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Research Article

SCANNING ELECTRON MICROSCOPIC INVESTIGATIONS OF ERYTHROCYTES IN MONOCROTOPHOS INTOXICATED RATS

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ABSTRACT
The activity of acetyl cholinesterase in the serum of monocrotopos treated rats i.e. TI, TII and R (Recovery) groups were observed. The percent inhibition was more than 40% in TII group whereas it was more than 30% in TI group. The recovery study suggested that the activity of this enzyme could be restored to 90% after 15 days of rest. Serum concentration of phospholipids showed the statistically significant increase in TI and TII as compared to their controls respectively, whereas in R group it recovered back to almost normal level. The changes in topography of rat erythrocytes of all groups were studied by scanning electron microscope (SEM). It was observed that control animals had typical appearance of erythrocytes where most of the cells were perfect discocytes (D) and few cup shaped stomatocytes (St). A significant effect on SEM appearance of erythrocytes in rats of group TI was observed. Most of cells were changed to stomatocytes, echinocytes (irregularly crenated cells with numerous projections) and acanthocytes (characterized by a few spicules of varying length, irregularly distributed over the entire cell surface with knobby ends). Rats of R group had normal discocytes, barring few stomatocytes and acanthocytes.

Keywords: *Monocrotopos, Erythrocytes, Stomatocytes, Acanthocytes*

INTRODUCTION
Pesticides occupy a rather unique position among chemicals that man encounters daily, in that they are deliberately added to the environment for the purpose of killing or injuring some forms of life. Monocrotophos (MCP), an organophosphorus insecticide has gained wide application in the field of agriculture. It has high insecticidal activity and low mammalian toxicity (Janardhan and Sisodia, 1990). It is a direct acting anticholinesterase agent and as such requires no activation in the body. Among the various tissues of animals, blood may be considered as target and carrier of insecticides since the lipid moiety of erythrocytes is likely the site of interaction (Misra *et al.*, 1982). According to Arutjunov *et al.*, (1981), the study of morphological features of cellular elements has great importance for assessing their functional state, vitality and kinetics. Therefore, to explore the toxic effects of monocrotophos, the peculiarities of shape, size and surface of erythrocytes studies by scanning electron microscope offer good parameter for determining the many pathological states.

MATERIALS AND METHODS
LD_{50} value of monocrotophos (technical grade) was standardized and was found to be 14mg/kg body weight. Dose of $1/5^{th}$ of LD_{50} i.e. 2.8 mg/kg body weight in distilled water was administered by intragastric intubation to three groups of female albino rats in proestrous phase of estrus cycle (8 rats in each group, each weighing between 125-150 g) for 15 days (TI), 30 days (TII), 30 days and then kept without dosage for 15 days (R). Another three groups CI, CII and CIII (8 rats in each group) were kept as corresponding controls for all the treatment groups. All the rats were kept on the commercial standard diet and tap water *ad libitum*. The weight of the animals was recorded weekly. At the end of the treatment period, one or two drops of blood were fixed from one rat of each group in 2.5% glutaraldehyde made in phosphate buffer and processed for scanning electron microscope. For serum, preparation, blood was collected in test tubes and allowed to clot at room temperature. The clotted blood samples were centrifuged at 2000 rpm for 10-15 minutes to separate the clot. Serum was used for the estimation of activity of acetyl cholinesterase and levels of phospholipids.

RESULTS AND DISCUSSION

The activity of acetyl cholinesterase in the serum of monocrotopos treated rats i.e. TI, TII and R (Recovery) groups are summarized in table1. The percent inhibition was more than 40% in TII group whereas it was more than 30% in TI group. The recovery study suggested that the activity of this enzyme could be restored to 90% after 15 days of rest. Serum concentration of phospholipids showed the statistically significant increase in TI and TII as compared to their controls respectively, whereas in R group it recovered back to almost normal level (Table1).

Table 1: Effect of monocrotophos on activityof acetylcholinesterase and phospholipids level in serum of female albino rats of proestrus phase of estrous cycle

Parameters	**2.8 mg/kg body weight Monocrotophos /day**					
	15 days treatment (TI)		**30 days treatment (TII)**		**15 days recovery (R)**	
	Control	**Exptl**	**Control**	**Exptl**	**Control**	**Exptl**
AChE (µmol /min /ml)	0.527±0.05	0.359±0.03ˣˣˣ	0.523±0.06	0.297±0.04ˣˣˣ	0.529±0.03	0.469±0.05ˣ
% Change	(-) 31.87%		(-) 43.21%		(-) 11.34%	
Phospholipids	6.35±0.69 (+)9.92%	6.98±0.84ˣ	6.59±0.72 (+)14.71%	7.56±0.37ˣ	6.30±0.69	6.72±0.28
% Change					(+)6.67%	

Values are expressed as Mean ± S.D. (n=6)
ˣP<0.05; ˣˣp<0.01; ˣˣˣp<0.001, when the values are compared with respective controls.

The changes in topography of rat erythrocytes of all groups were studied by scanning electron microscope (SEM). It was observed that control animals had typical appearance of erythrocytes where most of the cells were perfect discocytes (D) and few cup shaped stomatocytes (St) (Figures 1- 2). A significant effect on SEM appearance of erythrocytes in rats of group TI was observed (Figures 2-4). Most of cells were changed to stomatocytes, echinocytes (irregularly crenated cells with numerous projections) and acanthocytes (characterized by a few spicules of varying length, irregularly distributed over the entire cell surface with knobby ends) (Figure 2). Echinocytes observed were of different stages (Figure 3) as stage I (a) characterized by irregularity of edges, stage II (b) echinocytes are characterized by spicules in still flat cells and stage III (c) echinocytes are characterized by spicules uniformly distributed over the surface of round cells. The changes might be due to the disturbed lipid microenvironment of membrane and more so due to increased lipid peroxidation as explained by many workers while studying the effect of organophosphorus compounds and other chemicals as under: Sherman (1979) illustrated that the changes in membrane lipids composition was the key reason for such deformities in shape of blood cells in response to various chemical treatments. Sidorov and Erochin (1983) reported that chemical agents, ionizing radiations and pesticides have been shown to initiate lipid peroxidation in biological membranes. Agarwal (1989) while studying the effect of parathion and other chemicals on rat erythrocytes *in vitro* reported significant variations due to lipid peroxidation.

A significant inhibition of acetylcholinesterase activity and rise in phospholipid level in serum of monocrotophos intoxicated rats were observed during present studies may be responsible for the altered membrane structure of RBC's as according to Datta *et al.,* (1994), acetylcholinesterase is supposed to form the link between lecithin and protein in the erythrocyte membrane and contribute to the maintenance of membrane integrity, whereas the phospholipid component of biomembrane is believed to be site of

action of organophosphorus insecticides. Besides these types of alterations, some erythrocytes of atypical shape (irregularly outlined with sharply changed topography) were also found (Figure 3). These might be due to the process of physiological ageing and destruction as reported by Arutjunov *et al.*, (1981) after lead and chlorobenzene intoxication in blood of human beings. A few erythrocytes showed central as well as peripheral protuberances or blebbings. Increased anisocytosis was the predominant feature which was noticed by variation in size and shapes of cells (Figure 3). Moreover erythrocytes with central and peripheral protuberances showed the phenomena of fragmentation and sequestration of cell parts in pathological conditions. Another type of deformities in erythrocytes i.e. sphero- echinocytes having an increased volume and progressively less prominent spicules were observed (Figure 4). The exposure of rats for an even longer period i.e. for 30 days at the same concentration resulted in increased frequency of occurrence of echinocytes and spherocytes (Figure 5). The disruption of plasma membrane was accompanied by clubbing of irregularly shaped erythrocytes (Figure 6). Another type of altered erythrocytes designated as acantho-echinocytes (characterized by acanthocytes with secondary spicules superimposed by echinocytogenic factors) were also observed (Figures 7, 8, 9). These types of morphological alterations i.e. acantho-echinocytes and sphero-echinocytes were observed for the first time in present studies. This was not reported by any other worker previously. So it seems that monocrotophos is more hazardous compound than other organophosphates. Weed and Bessis (1973) while explaining such pathological erythrocytes reported that normal RBCs can respond to same echinocytogenic agents and develop echinocytic forms. The underlying pathologic shapes persist and secondary (new) spicules may be superimposed on primary spicules so as to give rise to acantho-echinocytes, whereas according to Brecher and Bessis (1972) sphero-echinocytes are altered forms of echinocytes. Rats of R group had normal discocytes (Figures 10, 11, 12) barring few stomatocytes and acanthocytes. The protective effects of withdrawal of treatment in R group could be possibly attributed to the spontaneous removal of insecticide from the body and also the enzyme inhibition through hydrolysis of phosphorylated cholinesterase and thus regeneration of acetylated cholinesterase. Therefore, it may be pointed out that changes observed during the present study had direct correlation with anticholinergic properties of monocrotophos as suggested by the significant inhibition of AChE activity as well as the various parameters studied was possible. Hence the workers exposed to oranophosphorus sprays are required to take a brief period of rest to cope up with any kind of abnormality with acetylcholinesterase activity.

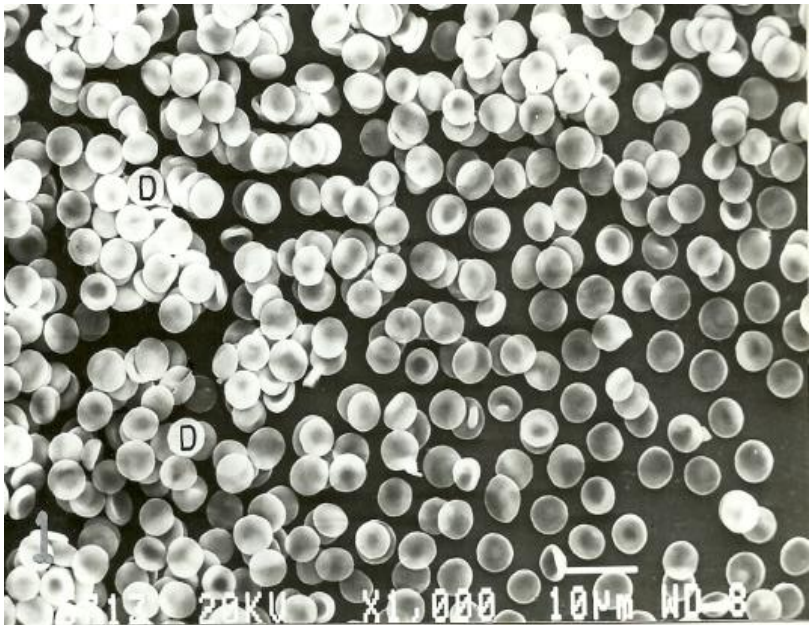

Figure 1: Erythrocytes from a control rat showing numerous biconcave discocytes (D)

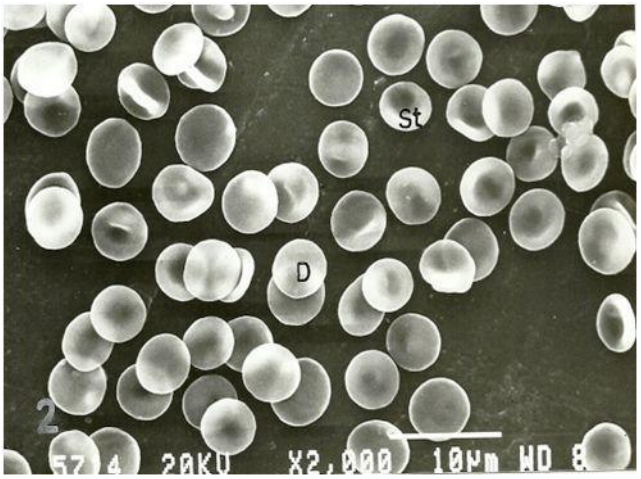

Figure 2: Erythrocytes from a control rat showing typical biconcave discocytes (D) and few cup shaped stomatocytes (St).

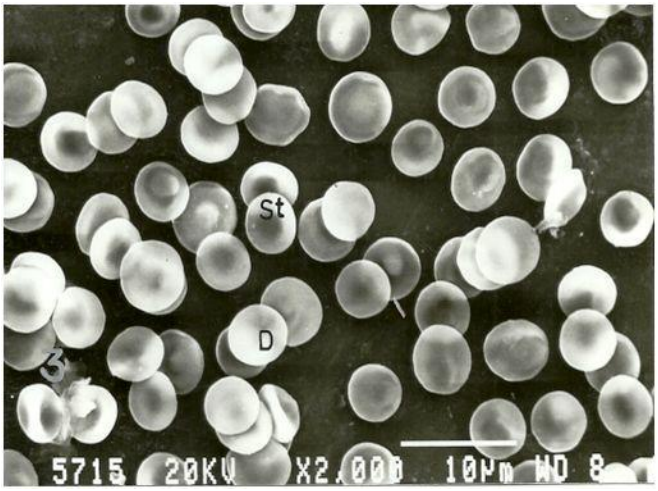

Figure 3: Erythrocytes from a control rat showing discocytes (D) and stomatocytes (St).

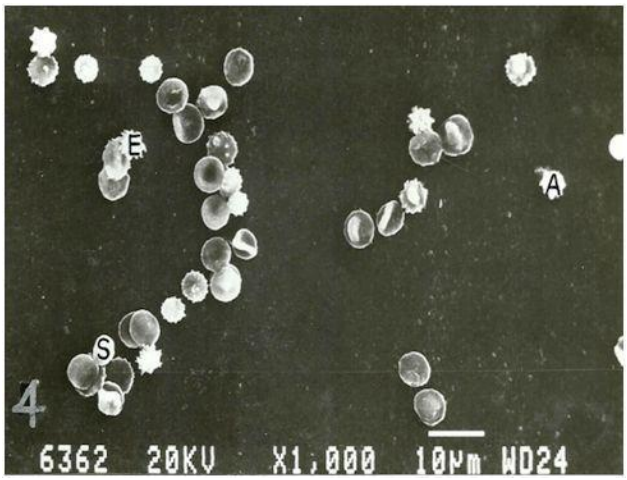

Figure 4: Erythrocytes from rat of TI group showing numerous acanthocytes (A) with small spicules, a few contracted echinocytes (E), spheroechinocytes, spherocytes (S) and acantho-echinocytes

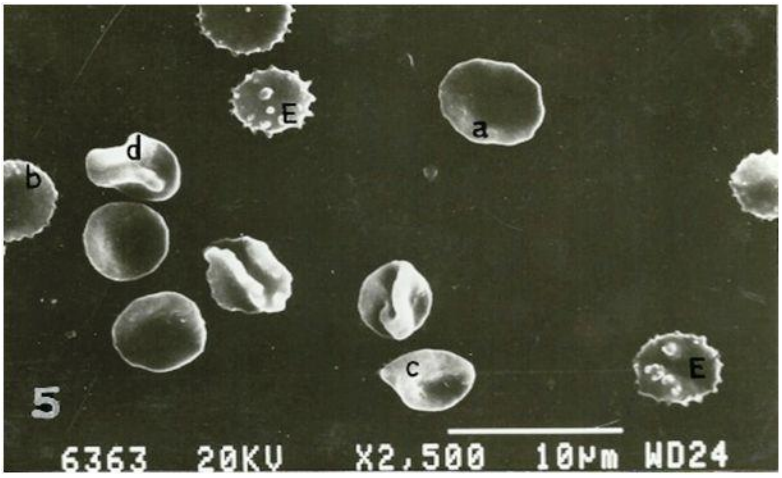

Figure 5: Erythrocytes from rat of TI group showing echinocytes of stage I (a), stage II (b), stage III (E) ; erythrocytes with peripheral protuberances (c); and of atypical shape (d).

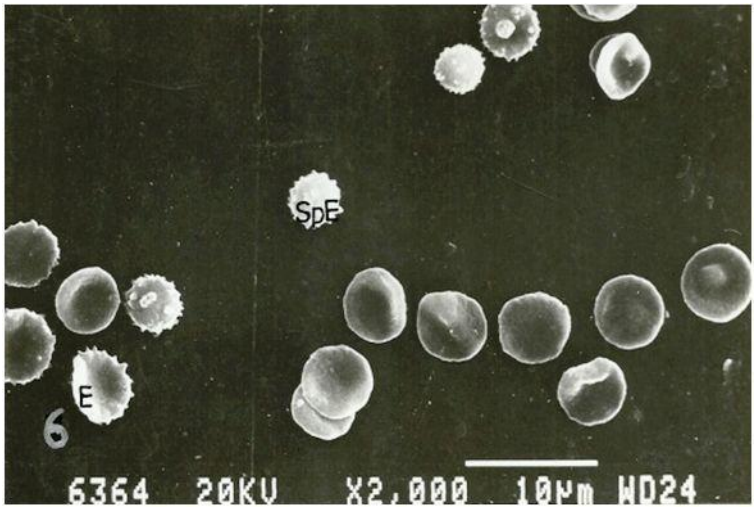

Figure 6: Erythrocytes from rat of TI group showing a few echinocytes (E) and sphero- echinocytes (SpE).

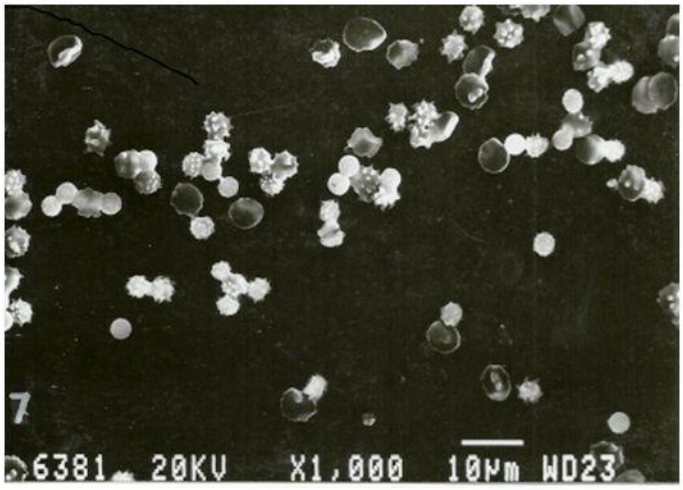

Figure 7: Erythrocytes from rat of TII group showing increase in number of echinocytes, spherocytes as well as clubbing of altered forms of erythrocytes.

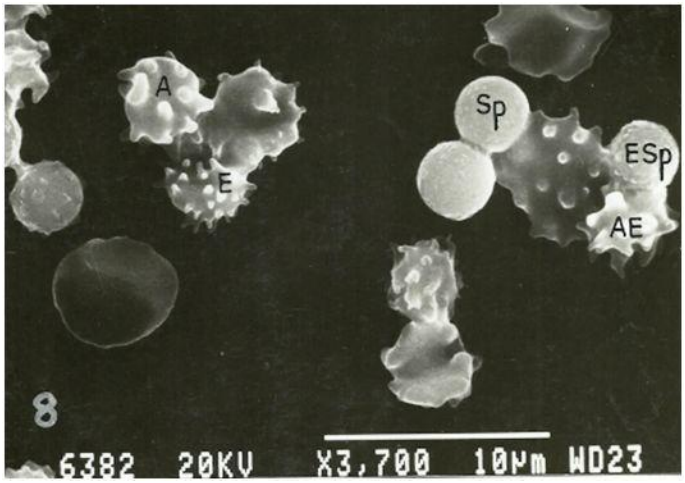

Figure 8: Erythrocytes from rat of TII group showing clubbing of echinocytes (E), acanthocytes (A), spherocytes (Sp), sphero-echinocytes (SpE) and acantho- echinocytes (AE).

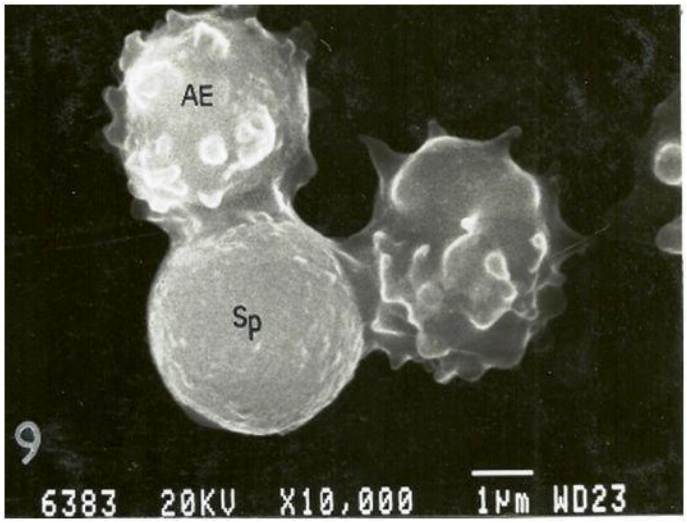

Figure 9: Blood cells from rat of TII group showing clubbing of acantho-spherocytes (AE) and spherocytes.

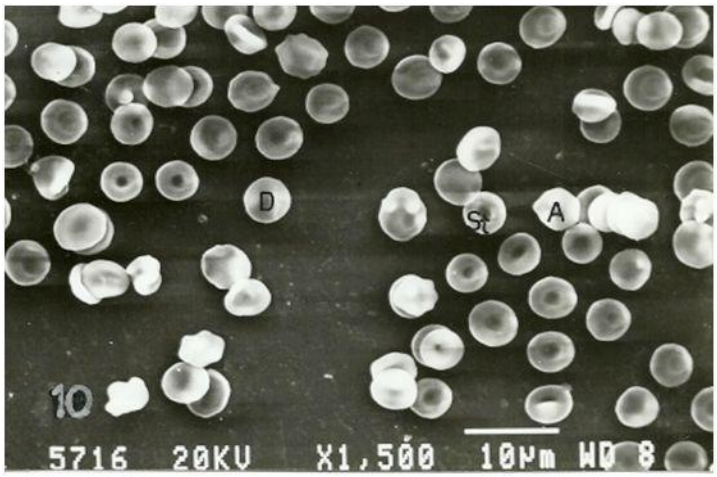

Figure 10: Blood cells from rat of R group showing discocytes (D), a few acanthocytes and moderate number of cup shaped stomatocytes (St).

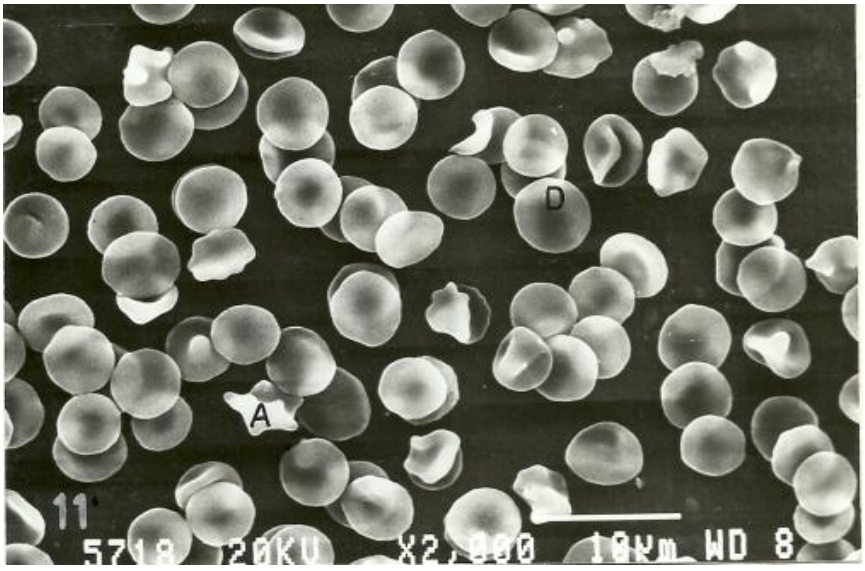

Figure 11: Erythrocytes from rat of R group showing discocytes (D) and acanthocytes (A)

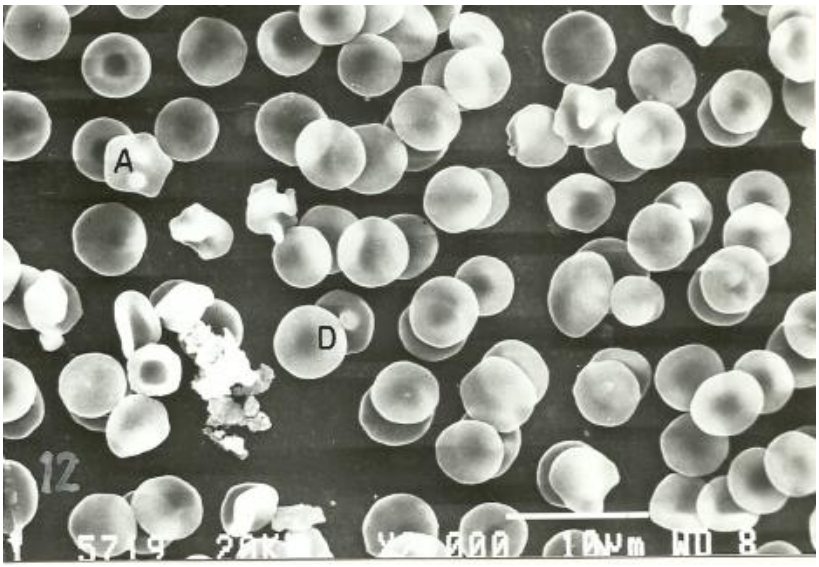

Figure 12: Erythrocytes from rat of R group showing moderate number of discocytes (D) and a few acanthocytes (A)

The study, therefore, is of great importance and suggests that the recovery could be possible due to non-persistent nature and reversible inhibition of the organophosphates which due to hydrolysis leads to the restoration of the active acetylcholinesterase and hence of inhibitory effect on the various receptors from accumulated acetylcholine in the synapses and hence resulting in restoration of normal metabolic activities and histoarchitecture. It can be safely recommended that if occupational workers exposed to organophosphate pesticides containing monocrotophos are given rest for about two weeks, the cytotoxic effects in the blood could be reversed.

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