STUDY OF ELECTROKINETIC POTENTIAL OF HUMAN ERYTHROCYTES IN PRESENCE OF ANTICOAGULANTS

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ABSTRACT

Electrokinetic potential (EKP) of human erythrocytes of blood group "O" was measured in dextrose isotonic medium. The effect of various concentrations of heparin or potassium citrate on EKP of red blood cells was studied. A linear correlation between EKP and logarithm of heparin concentration was revealed. However, no such relation could be seen in the case of varying concentrations of potassium citrate. In the case of potassium citrate, a slow increase in EKP up to the concentration of 28 mg % was observed, and this suddenly increased at higher concentrations. Probable reasons for the increase in EKP with the increasing concentration of the heparin and the peculiar EKP changes with potassium citrate have been suggested.

INTRODUCTION

RIDDICK[1] has recently put forward the physicochemical mechanism of blood coagulation, which implies the presence of substantial amount of electrical repulsive energy for maintaining the discreteness of blood cells without any coagulation. It is well known that EKP, which is a measure of diffuse double layer charge density, is mainly responsible for such repulsion[2]. Although EKP of erythrocytes has been studied under varying conditions[3] of pH, ionic strength,[4–7] and the agents modifying their surface characteristics[8–13], no information is available on the effect of anticoagulants. In this paper EKP changes of red blood cells in presence of anticoagulants—heparin or potassium citrate—are reported.

MATERIALS AND METHODS

Heparin Injection I.P.** (Biological Evans).
Potassium citrate I.P.**

For the present study we have examined the effect of blood groups on the EKP of erythrocytes. Our preliminary results suggested that there is an effect of blood group substances on the EKP of erythrocytes. This finding we are not reporting here only with a view to examine such differences by statistical analysis on a large population. In order to avoid an interference by blood groups it was decided to restrict the present investigation to only one specific blood group. For the present study, five healthy men belonging to blood group "O", between the age group of 18–25 years, were therefore considered.

Preparation of erythrocyte suspension

After pricking the finger tip of a volunteer with a sharp needle 0·1 ml of blood was drawn into a RBC pipette and was immediately transferred into 50 ml of freshly prepared isotonic dextrose solution. A gentle swirling motion was then given to ensure uniform distribution. This procedure gave 0·2% v/v of blood cell suspension. This suspension was considered as control sample. Following the similar procedure, erythrocyte suspensions containing required concentrations of anticoagulants were prepared. The concentration ranged from 0·1 to 10,000 IU† % and from 0·1 mg to 200 mg% for heparin and potassium citrate, respectively. A period of two minutes was allowed for erythrocytes to equilibrate with new bulk concentration of anticoagulant. The EKP determinations were then performed.

Measurement of electrophoretic mobility and calculation of EKP

A cylindrical microelectrophoresis cell of 4 mm diameter, equipped with platinum-irridium electrodes, was used for the measurement of electrophoretic mobility of erythrocytes.

The electrophoretic mobility readings were taken at the stationary level of the microelectrophoresis cell applying the required emf ranging from 50 to 150 volts. The voltage across the platinum iridium electrodes in the microelectrophoresis cell was kept constant throughout the measurement and was recorded. The direction of current flow was reversed with the help of reversing switch after every five measurements so as to minimize electrode polarization effects. Depending upon degree of homogeneity of the sample, 20 to 100 erythrocytes were tracked. From such data the average electrophoretic mobility was determined and used for calculating EKP by the Helmholtz Smouluchowski equation.

$$\zeta = \frac{4\Pi\eta V}{DE}$$

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** Indian pharmacopoeial grade.

† 100 IU = 1 mg.

where,

ζ = Electrokinetic potential,

η = Bulk viscosity,

D = Dielectric constant of suspending medium, and

V = Average electrophoretic mobility of erythrocyte in the field strength E.

Results and Discussion

The average EKP value of the five individuals is plotted against the concentration of anticoagulant (Fig. 1). From this figure, it can be seen that EKP of the erythrocytes is a linear function of the log.

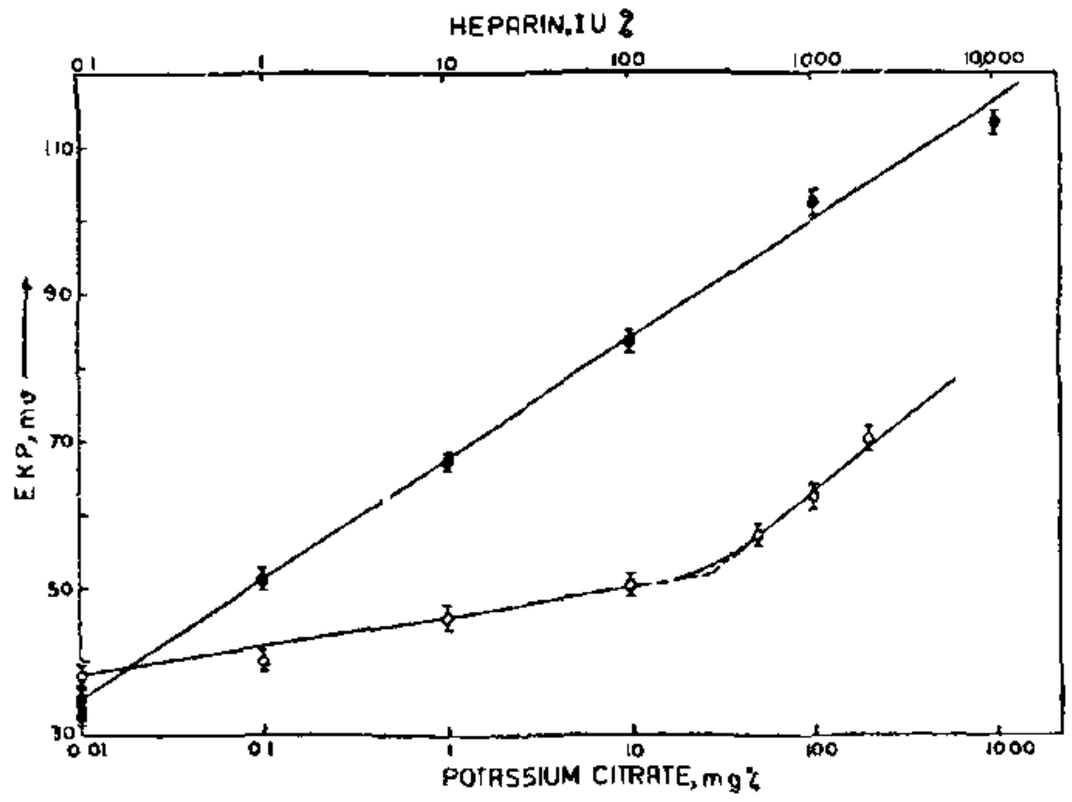

Fig. 1. A semilog. Plot of EKP *vs.* concentration of anticoagulants. ■; Control. ●—●; Heparin; ○—○ Potassium citrate. Bars indicate standard deviation.

heparin concentration. However, such linear relation cannot be seen in the case of potassium citrate because of the sudden change in the slope value after 28 mg %.

As there are no concrete reports available about the nature of surface ionogenic groups of erythrocyte[14], it is difficult to pinpoint the exact mechanism of adsorption of potassium citrate or heparin so as to explain the characteristic EKP changes of red blood cells in presence of these agents. However, similar type of characteristic EKP changes were observed by Riddick[15] for a solid/liquid colloidal system of minusil dispersed in water, where incremental addition of heparin enhanced the electronegative EKP of the system. In the other study[16], where potassium hexametaphosphate, 1 : 3 electrolyte, was added to the minusil suspension previously treated with ovalbumin, EKP increased progressively up to a certain concentration and then it showed a drastic increase. This was attributed to the multilayer adsorption in such a system. As potassium citrate, which is also a 1 : 3 electrolyte, showed a similar behaviour, we are tempted to believe that such a multimolecular adsorption might be taking place in the present system.

Riddick[1] has put forward a physico-chemical approach to the mechanism of blood coagulation. According to this mechanism, so long as the EKP of system remains constant, the fluidity of the system will also remain constant. There is no need for any enzyme action and unless there is a change in the EKP, due to platelets discharging cationic electrolytes or polyelectrolytes to the system, blood coagulation is not possible. If the EKP of the system is progressively lowered by the introduction of cationic electrolytes or polyelectrolytes, the stability of the system will undergo a progressive change from simple agglomeration to fluid gel formation and finally to rigid gel (blood clot).

As per the above physico-chemical approach to the mechanism of blood coagulation, it appears that EKP could be a substantially contributing factor, involved in explaining the role of heparin and potassium citrate as anticoagulants. As can be seen from Fig. 1, heparin and potassium citrate are anionic dispersants. Both of them increase the EKP of erythrocytes, thereby increasing the degree of repulsive forces and thus preventing the erythrocytes to approach the short range of Van der Waals forces of attraction for agglomeration, which is essentially a first step in the coagulation of blood.

From these studies, it also appears that by employing the known concentrations of heparin one may be able to develop a standard curve for heparin assay based on EKP measurements. With the help of this curve, it would then be possible to know the concentration of heparin in an unknown sample by determining the EKP of erythrocytes treated with it.

Acknowledgements

Financial assistance rendered by University Grants Commission to one of us (GMP) is gratefully acknowledged.

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INSECTICIDE IMPACT ON CONTRACTILE PATTERN OF AMPHIBIAN SKELETAL MUSCLE

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ABSTRACT

Effect of different concentrations of organochlorine pesticide (DDT) on twitch properties of frog gastrocnemius muscle showed significant decrease in the shortening length, twitch duration, and half contraction time (HCT), and half relaxation time (HRT), during direct (DS) and indirect (IDS) stimulations, indicating inhibitory modulation of DDT on contractile kinetics of the normal muscle.

INTRODUCTION

THOUGH *organochlorine pesticide DDT has been* extensively used, the detailed mechanism of its action and toxicity remains obscure[1]. DDT is primarily a neurotoxicant which inhibits the acetylcholinesterase activity, leading to atoxia, convulsion and eventual paralysis[2,3]. It has also been reported that DDT increases the nerve acetylcholine, not by inhibiting the enzyme but by enhancing the liberation of neurotransmitter from bound reserves, which play a dynamic role in the contraction characteristics of the excitable tissues[4]. Since DDT is known to inhibit ATPase reacting system[5] and oxidative metabolism[6,7] involved in the energy production of the contractile machinery, an attempt is made in the present investigation, to study the specific modulatory effect of this organochlorine pesticide, on the twitch properties and related contraction kinetics in the skeletal muscle of the frog.

MATERIALS AND METHODS

Healthy medium sized frogs, *Rana hexadactyla* were double pithed and the gastrocnemius muscles from both the legs were excised with least injury. The muscles were washed 3 to 4 times in amphibian Ringer[8] and allowed to stand in fresh Ringer medium for 10 minutes to recover from the shock effects. Single muscle twitches were recorded before and after presoaking the muscle for 10 minutes in 20, 40, 60 and 80 ppm DDT mixed in Ringer solution as per the method suggested by Matazaeman Uchida[9]. Single stimulus of 6 volts D.C. was found to elicit optimal response for both DS and IDS; this voltage was considered to be the threshold voltage. The twitch properties and the associated contraction kinetics were analysed by using a calibrated kymographic unit[10]. Contractile patterns of the muscle were recorded on smoked paper and the recordings were made permanent in 2 : 1 turpentine-varnish mixture, The amplitude of contraction or shortening length, twitch duration, HCT and HRT were calculated. Average values of six analyses were considered for the present study.

RESULTS AND DISCUSSION

The *twitch properties of gastrocnemius muscle subjected* to DS and IDS were analysed from the contraction pattern recordings (Table I).

When compared to DS, the HCT and HRT decreased during IDS, indicating that IDS elicits quicker