des Volkes, Alter der Königin, als auch *bleibend* verschieden durch erbliche Unterschiede zwischen den Rassen oder einzelnen Völkern.

G. HEIN

Institut für vergleichende Physiologie der Universität Utrecht, den 11. August 1953.

Summary

Bee dances indicating the position of feeding places situated at short distances from the hive show a great variability. They have only one element in common which seems suitable to this purpose: the "pull-dance" (Rucktanz), described by us earlier.

Method for the Quantitative Evaluation of Platelet Function in Blood Coagulation

The role of platelets in blood coagulation has been characterized in connection with the identification of several active agents supplied by the platelets themselves: (1) platelet thromboplastic factor[1]; (2) platelet factor 1, presenting Ac-globulin activity[2]; (3) platelet factor 2, which accelerates the conversion of fibrinogen into thrombin[2]; (4) platelet factor 3, or antiheparinic factor, which is probably similar or identical with the thromboplastic factor[3].

Quantitative or qualitative alterations of platelets may be revealed by the incomplete transformation of prothrombin into thrombin (positivity of the prothrombin consumption test). This test is, however, positive also in various other conditions, and its limited specificity is often the cause of diagnostic difficulties.

For the quantitative evaluation of platelet function as a whole, a method has been worked out which is based on the functional study of isolated platelets in a clotting system. The principle of the method is the following: all factors are kept constant, except the factor to be determined, i.e. platelet functional activity, according to this scheme:

Constant
Prothrombin
Antihemophilic globulin and allied factors
Ac-globulin or proaccelerin
Factor VII or proconvertin or SPCA
Calcium
Platelet number (300,000/cu.mm)

Variable
Platelet functional activity

The substrate containing all factors to be kept constant is obtained by preparing a strictly platelet-free plasma (high speed centrifugation, cold, siliconed glassware and needles[4]). Calcium is supplied in known amounts.

The most important point of the method is the preparation of the platelet suspension. In previous research, exact quantitative tests have not been employed[1], partly because the preparation of non-agglutinated platelet suspensions containing a fixed amount of platelets presents some technical difficulties. To avoid agglutination and to perform an accurate count of the platelets, it is indispensable to use throughout: (*a*) arquad coated needles; (*b*) siliconed coated glassware; (*c*) sequestrene as anticoagulant; (*d*) triton and sodium acetate for preserving the platelets. The details of this technique are described by STEFANINI *et al.*[2] and have been followed exactly. Differential centrifugation and repeated washings of platelets have to be carried out rapidly.

By employing such methods, it is possible to prepare suspensions of non-agglutinated platelets and to bring their concentration to 300,000/cu.mm with sufficient accuracy. The test for the quantitative evaluation of platelet function in blood coagulation is carried out as follows; 0·1 cm³ of the platelet suspension is added to 0·1 cm³ of platelet-free plasma. One tenth cm³ of $CaCl_2$ 0·025 *M* is added to this mixture and the clotting time at 37°C is recorded. By using serial dilutions of the platelet suspension, a standard curve is obtained which makes it possible to evaluate the percentages of platelet activity. The recalcification time of the platelet-free plasma, to which the undiluted platelet suspension has been added, is considered as 100%. If saline is added instead of platelet suspension, the values of the recalcification time refer to 0% platelet activity, and should be more than 6–8 min at least. The percentage of reduction of the clotting time is converted into percentage of platelet functional activity (Fig. 1).

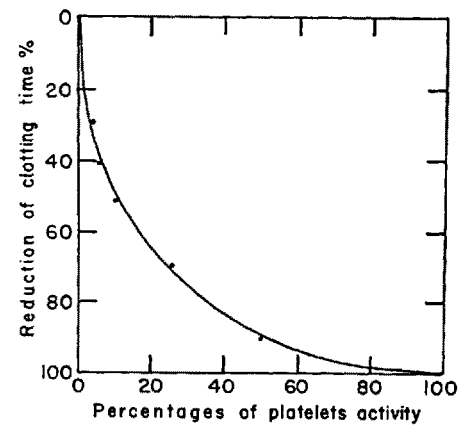

Fig. 1.–Standard curve for the evaluation of the percentages of platelets activity from the reduction of clotting time.

The same test might be carried out by using a two-stage method, as already observed by one of us[3]. High concentrations of purified prothrombin (3000 units/cm³), platelet-free plasma and optimal calcium amounts are used for this purpose. Platelet suspensions may be prepared according to the same procedure.

Preliminary observations carried out by using this method[4] have shown that variations of platelet func-

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tional activity as a whole can be detected not only in thrombocytoasthenias but also in other conditions, as for instance during menses[1]. (See previous researches on the positivity of the prothrombin consumption test during menses[2].) In such cases, as well as in other conditions, significant modifications can be detected, regardless of the actual platelet count, which might influence the results in other methods.

P. DE NICOLA and P. ROSTI

Department of Internal Medicine, University of Pavia, October 16, 1953.

Zusammenfassung

Eine Methode zur Auswertung der funktionellen Plättchenaktivität wird beschrieben. Isolierte Plättchen in konstanter Zahl (300000/cu.mm) werden einem alle übrigen Gerinnungsfaktoren in konstanter Menge enthaltenden Substrat zugesetzt. Prozente können von den Gerinnungszeiten durch eine Eichkurve ermittelt werden.

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The Influence of the Nucleus and of Heat Regulation on the Dipeptidase Activity of the Nucleated and Anucleated Erythrocytes of Vertebrates

Researches in Comparative Cytochemistry

The study of the peptidases—hydrolytic enzymes which split the CO-NH bond of the amino acids combined to form the peptides—has been greatly advanced by the work of the CARLSBERG Group (LINDERSTRÖM-LANG, HOLTER an others[1]). These workers have put forward the hypothesis that these enzymes, known to be ubiquitous, may play a part in the process of protein synthesis which occurs in the cells; such a suggestion has arisen from the fact that the peptidases are found in very considerable quantities in cells with notable power of synthesis (DUSPIVA[1], SMITH[2]). It has moreover been possible to locate the peptidases in the cytoplasm of the cells (DUSPIVA, HOLTER and LÖVTRUP[3]), and not in the nucleus. URBANI[4] was able to notice that in amoebas experimentally enucleated, the dipeptidases are partially affected by the removal of the nucleus, from which one must conclude that the nucleus, although it does not itself contain the enzyme, partly controls its presence in the cytoplasm.

We may recall briefly that researches on dipeptidase activity have even been carried out on the cells of human haematopoietic tissues, in normal conditions and in connection with the relation between *d-peptides* and malignant processes (MERTEN, ADAMS, SALVIDIO, and PARREIRA[1]). Recently ADAMS *et al.*[2] have assayed Mammalian and Avian red blood corpuscles for four separate peptidases; they found that each peptidase varied only a 3-fold range from species to species, and that nucleated avian erythrocytes showed no distinctive differences in activity from mammalian corpuscles.

To explain more clearly the relationship between nucleus and cytoplasm, G. COTRONEI suggested to us that a piece of comparative research, extended to all the Vertebrates, might be of interest in the evaluation of the influence of the nucleus on the enzymatic content of haematic cells.

We have therefore undertaken an analysis of the dipeptidases in the erythrocytes of the Vertebrates, from Fish to Man; we have, however, not been able for the moment to carry out research on the Cyclostomata, owing to lack of material.

Material and Method. Erythrocytes of the following species: *Carassius auratus, Triton cristatus, Bufo vulgaris, Rana esculenta, Lacerta viridis, Anser anser, Gallus domesticus, Mus musculus,* and *Homo sapiens.* Determination of the dipeptidase activity (substrate *dl*-alanylglycine 0·2 m) was carried out according to the titrimetric micromethod of LINDERSTRÖM-LANG and HOLTER[3]. After taking the sample the red corpuscles were washed, counted and hemolysed with distilled water. We calculated the standard cleavage of alanylglycine 100,000 cells and for one hour's incubation at 38°C, and the cleavage per cellular volume unit, obtaining this latter by means of a microhematocrit.

Results. Having ascertained that in the erythrocytes of the Urodela also, the enzymatic activity occurs almost entirely in the cytoplasm, we established the pH opimum of the peptidases in the various classes of Vertebrates, and by comparing them, determined the enzyme content of the erythrocytes of the Vertebrates examined.

As one can see from the table, the optimum pH in the various species examined is around 7·70, though it has an optimum of 7·40 in Fish, and 7·90 (with a secondary maximum at 7·10) in Man. Quantitatively, on a superficial examination of the data, it would seem that there exists a *direct proportionality* between cellular volume and dipeptidase activity. On a more careful examination, however, one notes that while the relationship between the volume of the erythrocytes of Triton and Mouse is 50 to 1 (2500 micron^3 to 51 micron^3), the relationship between the respective standard cleavages is 103 to 1, and the cleavage per volume is 1·46 for the Triton, 0·60 for the Mouse, and 0·41 for Man. These facts show that the enzymatic activity of the erythrocytes in Mouse and Man is *less* that one would have expected from the relationship to cellular volume. An examination of the connection between cellular volume and enzymatic activity per volume unit of the erythrocytes of the cold blooded animals shows, on the other hand, that the dipeptidase activity is proportional to the cellular size and practically equal in the various cold blooded species, in relation to the unit of cellular volume (seeTable). From the Figure it can be seen that the cleavage per volume unit (in grey) is almost the same in the cold blooded Vertebrates, while there is a clear difference between cold and hot blooded species. Between

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