possible diagnostic import. Despite the limited number of abnormal cases studied (Table III), the results show only minor variations from the normal which could be explained on the grounds of changes in maternal serum proteins; for instance, the low value of serum albumin in toxæmia may well be responsible for the correspondingly low albumin reading in the liquor, and this may also apply to the high α_1 -globulin levels in the liquor of these patients.

The significance of the slightly elevated total protein in the liquor in anencephaly, hydramnios, and hydrops foetalis, and its possible relationship to foetal swallowing, can only be a matter of conjecture. On the other hand, the findings of identical protein patterns in the two amniotic sacs of uniovular twins where hydramnios was limited to the upper of the two sacs, as confirmed on abdominal paracentesis, shows that there is more to it than foetal swallowing, and protein dialysis in the complex processes involved in the formation and elimination of all the constituents of the amniotic fluid.

Summary

Proteins present in maternal serum, umbilical-cord blood, liquor amnii, and dialysates from maternal serum across "foetal membranes" have been studied with the aid of paper electrophoresis.

The protein pattern seen in the liquor amnii has the characteristics of a simple protein dialysate, and can be reproduced *in vitro*.

Evidence is presented to suggest that the swallowed liquor amnii contributes to foetal nutrition and may well provide a source of γ -globulin and antibodies to the foetus.

Despite minor variations, no diagnostically significant changes were detected in the electrophoretic patterns of the liquor in toxæmia, anencephaly, twins, hydramnios, and hydrops foetalis.

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DEMONSTRABLE DEFICIENCY OF CHRISTMAS FACTOR IN TWO SISTERS

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Evidence indicates that Christmas disease is inherited as a sex-linked recessive defect. This was suggested by one of the family trees in the first seven cases of Christmas disease reported by Biggs *et al.* (1952). Numerous family trees published since that time have confirmed this. Koller (1954), for example, demonstrated that four members of the famous Tenna family of bleeders in Switzerland, where the inheritance is of this pattern, are affected with Christmas disease.

In a genetic defect of this type the disease is transmitted by apparently normal carrier females to an affected male. All the daughters of an affected man are carriers. In the family of a carrier there is a 50% chance of a daughter being a carrier and of a son being affected. Biggs *et al.* (1952) noted a prolonged clotting-time and defective prothrombin consumption in the mother of one of their cases. Brinkhous *et al.* (1954) reported P.T.C. (Christmas factor) deficiency in a woman aged 49. Such cases suggested that the Christmas-disease gene might be less completely recessive than that producing haemophilia (Pitney and Dacie, 1955).

The object of the present paper is to record a demonstrable deficiency of Christmas factor in two sisters, the daughters of a man suffering from Christmas disease.

The Patients

Case 1 (IV, 2, see family tree).—A schoolgirl aged 13 had possibly excessive loss of blood at the onset of menstruation, but the periods thereafter were normal. On two occasions she had bled for 48 hours after dental extractions. Physical examination was negative. In hospital she had further dental extractions, with the insertion of acrylic resin splints, without any haemorrhage.

Family tree of the two cases studied. Case 1 was IV, 2 and Case 2 was IV, 1.

Case 2 (IV, 1).—A typist aged 16, she had not had any incidents to raise a suspicion of abnormal haemorrhage.

The Relatives

III, 4.—This uncle was first investigated after a severe episode of melaena necessitating transfusion of 4½ pints (2.3 litres) of whole blood. There had been five previous incidents of gastro-intestinal bleeding, each of which had necessitated blood transfusion. He had suffered a number of incidents apart from those of gastro-intestinal bleeding, and had been admitted to hospital in childhood after troublesome bleeding from his lower lip. He had required transfusion after dental extraction, and occasional epistaxis was severe. Laboratory investigation established that he was suffering from Christmas disease.

III, 1.—This uncle had died from gastro-intestinal bleeding before we investigated the family. It is probable, though not certain, from the available history that he also had the defect.

III, 3.—This uncle gave no history of abnormal haemorrhage.

III, 2.—The father of the two sisters. He was investigated as part of the family study resulting from the discovery of *III, 4*. He had also bled after dental extraction for periods exceeding two days. Severe epistaxis had also occurred. Laboratory study showed that he also had Christmas disease.

In none of these brothers was there any history of haemarthrosis. None of the other relatives gave any history of abnormal bleeding.

Methods

The bleeding-time was measured by Ivy's method as described by O'Brien (1951); the tourniquet test was that described by Biggs and Macfarlane (1953); and the platelet count as described by Rees and Ecker (1923), using edetic acid as anticoagulant. The whole-blood coagulation time was measured by the method of Lee and White (1913) as described by Biggs and Macfarlane (1953).

Recalcification times: Mixtures were made of one part of control or test plasma in nine parts of Christmas-disease plasma. Doubling dilutions of this were made in further volumes of Christmas-disease plasma; this plasma was obtained from a patient severely affected with Christmas disease. The recalcification times were assessed by the method described by Biggs and Macfarlane (1957). The one-stage "prothrombin" test was performed as described by Biggs and Macfarlane (1953), using saline brain thromboplastin as prepared by Toohey (1950).

Assessment of the blood thromboplastin system (prothrombin consumption) was carried out (a) on whole blood by the method described by Merskey (1950), and (b) on recalcified plasma. The use of the prothrombin consumption of recalcified haemophilic or Christmas-disease plasma for the detection and assay of anti-haemophilic globulin and Christmas-factor deficiencies has previously been described (Douglas and Cook, 1957; Douglas and Mair, 1958; Douglas, 1958a).

The thromboplastin generation technique was carried out as described by Biggs and Douglas (1953). In some of the tests chloroform extract of dried brain was used in place of platelets, as described by Bell and Alton (1954). The test was modified to give an assay of Christmas factor in the same way as it can be used to assay anti-haemophilic globulin as described by Douglas (1958b). Normal adsorbed plasma and platelets are kept

constant, and the variable consisted of mixtures of normal or test sera in Christmas-disease sera.

Results

The preliminary laboratory investigations of Cases 1 and 2 are set out in Table I.

TABLE I.—Preliminary Laboratory Investigations in Cases 1 and 2

Tests	Case 1	Case 2	Normal
Bleeding-time (mins.)	3½	3	2½–7
Platelet count (per c.mm.)	240,000	280,000	150,000–500,000
Tourniquet test	Negative	Negative	Negative
Whole-blood coagulation time (mins.)	9½	9	5–10
One-stage prothrombin time (secs.)	13	13	13
Prothrombin consumption index (average of 2 observations)	27%	17%	Less than 20%
Thromboplastin generation test:			
Patient's adsorbed plasma	Normal	Normal	
" serum	Abnormal	Abnormal	

Thromboplastin Generation Technique

The significant findings were in the thromboplastin generation technique, where there was a defect in the serum side of the system.

The results in Case 1 are shown in Table II. It will be seen that the difference from the normal serum was not marked, but was a consistent finding on six other

TABLE II.—Serum Defect in Thromboplastin Generation

Variable Reagents	Incubation Time in Minutes					
	1	2	3	4	5	6
Normal adsorbed plasma	46	20	11	9	10	10
" serum						
Patient's adsorbed plasma	48	16	14	13	12	14
" serum						
Normal adsorbed plasma	44	16	14	13	14	13
Patient's adsorbed plasma						
Normal serum	25	11	11	11	10	10
" serum						

Thromboplastin generation technique with M/40 calcium chloride and chloroform brain extract constant.

separate observations. The essential part of this study was to establish the nature of this serum thromboplastin defect.

In Table III the results are shown of the failure of Case 1 serum to correct the serum defect in a known case of Christmas disease as effectively as the normal

TABLE III.—Inability of Serum to Correct Christmas-disease Serum in Thromboplastin Generation

	Incubation Time in Minutes					
	1	2	3	4	5	6
Normal serum	55	31	11	12	11	12
Case 1	70	76	51	35	13	13
Christmas-disease serum	96	100	76	47	25	23
10% normal serum	84	70	35	16	13	13
90% Christmas						
10% Case 1 serum	80	67	56	28	22	20
90% Christmas						

Thromboplastin generation technique with serum as variable reagent; M/40 calcium chloride, normal adsorbed plasma, and chloroform brain extract are constant.

serum. In this particular experiment the normal reagents were not yielding such a powerful thromboplastin as that in Table I, and the serum defect in Case 1 is even more obvious.

Comparison of sera from Case 1 and Case 2 in the same experiment demonstrated that the sisters had an identical defect and that the degree of this was the same.

Prothrombin Consumption Technique on Recalcified Christmas-disease Plasma

The results of this are shown in Table IV. It will be seen that the addition of plasma from Case 1

TABLE IV.—*Prothrombin Consumption of Recalcified Christmas-disease Plasma. Clotting-times of Fibrinogen in Seconds*

Plasma Addition	Time in Minutes after Addition of M/10 Calcium Chloride					
	10	20	30	40	50	60
Normal	40	46	42	40	39	42
Case 1	16	19	19	18	17	21
Christmas disease	12	12	12	11	12	13

is not nearly so powerful in correcting the defective prothrombin consumption of Christmas-disease plasma as is the normal plasma addition.

Assays of Christmas Factor and Antihaemophilic Globulin

These assays were performed by the thromboplastin generation method and by the prothrombin consumption technique in recalcified Christmas disease or haemophilic plasma.

In tables V and VI are shown the results, expressed in clotting-times, of the Christmas-factor assays by the thromboplastin generation technique and the prothrombin consumption method on recalcified Christmas-disease plasma.

The results of all the assays on Case 1 and Case 2 and their parents are shown in Table VII. As the father

TABLE V.—*Assay of Christmas Factor Content of Serum by the Thromboplastin Generation Technique*

Percentage of Normal Serum in Christmas-disease Serum	Clotting-time in Seconds after 6 Minutes' Incubation
50	16
25	17
12	21
6	24
3	32
0	43
Percentage of Case 1 Serum in Christmas Disease serum	
50	23

Thromboplastin generation technique with normal adsorbed plasma and platelets constant, and the serum component of the system variable. The clotting-times represent the mean of five observations.

TABLE VI.—*Assay of Christmas Factor Content of Plasma by the Prothrombin Consumption Method*

Percentage in Terms of Standard Addition	Clotting-times of Fibrinogen in Seconds
100	61
50	52
25	45
12	32
6	23
3	16
0	14
Case 1 plasma: 100	29

Clotting-times of fibrinogen represent the mean of 11 observations.

TABLE VII.—*Assays of Christmas Factor and Antihaemophilic Globulin in the Two Sisters and Their Parents*

	Christmas Factor	Antihaemophilic Globulin
Case 1 ..	13	74
.. 2 ..	18	82
Father ..	3	79
Mother ..	84	92

The assays are expressed as percentages in comparison with a mixture of 4 normals. In Case 1 the figures expressed are the mean of 11 assays on the prothrombin consumption method and 5 assays on the thromboplastin generation technique. In the others only single assays were made.

had Christmas disease of only moderate severity the assays were made in comparison with plasma and serum from a severely affected patient with Christmas disease.

Recalcification Times.—The impaired ability of plasma from Case 1 and her father to shorten the recalcification time of plasma from a patient with severe

TABLE VIII.—*Recalcification Times. (Clotting-times of Plasma in Seconds)*

	Proportion of Addition to Christmas-disease Plasma					
	1/10	1/20	1/40	1/80	1/60	0
Control	114	156	201	270	319	545
Case 1	316	320	361	401	433	
Father	363	392	408	476	492	

Christmas disease is demonstrated in Table VIII. Their plasmas corrected plasma from a severe haemophilic as well as did normal plasma.

Discussion

The object of this paper is to report the cases of two sisters, heterozygous for the defect of Christmas disease. As daughters of a man with this condition they are necessarily transmitters of the condition. These two sisters have a demonstrable deficiency of Christmas factor; this abnormality can be shown on recalcification time, and thromboplastin generation and prothrombin consumption techniques. On clinical grounds it was impossible to tell with certainty that these two sisters had an abnormality. The older sister gave no history of abnormal tendency to bleed and the younger sister gave only a borderline history of abnormality.

The inheritance of the Christmas-factor deficiency in the two sisters is likely to be due to the incomplete recessivity of the gene, as there was no evidence to suggest that either patient was homozygous for the abnormality.

Abnormal prothrombin consumption and/or abnormal thromboplastin generation in transmitter females has also been reported by Rosenthal and Sanders (1954), Ramot *et al.* (1955), Reed (1955), Hule and Nešpůrková (1956), and Firkin (1958). Hardisty (1957) has also reported Christmas-factor deficiency as low as 8% in a woman whose sister had a Christmas factor level of 65%. He considered that the wide difference in the two Christmas-factor levels was evidence of a variable expressivity in the gene apart from its incomplete recessivity.

Summary

A deficiency of Christmas factor in two sisters has been shown by several techniques (recalcification times, thromboplastin generation, and prothrombin consumption).

The father of the two sisters suffers from Christmas disease of relatively mild severity.

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CASE 2

A man aged 59, with severe anaemia due to myelomatosis, had been treated for 2½ years with vitamin B₁₂, urethane, and blood transfusions. Treatment with M & B 938 was begun on May 14, 1958, at Charing Cross Hospital. The dosage was 0.1 g. twice weekly to a total of 0.9 g. During the course two blood transfusions were given without a reaction. On June 8 the patient had vomiting and shivering, and three days later developed jaundice associated with pale stools and dark urine. The liver was enlarged but the spleen was not palpable. On June 15 the serum bilirubin was 5.3 mg./100 ml., the alkaline phosphatase was 6 K-A units, and the prothrombin time was prolonged. Bile salts and bilirubin were present in the urine but urobilinogen was absent. On June 27 the thymol turbidity was 1 unit. Prednisolone, 10 mg. thrice daily, was given for a week. On July 18 the jaundice had faded and the serum bilirubin was 1.9 mg./100 ml. Liver biopsy showed marked distension of the bile canaliculi by bile. This change was mainly localized to the centrilobular zones. The parenchyma and portal tracts appeared healthy.

COMMENT

It seems to be very probable that the jaundice in both these cases was due to the action of M & B 938 upon the liver. The elevation of alkaline phosphatase in Case 1 and the histological picture of bile stasis without parenchymal damage together with a negative thymol turbidity test in Case 2 are suggestive of jaundice due to cholangiolitic obstruction rather than of jaundice due to parenchymal liver damage. Although it is unjustifiable to generalize from only two cases, the analogy between the occurrence of jaundice in these two patients and jaundice due to chlorpromazine is obvious. A further point of similarity is that jaundice in Case 1 was preceded by marked systemic upset, including the appearance of an urticarial rash: an allergic basis for chlorpromazine jaundice, as well as for jaundice following the administration of thiouracil, methyltestosterone, and sulphadiazine, has been suggested (Werther and Korelitz, 1957).

It has been shown by Henry and Grindley (1942) and Hampton (1947) that the chemically related drug stilbamidine is highly cumulative. Although no definite information is available at the moment, this may well be true of M & B 938. Moreover, experience suggests that nothing is lost therapeutically by spreading treatment over many weeks.

We consider it is wise not to exceed a dose rate of 0.1 g. twice weekly or a total of 2 g. in any one course of treatment. Our current practice is to give 0.1 g. twice weekly for two weeks and thereafter once weekly.

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Medical Memoranda

Jaundice during Treatment of Myelomatosis with M & B 938

The use of M & B 938 (4-4'-diamidinodiphenylamine dihydrochloride) for the first time in myelomatosis has been reported (Ward, 1958a, 1958b). Of the first ten patients treated one had complete relief of bone pain, a fall in the E.S.R., and recalcification of osteolytic lesions. He is still well five years after treatment. In a second patient previous treatment with urethane had been without effect, but during treatment with M & B 938 a palpable rib-tumour disappeared and pain was relieved. A third patient had severe pain in the back despite previous radiotherapy to the area. The pain vanished during treatment with M & B 938. In the other patients there was no obvious benefit.

It is worth noting that jaundice has occurred in two out of these 10 patients who have received M & B 938 for myelomatosis.

CASE 1

A man aged 36 had myeloma deposits in the skull and sacrum. He was given six intravenous injections of M & B 938 daily at Charing Cross Hospital, starting on August 17, 1953. The doses varied from 0.1 g. to 0.25 g. A further dose of 0.1 g. was given at home on August 29. The total dosage was 1.27 g. The last injection was followed by a rigor, and the patient developed pyrexia and an urticarial rash on the neck. On September 3 he had jaundice associated with pale stools and dark urine. The liver was enlarged, but the spleen was not palpable. On September 8 the thymol turbidity was over 10 units, but the alkaline phosphatase was only 4 King-Armstrong units. On September 25 the jaundice was fading, but the alkaline phosphatase had risen to 20 K-A units.

By mid-October the jaundice had cleared, and on December 9 the thymol turbidity was 2 units and the alkaline phosphatase 6 units.

Another course of M & B 938 was begun in January, 1955. On this occasion a total dosage of 1.7 g. was spread over five weeks. There was no jaundice.