

# CELL SURFACE CHANGES DURING DEDIFFERENTIATION IN THE METAPLASTIC TRANSFORMATION OF IRIS INTO LENS

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## ABSTRACT

Changes at the cell periphery during the dedifferentiative phase of the metaplastic transformation of iris into lens have been studied in *Notophthalmus viridescens* and *Taricha granulosa* using cell electrophoresis. Cell surface charge density increases as early as 1–3 days after lens removal. Cells of regenerates at 10–15 days after lentectomy have significantly lower electrophoretic mobilities than those of the irises of nonlentectomized newts. Decrease in surface charge density is due, at least in part, to the loss of ribonuclease- and neuraminidase-sensitive groups from the cell periphery. Loss of negatively charged groups from the cell surface appears to occur as cells go through dedifferentiation. Loss of cell surface components also occurs in the cells of the ventral iris which also undergo dedifferentiation but do not regenerate a lens.

## INTRODUCTION

During the regeneration of the lens in some urodeles the pigmented cells of the dorsal iris undergo a metaplastic change in which they lose their original tissue specificity and redifferentiate into lens cells. That the regenerated lens is derived from pigmented cells of the dorsal iris is indicated by experiments in which thymidine-<sup>3</sup>H-labeled irises gave rise to labeled lenses in lentectomized eyes (Eisenberg and Yamada, 1966; Zalik and Scott, 1971). As early as 3 days after lentectomy the pigmented cells of the dorsal iris undergo an increase in RNA synthesis (Yamada and Karasaki, 1963; Reese et al., 1969), which is followed in time by that of protein (Yamada and Takata, 1963) and initiation of DNA replication (Eisenberg and Yamada, 1966; Reyner, 1971). Concomitantly with these processes the cells lose their pigment granules and give rise to a depigmented, dedifferentiated cell population, which undergoes at least two cell divisions (Takata et al., 1965; Eisenberg-Zalik and Yamada, 1967) before redifferentiating into lens cells.

Although changes at the cell surface have been described in many cases of neoplastic cell transformations (see Weiss, 1970 *a, b* for reviews), no studies of the cell surface have been made in this example of well-regulated cell metaplasia. The objective of this work was to study whether changes at the cell periphery occur during metaplastic transformation of iris into lens. In this paper we report mainly on some of the surface transformations occurring during the depigmentation stage of the iris cells. It was found that between 10 and 15 days after lens removal, when most of the cells of the regenerate are in the dedifferentiative phase, transformations occur which may be interpreted as alterations of material at the cell surface.

## MATERIALS AND METHODS

Newts of the species *Notophthalmus viridescens* and *Taricha granulosa* were used. Animals were lentectomized as described previously (Eisenberg and Yamada, 1966) and kept at room temperature for different time intervals after lens removal. Animals were fed twice weekly with *Enchytraeus* worms.

### Cell Dissociation

Irises were dissected as described previously (Eisenberg-Zalik and Scott, 1969). In the first series of experiments dorsal or ventral irises obtained from normal or lentectomized newts at 1, 3, 5, 7, 10, 15, and 20 days after lentectomy were used; a minimum of 50 irises was used for each experiment. After dissection, tissues were washed twice in Tris-buffered amphibian Ringer's with 0.2% glucose at pH 7.5 (G.A.R.) and placed in 0.25% purified trypsin solution (Fisher Scientific Company, Pittsburg, Pa.) in  $\text{Ca}^{++}$ -free amphibian Ringer's with  $5 \times 10^{-5}$  M sodium citrate. Tissues were incubated at 37°C in a rotating shaker for 30 min. After this time, dissociation was aided by gentle pipetting. The cell suspension thus obtained was removed and cells were collected by gentle centrifugation (250 g) for 5 min. They were washed twice in G.A.R. and suspended in 0.118 M NaCl adjusted to pH 7.2 with 0.5 M  $\text{NaHCO}_3$  for determination of their electrophoretic mobilities (E.P.M.).

### Measurement of Electrophoretic Mobilities

For this purpose the cylindrical cell electrophoresis apparatus (Bangham et al., 1958) equipped with the 4 ml chamber was used. The stationary layer was standardized with erythrocytes from healthy donors as described by Seaman (1965). Measurements were performed at 25°C in a 4 v/cm gradient with a current of 1.8 ma, and the time taken for cells to travel 45  $\mu$  was recorded. Experiments were also designed to determine whether changes in E.P.M. occurred with time. Cell suspensions were obtained as described previously and E.P.M. were measured immediately after isolation and at subsequent time intervals varying from 30 min to 24 hr after tissue dissociation. For this purpose portions of the cell suspension were stored at room temperature in G.A.R. between mobility determinations. These experiments were performed in normal irises and in regenerates at 3 and 10 days after lentectomy.

### Enzyme Treatment

Normal irises as well as regenerates at 3, 10, and 15 days after lentectomy were used. At least 150 irises were used per experiment. For the neuraminidase treatment cells were dissociated and placed in a solution of 0.118 M NaCl, 0.005 M  $\text{CaCl}_2$  adjusted to pH 7.2 with 0.5 M  $\text{NaHCO}_3$ . Neuraminidase from *Vibrio comma* (Behringwerke AG, Marburg-Lahn, West Germany) was added at a concentration of 20 units per ml. This enzyme was found to be free of protease activity by the casein assay method (Kakade et al., 1970) and is reported to be free of lecithinase activity by the manufacturers. Cells were incubated in the enzyme solution at 37°C for 30 min, washed twice in

G.A.R., and resuspended in 0.118 M NaCl, pH 7.2, for determination of the E.P.M. Ribonuclease (pancreatic) was added to a solution of 0.118 M NaCl, pH 7.2 at a concentration of 0.1 mg/ml, and a volume of the cell suspension was added v/v. The enzyme preparation (R. A. F., Worthington Biochemical Corp., Freehold, N. J.) is monophoretic, and protease activity was undetectable by the casein assay (Kakade et al., 1970) and by the method of Schwert and Takenaka (1955). Controls for enzyme treatments consisted of cells whose E.P.M. was determined immediately after isolation, and cells incubated in saline less enzyme or G.A.R. for 30 min at 37°C. Additional control consisted of cells treated with enzyme that had been heated previously at 100°C for 45 min.

In order to determine whether the enzyme effects on E.P.M. were additive, cells from normal irises were treated with neuraminidase for 30 min at 37°C, they were washed twice in G.A.R., treated with RNase at 37°C for 30 min with mild agitation, washed twice, suspended in 0.118 M NaCl pH 7.2, and their E.P.M. were measured. Experiments were also performed in which the order of the enzymatic treatment was reversed.

Electrophoretic mobilities of macrophage-like cells of *Taricha granulosa* were also measured. For this purpose, animals were injected intraperitoneally with 1 ml of a 0.5% solution of oyster glycogen (Fisher Scientific Company). After 48 hr, 1 ml of amphibian Ringer's was injected intraperitoneally, the peritoneal cavity was opened, washed exhaustively, and the exudate was collected. Cells were obtained from this suspension by centrifugation, washed twice in G.A.R., resuspended in 0.118 M NaCl, pH 7.2, and their mobilities were determined.

After enzyme treatments, the supernatants obtained from the cell suspensions were stored frozen at 0°C until the assays were performed. Sialic acid was determined in them by a modification of the method of Warren (1959), and ribose was determined by the method of Dische (1949).

### RESULTS

The suspensions obtained from irises after trypsinization consisted of cells varying from round to slightly irregular in outline. Cell suspensions obtained from normal irises, examined under the phase-contrast microscope as well as in smear preparations, appeared to be composed, in the majority, of pigment cells. A few iridophores as well as nonpigmented cells presumably derived from the iris stroma were also present but were relatively infrequent. Dye exclusion tests with erythrosine B in depigmented cells of 10- and 15-day regenerates showed that 85-97% of the cells

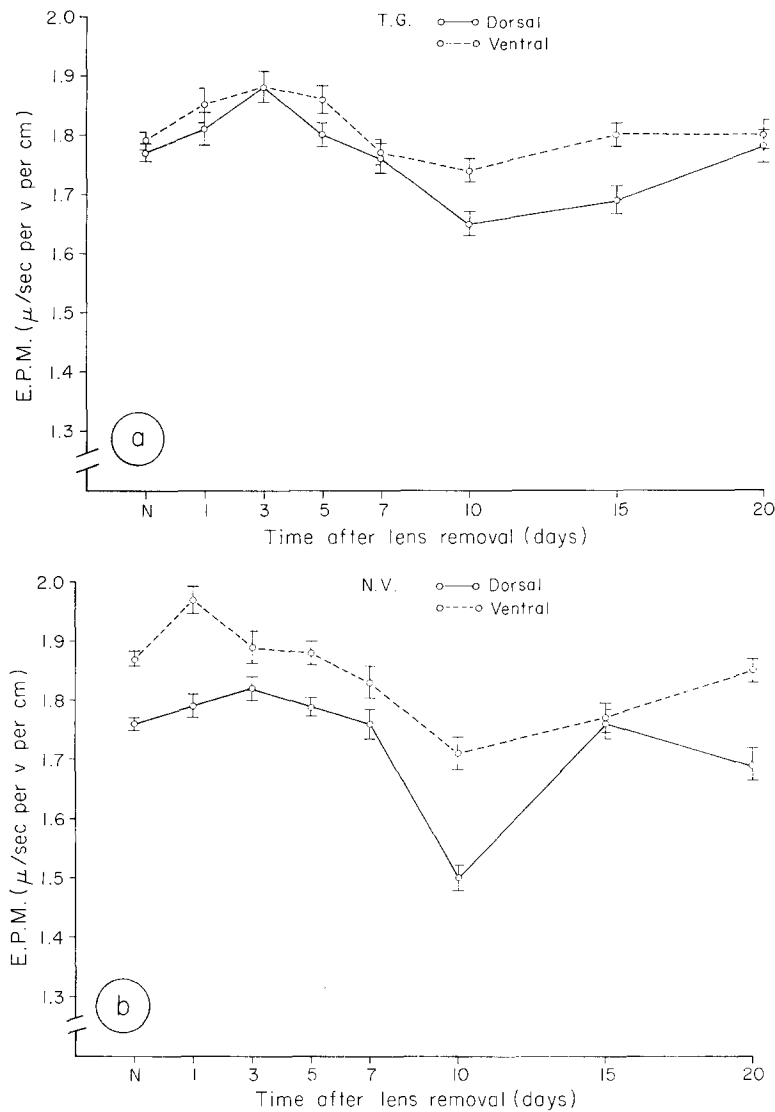


FIGURE 1 Mean electrophoretic mobilities of dorsal and ventral iris cells from (N) the normal eye and at various intervals (days) after lens removal. Each point represents mean E.P.M. and standard error. Average E.P.M. values were obtained from 150 or more measurements gathered from three to four experiments. (N.V.) *Notophthalmus viridescens*; (T.G.) *Taricha granulosa*.

did not take up the stain. This criterion for viability could not be used in the normal iris and earlier regenerates due to the fact that the presence of large numbers of pigment granules makes detection of color in the cytoplasm impossible. However, cells obtained by this method grow and form monolayers under culture conditions. The average yield of cells was 3600 per dorsal iris, with values ranging between 2600 and 4000 cells. The average

cell concentration used for E.P.M. determinations varied between 30,000 and 40,000 per ml.

The mean E.P.M. values in the cells of the dorsal iris at intervals subsequent to lens removal in *Notophthalmus viridescens* and *Taricha granulosa* are presented in Table I and Fig. 1. This figure shows that E.P.M. values in cells of the dorsal iris begin to rise as early as 1 day after removal of the lens and to reach a maximum value at 3 days; the

TABLE I  
*Mean Electrophoretic Mobility ( $\mu$ /sec per v per cm) of Iris Cells at Subsequent Stages of Regeneration*

Days after lentectomy	<i>Notophthalmus viridescens</i>						<i>Taricha granulosa</i>					
	Dorsal (Mean $\pm$ sd)			Ventral (Mean $\pm$ sd)			Dorsal (Mean $\pm$ sd)			Ventral (Mean $\pm$ sd)		
0	(533)*	1.757	(0.267)	(400)*	1.866	(0.262)	(300)*	1.773	(0.264)	(300)*	1.789	(0.247)
1	(100)	1.786	(0.213)	(100)	1.967	(0.236)	(100)	1.814	(0.271)	(100)	1.854	(0.282)
3	(140)	1.821	(0.244)	(100)	1.886	(0.272)	(100)	1.879	(0.257)	(100)	1.879	(0.267)
5	(221)	1.794	(0.259)	(150)	1.881	(0.300)	(150)	1.797	(0.239)	(150)	1.862	(0.276)
7	(100)	1.764	(0.256)	(100)	1.835	(0.274)	(150)	1.758	(0.305)	(150)	1.767	(0.265)
10	(140)	1.498	(0.268)	(100)	1.714	(0.283)	(200)	1.646	(0.303)	(200)	1.736	(0.304)
15	(150)	1.757	(0.309)	(150)	1.768	(0.303)	(150)	1.686	(0.284)	(150)	1.798	(0.254)
20	(160)	1.686	(0.328)	(160)	1.848	(0.261)	(150)	1.775	(0.337)	(150)	1.795	(0.317)

Each value represents data gathered from three or more experiments. Least significant difference at the 1% level is 0.096 for *Notophthalmus viridescens* values and 0.1028 for those of *Taricha granulosa*.

\* Figures in parentheses indicate number of determinations on which each value is based.

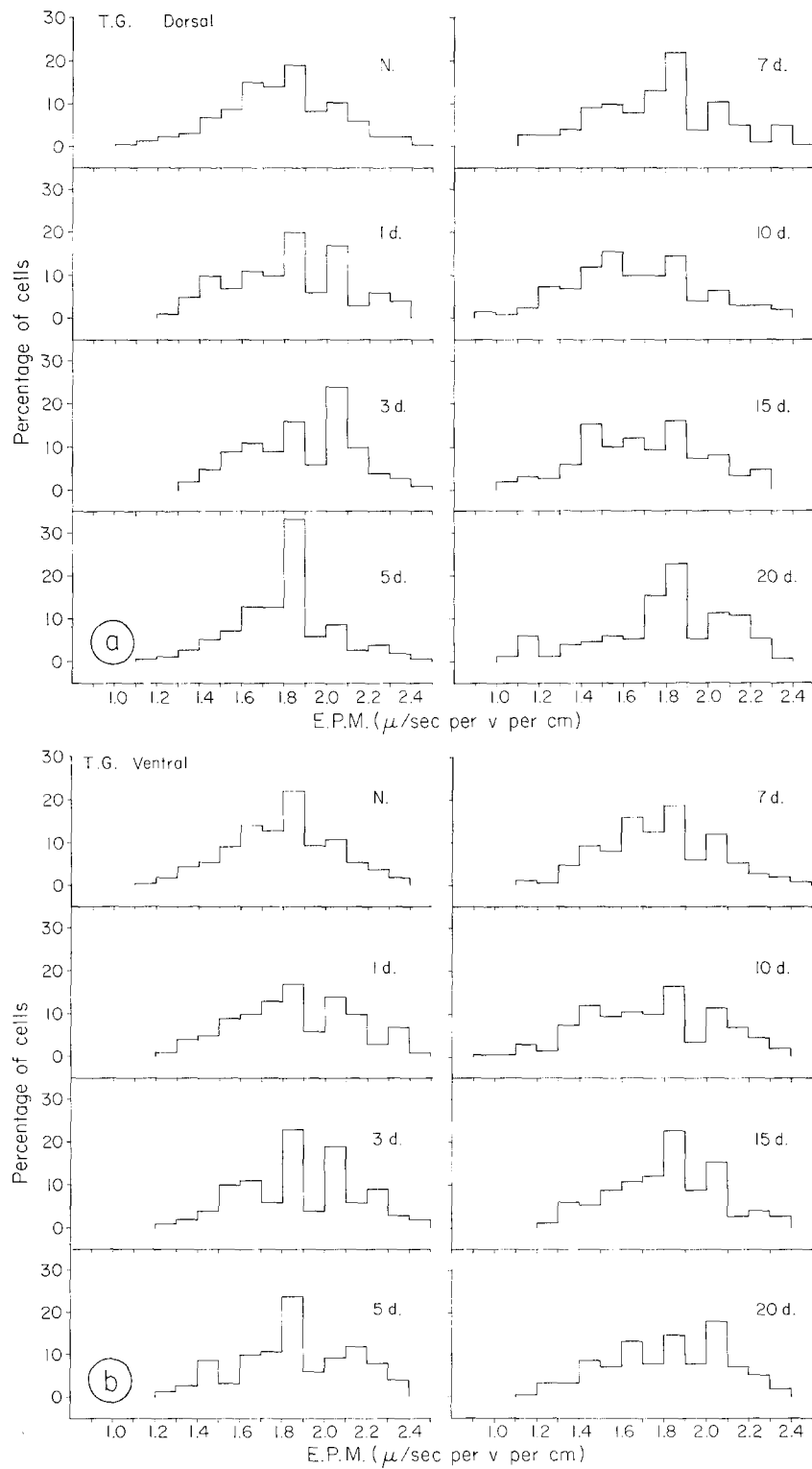
mobility values, however, are not significantly different from those of the controls. The mean E.P.M. decreases from this day to attain low values in cells of 10-day regenerates, which are significantly different from those of controls and earlier regenerates. In *Notophthalmus viridescens* the mobility then increases significantly at 15 days after lens removal; however, in *Taricha granulosa* the increase is not significant until 20 days after lentectomy (see Table I for values of least significant difference). Similar changes appear to occur in the ventral iris which does not regenerate a lens; however, the decrease in E.P.M. at 10 days after lentectomy is not so marked as that undergone by cells of the dorsal iris which are involved in cell metaplasia.

A frequency histogram of mobilities of iris cells in the normal as well as in the lentectomized eyes at different times after lens removal is presented in Fig. 2. Although there is a wide variation in the mobilities of the cell population, it appears that the mean E.P.M. values presented in Fig. 1 are due to a change in mobility undergone by a large proportion of cells in the regenerates. There does not appear to be a group of very rapidly or very slowly migrating cells present in the 3- and 10-day regenerates, respectively, which would affect the mean E.P.M. values significantly.

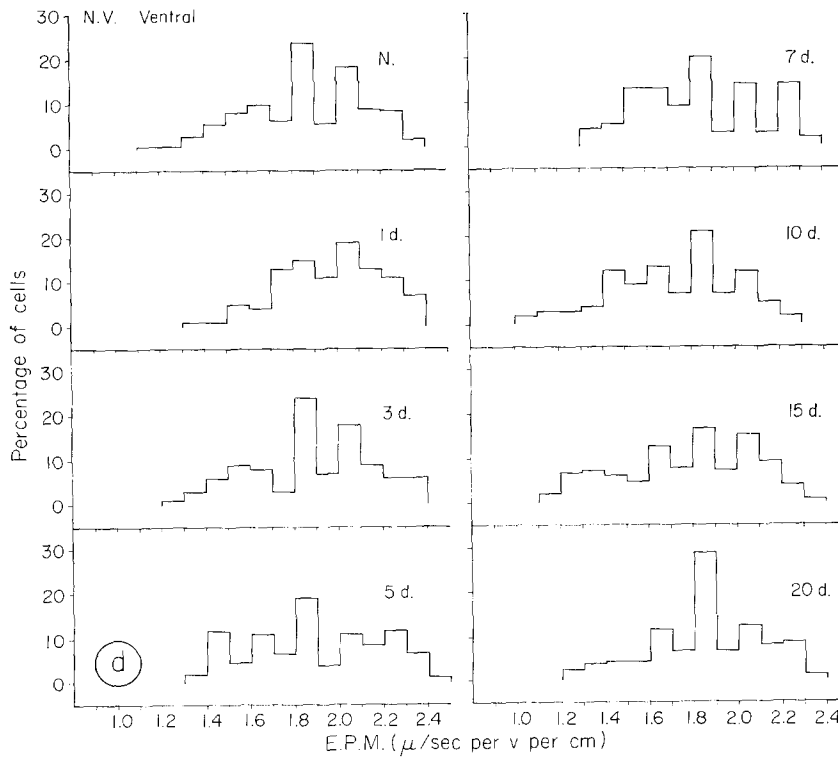
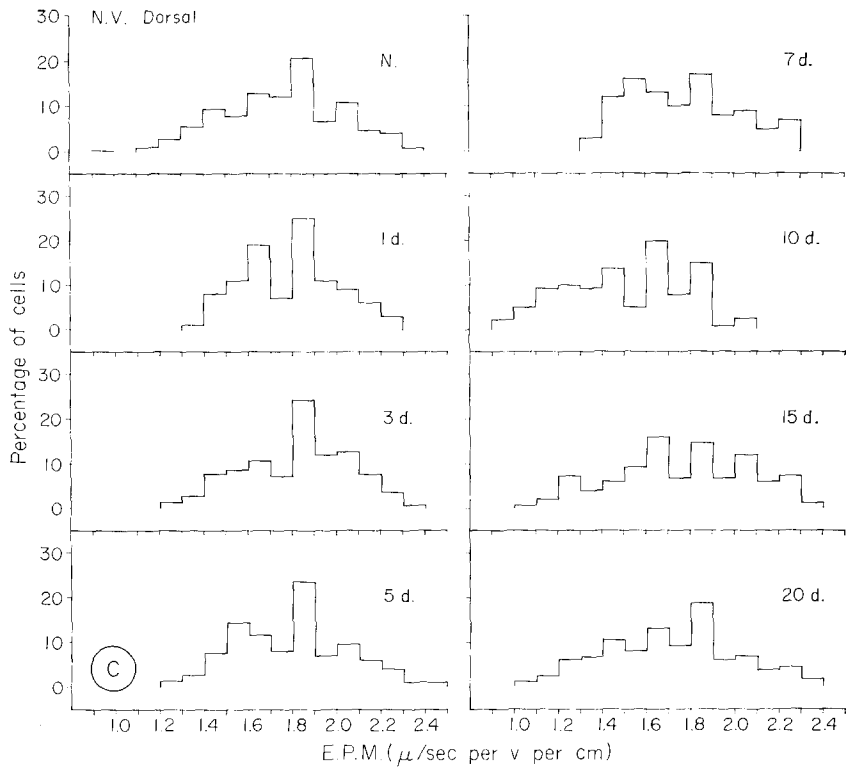
Since changes in E.P.M. with time after dissociation have been reported in liver (Maslow, 1970) and retina cells (Barnard et al., 1969), experiments were performed to determine mobilities at different time intervals after preparation of the cell suspen-

sion. Normal irises as well as 3- and 10-day regenerates were used. From Table II it can be observed that the mobilities of 3-day regenerates remained constant up to 24 hr, and that those of normal and 10-day regenerates did not differ significantly with time.

The appearance of ameboid-type cells presumed to be involved in phagocytosis of pigment granules released from iris cells during depigmentation has been reported by various investigators (Eguchi, 1963; Yamada, 1972; Yamada and Dumont, 1972). These cells appear to be more abundant during the stages or at the regions of the regenerates where extensive depigmentation of the iris cells is occurring. To determine whether the decrease in E.P.M. which starts in cells of 5-day regenerates and is significant in 10-day regenerates was due to the contamination of the iris cell suspension with macrophage and leukocytic-like cells, the E.P.M. of cells of peritoneal exudates was measured. Under phase-contrast microscopy, cells appeared with a ruffled membrane and were involved in active phagocytosis of glycogen granules. Smears of these cells treated with Giemsa's stain showed a large number of cells with oval or slightly lobulated nucleus, with large numbers of engulfed glycogen granules in their cytoplasm, and with abundant filiform projections. Their size varied from 18 to 38  $\mu$ , and a few measured up to 48  $\mu$  in diameter. These cells are similar to the macrophages involved in phagocytosis of melanosomes present in the depigmenting iris epithelium described by Yamada and Dumont (1972). Some



**FIGURE 2** Histograms depicting the electrophoretic mobilities of the dorsal and ventral iris cells, (*N*) before and at various time intervals in days (*d*) after lens removal. The histograms represent the cell population whose average mobilities are presented in each point of Fig. 1. (*N.V.*) *Notophthalmus viridescens*; (*T.G.*) *Taricha granulosa*.



cells with a highly lobulated nucleus, presumably heterophil leukocytes containing large numbers of glycogen granules, were also present in our preparations. A histogram showing the migration rates of cells from peritoneal exudates of *Taricha granulosa* is presented in Fig. 3. In this figure a histogram of a 10 day regenerate with the lowest mobilities recorded is also shown. It can be seen that the migration rates of these two cells are distinctly different from each other, mobilities of cells from peritoneal exudates being considerably lower than those of 10-day regenerates. The mean E.P.M. of the former was 0.653, while the lowest mean value obtained for 10-day regenerates was 1.360  $\mu$ /sec per v per cm.

TABLE II  
*Electrophoretic Mobilities ( $\mu$ /sec per v per cm) of Cells from Dorsal Iris and Different Stage Regenerates at Subsequent Time Intervals after Dissociation*

Time after dissociation	Normal iris	3 day	10 day
0-20 min	1.861	1.998	1.697
90 min	1.948	2.023	1.639
3 hr	1.851	2.023	1.688
6 hr	1.905	2.013	1.565
10 hr	1.859	—	1.648
22 hr	—	2.003	—
24 hr	1.899	—	1.625

Cell suspensions were obtained by trypsinization and stored at room temperature in G.A.R. for the duration of the experiment. The differences between time intervals for the same regenerate stage are not significant. Each mean E.P.M. represents an average of 30-50 measurements.

The effect of neuraminidase on the mean E.P.M. of dorsal and ventral iris cells before and at different time intervals after lentectomy is shown in Table III. While the mobilities of cells from the normal dorsal iris and from 3-day regenerates are decreased 10-16% by neuraminidase, those of cells from the 10-day and 15-day regenerates were not affected by this enzyme. A similar effect was observed for cells of the ventral iris; however, in the latter case, E.P.M. were reduced again by neuraminidase by 15 days after lentectomy. Fig. 4 presents a histogram of control and enzyme-treated cells of the normal dorsal iris and the 3 day regenerate. It can be observed that this enzyme appears to affect a large number of cells present in the population. The differences between control and neuraminidase-treated cells in normal and 3-day regenerates are statistically significant. The amount of neuraminic acid released from the cell suspension into the supernatant varied between 3.8 and  $6.75 \times 10^{-4} \mu\text{M}$ . Since in this series of experiments no cell counts were performed, no quantitative conclusion could be reached.

The effect of ribonuclease on the E.P.M. of the normal dorsal iris as well as of regenerate cells at 3, 10, and 15 days after lentectomy can be seen in Table IV. Also, in this case the cells of the normal iris and 3-day regenerates were decreased 10-17% in their E.P.M. by the action of this enzyme. This effect is significantly different. Mobilities of cells obtained from the dorsal iris of 10- and 15-day regenerates did not appear to be affected by RNase treatment under our experimental conditions. The ventral iris of the nonlentectomized eye as well as that at 3 days after lentectomy was

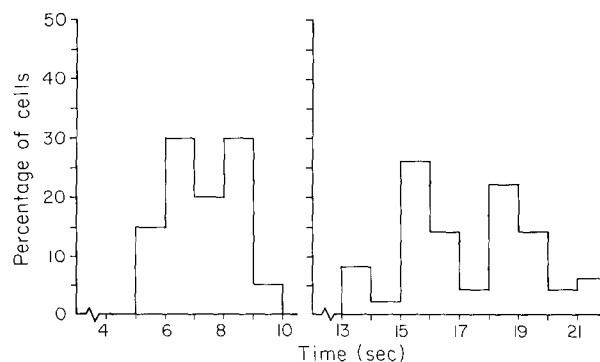


FIGURE 3 Histograms of migration rates (time required to travel  $45 \mu$ ) of macrophage-like cells from peritoneal exudates (right) and from an experiment of cells of 10-day regenerates with the lowest mobilities recorded (left). Erythrocytes were excluded from mobility determinations, since they are easily identified in the electrophoresis chamber.

TABLE III  
Electrophoretic Mobilities of Dorsal Iris and Lens Regenerate Cells after Neuraminidase Treatment

Stage	Dorsal				Ventral			
	Control	(S) Saline	(E) Enzyme	(S-E) % of decrease	Control	(S) Saline	(E) Enzyme	(S-E) % of decrease
Normal	1.633	1.660	1.472	11.4*	2.015	1.963	1.571	19.9*
	1.958	1.870	1.632	12.8*				
	1.825	1.850	1.533	17.3‡				
	—	1.867	1.571	16.0‡				
	1.865	1.822	1.612	11.5*				
	1.724	1.736	1.548	10.9*				
	1.845	1.907	1.638	14.1‡				
	1.767	1.722	1.448	15.9‡				
	1.808	1.814	1.626	10.4§				
3 day	1.852	1.826	1.660	12.0*	2.073	1.962	1.809	7.7
	2.020	1.945	1.736	10.8§				
10 day	1.615	1.667	1.657		1.847	1.780	1.902	
	1.836	1.814	1.826					
15 day	2.010	1.867	1.891	5.0	1.999	1.940	1.815	6.2
	1.650	1.716	1.630	0.05	1.912	1.840	1.579	12.5§
	1.647	1.667	1.650		1.657	1.729	1.481	14.1*

Controls represent cells in which mobilities were determined immediately after cell suspensions were obtained. Saline represents a second control in which cell suspensions were placed in saline less enzyme for the time interval of enzyme treatment. Each mean E.P.M. ( $\mu$ /sec per v per cm) represents an average of 30-40 determinations.

\*  $P > 0.01$  or less.

‡  $P > 0.001$  or less.

§  $P > 0.05$  or less.

Where probability values are missing, the differences are not significant.

affected by this enzyme. This susceptibility is lost at day 10 but appears, in some experiments, at 15 days after lens removal. As in the case with neuraminidase, ribonuclease did not appear to affect preferentially a small group of cells, but rather a great proportion of the cell population of the normal iris and 3-day regenerates seemed to be affected (Fig. 5). Under our conditions no ribose could be detected in the cell supernatants after treatment with this enzyme, when compared to those of controls. Heat-treated enzymes had no effect on the E.P.M. of normal dorsal iris cells (Table V). The effects of ribonuclease and neuraminidase, regardless of their sequence, were consistently additive in all experiments performed with normal dorsal iris cells. Fig. 6 presents a frequency histogram showing the additive effects of these enzymes.

#### DISCUSSION

It is evident from the data reported in this paper that during metaplasia of iris into lens, transformations at the cell periphery occur as evidenced by changes in the E.P.M. These changes occur in the majority of cells of the iris. As mentioned previously, our cell suspensions mainly consisted of pigmented iris cells. However, the possibility remains that the average E.P.M. values reported here represent, to a small extent, changes also occurring in the iridophores as well as iris stroma cells.

The cell preparations used in these experiments were obtained by trypsinization of cells originally present in iris or lens regenerate tissue. In consequence the difficulty of interpreting the results arises. As pointed out by Kemp (1969), "one of



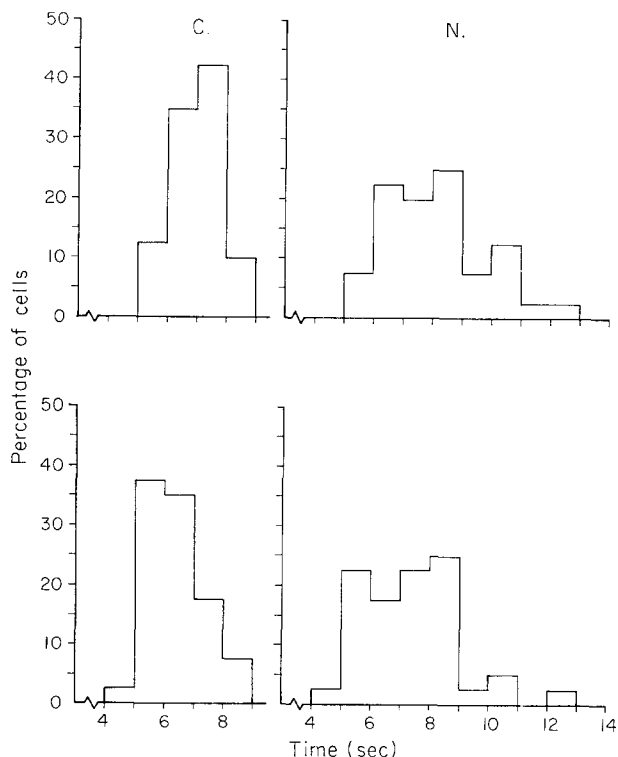


FIGURE 4 Histograms showing the effect of neuraminidase on the migration rates of cells from the dorsal iris (upper) and the 3-day regenerates (lower). (C) Control (30 min in saline less enzyme); (N) neuraminidase.

the difficulties in performing electrokinetic studies on tissue cells is the absence of an absolute reference point with which to compare the effects of agents used for disaggregation." Attempts to obtain cell suspensions from irises by mechanical means or by use of chelating agents have resulted in a very low cell yield unsuitable for electrophoretic measurements. The best results with regard to effectiveness in disaggregation, healthy appearance of the cells, absence of sliminess in the cell suspensions, and high proportion of unstained cells in dye exclusion tests, were obtained when a crude trypsin preparation was used for dissociation, and therefore this method was used in all of the experiments reported herein.

Although trypsinization is used quite widely as a method to obtain individual cell suspensions, the actual mechanism by which this enzyme separates tissue cells is not clearly understood. Trypsinization is supposed to act by splitting protein in the glycoprotein present in the intercellular cement; on the other hand, it has been reported to uncover plant agglutinin-binding groups at the cell

periphery of cultured cells (Burger, 1970; Inbar and Sachs, 1969). The effects of trypsin on electrophoretic mobility are also conflicting. While Häyry et al. (1965) and Kemp (1969) report lower E.P.M. in trypsin-dissociated cells, Simon-Reuss et al. (1964) and Weiss (1966) did not find a decrease in E.P.M. after trypsin treatment of cultured cells, and Maslow (1970) reported a decrease in E.P.M. with time after dissociation in liver cell suspensions obtained by trypsinization. It appears that changes in E.P.M. with time vary with the cells and tissues used in the experiments. Under our experimental conditions cells derived from 3-day regenerates maintained a constant E.P.M. up to 24 hr after dissociation, while the slight increase and decrease in E.P.M. of cell suspensions of normal irises and 10-day regenerates were not significantly different from controls. If we assume similar effects of trypsin during dissociation of the normal iris and the iris involved in regeneration, it would appear that the measured changes in E.P.M. during regeneration do reflect transformations at the cell periphery during this

TABLE IV  
Effect of Ribonuclease on Electrophoretic Mobilities of Normal Iris and Lens Regenerates

Stage	Dorsal				Ventral			
	Control	(S) Saline	(E) Enzyme	(S-E) % of decrease	Control	(S) Saline	(E) Enzyme	(S-E) % of decrease
Normal	1.815	1.785	1.525	14.6*				
	1.878	2.044	1.782	12.7‡	2.052	2.229	1.785	19.9*
	—	1.867	1.585	15.0*				
	1.724	1.736	1.476	15.0*				
	1.845	1.907	1.577	17.3*				
	1.799	1.729	1.495	13.3‡				
	1.808	1.814	1.634	9.9§				
	1.884	1.857	1.645	11.4‡				
3 day	1.932	1.832	1.541	15.9*				
	1.929	1.962	1.764	10.2§	1.920	1.978	1.833	7.3§
	2.031	1.929	1.649	14.5*				
10 day	1.605	1.674	1.618	3.0				
	1.786	1.827	1.881	—	1.962	1.884	1.862	1.2
15 day	1.865	1.888	1.923	—	1.953	2.04	1.841	9.8§
	1.828	1.866	1.905	—	1.870	1.827	1.792	2.0
	1.864	1.977	1.885	4.7	1.927	1.893	1.755	7.3§
	1.726	1.724	1.716	—	1.736	1.591	1.549	2.6

Each mean E.P.M. ( $\mu$ /sec per v per cm) reported in the table represents an average of 30–40 readings. For further information, see text and legend for Table III.

\*  $P > 0.001$ .

‡  $P > 0.01$  or less.

§  $P > 0.05$  or less.

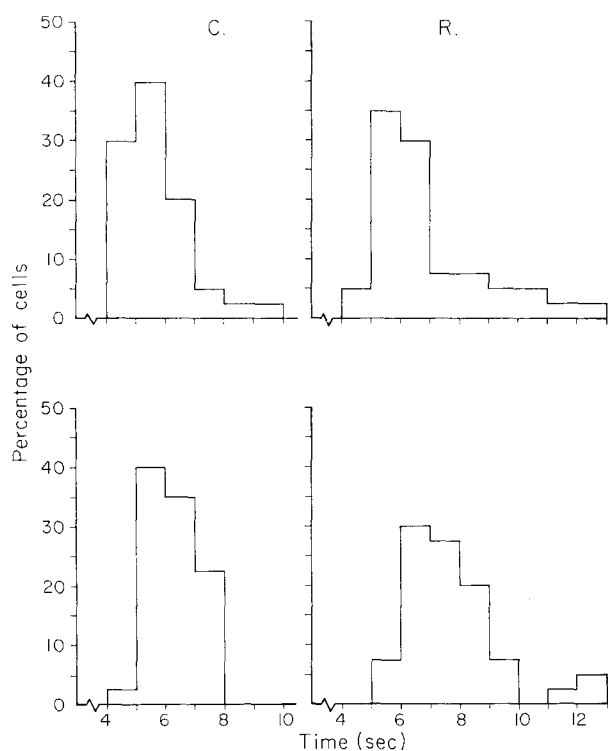
Where probability values are missing, the differences between saline- and enzyme-treated cells are not significant.

particular case of cell metaplasia. That this seems to be the case is supported by the different susceptibility of cells from different stage regenerates to enzyme treatment. However, the possibility of RNase- and neuraminidase-susceptible groups being released preferentially from the cell surface in some regenerates during tissue dissociation cannot be excluded.

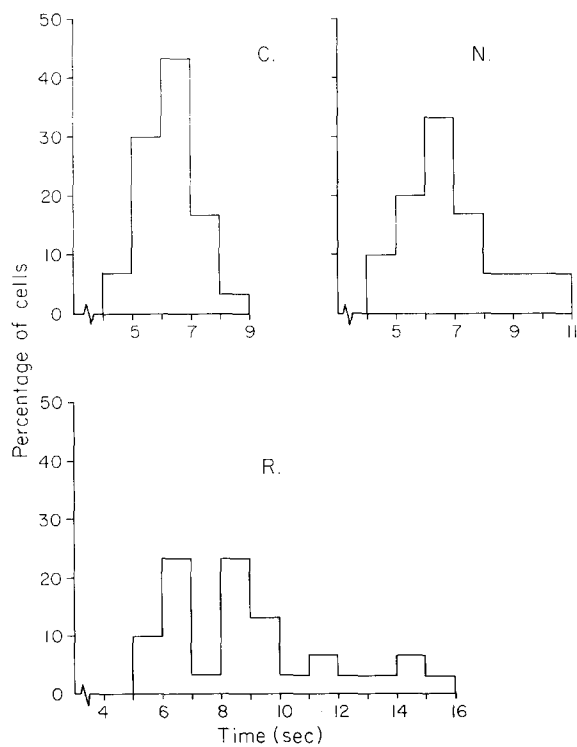
After lentiectomy, during the dedifferentiation phase, extensive depigmentation occurs in the dorsal iris cells. At the same time macrophages surround the dorsal iris and phagocyte pigment granules and cell debris. According to Yamada and Dumont (1972), the macrophage population is most numerous from 7 to 10 days after lentiectomy. The possibility of macrophage cells being present as an important contaminant of the regenerate cell suspensions was tested by measuring E.P.M. of macrophage-like cells from the peritoneal cavity

which may conceivably be involved also in phagocytosis of the glycogen granule. Our data show that for *Taricha granulosa* the mobilities of macrophages are substantially lower than those of 10-day regenerates, thereby eliminating the possibility that the low E.P.M. recorded for 10-day regenerates are due to macrophage contamination of the iris cell suspension.

A decrease in cell E.P.M. could be due to the presence of additional positive charges or disappearance of negatively charged groups from the surface of the cell. The lack of RNase and neuraminidase susceptibility of the surface-charged groups of cells from 10- and 15-day regenerates suggests that, at least in part, surface charge density decrease may be due to the disappearance of these groups from the cell periphery. Whether these groups become cryptic in the cell surface, or are lost due to shedding of the cell membrane



**FIGURE 5** Frequency histogram showing the effect of ribonuclease on the migration rates of cells from normal iris (upper) and from irises at 3 days after lentectomy (lower). (C) Control (30 min in saline less enzyme); (R) Ribonuclease treated.



**FIGURE 6** Histogram depicting the additive effects of neuraminidase and ribonuclease on the migration rates of cells from normal dorsal iris. (C) Control (1 hr in G.A.R.); (N) neuraminidase (30 min); (R) neuraminidase (30 min) followed by ribonuclease treatment (30 min).

TABLE V  
Electrophoretic Mobilities of Dorsal Iris Cells  
after Several Enzyme Treatments

1st (30 min)	2nd (30 min)	E.P.M. ( $\mu$ /sec per v per cm)
—	—	1.810
Neuraminidase	—	1.612
G.A.R.	—	1.822
Neuraminidase	RNase	1.378
Neuraminidase	G.A.R.	1.776
G.A.R.	G.A.R.	1.902
—	—	1.808
RNase	—	1.634
Neuraminidase	—	1.626
G.A.R.	—	1.814
RNase	Neuraminidase	1.409
RNase	G.A.R.	1.660
Neuraminidase	RNase	1.445
Neuraminidase	G.A.R.	1.579
G.A.R.	G.A.R.	1.838
Saline	—	1.867
RNase	—	1.585
RNase 100°C*	—	1.884
Neuraminidase	—	1.571
Neuraminidase 100°C*	—	1.941

E.P.M. values depicted to the right represent an average of 30 measurements. Cell suspensions were obtained from a minimum of 150 irises.

\* Enzyme solutions were treated for 30 min at this temperature.

as suggested by Yamada (1972), remains to be established. Alternatively, the possibility that neuraminidase- and RNase-susceptible groups conceal some other surface groups in the iris cell periphery (Winzler, 1969) which may be either exposed or removed concomitantly during metaplasia also has to be considered.

The increase in E.P.M. for 1- and 3-day regenerates may be due to loss of positively charged groups or to the appearance of new negatively charged groups at the cell periphery. The data obtained so far do not exclude either possibility. This increase in mobility could be related to nucleolar activation (Dumont et al., 1970), synthesis of ribosomal precursors (Reese et al., 1969), and entrance into the cell cycle (Eisenberg-Zalik and Yamada, 1967), which appear as early as 1-3 days after lentectomy.

It is interesting to note that the population of 10- and 15-day regenerates consists mostly of cells in the dedifferentiating phase which are depigmented but are not yet involved in synthesis of lens-specific proteins (Yamada, 1967). The possibility thus exists that an event in dedifferentiation is the loss of certain negative groups at the cell periphery which would enable the cell to change its differentiative route.

Although neuraminic acid residues appear to occur at the surface of many mammalian cells (see Weiss, 1970 *a*, and Winzler, 1969 for a review), ribonuclease-sensitive groups have rarely been detected. Nevertheless, these groups have been reported on *Arbacia* eggs and *Elodea* (Lansing and Rosenthal, 1952), on several mammalian cell lines (Weiss and Mayhew, 1966; Mayhew and Weiss, 1968), and on supernatants of amphibian gastrulae dissociated with ethylenediaminetetraacetate (Curtis, 1958). Weiss and Mayhew (1966) have reported that phosphate esters are released by this enzyme. While in some experiments material with high absorbance at 250 and 270  $\mu$ , which suggests the presence of guanine, appears in the supernatant of ribonuclease-treated cells, our ribose determinations gave inconclusive results. Experiments in our laboratory (Zalik and Scott, in preparation) indicate that RNase-sensitive groups are also removed by hyaluronidase. This would imply the presence of RNase-sensitive groups associated with a mucopolysaccharide or glycoprotein, as suggested by Brachet (1960), and recently by Weiss (1970 *b*). The nature of this material is currently under investigation.

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