THE PARTIAL CYTOLYSIS OF THE AMPHIBIAN ERYTHROCYTE AND LIVER PARENCYHMA CELL BY A NON-IONOGENIC SURFACE ACTIVE AGENT

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INTRODUCTION

The isolation of the nucleus and the study of its structure and metabolism form a rapidly expanding field of cell research. Excellent reviews by Allfrey (1), Mirsky and Osawa (2), and Allfrey and Mirsky (3) indicate that under certain circumstances the intact isolated nucleus has a specific enzymatic architecture which can synthesize ATP, incorporate amino acids into nuclear protein, and incorporate RNA bases into nuclear RNA. It appears, however, that the metabolic activity of the isolated nucleus considered as a whole, although significant, is rather low. Factors which may contribute to this relative inactivity are the time involved in the isolation and the structural damage which may well occur during homogenisation and multiple centrifugal fractionation.

Homogenisation would seem to be the only practicable first step for isolation from an entire tissue in which there is a dense connective tissue component. On the other hand, any tissue which readily allows enzymatic or mild mechanical dissociation, such as thymus and embryonic liver, or any free cell suspension such as that of the nucleated erythrocytes, ascites cells, and some tissue culture preparations may be partially cytolysed by chemical rather than mechanical means. A chemical lysis of the plasma membrane must be rapid, controllable, and result in a "clean" nucleus. Until these requirements are met, the accurate assessment of activity in the isolated nucleus will be difficult.

The phenomena of surface activity and the known application of surface active compounds in the delamination of colloidal and other micelles suggested that the amphipathic substances and, in particular, the non-ionogenic surface active agents, first exploited in German industry about 1938, might be used to lyse the plasma membrane and liberate the nucleus.

METHODS

This investigation was undertaken to test the effect of one non-ionogenic surface-active agent—an iso-octylphenol condensed with an average of six ethylene oxide groups, the commercial designation being Nonidet P 40 (NP40) (Shell (Australia) Ltd.). Amphibian erythrocytes were obtained from the aorta of the Queensland cane toad (*Bufo floridensis*), suspended, and washed twice in amphibian Ringer's solution containing 0.01 M sodium citrate, and again three times in amphibian Ringer-phosphate solution. Subsequently, some cells were suspended in this solution (pH 6.8), and others in 0.25 M sucrose (pH 7.0). Liver removed from the toad was immersed in ice-cold Ringer's solution, the capsule was then stripped off, and the remaining tissue was cut into small pieces which were teased and washed in cold Ringer-phosphate solution. The teased liver parenchyma was further macerated with fine glass rods, shaken, and washed several times; after this treatment, it was allowed to stand at 4°C until the

TABLE I

Lysis of the Erythrocyte Plasma Membrane in Media Containing a Non-Ionogenic Surface Active Agent, NP40.

Amphibian erythrocytes were suspended in both Ringer's solution and sucrose media at different concentrations of NP40 in the medium; room temperature 22 °C. (see text). At the periods indicated a sample of the suspension was examined with phase contrast and the degree of cytolysis was noted. For convenience, arbitrary stages from 1 to 7 (see text) describe the degree of cytolysis. The figure in brackets represents the percentage of cells per field (Pv. Apo. L40/0.70 obj. & x10 eyepiece) that has reached a particular stage of lysis; *e.g.* 4(10) means that 10 per cent of cells are at stage 4 whereas 90 per cent are at prior stages.

Time in medium		Degree of cytolysis, stages 1 to 7				
		1 min.	15 min.	20 min.	60 min.	80 min.
Medium	Conc. NP40					
	per cent					
Amphibian Ringer's solution pH 6.8	1	7 (100)	7 (100)	7 (100)	cytol.	cytol.
	0.1	7 (100)	7 (100)	7 (100)	7 (100)	cytol.
	0.01	1 (100)	3 (10)	3 (40)	4/5 (20)	5 (100)
	0.001	1 (100)	1 (100)	1 (100)	2 (10)	3 (10)
	Control	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	per cent					
0.25 M sucrose, pH 7.0	1	7 (100)	7 (100)	7 (100)	7 (100)	cytol.
	0.1	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)
	0.01	1 (100)	2 (20)	3 (20)	4/5 (10)	5 (100)
	0.001	1 (100)	1 (100)	1 (100)	1 (100)	3 (20)
	Control	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

bulk of the material had settled. Samples of suspended cells were taken from the supernatant fluid.

Erythrocyte and liver cell suspensions were treated with different concentrations of NP40 (Table I). The effect of this substance on cell structure was followed microscopically with Leitz phase-contrast equipment. Erythrocyte suspensions were treated at both room temperature and 4°C; liver cell suspensions were treated only at room temperature.

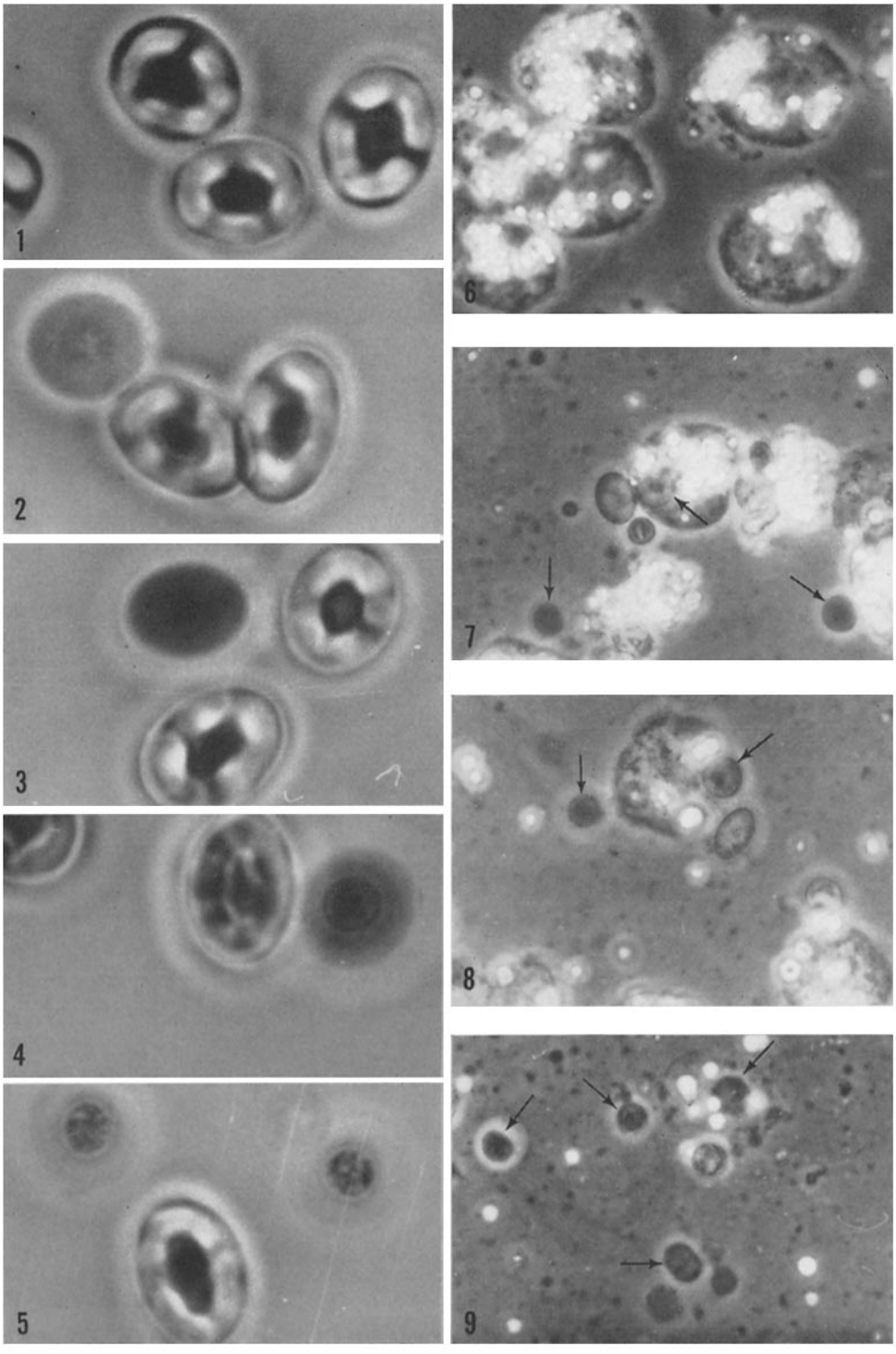
OBSERVATIONS

Within the range of concentrations of NP40 that were employed, it was found possible to obtain lysis of the plasma membrane. Nuclei in free suspension in the medium appeared morphologically normal, both under phase contrast microscopy and after fixation and staining.

Whereas lysis of the plasma membrane was apparently instantaneous at NP40 concentrations of 0.1 per cent and above, at a concentration of 0.01 per cent the process was gradual and could be easily followed. The stages of cytolysis of erythrocytes by 0.01 per cent NP40 were regular in sequence (Figs. 1 to 5). Erythrocytes suspended in 0.25 M sucrose passed through the same stages

FIGURES 1 to 5 Stages 1 to 5 during the partial cytolysis of the amphibian erythrocyte suspended in amphibian Ringer's solution containing 0.01 per cent NP40. The figures are selected to demonstrate the appearance of the erythrocyte with phase contrast during the cytolytic process and, at the same time, to compare this appearance with that of a cell which appears normal. × 1500.

FIGURES 6 to 9 Liver parenchyma cells undergoing the same treatment as above. Fig. 6 shows cells at the beginning of the process. The cell at lower right is followed in Figs. 7 to 9. Arrows indicate the nuclei of the cells. Phase contrast. × 800.


1
2
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in approximately the same time (Table I). Refrigeration did not slow the process significantly.

Degrees of cytolysis of the nucleated erythrocyte have been classified for convenience as stages 1 to 7. These stages are clearly defined both in phase contrast microscopy and after fixation and staining. The classification, however, is purely arbitrary in nature and carries with it no special significance. The stages are as follows:

1. No cytolysis (Fig. 1).
2. The cell has become rounded, the cytoplasm darker, the nucleus more clearly defined and spherical (Fig. 2). In both Ringer's solution and sucrose media (NP40 0.01 per cent or less), stage 2 may appear within minutes in some cells but not until much later in others: it lasts for from 5 to 20 minutes.
3. The cell is much darker (Fig. 3), rounded, and the nucleus is faint but clearly defined. The onset of this stage and its duration are variable.
4. The transformation from stage 3 to 5 is relatively rapid, taking from 5 to 10 seconds. Stage 4 represents the cell when the cytoplasm has become more translucent, and because of this the nucleus becomes clearly visible (Fig. 4).
5. The cells remain in stage 5 (Fig. 5) for some hours under these conditions. There does not appear to be any cytoplasm within the plasma membrane, although the latter is clearly defined and will withstand centrifugation.
6. Appearances are similar to those of stage 5. The plasma membrane becomes very faint and will not withstand shaking or centrifugation, and, depending upon the tonicity of the medium, nuclear enlargement may begin.
7. The nucleus is isolated. In media containing 0.1 per cent NP40 the isolation is almost instantaneous and the isolated nucleus appears structurally normal. Nuclear enlargement and cytolysis begin after 10 to 20 minutes if the nuclei remain in contact with NP40.

The cytoplasmic components of the liver parenchyma cell are more numerous and more diverse than those of the erythrocyte, and although similar cytolytic changes occur, the same description is not appropriate. The plasma membrane is at first well defined, the nucleus with its characteristic nucleolus clearly visible, and the highly refractile material retained within the cell (Fig. 7). After a time the cytoplasm becomes less dense, the plasma membrane somewhat indistinct, and most of the refractile material leaves the cell (Fig. 8). The plasma membrane remains visible for a time although it appears incomplete (Fig. 9). Finally, it disappears and the nucleus remains freely suspended in the medium.

COMMENTS

A discussion of the possible mechanism underlying the lytic changes described would be premature at this stage. Little or no information is available concerning the kinetics of interfacial adsorption, insofar as the non-ionogenic agents are concerned; few data are obtainable concerning their range of chemical structure or their physicochemical properties. There appear to be no data on the biological or biophysical properties of the polyethenoxy agents at the cell level.

The observation that the plasma membrane in both the erythrocyte and the liver parenchyma cell reacts quite differently than the nuclear membrane suggests two immediate possibilities: (*a*) that the plasma membrane is lysed by the lowering of one or more boundary tensions and the nuclear membrane temporarily stabilised by layer absorption—this would be compatible with concepts about interfacial adsorption in surface active agents; (*b*) that the two membranes differ more widely, chemically or structurally, than is generally accepted.

The amphipathic substances may prove to be a useful chemical tool in the analysis of biophysical properties of the cell membrane.

Finally, inherent in these results is a method of rapid nuclear isolation which may prove to be valuable, especially in the field of microtechniques.

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