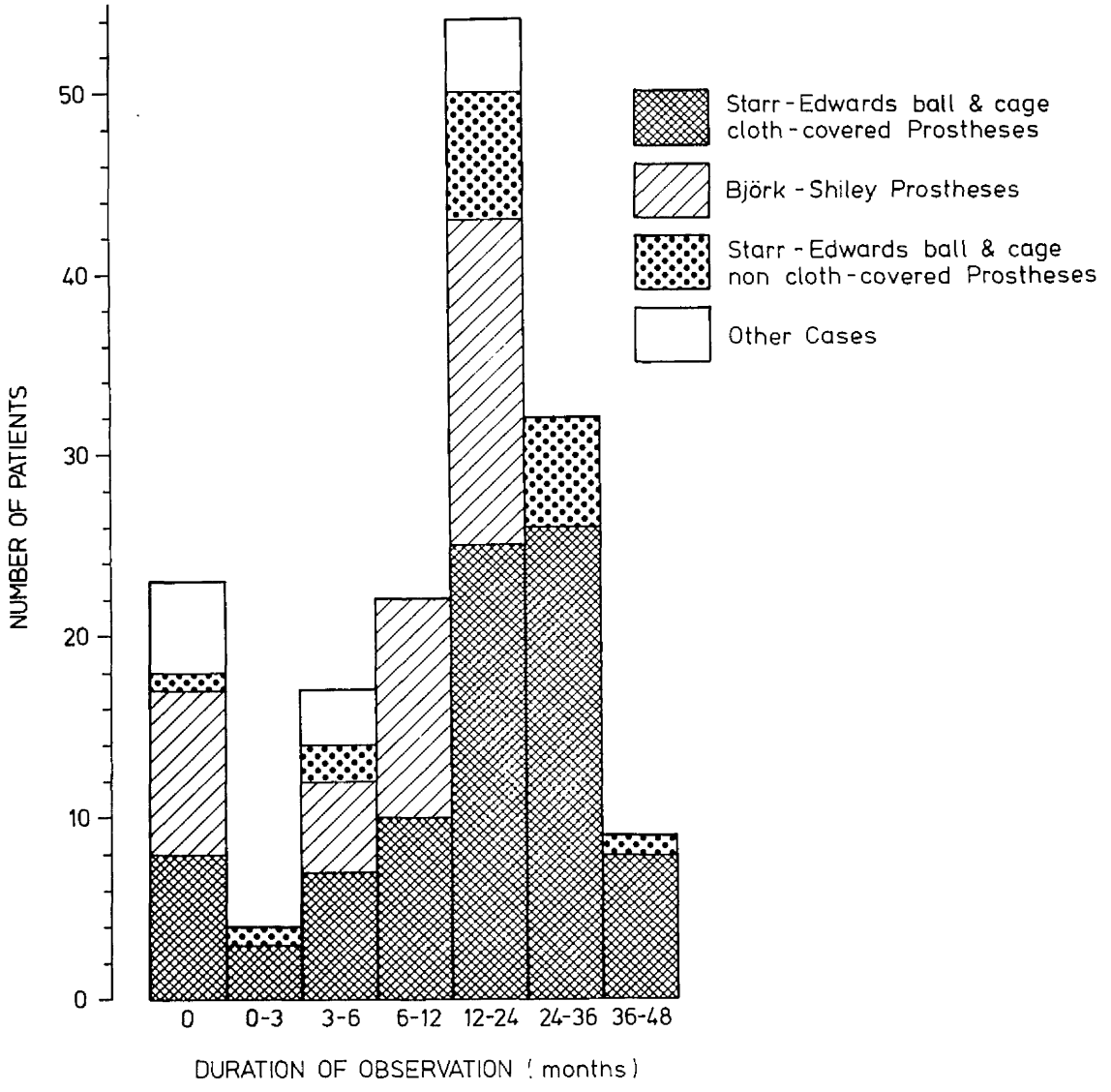


Fig. 41 Distribution of duration of observation according to prosthetic valve type.

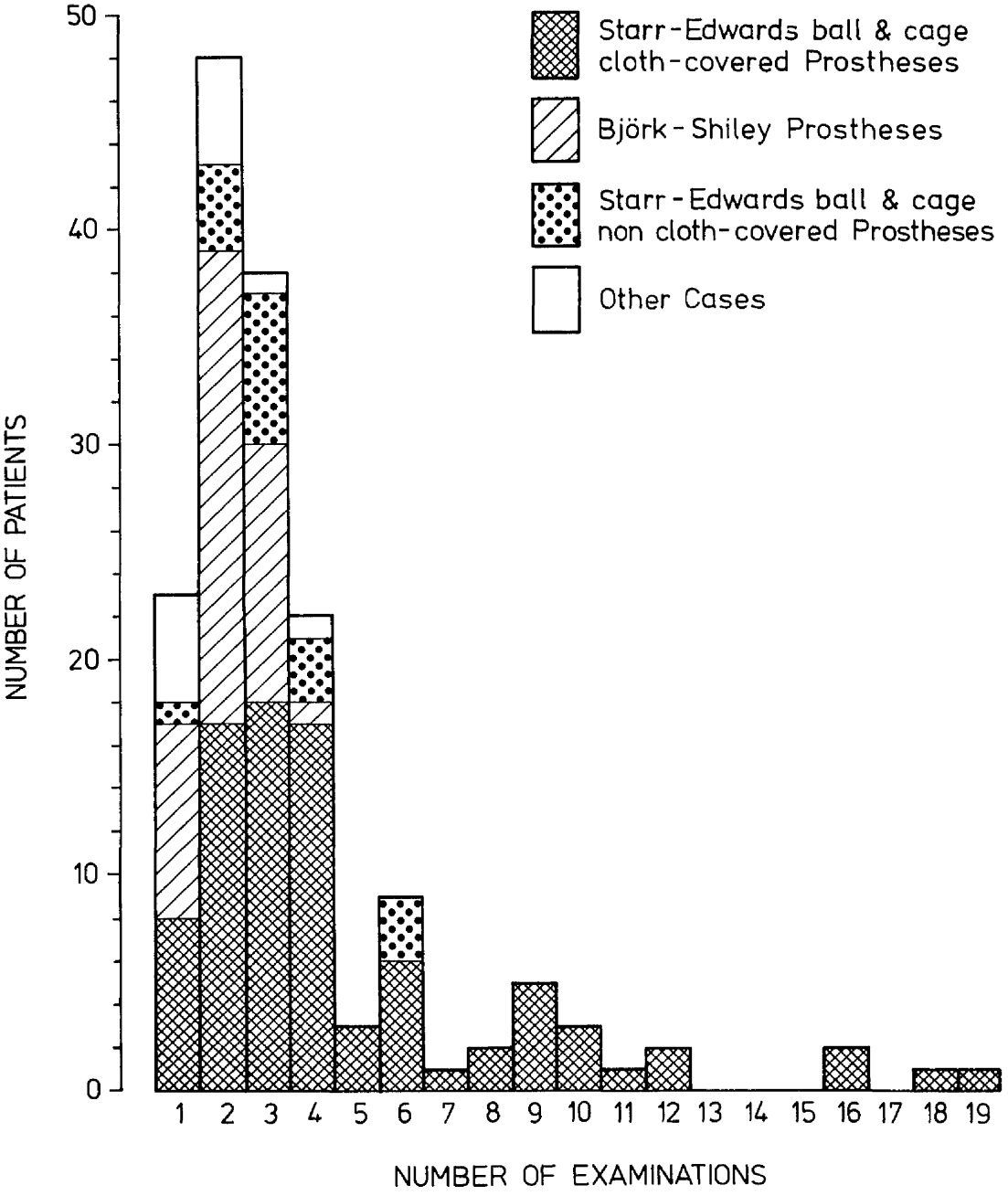


Fig. 42 Distribution of the number of peripheral blood and/or urinary haemosiderin examinations according to prosthetic valve type.

possibly as a consequence of a sudden arrhythmia.

The times at which investigations were completed relative to the date of operation also varied significantly, and these have been detailed in Table VI (p. 87) for all patients and in Table VII (p.88) according to prosthesis model. These times are presented in histogram form here in figure 43 related to the main prosthetic valve types. The great majority of patients had investigations carried out at least one year after surgery, and in 50% post-operative follow-up exceeded two years. The longer periods of time during which the non cloth-covered Starr-Edwards ball and cage valves had been in situ can be seen, contrasting with the more recently introduced Björk-Shiley prostheses.

(i) Persistence of Haemolysis

In 90 of the total of 102 patients with haemolysis repeat investigations were carried out, and in 86 haemosiderinuria was persistently detected. There was additional evidence of continuing active haemolysis at the end of the observation periods in 77 of these patients from the presence of red cell fragmentation with or without anaemia, and/or an elevated serum lactate dehydrogenase level. Serum LDH levels were not estimated in the remaining nine patients in whom haemosiderinuria was the only haematological abnormality at the time of the last check except for a reticulocytosis in two patients.

In four patients haemosiderinuria previously detected on more than one occasion was not found at the time of their last investigation. Serum LDH levels were, however, concurrently elevated in three and serum haptoglobin levels were not estimated, so that a persisting minor degree of haemolysis cannot be excluded.

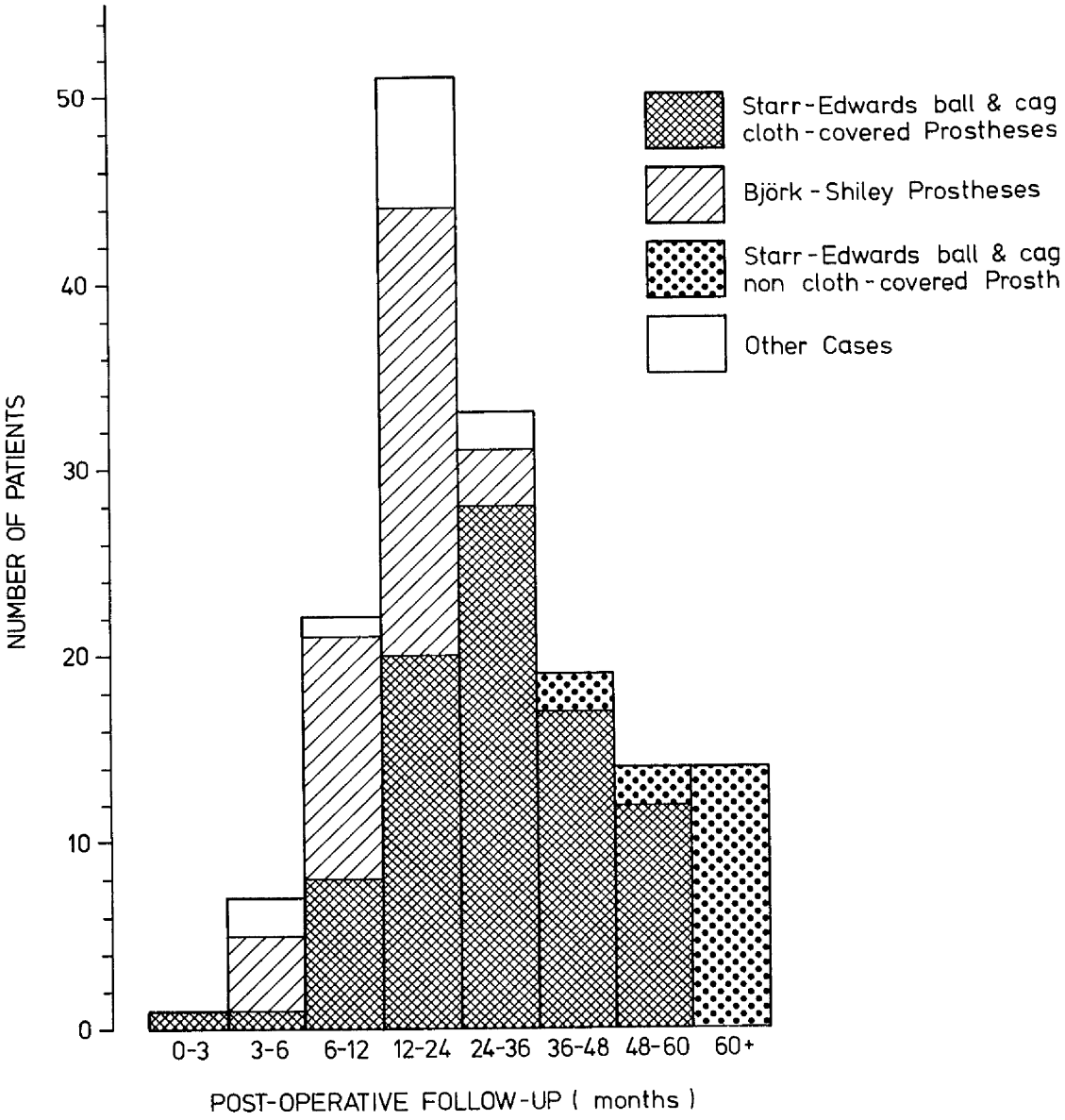


Fig. 43 **Distribution of post-operative follow-up time according to prosthetic valve type.**

(ii) Development and Outcome of Haemolytic Anaemia

Altogether 30 patients in this series have developed haemolytic anaemia, and Table XXIII details the results of investigations and the outcome. In many cases (14/30) the anaemia was detected at the time of the first post-operative investigation, in a few of these a clinically obvious anaemia itself determining the need for urgent investigation. In 14 of the patients the anaemia has been mild (Hb 9+ g/100 ml) and in 12 very mild (Hb 12+ g/100 ml in males; Hb 10.5+ g/100 ml in females). Nearly all had marked haemosiderinuria and high measured urinary iron losses. Associated iron deficiency changes were detected in half of the patients but almost all received oral iron supplements.

Two-thirds of the patients were followed for periods of one to three years after the diagnosis of anaemia. In eight patients (three of whom were known to have iron deficiency) the haemoglobin returned to and remained normal. In another 15 patients (nine of whom had iron deficiency) there was despite iron a persistent or recurrent anaemia which was, however, mild or very mild in all but one case. Two patients developed a more severe anaemia for which re-operation was eventually undertaken because of a limited response to oral or parenteral iron, blood transfusion, and rest. One other with a mild anaemia was re-operated upon because of severevalvar incompetence with cardiac failure. In the remaining four patients no further follow-up is available. Table XXIV summarizes the outcome in these 30 cases according to prosthetic valve type. The associated assessments of valve function listed in TableXXIII are discussed below in section III.

No.	Sex	Time of Diagnosis of Haemolysis/ Anaemia (months post-op)	Lowest Hb. (g/100 ml)	Evidence of Iron Deficiency	Degree of Haemosiderinuria (grade)	Urinary Iron[a] (mg/24h)	^{51}Cr Red Cell Survival ($T_{\frac{1}{2}}$ - days)	Valve Function[b]	Outcome[c] of Anaemia	Follow-up Time after Diagnosis of Anaemia (months)
AORTIC PROSTHESES - 2300 MODEL										
1	F	12 / 12	7.1	+[d]	5	2.04	9	Syst.E.M.	Re-operation.Valve cloth wear & tear.Died post-op.	14
2	F	8 / 8	7.5	+	5	3.20	13.5	Syst.E.M.	Persisting mild/moderate anaemia	38
3	M	13 / 13	7.9	+	3	3.80	10	Syst.E.M.	Re-operation.Narrowed valve orifice.Recovery	18 to Re-op. 11 after
4	M	10 / 10	10.1	+	5	4.25	14	Leak	Resolved, but recurs if iron stopped	37
5	F	16 / 54	10.1	-[d]	4	5.62	16 → 9.5	Syst.E.M.	Persisting mild anaemia[e]	4
6	M	14 / 34	11.1	-	5			Syst.E.M.	No follow-up	00
7	M	17 / 17	11.2	+	5	2.32	12	Normal	Recurring very mild anaemia	34
8	M	11 / 11	11.3	+	5	5.23		Syst.E.M.	Persisting very mild anaemia	42
9	M	25 / 41	12.4	-	4	1.72	19.5	Normal	Recurring very mild anaemia	10
10	M	14 / 14	13.0	-[d]	5	2.17		Syst.E.M.	Resolved	34
- 2310 MODEL										
11	M	2 / 2	9.4	-[d]	2		8	Leak	Re-operation.Prosthetic leak.Died post-op.	1
12	M	5 / 16	11.2	-[d]	3	0.46		Normal	Persisting very mild anaemia	13
13	M	3 / 29	11.2	-[d]	5			Normal	Persisting mild anaemia	10
14	M	4 / 7	11.6	+[d]	5	5.00	20	Normal	Persisting very mild anaemia	15
15	M	7 / 10	12.2	-[d]	5	5.07	11.5	Leak	Resolved	31
- B/S MODEL										
16	M	4 / 4	12.3	-[d]	2			Leak	Resolved	16

TABLE XXIII DETAILS OF INVESTIGATION AND OUTCOME IN PATIENTS WITH HAEMOLYTIC ANAEMIA/CONTINUED

No.	Sex	Time of Diagnosis of Haemolysis/ Anaemia (months post-op)	Lowest Hb. (g/100 ml)	Evidence of Iron Deficiency	Degree of Haemosiderinuria (grade)	Urinary Iron[a] (mg/24h)	^{51}Cr Red Cell Survival ($T\frac{1}{2}$ - days)	Valve Function[b]	Outcome of Anaemia[c]	Follow-up Time after Diagnosis of Anaemia (months)
MITRAL PROSTHESES - 6300 MODEL										
17	F	8 / 13	7.7	+	4	1.54	11.5	Normal	Resolved	12
18	M	14 / 24	12.1	+	5			Leak	Recurring very mild anaemia	16
- 6310/20 MODELS										
19	M	7 / 11	12.6	+	5	2.28	17.5	Normal	Resolved	25
20	M	18 / 18	12.6	+[d]	4	0.70		Normal	No follow-up	0
21	M	4 / 12	12.7	-[d]	3	1.64	20.5	Normal	Resolved	13
22	F	6 / 29	11.2	-[d]	5			Leak	No follow-up	0
- 6520 MODEL										
23	F	9 / 31	11.2	-[d]	4			Normal	No follow-up	0
AORTIC plus MITRAL PROSTHESES - 2300 + 6300/10 MODELS										
24	F	15 / 15	9.0	+	4	0.88		Aortic Syst. E.M.& Mitral leak	Persisting mild anaemia	35
25	F	3 / 3	9.3	+	5	0.92	19.5	Aortic Syst. E.M.	Persisting very mild anaemia	28
26	F	5 / 5	9.8	+[d]	5	2.94	14	Aortic Syst. E.M.	Recurring mild anaemia	38
27	F	3 / 13	9.9	-[d]	5	2.05	13	Aortic leak	Resolved	27
28	F	12 / 41	10.9	-[d]	4	1.61	17	Mitral leak	Recurring very mild anaemia	8
29[f]	F	6 / 13	10.0	+[d]	5	1.56		Mitral leak	Persisting mild anaemia. Died suddenly	2

TABLE XXIII DETAILS OF INVESTIGATION AND OUTCOME IN PATIENTS WITH HAEMOLYTIC ANAEMIA /CONTINUED

No.	Sex	Time of Diagnosis of Haemolysis/ Anaemia (months post-op)	Lowest Hb. (g/100 ml)	Evidence of Iron Deficiency	Degree of Haemosiderinuria (grade)	Urinary Iron[a] (mg/24h)	^{51}Cr Red Cell Survival ($T_{\frac{1}{2}}$ - days)	Valve Function[b]	Outcome of Anaemia[c]	Follow-up Time after Diagnosis of Anaemia (months)
MITRAL plus TRICUSPID PROSTHESES - 6310 + 6520 MODELS										
30	F	9 / 9	10.6	-	5			Normal	Persisting very mild anaemia	9

Notes:
a Urinary iron normally not > 0.37 mg/24 h - see p.169
b Syst.E.M. = very loud and long aortic systolic ejection murmur audible.
c All patients except subjects 9 & 18 given or already receiving oral iron.
d Patients already receiving oral iron.
e Re-operation subsequently undertaken because of progression of anaemia. Cloth wear and tear found. Valve replaced with Bjork-Shiley prosthesis and patient made full recovery.
f Also has a tricuspid Starr-Edwards 6500 disc prosthesis.

TABLE XXIII DETAILS OF INVESTIGATION AND OUTCOME IN PATIENTS WITH HAEMOLYTIC ANAEMIA.

Prosthesis Type[a]	Resolved	Recurrent	Persistent	Re-operation	No follow-up
2300(A) (10)	2	2	3[b]	2	1
2310(A) (5)	1	-	3	1	-
B-S (A) (1)	1	-	-	-	-
6300(M) (2)	1	1	-	-	-
6310/20(M) (4)	2	-	-	-	2
6520(M) (1)	-	-	-	-	1
S-E A + M + T (6)	1	2	3	-	-
S-E M + T(1)	-	-	1	-	-
Total (30)	8	5	10	3	4

Notes: a A = aortic, M = mitral, T = tricuspid valve replacement; B-S = Bjørk-Shiley; S-E = Starr-Edwards. Total number of anaemic patients for each prosthetic type given in brackets.

b One of these has subsequently required re-operation

TABLE XXIV OUTCOME OF HAEMOLYTIC ANAEMIA ACCORDING TO PROSTHETIC VALVE TYPE.

(iii) Other Cases of Anaemia

During the course of this study seven other patients have developed a mild anaemia. This was detected in one, five months after operation; it was normocytic and normochromic in nature, there was no evidence of haemolysis, it quickly resolved in association with oral iron and may have been due to operative blood loss. In the six other patients haemolysis was present but they have not been included in the group of haemolytic anaemia cases since a cause other than their haemolytic state was considered to have precipitated the anaemia.

In five of these patients there was marked blood film and serum evidence of iron deficiency which appeared out of proportion to the minor degree or absence of concurrent red cell fragmentation and to the extent of haemosiderinuria, and intravascular haemolysis alone was judged insufficient to explain the iron deficiency anaemia. This led to the finding that two patients were suffering from menorrhagia and one from bleeding haemorrhoids none of which had been spontaneously complained of. In the other two no cause for the iron deficiency could be found but it resolved with iron therapy. In all five patients concurrent anticoagulant control was very satisfactory with the Thrombotest within the therapeutic range of 5 - 15%.

In the sixth patient, who had a mitral cloth-covered Starr-Edwards 6310 model, a mild compensated haemolytic state was detected four months after operation, there being very slight haemosiderinuria and a normal blood film and reticulocyte count. An elevated mean corpuscular volume (M.C.V.) of 110 μm^3 was, however, noted at that time. Three months later she presented with a haemoglobin of 9.7 g/100 ml with

oval macrocytes and hypersegmented neutrophils and an M.C.V. of 118 μm^3. There was moderate red cell fragmentation, normochromia, the reticulocyte count varied from 2% to 5%, and only very occasional clumps of haemosiderin could be found in specimens of urine. Iliac crest marrow revealed megaloblastic erythropoiesis with little stainable iron. Her serum folate was low (2.6 & 3.0 ng/ml; normally not < 4.0 ng/ml), serum B_{12} was normal (200 - 300 pg/ml; normally not < 120 - -180 pg/ml),[219] and serum iron was normal (128 μg/100 ml). Serum lactate dehydrogenase (1360 iu/l) and plasma haemoglobin (9.0 mg/100 ml) were elevated. Methaemalbuminaemia was not detected, nor was there haemoglobinuria. Her measured total 24-hour urinary iron level was high at 1.84 mg (normally not > 0.37 mg; see p.169). The half-life of ^{51}Cr-labelled red cells was 14.5 days. Xylose excretion and faecal fat estimation were normal, and the patient refused to undergo jejunal biopsy. There was free gastric acid. An excellent (16%) reticulocyte response was obtained with folic acid together with oral iron and the anaemia and macrocytosis resolved.

The possibility of a folate deficiency arising as a consequence of chronic intravascular haemolysis was considered. While this remains a possible contributory factor the elevation of M.C.V. with little other evidence of haemolysis when first investigated, the persistent only very slight degree of haemosiderinuria contrasting with the almost invariably large amounts seen in other patients with haemolytic anaemia, suggests another primary cause for the folate deficiency. Furthermore, there was no clinically detectable evidence of valve malfunction although this does not appear from the results in this study to be a prerequisite for the development of haemolytic anaemia. There was no history of alcoholism, but her dietary intake of folate

was assessed at approximately 30 μg daily (normal daily requirements probably > 100 μg[210]). She is a thin woman and malabsorption has by no means been excluded. She remains well on folic acid with a normal haemoglobin and persisting slight haemosiderinuria $2\frac{1}{2}$ years after this event.

III STRUCTURAL AND FUNCTIONAL PROSTHETIC FACTORS OPERATIVE IN THE GENESIS OF HAEMOLYSIS

(1) The Influence of Valve Orifice Size on the Severity of Haemolysis

The relationship between the diameter and area of the valve orifice and the development of haemolytic anaemia in patients with cloth-covered Starr-Edwards aortic prostheses, single or combined with mitral replacements, is demonstrated in Table XXV.

Valve Size[a]	Starr-Edwards Aortic Valve Models							
	2300				2310			
	Internal Orifice		No. of Patients		Internal Orifice		No. of Patients	
	Diameter (mm)	Area (mm^2)	Total	Anaemic	Diameter (mm)	Area (mm^2)	Total	Anaemic
8(21)	12+	121	1	1	14-	143	-	-
9(22)	14-	145	7	7	15-	170	2	-
10(24)	14+	161	5	5	16-	189	2	-
11(26)	15+	182	3	3	17-	215	3	1
12(27)	16+	206	2	-	17+	236	4	1
13(29)	17+	240	1	-	18+	268	5	1
14(31)	18-	251	2	-	19+	293	3	2

Notes: a - annulus (external, tissue) diameter in millimeters given in brackets.

TABLE XXV RELATIONSHIP BETWEEN VALVE ORIFICE SIZE AND HAEMOLYTIC ANAEMIA

There appears to be an inverse correlation between valve orifice size and the incidence of anaemia in patients with the 2300 aortic model. Haemolytic anaemia was much less common with the 2310 model and there was no obvious relationship between it and orifice size. The larger internal orifice diameter and area for a given total valve size (i.e. external, tissue, or annulus diameter) with the 2310 models is also evident from the figures, such that while most of the anaemic patients with the 2300 prosthesis had valves of internal orifice area less than $170mm^2$, the 2310 valves studied of similar overall size had larger orifice areas. If a figure of 200 mm^2 is arbitrarily taken as differentiating between smaller and larger orifice valves, the association between the presence of haemolytic anaemia and a "small" valve and its absence and a "large" valve is found to be significant in this total group of patients with

Starr-Edwards cloth-covered aortic valves. (X^2 = 10.03, d.f. = 1, $0.005 > P > 0.001$).

There were seven patients with mitral cloth-covered, ball and cage, Starr-Edwards prostheses, single or combined with tricuspid valve replacements, who developed haemolytic anaemia. Six had medium sized, and one a large sized valve (i.e. sizes 3 & 4, internal orifice diameter 18- to 19+ mm and area 251 to 294 mm^2), and no association between the development of anaemia and valve orifice size was evident in patients with these prostheses.

(2) Findings at Re-operation

Re-operation undertaken in four cases with haemolytic anaemia yielded the following results.

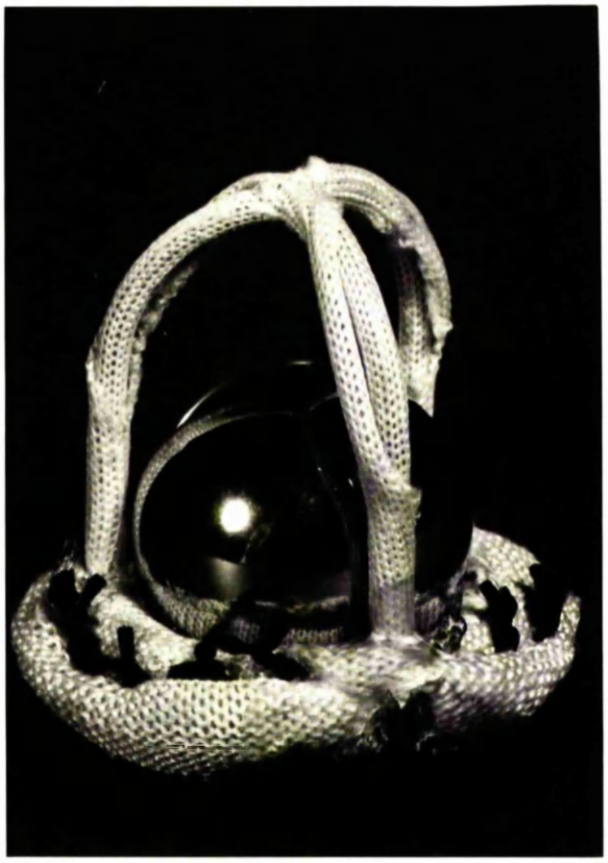
The presence of a paravalvular leak in the aortic 2310 Starr-Edwards cloth-covered model of the patient with mild anaemia and cardiac failure (Subject 11, Table XXIII) was confirmed. The cloth on the sewing margin of the valve ring had run at one point allowing the sutures to loosen, and a crescent-shaped hole had developed between the valve and the aortic wall. The valve was replaced but the patient died shortly after operation in renal failure after an episode of cardiac arrest.

In one patient re-operated upon for severe anaemia (Subject 3, Table XXIII), the endothelialized valve orifice of his aortic 2300 Starr-Edwards cloth-covered prosthesis was found to be roughened and narrowed by pale ?fibrinous deposits (Fig. 44). The effective valve orifice diameter was approximately 11 mm with a calculated orifice area of 95 mm^2, compared to the corresponding figures at the time of implant of 14+ mm and 161 mm^2. The left ventricle was capacious and muscular and it seemed likely that the haemolysis was the result of a relatively large stroke volume being forced through this narrow outlet. The valve was replaced by a Björk-Shiley tilting disc prosthesis and the patient made an excellent recovery. Standard blood count,

Fig. 44.
Aortic 2300 Starr-Edwards ball and cage valve viewed from the ventricular aspect showing the orifice to be irregularly narrowed and roughened by pale ? fibrinous nodules and tiny protuberances. Ignore the brown reflection of orifice tissue on the ball.

reticulocyte count, blood film and serum lactate dehydrogenase levels have reverted to normal but moderate haemosiderinuria persists after 11 months. While serum haptoglobins are absent, the normality of the other indices of haemolysis suggests that this degree of haemosiderinuria is at least partly due to the continued release of haemosiderin from renal tubular cells heavily loaded with iron as a consequence of the original severe chronic intravascular haemolysis.

In the other patient re-operated upon for severe anaemia (subject 1, Table XXIII) the Teflon cloth on the cage struts of her aortic Starr-Edwards 2300 prosthesis was found to be badly split, worn and shredded. Figures 45 & 46 show these changes from photographs taken of the valve, and the appearances of the cloth on the struts are as were encountered at operation and have not been modified by the process of removal or by subsequent handling. The damage can be seen to have occurred mainly on the inner and upper aspects of the struts against which theball strikes in systole. A lesser degree of



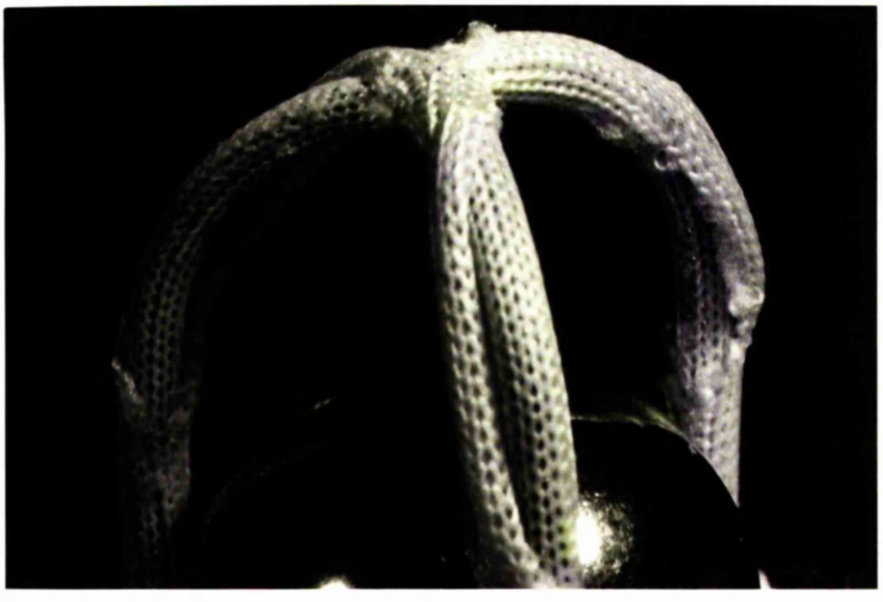


Fig. 45 Aortic 2300 Starr-Edwards valve showing split and worn cloth on the cage struts particularly the inner and upper aspects.

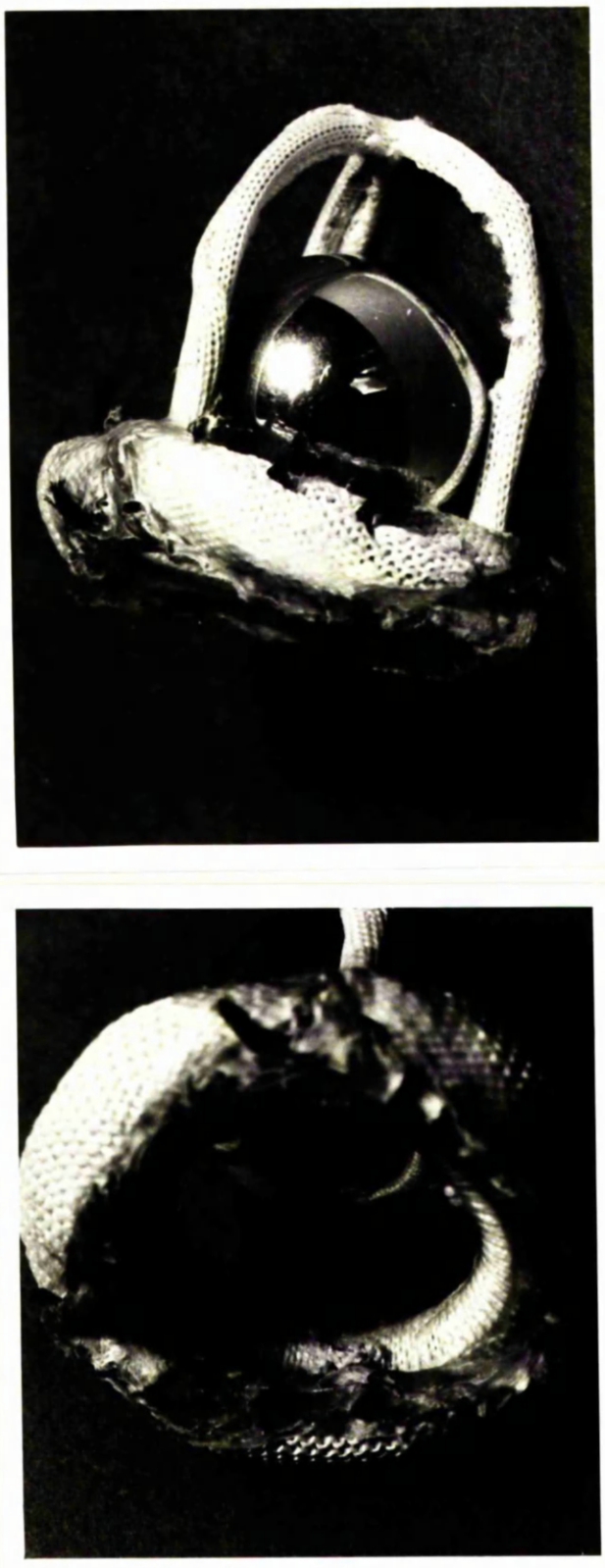


Fig. 46 Further views of the aortic 2300 Starr-Edwards valve showing cloth wear and tear on the cage struts and, viewed from the ventricular aspect (bottom photograph), on the outflow side of the valve ring (ignore tissue sticking to the sewing ring).

cloth wear is present on the outflow side of the valve ring upon which the ball falls in diastole. There was no valvar incompetence but a very loud and long aortic systolic ejection murmur had been audible for several months prior to operation. The prosthesis was replaced by a Björk-Shiley disc valve but the patient died suddenly shortly after operation.

During the preparation of this manuscript one other patient (subject 5, Table XXIII) has come to re-operation because of the progression of her anaemia, and almost identical appearances of cloth wear and tear were found in her Starr-Edwards 2300 valve. There was no prosthetic leak but a marked aortic systolic ejection murmur had also been noted before operation. Recovery was uncomplicated following the insertion of a Björk-Shiley valve and the patient is currently in the early post-operative period with a normally maintained haemoglobin.

(3) Relationship between Prosthetic Leaks and other Auscultatory Abnormalities and the Severity of Haemolysis

The numbers of patients with aortic prostheses who have prosthetic leaks or other auscultatory abnormalities are given in Table XXVI divided according to the presence or absence of haemolysis and haemolytic anaemia. These findings related specifically to individual patients with anaemia have been listed in Table XXIII.

The first point of note is the relatively small number of anaemic patients with a prosthetic leak, and half of those with leaks were not anaemic. Second, the most frequent auscultatory finding in the anaemic patients was a systolic ejection murmur which was considered unusually loud and long in comparison to the short and moderately loud systolic murmurs that are an almost invariable finding with prosthetic aortic valves. This was limited to those with the 2300 Starr-Edwards valve, and systolic murmurs of similar intensity were not heard in non-anaemic patients. Finally, in approximately half of the patients with haemolysis including some with anaemia no clinical evidence of valve malfunction could be detected.

Valve Type[a]	Valve Function[b]	Haemolysis		No Haemolysis
		Anaemic	Non-anaemic	
2300 (15)	leak	1	2	-
	syst.E.M.	7	-	-
	normal	2	3	-
2310 (17)	leak	2	2	-
	syst.E.M.	-	-	-
	normal	3	8	2
B-S (16)	leak	1	-	1
	syst.E.M.	-	-	-
	normal	-	4	10

Notes: a B-S = Bjork-Shiley. Total number of patients for each prosthetic type given in brackets.

b syst.E.M. = very loud and long aortic systolic ejection murmur audible.

TABLE XXVI VALVE MALFUNCTION IN RELATION TO HAEMOLYTIC STATE IN PATIENTS WITH AORTIC PROSTHESES.

A similar pattern of findings obtained in the patients with aortic plus mitral (± tricuspid) prostheses (Table XXVII).

Valve Type[a]		Valve Function[b]	Haemolysis[c]		No Haemolysis[c]
			Anaemic	Non-anaemic	
S-E (11)	aortic	leak	D	K	
		syst.E.M.	ABC		
		normal	EF	GHIJ	
	mitral	leak	A EF	JK	
		normal	BCD	GHI	
B-S (9)	aortic	leak		A^1	F^1
		syst.E.M.			
		normal		B^1C^1	$D^1E^1F^1G^1H^1$
	mitral	leak		A^1	H^1I^1
		normal		B^1C^1	$D^1E^1F^1G^1$

Notes: a S-E = Starr-Edwards; B-S = Bjork-Shiley. Total number of patients for each prosthetic type given in brackets.

b syst.E.M. = very loud and long aortic systolic ejection murmur audible. All tricuspid prostheses were functioning normally.

c Each individual patient is denoted by a letter of the alphebet.

TABLE XXVII VALVE MALFUNCTION IN RELATION TO HAEMOLYTIC STATE IN PATIENTS WITH AORTIC PLUS MITRAL (± TRICUSPID) PROSTHESES.

Three of the six anaemic patients with Starr-Edwards prostheses had a very loud aortic systolic ejection murmur and in each case the aortic prosthesis was a 2300 cloth-covered model. All of the total of 10 patients with the aortic 2300 prosthesis, single or combined with mitral replacement, who had very loud ejection systolic murmurs had small sized valves, six having a size '9' and four a size '10' prosthesis (see Table XXV).

No consistent association between malfunction of mitral prostheses and the presence of haemolysis or haemolytic anaemia was found. In most patients with mitral (± tricuspid) replacements who had haemolysis there was no clinical evidence of valve malfunction, although most but not all of those with leaks were haemolysing (Table XXVIII). In only two of the eight anaemic patients was a prosthetic leak detected, and the episode of severe anaemia seen in one patient (subject 17, Table XXIII) remains unexplained. In another two anaemic patients with aortic plus mitral prostheses, a mitral leak was the only valvular abnormality (Tables XXIII & XXVII).

Valve Type[a]	Valve Function[b]	Haemolysis		No Haemolysis
		Anaemic	Non-Anaemic	
600/6120 (15)	leak	–	–	1
	normal	–	3	11
6300 (20 + 1)	leak	1	3 + ?2	–
	normal	1	10	4
6310/20 (15+5)	leak	1	1 + ?1	–
	normal	4	12	1
6520 (6)	leak	–	–	–
	normal	1	3	2
B-S (13 + 5)	leak	–	1 + ?1	3
	normal	–	–	13

Notes: a B-S = Björk-Shiley. Total number of patients for each prosthetic type given in brackets; second figure refers to number who also have a tricuspid valve replacement. Two cases (one a 6300 mitral, the other a B-S mitral + tricuspid replacement) were lost to follow-up, and no information on their valve function could be obtained.

b Tricuspid valve replacements all functioning normally in 11 patients with both mitral and tricuspid prostheses.

TABLE XXVIII VALVE MALFUNCTION IN RELATION TO HAEMOLYTIC STATE IN PATIENTS WITH MITRAL (± TRICUSPID) PROSTHESES.

The degree of incompetence detected with aortic or mitral prostheses in this study has in most cases been slight and only three patients have required re-operation, one each with an aortic 2310 model, a mitral 6310, and a mitral Björk-Shiley prosthesis.

CHAPTER 6
DISCUSSION AND CONCLUSIONS

The value of the urinary haemosiderin test in the diagnosis of intravascular haemolysis has been discussed in the previous section, and it has been used to determine the incidence of haemolysis in patients with different types of cardiac valve prosthesis. The severity of haemolysis has been assessed from haemoglobin levels and the frequency of occurrence of anaemia, from the height of the reticulocyte count and from the degree of elevation of serum lactate dehydrogenase (LDH). Where anaemia developed its haemolytic nature was substantiated in all cases by the concomitant presence of red cell fragmentation, marked haemosiderinuria and, measured in most cases, reduced ^{51}Cr erythrocyte survival, as well as by the exclusion of any other unrelated precipitating cause of anaemia.

The results show the very common occurrence of haemolysis after valve replacement with a metal ball and cloth-covered cage Starr-Edwards prosthesis, and demonstrate an equally high incidence of haemolysis with either aortic, mitral or multiple replacement (94%, 92% and 88% of cases respectively). Furthermore, no significant difference was found in the frequency of haemolysis between the two types of Starr-Edwards prosthesis studied, the earlier 2300 aortic and 6300 mitral valves compared to the corresponding 2310 and 6310/20 models. By contrast, haemolysis appears to be a relatively uncommon complication of the Björk-Shiley tilting disc prosthesis at any site, occurring in 31% of aortic, 15% of mitral and 20% of multiple replacements respectively, the differences in frequency between the sites not being of statistical significance in the numbers studied. It was equally less common with the Starr-Edwards Silastic ball and non cloth-covered cage mitral prostheses, models 6000 and 6120, the incidence amounting to 20%. There

were insufficient patients available for study who had the corresponding non cloth-covered aortic models. There were also few patients with the non tilting disc Starr-Edwards mitral valve, although the results suggest a possible lesser tendency for haemolysis than with the cloth-covered ball and cage types.

The severity of haemolysis was also correspondingly greater with the Starr-Edwards cloth-covered, ball and cage valves than with the other prosthetic types as evidenced by the more frequent occurrence of red cell fragmentation and haemolytic anaemia, the lower haemoglobin levels, and the higher reticulocyte counts and LDH levels. Haemolysis was particularly marked with the Starr-Edwards 2300 aortic valve, anaemia occurring in 10 out of 15 patients, whereas only one case of haemolytic anaemia developed with Björk-Shiley valves, an aortic replacement, and this was transient and mild. Elevation of serum aspartate amino-transferase levels, too, was limited almost exclusively to patients who had a 2300 aortic valve. The degree of haemolysis, if not its frequency, was also related to valve site, being more severe with the 2300 aortic than with the 6300 mitral valve, and equally severe with Starr-Edwards aortic plus mitral ($\pm$ tricuspid) replacements. There were, however, no significant differences in the lesser degrees of haemolysis between the 2310 aortic and 6310/20 mitral valves, or between the Björk-Shiley aortic and mitral valves.

The times at which investigations were commenced in these patients relative to the date of operation, the lengths of the observation periods, and the final post-operative follow-up times varied. The results suggest, however, that with the cloth-covered prostheses haemolysis will develop within three to six months of operation and that it is likely to persist indefinitely. Furthermore, most of the cases of haemolytic anaemia developed within about one year of operation. Since virtually all patients had investigations performed at least six months after operation and in most cases at a year or more, it is considered that a

comparison of the haemolytic properties of the different prostheses is appropriate, and that any differences found represent real differences between the valves and are not due to a disparity in the timing of investigations.

Although the high incidence of haemolysis is very similar, the frequency of anaemia in patients with Starr-Edwards cloth-covered prostheses found in this study (67% with the 2300 and 29% with the 2310 aortic; and 10% with the 6300, and 27% with the 6310/20 mitral prostheses) greatly exceeds that previously documented. An average incidence of haemolytic anaemia of 8% for aortic prostheses and 1.5% for mitral prostheses can be determined from previous reports and these have been detailed in the literature review (p.45,49). This contrast is probably partly due to differences in the definition of anaemia. In this thesis all cases with haemoglobin and packed cell volume levels consistently below the lower limit of normal according to sex, as defined by Dacie and Lewis,[167] have been recorded as anaemic. In most other studies anaemia has either not been clearly defined or reference has been made to "clinically significant" anaemia without further comment. However, the disparity in the frequencies of anaemia is almost certainly mainly a consequence of differences in the types of prosthesis studied, the great majority of previous reports dealing with the Silastic ball and non cloth-covered cage Starr-Edwards valves.

The possibility that aortic metal ball and cloth-covered cage models might be attended by a greater degree of haemolysis than Silastic rubber ball and non cloth-covered types has previously been raised. Hodam et al.[47] had found an unusually high incidence of haemolytic anaemia of 31% in 39 patients with the 2300 series Starr-Edwards valve, although this was not a severe problem in the absence of a peri-prosthetic leak. Rees et al.[51] found anaemia in four of nine patients with this model, and Milam et al.[52] reported haemolytic anaemia in three of five patients with

another type of cloth-covered prosthesis in contrast to one of 15 with a non cloth-covered valve. However, Walsh et al.[50] could find no difference in the severity of haemolysis between 13 patients with the cloth-covered Starr-Edwards aortic valve and 32 with the non cloth-covered type, and their conclusion has been accepted in a recent monograph on the long-term prognosis following valve replacement[211].

Total cloth-covering of ball and cage valves along with the substitution of hollow metal for Silastic rubber balls was introduced with the aim of reducing the frequency of thrombo-embolism by encouraging tissue ingrowth and eventual complete encapsulation by neo-intima, and of obviating the development of ball variance. While these goals appear to have been achieved,[195,212-215] the results in this study suggest that there has been an associated increase in the degree of haemolysis. This would appear substantiated by the considerably greater incidence of haemolytic anaemia with aortic models in comparison to previous reports relating to the non cloth-covered type, as well as by a direct comparison in this study of the haemolytic effects of both types of valve in the mitral area. These findings are supported by those of Myhre, Dale and Rasmussen[216] who have reported significantly lower ^{51}Cr-labelled red cell survival and higher LDH levels in patients with the aortic 2300 Starr-Edwards model compared to the corresponding Silastic ball and non cloth-covered 1200 valve. A similar high figure (50%) for the incidence of haemolytic anaemia with the 2300 valve has been more recently documented,[207] and the greater severity of haemolysis with the mitral as well as with the aortic cloth-covered model in comparison with the non cloth-covered valves has also been confirmed[217].

The high incidence of anaemia found in this study has not constituted a troublesome clinical problem. In nearly all cases (26/30) it has been mild, although has been persistent or recurrent over periods of up to 3 years in half of the patients. In some (8/30) it has resolved in

association with oral iron. In only three patients, all with aortic cloth-covered 2300 models, has more severe and resistant anaemia necessitated re-operation. Nonetheless, the frequent and often persistently less than optimum haemoglobin levels found in these patients, cannot be regarded as very satisfactory. An even mild anaemia in patients with cardiovascular changes may be considered of greater significance than a similar level of anaemia in an otherwise normal patient. Prosthetic valve patients also generally view with considerable regard the clinical improvements brought by operation and do not easily complain.

From the results in this study and the findings of other workers there appear two likely explanations for the increased severity of haemolysis with cloth-covered valves. Haemodynamic measurements[218,219] in patients with the 2300 aortic and 6300 mitral valve have demonstrated persistent elevation in transvalvar pressure gradients, and effective valve orifice areas calculated from hydraulic data were found to be considerably less than the actual areas at the time of implant. These features compared unfavourably with the haemodynamic characteristics of non cloth-covered valves. The peak systolic pressure gradient across aortic 2300 valves ranged from 4 to 76 mm Hg at rest and was higher over smaller orifice valves (sizes 8 - 10) with which there was an average peak systolic gradient of approximately 40 mm Hg.[219] These higher pressure gradients are at least partly due to the restriction imposed on the final orifice diameter and area by the teflon covering and the eventual layer of neo-intima, and would be accentuated by any further reduction in diameter produced by more exuberant tissue ingrowth or by thrombotic deposits[218,219]. It has been suggested that the greater severity of haemolysis with the 2300 aortic valve is related to increased shearing stresses set up by these relatively high transvalvar pressure gradients[207]. Certainly, an average peak systolic pressure gradient of

40 mm Hg at rest would be sufficient to result in shearing stresses that exceed the critical tolerance of the red cell [128] (p.55), and exercise would have the effect of further increasing this gradient. Furthermore, in this study the incidence of haemolytic anaemia was found to be significantly higher in patients with smaller orifice valves, and an inverse relationship between valve orifice size and the severity of haemolysis has been reported by other workers[206,216,217,220]. The severe haemolytic anaemia encountered in the patient described in the previous chapter (p.149) whose orifice diameter was particularly narrowed by ?fibrinous deposits serves as an example of this mechanism.

The other factor of aetiological importance is suggested by the marked wear and tear of the cloth covering, especially that over the cage struts, which was found in two patients in this study whose severe haemolysis necessitated re-operation (p.150-). The major site of the cloth damage on the inner and upper aspect of the struts and on the valve ring indicates that this was due to trauma from the metal ball. Haemolysis might then occur as a consequence of trapping of the red cells within the interstices of the cloth or between the cloth and the underlying metal rendering them vulnerable to direct trauma from the ball. It might also be due to forced contact of the cells with bare Teflon fibres. This would be analogous to the red cell fragmentation and distortion produced by intravascular fibrin strands in conditions causing microangiopathic haemolytic anaemia[130]. Local turbulence set up by badly shredded and loose portions of cloth may also contribute to haemolysis. Three other cases of severe haemolysis attributed at least in part to similar cloth damage have recently been reported[220-222]. In one of these[221] the cloth on the valve ring was so worn that during diastole a loose seal was created and regurgitation through the valve orifice occurred. It may be, therefore, that lesser degrees of cloth damage are instrumental in the very frequent development of mild haemolysis with cloth-covered prostheses.

Red cell damage against intact but non-endothelialized Teflon has also been suggested as a cause of haemolysis with cloth-covered valves[216,217] and likened to the classical case of Sayed et al.[6] (p.28), and simple trauma from the metal ball of these valves may be more damaging than that from a Silastic rubber ball[216,217].

All these possible haemolytic factors are likely to be accentuated in the high pressure system over the aortic valve, and whereas initial experience with prosthetic valves suggested that severe haemolysis was unlikely to develop in the absence of significant aortic regurgitation, it is becoming clear that with the cloth-covered metal ball models as much attention should be paid to systolic factors in this area. Only four of 21 anaemic patients in this study who had an aortic cloth-covered prosthesis had a clinically detectable prosthetic leak. However, ten patients had an aortic systolic ejection murmur judged independently by experienced observers to be unusually loud and long in comparison to the systolic murmur that is an almost invariable accompaniement of aortic prostheses. They all had an aortic 2300 model and the valve orifice size was small. In the three re-operated upon, stenosis of the valve was found in one and marked cloth wear and tear in two. This prominent murmur, aside from any accentuation due to a hyperdynamic circulation associated with anaemia, must at least partly be a consequence of an increased aortic systolic gradient but might also arise from vibrations of torn cloth. Its evaluation may therefore be of diagnostic importance, and phonocardiographic studies would be of interest. A metallic opening click suggestive of significant strut cloth wear has also been described[214].

The 2310/2320 aortic and 6310/6320 mitral Starr-Edwards cloth-covered valves were constructed with a wider orifice which has had the desired effect of reducing transvalvar pressure gradients to levels similar to those with non cloth-covered valves[215,223,224]. In addition, the small metal studs that alternate with the cloth on the outflow side

of the valve ring constituting a "composite-seat" for the ball protect the cloth from wear[214]. Latterly, to increase cloth durability an outer layer of tubular knitted polypropylene cloth with an inner of Teflon have been used for the cage struts in place of two layers of Teflon, and a composite yarn of Teflon and polypropylene filaments has been used for the valve ring[195,214]. The results in this study suggest that, while these modifications have not reduced the incidence of haemolysis in comparison with the 2300 and 6300 valves, the severity of haemolysis with the aortic 2310 prosthesis is less, and this finding is confirmed by other recently published work[217,220].

In comparison with the haemolytic properties of either of the Starr-Edwards cloth-covered valves at any site, the results demonstrate a striking advantage in patients with a Björk-Shiley prosthesis. Haemolysis has been detected in only a minority of cases ($< 25\%$) and there has been only one instance of haemolytic anaemia and this was transient and very mild. The Björk-Shiley tilting disc prosthesis was introduced clinically by Björk in 1969,[196] and so far there has been no detailed published study of its haemolytic properties, although they have been reported as minimal and without clinical significance on the basis of normal haemoglobin values and detectable plasma haptoglobin levels[225]. Good clinical and experimental haemodynamic results are well documented,[196, 225-229] although the incidence of thrombo-embolic complications may be slightly greater than with the Starr-Edwards cloth-covered valves.

The infrequent and very mild haemolysis that has been encountered in patients with the Björk-Shiley valve seems likely to be related at least partly to the absence of high pressure gradients due to the large orifice area and the central flow design that are its distinctive haemodynamic features, compared to the central occluding and lateral flow, ball-valve type. Figure 47 and Table XXIX illustrate the considerably larger orifice diameter

Valve Annulus (External, Tissue) Diameter (mm)	Starr-Edwards Aortic Valve Models[a]				Bjork-Shiley Aortic Prosthesis	
	2300		2310			
	Internal Orifice		Internal Orifice		Internal Orifice	
	Diameter (mm)	Area (mm^2)	Diameter (mm)	Area (mm^2)	Diameter (mm)	Area (mm^2)
17	11-	85			12	113
19	11+	102	12+	120	14	154
21	12+	121	14-	143	16	201
22	14-	145	15-	170		
23					18	255
24	14+	161	16-	189		
25					20	314
26	15+	182	17-	215		
27	16+	206	17+	236	22	380
29	17+	240	18+	268	24	450
31	18-	251	19+	293	26	530

Notes: a. The corresponding figures for the Starr-Edwards non cloth-covered aortic 1200 model approximate very closely to those with the 2310 valve.

TABLE XXIX: COMPARATIVE VALVE ORIFICE SIZES IN DIFFERENT TYPES OF AORTIC VALVE PROSTHESIS.

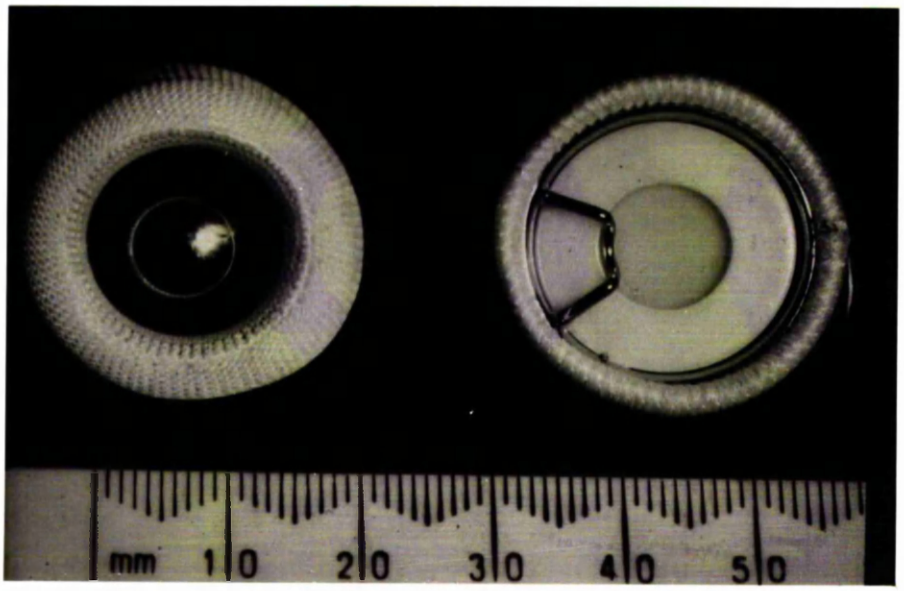


Fig. 47.
Comparative valve orifice sizes in Starr-Edwards 2300 (left) and Björk-Shiley (right) aortic prostheses. Both have the same annulus (tissue) diameter but the Björk-Shiley valve has the larger orifice.

and area for a given external tissue diameter with aortic Björk-Shiley valves compared to the Starr-Edwards cloth-covered models. Turbulence is also less with Björk-Shiley valves because of the more laminar flow allowed by the design. The disc does not overlap the valve ring but fits within the orifice and has a low closing velocity, and therefore any direct trauma to red cells between opposing surfaces is largely avoided compared to the ball and cage arrangement. Furthermore, apart from the outer sewing ring of Teflon there is no cloth incorporated in the model.

The equally slight haemolysis produced by the Starr-Edwards Silastic ball and non cloth-covered cage mitral prostheses, models 600/6120, compared particularly to the 6310/20 composite seat cloth-covered valves which have the same orifice to annulus diameter ratio, suggests that in the absence of regurgitation or functional stenosis it is primarily the presence of cloth or a Stellite metal ball that determines the degree of haemolysis rather than the basic turbulence characteristic of the flow

pathway. This observation is in agreement with the view expressed by Myhre and associates[216,230] of the importance of direct red cell trauma between the ball and cloth, and lends clinical support to the experimental studies of Blackshear et al.[132] (p.56). Indeed, anaemia developed in four patients with the mitral 6310 prosthesis and in three with the aortic 2310 prosthesis despite clinically normal valve function. Whether lack of endothelial covering, cloth wear and tear, or the action of the metal ball is more important is impossible to say. Probably all three factors are operative, but it would seem unlikely from the work of Fok and Schubothe[131] (p.56) that the ball alone acting on intact endothelialized cloth could be responsible for significant haemolysis.

Finally, patients with prosthetic valves are not immune from other causes of anaemia, and being on long-term anticoagulant therapy are more susceptible to the effects of any bleeding lesion. Abnormalities in haemoglobin or reticulocyte count must be checked by blood film examination and other confirmatory tests for the presence and degree of intravascular haemolysis and by clinical examination. In this regard the urinary haemosiderin test has proved useful, in that where marked blood film evidence of iron deficiency changes has appeared out of proportion to the degree of haemosiderinuria, and knowledge of sequential haemosiderin tests allows more certain assessment, this has raised the possibility of a primary cause of iron deficiency. The urinary iron loss and iron deficiency that may follow intravascular haemolysis in patients with prosthetic valves is discussed in the next Section.

Conclusions

In this Section the incidence and severity of haemolysis in 161 cases of heart valve replacement with different types of prosthesis has been studied, and an assessment made of factors of possible aetiological importance. The following conclusions can be drawn:-

1. Starr-Edwards, Stellite ball and cloth-covered cage prostheses irrespective of the site of valve replacement are almost invariably associated with haemolysis as detected by the presence of

haemosiderinuria. The haemolysis is most marked in patients who have an earlier 2300 aortic model in whom a consequent anaemia is common although infrequently serious. In all groups, however, it appears to be more prominent than the haemolysis associated with the Silastic ball and non cloth-covered cage valves.

2. Björk-Shiley tilting disc prostheses are infrequently associated with haemolysis as detected by the presence of haemosiderinuria, and where present it is of minimal degree.

3. Haemolysis is promoted by prostheses of small orifice size probably through high shearing stresses set up as a result of increased transvalvar pressure gradients, by the incorporation of cloth in the flow pathway and its subsequent wear and tear, and possibly by the direct traumatizing effect of a Stellite metal ball. Its development is not dependent upon valve malfunction, and in the absence of regurgitation or functional stenosis the presence of cloth and a metal ball is probably of greater significance than any factor of turbulence determined by the geometry of the valve.

4. All these haemolytic factors will be accentuated in the high pressure system over the aortic valve in systole, and with cloth-covered prostheses they are likely to be of equal importance in the causation of severe haemolysis as prosthetic leaks in this area. The evaluation of the systolic murmur almost invariably present with aortic prostheses may, therefore, be of diagnostic importance, and an unusually loud and long ejection murmur may be a sign of significant cloth wear and tear.

5. The introduction of the wider orifice, composite-seat, and cloth-strengthened Starr-Edwards prostheses, models 2310/20 and 6310/20 has led to some reduction in the degree of haemolysis at the aortic area though not in its frequency. The minimal haemolysis produced by the Björk-Shiley valve is almost

certainly a consequence of its even bigger orifice area, the absence of cloth, the little direct trauma between opposing surfaces, and the lesser turbulence of the more laminar blood flow through the valve.

SECTION III

THE POTENTIAL COMPLICATIONS OF CHRONIC INTRAVASCULAR HAEMOLYSIS

CHAPTER 7

RESULTS

I URINARY IRON LOSS

The 24-hour urinary iron excretion was measured in patients and controls by a routine autoanalyser technique employed for the determination of serum iron, and the method has been detailed in Chapter 2, p77 . In females urinary collections were obtained only in the intermenstrual period.

(1) Normal Urinary Iron Output

Thirty-one healthy hospital employees served as control subjects (19 men and 12 women; mean age 26 years, range 18-38 years), and the results of 24 h urinary iron estimations are given in histogram form in figure 48. While the values range from 0.02 to 0.37 mg/24 h with a mean ($\pm$ s.d.) of 0.135 ($\pm$ 0.09) mg/24 h, the distribution is positively skewed and most results (84%) lie below 0.20 mg/24 h. For comparative purposes, however, the upper limit of normal has been taken as 0.37 mg/24 h.

(2) Urinary Iron Levels in Patients with Prosthetic Valves

A highly significant ($P < 0.0005$) association was found between the presence of haemosiderinuria and a high 24 h urinary iron output, and the absence of haemosiderinuria and a normal urinary iron output (Table XXX). There were only six patients with high measured urinary iron levels in whom haemosiderinuria was not detected. Conversely, in 17 patients urinary iron levels were normal despite persistent but usually slight haemosiderinuria, although they exceeded 0.20 mg/24 h in all but four cases. A highly significant ($P < 0.0005$) direct quantitative relationship between the degree of haemosiderinuria and the amount of urinary iron excreted was also observed(Table XXXI).

The incidence of elevated urinary iron levels correspondingly very closely paralleled the incidence of haemolysis in these patients, being high with the Starr-Edwards cloth-covered valves (2300 & 6300/10) and low with the non cloth-covered models (600/6120) and in patients with Björk—

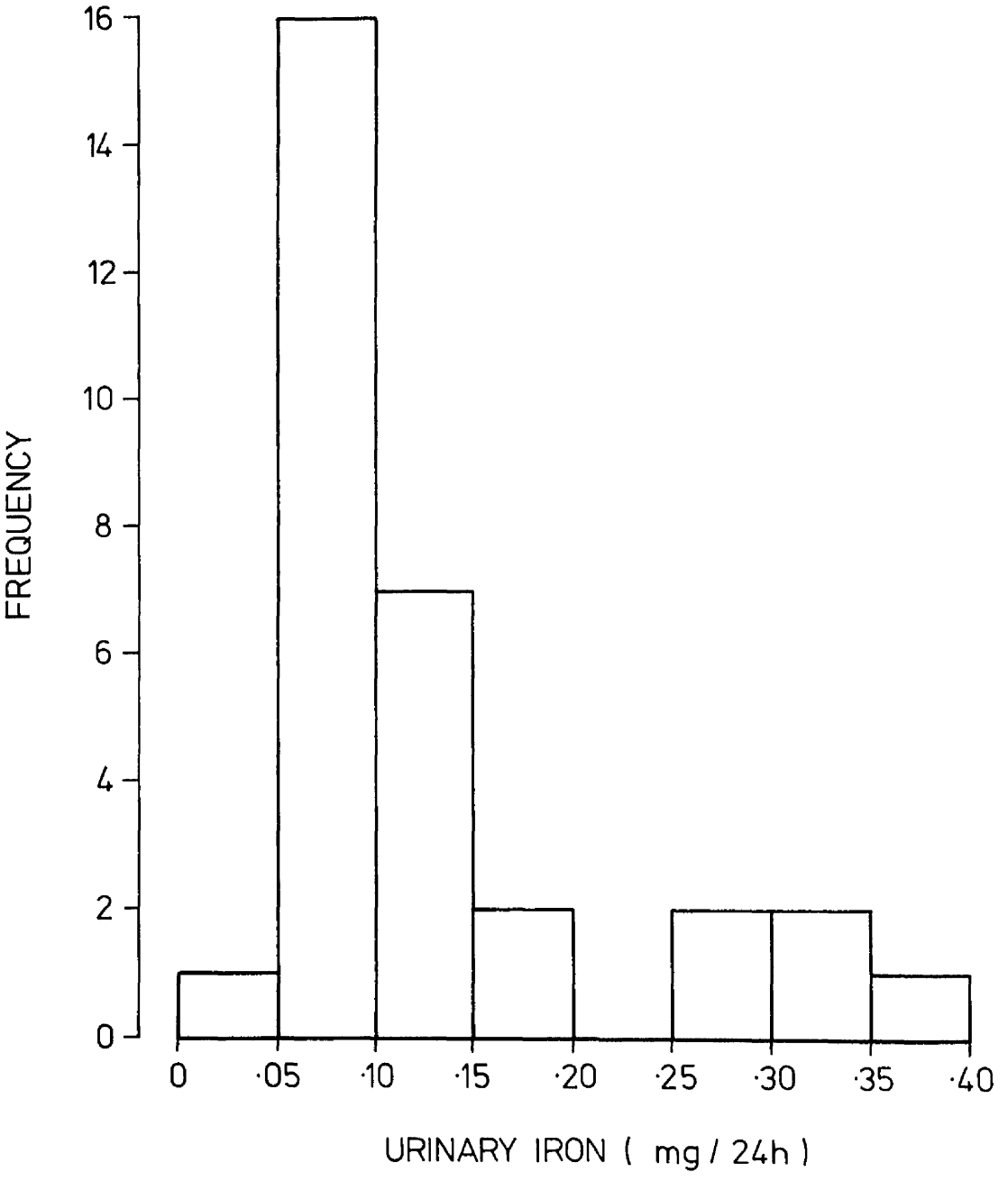


Fig. 48 Distribution of 24 h urinary iron levels in normal subjects.

Test		24 h Urinary Iron Elevated*	Normal
Haemosiderinuria	Present	67	17
	Absent	6	42

*i.e. > 0.37 mg/24 h

X^2 = 53.22, d.f. = 1, $p < 0.0005$

TABLE XXX ASSOCIATION BETWEEN MEASURED URINARY IRON AND THE PRESENCE OR ABSENCE OF HAEMOSIDERINURIA

Urinary Iron Levels (mg/24 h)	Number of Cases in Haemosiderinuria Grades 1 & 2 (slight)	3 (moderate)	4 & 5 (marked)
< 1 mg	16	10	8
1 - 2 mg	3	8	17
> 2 mg	-	1	21

X^2 = 32.91, d.f. = 4, $P < 0.0005$

TABLE XXXI ASSOCIATION BETWEEN URINARY IRON LEVELS AND THE DEGREE OF HAEMOSIDERINURIA.

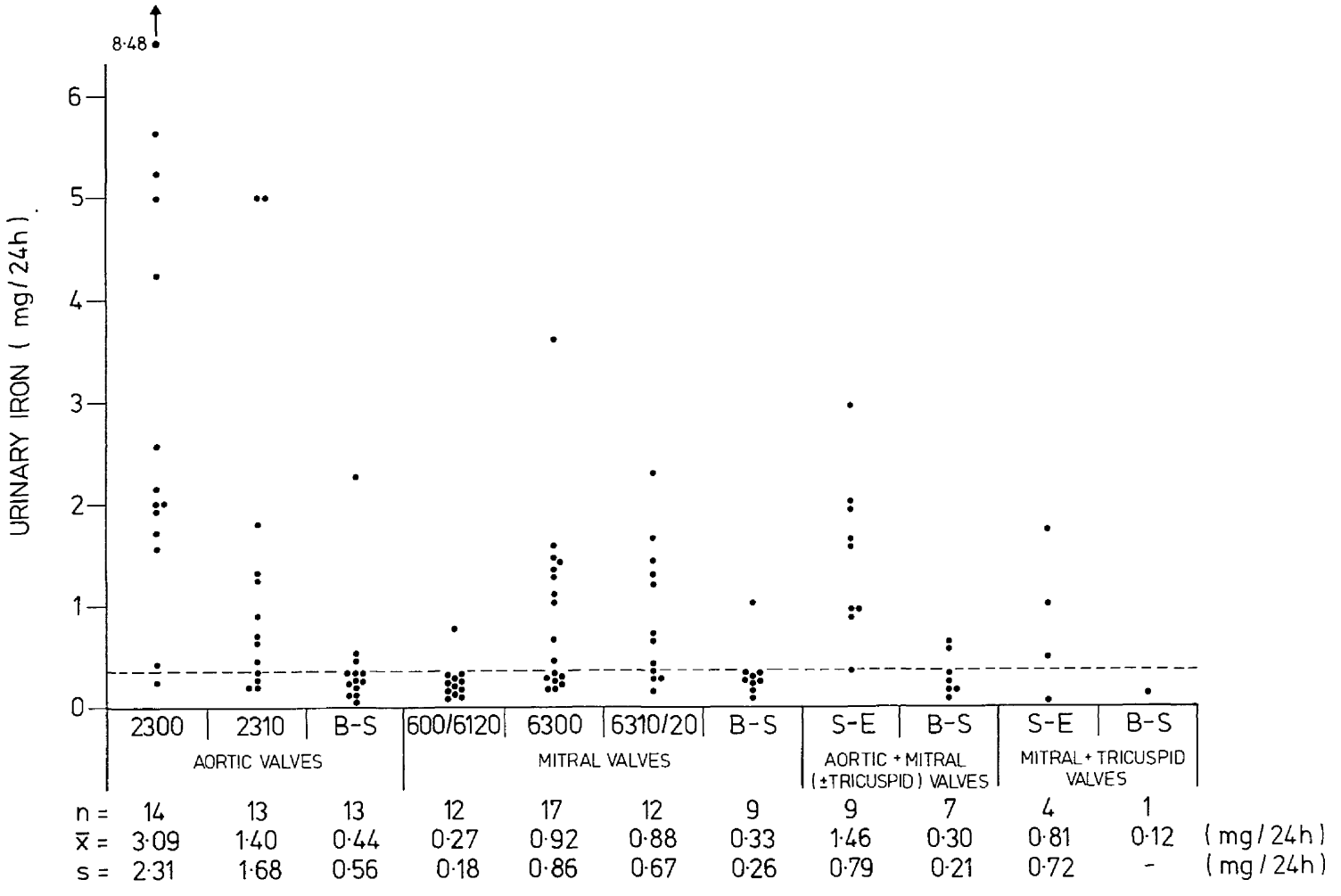


Fig. 49 Urinary iron levels according to prosthetic valve type and site. Mean ($\bar{x}$) and standard deviation (s) values given. (B-S = Bjork-Shiley; S-E = Starr-Edwards; interrupted line = upper limit of normal at 0.37 mg/24 h).

Shiley prostheses. Figure 49 gives the results of urinary iron estimation according to prosthetic valve type and site in the main group of patients, and includes the highest recorded levels in 10 patients in whom estimations were performed on two separate occasions. Particularly high levels were detected in patients with aortic 2300 valves, and are significantly higher than those found in association with the corresponding 6300 mitral valve or the 2310 composite-seat aortic valve (2300 v 6300, $t = 3.32$, d.f. = 16, $0.005>P>0.001$; 2300 v 2310, $t = 2.16$, d.f. = 25, $0.05>P>0.025$). There is no significant difference in levels between the composite-seat valves at aortic and mitral areas (2310 v 6310/20), or between either type of mitral cloth-covered valve (6300 v 6310/20). There is also no significant difference in urinary iron levels when the results in patients with Starr-Edwards aortic plus mitral ($\pm$ tricuspid) valves are compared to those in the combined group of patients with single aortic replacements (2300 & 2310), as both types of cloth-covered aortic prostheses were used in the multiple replacement group. Urinary iron levels were normal in the three patients who had an aortic 1200 Silastic ball and bare metal cage prosthesis, and were elevated to a maximum of 1.22 mg/24 h in five of the six patients with mitral 6520 Starr-Edwards disc valves.

(3) Influence of Oral Iron on Urinary Iron Levels

Sixteen healthy volunteer hospital employees (11 men and 5 women) from whom true consent for the investigation had been obtained received oral iron supplements for 14 days. Their urinary iron levels were measured in two separate 24 h collections obtained immediately before commencing iron and in two taken during the last 2 days of treatment. Estimation of serum iron and total iron binding capacity (T.I.B.C.), standard blood and reticulocyte counts, blood film examinations and urinary haemosiderin tests were also carried out before and at the end of treatment. The iron preparation used was ferrous sulphate in a dose

of 200 mg three times daily after food. The female subjects were all studied during the intermenstrual phase.

The results are detailed in TableXXXII

Investigations	Before Iron (mean ± s.d.)	During Iron[a] (mean ± s.d.)	Significance of the Difference[b]
Urinary Iron (mg/24h)	0.14 ± 0.08	0.13 ± 0.10	N.S.
Serum iron (μg/100 ml)	97.5 ± 23	103 ± 32	N.S.
Saturation of T.I.B.C. (%)	27 ± 8.5	32 ± 11	N.S.

Notes: a i.e. During the last two days of 14 days of oral iron administration.
b Comparison by means of the paired Student's t test; N.S. = not significant, i.e. $P > 0.05$

TABLE XXXII INFLUENCE OF ORAL IRON ON 24 h URINARY IRON OUTPUT

No significant difference was found in the urinary iron levels before or during treatment. There was also no significant change in the serum iron or percentage saturation of the iron binding capacity. Six subjects had slightly lower than normal pre-treatment serum iron levels accompanied by a slight increase in serum T.I.B.C. in two, one of whom also showed a minor degree of hypochromia. All other investigations carried out were normal.

(4) Iron Deficiency Changes

Of the 30 patients with haemolytic anaemia detailed in Table XXIII (p141-3), 15 had evidence of superimposed iron deficiency in that serum iron levels were reduced and/or there was hypochromia of the red cells, both changes being present in the majority (Fig.50). All but one had marked haemosiderinuria and urinary iron levels were elevated to a mean of 2.62 mg/24 h. Despite persistence of haemolysis and marked haemosiderinuria the iron deficiency changes were reversed in eleven of the 15 patients with oral iron supplements, and anaemia resolved or improved with increments in haemoglobin of up to 4 g/100 ml in six. In two of these

six patients a fall in haemoglobin of approximately 3 g/100 ml and a recrudescence of iron deficiency developed within six months when iron was stopped; recommencing iron reversed these changes.

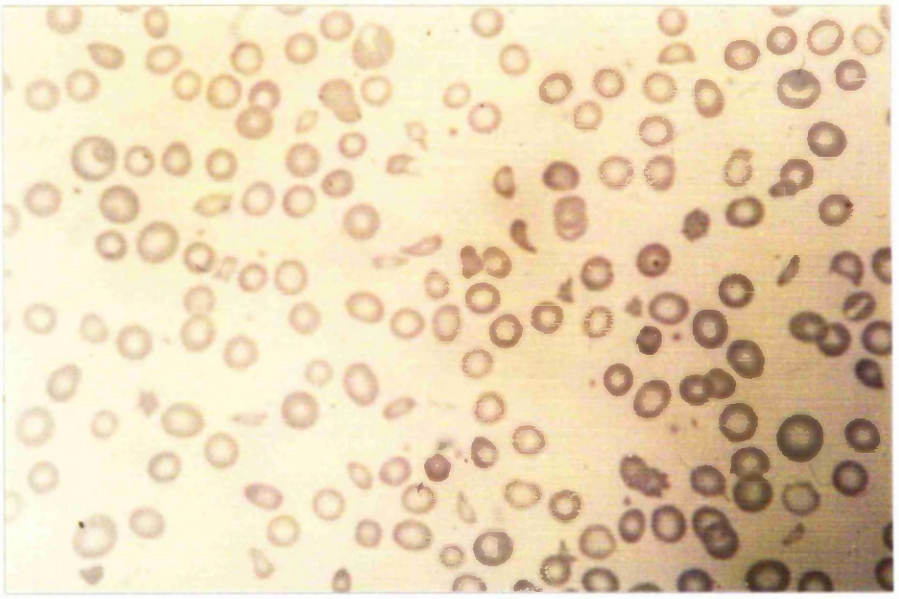


Fig. 50 Hypochromia and microcytosis together with red cell fragmentation and distortion in a case of severe haemolytic anaemia associated with a prosthetic aortic 2300 Starr-Edwards valve. A number of reticulocytes are also present.

II RENAL FUNCTION

An investigation of the effect of chronic intravascular haemolysis on renal function was carried out in eight patients who had undergone aortic valve replacement with the Starr-Edwards 2300 cloth-covered prosthesis 21 to 45 months previously. They all fulfilled the following criteria. They had evidence of chronic and continuing intravascular haemolysis with persistent gross haemosiderinuria for no less than the previous one year. There was no past history of renal disease, there had been no renal problems associated with the valve replacement operation, and there was no history or evidence of systemic disease. They were not hypertensive or in cardiac failure, had no severe anaemia at the time of investigation, and were under good anticoagulant control with warfarin. Five were also taking oral iron but no other drugs, in particular no diuretics, were being given, and no patient was on a restricted sodium intake. A full explanation of the purpose and nature of all the proposed investigations was given to each of the patients and true consent for the study was obtained from them all.

Table XXXIII details the background haematological and other findings. All patients were known to have had persistent gross haemosiderinuria for at least 15 months, and ^{51}Cr red cell survival studies performed 11 to 22 months previously had shown reduced survival in all cases measured. Present studies demonstrated an absence of plasma haptoglobins and high serum lactate dehydrogenase levels. All patients except case 1 had obvious red cell fragmentation and distortion on blood film examination, and in six there was mild anaemia. Urinary haemoglobin was not detected in any patient but total daily urinary iron excretion was considerably elevated in them all.

The results of the renal investigations are listed in Table XXXIV. Plasma urea and serum creatinine levels were normal. Two of the patients (cases 1 & 6) had slightly impaired endogenous creatinine clearance rates

Subject	Age and Sex	Time Since Operation (months)	Known Duration of Haemosiderinuria (months)	Hb. (g/100 ml)	P.C.V. (%)	Reticulocytes (%)	Red Cell Fragmentation (grade)	Plasma Haptoglobin (mg/100 ml)	Serum Lactate Dehydrogenase (iu/l)	Urinary Haemoglobin	Urinary Iron (mg/24 h)	^{51}Cr Red Cell Survival ($T\frac{1}{2}$ - days)
1	49 M	30	17	15.3	45	2	-	NIL	1000	NIL	1.43	22
2	48 M	21	18	12.1	36	3	2	NIL	1520	NIL	5.00	20
3	40 M	45	20	13.6	42	5	1	NIL	800	NIL	1.42	19.5
4	41 M	31	22	13.0	39	4	2	NIL	1200	NIL	1.66	14
5	39 M	31	21	11.3	32	4	1	NIL	1920	NIL	5.23	
6	53 M	38	21	13.4	39	2	1	NIL	920	NIL	2.58	12
7	51 F	29	22	10.5	29	8	3	NIL	1560	NIL	5.00	13.5
8	49 M	22	15	12.2	34	6	3	NIL	1800	NIL	5.07	11.5
Normal Values =				M $\not<$ 13.5 F $\not<$ 11.5	$\not<$ 40 $\not<$ 35	0.2 - 2.0	-	48 - 216	116 - 467	NIL	Not > 0.37	25 - 33

* Results of ^{51}Cr-labelled red cell survival studies performed previously.

TABLE XXXIII HAEMATOLOGICAL INFORMATION IN EIGHT CASES OF AORTIC VALVE REPLACEMENT IN A STUDY OF RENAL FUNCTION.

Subject	Plasma Urea (mg/100 ml)	Serum Creatinine (mg/100 ml)	Creatinine Clearance (ml/min/1.73m^2)	Proteinuria (mg/24 h)	Urinary W.B.C. Excretion (cells/hour)	Urinary R.B.C. Excretion (cells/hour)	Urine Culture
1	25	1.05	69	NIL	80000	NIL	-
2	34	0.55	141	180	285000	NIL	-
3	38	0.96	111	NIL	147000	NIL	-
4	21	0.75	132	NIL	80000	NIL	-
5	31	1.17	80	NIL	386000	NIL	-
6	28	0.90	63	NIL	386000	NIL	-
7	30	0.73	128	NIL	NIL	NIL	-
8	32	0.13	98	NIL	200000	NIL	-
Normal Values	15 - 35	0.8 - 1.4	Not < 80	0 - 90	0 - 400000	0 - 200000	-

		Acid Load Test				
Subject	Urine Osmolality post-dehydration (mosmol/kgH_2O)	Lowest Urinary pH	Highest titratable acidity (μEq/min)	Highest ammonium excretion (μEq/min)	Total hydrogen ion excretion (μEq/min)	Urinary Amino Acids
1	912	4.95	62.5	66.0	128.5	Normal
2	944	5.00	45.0	74.7	119.7	Normal
3	1002					Normal
4	785	5.20	33.3	100.0	133.3	Normal
5	820	5.10	53.0	100.2	153.2	Normal
6	918	4.80	37.0	95.0	132.0	Normal
7	805	4.70	29.3	52.5	81 .8	Normal
8	937	5.00	36.0	80.0	116.0	Normal
Normal Values	Not < 800	4.60-5.24	24 - 51	33 - 75	60 - 124	Normal

TABLE XXXIV RESULTS OF RENAL FUNCTION STUDIES IN PROSTHETIC VALVE PATIENTS WITH CHRONIC HAEMOLYSIS.

after these values had been adjusted for surface area. One patient had a slightly increased 24 h urinary protein level. Urinary red and white blood cell excretion rates were normal and all urine cultures were sterile. The response to water deprivation was normal in all cases except for a marginally abnormal result in subject 4. The short acid load test showed no impairment in the excretion of acid in any of the seven patients in which it was carried out. The total urinary amino acid concentration as well as the chromatographic pattern of amino acid excretion was normal in all.

III INTRAVASCULAR COAGULATION

To examine the possibility that intravascular haemolysis in prosthetic heart valve patients might lead to or potentiate the development of intravascular thrombosis through the release of pro-coagulant factors contained within red cells, the association between the presence of haemolysis and the occurrence of thrombo-embolic complications was investigated in the 161 cases studied in this thesis. Serum fibrinogen-fibrin degradation product levels (F.D.P.) were also estimated in 56 of these patients selected at random. All patients were on long-term anticoagulent therapy with warfarin.

No clinical association was found between the presence of haemolysis and the occurrence of thrombo-embolic incidents. Fifteen patients out of the 161 cases studied were considered to have sustained an episode of thrombo-embolism as judged from a history of a sudden well-defined neurological deficit such as dysphasia, motor paresis of a limb, a clear-cut hemi-sensory disturbance, or a visual field loss. The majority of these were transient and some were recurrent. Ill-defined episodes of numbness, paraesthesiae or diplopia were not taken as evidence of thrombo-embolism, and embolic incidents occurring in the immediate post-operative period are not included in this analysis. Only seven of the 15 patients with thrombo-embolic complications had evidence of haemolysis which was of minimal degree. Eight of the 15 had the Starr-Edwards non cloth-covered mitral 600/6120 valves (one with haemolysis), but no preponderance of any other single valve type was noted amongst the remaining seven patients (six with haemolysis).

Figure 51 shows the results of serum F.D.P. assay according to the degree of haemolysis as determined by the presence of haemosiderinuria (grade 1), red cell fragmentation (grade 2), and haemolytic anaemia (grade 3) (see Ch. 3, p.92 & Table IX, p.92). For comparison, F.D.P. levels previously detected in this department in patients with micro-

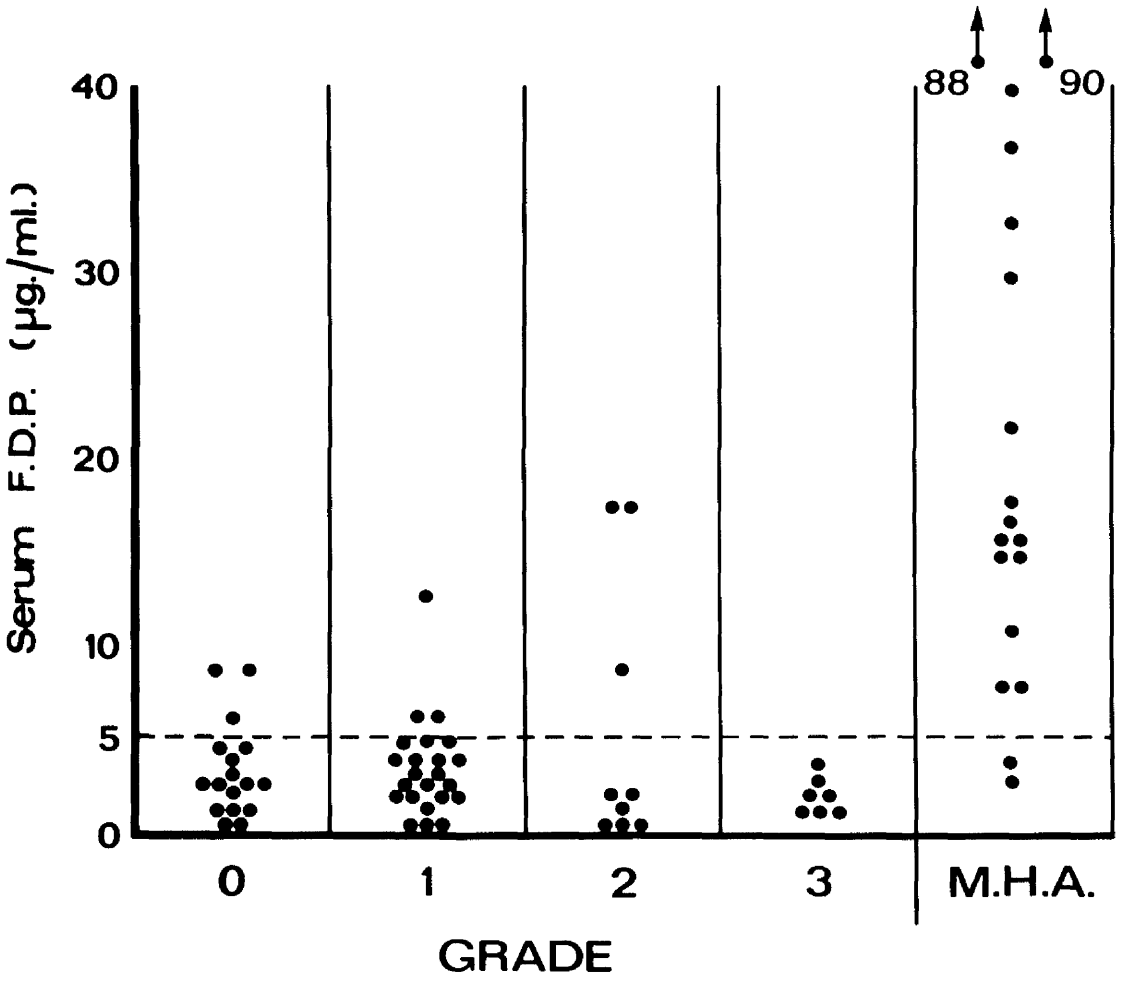


Fig. 51 Serum F.D.P. levels in prosthetic valve patients with different grades of haemolysis and in patients with microangiopathic haemolytic anaemia (M.H.A.)

Subject	Sex	Valve Prosthesis	Hb. (g/100 ml)	P.C.V. (%)	Reticulocytes (%)	Red Cell Fragmentation (grade)	Urinary Haemosiderin (grade)	Serum LDH[a] (iu/l)	^{51}Cr Red Cell Survival[b] ($T_{\frac{1}{2}}$ - days)	F.D.P.[c] (µg/ml)
1	F	Aortic 2300	7.5	22	13	3	5	1560	13.5	1.4
2	M	Aortic 2300	7.9	22	9	2	3	2700	10	2.7
3	F	Aortic 2300	9.0	27	13	3	5	5360	9	1.4
4	M	Aortic 2300	10.1	30	9	2	5	1330	14	2.7
5	F	Aortic 2300 + Mitral 6310	10.9	32	8	3	5	1480	14	3.2
6	M	Aortic 2300	11.8	32	4	2	5	1220	12	1.4
7	M	Aortic 2300	13.3	39	5	1	5	1920		2.8

Notes: a LDH = lactate dehydrogenase; normally 116–467 iu/l.
b $T_{\frac{1}{2}}$ normal 25–33 days.
c F.D.P. normally not >5µg/ml.

TABLE XXXV FIBRINOGEN-FIBRIN DEGRADATION PRODUCT LEVELS (F.D.P.) IN PROSTHETIC VALVE PATIENTS WITH HAEMOLYTIC ANAEMIA.

angiopathic haemolytic anaemia (M.H.A.) complicating malignant hypertension or renal disease[231] are shown.

In only nine prosthetic valve patients were raised F.D.P. levels found and there were no significant differences in the incidence of high F.D.P. values between the different grades of haemolysis. By contrast, F.D.P. levels were elevated in 16 of the 18 patients with M.H.A. Details of the parallel investigations performed in the seven patients with haemolytic anaemia in grade 3, none of whom had abnormal F.D.P. levels, are given in Table XXXV. All of them had pronounced intravascular haemolysis with red cell fragmentation. Of the nine patients with high F.D.P. levels, repeat estimation in three gave normal results, and no explanation for the high values was apparent; there was no clinical evidence to suggest that they may have been due to embolic episodes.

CHAPTER 8

DISCUSSION AND CONCLUSIONS

Urinary Iron Loss

A generally available method has been applied to the measurement of urinary iron excretion, and a similar procedure has been described by other workers[232]. This automated analysis, working at a rate of forty samples per hour, is simple, requires no sample preparation, and has been found to be accurate and precise within the working limits of 0.2 - 3.0 mg Fe/l. Results in the control group agree well with published values for normal urinary iron output[40,233-235]. The mean urinary iron level (0.135 mg/24 h) is, however, slightly higher than that usually reported, and while 0.37 mg/24 h has been taken as the upper limit of normal, the positive skewness of the distribution suggests that levels in excess of 0.20 mg/24 h should be regarded with caution.

Previous reports have documented high urinary iron levels in patients with chronic intravascular haemolysis due to prosthetic heart valves[34,37,40,46,50,118]. They relate almost exclusively, however, to the non cloth-covered Starr-Edwards valves and particularly to aortic prostheses. Urinary iron levels have been found to be normal in almost all of the very few mitral valve replacement patients examined[37,50].

The results in this study demonstrate that in patients with cloth-covered, ball and cage, Starr-Edwards valves excessive urinary iron loss frequently occurs, and is equally common irrespective of the site of replacement. It is also of equal magnitude with the current cloth-covered composite-seat models, aortic or mitral, but particularly high levels were encountered in patients with the aortic 2300 prosthesis corresponding to the more severe haemolysis associated with this earlier cloth-covered ball-valve type. The usually normal levels in patients with mitral non cloth-covered valves has been confirmed, and equally low levels have been found in association with the recently developed Björk-

Shiley tilting disc prosthesis. As is to be expected there was a significant association between the presence of haemosiderinuria and a high 24 h urinary iron level. In addition the degree of haemosiderinuria was found to correlate directly with the amount of urinary iron excreted. Many patients with marked haemosiderinuria had levels of urinary iron exceeding 2 mg/24 h whereas in those with slight haemosiderinuria the urinary iron output was nearly always less than 1 mg/24 h.

About half of the patients with elevated urinary iron levels were taking oral iron when the estimation was carried out. There is evidence of a modest but restricted alteration in iron losses in parallel with total body iron content[237], presumably relating to the concentration of iron in desquamated cells, but there appears to be little available information specifically on the influence of iron intake upon urinary iron excretion. Barer and Fowler[238] found no significant alteration in urinary iron levels with oral iron in 10 patients, but more recently Man and Wadsworth[236] reported a direct relationship between dietary iron intake and urinary iron in two subjects. Finch[237] has stated that "after a dose of iron by mouth, the urinary iron excretion increases appreciably" but gave no further details. In this study, no increase in urinary iron levels was detected by the method employed at the end of a two week period of standard oral iron medication in 16 normal subjects. This, together with the correlation observed between urinary iron levels and the degree of haemosiderinuria, is evidence that in the prosthetic valve patients studied in this thesis oral iron has had no significant effect upon the results of urinary iron estimation, and has not been responsible for the high urinary iron levels found in those already on iron.

Excessive urinary iron losses in chronic intravascular haemolysis may result in iron deficiency which may precipitate or aggravate anaemia (Fig. 52), and a beneficial response to oral or parenteral iron has frequently been reported[34,37,46,47,49,50,58]. Half of the 30 patients with haemolytic anaemia in this study, including four with a mitral cloth-covered prosthesis,

were iron deficient. All but one had persistent marked haemosiderinuria, urinary iron losses exceeded 2 mg per day in most, and no other reason for the iron deficiency was evident. A good response to oral iron was obtained in six. In two of these iron deficiency changes and anaemia recurred when iron was stopped,but restarting it led to complete recovery despite persistence of the haemolysis. Conversely, three patients ultimately required re-operation because of the progression of severe anaemia despite full iron repletion. Depending on the virulence of the underlying haemolytic process, therefore, an adequate supply of iron may prevent the occurrence of anaemia and allow the establishment of a compensated haemolytic state, and oral iron should be given prophylactically if there is marked haemosiderinuria or a measured urinary iron loss of over 2 mg per day.

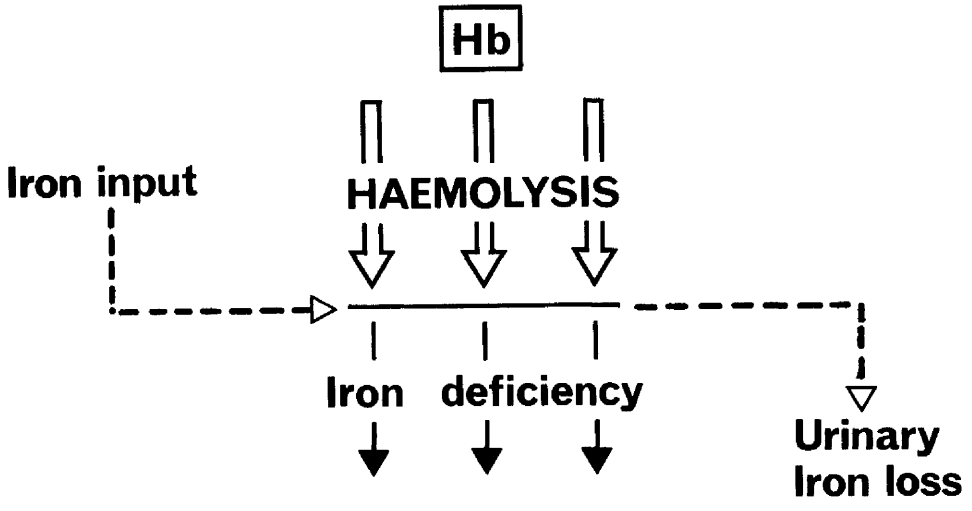


Fig. 52 Diagrammatic representation of iron balance in patients with prosthetic valve haemolysis.

Renal Function

Chronic intravascular haemolysis irrespective of aetiology may lead to heavy and selective deposition of iron in the kidneys, but the effect of renal haemosiderosis upon renal function has not been studied in detail.

Renal biopsy and post-mortem studies of affected kidneys[25,116,117,133] have shown that the haemosiderin deposits are most abundant in the cells of the proximal convoluted tubules, less pronounced in the loops of Henle, and only occasionally and scantily present in the cells of the distal convoluted tubules and in the interstitial tissue. Small particles of haemosiderin were also found very occasionally in the epithelial cells of the glomerulus and in Bowman's space, and haemosiderin was present in the lumina of the convoluted and collecting tubules but not apparently obstructing them. No evidence of significant fibrosis or tubular atrophy was found in these studies. The only evidence of tubular damage noted was cellular degeneration followed by slow elimination of the cells most infiltrated by haemosiderin[116], but it was considered that this loss could probably be compensated for by regeneration.

Occasional cases of structural renal damage with renal failure considered likely to have been directly related to renal haemosiderosis associated with paroxysmal nocturnal haemoglobinuria (P.N.H.) or paroxysmal cold haemoglobinuria have been described[138-140]. In these, however, other features such as renal infection, diabetes, or repeated spontaneous or post-transfusional acute intravascular haemolytic episodes were clearly important and complicating aetiological factors.

These generally benign histological findings may explain the experience, mainly related to cases of P.N.H., that serious or even significant renal functional impairment seldom if ever develops[25,134-136]. However, formal investigations of renal function have been limited in extent, often confined to isolated cases, and altogether appear inconclusive. Thus, Bradley and Bradley[137] in an investigation of renal

function in anaemia studied two cases of P.N.H., and while finding abnormalities in glomerular and tubular function and in renal blood flow, concluded that these were due to the anaemia per se and not to the renal haemosiderosis. Leonardi & Ruel[116] reported on eight patients with renal haemosiderosis of varying aetiology, all of whom were severely or moderately anaemic. They found that renal concentrating power was at the lower limit of normal, renal dilution tests were normal, and the urea clearance varied from 77 - 110% of normal. More detailed study of glomerular filtration rate along with estimation of renal plasma flow gave increased values in two out of three of these patients, and the authors suggested that perhaps a more obvious functional tubular defect was being masked by compensatory renal hypertrophy. Their conclusion was, however, that renal haemosiderosis caused little or no functional damage. Hutt, Reger and Neustein[117] found no definite abnormality of renal function in two patients with P.N.H. but employed very limited tests of renal function.

Paroxysmal nocturnal haemoglobinuria has previously been considered the most common condition causing chronic intravascular haemolysis[117] but is itself a rare disease, and so the opportunity for studying an uncomplicated uniform group of such cases has been small. Prosthetic cardiac valves probably now constitute the most common cause of chronic intravascular haemolysis and a fairly uniform group of such cases in whom likely complicating features had been excluded as far as possible has been investigated. All of the patients were known to have gross haemosiderinuria for 15 to 22 months on repeated testing of their urine, often to the extent that the blue of the positive iron stain was visible on naked-eye inspection of the slide, and their total daily urinary iron excretion was considerably elevated. Red cell fragmentation was repeatedly seen in all but one patient, and ^{51}Cr erythrocyte survival studies performed early in

these observation periods had shown reduced erythrocyte survival. Current investigations demonstrated elevated serum lactate dehydrogenase and zero plasma haptoglobin levels in every patient indicating active haemolysis. These findings taken together confirm that significant intravascular haemolysis was occurring at the time of this study of renal function and had been in operation for at least 15 to 22 months, and indicate that an appreciable degree of renal haemosiderosis was very likely to have been present in these patients for equal periods of time. In this group as a whole no evidence of impairment of either glomerular or tubular function has been detected. The reduction in creatinine clearance found in two patients was slight as was the elevation in urinary protein in a third patient, and these abnormalities are of doubtful significance. The 8-hour period of dehydration was found to constitute a very adequate stimulus to water concentration although in the one patient with a very marginally abnormal result more prolonged dehydration would probably have led to an unequivocally normal value.

The question of possible eventual renal impairment in patients with haemolysis secondary to prosthetic heart valves has frequently been raised[29,45,118,141], but no study of renal function in them has been reported. The results in this investigation support the view that chronic intravascular haemolysis with renal haemosiderosis does not interfere with renal function. Before the question can be answered with certainty, however, serial investigations over a more prolonged period of time are required.

Intravascular Coagulation

Red cell haemolysate contains adenosine diphosphate[239] which is a powerful platelet aggregating agent[240]. It has thromboplastic activity[241], inhibits fibrinolysis[242], and appears likely to have a physiological role in haemostasis[243]. It has been suggested, therefore, that intravascular

haemolysis may be associated with a tendency to intravascular coagulation. The possibility that this mechanism may be partly responsible for the thrombotic complications of paroxysmal nocturnal haemoglobinuria has been discussed[136]. It has also been proposed that in disorders associated with microangiopathic haemolytic anaemia (M.H.A.), in which the red cell fragmentation and haemolysis are considered secondary to damage from intravascular fibrin deposits (p. 64), a vicious cycle of recurrent fibrin deposition in small arterioles may be set up[164,244,245]. In conditions with slow capillary blood flow such as shock there is clinical and experimental evidence that intravascular haemolysis may be a cause rather than a sequal of disseminated intravascular coagulation[246].

In patients with prosthetic heart valves thrombo-embolism is a major potential complication and may occur despite well controlled anticoagulant therapy[247]. The possibility of a causative relationship between the haemolytic and thrombo-embolic complications has not previously been explored. In this study, however, no direct clinical association between the two has been apparent. Indeed, an almost inverse relationship has obtained but this is almost certainly due to a third factor, namely the design characteristics of the valve. The lesser incidence of thrombo-embolic complications with the completely cloth-covered Starr-Edwards models compared to the non cloth-covered valves appears established[195, 212-215]. On the other hand, the cloth-covered valves, as has been determined in this thesis, have undoubtedly greater haemolytic properties.

In a proportion of the patients serum fibrinogen-fibrin degradation product levels (F.D.P.) were estimated, as they are often elevated in association with intravascular fibrin formation probably as a consequence of secondary fibrinolytic action[169,248]. However, in only a few patients were elevated levels found and there was no correlation between the incidence of high levels and the degree of haemolysis.

Indeed, in those patients with the most severe degrees of haemolysis F.D.P. levels were uniformly normal. This contrasted with the almost invariably high levels in the patients with M.H.A. in whom the degree of haemolysis was no more severe than in the prosthetic valve patients, emphasising the significance of the underlying coagulation disturbance in M.H.A.

Measurement of radioactive fibrinogen turnover may be a more sensitive parameter of fibrin formation than either F.D.P. assay or clinical findings. In the few prosthetic valve patients in whom this investigation has been carried out fibrinogen catabolism has not differed significantly from normal[123,163]. No parallel information was given, however, regarding the presence or absence of haemolysis in the 20 patients studied by Harker and Slichter[123] except that red cell fragmentation was noted in only one case. All of the three patients investigated by Baker et al.[163] had a complicating haemolytic anaemia.

It would seem, therefore, that haemolysis in prosthetic valve patients is itself not of importance in the genesis of intravascular coagulation. This does not discount the possibility that intravascular haemolysis in general may have a pro-coagulant action. It may be that as in prosthetic valve patients the red cell fragmentation occurs at the valve replacement site, the thrombus-promoting factors released are diluted or inhibited in the fast flowing blood stream of the large arteries.

Conclusions

Potential complications of chronic intravascular haemolysis have been examined in this section and include the assessment of urinary iron losses and accompanying iron deficiency, the effect of renal haemosiderosis upon renal function, and the possible pro-coagulant action of red cell haemolysate. The following conclusions are drawn:-

1. Urinary iron losses are very commonly excessive in patients with cloth-covered ball and cage, Starr-Edwards valves irrespective of the site of valve replacement, and are particularly high with the earlier 2300 aortic valves. By contrast, they are usually normal in patients with the mitral non cloth-covered prosthesis and in those with a Björk-Shiley tilting disc valve.
2. The degree of haemosiderinuria correlates directly with the 24-hour urinary iron output.
3. Iron deficiency may consequently ensue and precipitate or aggravate anaemia, and this may be reversed with oral iron which should be given prophylactically in the presence of marked haemosiderinuria or a urinary iron excretion exceeding 2 mg per day. Concurrent oral iron therapy has no significant effect on the results of urinary iron estimation when carried out by an auto-analyser method.
4. Renal haemosiderosis does not interfere with renal glomerular or tubular function at least over a period of two years.
5. Intravascular haemolysis in prosthetic valve patients does not appear to result in intravascular coagulation or to potentiate the occurrence of thrombo-embolism in these patients.

SECTION IV

IMMUNE HAEMOLYTIC FACTORS FOLLOWING PROSTHETIC VALVE REPLACEMENT

CHAPTER 9

RESULTS

Serological investigations were carried out in 156 of the 159 patients studied in this thesis to determine the frequency with which irregular serum antibodies (alloantibodies) develop following the massive transfusion of blood often required during cardio-pulmonary by-pass for prosthetic valve replacement. Evidence was also sought from the results of the direct anti-human globulin (Coombs') test performed on all specimens for the possibility of an autoimmune component to the high incidence of haemolysis in these patients. The timing of the investigations relative to the date of operation is given in Table XXXVI.

Months after Operation	Number of Patients
0 - 3	3 (2%)
3 - 6	49 (31%)
6 - 12	37 (24%)
12 - 24	41 (26%)
24 - 36	13 (8%)
36 - 48	3 (2%)
48 - 60	3 (2%)
60 +	7 (4%)

TABLE XXXVI TIMING OF INITIAL POST-OPERATIVE SERO-LOGICAL TESTS.

In the majority the operations had been performed within the previous 24 months, and within the previous 12 months in many.

I. DISTRIBUTION OF BLOOD GROUP ANTIGENS.

The Rhesus (D) status was identified in all 156 patients, the full Rhesus phenotype in 137, and the Kell and Duffy^a blood group in 133, and Table XXXVII details the distribution in them of the various blood group antigens. The results compare very well with published data for the incidence of these blood group antigens,[249,250] and are used in calculations below to estimate the antigen status of the few patients in

whom the full Rhesus phenotype or Kell and Duffy^{a} group was not determined.

Antigen*	+		-	
C	93	(68%)	44	(32%)
$\bar{c}$	116	(85%)	21	(15%)
D	135	(87%)	21	(13%)
E	45	(33%)	92	(67%)
e	132	(96%)	5	(4%)
K	14	(11%)	119	(89%)
Fy^{a}	80	(60%)	53	(40%)

* The number (and % No.) of patients possessing (+) or lacking (-) the corresponding antigen is given.

TABLE XXXVII DISTRIBUTION OF BLOOD GROUP ANTIGENS.

II. ALLOANTIBODIES.

Of the 156 patients examined 12 (7.7%) were found to have developed alloantibodies post-operatively (Table XXXVIII).

In nine cases (5.8%) a Rhesus antibody was detected, being Rhesus anti-E in every instance although one patient (case 7) had both anti-E and anti-$\bar{c}$. However, a more meaningful expression is to relate the incidence of anti-E to the number of Rh (E)-negative persons potentially exposed to the E antigen. Excluding 20 of the 21 patients who were also Rh (D)-negative and had received only Rh (D)-negative blood (which as supplied in this region is also Rhesus C and E negative), and one patient who developed anti-E pre-operatively (vide infra), 71 were found to be Rh (E)-negative. A further 12 were estimated to be E negative in the group of 19 patients with unknown full Rhesus phenotypes. Of this total of 83, therefore, 10.8% had developed anti-E.

Anti-Kell was detected in four patients. Excluding three Kell-negative patients who developed anti-Kell or unidentified antibodies pre-operatively (v.i.), this represents an incidence of 2.9% out of 136 Kell-negative cases (116 known plus 20 estimated).

Antibody tests were repeated at intervals over periods of 8 to 40 months in all but one of the affected patients (Table XXXIX). In nine of these detectable antibody persisted, although in two (cases 4 & 11) there was an interim period when negative tests were obtained, and in one (case 7) there was persistent loss of detectable activity in respect of one (anti-$\bar{c}$) of the two antibodies initially present. In two patients (cases 8 & 9) follow-up investigations were repeatedly negative for the antibody originally identified, but case 8 developed another antibody (anti-E) after his second aortic valve replacement, and another antibody (anti-Kell) was also detected in case 9. There was no consistent pattern of change in the titre of the antibodies that were persistently detected.

In 42 patients without alloantibodies serological investigations were repeated on one or more occasions over the course of 12 to 36 months

Case	Blood Group[a]	Operation[b]	Volume of Blood Used (units)	Pre-operative Antibody Tests	Post-operative Antibody Tests			
					Time (months post-op.)	Result	Method[c] and Titre	
1	O Rhesus $\overline{Cc}Dee$ Kell +ve Fy (a +ve)	A V R	14	Negative	6	Anti-E	Enzyme	1:2
2	O Rhesus CCDee Kell +ve Fy (a -ve)	M V R		Negative	23	Anti-E	Enzyme	1:4
3	O Rhesus $\overline{Cc}Dee$ Kell -ve Fy (a +ve)	M V R	6	Negative	5	Anti-E	Enzyme I A H G T	1:16 1:1
4	A Rhesus CCDee Kell -ve Fy (a +ve)	A V R	20	Negative	4	Anti-E	Enzyme I A H G T	1:8 1:2
5	A Rhesus CCDee Kell-ve Fy (a +ve)	M V R + Aortic valvotomy	16	Negative	14	Anti-E	Enzyme	1:8
6	O Rhesus $\overline{Cc}Dee$ Kell -ve Fy (a -ve)	M V R	9	Negative	6	Anti-E	Enzyme	1:8
7	O Rhesus CCDee Kell -ve Fy (a -ve)	A + M V R	13	Negative	9 9	Anti-E Anti-$\overline{c}$	Enzyme Enzyme I A H G T	1:4 1:16 1:2
8	A Rhesus $\overline{Cc}Dee$ Kell -ve Fy (a -ve)	A V R A V R (repeat)	11 16	Negative Negative	13 6	Anti-P_1 Anti-E	Room temp. Enzyme	Saline 1:2

TABLE XXXVIII DETAILS OF RESULTS IN PATIENTS WITH ALLOANTIBODIES/CONTINUED

Case	Blood Group[a]	Operation[b]	Volume of Blood Used (units)	Pre-operative Antibody Tests	Post-operative Antibody Tests			
					Time (months post-op.)	Result	Method[c]	and Titre
9	A Rhesus $C\bar{c}Dee$ Kell -ve Fy (a +ve)	M V R	13	Negative	4 28	Anti-E Anti-Kell	Enzyme Enzyme I A H G T	1:4 1:16 1:8
10	B Rhesus $C\bar{c}Dee$ Kell -ve Fy (a -ve)	M V R	24	Negative	71	Anti-Kell	I A H G T	1:8
11	B Rhesus $C\bar{c}Dee$ Kell -ve Fy (a +ve)	M V R	8	Negative	7	Anti-Kell	Enzyme	1:2
12	O Rhesus $C\bar{c}Dee$ Kell -ve Fy (a +ve)	A + M V R		Negative	16	Anti-Kell	I A H G T	1:8

Notes: a Rhesus phenotypes were determined by tests with anti-C, anti-$\bar{c}$, anti-D, anti-E and anti-$\bar{e}$.
b A V R = aortic valve replacement, M V R = mitral valve replacement.
c Only the method(s) yielding positive reactions are tabulated; I A H G T = indirect anti-human globulin test.

TABLE XXXVIII DETAILS OF RESULTS IN PATIENTS WITH ALLOANTIBODIES.

Case	Antibody Test	Times Post-operative (months) 3-6	6-12	12-24	24-36	36-48	48-60	60+	Antibody Titre Change[a]
1	Positive (anti-E)		xx		x				incr. (enzyme)
2	Positive (anti-E)			x		x	x		incr. (enzyme)
3	Positive (anti-E)	x		xx		x			decr. (enzyme) incr. (I.A.H.G.T.)
4	Positive (anti-E)	x		x					decr. (enzyme)
	Negative (anti-E)			xx					absent (I A H G T)
5	Positive (anti-E)			x					
6	Positive (anti-E)	x		x					decr. (enzyme)
7	Positive (anti-E)		xx			xx			incr. (enzyme)
	Positive (anti-c)		xx						decr. (enzyme &
	Negative (anti-c)					xx			I A H G T)
8	Positive (anti-P_1)			x		x[b]			
	Negative (anti-P_1)			xxx	xxx				
	Positive (anti-E)		x[c]						
9	Positive (anti-E)	x							
	Negative (anti-E)				xx				
	Positive (anti-Kell)				xx				same (enzyme & I A H G T)
10	Positive (anti-Kell)							xx	decr. (I A H G T)
11	Positive (anti-Kell)		x		xx				same (enzyme)
	Negative (anti-Kell)		x	x					
12	Positive (anti-Kell)			xx					decr. (I A H G T) enzyme +ve 1:2

Notes: a incr. = increased; decr. = decreased; I A H G T = indirect anti-human globulin test.
b test done immediately before repeat aortic valve replacement.
c test done six months after repeat aortic valve replacement.

TABLE XXXIX RESULTS OF FOLLOW-UP STUDIES ON ALLOANTIBODY POSITIVE PATIENTS.
(Each cross represents a separately timed investigation).

with negative results.

In addition to the 12 patients who developed antibodies post-operatively a further seven gave positive results. In five the antibodies detected were anti-C (1 patient), anti-C plus anti-D plus anti-Kell (1 patient), anti-E (1 patient), and unidentified antibodies (2 patients). These had all, however, been detected prior to the valve replacement operation and so cannot be attributed to it. Another patient, a Rh (D)-negative male, developed anti-D following operation as a consequence of the transfusion of Rh (D)-positive blood. In one other patient anti-C was detected 25 months after operation but 2 months following a six unit blood transfusion for severe traumatic haemolytic anaemia. Previous post-operative investigations for alloantibodies had been negative so that the anti-C was presumed secondary to the later transfusion.

III. DIRECT ANTI-HUMAN GLOBULIN (COOMBS') TEST.

Direct anti-human globulin (Coombs') tests carried out in all 156 patients were negative. Repeat tests performed over the course of 12 to 36 months in 42 patients remained negative.

CHAPTER 10

DISCUSSION AND CONCLUSIONS

Antibodies to red cell antigens may develop following ABO and Rhesus (D) compatible blood transfusion since it is virtually impossible to match donor and recipient blood completely. However, the remaining blood group antigens appear to be relatively weak antigenic stimuli, and the overall incidence of alloimmunization following ABO and Rhesus (D) compatible transfusion has been reported as approximately 1%[251], and is probably less with single transfusions[249]. Massive transfusions contain a potentially greater variety as well as a larger volume of foreign antigen, but there have been few studies of their effect on allo-immunization and results are conflicting.

Wallace and Henry[103] found alloantibodies in two of 65 (3%) open heart surgery patients (average transfusion 17 units), and concluded that massive transfusion probably does not increase the risk of alloimmunization. Perkins[87] found an incidence of 5.2% in 539 open heart surgery patients (average transfusion 13 units), but after exclusion of possible "naturally occurring" and cold antibodies considered that only 1.5% of cases had developed clinically significant antibodies after operation. Polesky, Smith and Weirich[88] in a study of 100 post-operative cardiac surgery patients detected two with blood group specific antibodies. On the other hand, Lostumbo, Holland and Schmidt[86] reported an incidence of post-transfusion alloimmunization of 34% in 127 patients who had been exposed to an average of 24.9 units of blood during open heart surgery. After exclusion of non-specific and cold panagglutinating antibodies they found the incidence of blood group specific antibodies to be 24%, and concluded that the risk of alloimmunization is additive and approximately 1% per unit of transfused blood.

In this study, 7.7% of 156 patients developed blood group specific antibodies after open heart surgery. Anti-E was found in 10.8% of 83 Rh (E)-negative patients, and anti-Kell in 2.9% of 136 Kell-negative cases. The total quantity of blood used during operation and in the immediate post-operative period in the affected patients varied from 6 to 24 units (mean $\pm$ s.d. = 13.6 $\pm$ 5.3), and similar volumes were used in the unaffected patients. Three patients had had blood transfusions of up to 4 units six to 10 years before the valve operation. One other (case 8) received a further 11 units of blood between his first and corrective second operation because of the development of haemolytic anaemia. Six patients had had one or more pregnancies between four and 24 years before operation. However, all of the affected cases had been investigated for alloantibodies more than once pre-operatively, including in the immediate pre-operative period, with negative results. It is most likely, therefore, that the antibodies detected post-operatively were a consequence of the transfusion at operation. One probable exception is the first antibody (anti-P_1) found in case 8 which, while not detected before his first operation, is by its nature and serological reaction unlikely to have been immune in origin[252]. The high incidence of allo-immunization found in this study suggests that its development may be directly related to the volume of blood used, although the interesting possibility has been raised that an extracorporeal circulation may enhance the antigenicity of donor red cells[253].

There are a number of possible explanations for the wide divergence of results in these studies. Wallace and Henry[103] did not employ an enzyme-treated red cell method which is considered the most sensitive technique for detecting Rhesus antibodies[254-256]. Lostumbo et al.[86] included in their final calculation several antibodies often regarded as "naturally occurring"[249,252]. The timing and frequency of the anti-body screening procedure is also of importance. From observations on

the production of anti-D in Rhesus (D)-negative recipients[104], it has been suggested that the best time to detect alloimmunization is 20 weeks after transfusion, and it was found that anti-D was unlikely to be detected before 8 or 9 weeks had elapsed. Screening only in the early post-operative period, as in the study of Polesky et al.,[88] is therefore unlikely to detect a developing immune antibody. There is also, conversely, the risk that positive results in the immediate post-operative period will be due to passively administered antibody. Rhesus antibodies once formed usually persist for many years[252], but loss of previously detectable antibody activity by in-vitro tests is not necessarily paralleled by a disappearance of agglutinating or haemolytic potency in-vivo[257,258]. In this study, antibodies initially detected in five patients were not found at subsequent screens, although this was a temporary phenomenon in two of them. It is clear that one negative antibody investigation does not exclude the possibility of alloimmunization.

The antibodies that most frequently develop after transfusion of ABO and Rh (D) compatible blood are anti-Kell, anti-E, and anti-$\bar{c}$ [249,251,259]. In this study, four examples of anti-Kell, nine of anti-E and one of anti-$\bar{c}$ were found. Giblett[249], from a consideration of data on the population incidence of the various blood group antigens and their corresponding antibodies, has estimated the relative potency of a given antigen by comparing it with the potency of the Kell antigen. This she has taken to be 0.05, that is, that anti-Kell may be expected to develop in 5% of transfusions involving a Kell-positive donor and a Kell-negative recipient. The corresponding figure for anti-E was 1.69%, the E-antigen being calculated to be about one-third as potent (0.0169) as the Kell antigen. Using Giblett's method of calculation (Appendix B), the results in this study give a figure for the relative potency of the E-antigen of 0.0375. Any comparison of estimates of antigen potency, however, must take into account possible differences in the manner, amount and frequency of

exposure to the foreign antigen. It appears likely, nonetheless, that after massive transfusion Rh (E)-negative patients may be at greater risk of developing anti-E than has perhaps been realized.

Alloantibodies may be responsible for haemolytic transfusion reactions and for difficulty obtaining adequate supplies of compatible blood. Patients with prosthetic heart valves are at particular risk of requiring further transfusion since most are on anticoagulants, a few will require blood for secondary haemolytic anaemia, and some may ultimately come again to cardiac surgery. While it would be desirable for patients being prepared for open heart surgery to receive blood which was, for example, Rhesus E negative and/or Kell-negative if they lacked these antigens, in routine blood transfusion service this is generally impractical. However, following operation, or in any patient after massive transfusion, investigations for alloantibodies should become part of the routine follow-up. In the event of a positive result the patient should be informed and should carry a record of the serological details.

The direct anti-human globulin (Coombs') tests carried out in all 156 patients, and repeated over the course of one to three years in some, were all negative. Pirofsky et al.,[84] from the observation of a positive direct Coombs' test with haemolytic anaemia in patients after heart valve replacement, suggested that there may be an autoimmune component to the mechanical haemolysis associated with prosthetic valves. As has been fully discussed in the literature review chapter (p.41) this possibility remains unproven, and cytomegalovirus infection with an associated Coombs-positive haemolytic anaemia appears a more likely explanation for at least some of Pirofsky's patients. The finding in this study of uniformly negative Coombs' tests despite a high incidence of haemolysis lends no support to the existence of an auto-immune factor.

Conclusions

In this section serological investigations were carried out to determine the frequency with which alloantibodies develop following the massive transfusion that is often required with heart-lung by-pass for prosthetic valve replacements. Evidence was also sought from the results of the direct Coombs' test for the possibility of an autoimmune component to the haemolysis encountered in these patients. The following conclusions can be made:-

1. Massive transfusion at prosthetic valve replacement operations appears to be associated with an increased risk of allo-immunization.
2. Rhesus E negative persons may be at particular risk, and the potency of the E antigen may be greater than previously suspected.
3. Investigations for alloantibodies should be part of the routine follow-up in patients with prosthetic valves, and reliance must not be placed on the finding of one negative result.
4. Autoimmune factors do not appear to be operative in the genesis of the traumatic haemolysis that occurs with prosthetic valves.

CHAPTER 11
FINAL COMMENTARY

There are few, if any, advances in Medicine that are not attended by potential adverse effects, and prosthetic cardiac valve replacement is no exception. However, the ability to replace damaged cardiac valves has transformed the outlook in many patients with rheumatic or congenital heart disease, and any complications must be viewed against this perspective. In Starr's experience[195] the 5-year survival for aortic or mitral replacement with the composite-seat, cloth-covered valves is approximately 80%, with a re-operation rate at 5 years of 10% for aortic prostheses. In this thesis the haemolytic complications have been examined, and notwithstanding a very substantial incidence of haemolysis and haemolytic anaemia with some prostheses, the majority of patients studied have benefitted from operation. Nonetheless, while haemolysis and haemolytic anaemia may be an acceptable price to pay for an overall clinical improvement, their existence cannot be regarded as satisfactory. The development or progression of haemolysis may, furthermore, parallel or herald a breakdown in some aspect of prosthesis structure or function. A regular haematological assessment has, therefore, prognostic as well as diagnostic value.

The basic requirements of an ideal valve substitute were defined by Harken and his associates in 1962[260], but as yet no valve of prosthetic or natural material fulfils all of their criteria. The Starr-Edwards ball and cage prostheses have tended to act as yardsticks against which other valve substitutes are compared, as they are probably still the most widely used form of valve replacement. With regard to the totally cloth-covered metal ball models there is no doubt that an increase in the haemolytic complications has occurred. However, this has gone hand in hand with a diminution in the thrombo-embolic complications, a potentially greater source of morbidity and mortality. The introduction of the wider orifice composite-seat valves appears to have had a favourable

influence on the haemolytic problems, and insofar as these may be related to cloth wear and tear, a recent Starr-Edwards development may lead to a further reduction in haemolysis. This is a cloth-covered, composite-seat model, in which a protective metal track has been added to the cloth on the inner aspect of the cage struts[195]. By contrast, the Björk-Shiley tilting disc valve undoubtedly represents an advance as far as the haemolytic properties of prosthetic valves are concerned, but it still requires overall evaluation in the long-term. The problems of haemolysis and especially of thrombo-embolism are much less with homograft or heterograft valves, or with valves made from autologous tissue such as fascia lata[28,73,206,261-264]. These, are, however, not so readily available and are technically more difficult to insert, nor is their long-term function and durability yet quite so relatively well established in general as that of prosthetic valves.

The controversy for and against the various types of valve prosthesis, or over natural as opposed to prosthetic valve replacement continues[265,266]. Hopefully, from this will emerge a readily available valve substitute which approximates more closely to the ideal. Close scrutiny of the haemolytic properties both by in-vitro techniques and by continued clinical observation is essential to the identification of such a structure.

APPENDIX A

RESULTS OF STATISTICAL ANALYSES

Prosthesis Models[a]	Haemolysis	Red Cell Fragmentation	Haemolytic Anaemia
AORTIC VALVES			
2300 v[b] B-S	$X^2_2 = 13.12$, $P<0.0005$	$X^2_2 = 9.31$, $0.005>P>0.001$	$X^2 = 9.85$, $0.005>P>0.001$
2310 v B-S	$X^2 = 8.95$, $0.005>P>0.001$	$X^2 = 3.99$, $0.05>P>0.025$	N.S.
2300 v 2310	N.S.	N.S.	N.S.[c]
MITRAL VALVES			
6300 v B-S	$X^2_2 = 13.62$, $P<0.0005$	N.S.	N.S.
6310/20 v B-S	$X^2 = 17.51$, $P<0.0005$	$X^2 = 4.72$, $0.05>P>0.025$	N.S.
6300 v 6310/20	N.S.	N.S.	N.S.
600/6120 v B-S	N.S.	N.S.[d]	N.S.
600/6120 v 6300	$X^2_2 = 12.96$, $P<0.0005$	N.S.	N.S.
600/6120 v 6310/20	$X^2 = 16.81$, $P<0.0005$	$X^2 = 5.71$, $0.025>P>0.01$	N.S.
MULTIPLE VALVES			
S-E v B-S	$X^2 = 12.43$, $P<0.0005$	$X^2 = 8.42$, $0.005>P>0.001$	$X^2 = 5.68$, $0.025>P>0.01$

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards
b All X^2 tests have one degree of freedom; N.S. = not significant, i.e. $P>0.05$, but probabilities between 10% and 5% ($0.1>P>0.05$) are noted
c $X^2_2 = 3.07$, $0.1>P>0.05$
d $X^2 = 3.09$, $0.1>P>0.05$

TABLE XL STATISTICAL COMPARISON (CHI-SQUARED TEST) OF THE INCIDENCE OF HAEMOLYSIS, RED CELL FRAGMENTATION, AND HAEMOLYTIC ANAEMIA BETWEEN DIFFERENT VALVE TYPES.

Prosthesis Models[a]	Haemolysis	Red Cell Fragmentation	Haemolytic Anaemia
Aortic v Mitral Valves			
2300 v[b] 6300	N.S.	$X^2 = 4.64$, $0.05 > P > 0.025$	$X^2 = 10.41$, $0.005 > P > 0.001$
2310 v 6310/20	N.S.	N.S.	N.S.
B-S Aortic v B-S Mitral	N.S.	N.S.	N.S.
Total AVR v Total MVR	N.S.	$X^2 = 6.76$, $0.01 > P > 0.005$	$X^2 = 7.84$, $0.01 > P > 0.005$
Aortic v Aortic plus Mitral (± Tricuspid) Valves			
S-E Aortic (2300/10) v S-E Aortic + Mitral (± Tricuspid)	N.S.	N.S.	N.S.
B-S Aortic v B-S Aortic + Mitral (± Tricuspid)	N.S.	N.S.	N.S.
Total AVR v Total A + M (± T) VR	N.S.	N.S.	N.S.
Mitral v Mitral plus Tricuspid Valves			
Total MVR v Total M + TVR	N.S.	N.S.	N.S.

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards; AVR = aortic valve replacement; MVR = mitral valve replacement; TVR = tricuspid valve replacement.

b All X^2 tests have one degree of freedom; N.S. = not significant, i.e. $P > 0.05$

TABLE XLI STATISTICAL COMPARISON (CHI-SQUARED TEST) OF THE INCIDENCE OF HAEMOLYSIS, RED CELL FRAGMENTATION, AND HAEMOLYTIC ANAEMIA BETWEEN DIFFERENT VALVE SITES.

Prosthesis Models[a]	Haemoglobin Level	Reticulocyte Count
Aortic Valves		
2300 v B-S[b]	t = 3.59, d.f. = 21, 0.005>P>0.001	t = 3.64, d.f. = 15, 0.005>P>0.001
2310 v B-S	t = 3.07, d.f. = 31, 0.005>P>0.001	t = 2.71, d.f. = 24, 0.02 > P>0.01
2300 v 2310	N.S.	t = 2.79, d.f. = 16, 0.02 > P>0.01
Mitral Valves		
6300 v B-S	N.S.	N.S.
6310/20 v B-S	t = 2.42, d.f. = 26, 0.025>P>0.02	t = 3.24, d.f. = 21[c], 0.005>P>0.001
6300 v 6310/20	N.S.	N.S.
600/6120 v B-S	N.S.	N.S.
600/6120 v 6300	N.S.[d]	N.S.
600/6120 v 6310/20	t = 2.49, d.f. = 28, 0.02 > P>0.01	t = 3.22, d.f. = 16, 0.01 > P>0.005
Aortic plus Mitral (± Tricuspid) Valves		
S-E v B-S	t = 4.13, d.f. = 18, P<0.001	t = 3.48, d.f. = 11, 0.01>P>0.005

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards
b d.f. = degrees of freedom; N.S. = not significant, i.e. P>0.05, but probabilities between 10% and 5% (0.1>P>0.05) are noted.
c t = 1.72, d.f. = 34, 0.1>P>0.05
d t = 1.70, d.f. = 34, 0.1>P>0.05

TABLE XLII STATISTICAL COMPARISON (STUDENT'S t TESTS) OF HAEMOGLOBIN LEVEL AND RETICULOCYTE COUNT BETWEEN DIFFERENT VALVE TYPES

Prosthesis Models[a]			Elevated LDH	Elevated AsT	Elevated Bilirubin
Aortic Valves					
2300	v[b]	B-S	$X^2_2 = 9.79$, $0.005 > P > 0.001$	$X^2 = 7.34$, $0.01 > P > 0.005$	$X^2 = 4.66$, $0.05 > P > 0.025$
2310	v	B-S	$X^2 = 4.77$, $0.05 > P > 0.025$	N.S.	N.S.
2300	v	2310	N.S.	N.S.	N.S.
Mitral Valves					
6300	v	B-S	$X^2_2 = 14.00$, $P < 0.0005$	N.S.	N.S.
6310/20	v	B-S	$X^2 = 15.60$, $P < 0.0005$	N.S.	N.S.
6300	v	6310/20	N.S.	N.S.	N.S.
600/6120	v	B-S	N.S.	N.S.	N.S.
600/6120	v	6300	$X^2_2 = 9.96$, $0.005 > P > 0.001$	N.S.	N.S.
600/6120	v	6310/20	$X^2 = 11.59$, $0.001 > P > 0.0005$	N.S.	N.S.
Multiple Valves					
S-E	v	B-S	$X^2 = 12.60$, $P < 0.0005$	$X^2 = 4.55$, $0.05 > P > 0.025$	N.S.

Notes: a B-S = Bjork-Shiley; S-E = Starr-Edwards
b All X^2 tests have one degree of freedom; N.S. = not significant, i.e. $P > 0.05$

TABLE XLIII STATISTICAL COMPARISON (CHI-SQUARED TEST) OF THE INCIDENCE OF ELEVATED SERUM LACTATE DEHYDROGENASE (LDH), ASPARTATE AMINOTRANSFERASE (AsT), AND TOTAL BILIRUBIN LEVELS BETWEEN DIFFERENT VALVE TYPES.

APPENDIX B

METHOD OF CALCULATING THE RELATIVE ANTIGENIC POTENCY OF THE RHESUS E ANTIGEN (after Giblett[249])

Estimated Potency of Rh (E)

$$= \frac{\text{No. of cases of anti-E}}{\text{No. of cases of anti-Kell}} \times \frac{\text{Probability of exposure to the Kell antigen}}{\text{Probability of exposure to the Rh (E) antigen}} \times \text{Potency of the Kell antigen}$$

Probability of exposure to antigen x = Proportion of the Population ("donors") with x X Proportion of the Population ("recipients") without x

In this study the relative antigenic potencies can be more precisely estimated in populations all of whose members lack the antigens, as all of the recipients without antigens Rh (E) or Kell have been identified; the Probability of Exposure to these antigens in them, therefore, simply = the Proportion of the Population ("donors") with the antigens. These proportions are taken from the results tabulated in Table XXXVII, P. 192

Thus Estimated Potency of Rh (E)

$$= \frac{9 \times 0.11 \times 0.05 \text{ (from Giblett)}}{4 \times 0.33}$$

$$= 0.0375$$

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