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Haemorrhagic Smallpox

1. Preliminary Haematological Studies *

J. F. ROBERTS,[1] G. COFFEE,[1] S. M. CREEL,[1] A. GAAL,[1] J. H. GITHENS,[2]
A. R. RAO,[3] B. V. SUNDARA BABU [4] & C. H. KEMPE [5]

In an investigation of specific haematological defects that might account for the haemorrhagic diathesis in certain smallpox patients, 93 patients with haemorrhagic and non-haemorrhagic forms of the disease were subjected to a variety of bleeding and coagulation studies. The findings indicate that smallpox patients with no clinical evidence of haemorrhage have no significant clotting defects although many have decreased platelets and clot retraction abnormality. Patients with the late haemorrhagic form of smallpox consistently show thrombocytopenia and associated abnormalities in the bleeding time, tourniquet test and clot retraction; some also have slightly depressed specific prothrombin activity.

Patients with the severe, and uniformly fatal, early haemorrhagic form have severe thrombocytopenia, a marked decrease in specific prothrombin activity and prolongation of the prothrombin complex times. They also have a marked prolongation of the thrombin time, suggesting the presence of a circulating antithrombin.

Both early and late haemorrhagic smallpox patients also have a marked abnormality of prothrombin consumption, indicating impaired plasma thromboplastin production. This finding could be explained by the thrombocytopenia present in all haemorrhagic cases.

Investigations into various aspects of smallpox have been carried out over the past several years in the WHO-supported research laboratory at the Infectious Disease Hospital, Madras, India. One of the main areas of study has been the haematological investigation of the varieties of variola major in which a bleeding diathesis occurs. The mortality among patients with " haemorrhagic smallpox " has been extremely high and has been universally fatal in patients in whom the haemorrhage develops at an early stage (Curschmann, 1875; Dickson, 1962).

Smallpox cases have been classified clinically by Rao et al. (1963) (and unpublished studies of A. R. Rao) as haemorrhagic, flat, ordinary, and modified The haemorrhagic cases can be divided into two distinct clinical types—the early and the late. Patients with early haemorrhagic smallpox are extremely ill and present with a history of several days of high fever followed by the appearance of haemorrhages. These are usually in the mucous membranes and conjunctiva but may be in the skin. The bleeding almost invariably appears before the rash develops. Severe blood loss may occur from the gastrointestinal or genito-urinary tract, and massive pulmonary haemorrhage may be a complication. Death usually occurs before the sixth day of illness.

* This is one of a series of studies carried out in Madras, India, with the purpose of shedding new light on a number of still unsolved problems in the epidemiology, immunology, pathogenesis, prevention and treatment of smallpox. The studies were conducted under the sponsorship of the World Health Organization; the Indian Council for Medical Research; the Director of General Health Services, Government of Madras; the Commissioner, Corporation of Madras; the Health Officer, Corporation of Madras; the King Institute, Guindy; and the Infectious Disease Hospital, Madras. They were supported by grants from the World Health Organization; the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., USA (Grant AI 01632-09 VR); and the Marcus Tullius Reynolds III Fund.

[1] Medical Student Research Fellow, University of Colorado Medical School, Denver, Colo., USA.

[2] Professor of Pediatrics and Associate Dean, University of Colorado Medical School, Denver, Colo., USA.

[3] Health Officer, Corporation of Madras, Madras, India.

[4] Formerly, Health Officer, Corporation of Madras, Madras, India (deceased).

[5] Professor and Head, Department of Pediatrics, University of Colorado Medical Center, Denver, Colo., USA.

In late haemorrhagic smallpox the bleeding manifestations usually do not occur until the typical skin lesions of variola have appeared. The course of the disease is characterized by high fever with haemorrhage into the skin lesions and mucous membranes, with death usually occurring before the twelfth day.

The non-haemorrhagic types of smallpox have been classified by Rao into flat, ordinary and modified, these being subdivided into confluent, semi-confluent and discrete depending upon the distribution, the number and the clinical appearance of the lesions.

The existing literature reveals very few bleeding or clotting studies in the various types of smallpox. Early reports by Simon in 1918 and Ikeda in 1925 have indicated that thrombocytopenia might occur at various stages of the disease in all forms of variola major. The purpuric form was found by them to have a persistent thrombocytopenia until the patient's demise, while a rise in platelets occurred after the vesicular stage in the other clinical varieties. Haviland (1952) reported on six cases of " purpura variola " that occurred in an outbreak in the USA in 1946. He found platelet counts ranging from 10 000 to 24 000 in four of the patients. Further studies of these patients indicated that the bleeding time was normal in all six. The clotting time was 6 minutes in one and greater than 10 minutes in another patient; the prothrombin time was taken for three of the patients and was 89% and 61% in two cases while one sample did not clot.

Thus at the present time, the literature contains reports of platelet counts in several series, a clotting time and bleeding time in two patients and prothrombin determinations in three patients. The present study was initiated as a preliminary investigation to determine the general areas of haematological defect in both the early and late clinical forms of haemorrhagic smallpox. The findings in the haemorrhagic forms of the disease were compared with those in non-haemorrhagic smallpox and with those in a few patients with chickenpox.

MATERIALS AND METHODS

All the patients in this study were hospitalized in the Infectious Disease Hospital, Madras, India. A total of 106 patients were studied, various bleeding and coagulation tests being made during the period from December 1962 to March 1964. Ninety-three patients in the group had smallpox; 46 developed the haemorrhagic manifestations and 47 did not. The remaining 13 patients had chickenpox; control plasma samples were run daily for each test, the control samples being drawn primarily from the laboratory personnel. An attempt was made to carry out bleeding and clotting studies on all haemorrhagic patients admitted to the hospital during the period of study. The non-haemorrhagic patients were chosen at random.

The following preliminary studies were carried out in this group of patients. Vascular integrity and platelet number and function were evaluated by the bleeding time, the tourniquet test, clot retraction, and estimation of platelets by examination of the blood smear or by a platelet count. Blood coagulation was screened by a venous clotting time, one-stage prothrombin time, specific prothrombin assay, and the prothrombin consumption test. Evidence for a possible circulating antithrombin was evaluated by the thrombin time. An effort was made to complete all these studies on each patient, but for technical reasons this was not always possible.

The bleeding time was performed by the Ivy technique, using a sphygmomanometer cuff on the upper arm inflated to 40 Torr. The incision was made on the forearm with a 2-mm haemolet lance. The normal time by this method was found to be generally less than 6 minutes.

The tourniquet test was performed by observing for petechia after the sphygmomanometer cuff had been inflated to 40 Torr for 5 minutes. Platelet numbers were estimated from blood smears and reported as either decreased or normal. Platelet counts were performed by the direct method of Skirmont, Marks & Jacobsen (1949). The normal range by this method is 275 000 $\pm$ 100 000 per mm^3.

Venous blood for plasma, serum, clotting time, and prothrombin consumption was drawn using a three-syringe technique. Blood in the initial syringe was discarded. Plasma was collected by drawing 9 ml of blood into a siliconized syringe containing 1.0 ml of 3.2% sodium citrate. This blood sample was immediately transferred to siliconized tubes and spun for 10 minutes at 3000 rev/min in a cold centrifuge. Samples to be used at a later date were labelled, frozen immediately and stored at —10°C. Blood for the clotting time and prothrombin consumption were drawn into a third siliconized syringe.

The clotting time was performed by placing 1.0 ml of whole blood into each of two clean, dry,

glass tubes (13 mm × 100 mm). These were incubated at 37°C throughout the test and tipped approximately 10°-15° every 30 seconds until clotting occurred. The normal by this method was found to be less than 10 minutes.

Clot retraction was observed at one hour and at 24 hours in the two tubes that had been used for the clotting time after incubation at 37°C. Results were classified as good, poor or absent.

The one-stage prothrombin test was performed according to the method of Quick (1940) by adding 0.1 ml of thromboplastin (Difco) and 0.1 ml of 0.02 M calcium chloride to 0.1 ml of test plasma in a plain glass tube (13 mm × 100 mm). Results were recorded in seconds. The normal varied from 12 to 18 seconds under the conditions of the laboratory in Madras, but was usually between 12 and 14 seconds.

The thrombin time was performed by a modification of the method of von Kaulla & von Kaulla (1964). One thousand units of bovine thrombin (thrombin, topical, Parke-Davis) were dissolved in 5 ml of 50% glycerol and stored at —10°C. For the test 0.5 ml of the thrombin-glycerol solution was diluted with 1.8 ml of buffered saline (at pH 7.35); 0.15 ml of the diluted thrombin was added to 0.15 ml of undiluted citrated plasma and the rate of clotting was timed in a water-bath at 37°C.

The specific prothrombin determination was performed by the method of Owren & Aas (1951), using commercially available prothrombin and proconvertin-free beef plasma (Difco), normal human serum and thromboplastin (Difco) as reagents. The specific prothrombin activity of the test plasma was calculated as the percentage of normal activity by using a dilution curve of normal plasma.

The prothrombin consumption test was performed by measurement of the specific prothrombin remaining in the serum after one hour of clotting at 37°C by a similar method (Rapaport et al., 1955). The amount of prothrombin in the serum was calculated on the basis of the percentage of that originally present in the patient's plasma.

RESULTS

As noted above, it was not always possible to complete all the studies on every patient. Of the 106 patients, 93 in the group had smallpox, 46 with haemorrhagic disease and 47 with no clinical evidence of haemorrhage. Certain of the studies were also completed on 13 patients with chickenpox.

Vascular integrity and platelet function

These studies are summarized in Table 1.

Bleeding time. The bleeding time was prolonged in all 30 of the patients with haemorrhagic smallpox on whom this test was performed. This included patients with both the early and late forms of the disease. It was abnormal in 6 of 15 with non-haemorrhagic disease.

Tourniquet test. The tourniquet test was positive in 15 of 31 patients with haemorrhagic smallpox. It was abnormal in all of those with the early form of the disease while it was positive in only 9 of 25 patients with the late hemorrhagic manifestations. Only one of 15 patients with non-haemorrhagic smallpox had a positive tourniquet test.

Clot retraction. The clot retraction was abnormal in all 29 patients with haemorrhagic smallpox on whom it was performed. The abnormality was present in both the early and late forms of the disease. Clot retraction was poor or nil in 3 of 14 patients with non-haemorrhagic smallpox.

Platelets. Platelet counts were not performed in the first group of patients in this study. However, blood smears were examined for estimation of platelets, and showed reduced thrombocytes in all the cases of haemorrhagic smallpox (both early and late forms) in which this observation was made. Platelets were estimated to be normal to slightly reduced in individuals with non-haemorrhagic smallpox.

Platelet counts were completed in 19 patients in the second group studied. The range of the findings is summarized in Table 1. All patients with early and late haemorrhagic smallpox demonstrated significant thrombocytopenia (less than 40 000) while the platelets varied from a moderate decrease of 48 400 to a low normal of 166 100 in patients without clinical evidence of haemorrhage.

Coagulation studies

The following coagulation studies were performed on a large proportion of the 46 haemorrhagic patients, most of the 47 patients with non-haemorrhagic smallpox, and 9 of the chickenpox patients. Fresh controls were run daily for each test.

Clotting time. Results of the whole-blood clotting time are shown in Table 2. It was abnormal in 12 of 27 haemorrhagic patients, being prolonged in a significant number of both early and late patients.

TABLE 1
VASCULAR INTEGRITY AND PLATELET FUNCTION

Study	Non-haemorrhagic smallpox	Haemorrhagic smallpox		
		Early	Late	Total
Bleeding time (Ivy): [a]				
0-6 min	9	0	0	0
6-10 min	5	0	1	1
>10 min	1	6	23	29
Tourniquet test:				
Negative	14	0	16	16
Positive	1	6	9	15
Clot retraction:				
Good	11	0	0	0
Poor	2	1	1	2
Nil	1	5	22	27
Platelets:				
Platelet estimation on blood smear	Normal to slightly decreased	Decreased	Decreased	Decreased
Platelet counts (range)	48 400 to 166 100	23 000 to 31 000	12 300 to 39 600	12 300 to 39 600

[a] Normal = 6 min.

It was normal in all of the non-haemorrhagic smallpox patients who were studied.

One-stage prothrombin (Quick, 1940). The one-stage prothrombin times are illustrated in Fig. 1, with the results shown in seconds. The upper range for the controls is slightly above that which is considered normal in most laboratories but is probably explained by the exposure of the reagents to the hot climate in Madras. The findings demonstrate a marked prolongation in all cases of early haemorrhagic smallpox, with a slight prolongation in a few of the late haemorrhagic and non-haemorrhagic smallpox patients. The chickenpox cases fall within the normal range. These data were obtained on 10 early and 32 late haemorrhagic smallpox patients, 47 non-haemorrhagic smallpox patients and 10 chickenpox patients.

TABLE 2
VENOUS CLOTTING TIME [a]

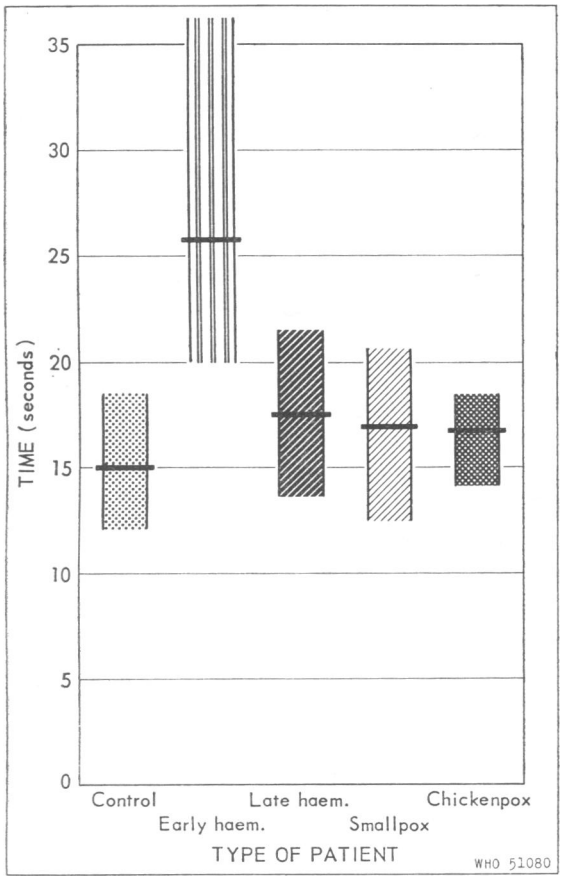
Time	Non-haemorrhagic smallpox	Haemorrhagic smallpox		
		Early	Late	Total
<10 min	10	1	14	15
10-20 min	0	1	2	3
>20 min	0	3	6	9

Specific prothrombin activity. The plasma level of specific prothrombin is illustrated in Fig. 2, with the results presented as a percentage of normal activity. A definite decrease of prothrombin is noted in the early haemorrhagic patients and in a few of the late haemorrhagic patients. Levels in non-haemorrhagic smallpox and chickenpox patients fall essentially within the normal range. Data include results on 5 early and 22 late haemorrhagic patients 23 non-haemorrhagic smallpox patients, and 8 chickenpox patients.

Serum prothrombin time (prothrombin consumption test). This determination evaluates the activity of platelets as well as all first-stage clotting factors.

FIG. 1

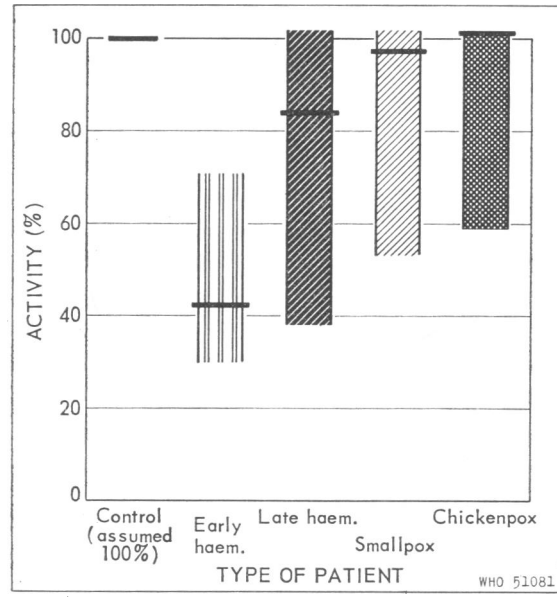
ONE-STAGE PROTHROMBIN TIMES (RANGES AND MEDIANS) [a]



[a] Marked prolongation in patients with early haemorrhagic smallpox. Findings indicate a defect in thrombin formation or activity. Method of Quick (1940).

FIG. 2

SPECIFIC PROTHROMBIN (RANGES AND MEDIANS) EXPRESSED AS PERCENTAGE OF CONTROL PLASMA ACTIVITY [a]



[a] Decreased activity in most patients with early haemorrhagic smallpox and in a few with late haemorrhagic smallpox.

The results are illustrated in Fig. 3, which demonstrates a significant abnormality in essentially all the patients with haemorrhagic smallpox. The early haemorrhagic patients had a more marked defect than the late haemorrhagic ones. A few patients with non-haemorrhagic smallpox also showed an abnormality in their serum prothrombin time, while the chickenpox patients were all within the normal range. These data were obtained on 5 early and 21 late haemorrhagic smallpox patients, 32 non-haemorrhagic smallpox patients, and 7 chickenpox patients.

Thrombin time. The thrombin time, which is primarily a measure of antithrombin activity,[1] was performed in the same group of patients. The results (Fig. 4) showed a marked abnormality in all patients with early haemorrhagic smallpox while there was no evidence of increased antithrombin activity in the late haemorrhagic patients. A few patients with non-haemorrhagic smallpox, however, had slight prolongation of the thrombin time. These data include 10 early and 30 late haemorrhagic smallpox patients, 31 non-haemorrhagic smallpox patients and 10 chickenpox patients.

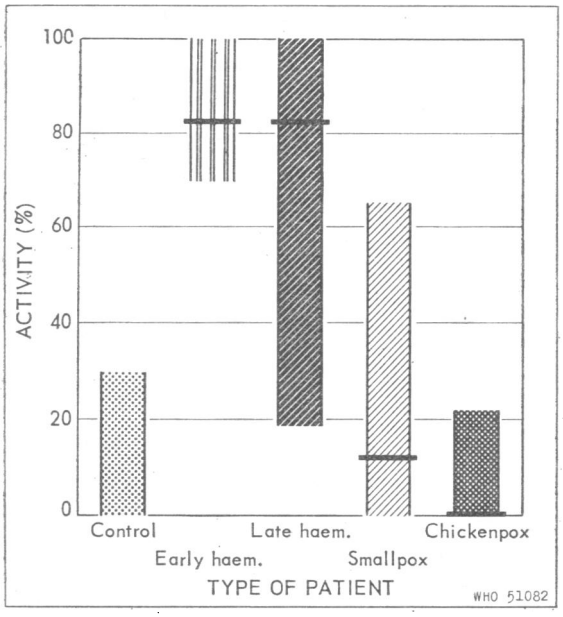
DISCUSSION

The results of these determinations revealed that all patients with haemorrhagic smallpox had a prolonged bleeding time and an abnormal clot retraction. Many also had a positive tourniquet test.

[1] The thrombin time would also be prolonged in the presence of a markedly diminished fibrinogen. Therefore a screening test for fibrinogen was carried out on 22 of the plasma samples that were available in the frozen state. The fibrinogen titre method of Bowman & Yelito (1957) was used, and all but one of the patients tested were found to have fibrinogen activity within normal levels.

FIG. 3

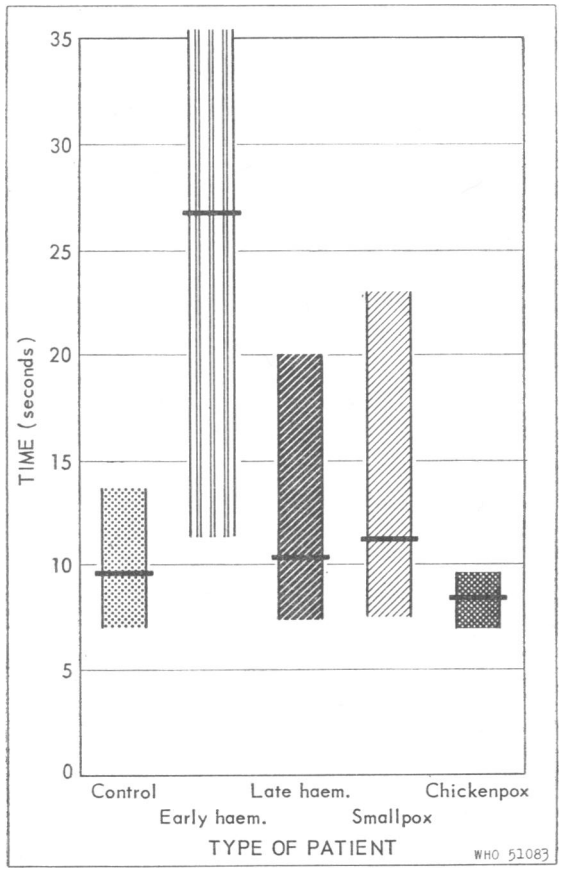
PROTHROMBIN CONSUMPTION (RANGES AND MEDIANS) EXPRESSED AS PERCENTAGE OF PATIENT'S PLASMA ACTIVITY [a]



[a] Abnormal in haemorrhagic smallpox and in a few patients with non-haemorrhagic smallpox. Findings indicate a defect in plasma thromboplastin formation.

FIG. 4

THROMBIN TIMES [a]



[a] Marked abnormality in early haemorrhagic and non haemorrhagic smallpox. Findings indicate increased antithrombin activity.

Platelet counts were less than 40 000 and examination of the blood smear revealed reduced thrombocytes in all haemorrhagic patients on whom this observation was made. These findings are all compatible with the presence of thrombocytopenia and are in agreement with the previously reported studies (Simon, 1918; Ikeda, 1925; Haviland, 1952). The slight reduction of platelets and an abnormal clot retraction in a few patients with non-haemorrhagic smallpox also support the finding of Ikeda (1925), who suggested that a temporary thrombocytopenia may be present in non-haemorrhagic patients who later recover.

The coagulation studies revealed an abnormal serum prothrombin time (prothrombin consumption test) in all haemorrhagic cases; this study was normal in most of the non-haemorrhagic smallpox patients and in all the chickenpox patients. This abnormality could be produced by thrombocytopenia alone or by any first-stage clotting defect. It is not possible from the present data to draw any further conclusions regarding the possibility of other coagulation factor defects in the first stage of clotting (production of plasma thromboplastin) in these patients since the abnormality could be produced entirely by the thrombocytopenia.

Evidence of definite impairment in the second stage of coagulation (thrombin formation) was found in all patients with the early haemorrhagic form of the disease. This group showed a markedly abnormal one-stage prothrombin time, a very low level of specific prothrombin and a marked increase in antithrombin activity. In addition, most of the late haemorrhagic patients showed a moderate decrease in specific prothrombin.

These clotting deficiencies and the evidence for

increased antithrombin activity have not been previously described in patients with haemorrhagic smallpox. These results demonstrate the need for further assays of all coagulation factors in patients with haemorrhagic smallpox and in addition provide clues to treating the haemorrhagic manifestations not only with platelet concentrates but also with plasma or whole-blood transfusions.

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RÉSUMÉ

En vue de préciser la nature des altérations sanguines responsables de la diathèse hémorragique qui caractérise certaines formes de variole, toute une série d'épreuves hématologiques ont été pratiquées chez 93 varioleux, dont 46 atteints de variole hémorragique, hospitalisés à Madras, Inde.

Chez les malades qui ne présentent aucune manifestation hémorragique cliniquement apparente, la coagulation sanguine ne montre pas d'altérations notables, bien qu'une réduction du nombre des plaquettes et des anomalies de la rétraction du caillot s'observent dans plusieurs cas. Dans les formes hémorragiques tardives, on note de la thrombocytopénie et la recherche du temps de saignement, du signe du lacet et des modalités de la rétraction du caillot fait ressortir des troubles de l'intégrité vasculaire et de la fonction plaquettaire. Dans certains cas, on constate un léger déficit de l'activité prothrombinique spécifique. Chez les malades atteints de formes hémorragiques précoces, lesquelles sont par ailleurs d'une extrême gravité et invariablement mortelles, il existe une forte thrombocytopénie ainsi qu'une diminution marquée de l'activité prothrombinique spécifique et une prolongation du temps de Quick caractéristique d'anomalies du complexe prothrombinique. Dans toutes les formes précoces, le temps de thrombine est nettement prolongé. Enfin, dans toutes les formes hémorragiques, précoces ou tardives, on observe des anomalies de la consommation de prothrombine, témoin d'un déficit de la production de thromboplastine plasmatique.

Ces observations préliminaires montrent la nécessité de poursuivre l'étude des divers facteurs intervenant dans la coagulation sanguine chez les varioleux atteints de formes hémorragiques et sont susceptibles de fournir des renseignements utiles pour le traitement de ces états.

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