The Inhibiting Effect of High Plasma Concentrations on Blood Coagulation. Observations during the Treatment with Dicoumarin Derivatives and in Other Conditions

The recent investigations of TOCANTINS *et al.*[1] on the actual plasma concentration in the clotting mixtures have shown that: (1) the usual dilution curves of normal plasma mixed with thromboplastin and calcium (one-stage Quick's method) only refer to the original plasma concentration but not to the final concentration in the mixture; (2) for a mathematical evaluation of the complete curve at all plasma dilutions; i.e. also at high concentrations, a formula is needed which corresponds to the parabolic and not to the hyperbolic course; (3) in hemophiliacs the parabolic course is very evident, whereas in asbestos plasmas the curve will approach a hyperbolic course; (4) in hemophiliacs the response to thromboplastin is different from the response in normal subjects.

The observations of TOCANTINS have been carried out with a particular technique, which is necessary for the study of hemophilic plasmas. For the investigation of other physiopathological conditions, and in order to establish some parallels with the usual tests (one-stage method), we tried to unify the experimental conditions, to characterize the influence of high plasma concentrations on the blood coagulation, and to make it possible to apply this method currently.

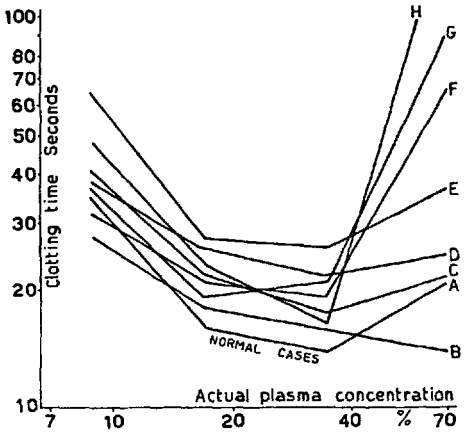

Fig. 1.—Clotting times of a mixture containing: 0·25 ml plasma at various dilutions, 0·025 ml calcium chloride 0·2 M, 0·025 ml thromboplastin. Actual plasma concentrations are given (see details in the paper). *A:* normal values; *B, C, D, E:* subjects treated with dicoumarin derivatives (Tromexan); *F, G, H:* other conditions (liver diseases).

Methods. Oxalated plasma is used as in the one-stage method[2]. The determinations are carried out by mixing plasma, thromboplastin and calcium chloride according to the following schema:

(a) Determinations according to Quick's original method: 0·1 ml plasma + 0·1 ml high active thromboplastin + 0·1 ml 0·02 M calcium chloride, at 37°C. Normal values : 10·5–11·5″.

(b) Determinations with concentrated plasma: 0·25 ml undiluted and diluted plasma (50%, 25% and 12·5% in saline) + 0·05 of a mixture containing 0·2 M calcium chloride and high active thromboplastin in equal parts.

The graphic representation of the values obtained by the latter procedure is made on a logarithmic chart. The diluting effect of the oxalate and of the calcium and thromboplastin has been calculated. In the normal cases the final plasma concentration in the mixture is about 70% for the non-diluted plasma and of 35%, 17·5%, 8·725% for the diluted plasma. In the clotting mixture of the original Quick's method, the actual concentration of the non-diluted plasma is about 26–28% under normal conditions (Fig. 1).

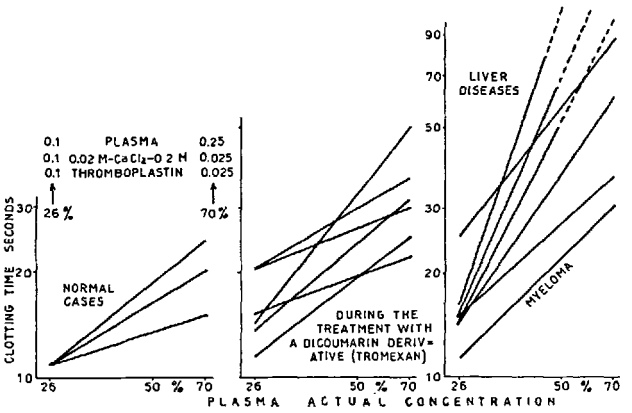

Fig. 2.—Comparison of the usual prothrombin time with the prothrombin time at high plasma concentration.

As in the original one-stage method the variations of the hematocrit are not considered. The comparison of the usual method with the high concentrations method has been done without considering such variations, in order to obtain a standardization of the methods.

For the numerical evaluation of the correlations between the two methods, we suggest the comparison of the clotting times obtained with non-diluted plasma at the actual concentrations of 26 and 70% and the connection of the values in a logarithmic chart (Fig. 2).

Discussion. In the graphs some of the results are presented which have been obtained in normal subjects, in subjects treated with dicoumarin derivatives and in subjects suffering from various diseases, especially liver diseases, and, in one case, myeloma. The left portion of the curve shows a course which is similar to the course observed in the conventional curve of the one-stage method. The differences between the various plasmas are not very marked. The right portion of the curve, on the contrary, shows a very characteristic behaviour, because the differences between the various plasmas are very marked[1]. In some cases of severe liver diseases we observed values higher than 300″ (determinations on concentrated plasma), although the clotting time of the diluted plasma was not very much prolonged. The dilution probably eliminates the influence of inhibiting substances (antithromboplastin substances, according to TOCANTINS *et al.*[2]). It is probable, however, that the inhibition is due, in some cases, to the presence of protein anticoagulant substances, and generally to a dysproteinaemic condition, as it has been shown in numerous physiopathological observations. As similar results can

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be observed also in some subjects treated with dicoumarin derivatives, it is not yet possible to establish the value of such an interpretation.

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Zusammenfassung

Der Einfluß hoher Plasmakonzentrationen auf die Blutgerinnung wurde während der Behandlung mit Dikumarinderivaten und bei anderen Krankheitszuständen untersucht. Eine einfache Methode zur klinischen Auswertung solcher Ergebnisse wird vorgeschlagen. Es wird die Möglichkeit besprochen, durch solche Methoden zirkulierende Antikoagulantien zu bestimmen.

Research on the Proliferation Activity of Erythroblasts at Low Atmospheric Pressure

It is now an established fact that when living under conditions of low atmospheric pressure animals (as well as human beings) show an increase in erythrocytes and haemoglobin (BERT[1], VIAULT[2], BARCROFT[3], SEYFARTH[4], MONGE *et al.*[5], WOLFER[6], HURTADO[7], HEILMEYER *et al.*[8], KRUPSKY and ALMASY[9], TALBOTT and DILL[10], HURTADO *et al.*[11], FARBER[12], VERZÁR[13], ROBLES and GONZALES[14], GRANDJEAN[15], MERINO[16], LAWRENCE[17]). The general assumption is that this hyperglobulia is due to the enhanced erythroblastic activity of the bone marrow, rather than to a decreased hemocatheresis. Such an assumption is supported by the finding of a peripheral reticulocytosis (SEYFARTH[4], WOLFER[6], BARCROFT[18], HEILMEYER *et al.*[8], KRUPSKY and ALMASY[9]) and, even more, of a medullary erythroblastosis (SCHACKE and MCDUPPE[19], REISSMANN[20]) From these findings most authors infer that hypoxia may act as a stimulant of erythropoiesis. According to such a concept, polyglobulia would be the ultimate condition brought about by the following gradual stages: hypoxia of the surrounding atmosphere → hypoxemia (peripheral) → medullary hypoxia → erythropoiesis enhanced by hypoxia → polyglobulia.

Some facts, however, seem to be inconsistent with such an assumption.

First of all, ROSIN and RACHMILEWITZ[1] have noted that in bone marrow cultures *in vitro* the mitotic index decreases as the O_2 tension decreases. Under similar conditions, MAGNUSSEN[2] observed a decrease in erythrocyte formation. BULLOUGH[3], as well as ASTALDI *et al.*[4] noticed that under anoxaemic conditions, the cells do not enter mitosis, although the mitoses, which are already under way, continue to cytodieresis. GRANT and ROOT[5] have observed a normal O_2 saturation of medullary blood in some cases of enhanced erythropoiesis. VANNOTTI and MARKWALDER[6] as well as VERZÁR[7], reported that polyglobulia under low atmospheric pressure is preceded by an incease in erythrocyte destruction, and consequently assumed that the stimulus toward erythropoiesis may be due to the products of erythrocatheresis, rather than to an O_2 decrease at the marrow level. Finally, REISSMANN[8] showed that polyglobulia occurs not only in the animal under low atmospheric pressure, but also in an animal under normal atmospheric pressure when it is parabiotically connected through a tissular ear-tail anastomosis with the animal under low atmospheric pressure. This phenomenon is explained by REISSMANN assuming that erythro-stimulating substances (erythropoietins) are formed in the animal under low pressure as a consequence of hypoxia, and that such substances pass through the anastomosis to the other animal.

In our opinion, all the foregoing facts are markedly inconsistent with the assumption that hypoxia may by itself be able directly to stimulate erythropoiesis, as most haematologists think. Consequently we felt that the problem was worth examining from various standpoints, and this paper is a report on the functional activity shown by bone marrow surviving under low atmospheric pressure, with special reference to the proliferation activity of erythroblasts of human bone marrow.

The Astaldi-Mauri stathmokinetic test has been used to determine the amount of proliferation. Such a test consists in transplanting bone marrow fragments to a plasma culture containing optimum concentration of colchicine. Owing to the stathmokinetic effect of colchicine, all the cells entering mitosis are arrested in the pre-metaphase, whereby the amount of proliferation activity can be inferred from the number of mitosis occurring in a given period of time (stathmokinetic index).

This research has been carried out on the bone marrow (from the sternum) of 10 subjects suffering from secondary anaemia, in order to obtain a marrow rich in erythroblasts. The culture medium was composed as follows: 1·4 cm^3 plasma taken from the marrow donor himself (this plasma had been obtained as fluid by the addition of 10 A.U. of heparin per cubic centimeter blood); 0·2 cm^3 of 1/50000 colchicine in Tyrode solution; 0·4 cm^3 of 1/500 protamin sulphate. Protamin, which neutralizes the heparin, making possible the coagulation of plasma (ASTALDI and FRANKO, 1950), had been used to coagulate the culture medium, and should therefore be added just before transplanting.

By using a particular culture apparatus, the bone marrow of each subject has been explanted under the

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