THE COMPLEMENTARY EFFECTS OF 2-MERCAPTOETHANOL AND TRYPSIN IN THE LYSIS OF HeLa CELLS

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INTRODUCTION

Considerable interest has been expressed in recent years in the relative effects of different methods of DNA isolation on the physical, chemical, and biological properties of the material isolated. In particular, interest has been centered on methods of disrupting cells. Of these methods, mechanical abrasion, sonication, and chemical lysis have been most often reported. The effectiveness of mechanical methods in producing intact DNA is in doubt, inasmuch as DNA molecules

have been shown to be extremely susceptible to shear forces (1). In like manner, sonication has proven to be of dubious value in the preparation of intact DNA. Overend and Stacey (2) demonstrated the sonic disaggregation of dilute aqueous solutions of DNA as early as 1952. Litt *et al.* (3) have shown that pneumococcal transforming DNA suffers a degradation from molecular weight 8,000,000 to molecular weight 320,000 after 3 hours' exposure to 9 kc sonication, simultaneously and proportionately losing its transforming activity. Hall and Litt (4), using the technique of electron microscopy, have observed morphological changes occurring in salmon sperm DNA which has been exposed to 5 to 9 kc sonication.

TABLE I

Relationship of Channel Number to Particle Size

Channel no.	Particle size range (μ^3)
2	455–728
3	729–1092
4	1093–1456
5	1457–1820
6	1821–2184
7	2185–2548
8	2549–2912
9	2913–3276
10	3277–3640
11	3641–4004
12	4005–4368
13	4369–4732
14	4733–5096
15	5097–5460
16	5461–5824
17	5825–6188
18	6189–6552
19	6553–6916
20	6917–7280
21	7281–7644
22	7645–8008
23	8009–8372
24	8373–8736
25	8737–9210

Such observations have led to the development of methods of cell disruption which are less likely to cause mechanical damage to released nucleic acid molecules. The use of sodium dodecyl sulfate and other surface-active agents has become the method of choice (5). The development of further non-destructive methods of cell lysis would be of value, not only for their use as a tool for obtaining cellular contents, but for their possible applicability to a wider variety of cells. Such a method has been reported by Borenfreund *et al.* (6) in the course of isolating DNA from mammalian sperm. The sperm, seemingly impervious to most of the usual methods for disrupting cells, yielded DNA upon treatment with 2-mercaptoethanol followed by trypsinization. It has been postulated that the thiol compound reduces protein disulfide bridges (7, 8), thus rendering the proteins susceptible to lysis by trypsin. A study was made of the morphological effects of 2-mercaptoethanol and of trypsin on HeLa cells with the ultimate object of preparing DNA from cells which have not been subjected to sonication or to mechanical disruption.

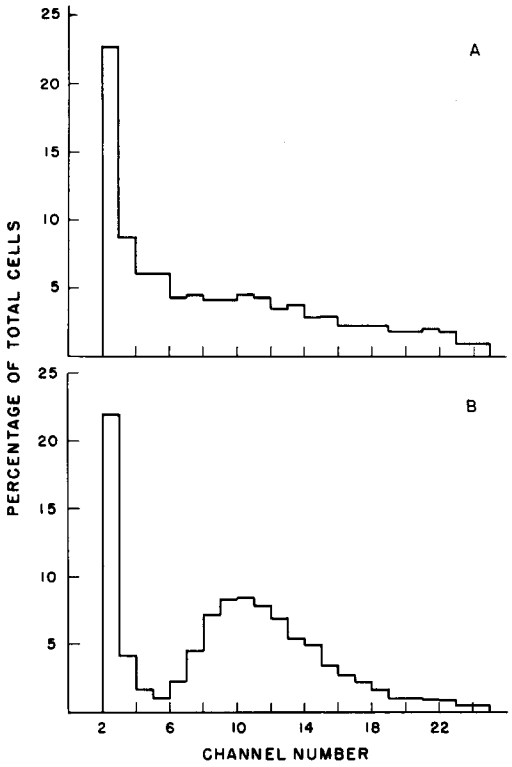


FIGURE 1

The effect of trypsin upon cultured HeLa cells. *A*, saline suspension of cultured HeLa cells; *B*, saline suspension of HeLa cells treated with trypsin.

MATERIALS AND METHODS

HeLa S3 strain cells were grown in agitated-fluid medium (9). Cell size distribution data were obtained using the Coulter Particle Size Distribution Plotter

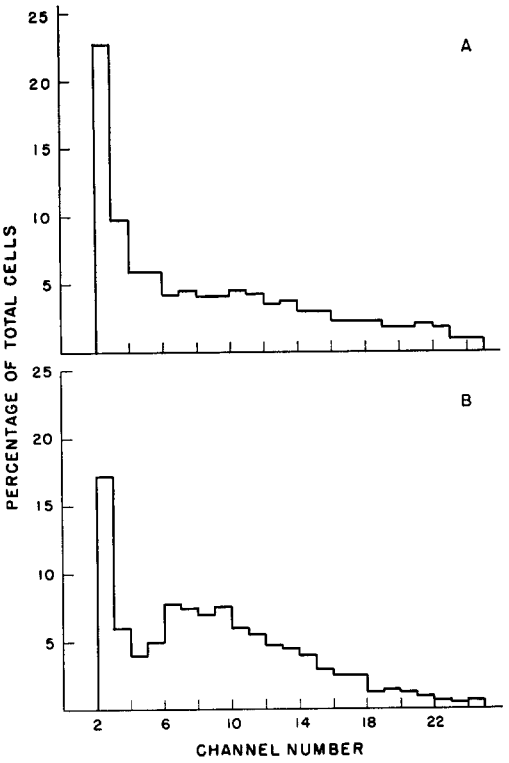


FIGURE 2

The effect of 2-mercaptoethanol upon HeLa cells. *A*, saline suspension of cultured HeLa cells; *B*, saline suspension of HeLa cells treated with 2-mercaptoethanol.

designed for use with the Coulter Model B[1] (10). The relationship of particle size to channel number may be found in Table I. Photomicrographs were obtained by injecting HeLa cell suspensions into Rose chambers (11) and photographing the suspension under phase contrast conditions.

RESULTS

The effect of trypsin on the size distribution of an aliquot of washed HeLa cells was determined in the following manner. Two aliquots of a suspension of cells in medium were centrifuged and the medium was removed. The packed cells of the first sample were suspended in physiological saline, while those of the second were suspended in 0.05 per cent trypsin in saline G (9). Both samples were incubated at 37°C for 12 minutes. The particle size distributions of both samples were then determined using the electronic size distribution plotter. The results are represented in Fig. 1.

It may be seen that trypsinization resulted in a distribution of cells more nearly approaching, in the size range of single cells, a normal distribution curve. Microscopic examination of an aliquot of suspended cells removed from a culture flask revealed a large number of multiple cell groupings. Although di-, tri-, and tetra-groupings of cells predominated, greater aggregates could be seen to occur. Since each group of aggregated cells would register on the cell plotter as a single large cell, the alteration of cell size distribution was assumed to be due to the disaggregation of clumped cells. This assumption was verified by a microscopic examination of an aliquot

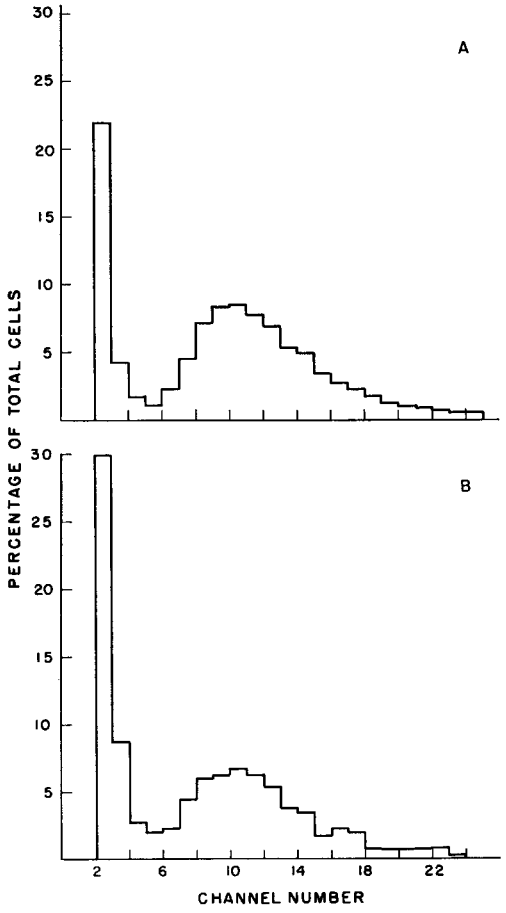


FIGURE 3

The effect of 2-mercaptoethanol upon trypsinized HeLa cells. *A*, saline suspension of trypsinized HeLa cells; *B*, saline suspension of trypsinized HeLa cells which have been treated with 2-mercaptoethanol.

[1] Available from Coulter Electronics, Chicago.

of a suspension of trypsin-treated cells, which revealed negligible numbers of aggregated cells.

In a similar manner, the effect of a saline solution of 5 per cent 2-mercaptoethanol on the size distribution of cultured HeLa cells was determined. Two aliquots of a suspension of HeLa cells in medium were centrifuged and the medium was removed. One sample of compacted cells was resuspended in physiological saline at 20°C for 60 minutes, while the second sample was resuspended in the 2-mercaptoethanol solution for the same length of time. The cell size distributions of both samples were determined, and the results are given in Fig. 2. It can be seen that the change in size distribution suggests disaggregation of clumped cells, although to a lesser degree than was obtained with trypsin. Microscopic examination of the treated cells verified this hypothesis. It was also noted during this examination that the cells had a curiously mottled, granular appearance.

The effect of 2-mercaptoethanol on previously trypsinized HeLa cells was determined in the following manner. Two aliquots of a suspension of HeLa cells in medium were centrifuged and the medium was removed. The packed cells of both samples were resuspended in 0.05 per cent trypsin in saline G and incubated at 37°C for 12 minutes.

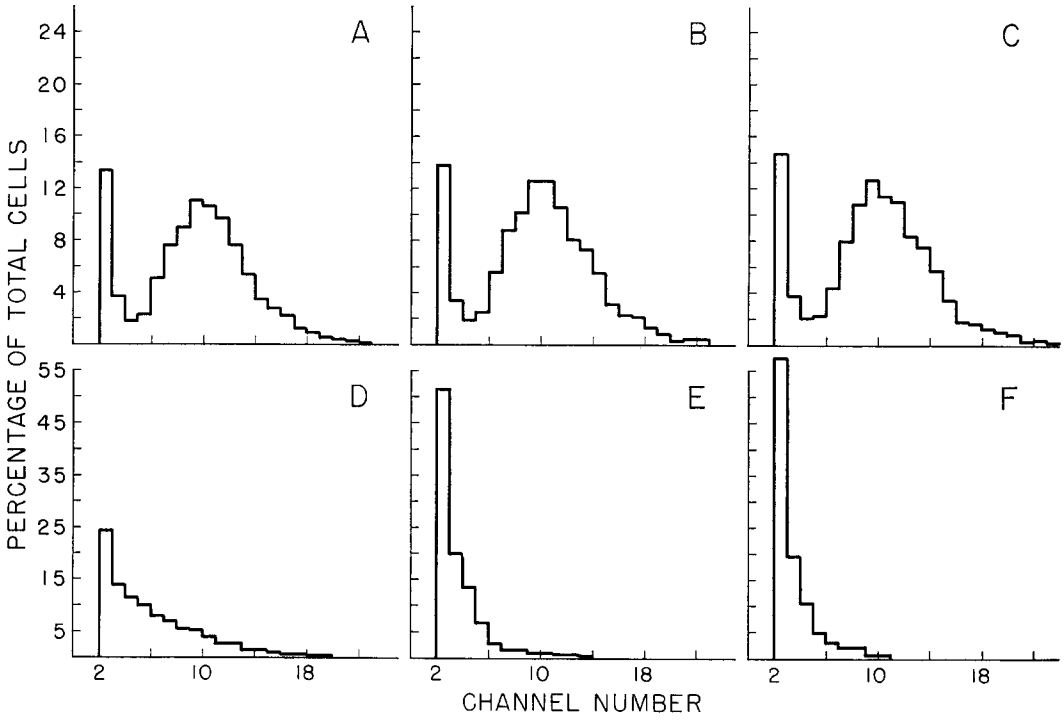


FIGURE 4

The effect of trypsin upon HeLa cells pretreated with 2-mercaptoethanol. *A*, suspension of HeLa cells in a saline solution of trypsin for 2 minutes; *B*, same as *A* at 10 minutes; *C*, same as *A* at 60 minutes; *D*, suspension of 2-mercaptoethanol–pretreated HeLa cells in a saline solution of trypsin; *E*, same as *D* at 10 minutes; *F*, same as *D* at 60 minutes.

Both suspensions were then centrifuged and the trypsin supernatant was discarded. One sample of packed cells was resuspended in physiological saline, and the second sample was resuspended in a 5 per cent saline solution of 2-mercaptoethanol. The particle size distribution of these suspensions was determined after incubation at room temperature for 60 minutes. It can be seen from Fig. 3 that mercaptoethanol treatment of previously trypsinized HeLa cells has relatively little effect on the particle size distribution of the cell suspension.

Having determined the effects of trypsin, of mercaptoethanol, and of trypsin followed by mer-

captoethanol on the particle size distribution of cultured HeLa cell suspensions, an attempt was made to determine the effects of trypsin upon cells which had been pretreated with 2-mercaptoethanol. Two aliquots of a suspension of cultured HeLa cells were centrifuged, the medium was discarded, and the packed cells were resuspended in a 0.05 per cent solution of trypsin in saline G. Both suspensions were incubated for 12 minutes at 37°C, centrifuged, and the trypsin supernatants discarded. The first sample of packed cells was resuspended in saline G for 1 hour at room temperature, while the second sample was resuspended in a 5 per cent solution of mercaptoethanol in physiological saline for 1 hour. Both suspensions were gently agitated periodically. Both suspensions were then centrifuged and the packed cells washed twice with physiological saline and centrifuged. Both samples were then resuspended in trypsin solution. The particle size distribution of both suspensions was determined at 2 minutes' time and at predetermined intervals thereafter. The results are illustrated in Fig. 4.

It can be seen from Fig. 4 that treatment with trypsin caused little change in size distribution of the suspension of HeLa cells which had received no pretreatment before trypsinization. However, the particle size distribution of the suspension of HeLa cells which had received prior treatment with 2-mercaptoethanol changed upon addition of trypsin. Within a short time, there were very few cells present within the normal size range of HeLa cells. At the same time, the suspension of the cells so treated became quite viscous. These data suggested the disruption of the HeLa cells present in the suspension with a consequent release of their contents.

Finally, photographic records were made of the process of mercaptoethanol-trypsin lysis of HeLa cells in order to support the particle size distribution data obtained on the same process. Four Rose chambers were set up for microphotography. An aliquot of a saline suspension of cells which had been treated with a 0.05 per cent trypsin solution for 12 minutes at 37°C was placed in the first chamber. In the second chamber was placed a saline suspension of washed cells which had been treated for 1 hour with a 5 per cent saline solution of mercaptoethanol at room temperature. In the third chamber was placed an aliquot of a suspension of cells which had been

FIGURES 5 to 10

Photomicrographs showing the effects of trypsin, mercaptoethanol, and combinations of these reagents upon HeLa.

FIGURE 5

Normal HeLa cells. × 180.

FIGURE 6

HeLa cells treated with 2-mercaptoethanol. × 180.

FIGURE 7

HeLa cells treated with trypsin solution for 2 minutes. × 180.

FIGURE 8

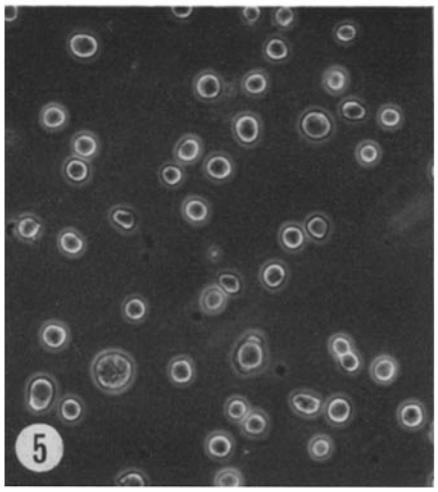
HeLa cells treated with trypsin solution for 20 minutes. × 180.

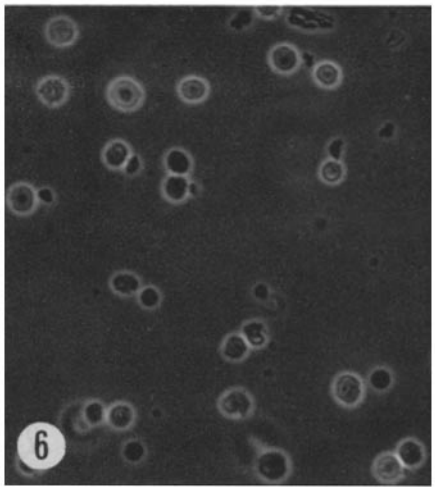
FIGURE 9

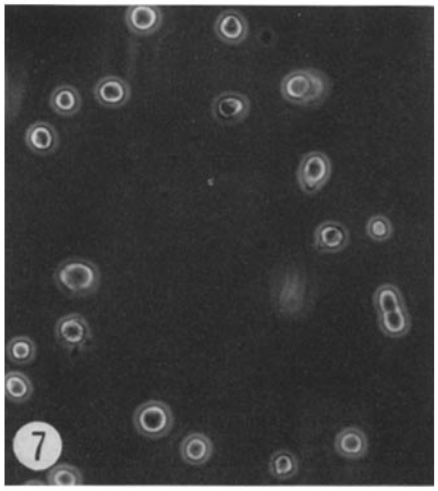
Suspension of 2-mercaptoethanol–pretreated HeLa cells in saline trypsin solution for 7 minutes. × 180.

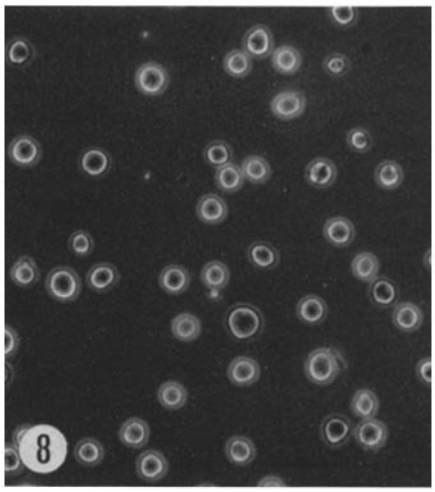
FIGURE 10

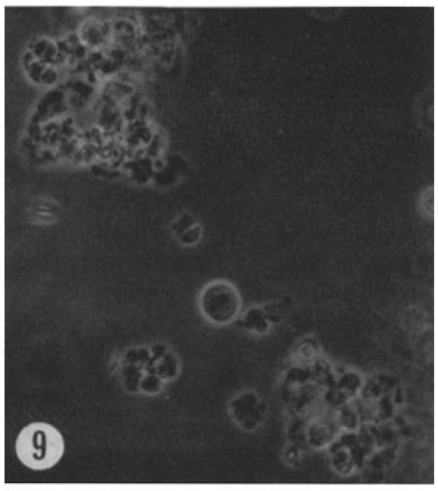
Suspension of 2-mercaptoethanol–pretreated HeLa cells in saline trypsin solution for 20 minutes. × 180.

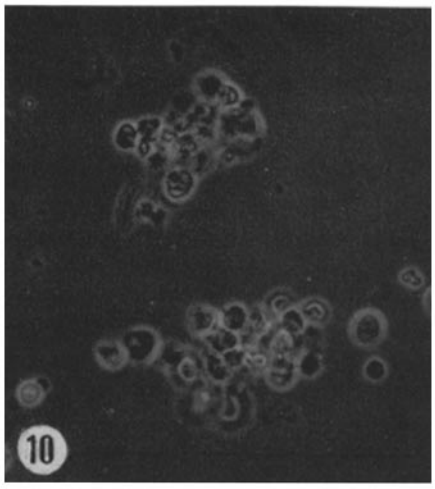












treated with a 0.05 per cent trypsin solution for 12 minutes at 37°C, washed in saline, and then resuspended in a 0.05 per cent saline solution of trypsin. The fourth chamber contained an aliquot of a suspension of cells which had been treated with a 0.05 per cent saline solution of trypsin for 12 minutes at 37°C, washed, treated for 1 hour at room temperature with a 5 per cent saline solution of mercaptoethanol, washed, and resuspended in 0.05 per cent saline trypsin solution. Photomicrographs were taken of chambers 1 and 2 at 2 minutes, and of chambers 3 and 4 at 2 minutes and at predetermined intervals thereafter. These photomicrographs are included as Figs. 5 through 10.

It can be seen that the cells serving as the first of the controls, Fig. 5, treated neither with prolonged trypsinization nor with mercaptoethanol, retained the configuration and appearance of normal cells. The cells serving as the second of the controls, Fig. 6 treated only with mercaptoethanol, may be seen to have a curious granular appearance while remaining intact. Figs. 7 and 8 represent HeLa cells which have been subjected to trypsinization for 60 minutes. They appear to have remained intact. This agrees with the size distribution data and suggests that neither mercaptoethanol nor trypsin is able to lyse HeLa cells.

Figs. 9 and 10 represent HeLa cells which have been treated with mercaptoethanol followed by trypsin. The earliest photograph available, taken at 7 minutes, shows that disruption of the cells has occurred. The majority of the cells present had been lysed, and there is reason to believe that this had occurred within 2 minutes after addition of trypsin. These photographic data confirm the hypothesis that pretreatment of HeLa cells with 2-mercaptoethanol renders them susceptible to tryptic lysis.

DISCUSSION

Treatment of HeLa (S3) cells with mercaptoethanol followed by trypsinization causes a disintegration of the cell walls and a subsequent release of cell contents into the surrounding medium. These results, obtained by an electronic cellular size distribution plotting method and confirmed by photomicrographs of the process, suggest a method of mammalian cell disruption which is free of degradative factors present in mechanical and ultrasonic disruption methods. Such a method might well be applied to the task of obtaining intact nucleic acid molecules from cells which are susceptible to this method of lysis.

SUMMARY

Studies were carried out upon the effects of trypsin, of 2-mercaptoethanol, and of combinations of these reagents upon the morphology of agitated-fluid cultured HeLa (S3) cells. Using an electronic device for plotting cell size distribution, it was observed that while either trypsin or 2-mercaptoethanol alone tended to disaggregate clumped cells, 2-mercaptoethanol treatment followed by trypsinization caused a disintegration of cellular walls. These observations were confirmed in a series of photomicrographs taken during this procedure. It is suggested that this technique may prove successful as a method of releasing labile cell contents, specifically high molecular nucleic acids, with a minimum of denaturation.

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