the patients are not clear, we would emphasize that in these the clinical picture and duration of the disability fall well within the limits of those whose aetiology is more certain.

Treatment

With regard to treatment, we would make the following observations. Patients should be warned against crossing legs when sitting. There is no justification for the admission of these patients to hospital. A toe-raising spring overcomes the essential disability and even in heavy industry there is no need for the patient to remain off work. Massage, coloured lights, and other forms of passive physiotherapy, not to mention the administration of vitamin B_1, play no part whatever in treatment. The patient should be instructed to carry out active movements of the affected muscles as often as possible when not wearing a spring, and, indeed, the spring itself is necessary only in the event of a severe paralysis of the tibialis anticus.

Summary and Conclusions

Paralysis of the external popliteal nerve, excluding the results of gross trauma, is not uncommon.

Such paralysis generally (if not always) results from local nerve ischaemia, and simple mechanical factors can usually be found; these include kneeling, bandaging, crossing the legs while sitting, lying on a hard surface, and the wearing of knee-pads. Previous loss of weight conduces to this type of damage.

Motor paralysis is often complete, but sensory loss is frequently absent and is never profound. The condition is always painless, and the onset sudden.

Complete recovery is common and partial recovery the rule.

We wish to thank Mr. J. M. P. Clark, Dr. E. W. Jackson, Dr. A. Leese, Mr. A. B. Pain, and Professor R. E. Tunbridge for referring some of the patients.

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Messrs. Williams and Wilkins (Baltimore, U.S.A.) announce the forthcoming publication of two new journals. The *Journal of Histochemistry and Cytochemistry* is to commence publication in January. It is to appear bi-monthly at an annual subscription of $7 (£2 10s.), and will contain original papers relating to the development and application of histochemical methods, with occasional review articles covering important aspects of histochemistry. *Applied Microbiology*, an official publication of the Society of American Bacteriologists, will publish papers concerned with the application of microbiology to the fields of food, sanitation, agriculture, antibiotics, and other subjects concerning the use or control of animal and plant disease. The journal will appear bi-monthly, commencing in January, 1953, and the annual subscription is $7.50 (£2 15s.).

CHRISTMAS DISEASE

A CONDITION PREVIOUSLY MISTAKEN FOR HAEMOPHILIA

BY

ROSEMARY BIGGS, M.D.

A. S. DOUGLAS, M.R.C.P.

R. G. MACFARLANE, M.D.

Radcliffe Infirmary, Oxford

J. V. DACIE, M.D., M.R.C.P.

W. R. PITNEY, M.D., M.R.A.C.P.

Postgraduate Medical School, London, W.12

C. MERSKEY, M.D., M.R.C.P.

University of Capetown

AND

J. R. O'BRIEN, D.M.

South Devon and East Cornwall Hospital, Plymouth

Haemophilia is a severe bleeding disease of males with a sex-linked recessive inheritance. Laboratory tests show a prolonged whole-blood clotting-time and deficient conversion of prothrombin to thrombin during clotting. When clinical, genetic, and the usual laboratory features are all present the diagnosis of haemophilia is generally believed to be simple. In some families haemophilia arises suddenly with no previous history of the disease; the clotting-time may not be greatly prolonged. To establish a diagnosis in these less clearly defined cases a new technique was used extensively by Merskey (1950). This test depends on the fact that normal blood added to haemophilic blood in small proportions shortens the clotting-time of haemophilic blood, whereas the addition of haemophilic blood is ineffective. The normal blood contains a substance—the antihaemophilic globulin—which is lacking in haemophilia. Thus to confirm the diagnosis of haemophilia the blood of the patient to be tested is added to the blood of a known haemophilic patient and the shortening of the clotting-time is compared with that obtained with normal blood. If the blood contains the antihaemophilic globulin its addition will shorten the haemophilic clotting-time.

This test has led to the discovery that occasionally a mixture of blood samples from two apparently classical cases of haemophilia has a shorter clotting-time than that of either specimen separately. We have now found seven patients who by ordinary tests would be said to have haemophilia. When a small proportion of the blood or plasma of these patients is added to haemophilic blood or plasma the clotting-time is greatly reduced. Similar instances of this phenomenon were recorded by Pavlovsky (1947), Koller *et al.* (1950), Aggeler *et al.* (1952), Schulman and Smith (1952), and Poole (1952). From these observations it must be concluded that within the general group of patients thought to have haemophilia there are at least two different conditions. To avoid confusion it is essential at this stage to make a restricted definition of the term "haemophilia."

Antihaemophilic globulin can be shown to be essential for the normal formation of blood thromboplastin (Biggs, Douglas, and Macfarlane, 1953). Haemophilia may therefore be defined as a recessive, sex-linked

hereditary bleeding diathesis due to a failure or delay in blood thromboplastin formation caused by absence or deficiency in the blood of antihaemophilic globulin. If this definition of haemophilia is accepted then the seven cases recorded in this paper are not those of haemophilia but a newly recognized condition which we propose to call "Christmas disease," after the name of the first patient examined in detail. The naming of clinical disorders after patients was introduced by Sir Jonathan Hutchinson and is now familiar from serological research; it has the advantage that no hypothetical implication is attached to such a name.

The details of the technical methods used in this study are all described by Biggs and Macfarlane (1953). In all the cases recorded below, the clinical histories were similar to those obtained from patients with haemophilia.

Case Reports

Case 1.—The patient was a boy named Christmas, aged 5 years. There was no history of haemorrhage in other members of the family. He had numerous episodes of haemorrhage dating from the age of 20 months, mostly resulting from injuries during play. He was transfused on numerous occasions; each transfusion resulted in abrupt cessation of bleeding.

Case 2.—A boy aged 7 had had numerous episodes of bleeding, including epistaxes, deep-tissue haemorrhages, and haemarthroses, since the age of 3 months. There was no history of haemorrhage in other members of the family. Doubt about the diagnosis of haemophilia was raised by Poole (1952) because additions of small proportions of haemophilic blood corrected the patient's clotting-time.

Cases 3 and 4.—These patients were members of the same family (see Fig. 1). From this diagram the condi-

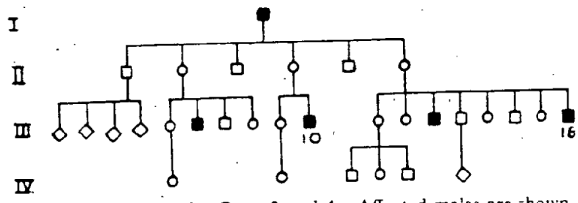

FIG. 1.—Family tree for Cases 3 and 4. Affected males are shown as solid black squares. Case 3 is III_{10}, and Case 4 III_{16}.

tion appears to be inherited as a sex-linked recessive character. The patients examined were III_{10} and III_{16}. III_{10} was a boy aged 14 with a history of numerous bleeding episodes, including haemarthroses dating from the age of 8 months. He was transfused on numerous occasions, and bleeding always stopped rapidly. III_{16} was a boy aged 6 who had suffered from numerous haemorrhagic episodes.

Case 5.—A man aged 28 had had numerous haemorrhagic episodes dating from infancy. These included haemarthroses, haematuria, melaena, retroperitoneal haemorrhage, and haemorrhage into the base of the tongue. He had been admitted to hospital on more than 20 occasions and had had numerous transfusions.

Case 6.—A Cape coloured boy aged 6 years had always bled excessively from minor injuries. He was admitted to hospital comatose from haemorrhage from a cut in the left foot. Transfusion of 650 ml. of three-days-old blood on admission brought a temporary but complete correction of his clotting defect. The child's brother had suffered from repeated epistaxis, had had a definite haemarthrosis, and had died in hospital of intraperitoneal haemorrhage following laparotomy for abdominal pain. No other members of the family had any haemorrhagic tendency.

Case 7.—A man aged 21 had had prolonged bleeding following teeth extractions on four occasions, one of which required a transfusion of 4 pints (2.3 litres) of blood. At 16 he had had haematuria for three weeks, and at 17 a haematoma of the buttock which was drained and took four weeks to heal. His younger brother, aged 3½, bled for three weeks from a cut lip, and the maternal great-grandfather died of haemorrhage. The patient had a relatively mild haemorrhagic diathesis, and the laboratory findings were less abnormal than in the other six cases. He was first seen before Christmas disease was recognized. A probable diagnosis of haemophilia was made at that time, although the clotting-time and prothrombin consumption test were normal, from the clinical and family history, and from the fact that the whole-blood clotting-time was recorded occasionally at the upper border of the normal range and the thrombin generation test was abnormal (Macfarlane and Biggs, 1953).

Experimental Results

The results shown in Table I are the same as those found in true haemophilic patients. The bleeding-time, tourniquet test, and platelet count are normal, and prothrombin times

TABLE I.—*Results of Various Tests*

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Normal
Clotting-time (min.) (Lee and White method)	10–15	39–72	14–16	13–16	28–45	9	7–10	5–10
Prothrombin consumption index (Merskey) %	100	150	100	75	160	136	20	Less than 40
One-stage prothrombin time (sec.)	12–15	15	15	19	15	17	15	15
Bleeding time, Tourniquet test, Platelet count	Normal in all cases							

are normal or at the upper limit of normal. The whole-blood clotting-time is lengthened in all but Cases 6 and 7, and the consumption of prothrombin during clotting is deficient in all but Case 7.

When the patients' plasma samples were added to known haemophilic plasma the clotting-time of the haemophilic plasma was shortened (Table II). The extent of the shorten-

TABLE II.—*Effect on Calcium Clotting-time of Adding Dilutions of Normal and the Patients' Plasma to Haemophilic Plasma. The Clotting-times are Recorded in Seconds*

Type of Plasma Added to Haemophilic Plasma	Dilution of Plasma Added to Haemophilic Plasma				
	1/2	1/10	1/50	1/100	0
Normal plasma	150	130	150	339	450
Case 1	165	165	195	—	450
,, 2	240	—	—	—	555
,, 3	—	177	—	329	895
,, 4	165	243	—	395	900
,, 5	115	120	—	315	600
,, 6	—	300	—	417	1,500
,, 7	140	175	220	240	780

ing was comparable to that caused by dilutions of normal plasma. Similarly, haemophilic and normal plasma dilutions shortened the patients' calcium clotting-times (Table III). When the plasma of Case 2 was mixed with that of

TABLE III.—*Effect on Calcium Clotting-time of Patients' Plasma of Adding Proportions of Normal or Haemophilic Plasma. The Clotting-times are Recorded in Seconds*

Substrate Used for Tests	Type of Plasma Added to Patients' Plasma							
	Normal				Haemophilic			
	1/10	1/50	1/100	0	1/2	1/10	1/100	0
Case 1	140	175	—	215	—	115	135	207
,, 2	285	—	—	900	240	—	—	555
,, 3	190	—	225	385	—	170	218	385
,, 4	150	—	175	265	—	158	227	265
,, 5	170	—	205	630	145	215	305	630

Cases 1, 3, and 5 and the plasma of Case 5 with that of Case 7 there was no shortening of clotting-time in the mixtures. When a potent preparation of antihaemophilic globulin in the fibrinogen fraction of normal plasma was added to the plasma of Case 1 there was no shortening of the clotting-time.

These results suggest that these patients do not lack the antihaemophilic globulin, and therefore, according to the definition already given, do not have haemophilia. Since small proportions of normal blood or plasma shorten the clotting-time of the plasma of patients with Christmas disease it is clear that these patients lack a substance which differs from the antihaemophilic globulin.

Preliminary tests with the plasma of Case 1 show some characteristics of the substance deficient in these cases (Table IV). The substance which shortens the clotting-time of the plasma is in the crude α- and β-globulin fraction of normal plasma and is precipitated at between 33 and 50% saturation with ammonium sulphate. It is present in haemophilic and normal serum, it is destroyed by heating plasma to 56° C. for 10 minutes, and is removed from plasma by Seitz filtration; it is stable on storage. Further tests have shown that the substance is adsorbed by $Al(OH)_3$ and that a substance closely resembling the Christmas factor is reduced in the blood of patients treated with the dicoumarol derivative ethyl biscoumacetate. As these results clearly show that the substance lacking from these cases is very different from the antihaemophilic globulin, it is called the Christmas factor. The properties of antihaemophilic globulin and Christmas factor are compared in Table V.

TABLE IV.—*Effect of Various Substances on Calcium Clotting-time of Plasma of Case 1 and a Haemophilic Patient*

Substance Added to Haemophilic or Patient's Plasma	Substance Added to Plasma of Case 1 in Concentration:			Substance Added to Plasma of a Haemophiliac in Concentration:		
	10%	2%	0	10%	2%	0
Normal plasma	105	130	210	120	150	450
Fibrinogen	230	240	237	150	195	450
Plasma heated to 56° C. for 10 min.	180	190	215	200	240	450
Plasma stored two weeks	140	175	217	130	155	450
Seitz-filtered plasma	230	250	225	100	115	450
Haemophilic serum	120	150	210	430	475	450
Normal serum	90	100	230	190	265	450
Crude α- and β-globulin, sample I	125	200	200	225	405	435
Crude α- and β-globulin, sample II	120	135	200	420	375	435
Albumin	165	210	200	450	520	450
α-globulin	200	210	200	455	420	455
0–25% sat. $(NH_4)_2SO_4$ from normal plasma	240	255	220	Corrects haemophilic defect		
25–33% fraction	225	215	205	Does not correct haemophilic defect		
33–50% ,,	125	180	215	Does not correct haemophilic defect		

TABLE V.—*Properties of Christmas Factor and Antihaemophilic Globulin*

Method of Differentiation	Christmas Factor	Antihaemophilic Globulin
Ammonium sulphate fractionation	Precipitated from normal plasma by 33–50% saturation	Precipitated from normal plasma by 25% saturation
Ether fractionation	Precipitated from normal plasma in crude α- and β- globulin fraction	Precipitated from normal plasma in fibrinogen fraction
Test for presence in normal serum	Present in large amounts	Almost absent
Test for presence in haemophilic serum	Present in large amounts	Absent
Stability to heat	Destroyed by heating to 56° C. for 10 minutes	When isolated from plasma resists heating to 56°C. for 10 minutes
Stability on storage	Stable	Often unstable
Effect of Seitz filtration	Adheres to the filter	Is unaffected
Effect of adsorption with $Al(OH)_3$	Very readily adsorbed	Not adsorbed

Samples of normal plasma, haemophilic plasma, and the seven patients' plasma were tested for their ability to form blood thromboplastin by the thromboplastin generation method (Biggs *et al.*, 1953). With this test it has been shown that when antihaemophilic globulin, platelets, and normal serum are incubated with calcium chloride a powerful thromboplastin is formed. If any of these factors are lacking the formation of thromboplastin is deficient. With $Al(OH)_3$-treated plasma as a source of antihaemophilic globulin it is easy to determine which of the components for thromboplastin formation is reduced in amount in any particular patient. Plasma and serum samples were collected from all seven patients and from normal and haemophilic subjects. Experiments carried out on these samples gave a constant pattern of results, illustrated from Case 1 in Figs. 2 and 3. In Fig. 2 antihaemophilic globulin preparations from a normal and a haemophilic subject and from Case 3 were tested with normal platelets and serum.

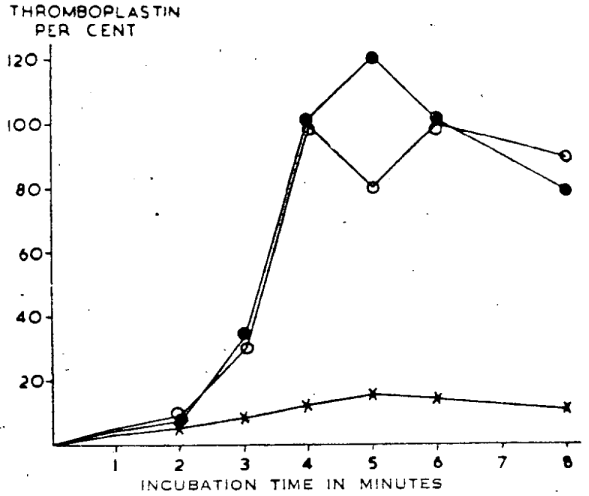

FIG. 2.—The curves represent the results of the thromboplastin generation test carried out on normal serum and platelets and $Al(OH)_3$-treated plasma from a normal person (O——O), from Case 3 (●——●), and from a haemophilic subject (X——X). The clotting-times have been converted to thromboplastin concentrations, using a dilution curve in which 100% thromboplastin corresponds to a clotting-time of 10 seconds.

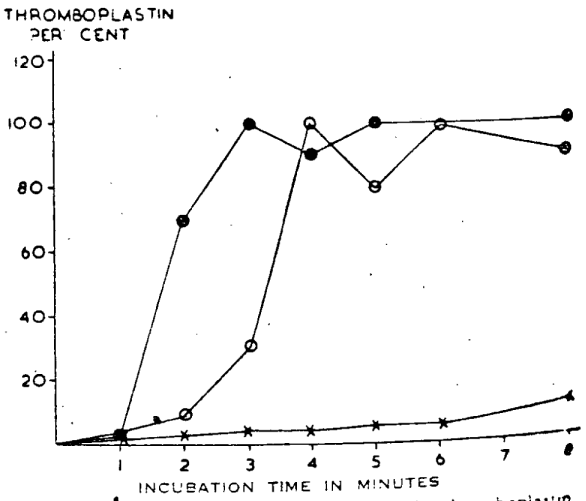

FIG. 3.—The curves represent the results of the thromboplastin generation test carried out with normal $Al(OH)_3$-treated plasma and platelets and serum from a normal person (O——O), from a haemophilic subject (●——●), and from Case 3 (X——X). The clotting-times have been converted to thromboplastin concentrations, using a dilution curve in which 100% thromboplastin corresponds to a clotting-time of 10 seconds.

It will be seen that, whereas the haemophilic subject lacks antihaemophilic globulin, the preparation from Case 3 behaves normally and therefore contains the normal amount of antihaemophilic globulin. In Fig. 3 the normal antihaemophilic globulin is tested with sera from a normal and a haemophilic subject and from Case 3. From these curves it is clear that the haemophilic and normal sera behave similarly, whereas the serum of Case 3 lacks a substance necessary for thromboplastin formation. Thus the Christmas factor is a factor required for normal blood thromboplastin formation. In the thromboplastin generation test the fundamental defect, the ability to form blood thromboplastin, is tested directly. Both those patients with haemophilia and those with Christmas disease fail to form thromboplastin normally, but in the two conditions a different factor is lacking.

Discussion

The condition described in these seven patients—Christmas disease—resembles haemophilia so closely in its clinical and laboratory findings that until recently cases of the disease were undoubtedly classified as haemophilia. These patients, and the three described by Koller *et al.* (1950), Aggeler *et al.* (1952), and Schulman and Smith (1952), are probably all examples of the same condition. In testing these 10 cases some 50 patients with true haemophilia or antihaemophilic globulin deficiency have been studied. It is not possible to suggest the true incidence of Christmas disease in so-called haemophilic patients from these figures, because in all instances the anomalous case was tested first. In 35 patients examined by us one additional patient (Case 6) was discovered. It is probable, therefore, that the disease is not very common.

Haemophilia is inherited as a sex-linked recessive character. All 10 cases of Christmas disease so far described were in males. There was no family history in five. The patient described by Koller *et al.* (1950) had a history resembling that of haemophilia. The only abnormal feature was that the female carriers in this family had abnormal prothrombin consumption tests. This finding suggests that in this family the factor controlling the disease was not completely recessive. Cases 3 and 4 described in this investigation were members of one family in which the inheritance was the same as that in haemophilia (Fig. 1). The mother of Case 3 showed no abnormality, but the mother of Case 4 had a lengthened clotting-time and deficient prothrombin consumption. These findings are similar to those of Koller *et al.* In the family of Case 7 also the inheritance was that of a sex-linked recessive character. Case 6 had an affected brother. From these findings it seems that the inheritance in Christmas disease is similar to that in haemophilia, but that the defect may not be completely recessive.

The fundamental defect in both haemophilia and Christmas disease is a failure to form intrinsic thromboplastin. The factors necessary for thromboplastin formation are platelets, antihaemophilic globulin, and the fraction of serum proteins which is adsorbed by $Al(OH)_3$. Patients with haemophilia lack antihaemophilic globulin and patients with Christmas disease lack a factor most readily obtained from serum. Biggs *et al.* (1953) showed that a "serum factor" was necessary for blood thromboplastin formation. This factor is probably identical with the factor VII of Koller *et al.* (1951) which is necessary for the action of brain thromboplastin. Both are present in serum, are adsorbed by inorganic precipitates, and are stable on storage. On separation of plasma fractions by the ether precipitation method both appear to be in the crude α- and β-globulin fraction. When a patient is treated with ethyl biscoumacetate there is a similar and parallel reduction in both the Koller factor VII and a factor required for blood thromboplastin formation. But the factor which is lacking in Christmas disease differs from Koller's factor VII in that the Christmas factor is not necessary for the action of brain thromboplastin, hence the normal one-stage prothrombin times. This difference can be interpreted in one of two ways: either the factor VII in Christmas disease is modified in some way, retaining its ability to react with brain thromboplastin but losing its ability to form blood thromboplastin, or it must be suggested that the two similar factors are both necessary for thromboplastin formation.

The best-known treatment for haemorrhage in cases of true haemophilia is transfusion with fresh blood or with the concentrated material prepared from the fibrinogen fraction of normal fresh plasma. Cases 1 and 5 were transfused with the fibrinogen fraction of normal plasma and, as would be expected, this had no effect. From *in vitro* tests with Case 1 it is clear that both haemophilic and normal plasma are more effective than the fibrinogen fraction. In general, haemorrhage in Cases 1 and 3 is said to cease rapidly after transfusion. On the other hand, Case 4, a child weighing only 45 lb. (20.4 kg.), was transfused with 300 ml. of fresh blood, and the laboratory tests showed no change after this relatively large transfusion. A patient with true haemophilia of the same age and weight was transfused at the same time with the same amount of fresh blood and showed the usual good response to transfusion. Case 6 was transfused with 650 ml. of blood three days old, and his clotting defect was temporarily abolished.

Case 5 required the extraction of five teeth. Before the first two were removed he was given a transfusion of 2 pints (1.1 litres) of stored plasma. During the 24 hours after this transfusion his whole-blood clotting-time, prothrombin consumption index, and thromboplastin generation test were within normal limits, and after the extractions he did not bleed. By three days after transfusion all of the tests showed a return almost to the pre-transfusion level. Two days after the removal of the first two teeth he was given 350 ml. of fresh serum, and on the next day three teeth were removed. The whole-blood clotting-time and prothrombin consumption index were reduced to the upper limits of normal following this transfusion, but the thromboplastin generation test remained far below the normal range. After these extractions he bled profusely, his haemoglobin being reduced to 52% of normal.

From these results it seems that the thromboplastin generation test may be the most sensitive index of the therapeutic effect of transfusion. Stored blood may be a more effective treatment for Christmas disease than quite fresh blood. The amount of blood likely to be required to control haemorrhage in an adult is of the order of 4 pints (2.3 litres) or 2 pints (1.1 litres) of plasma.

In conclusion it may be said that among cases usually classed as haemophilia are two distinct entities. Most "haemophilic" patients lack the antihaemophilic globulin; a smaller proportion lack a serum factor related to but differing from factor VII of Koller. This substance is referred to as the Christmas factor. A preliminary differentiation between these two conditions can readily be made by studying the *in vitro* effect of proportions of normal and haemophilic plasma on the calcium clotting-time of the patient's plasma. An exact distinction can be made by using the thromboplastin generation test. The existence of Christmas disease is of great academic interest, and the recognition of the condition in a specific patient is important because the treatment is not the same as that for haemophilia.

Summary

In this investigation haemophilia has been defined as a severe bleeding tendency in males dating from early infancy, inherited as a sex-linked recessive character in which there is a failure or delay in blood thromboplastin formation owing to a deficiency in antihaemophilic globulin.

Seven cases are recorded in which the clinical, usual laboratory, and in four cases the genetic features of haemophilia were present. In the blood of these patients antihaemophilic globulin was present in normal amounts, but blood thromboplastin formation was

grossly reduced because of the deficiency in the blood of a factor called the Christmas factor.

The Christmas factor can be obtained most readily from serum, and in some features resembles the serum factor VII of Koller *et al.* (1951). It differs greatly from the antihaemophilic globulin, and the blood from patients with true haemophilia (antihaemophilic globulin deficiency) is as effective as is normal blood in correcting the clotting abnormality in the blood or plasma of patients with Christmas disease.

In the treatment of haemorrhage in cases of Christmas disease concentrated preparations of antihaemophilic globulin are ineffective.

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SUPPRESSION OF MALARIA (P. BERGHEI) BY MILK

BY

B. G. MAEGRAITH, D.Phil., M.B., B.Sc., M.R.C.P.
Professor of Tropical Medicine, Liverpool School of Tropical Medicine

T. DEEGAN, M.A.
May and Baker Fellow

AND

E. SHERWOOD JONES, M.B., Ch.B., M.R.C.P.
Lecturer in Tropical Medicine, Liverpool School of Tropical Medicine

A year ago, during the course of experiments on the metabolism of haemoglobin derivatives in rats infected by blood passage with *P. berghei*, difficulty was experienced in infecting animals which were being maintained on a standard low-iron diet, consisting of milk to which minimal quantities of vitamins B_1 and B_6 and calcium pantothenate had been added (Copp and Greenberg, 1946). It was at first thought that the low iron content of the diet might be responsible for this phenomenon, but the addition of iron in adequate dietary amounts to the milk vitamin was found to have no effect. The results of further preliminary experiments, however, indicated that some degree of suppression of *P. berghei* infection occurred in animals which were infected while on a milk-vitamin diet. Recent experiments carried out under more carefully controlled conditions have confirmed this. Suppression of blood-transmitted *P. berghei* malaria in rats has been obtained in animals fed on diets of cow's milk, reconstituted proprietary dried milks, or human milk. Suppression of infection has also been observed in mice maintained on a diet of cow's milk and vitamins.

Experimental

The Parasite.—The strain of *P. berghei* used in these experiments was obtained originally from the London School of Hygiene and Tropical Medicine, and has been passaged in rats over the last three years by intraperitoneal blood injection. Frequent rapid passage of this strain through rats has provided us with a parasite of high virulence which commonly kills animals living on a normal laboratory diet.

In animals which survive, two waves of parasitaemia are commonly observed. The first wave is usually the more severe, reaching its maximum on about the tenth day after intraperitoneal injection with a standard inoculum of one million infected cells. The second, and usually much milder, wave appears somewhere about the twentieth day after injection.

Method of Infection of Rats.—In our early experiments the infecting dose of parasites was not calculated. Much more consistent results have been recently achieved by controlling the inoculum as follows. Blood is taken from an infected animal in which the parasitaemia is high and rising (usually 30% or more red cells infected). The number of parasites per 500 red cells is estimated and the blood diluted with citrated saline so that 0.2 ml. contains the standard inoculum of one million infected red cells. This volume is immediately injected intraperitoneally into each recipient rat.

Animals.—The rats used in the experiments were albinos bred in our laboratories from a strain obtained 18 months ago from the Sir William Dunn School of Pathology, Oxford. In each experiment the animals were of approximately the same age and weight (usually about 200 g.). In some cases litter mates were used. Before being placed on milk diets rats were maintained on the normal laboratory diet described below.

Diets

The diets referred to in the results were as follows.

(*a*) *Normal Laboratory Diet.*—This consisted of processed material obtained from Lever Bros. Ltd., containing wheat germ, skim-milk powder, dried yeast, fine bran, broad bran, molasses, coconut cake meal, groundnut cake meal, maize, fish meal, dried blood meal, limestone, common salt, bone flour. It was fed in the form of cubes with water *ad lib.* to the animals which served as controls for each experiment.

(*b*) *Cow's Milk.*—Retail whole milk was used. To each kilogram of milk was added 5 mg. each of vitamins B_1 and B_6 and 50 mg. of calcium pantothenate. Each animal was offered 150–160 ml. a day, equivalent to a protein intake of 4–5 g.

(*c*) *Reconstituted Dried Milk.*—Proprietary brands of dried milk were reconstituted according to instructions, and the same quantities of vitamins added per equivalent of reconstituted material. The volume offered was the same as in the cow's-milk diet.

(*d*) *Human Milk.*—A supply of human milk was obtained from the Liverpool Maternity Hospital. Before use it was stored in a deep freeze unit. No vitamins were added: each animal was offered 150–160 ml. a day, equivalent to a protein intake of approximately 3 g.

Experimental Conditions

All experiments were carried out in the same laboratory, which was controlled within a temperature range of 65–68° F. (18.3–20° C.). Animals were fed from sterilized inverted bottles stoppered by rubber bungs through which a sterilized glass feeding-tube was inserted and passed through the bars of the cage. Sterilization of feeding apparatus before filling was found to be necessary in order to avoid curdling.

In the earlier experiments small groups of animals were fed in the same cage from the same inverted bottles. We