

**FURTHER EXPERIENCE WITH A CONCENTRATE CONTAINING
HUMAN ANTIHAEMOPHILIC FACTOR**

BY

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Further Experience with a Concentrate Containing Human Antihaemophilic Factor*

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SINCE Kekwick and Wolf (1957) described the separation of a globulin fraction of human plasma rich in antihaemophilic factor and its use in six haemophilic patients, it has been possible to prepare this fraction on a limited scale and use it to control spontaneous and traumatic haemorrhage or haemorrhage occurring during surgical operations in patients suffering from classical haemophilia at Lewisham Hospital, Hammersmith Hospital and the Radcliffe Infirmary and Churchill Hospital between 1956 and 1960. The fraction has also been used on a few occasions at other hospitals. This paper describes the plasma fractions used, brief details of the patients, their response to treatment and the transfusion reactions observed in

CORRIGENDUM

For N. MCGIBBON, a co-author of this paper, read BARBARA H. MACGIBBON.

The method of Kekwick and Wolf (1957), modified in certain details so that it could be employed for plasma pools of 14–22 litres, was used to separate the globulin fraction rich in AHF from citrated blood specially collected by the North London, South London, Oxford and Cambridge Transfusion Centres. The fraction was dried in 100 ml. amounts from the frozen state in standard blood transfusion bottles and reconstituted before use by the addition of 100 ml. sterile distilled water. During the period 1957–59, the protein content of the wet preparation before drying averaged 2.01 g. per 100 ml. (range 1.17–2.53) and the fibrinogen content 65.7 per cent (range 35.3–89.0), as determined by the ratio of Clottable N : Total N.

* A report to the M.R.C. Working Party on Human Antihaemophilic Globulin.

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The concentration of antihaemophilic factor is expressed in one or other of two ways: (i) as a percentage of the normal plasma concentration taken as 100 per cent, (ii) as units, described by Wolf (1956), the average normal plasma concentration being 13.2 units per ml. (range 8–18 units per ml.).

The abbreviation AHF is used to denote antihaemophilic factor. The term human antihaemophilic globulin concentrate (AHG concentrate or HAHG) is used to denote the globulin fraction of human plasma rich in antihaemophilic factor. The clinical severity of the disease was graded according to the criteria described by Biggs and Macfarlane (1958).

HUMAN ANTIHAEMOPHILIC GLOBULIN CONCENTRATE

(Maycock, Evans, Vallet and Combridge)

The method of Kekwick and Wolf (1957), modified in certain details so that it could be employed for plasma pools of 14–22 litres, was used to separate the globulin fraction rich in AHF from citrated blood specially collected by the North London, South London, Oxford and Cambridge Transfusion Centres. The fraction was dried in 100 ml. amounts from the frozen state in standard blood transfusion bottles and reconstituted before use by the addition of 100 ml. sterile distilled water. During the period 1957–59, the protein content of the wet preparation before drying averaged 2.01 g. per 100 ml. (range 1.17–2.53) and the fibrinogen content 65.7 per cent (range 35.3–89.0), as determined by the ratio of Clottable N : Total N.

* A report to the M.R.C. Working Party on Human Antihaemophilic Globulin.

The AHF content was measured by the prothrombin conversion ratio assay of Wolf (1956) and more recently by a modification by Wolf (1959) of a method described by Hicks and Pitney (1957).

The AHF content differed from batch to batch. During the period 1957-59, the potency expressed in Wolf units varied from 2.1 to 8.13 units per mg. of nitrogen. The plasma equivalent per 100 ml. of AHG concentrate, before freeze-drying, of the batches prepared in the same period ranged from 300 to 900 ml. fresh citrated plasma.

Some loss of potency occurs during freeze-drying. Because the amounts of AHG concentrate that can be made are small, it is not thought justifiable to assay samples of all batches after freeze-drying, and examination was therefore restricted to 'short' bottles or batches rejected for one reason or another. Examination of 16 such samples showed an average loss of potency of 15 per cent in relation to the AHF content before freeze-drying. During the period of the survey, the plasma equivalent of AHG was calculated from the AHF content of the wet preparation before drying.

OBSERVATIONS FROM THE LEWISHAM HOSPITAL

(Wolf)

Between January 1957 and February 1960, 30 haemophiliacs were treated on one or more occasions. Reliance was placed mainly on AHG concentrate of which some 460 bottles were used. In addition, 49 bottles of blood* (36 of which were stored blood) were given to replace red cells lost by haemorrhage and about 24 litres of fresh citrated plasma were used when AHG concentrate was scarce.

METHODS

Administration of AHG Concentrate

The dried material was reconstituted by mixing with 100 ml. sterile distilled water. When more than 100 ml. were to be given, the contents of several bottles were pooled. The concentrate was usually given at a rate of 100 ml. in 15-30 minutes and the needle was removed immediately the transfusion was finished to reduce the risk of thrombophlebitis.

AHF Assay Technique

Two methods were used:

- (a) The prothrombin conversion ratio assay (Wolf, 1956);
- (b) A modified thromboplastin generation assay (Wolf, 1959) based upon the technique described by Hicks and Pitney (1957). The AHF potency is expressed in terms of units described by Wolf (1956).

PATIENTS AND THEIR TREATMENT

Of the 30 haemophiliacs treated, 19 had a clinically severe or moderately severe and 11 a clinically mild form of the disease. The observed plasma AHF levels corresponded with the degree of clinical severity, except in patient L.12 whose haemophilia was clinically mild; on five occasions only traces of AHF were found in his plasma. Fourteen of the 30 patients were aged 15 or less at the time of their first admission.

* One bottle of blood contains approximately 420 ml. of blood and 120 ml. ACD anticoagulant solution.

Of 13 patients who underwent dental extractions (see later), eight were between them admitted on 65 occasions for the treatment of various other episodes, four of these patients being admitted on 12, 22, 11 and 11 separate occasions respectively, mainly for the treatment of traumatic or spontaneous tissue or joint haemorrhage.

The remaining 17 patients who did not undergo dental extractions were between them admitted on 74 occasions (two on 18 and 10 separate occasions respectively) in the majority of instances for the treatment of traumatic or spontaneous tissue or joint haemorrhage. Below are brief notes on some of these episodes.

Operations

AHG concentrate was used to control haemorrhage in the following operations: second stage orchidopexy, removal of a perforated gangrenous appendix, gastrectomy, exploratory laparotomy, retroperitoneal evacuation of a psoas haematoma causing femoral nerve paralysis, excision of an encysted haematoma on forehead, arthrodesis of right subastragaloid joint together with tendon transplantation.

Torek's orchidopexy. Patient L.4 (mild). Haemophilia was diagnosed for the first time when a large haematoma formed after the first stage of the operation performed at another hospital. He was then treated by transfusion of fresh blood and remained in hospital 6 weeks. Immediately before the second stage, performed at Lewisham Hospital, 200 ml. of AHG concentrate were infused. There was no undue haemorrhage and recovery was uneventful; he was discharged 13 days after operation.

Appendicectomy. Patient L.17 (moderately severe). The patient was admitted with abdominal pain, vomiting, generalized abdominal tenderness and rigidity. He was immediately given 300 ml. of AHG concentrate, the abdomen being opened while the last 50 ml. were running in. A perforated gangrenous appendix, purulent haemorrhagic peritoneal exudate and haemorrhage in the wall of the caecum and over the right ilio-psoas muscle were found. The appendix was removed and the peritoneal cavity drained without undue haemorrhage occurring. The abdomen was closed without inserting a drain. On the 1st and 2nd post-operative days 200 ml. of concentrate were given, and on the 3rd, 4th and 5th days 100 ml. The sutures were removed on the 7th day and the patient discharged on the 15th day after an uneventful recovery. Haemorrhage was prevented during operation and post-operatively with a total of 1000 ml. of AHG concentrate, equivalent to about 8.1 litres of fresh plasma, no blood or fresh plasma being required.

Gastrectomy. Patient L.24 (mild). The operation was performed because of severe repeated haematemesis. Before operation 300 ml. of concentrate (equivalent to 2.7 litres of fresh plasma) were given and there was no undue haemorrhage. On the 1st, 2nd and 3rd post-operative days 200 ml. (altogether equivalent to 4.8 litres of fresh plasma) were given and, on the 4th day, 100 ml. (equivalent to 0.9 litres of fresh plasma). On the 8th day, melaena was observed and a small haematoma formed in the abdominal wall: 300 ml. concentrate (equivalent to 2.7 litres of fresh plasma) were given, followed by 200 ml. (equivalent to 1.8 litres of fresh plasma) on the 9th day. No further infusions were needed and the patient was discharged for convalescence 24 days after operation.

Retroperitoneal evacuation of a large haematoma in the left psoas muscle. Patient L.2 (severe). A recurrence of a spontaneous haemorrhage in the left psoas muscle was for 2 days treated inadvertently with a batch of AHG concentrate shown later to be inactive. This allowed a large haematoma to form, pointing below the inguinal ligament and involving the femoral nerve plexus, with consequent paresis of the quadriceps muscles and widespread sensory loss.

Before operation 300 ml. of AHG concentrate were given. Blood clot, some partly organized, was removed manually and about 150 ml. of blood were removed by suction and a drain left in the wound. Immediately after operation 300 ml. of AHG concentrate were given followed by two bottles of stored blood to correct anaemia (Hb pre-op; 8.5 g. per 100 ml.). On the 1st day after operation, 300 ml. of concentrate were given and the drainage tube was removed during this transfusion; no bleeding occurred. Motor power and sensation began to improve during the 1st week after operation. During the period of rehabilitation and convalescence, 1900 ml. of concentrate (equivalent to some 10 litres of fresh plasma) and 1200 ml. of fresh frozen plasma were transfused, the patient being discharged 6 weeks after operation. He attended as an out-patient for physiotherapy and 8 weeks after operation could walk and climb stairs without the aid of a stick or splint.

Laparotomy for haematemesis. Patient L.18 (mild). This patient was first diagnosed as haemophilic after admission for acute haematemesis. Eleven bottles of stored blood and 1000 ml. of fresh plasma were used in trying to control the haematemesis. After the diagnosis of haemophilia was made, 300 ml. of AHG concentrate were infused during laparotomy. Gastrectomy was not performed as no bleeding point was found. During the first 4 days after operation, 200 ml. of concentrate and 3000 ml. of fresh plasma were infused. Recovery was uneventful and the patient was discharged 6 weeks after operation. A large volume of plasma was required to treat this patient, not only because of a shortage of AHG concentrate, of which 500 ml., equivalent to 4 litres of fresh plasma were used, but also because after the initial haematemesis he had developed a double deficiency of AHF and Christmas factor; at the time of discharge, however, only the AHF defect persisted.

Arthrodesis of subastragaloid joint with tibial graft and tendon transplant. Patient L.30 (mild). This patient suffered from a severe valgus foot deformity with paralysis of tibialis anterior and tibialis posterior muscles following poliomyelitis in infancy. A total of 700 ml. of AHG concentrate (equivalent to 5.5 litres of fresh plasma) and two bottles of fresh frozen plasma were required to prevent haemorrhage during the operation and convalescence. Recovery was uneventful and the patient was discharged from hospital after 15 days; he remained in plaster for 10 weeks. The final result was satisfactory and the X-rays showed bony union.

Accident. Patient L.5 (mild). This patient was admitted deeply unconscious after his motor cycle had collided with a car. Examination revealed a large deep bleeding scalp wound, heavily contaminated with dirt; a double fracture of the right humerus and a haematoma extending over the right arm. He remained unconscious for the first 2 days and during the following 7 days was partly conscious with prolonged periods of restlessness. Immediately on admission, 300 ml. of AHG concentrate were infused and a further 300 ml. later the same day. To control possible intra-cranial haemorrhage, 300 ml. were given on the 2nd and 3rd days and 200 ml. on the 4th and 5th days. During this period the scalp wound did not bleed and the haematoma of the arm diminished. Later, several secondary haemorrhages occurred from the infected scalp wound and to control these, 1300 ml. of AHG concentrate and three bottles of stored blood were given. Altogether AHG concentrate equivalent to some 19 litres of fresh plasma were used for treating this patient. He was discharged 8 weeks after admission with nystagmus and diplopia which gradually improved. X-ray, 1 month later, showed the fractures to be firmly united.

Haematemesis. Patient L.19 (mild)

This patient was treated for acute haematemesis with two bottles of stored blood and 200 ml. of concentrate. He made an uninterrupted recovery and has had no further attacks since 1958.

Non-operative Treatment of Sudden Abdominal Pain

In three patients, L.2 and L.17 (severe), and L.8 (moderately severe), attacks of abdominal pain were treated by infusions of AHG concentrate on the assumption that, if the pain were caused by intra-abdominal bleeding and not by inflammation or perforation, it would subside after infusion of the concentrate. If, on the other hand, the pain persisted, and laparotomy seemed advisable, the patient would be ready for operation, during which more concentrate could be given, if necessary. In all three patients, pain disappeared after infusion of between 200 and 600 ml. of concentrate. Case L.8 had two such attacks with vomiting, both of which responded to this treatment.

Joint and Tissue Haemorrhages

About 70 per cent of the incidents were tissue haemorrhages or haemarthroses. About half the total amount of AHG concentrate available during the period under review was used for treating these incidents in 1958 and 1959. Later, to conserve the concentrate, more reliance was placed on treatment with fresh frozen plasma. The arrest of bleeding was not as quick with plasma as with AHG concentrate and generally multiple transfusions of plasma, instead of a single transfusion of concentrate, were necessary to achieve lasting haemostasis. In two instances where treatment with plasma failed, AHG concentrate achieved haemostasis. In 1957 all larger tissue and joint haemorrhages were treated with AHG concentrate and, on an average, 100–200 ml. for a child and 200–400 ml. for an adult were needed to stop bleeding. Later it was found that not all larger tissues and joint haemorrhages needed transfusion, certain haemorrhages being followed by complete functional recovery after treatment by bed rest alone: these haemorrhages were usually slow ones in which there was no nerve paralysis and pain and muscle spasm were not intense. Of 20 patients treated for joint and tissue haemorrhages, 13 had no permanent crippling before treatment and the normal range of joint mobility has been maintained and seven patients showed some previous crippling. The degree of disablement in six of these has been diminished, in several of them to a considerable extent. One patient with recurrent haemarthrosis of the knees, with very little accompanying peri-articular haemorrhage has deteriorated, possibly due to inadequate immobilization.

Improvement of previously affected joints was accomplished by judicious use of periods of immobilization, physiotherapy and manipulation performed under the protection of AHG concentrate. Treatment of these tissue and joint haemorrhages is described in detail elsewhere (Wolf, 1960).

Deaths

There were two deaths in children under 3 years of age (patients L.20 and L.29). Patient L.20 died of mediastinal compression from a haemorrhage which had spread rapidly from the neck. Patient L.29 died of an aspiration pneumonia following a trickle of blood from a gum injury.

OBSERVATIONS FROM THE HAMMERSMITH HOSPITAL

(McGibbon, French, Wallett and Dacie)

The principle governing the treatment of haemophiliac patients admitted to Hammersmith Hospital during the period July 1956 to March 1960 has been to use fresh plasma or blood or both whenever possible and most patients have been treated in this way. AHG concentrate

has been used (a) to control bleeding after dental extractions and operations or because the site or degree of haemorrhage made rapidly successful therapy imperative and (b) when treatment with fresh plasma or blood proved inadequate.

METHODS

Administration of AHG Concentrate

Each bottle of concentrate was reconstituted by gentle mixing with sterile distilled water, for 10–20 minutes, immediately before use. The usual time taken to infuse 100 ml. was about 10 minutes, but this rate was slowed if a reaction occurred.

Administration of Fresh Citrated Plasma or Whole Blood

Fresh donor blood was sometimes used as whole blood, but more often the plasma was rapidly separated and given by itself and then followed by a slower transfusion of the packed red blood cells, when restoration of haemoglobin concentration was also indicated.

AHF Assay Technique

The AHF assay technique employed was a modified form of Pitney's method (Pitney, 1956), in which at first rabbit, and later beef, serum was substituted for adsorbed haemophilic plasma as a source of Factor V.

PATIENTS AND THEIR TREATMENT

Between July 1956 and March 1960, 23 incidents in 16 haemophiliacs were treated with AHG concentrate. Of these 16 patients, 12 were severely, one moderately severely and three mildly affected haemophiliacs. Only traces of AHF were detectable in the plasma of two of the mild cases. Seven patients, who underwent dental extractions, were treated with AHG concentrate (see later). Some of the other conditions in the other nine patients and their response to treatment with AHG concentrate are described below.

Haematuria

In three patients with clinically severe haemophilia, haematuria, persisting in spite of transfusing fresh plasma, was rapidly controlled by AHG concentrate. In two instances the bleeding was controlled by single infusions of concentrate, equivalent to 0.8 and 1.5 litres of fresh plasma respectively. In the third patient two infusions of concentrate, 200 and 300 ml. equivalent respectively to 1.7 and 2.4 litres of fresh plasma, and separated by 48 hours, were necessary to stop bleeding.

Injuries

AHG concentrate was valuable for controlling bleeding caused by injuries in dangerous sites. A laceration of the chin in a clinically severe haemophiliac, aged 4, caused a haematoma of the neck which threatened to obstruct respiration. Bleeding was controlled by the rapid infusion of 100 ml. of concentrate equivalent to 0.6 litres of fresh plasma. In another severely affected haemophiliac, aged 5, a progressing haematoma within the mouth, caused by a laceration of the mucous membrane, was rapidly controlled by giving 200 ml. of concentrate equivalent to 1 litre of fresh plasma.

Joint and Tissue Haemorrhage

Because of the policy outlined above, epistaxes and haemarthroses were treated with fresh blood or plasma or both unless there was some other indication for using AHG concentrate.

On the same principle, only two patients with spontaneous tissue haemorrhage were treated with AHG concentrate. In both there was bleeding into the tissues of the floor of the mouth with, in one patient imminent, and in the other likely, respiratory obstruction. Bleeding was rapidly controlled in each case by giving 300 ml. of AHG concentrate equivalent to 1.8 litres of fresh plasma.

Among the other similar incidents, in which bleeding was controlled by the concentrate, were bleeding from an osteomyelitic sinus, haematomata of thigh and of buttock and thigh, haemarthrosis of the hip accompanied by haematoma and drainage of an infected crural haematoma, all occurring in severely affected patients.

Operations

The operations 'covered' by AHG concentrate were nasal polypectomy, dilatation and curettage and, later, hysterectomy, removal of a chondroma and resection of a gangrenous volvulus of the ileum. There were some features of special interest in the latter three cases.

Removal of chondroma. Patient H.9 was a mild haemophiliac with an AHF level of 25 per cent. He had previously required blood transfusions following epistaxes, dental extractions and injury to his mouth, but had never had spontaneous haemarthroses. AHG concentrate equivalent to 1.4 litres of fresh plasma raised his AHF level to 62 per cent pre-operatively. Although no bleeding occurred during or after the removal of the chondroma from his right medial malleolus, a haematoma subsequently developed at the operation site and healing was slow. After 4 months there was still an 'open' area about $\frac{1}{2}$ in. in diameter.

Dilatation and curettage; hysterectomy. Patient H.6 was a clinically mild female haemophiliac (Merskey, 1951, Case V.8), with an AHF level of 0-1 per cent, who presented with post-menopausal bleeding. A dilatation and curettage, dental extractions and, later, a vaginal hysterectomy and pelvic floor repair, were successfully carried out under AHG cover. AHG concentrate was given daily for 9 days following hysterectomy, in amounts equivalent to 2.5 litres of fresh plasma at first, diminishing to the equivalent of 1 litre of fresh plasma. A secondary haemorrhage occurred on the 14th post-operative day. This was controlled by AHG concentrate equivalent to 2.23 litres of fresh plasma and during the early part of this infusion a severe reaction occurred with sweating, loss of vision and consciousness. The reaction ceased when the infusion was stopped and the infusion was then completed slowly. A further episode of vaginal bleeding occurred on the 21st post-operative day and on this occasion, during the infusion of concentrate (equivalent to 1.8 litres of fresh plasma and of the same batch as that given on the 14th post-operative day), the patient complained of blurring of vision and the pulse became feeble. Because of these reactions, the next (and last) episode of vaginal bleeding was controlled with 1 litre of fresh plasma. The patient was discharged on the 38th day after operation with excellent healing.

Resection of volvulus. Patient H.7* was a 19-year-old severe haemophiliac with an AHF level of 0 per cent. He presented as an emergency during the night, with a 3 day history of abdominal pain and symptoms and signs suggesting peritonitis originating from a perforated appendix. No improvement was effected by giving him 200 ml. of AHG concentrate, equivalent to 1.2 litres of fresh plasma. Laparotomy was therefore carried out, under cover of a further infusion of concentrate equivalent to 2.4 litres of plasma. There was a blood-stained peritoneal effusion and a non-viable volvulus of the ileum was resected. The first AHF assay was carried out next morning, about 9 hours after operation, when the level was 54 per cent. The AHF level was above 35 per cent for the first 24 hours. The amount of AHG concentrate

* This case will be reported more fully elsewhere.

and fresh whole blood or plasma used during the first 11 days after operation was equivalent to between 2.5 and 3 litres of fresh plasma daily. For the next 10 days, the daily equivalent was between 1.5 and 2.5 litres, and from the 22nd to the 30th day, between 1.0 and 1.5 litres. For the first week, when the concentrate was given once a day, the average minimum AHF level for each 24 hour period, estimated immediately before infusion, was 11 per cent. From the 8th to the 21st day, the daily therapy was given as two doses at 12 hour intervals and, even though the total amount of AHG concentrate given each day was less than in the first week, the average minimum AHF level for each 24 hour period was 22 per cent. The only post-operative complication was the early formation of a haematoma in the superficial layers of the abdominal wound, with subsequent wound breakdown and infection. This cause of delay in healing would perhaps have been prevented by using rather more AHG concentrate initially, or perhaps by giving it at 12 hour intervals throughout, and thereby saving much. The AHG concentrate (in all equivalent to 60 litres of fresh plasma) was supplemented with fresh blood or plasma from 62 donors. Each source of AHF eventually became exhausted, the human concentrate temporarily after 24 days, and fresh donors a week later. During the short period when none was available, the granulation tissue began to bleed. The patient was eventually discharged, 10 weeks after admission, with a small area in the centre of the wound unhealed but healthy.

OBSERVATIONS FROM OXFORD

(Biggs, Handley and Macfarlane)

The major portion of the AHG concentrate available for use at Oxford was reserved for dental cases, particularly those severely affected haemophiliacs requiring multiple tooth extractions. A certain amount of the concentrate was used to control bleeding after relatively minor operations or in cases where therapy with fresh plasma or blood had proved inadequate. Animal AHG was preferred for most patients undergoing major surgery, as much more is available.

METHODS

Administration of AHG Concentrate

Each bottle of concentrate was reconstituted with distilled water by gentle shaking. This process was sometimes rather lengthy (up to 45 minutes), before all the material dissolved. The resultant solution is turbid and, in a bright light, many small particles can be seen in suspension. The usual rate of infusion was about 15–20 minutes per 100 ml., but this rate was reduced if a reaction occurred.

Administration of Fresh Citrated Plasma or Whole Blood

Fresh donor blood was used as whole blood in patients requiring replacement of blood loss, particularly if this was considerable. In other cases, where blood loss was slight, or prophylactic cover was required, plasma was used. This plasma was separated soon after collection (within 2–3 hours) and either used fresh, or rapidly frozen and stored at -30°C . until used, when it was thawed immediately before use. The usual adult dosage of plasma was 1100–1200 ml. infused in from 45 minutes to 1 hour. The patient was given plasma of the same group, but no special tests for compatibility were carried out.

AHF Assay Technique

AHF was assayed by the method of Biggs (1957) which is based on the thromboplastin generation test, using plasma from severely affected hæmophiliacs as a source of Factor V.

PATIENTS AND THEIR TREATMENT

Between January 1957 and February 1960, 24 hæmophiliacs received human AHG concentrate. Nineteen of these were severely affected, three were moderately severely affected and two were mildly affected hæmophiliacs. AHF was not detected in the plasma of any of the clinically severe patients nor in the plasma of one of the mildly affected patients. Nine of the 24 patients were aged 15 or less at the time of admission.

Two women, not apparently hæmophiliacs, but who had low AHF levels, were successfully treated for severe postpartum hæmorrhages.

The major portion of the available AHG concentrate was used to treat ten patients who underwent dental extractions (see later). The responses to treatment with AHG concentrate in some of the other incidents are described below.

Operations

Being more readily available, animal AHG concentrate was used for major surgical procedures and only three relatively minor operations were carried out using the human concentrate.

Patient O.19 was a severely affected hæmophiliac who presented with a swelling over the upper anterior aspect of the left tibia, which proved on incision to be an extension from a chronic blood cyst of the calf. No attempt was made to excise the cyst and the wound was resutured. Daily infusions of 300–400 ml. of AHG concentrate were given for 7 days and, when the sutures were removed on the 10th day, the wound appeared to be healing. There was no bleeding immediately post-operatively, and the limb was immobilized in a full-length plaster cylinder. When the plaster was removed 6 weeks after operation, the wound had broken down and was discharging dark altered blood. Subsequently, infection necessitated amputation of the leg through the knee, which was done under cover of AHG concentrate.

Patient O.20, a mildly affected hæmophilic boy (AHF levels 15 per cent and 29 per cent on two occasions), had a keloid scar excised from his lip. Several operations for repair of his hare lip had previously been followed by excessive bleeding and, on this occasion, it was decided to start active treatment to prevent this. He was therefore given daily transfusions of plasma for the first 6 days with control of bleeding. Two days after cessation of this therapy there was brisk oozing and he was then given four daily infusions of 100 ml. of animal AHG concentrate. Bleeding was controlled for a period of about 36 hours after each dose, but did not stop completely until 16 days after operation.

In patient O.22, a severely affected hæmophiliac who suffered from chronic osteomyelitis, a sequestrum was removed from the femur by blunt dissection. Bleeding was completely controlled by three infusions of AHG concentrate, each of 300 ml.

External Bleeding following Trauma

Two young children (O.23 and O.24), both aged 2½ years and both severely affected, were admitted with bleeding from the mouth following trauma. In patient O.23, bleeding from a lacerated frenulum of the tongue was temporarily controlled by 100 ml. of AHG

concentrate, but two further similar doses were required before bleeding eventually ceased 10 days after injury.

Patient O.24 was bitten on the upper lip by a dog, and was admitted to another hospital where bleeding was temporarily controlled by transfusing three bottles of fresh whole blood. Two days later profuse bleeding started again and, on arrival at Oxford, his haemoglobin was 27 per cent. Bleeding was controlled by 100 ml. of AHG concentrate, following which he was given two bottles of blood and fresh plasma from two bottles of blood.

The small volume of human AHG concentrate required in young children is an advantage as transfusion is often difficult because of the small size of the veins. Every effort should be made to avoid cannulation by 'open operation', and usually a small needle (SWG 21) can be inserted into a vein percutaneously.

Intramuscular and Intra-abdominal Bleeding

Fresh plasma is usually found to be effective in most patients in this group, and only exceptionally has AHG concentrate been used.

Two patients (O.4 and O.13), both severely affected haemophiliacs, had tense intramuscular haematomata (thigh and calf respectively), with considerable fall in haemoglobin level. Both responded well to AHG concentrate followed by fresh whole blood. One severely affected patient (O.2) with retroperitoneal haemorrhage was given 600 ml. of AHG concentrate in addition to fresh plasma, with control of bleeding.

Sublingual Haematomata

This potentially dangerous type of bleeding usually responds well to fresh plasma and human AHG concentrate has only been used in one patient. Patient O.21, a moderately severe haemophiliac who had a very large haematoma when first seen, was given one infusion of 300 ml. of concentrate in addition to fresh plasma with good result.

Patients with Conditions other than Haemophilia

A woman aged 32 years (O.25) had severe von Willebrand's disease with a low AHF level (7 per cent). She was delivered of a female child by Caesarean section without undue bleeding. Five days post-partum there was severe vaginal bleeding and a total of 11 bottles of fresh blood were given over the course of 4 days. Neither fresh blood nor fresh plasma controlled the bleeding, but it eventually stopped after three infusions of human AHG concentrate (each 400 ml.). Thirteen days later severe bleeding followed the incision of a Bartholin's abscess. Two infusions of a Swedish human AHG preparation (Blombäck and Nilsson, 1958) were given and bleeding ceased after the second. Four bottles of blood were required to replace blood lost.

A 32-year-old woman (O.26), with no previous history suggestive of a bleeding state, had severe vaginal bleeding 9 days after the apparently normal delivery of twins. A low AHF level (10 per cent) was found and, therefore, 900 ml. of fresh plasma were given in addition to fresh blood, but there was no apparent effect on the rate of bleeding. An infusion of 600 ml. of human AHG concentrate, given on the 10th day of bleeding and accompanied by a moderately severe reaction, controlled the bleeding. Over the 10 days of bleeding, a total of 17 bottles of whole blood (mainly fresh), was given. The diagnosis is still uncertain in this case. The low AHF level persists, no evidence of a circulating anticoagulant can be detected and tests of capillary function are normal.

DENTAL EXTRACTIONS

The patients who received AHG concentrate for dental extraction at the three centres are described together because this makes a rational classification easier and the conclusions to be drawn are clearer. In all but four instances (L.10, L.22, L.26 and H.2) general anaesthesia was used. A dental splint was usually applied at Oxford and Hammersmith, but was used for only two out of 13 patients at Lewisham. Many of the patients were treated with fresh frozen plasma in addition to AHG concentrate. This was necessary because sufficient concentrate was not available to control bleeding for the whole post-operative period in all patients. This obscures the picture in some instances but in others AHG concentrate was shown to be effective where plasma had failed to produce complete haemostasis. In all 30 patients received AHG concentrate in varying amounts for the prevention or treatment of bleeding after dental extractions. These included four children from whom deciduous teeth were removed (Table I), nine mildly affected patients from whom adult teeth were removed (Table II),

TABLE I
EXTRACTION OF DECIDUOUS TEETH

Patient	Age (yr.)	Wt. (kg.)	Teeth removed	HAHG given			Reactions	Bleeding
				× 100 ml.	Plasma equiv.			
					Litres	ml./kg./ infusion		
L.1(S)	6	21.6	2	2	1.7	79	Nil	None
L.3(S)	3	16.6	1	1	0.9	54	Nil	None
			2	4	2.2 (as 4 infusions)	—	Nil	None
L.11(S)	8	21.6	2	2	1.4	65	Nil	None
L.22(M)	8	—	1	1	0.9	—	Nil	None

S = Severely affected; M = Mildly affected.

six severely affected patients from whom one to three adult teeth were removed (Table III) and 11 severely affected patients from whom more than three teeth were removed (Table IV).

There was no bleeding after any of the extractions of deciduous teeth, even in severely affected patients, and one infusion of not more than 200 ml. of concentrate was sufficient. One patient (L.3) received four infusions because he was receiving treatment for a large haematoma of the thigh at the same time.

The mildly affected patients had one to ten teeth removed at the same operation and only one received any blood or plasma. Bleeding was usually slight and easily arrested by small infusions of concentrate. The exceptional case (L.19) was admitted with a history of toothache and gross swelling on both sides beneath the jaw. Two apical molar abscesses were diagnosed, one on each side. Before removal under general anaesthesia 200 ml. of concentrate were infused, together with penicillin. Post-operatively a total of 600 ml. of concentrate, one bottle of blood and 1200 ml. of fresh plasma were required.

In the severely affected patients who had one to three teeth removed, bleeding was severe

enough to require blood transfusion in two (L.26 and H.2) and only one patient was free from abnormal bleeding. One patient (O.11) who had three teeth removed was treated mainly with fresh frozen plasma which, though it reduced the amount of bleeding, failed to control it completely. After one infusion of concentrate the bleeding ceased and did not recur.

In Table IV are shown the results for 11 severely affected patients from whom more than three teeth were removed. Of these, six had bleeding sufficient to require blood transfusion and none was entirely free of abnormal bleeding. In only two patients (L.14 and H.10) was

TABLE II
EXTRACTION OF PERMANENT TEETH: MILDLY AFFECTED PATIENTS

Patient	Age (yr.)	Wt. (kg.)	Teeth removed	HAHG given			Reactions†	Bleeding
				× 100 ml.	Plasma equiv.			
					Litres	ml./kg./ infusion		
L.5	31	82.6	9	7 (as 4 infusions)	4.3	13*	Nil	Oozing on days 1, 5, 7 and 9
L.7	30	86	6+	3	2.3	27	Nil	Oozing days 3-7
L.10	17	58	4	2	1.6	28	Nil	Oozing for 2 days
L.12	17	43.5	2	2	1.8	42	— +	None
L.13	23	—	1	(1) 2	1.0	—	— +	Admitted post- operatively with bleeding Bleeding con- trolled
				(2) 1	0.5	—	Nil	
L.19	10	32	2	(1) 200	1.8	56	Nil	Oozing on days 2, 8, 9, 11 and 14
				(2) 100	0.9	28	Nil	
				(3-6) 100	0.6	19*	Nil	
H.4	41	70	2	4	3.5	50	+	No bleeding
H.6	70	76	7	(1) 3	1.7	22	Nil	Bleeding on 17th day stopped by dose 3
				(2) 2	1.1	15	Nil	
				(3) 3	1.7	22	Nil	
O.12	52	80	10	(1) 3	1.8	23	++	No bleeding
				(2) 2	1.0	13	Nil	
				(3) 2	1.0	13	Nil	

* Average volume per kg. of each infusion.

† Reactions: - +, mild; +, moderately severe; ++, severe.

AHG concentrate the only form of antihaemophilic treatment given. One of these patients (H.10) had 14 teeth and some roots removed and did not require any blood transfusion; a total of 3700 ml. of AHG concentrate were used to achieve this result. The rest of the patients received fresh frozen plasma in addition to the concentrate. In patients O.5, O.6, O.7 (first and second extractions), O.8 (first and second extractions) fresh frozen plasma was the main therapeutic agent, the concentrate being held in reserve and used only to control bleeding which was very severe and not greatly affected by plasma. Used in this way the concentrate always stopped the bleeding. Patient O.9 was an immensely fat and very severely affected

patient, with no visible or palpable superficial veins, who needed extraction of all remaining 20 teeth. It was necessary to insert a polythene catheter into a large vein to administer fluids and it was felt that all teeth should be removed within as short a period as possible. Ten teeth were removed at the first operation and since the sockets had remained entirely dry for 5 days the remaining ten were then removed. One day after the second extraction a circulating anticoagulant appeared and thereafter all treatment was ineffective and the patient was treated by blood replacement.

TABLE III
EXTRACTION OF ONE TO THREE PERMANENT TEETH: SEVERELY AFFECTED PATIENTS

Patient	Age (yr.)	Wt. (kg.)	Teeth removed	HAHG given			Reactions†	Bleeding	'Units' of FB, SB, RBC or FP‡
				× 100 ml.	Plasma equiv.				
					Litres	ml./kg./ infusion			
L.15	24	61.5	1	4 (as 3 infusions)	2.7	15*	Nil	Oozing on day 3	—
L.26	23	61.5	3	9 (as 5 infusions)	5.4	18*	+	Bleeding on days 1, 3 and 11	2 FB 3 SB
H.1	41	50	1	1.8	1.8	36	Nil	None	—
H.2	18	64	1	3 1.5	2.7 1.6	42 25	Nil Nil	Oozing Frank bleeding controlled by HAHG	— 1 FB, 1 FP
H.7	17	46	1	3 3 3 4	1.8 1.8 1.8 3.0	39 39 39 65	Nil Nil Nil +	Bleeding on days 1 and 5	— — — 2 FP
O.11	26	67	3	4	3.2	48	++	Bleeding not controlled by FP, stopped by HAHG	10 FP

* Average volume per kg. of each infusion. † Reactions: +, moderately severe; ++, severe.

‡ 'Units' are defined as follows:

FB = One bottle of freshly collected citrated whole blood (approx. 420 ml. blood and 120 ml. ACD).

SB = One bottle of stored citrated whole blood.

RBC = Packed red cells from one bottle of citrated whole blood.

FP = Plasma from two 'units' FB.

As would be expected, the extraction of deciduous teeth gave rise to no bleeding and in general the mildly affected patients had less bleeding than those clinically severely affected. The ideal result of complete freedom from bleeding was seldom achieved in the severely affected patients. The results have shown, however, that almost without exception infusion of AHG concentrate in amounts approximately equivalent to 25–50 ml. of fresh plasma per kg. body weight controlled bleeding for 24 hours and when such doses were given daily bleeding was absent or slight. The dosage given at the different centres was left to the discretion of the various workers and the observations do not allow of any conclusions about the minimum

amounts of AHG concentrate required for haemostasis nor have values for plasma AHF levels after treatment been included in the series. These figures were usually recorded but different methods of assay were used at the different centres; the results were not directly comparable and therefore, in the series as a whole, do not provide useful information.

TABLE IV
EXTRACTION OF MORE THAN THREE PERMANENT TEETH: SEVERELY AFFECTED PATIENTS

Patient	Age (yr.)	Wt. (kg.)	Teeth removed	HAHG given			Reactions†	Days of bleeding	'Units' of FB, SB, RBC or FP‡
				× 100 ml.	Plasma equiv.				
					Litres	ml./kg./ infusion			
L.14	54	51	10	10 (as 5 infusions)	6	24*	+	On days 2-6	3 SB
H.5	50	57	8+	10.5 (as 3 infusions) 0.25	6.3 —	37* —	+ to 1 ++	On day 2 stopped by HAHG Bled day 6	2 FP, 2 RBC 1½ SB
H.10	33	60	3	14 (as 5 infusions)	11.4	38*	+ to 3 doses	On days 3-8 controlled by HAHG	
			7	13 (as 3 infusions)	7.2	40*	+ to 1 dose	On day 6	
			6+	10 (as 2 infusions)	6.4	53*	+	Oozing days 5-10	
O.5	44	—	8 on day 1	5 (as 1 infusion)	3.5	—	Nil	Oozing on day 7 stopped by HAHG	6 FP
			9 on day 15	5 (as 1 infusion)	3.25	—	Nil	Oozing on day 7 stopped by HAHG	8 FP
O.6	51	95	14	4 (as 1 infusion)	3.6	38	++	Oozing on day 4 stopped by HAHG	10 FP
O.7	29	47	6 on day 1	—	—	—	—	Nil	4 FP
			10 on day 3	12 (as 3 infusions)	8.0	57*	+ to 3 infusions	Days 1-5 stopped temporarily by HAHG	10 FP 11 FB
			16 on day 17	30 (as 6 infusions)	23.5	83*	+ to 5 infusions	Oozing days 2-10 stopped temporarily by HAHG	9 SB

DISCUSSION

Human AHG concentrate was at one time considered of doubtful value for treating bleeding in haemophilia (e.g. Aggeler, Alexander, Rosenthal, Tocantins and Dameshek, 1956) because of the variable potency of the preparations available and because there was evidence suggest-

ing that repeated transfusions might lead to a progressively diminishing clinical response (Pohle and Taylor, 1938). Resistance to the action of AHG concentrate might be caused by (a) the development of an inhibitor in the patient's plasma, (b) the presence of severe tissue damage (Brinkhous and Penick, 1954) in the presence of which, it was suggested, the trans-

TABLE IV—continued

Patient	Age (yr.)	Wt. (kg.)	Teeth removed	HAHG given			Reactions†	Days of bleeding	'Units' of FB, SB, RBC or FP‡
				× 100 ml.	Plasma equiv.				
					Litres	ml./kg./ infusion			
O.8	45	74	5+ on day 1 6+ on day 4 4+ on day 20	4 (as 1 infusion) 28 (as 6 infusions)	3.8 19.8	51 45*	+ + -	Oozing 2 days Oozing 7 days stopped by HAHG None	6 FP 10 FP 15 SB —
O.9	43	92	10+ on day 1 10 on day 5	24 (as 3 infusions) 32 (as 4 infusions)	17.6 21.7	64* 59*	+ +	No bleeding till day 5 when inhi- bitor ap- peared then oozing 37 days	22 FB
O.10	21	59	4 2	10 (as 2 infusions) 10 (as 2 infusions)	6.5 5.45	55* 46	+ + + -	Bleeding stopped by HAHG Bleeding stopped by HAHG	2 FP
O.14	36	76	16 6	16 (as 4 infusions) 4 (as 1 infusion)	8.6 2.8	28* 35	+ + to 2 doses Nil	Bleeding stopped by HAHG Bleeding stopped by HAHG	4 FP 9 FB 4 FP
O.16	24	64	6 16	10 (as 3 infusions) 18 (as 6 infusions)	6.2 9.8	32* 25*	+ -	Bleeding not controlled by FP but stopped by HAHG	4 FP 8 FP 6 FB

* Average volume per kg. of each infusion.

† Reactions: + - , mild; + , moderately severe; ++ , severe.

‡ 'Units' as in footnote to Table III.

fused AHG is inactivated by thrombin liberated at the site of injury, or (c) the development of multiple coagulation defects after severe hæmorrhage or injury (Brinkhous and Penick, 1954).

The experience gained from treating 69 hæmophiliacs on 202 occasions in three different centres confirms the original clinical observations of Kekwick and Wolf (1957). When the present observations were made the three centres were using different assay methods and a unified policy of dosage had not been established. The results from the three centres cannot therefore be compared directly. But all the workers are agreed that in controlling bleeding

in severely and mildly affected haemophiliacs in a wide variety of circumstances, and particularly in dental extractions, haematomata and haemarthroses, human AHG concentrate prepared by the method of Kekwick and Wolf was more effective than fresh plasma since a haemostatic AHF level could be more quickly attained because the small volumes necessary could be rapidly injected. Moreover the concentrate was used during 12 major surgical procedures and the haemostatic effect was undoubtedly excellent.

TABLE V
AMOUNT OF HUMAN AHG CONCENTRATE REQUIRED TO PROVIDE A HAEMOSTATIC LEVEL OF AHF IN DIFFERENT HAEMOPHILIACS
(HAMMERSMITH HOSPITAL JULY 1956 TO FEBRUARY 1960)

Patient	Clinical severity	HAHG given			AHF level (%)		Procedure and result
		× 100 ml.	Fresh plasma equivalent		Pre-infusion	Post-infusion	
			Litres	ml./kg. body wt.			
H.1	Severe	1.8	1.8	36	0.5	34	Dental extraction. Haemostasis.
H.2	Severe	3.0	2.7	42	0.1	21	Dental extraction. Initial oozing. Frank bleeding after 48 hours.
H.4	Mild	3.0	2.5	35	0.1	47	Nasal polypectomy. Further 200 ml. HAHG given during first 24 hrs. No bleeding after pack removed on second day. Dental extractions. Haemostasis.
		4.0	3.5	50	0.1	46	
H.5	Severe	4.0	2.4	42	0.1	42	Dental extractions. No bleeding until 24 hours.
H.6	Mild	3.0	1.7	22	0.1	50	Dental extractions. Haemostasis. D. and C. Haemostasis. Hysterectomy. ?Bleeding more than normal during operation, therefore second infusion of HAHG given. Post-operative haemostasis.
		4.0	2.2	29	0.1	66	
		4.0+	3.2+	42+	0.1	21	
		4.0	2.2	29		30	
H.7	Severe	3.0+	1.8+	39+	0	21	Dental extraction. Immediate post-extraction bleeding necessitated second infusion of HAHG. Then satisfactory until fifth day. Resection of volvulus of ileum. Haemostasis.
		3.0	1.8	39		46	
		6.0	3.6	78	0	54 (9 hours after)	
H.10	Moderately severe	(1) 3.0	2.7	45	0.1	18	Dental extractions Upper jaw (1) Slight, becoming frank, bleeding. (2) Haemostasis. Lower jaw (3) } Haemostasis for 5 days. (4) }
		(2) 5.0	4.0	67		25	
		(3) 5.0	3.4	57	0.1	36	
		(4) 5.0	3.0	50	5-6	53	

Response to Human AHG Concentrate

The haemostatic effect of AHG concentrate was closely related to the rise in plasma AHF level obtained. When carrying out operative procedures, it was found that the pre-operative AHF level must be raised to about 25 per cent to secure haemostasis (Table V). This table also shows that the amounts of concentrate, expressed as the litre-equivalents of fresh plasma, needed to achieve this level in different patients varied considerably. It was also observed

that the same patient might exhibit considerable variability in response to dose at different times.

It is probable that discrepancy between the stated and the true potency of the concentrate may have been responsible for some of these variable results. At one centre, particularly during the early part of this investigation, there was considerable disparity between the results of assay of batches of AHG and their stated potency, although at another centre, providing the assays were done immediately after reconstitution of the dried material, the values obtained in a small comparative series (six samples) were approximately those stated for each batch. At the third centre, there was general agreement between the measured and the stated potencies. Although discrepancy between the measured and stated potencies may have been responsible in some instances for the apparent variability of response, that this was not so in all cases is supported by the fact that the same batch would produce good or poor responses in different patients. The difficulty in raising the AHF level in some patients seemed more likely to be related to the clinical severity of the haemophilia or to the extent of the tissue injury responsible for the haemorrhage (see below under 'Resistance to AHG').

The level of plasma AHF produced by an infusion of AHG concentrate fell rapidly and, even after a large dose, the haemostatic effect was seldom prolonged for much over 24 hours. Thirteen series of observations in five patients at Hammersmith failed to disclose any correlation between the initial ease or difficulty in raising the level of plasma AHF and the subsequent rate of disappearance of AHF activity from the plasma. On the assumption that the rate of 'loss' of plasma AHF follows an exponential pattern, as was suggested by observations made on patient H.4, the time at which only 50 per cent of the initial activity was detectable in the plasma was calculated from the results of assays carried out at, for example, 0, 4, 9, 12, 24 and 48 hours after infusion. This value ranged from 7 to 17 hours, average approximately 12 hours. On this basis, if sufficient AHG concentrate were given to achieve a plasma level of at least 50 per cent, 12-hourly infusions of smaller amounts should prove sufficient to maintain the plasma AHF at a haemostatic level, and such a regimen might allow a more economical use of concentrate. In practice, as the concentrate may have to be given intravenously over relatively long periods, it was found more convenient to give it at 24 hour intervals.

The concentrate on one occasion was given subcutaneously and on another intraperitoneally: on neither occasion did the plasma AHF level rise.

Resistance to AHG

In general there was no evidence of resistance or diminished clinical response as observed after animal AHG concentrate. Although many patients received repeated infusions, often at intervals exceeding 4 weeks, the expected rise in AHF level after each infusion was observed. In patient H.6, two satisfactory responses of 30 per cent in plasma AHF level per litre equivalent of AHG concentrate given were initially obtained on the occasions of a dilatation and curettage and dental extractions. Subsequently, but before hysterectomy, the corresponding values were 11 and 12 per cent. There was no evidence of a further progressive diminution in response to infusion given after hysterectomy, plasma AHF values ranging from 6 to 12 per cent. The change in response from 30 to 12 per cent occurred over a period of 19 days from the first transfusion for dilatation and curettage. Neither antibody nor anti-coagulant activity could be demonstrated in the plasma. In patient H.7, although the response per litre equivalent of concentrate varied from 12 to 24 per cent, there was no trend indicative of developing resistance.

In patient L.1 temporary resistance to AHG concentrate was associated on one occasion

with severe tissue damage caused by multiple large haemorrhages, only 11 per cent of the infused material being accounted for 20 minutes after infusion, whereas on three other occasions (in the presence of less severe tissue damage (two occasions) and once after an infusion given before dental extraction) from 42 to 52 per cent of the concentrate given was accounted for. Temporary resistance was also observed in haemophiliacs with a moderately severe form of the disease (e.g. patients L.5 and L.19); in the presence of severe tissue damage as much concentrate was then needed to control bleeding as in a severe haemophiliac.

Apart from these findings of a diminished clinical response, it was observed at Lewisham in patients L.1, L.2, L.5 and L.24 that the amount of AHG accounted for might progressively diminish during a period of successive daily infusions although the clinical efficacy of the concentrate was apparently unimpaired. After an interval of 4-6 days without infusion, post-infusion plasma AHF levels again reach the expected values.

Of the 69 patients with haemophilia, some of whom received multiple infusions of human AHG, two developed an inhibitor in their plasma. One patient (O.9) has failed to respond to human or animal AHG for 2 years since an inhibitor was found in his plasma after the second infusion of human AHG. An inhibitor, first detected in patient L.3 in April 1959, is still present, but since his last dental extraction in October 1959, this patient has not suffered from spontaneous haemorrhagic episodes.

Reactions

Reactions to human AHG concentrate were frequent, occurring in 17 out of 30 patients at Lewisham, 10 out of 16 patients at Hammersmith and 16 out of 26 patients at Oxford and were accompanied by an even wider variety of signs and symptoms than those described. The mildest form consisted of headache, with perhaps flushing, tachycardia, paraesthesiae and complaint of pain at the site of operation (e.g. in dental extractions). More severe reactions were accompanied by nausea, vomiting, pain in the back, a feeling of constriction in the chest with or without pain. In the most severe reactions, there were hypotension, clouding or loss of consciousness and sometimes disturbance of vision. Rigors also occurred but a rise of temperature was seldom observed.

Antihistamines did not prevent the reactions nor did hydrocortisone given intravenously before the infusion modify them. It was soon noted that their occurrence was related to the rate of infusion and that by reducing this at the first appearance of any evidence of a reaction, the more serious forms of reaction were prevented. In one centre it became the practice to administer the concentrate at a rate of 100 ml. in 20 minutes or more.

Reactions were not associated more with certain batches than with others. Material of one batch associated with a reaction in one patient on one occasion was not necessarily accompanied by a reaction when given again to the same or another patient. A patient who had had a severe reaction on one occasion did not necessarily react to subsequent infusions. Occasionally, however, patients reacted to almost all infusions of AHG concentrate.

In all cases the signs and symptoms of the reactions disappeared quickly after the end of the infusion. On no occasion has any lasting harmful effect been noted, nor any subsequent diminution in response to infusion of AHG concentrate. It is perhaps noteworthy that the occurrence of a severe reaction has never made a patient reluctant to receive a further infusion of concentrate.

In only two instances did a reaction contra-indicate further treatment with concentrates. Patient H.6 has been described above. Patient H.16 had a cerebral lesion (? cerebral abscess with or without haemorrhage). The first infusion of concentrate caused an intense exacer-

bation of headache and was possibly responsible for temporarily converting a condition of almost complete to complete aphasia. He was subsequently treated with daily transfusions of fresh plasma and recovered completely. This may have been similar to the type of reaction in patient H.7 after infusions of concentrate. This patient complained of abdominal pain and flushing of the abdomen was observed round the vascular infected wound area.

There was no evidence that the occurrence of reactions in a given patient was related to the number of infusions of AHG concentrate he received. A reaction was observed in several patients (e.g. patients L.12, L.13, L.15, L.16) during the first infusion, but not during subsequent infusions, perhaps spread over a period of 18 months or more (e.g. patient L.16). In others, reactions were interspersed over a long series of incidents, e.g. patient L.1, in whom reactions were observed to two out of 13 separate infusions given between August 22nd, 1956 and February 14th, 1960. In contrast, patient L.2 had reactions following 11 infusions in a series of some 50 infusions between September 10th, 1956 and November 13th, 1959. Attempts to demonstrate precipitating antibody to the concentrate in the sera of patients who have received many infusions have been unsuccessful. It seems more likely that the reactions have a pharmacological rather than an immunological basis.

Homologous Serum Jaundice

Particular attention has been paid to the occurrence of this complication in recipients of AHG concentrate. Among the patients reported here, three possible cases have been observed. Patient L.17 was infused with 1 litre in April 1958 when a perforated gangrenous appendix was removed and again in May 1958 for perirenal haemorrhage and haematuria. No blood or plasma was given. He became jaundiced 104 days after the first infusion in April and 73 days after the second infusion in May. There was no history of contact with infectious hepatitis. Patient L.9 was infused on three occasions in June and August 1959 for the treatment of tissue haemorrhage and during manipulative treatment of a contracture. On the first occasion he also received one bottle of blood from a donor, transfusion of whose blood on several previous occasions had not been associated with the development of jaundice. He became jaundiced in October 1959, 118 days after the first and 65 days after the last infusion of AHG. There was no history of contact with infectious hepatitis. Patient L.8 was infused on four occasions in October and December 1957 and January 1958. On the third occasion he also received two bottles of blood. Jaundice was noticed in April 1958, 85, 93, 110 and 179 days respectively after the four episodes. There was no history of contact with infectious hepatitis.

None of the batches given to these three patients was associated with the occurrence of jaundice in other recipients.

General

The use of human AHG concentrate has certain advantages. Compared with fresh blood or plasma, smaller volumes are needed to control haemorrhage and haemostasis is therefore achieved more rapidly, a fact of importance when haemorrhage is rapid and is causing extending tissue damage, particularly in sites where pressure may be exerted upon vital structures. The clinical effect of AHG concentrate, since it has a known AHF content, is usually more accurately predictable than that of fresh frozen plasma, the AHF content of which is variable and usually unknown to the user. As the volume of AHG concentrate is usually one-third (sometimes less) and the protein concentration about one-half that of normal plasma containing the corresponding amount of AHG, circulatory overloading, often a hazard when treating

haemophiliacs solely with whole blood or plasma, does not occur. In the series of patients treated, fresh blood was valuable in the presence of substantial external haemorrhage with severe anaemia. However, in the majority of instances, stored blood was used effectively to replace lost red cells.

The preparation of human AHG concentrate on a large scale will be technically very difficult. Because of the lability of AHF, the plasma must be separated and the aseptic fractionation begun with the least possible delay after the collection of the blood, in order to obtain maximum yields. The greater the amount of plasma to be fractionated the larger this delay inevitably becomes. In addition unavoidable losses occur during the fractionation process itself. At present only limited amounts can be prepared and these are being used for further clinical trials by the M.R.C. Working Party on Human Antihaemophilic Globulin with the purpose of investigating the most economical ways of using human AHG concentrate and the cause of the reactions sometimes associated with its use.

In this series of 69 patients, the average number of incidents per patient per year was slightly less than one and the average amount of AHG concentrate infused for each incident was approximately 5×100 ml. bottles. This amount, together with the samples of concentrate used up in assay and sterility tests is derived from some 20 to 25 bottles of citrated blood. If it were assumed that each of the haemophiliacs in the country, thought to number about 2000, suffered one incident per year requiring treatment with this amount of AHG concentrate, some 40,000 to 50,000 blood donations per year would be needed.

More human AHG concentrate is likely to be needed to control bleeding during major operations and the surgical treatment of severe accidents than in other types of incident. The question arises whether this scarce material should be used for first major operations in preference to animal AHG concentrate, in spite of the sensitization caused by this material, since the large amounts of human AHG concentrate needed to 'cover' such operations restrict its use in other types of incident for which it has proved so valuable. The chances that a given patient will have to undergo more than one major operation are small compared with the likelihood of his suffering relatively frequent minor incidents in which bleeding occurs. It would thus seem reasonable to use animal AHG concentrate to 'cover' first major operations: it is more readily available and, because of its greater potency per mg. of nitrogen, the high plasma AHF concentrations necessary to prevent abnormal bleeding are usually more quickly attained than with human AHG concentrate. Should a patient have to undergo a second operation or should the post-operative course of the first operation be accompanied by long continued bleeding so that the use of animal AHG concentrate has to be abandoned because the patient has become sensitive to it, human AHG concentrate could then be used to control bleeding.

SUMMARY

A preparation of human AHG concentrate was used to control bleeding in 69 haemophiliacs, of whom 36 suffered from a clinically severe form of the disease. The indications included a wide range of operations from dental extractions to hysterectomy and gastrectomy, spontaneous and traumatic tissue and joint haemorrhage, and 'cover' during the manipulative treatment of limb deformities.

Haemostasis was usually achieved when the plasma AHF level was raised to and maintained at or above 25 per cent of normal. The concentrate was given repeatedly without affecting the patients' responsiveness, save in one patient undergoing hysterectomy and in certain

patients with severe tissue damage caused by haemorrhage, in whom there was, temporarily, a diminished response to infusion of AHG concentrate. A circulating anticoagulant developed in two patients who became refractory to treatment with human or animal AHG.

The plasma level of AHF brought about by infusion of AHG fell rapidly, reaching about 50 per cent of its post-transfusion value in 12 hours. The haemostatic effect was seldom prolonged for more than 24 hours, even after large doses.

Reactions to the preparation of AHG used were observed in some patients. They could be largely eliminated by slowing the rate of infusion.

No permanent harm was caused by the patients' reactions, the occurrence of which was not associated with any change in response to the AHG concentrate. Their cause was not discovered.

The experience gained during the use of human AHG concentrate prepared by the method of Kekwick and Wolf shows that it is a reliable means of controlling bleeding in haemophiliacs and that there are no contra-indications to its prolonged or repeated use.

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