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HeLa CELL RNA AND PROTEIN SYNTHESES

EFFECTS OF LONG-TERM TREATMENT WITH 5,6-DICHLORO-1-β-D-RIBOFURANOSYLBENZIMIDAZOLE (DRB)

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Abstract—The population growth rate of HeLa cells treated with 60 μM 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) in reinforced Eagle's minimum essential medium was directly related to the concentration of fetal bovine serum in the range from 5 to 30%. Control cells proliferated at a maximal rate (doubling time: 18–19 hr) at serum concentrations in this range. In 60 μM DRB-containing medium supplemented with 5% serum, the inhibition of hnRNA synthesis after 1,5, and 48 hr of treatment was equivalent to 78, 84, and 51%, respectively, whereas that of protein synthesis was equivalent to 5, 41, and 49%. In 15% serum, the inhibition of hnRNA synthesis after 1, 5, and 48 hr of treatment was equivalent to 75, 79, and 40%, whereas that of protein synthesis was equivalent to 13, 30, and 21%. Thus, after 48 hr of treatment of cells with DRB in either 5 or 15% serum, hnRNA synthesis was less inhibited than it was after 1 or 5 hr of treatment, and the level of protein synthesis in DRB-treated cells did not decrease between the 5th and 48th hr. After 48 hr of treatment of cells with DRB in 15% serum, hnRNA and protein syntheses were both somewhat less inhibited than after treatment in 5% serum. These results suggest that, on prolonged treatment, HeLa cells undergo a partial, serum-dependent adaptation to the inhibitor.

Previous studies of the effects of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on hnRNA and mRNA syntheses and on proliferation of HeLa cells have indicated that the inhibition of cell proliferation after 1 or more days of treatment is less than might be expected based on the marked inhibition of RNA synthesis that is observed in short-term experiments [1–3]. For example, 40 μM DRB inhibits hnRNA synthesis by ~75% [2, 3] and mRNA synthesis by ~80% [4–8] as measured after short-term treatment of cells; yet, populations of cells treated with DRB for 24 hr or longer are able to proliferate at a substantial and exponential rate [1]. Moreover, the rate of population growth in DRB-treated cultures of HeLa cells is directly dependent on serum concentration; increasing the serum concentration from 5 to 15% enhances both the cycling and the survival of the treated cells, whereas it has no effect on the proliferation of control cells [1]. It appears that the cells may undergo a partial adaptive change during treatment with DRB, as a result of which the syntheses of mRNA and, secondarily, of protein may be less inhibited after prolonged treatment than they are after short exposure to DRB, and that this change may be dependent on serum components.

In this report we present evidence that the rate of population growth in DRB-treated HeLa cell cultures is directly related to serum concentration in the range from 5 to 30%, although untreated cells proliferate at the same maximal rate in 2.5 to 30% serum. The growth-supportive activity of serum for DRB-treated cells was, in part, dialyzable and, in part, nondialyzable, whereas that for untreated cells was nondialyzable. Serum protected cells in a concentration-dependent manner against the inhibitory action of DRB on uridine and leucine uptake in long-term experiments. The extent of inhibition of hnRNA synthesis by DRB was similar after treatment for 1 or 5 hr, but it was less after 24 or 48 hr. Protein synthesis was only slightly affected after 1 hr; it was substantially inhibited by 5 hr, but the inhibition was no greater after 48 hr of treatment. The effects of DRB on hnRNA and protein syntheses after prolonged treatment in 15% serum appeared to be somewhat less than in 5% serum.

MATERIALS AND METHODS

Cells and culture conditions. HeLa cells were grown in monolayer cultures as described previously [1]. The medium was reinforced Eagle's minimum essential medium [9], supplemented with GIBCO fetal bovine serum [3]. Lot No. C184419 was used in all experiments represented in the figures and tables; Lot No. 31PG401 was used in one additional experiment.

Population growth curves. Cultures were photographed, and cells were enumerated daily as previously described [10, 11].

Viable and total cell number determinations. Cells in monolayer cultures were stained with erythrosine B [12], and both the unstained and stained cells were counted in several fields. This gave a ratio of viable to nonviable cells. The cells were then trypsinized, and the total number was determined.

[^{3}H]Uridine uptake and syntheses of total RNA and heterogeneous nuclear RNA (hnRNA). At various intervals after the beginning of treatment with DRB, HeLa cells in 60 mm petri dishes (three dishes per variable) were pulse-labeled for 15 min with [^{3}H]uridine (25–29 Ci/mmole; New England

Nuclear), 2 μCi/ml, in 5 ml of reinforced Eagle's medium [12] supplemented with 5 or 15% fetal calf serum. The pulse was stopped by washing cultures four times with cold phospate-buffered saline (PBS). The dishes were drained and frozen. At convenient times they were thawed, 1.5 ml of 1% sodium dodecyl sulfate (SDS) was added per dish, and the cells were detached and lysed. Aliquots of the lysate were spotted on filters for determination of total cell-associated radioactivity. The lysate was then precipitated with 10% trichloroacetic acid (TCA) at 4°, and the precipitate was collected on filters for determination of acid-precipitable radioactivity. The ratio of acid-precipitable to total radioactivity was calculated for control and DRB-treated cells. To obtain an estimate of inhibition of RNA synthesis by DRB, this ratio for DRB-treated cells was expressed as a percentage of that for control cells [1, 2, 4]. Such estimates of inhibition of RNA synthesis are independent of variations in the level of RNA synthesis due to culture conditions [2].

In experiments in which the effect of DRB on hnRNA synthesis was investigated, actinomycin D (0.04 μg/ml) was included in the medium for 25 min before pulse-labeling and during the 15 min pulse to inhibit preribosomal RNA synthesis.

[^{3}H]Leucine uptake and protein synthesis. Cells were pulse-labeled for 15 min with [^{3}H]leucine (5 Ci/mmole; New England Nuclear), 8 μCi/ml, and processed as described for [^{3}H]uridine, except that before precipitation with 10% trichloroacetic acid at 4° the samples were first incubated at 37° for 2 hr.

RESULTS

Dependence of HeLa cell proliferation in 60 μM DRB on serum concentration. Figure 1 demonstrates that the rate of population growth in cultures of HeLa cells continuously treated with 60 μM DRB was strikingly dependent on serum concentration, in spite of the fact that control cells incubated without DRB proliferated at closely similar rates in 5, 10, 20 and 30% fetal bovine serum. We have found that the control cells proliferate at a maximal rate even in 2.5% serum. In contrast, serum concentrations of 5% or less failed to support a net increase in cell number in cultures treated with 60 μM DRB. However, in 30% serum the DRB-treated population increased with an approximate doubling time of 41 hr, and reached confluence.

Roles of nondialyzable and dialyzable serum components in promoting the growth of DRB-treated cell populations. Fetal bovine serum was dialyzed against 100 vol. of Eagle's medium containing $NaHCO_3$ and antibiotics and filtered. Figure 2 shows that untreated HeLa cells proliferated at closely similar rates in reinforced Eagle's medium supplemented with undialyzed or dialyzed serum at either a 5 or 15% concentration. In sharp contrast, the growth of 40 μM DRB-treated populations was dependent on both serum concentration and whether undialyzed or dialyzed serum was used. Dialyzed serum at a concentration of 15% supported substantially greater population growth than dialyzed serum at 5%. It was also evident that undialyzed serum was considerably more active than dialyzed serum in supporting the growth of DRB-treated cell populations.

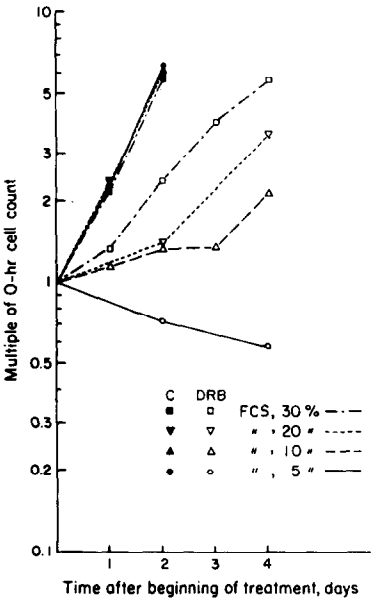

Fig. 1. Relationship between serum concentration and proliferation of control and 60 μM DRB-treated HeLa cells. DRB was present for the duration of the experiment. Cultures were planted at a density of 3.6×10^3 cells/cm^2 (1.8×10^4 cells/ml) in reinforced Eagle's medium containing 5% serum. Twenty-four hours later, i.e. at 0 time, the cell counts per cm^2 were as follows: for cultures to be incubated in 30, 20, 10 or 5% serum: 3.5, 3.4, 3.0, and 3.5×10^3 cells, respectively, for controls, and 4.1, 2.8, 3.8 and 3.9×10^3 cells for DRB. The 0-time values were used in normalizing consecutive cell counts, which are plotted as multiples of the respective 0-hr cell counts.

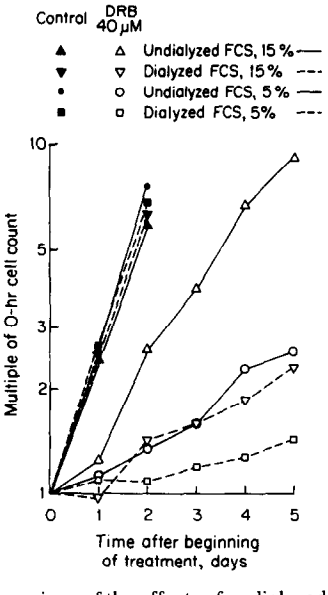

Fig. 2. Comparison of the effects of undialyzed and dialyzed serum on the proliferation of 40 μM DRB-treated and control HeLa cells. DRB was present for the duration of the experiment. Cultures were planted at a density of 3.6×10^3 cells/cm^2. Twenty-four hours later, i.e. at 0 time, the cell counts per cm^2 were as follows: for cultures to receive undialyzed or dialyzed 15% serum or undialyzed or dialyzed 5% serum: 5.2, 4.6, 3.2, and 4.5×10^3, respectively, for controls, and 2.9, 4.0, 3.9, and 4.6 for DRB.

Table 1. Viability of HeLa cells after treatment with 60 μM DRB for 48 hr*

Expt.	0-hr Cell number per $cm^2 \times 10^{-4}$ Con	DRB	Multiple of 0-hr cell number 5% Serum Con	DRB	15% Serum Con	DRB
A	0.64	2.6	4.80	0.79	4.88	1.03
B	2.6	2.6	4.29	0.60	4.58	1.07
	Mean		4.54	0.70	4.73	1.05
			Viable cells (% of total)			
A	0.64	2.6	99.5	85.0	99.5	89.0
B	2.6	2.6	99.7	76.9	99.6	88.9
	Mean		99.6	81.0	99.5	89.0

* A and B each represent mean results of two experiments. For procedure, see Materials and Methods.

Cell viability as measured by erythrosine B exclusion. After 5 hr of DRB treatment (60 μM) in either 5 or 15% serum, under the conditions of the experiments summarized in Tables 2–4, 97% of cells present were viable compared to 98% in untreated controls.

The results obtained after 48 hr of treatment are summarized in Table 1. Cultures were planted either at two different densities (Expt. A, control at 0.32×10^4 and DRB at 1.33×10^4 cells/cm^2), as in the 48-hr experiments summarized in Tables 2–4, or at the same cell density (Expt. B, control and DRB both at 1.3×10^4 cells/cm^2). As can be seen, the viability of control cells at the termination of the experiment was >99% in both 5 and 15% serum. In cultures that had been treated with DRB for 48 hr in 5% serum, the mean percentage of viable cells was ~81, and in 15% serum it was ~89. Thus, at the end of a 48-hr treatment period, most of the cells present were viable. Table 1 also shows that, relative to the 0-hr cell number, there was a *net* decline of 30% in total cell number after 48 hr of treatment with DRB in 5% serum, and a *net* 5% increase in 15% serum.

Serial time-lapse cinemicrographic observations of single cells have shown that both mitoses and deaths occur in 60 μM DRB-treated cultures of HeLa cells [1]. Cells that die lose identifiable characteristics as cells after a time and would no longer be detected as cells in the dye exclusion assay. Thus, this assay provides an estimate of the fraction of nonviable cells present at a given time, but not a cumulative estimate of how many cells have died over a time period. This is the basis for the very high proportion of viable cells in DRB-treated cultures in which numerous deaths, as well as mitoses, occurred during 48 hr of treatment [1].

Uridine uptake and total RNA and hnRNA syntheses. Tables 2 and 3 summarize results of experiments in which the effects of treatment with 60 μM DRB for 1, 5 or 48 hr in 5 or 15% serum were determined with respect to uridine uptake, and total RNA (Table 2) or hnRNA (Table 3) synthesis. To measure the effect of DRB on hnRNA synthesis, actinomycin D (0.04 μg/ml) was used to suppress preribosomal RNA synthesis during the assay. As the table footnotes indicate, different initial cell densities were used for different experimental conditions so that cell densities would not be widely different during pulse-labeling at the end of the treatment periods of varying length. To evaluate inhibition of RNA biosynthesis by an agent such as DRB, which inhibits the uptake into cells of

Table 2. Inhibition of [^{3}H]uridine uptake and total RNA synthesis in HeLa cells after treatment with 60 μM DRB in 5 or 15% fetal calf serum*

Time (hr)	Medium Serum (%)	DRB (μM)	[^{3}H]Uridine incorporation Total (cpm)	Acid-prec. (cpm)	Prec./Total (%)	DRB (% of control) Total	Acid-prec.	Prec./Total (total RNA synthesis)
1†	5	0	429,897	30,599	7.12			
1	5	60	177,284	4,184	2.36	40.2	13.7	33.1
1	15	0	506,027	34,883	6.88			
1	15	60	195,333	4,424	2.26	38.6	12.7	32.8
5‡	5	0	559,760	16,678	2.98			
5	5	60	131,157	1,266	0.965	23.4	7.59	32.4
5	15	0	556,380	19,518	3.51			
5	15	60	158,953	1,767	1.11	28.6	9.05	31.6
48§	5	0	721,470	11,059	1.53			
48	5	60	58,033	609	1.05	8.04	5.51	68.6
48	15	0	719,360	12,592	1.75			
48	15	60	170,427	2,338	1.37	23.7	18.6	78.3

* Mean results of two experiments. For procedure, see Materials and Methods.

† Sixty-millimetre plastic dishes (growth area of 19.6 cm^2) were used and the volume of medium was 5 ml. Cultures were planted at 0.64×10^4 cells/cm^2 (2.5×10^4 cells/ml), and the experiments done 3 days after planting.

‡ Cultures were planted at 1.3×10^4 cells/cm^2 (5×10^4 cells/ml), and the experiments started 24 hr later.

§ Cultures for controls were planted at 0.32×10^4 cells/cm^2 (1.25×10^4 cells/ml) and those for DRB at 1.3×10^4 cells/cm^2 (5×10^4 cells/ml), and the experiments were started 24 hr later.

Table 3. Inhibition of [^{3}H]uridine uptake and hnRNA synthesis in HeLa cells after treatment with 60 μM DRB in 5 or 15% fetal calf serum*

Time (hr)	Medium Serum (%)	DRB (μM)	[^{3}H]Uridine incorporation Total (cpm)	Acid-prec. (cpm)	Prec./Total	DRB (% of control) Total	Acid-prec.	Prec./Total (hnRNA synthesis)
1†	5	0	543,617	23,341	4.29			
1	5	60	191,727	1,792	0.935	35.3	7.68	21.8
1	15	0	543,944	23,260	4.28			
1	15	60	211,700	2,245	1.06	38.9	9.65	24.8
5‡	5	0	552,838	8,679	1.57			
5	5	60	125,210	321	0.256	22.6	3.70	16.3
5	15	0	554,580	8,177	1.47			
5	15	60	153,863	480	0.312	27.7	5.87	21.2
48§	5	0	713,857	5,782	0.810			
48	5	60	57,323	225	0.393	8.03	3.89	48.5
48	15	0	749,633	6,235	0.832			
48	15	60	175,930	873	0.496	23.5	14.0	59.6

* Mean results of two experiments. For procedure, see Materials and Methods.
† See (†) footnote, Table 2.
‡ See (‡) footnote, Table 2.
§ See (§) footnote, Table 2.

[^{3}H]uridine used as the labeled precursor, the acid-precipitable radioactivity was corrected for the reduced amount of isotope taken up by the treated cells (see Materials and Methods). Estimates of inhibition of RNA synthesis by this procedure agree with those obtained by the use of ^{32}P, whose uptake into cells is not inhibited by DRB [13]. Inhibition of RNA synthesis by DRB is not dependent on inhibition of uridine transport as DRB inhibits RNA synthesis to the same extent in the presence or absence of exogenous uridine [2]. The biochemical target for DRB has not been identified yet, but it appears to be a component of the mechanism for transcription of DNA by RNA polymerase II. The last columns in Tables 2 and 3 give estimates of total RNA or hnRNA synthesis, respectively, in DRB-treated cells expressed as a percentage of synthesis in untreated control cells.

Table 2 shows that 1-hr DRB treatment caused approximately 60% inhibition or uridine uptake and 65% inhibition of total RNA synthesis. These results are similar to those previously reported for even shorted periods of treatment [2]. It is apparent that serum concentration (5 vs 15%) did not affect significantly the extent of inhibition of uridine uptake and RNA synthesis by DRB in short-term experiments, which confirms earlier results [2]. We also measured inhibition of these processes by DRB after a 1-hr incubation of cells in medium containing no serum or supplemented with 30% serum, and obtained similar results.

After 5 hr of DRB treatment in either 5 or 15% serum, inhibition of uridine uptake was more marked than after 1-hr treatment (Table 2). At 5 hr there was no apparent change in the extent of inhibition of RNA synthesis. The inhibition of uridine uptake by DRB became more marked between 5 and 48 hr in 5% serum and remained approximately the same in 15% serum. In both 5 and 15% serum, the extent of inhibition of RNA synthesis after 48 hr of treatment was much less than after 5 hr of treatment with DRB. In 15% serum, RNA synthesis was inhibited 68% at 5 hr and only 22% at 48 hr. This difference is of particular interest as it was unaccompanied by any significant change in uridine uptake. Thus, after prolonged treatment of cells with DRB, the inhibitory effect of the compound on RNA synthesis becomes markedly reduced. The results also suggest that, after 48 hr of incubation in 15% serum, the inhibition of RNA synthesis in DRB-treated cells was somewhat less than that after incubation in 5% serum.

In the experiments in Table 2 no actinomycin D was used, whereas in the experiments in Table 3 actinomycin D (0.04 μg/ml) was present during the RNA synthesis assay to suppress preribosomal RNA synthesis. It is evident, by comparing the total cpm in Tables 2 and 3, that actinomycin D itself had no effect on [^{3}H]uridine uptake into cells. The RNA synthesis that continued in the presence of actinomycin D (0.04 μg/ml) predominantly represented hnRNA synthesis and was, therefore, inhibited by DRB more than total RNA synthesis because the action of DRB is selective for hnRNA (compare values in the last column of Table 3 with those in Table 2). Conversely, the greater inhibition of RNA synthesis by actinomycin D in DRB-treated (63% on the average) than control cells (47%) was due to the fact that preribosomal RNA synthesis as a fraction of total is greater in DRB-treated than in control cells. At the same time, the data presented in Tables 2 and 3 are reflective of the facts that not all of hnRNA synthesis is sensitive, and not all of preribosomal RNA synthesis is resistant, to inhibition by DRB [2, 13].

Table 3 confirms all the findings in Table 2 con-

Table 4. Inhibition of [^{3}H]leucine uptake and protein synthesis in HeLa cells after treatment with 60 μM DRB in 5 or 15% fetal calf serum*

Time (hr)	Medium Serum (%)	DRB (μM)	[^{3}H]Leucine incorporation Total (cpm)	Acid-prec. (cpm)	Prec./Total (%)	DRB (% of control) Total	Acid-prec.	Prec./Total (total protein synthesis)
1†	5	0	125,053	5,707	4.56			
1	5	60	121,807	5,255	4.31	97.4	92.1	94.5
1	15	0	125,528	6,356	5.06			
1	15	60	130,296	5,707	4.38	104	89.8	86.6
5†	5	0	50,483	1,764	3.49			
5	5	60	36,389	686	1.89	72.1	38.9	54.2
5	15	9	49,733	1,333	2.68			
5	15	60	39,804	747	1.88	80.0	56.1	70.1
48‡	5	0	69,040	2,754	3.99			
48	5	60	23,880	487	2.04	43.1	17.7	51.1
48	15	0	69,284	2,589	3.74			
48	15	60	49,629	1,457	2.94	71.2	56.3	78.6

* One-hour treatment: one experiment; 5-hr treatment: mean of five experiments; 48-hr treatment: mean of two experiments. For procedure, see Materials and Methods.

† Cultures were planted at 0.64×10^4 cells/cm^2 (2.5×10^4 cells/ml), and experiments were done 2 days later.

‡ Cultures for controls were planted at 0.32×10^4 cells/cm^2 (1.25×10^4 cells/ml) and those for DRB at 1.3×10^4 cells/cm^2 (5.4×10^4 cells/ml), and experiments were started 2 days later.

cerned with the effects of 60 μM DRB on uridine uptake as a function of duration of treatment and serum concentration. The extent of inhibition of hnRNA synthesis after 1 hr of treatment (70–75%) agrees closely with previous findings obtained in HeLa-S_3 cells in suspension [2, 13] and in HeLa cells in monolayer [3]. The inhibition of hnRNA synthesis after 5 hr of treatment with DRB was similar to that observed after 1 hr, but after 48 hr of treatment the inhibition was much less. The results in Table 3 suggest that inhibition of hnRNA synthesis by DRB was somewhat less in 15% than in 5% serum after 48 hr and also after 5 hr of treatment. Relating the levels of hnRNA synthesis in the DRB-treated cultures, 5 vs 15% serum, directly to each other by means of the ratios of precipitable to total counts, it can be calculated that, after 48 hr of treatment, hnRNA synthesis in 5% serum was 79% of that in 15% serum and, after 5 hr of treatment, it was 77%.

In an experiment in which hnRNA synthesis was investigated after 24 hr of treatment with 60 μM DRB, hnRNA synthesis was inhibited 49 and 32% in 5 and 15% serum respectively. Thus, it appears that the effect of DRB on hnRNA synthesis was reduced between 5 (see Table 3) and 24 hr after the beginning of treatment.

Leucine uptake and protein synthesis. As shown in Table 4, treatment of HeLa cells with DRB for 1 hr had no significant effects on leucine uptake and possibly only a slight inhibitory effect on protein synthesis. These results agree with previous findings [2]. After 5-hr treatment of cells, both leucine uptake and protein synthesis were moderately inhibited. In five experiments in which cells were treated for 5 hr, the small (11%) difference in the inhibition of protein synthesis in 5 vs 15% serum was statistically significant ($P < 0.025$); however, the even smaller difference (8%) in the inhibition of leucine uptake was not ($P > 0.35$). In the interval between 5 and 48 hr, the inhibition of leucine uptake increased considerably in 5% serum and only slightly, if at all, in 15% serum. Of particular interest is the finding that the inhibition of protein synthesis by DRB did not become more marked when treatment was extended from 5 to 48 hr. After 48 hr of treatment, the inhibitory effects of DRB on protein synthesis appeared to be somewhat less in 15% than in 5% serum.

DISCUSSION

Figure 3 summarizes the results presented in Tables 2–4 and illustrates the following aspects of the action of 60 μM DRB on precursor uptake and hnRNA and protein syntheses in Hela cells incubated with 5 or 15% serum: (1) DRB rapidly and markedly inhibited the uptake of uridine into cells; leucine uptake was inhibited slowly and much less; the effects of DRB on precursor uptake were less in 15% than in 5% serum; (2) DRB caused very marked and

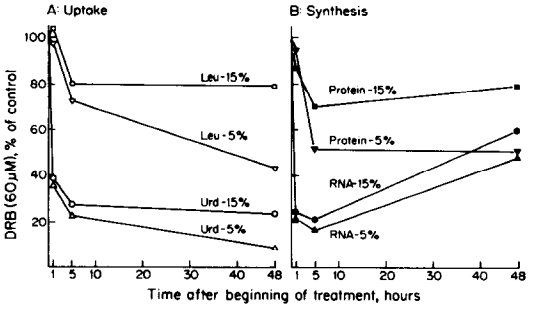

Fig. 3. Summary of the effects of 60 μM DRB on uridine and leucine uptake and on hnRNA and protein syntheses as a function of duration of treatment and serum concentration. Based on data in Tables 2–4.

rapid inhibition of hnRNA synthesis; when treatment was continued, the inhibition persisted at the marked level for at least 5 hr but, subsequently, inhibition was partially reversed and, apparently, more so in 15% than in 5% serum; (3) DRB caused little inhibition of protein synthesis after treatment of cells for 1 hr; by 5 hr, the inhibition was moderate, but showed no further increase on continued treatment in 15% serum, although it did in 5% serum.

A time-lapse study of the frequencies of cell divisions and deaths in DRB-treated cultures incubated with 5 or 15% serum has shown that deaths, as well as mitoses, are more frequent during the first 24 hr of incubation than later, and that there occur somewhat fewer deaths and more mitoses in 15% than in 5% serum [1]. The early relatively high frequency of deaths is readily understood on the grounds of the very rapid and marked inhibition of hnRNA and also mRNA synthesis [2, 13] in DRB-treated cells. The early relatively high mitotic frequency in the the DRB-treated cell population suggests that a considerable number of cells in later stages of their division cycle complete the cycle and divide in spite of severe inhibition of hnRNA synthesis. After the initial period of less than 24 hr, new steady-state conditions are established under which DRB-treated cells incubated in 15% serum divide more frequently and die less frequently than those incubated in 5% serum. The quantitative relationship between DRB and serum effects was illustrated by the similarity of action of 60 μM DRB in 15% serum and 40 μM DRB in 5% serum; at the same time in 5% serum, the action of 60 μM DRB on proliferation is much greater than that of 40 μM DRB [1].

In HeLa cell cultures treated in 15% serum, hnRNA synthesis was inhibited ~ 40% and protein synthesis ~ 20% at 48 hr from the beginning of treatment. For comparison, in cultures treated in 5% serum, the synthesis of both hnRNA and protein was inhibited ~50%. HeLa populations treated with 60 μM DRB in 15% serum showed a slow increase in cell number. In contrast, there was a net decrease or little change in cell number in populations treated in 5% serum (Fig. 1; [1, 3]).

It is evident that, in HeLa cells treated with DRB for a prolonged period, several biochemical and biological parameters change with time, and that serum concentration affects these changes. We have demonstrated previously that after replanting of HeLa cells which had been treated with 60 μM DRB for 3 or 5 days, and further incubation in the continued presence of DRB, the cell populations remain sensitive to inhibition of hnRNA by DRB, although the inhibition is less than in cultures treated for a short period only [1]. These results are consistent with present findings. When DRB treatment is discontinued after 3 or 5 days, hnRNA synthesis returns to the level observed in the untreated control cells [1]. After surviving cells in a previously DRB-treated culture resume proliferation at a rapid rate, the population is as sensitive to growth inhibition by DRB as control cells which have not been treated previously with DRB [14]. All of the results are consistent with the possibility that cells exposed to DRB undergo a partial, serum-dependent adaptive response to the inhibitor, which is expressed in their hnRNA synthesis, cycling, and survival. There is as yet no evidence as to how the alteration in hnRNA synthesis is brought about in DRB-treated cells or whether the change in hnRNA synthesis is responsible for the change in the proliferative behavior of the cells. It is possible that, during DRB treatment, access of DRB to its target may be restricted as a result of a serum-dependent cellular alteration. Alternatively, the cells may respond to the metabolic restriction imposed by DRB by amplification of their biosynthetic capacity. In either case, in the serum concentration range from 5 to 30%, the population growth rate of 60 μM DRB-treated cells over a period of 4 days or more is directly dependent on the concentration of serum (Fig. 1; [1, 3]). The action of serum was clearly related to DRB treatment as the proliferation of control cells was independent of serum concentration in the 2.5 to 30% range. In effect, these results can be interpreted as indicating an increased growth factor requirement of DRB-treated cells. This increased requirement appears to involve both dialyzable and nondialyzable serum components. The growth-promoting activity of dialyzed serum could be due to cellular growth factor, such as the platelet-derived growth factor and somatomedins, remaining after dialysis [15–18]. However, dialyzable components in serum, not present in reinforced Eagle's medium, appear also to be required for the proliferation of DRB-treated cells. These may include metals, vitamins, and hormones [19]. The identification of the specific factors required in abnormally high amounts by the biosynthetically depressed DRB-treated cells would be of considerable interest.

It is important to note that the cell growth-supporting and protective effects of serum are not due to inactivation of DRB by serum components, as DRB-containing culture medium retains full capability to inhibit RNA synthesis after several days of incubation with cells [3, 9]. This has been demonstrated in short-term assays with fresh cells. The available results are consistent with the possibility that serum is required for the expression of the postulated adaptive change of HeLa cells to the inhibitor; however, they do not exclude the possibility that the adaptive change itself may be dependent on serum components.

Whether the findings presented in this and related reports [1, 3] have applicability to other systems in which the metabolism and proliferation of cells are restricted by chemical agents remains to be determined. This is potentially an important issue as whole serum is the usual supplement in cell culture medium and is commonly used at a single concentration, which, although adequate to assure maximal proliferation of control cells, may not be sufficient to permit maximal proliferation of drug-treated cells.

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