Effect of sodium n-butyrate on induction of prostaglandin synthase activity in cloned mastocytoma P-815 2-E-6 cells

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Treatment of a cloned mastocytoma P-815 cell line (2-E-6) with 1 mM-sodium n-butyrate for 40 h induced prostaglandin synthase activity and arrested cell growth. The induction of enzyme activity by n-butyrate was not associated with suppression of DNA synthesis, since hydroxyurea had no effect on prostaglandin synthase induction. The effect of sodium n-butyrate was reversible. Experiments with cycloheximide and actinomycin D showed that the induction of prostaglandin synthase activity involved synthesis *de novo* of protein and RNA. The time of half-maximal induction of the newly synthesized RNA for prostaglandin synthase activity was estimated as about 15 h, which is similar to the generation time of the cells. Selective induction of fatty acid cyclo-oxygenase activity by sodium n-butyrate was suggested by the following two pieces of experimental evidence. (1) There was no significant difference between treated and untreated cells in the activities of radioactive prostaglandin H_2 conversion into individual prostaglandins. (2) The incorporation of [3H]acetylsalicylic acid into the fraction equivalent to protein of mol.wt. approx. 75 000 of sodium n-butyrate-treated cells was higher than that of untreated cells, on analysis of cell particulate fraction by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. A high concentration (5 mM) of sodium propionate also induced prostaglandin synthesis, but other short-chain fatty acids, such as isobutyrate and sodium acetate, had no effect.

n-Butyrate is a ubiquitous compound with a wide variety of morphological and physiological effects on mammalian cells grown in tissue culture (Plasad & Sinha, 1976) and inhibitory effects on DNA synthesis and cell division (Bombik & Burger, 1973; Schneider, 1976; Hagopian *et al.*, 1977). n-Butyrate has also been found to cause hyperacetylation of histones in cultured cells (Sealy & Chalkley, 1978), induction of new gene expression in Friend erythroleukaemic cells (Reeves & Cserjesi, 1979) and induction of cholera toxin receptors in several cell lines (Fishman & Atikkan, 1979).

Recently we reported that exposure of mastocytoma P-815 cells to sodium n-butyrate results in remarkable production of prostaglandins D_2 , E_2 and $F_{2\alpha}$, which are not produced by untreated cells (Koshihara *et al.*, 1980). We also observed that the effect of sodium n-butyrate is probably attributable to induction of cyclo-oxygenase, involved in the pathway of prostaglandin synthesis.

Here we report further studies on the effect of sodium n-butyrate on prostaglandin induction in a cloned mastocytoma P-815 cell line, 2-E-6, to

exclude the possibility of cell selection by sodium n-butyrate treatment.

Experimental

Materials

The source of the reagents used in these experiments were as follows; [$1-^{14}C$]arachidonic acid (sp. radioactivity 55.5 Ci/mol) and [$6-^3H$]thymidine (sp. radioactivity 21 Ci/mol), The Radiochemical Centre, Amersham, Bucks., U.K.; foetal bovine serum (mycoplasma- and virus-free), Grand Island Biochemical Co.; cycloheximide and sodium n-butyrate, Wako Chemical Co., Osaka, Japan; authentic prostaglandin standards, Ono Pharmaceutical Co., Osaka, Japan; thin-layer silica gel plates (60F $_{254}$; layer thickness 0.25 mm), Merck Co., Darmstadt, Germany; actinomycin D, Marker Chemicals, Jerusalem, Israel.

Cell culture

Mastocytoma P-815 cells, originally established by Dunn & Potter (1957) as a transformed mast-cell

line were kindly supplied by Dr. T. Odaka, Department of Genetics, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Cells were harvested from the ascites fluid of a female DBA/2 mouse bearing the tumour and cultured in suspension in Eagle's basal medium supplemented with 10% (v/v) foetal bovine serum under CO₂/air (1:19) at 37°C. Cloning of the cells was performed in a microtitration tray. Cloned cells were adapted to grow in suspension by using a gyrotory shaker (model-G2; New Brunswick Scientific Co.). In most experiments cloned mastocytoma P-815 2-E-6 cells were used. Their generation time was about 13–14 h. Portions of cultures in the exponential phase were treated with 1 mM-sodium n-butyrate for 40 h, or for an appropriate period. Cell numbers were counted in a Coulter Counter (model G; Coulter Electric Inc., Hialeah, FL, U.S.A.).

Assay of prostaglandin synthase in cell homogenates

Cells that had been incubated with or without sodium n-butyrate for an appropriate period were harvested by centrifugation at 200g for 5 min, washed once with phosphate-buffered saline (Ca²⁺- and Mg²⁺-free), pH 7.4, and stored as a pellet at –70°C until use.

Under the standard assay conditions, cells (1 × 10⁷) were suspended in 1 ml of 0.05 M-phosphate buffer, pH 8.2, and sonicated five times for 4 s periods in a Branson Sonifier (model W185; Ultrasonic, Plainview, NY, U.S.A.) at a setting of 3. The resulting cell homogenate was incubated with 0.05 µCi of [¹⁴C]arachidonic acid at 37°C for 30 min with shaking in an open tube. The reaction was terminated by adjusting the mixture to pH 3.0 with HCl. The prostaglandins synthesized were extracted with 6 vol. of ethyl acetate, condensed and subjected to t.l.c. with the upper phase of solvent C, consisting of ethyl acetate/2,2,4-trimethylpentane/acetic acid/water (11:5:2:10, by vol.) as described previously (Murota *et al.*, 1978; Koshihara *et al.*, 1980).

Labelled prostaglandins separated on the plates were scanned with a Berhold Dünnschicht Scanner II. The radioactive zones were each scraped off the plate for measurement of radioactivity in a liquid-scintillation spectrometer.

The activity of prostaglandin synthase of the cell is expressed as the total radioactivity of prostaglandin synthesized, i.e. c.p.m. of ¹⁴C-labelled prostaglandins per homogenate of 10⁷ cells.

Treatment with inhibitors of protein synthesis or RNA synthesis

Cells that had been treated with 1 mM-n-butyrate were incubated with 1.0 µg of cycloheximide or 0.5 µg of actinomycin D per ml of culture to inhibit protein synthesis or RNA synthesis completely.

Treatment with these concentrations for up to 15 h did not affect the cell viability. For measuring the initial enzyme activity of the cells, n-butyrate-treated cells were harvested just before addition of inhibitor. At 4 or 5 h intervals after treatment with inhibitors, cells (1 × 10⁷) were harvested and their prostaglandin synthase activity was determined as described elsewhere in the text.

DNA synthesis

Portions (2 ml) of cell suspension were withdrawn at intervals and incubated with 0.2 µCi of [³H]-thymidine at 37°C for 30 min. The following procedures were performed at 0°C unless otherwise mentioned. Incorporation of radioactivity was stopped by the addition of excess unlabelled thymidine (0.5 mM), and then the cells were washed once with 5 ml of phosphate-buffered saline containing excess unlabelled thymidine with centrifugation at 200g for 5 min and three times with 5% (w/v) trichloroacetic acid containing 1% (w/v) sodium pyrophosphate with centrifugation at 900g for 10 min. The radioactivity of the resulting acid-insoluble materials was counted in a liquid-scintillation counter.

Flow microfluorimetry

Sample cell suspensions were centrifuged at 200g for 5 min, and the cell pellets were resuspended in propidium iodide [2,7-diamino-10-(diethylamino-propyl)-9-phenylphenanthridinium iodide methiodide] solution (50 µg/ml in 0.1% sodium citrate) at a concentration of about 10⁶ cells/ml. The resulting suspensions were incubated at 37°C for 10 min. Results were not affected by keeping the cells on ice for several hours before microfluorimetric analysis of the DNA content per cell. The analysis was performed with a Fluorescence Activated Cell Sorter-III (Becton Dickinson Electronics Laboratories, Mountain View, CA, U.S.A.) with an argon ion laser at 488 nm (Herzenberg *et al.*, 1976; Horan & Wheelless, 1977).

Prostaglandin synthesis using [¹⁴C]prostaglandin H₂

Cells (1 × 10⁷) were treated with 1 mM-sodium n-butyrate for 40 h, harvested and homogenized as described above. The cell homogenates in 0.2 ml of 0.05 M-phosphate buffer, pH 8.2, were incubated with 74 000 c.p.m. of [1-¹⁴C]prostaglandin H₂ at 28°C for 3 min. The subsequent procedures were performed by the method of Ogino *et al.* (1977).

Incorporation of [³H]acetylsalicylic acid

[³H]Acetylsalicylic acid was synthesized by the method of Hemler & Lands (1976) from salicylic acid and [³H]acetic anhydride. The yield of [³H]-acetylsalicylic acid (sp. radioactivity 128 Ci/mol) measured as recovery of radioactivity was 5%. After

two separations by t.l.c., the purity of the [^3H]-acetylsalicylic acid synthesized was 98%.

Cells (1×10^7) with or without treatment with 1 mM-n-butyrate for 40 h were labelled with 33.5 μCi of [^3H]acetylsalicylic acid in serum-free minimum essential medium at 37°C for 2 h. Incorporation of [^3H]acetylsalicylic acid into 5% trichloroacetic acid-insoluble materials of whole cells increased for at least 2.5 h. Then cells were harvested and thoroughly washed with phosphate-buffered saline containing excess unlabelled acetylsalicylic acid. The cell pellet was suspended in 0.05 M-Tris-HCl, pH 7.4, containing 0.25 M-sucrose and 0.1 mM-phenylmethanesulphonyl fluoride (Sigma Chemical Co., St. Louis, MO, U.S.A.), and disrupted in a Branson Sonifier. The homogenate was centrifuged at 2000 rev./min for 10 min, and the supernatant fraction was re-centrifuged at 40000 rev./min for 60 min. The resulting pellet was dissolved in the above buffer containing 1% sodium dodecyl sulphate and subjected to electrophoresis on 5% polyacrylamide/0.1% sodium dodecyl sulphate gel by the method of Laemmli (1970). The radioactivity was measured by cutting the gel into 3 mm sections, solubilizing them with Soluene-350 (Packard Instrument Co., Downers Grove, IL, U.S.A.) and counting the radioactivity in a liquid-scintillation spectrometer.

Results

Effects of sodium n-butyrate and related short-chain fatty acids on induction of prostaglandin synthesis in cloned mastocytoma P-815 2-E-6 cells

The specificity of the effect of n-butyrate on induction of prostaglandin synthesis was examined by exposing cells in randomly growing cultures to various fatty acids at 1 mM or 5 mM for 40 h. This period of treatment was the most effective, as

described previously (Koshihara *et al.*, 1980). The results in Table 1 show that at a concentration of 1 mM, sodium n-butyrate was the most effective fatty acid, whereas at a concentration of 5 mM, sodium propionate induced more prostaglandins than sodium n-butyrate. These contradictory results seem to be due to the difference in toxicity of the reagents; treatment with 5 mM-sodium n-butyrate for 40 h killed the cells and thus induced less prostaglandin than a concentration of 1 mM, whereas sodium propionate had little effect on cell viability even at 5 mM, though like sodium n-butyrate it inhibited cell growth. Isobutyrate had no effect.

These results show that sodium n-butyrate was the most effective fatty acid for induction of prostaglandin in 2-E-6 cells.

Time-dependency and reversibility of the effect of sodium n-butyrate

The above experiment confirms our previous finding (Koshihara *et al.*, 1980) that 1 mM-sodium n-butyrate is the most effective concentration for prostaglandin induction. Therefore, 1 mM-sodium n-butyrate was used in all subsequent experiments. When cells (1.8×10^5 cells/ml of culture medium) were cultured with 1 mM-n-butyrate at 37°C, their prostaglandin synthase activity increased linearly for up to 40 h (Fig. 1). Longer treatment than 40 h slightly inhibited prostaglandin synthase activity (results not shown). Untreated cells were cultured in parallel. Untreated cells in either the growing or stationary phase had little prostaglandin synthase activity.

To determine whether the effect of n-butyrate was reversible, we discarded the medium containing n-butyrate after culture for 40 h, washed the cells by centrifugation and re-cultured them at low cell density (2×10^5 cells/ml of culture medium) in regular medium without n-butyrate for a further

Table 1. *Effects of various short-chain fatty acids on the induction of prostaglandin synthase activity*
Various short-chain fatty acid were added to 2-E-6 cell cultures (2.2×10^5 cells/ml) at a final concentration of 1 mM or 5 mM. After 40 h, the cells (1×10^7) were harvested and assayed for prostaglandin-synthesizing activity as described in the text. Untreated control cells did not show any prostaglandin-synthesizing activity (results not shown). Abbreviations used: PG, prostaglandin; n.g., conversion rate < 0.1%.

Short-chain fatty acid	Concentration (mM)	PG synthesized (c.p.m.)				PG synthesized (%)
		PG D ₂	PG E ₂	PG F _{2a}	Total	
Sodium acetate	1	n.g.	n.g.	n.g.	n.g.	n.g.
	5	264	n.g.	n.g.	264	3.4
Sodium propionate	1	97	114	154	365	4.8
	5	2165	3388	3599	9152	120.1
Isobutyrate	1	343	145	n.g.	488	6.4
	5	332	396	420	1148	15.1
Sodium n-butyrate	1	1690	2310	3619	7619	100.0

Fig. 1. Time dependency and reversibility of the effect of *n*-butyrate on induction of prostaglandin-synthesizing activity

2-E-6 cells were incubated with or without 1 mM-*n*-butyrate for up to 40 h. Then the *n*-butyrate was washed out and the cells were cultured in fresh medium free of *n*-butyrate. The cell number was counted at the indicated times. Prostaglandin synthase activity in samples of 10^7 cells was assayed as described in the text. R indicates the time of transfer to medium without *n*-butyrate. Prostaglandin synthase activity was measured in *n*-butyrate-treated cells (●) and untreated cells (○). Cell number was also measured in *n*-butyrate-treated cells (■) and untreated cells (□).

72 h. After a lag time of 20 h these *n*-butyrate-pretreated cells grew like untreated cells and their prostaglandin synthase activity gradually decreased to the value in control cells. Thus the effect of *n*-butyrate was reversible.

Inhibition of DNA synthesis is not primarily responsible for prostaglandin induction

The DNA contents of cells treated with 1 mM-*n*-butyrate for 40 h and of untreated control cells in randomly growing cultures were measured by microfluorimetry with a Fluorescence Activated Cell Sorter-III with an argon ion laser at 488 nm. The distribution of the untreated control cell population showed three phases with a major peak in G_1 , a plateau in S and a second peak in G_2 (Fig. 2a). In contrast cells grown with 1 mM-*n*-butyrate (Figs. 2b and 2c) showed an increased proportion of G_1 -phase cells after 24 h with markedly decreased numbers of S-phase cells and G_2 -phase cells. At 40 h, S-phase cells had almost completely disappeared. The decrease in the proportion of S-phase cells depended on the period of *n*-butyrate treatment. This result implies that *n*-butyrate inhibits DNA synthesis of mastocytoma. Actually DNA synthesis in cells treated with *n*-butyrate for 40 h was only 30% of that in control cells.

Fig. 2. Flow microfluorimetric determination of the DNA content of 2-E-6 cells

DNA was prepared from mastocytoma P-815 cells incubated with or without 1 mM-*n*-butyrate and analysed in a Cell Sorter as described in the text. (a) Randomly growing untreated control cells; (b) cells treated with 1 mM-*n*-butyrate for 24 h; (c) cells treated with 1 mM-*n*-butyrate for 40.5 h.

Since *n*-butyrate inhibited DNA synthesis and cell growth, we next examined whether inhibition of cell growth was associated with induction of prostaglandin synthesis. Randomly growing cells (2×10^5 cells/ml of culture medium) were treated with 0.1 or 0.2 mM-hydroxyurea, and prostaglandin synthase activity was measured in samples of 10^7 cells at intervals. At the same times DNA-synthesis activity was determined by measuring incorporation of [^3H]thymidine for 30 min into the acid-insoluble fraction of portions of cell culture. Hydroxyurea

Fig. 3. Effect of inhibition of DNA synthesis by hydroxyurea on prostaglandin-synthesizing activity

Hydroxyurea (0.1 mM and 0.2 mM) was added to an untreated control cell culture (2×10^5 /ml) at zero time. As a control, n-butyrate was added to a different culture at a final concentration of 1 mM. At the indicated times, cells (1×10^7) were assayed for prostaglandin synthase activity as described in the text (a). Simultaneously, portions of cell cultures were assayed for DNA synthesis by measuring [³H]thymidine incorporation into the acid-insoluble fraction (b). Symbols: ●, n-butyrate treatment; △, hydroxyurea (0.1 mM) treatment; □, hydroxyurea (0.2 mM) treatment; ○, untreated control cells (which did not have significant prostaglandin-synthesizing activity).

(0.2 mM) arrested cell growth completely, and inhibited DNA-synthesis activity by 83% throughout the experimental period (Fig. 3b). However, the inhibition of DNA synthesis activity was not associated with significant induction of prostaglandin synthase activity (Fig. 3a). The decline in DNA synthesis in Fig. 3(b) is due to cell-density-induced inhibition of growth after 23 h.

This result suggests that inhibition of DNA synthesis is not primarily responsible for the induction of prostaglandin synthesis by n-butyrate.

Induction of prostaglandin synthase activity by n-butyrate is coupled with new syntheses of RNA and protein

We used inhibitors of RNA and protein syntheses to determine whether induction of prosta-

Fig. 4. Effects of actinomycin D and cycloheximide on the effect of n-butyrate

2-E-6 cells cultured at a cell density of 2×10^5 cells/ml were treated with 1 mM-n-butyrate for up to 48 h. Arrows indicate the time of addition of actinomycin D ($0.5 \mu\text{g/ml}$) or cycloheximide ($1.0 \mu\text{g/ml}$). Cells (1×10^7) were assayed for prostaglandin synthase activity as described in the text. ●, n-butyrate treatment; △, actinomycin D treatment during n-butyrate treatment; ■, cycloheximide treatment during n-butyrate treatment.

glandin synthase activity in n-butyrate-treated cells involved synthesis of enzyme *de novo*. The inhibitors were added to cultures of cells in medium with n-butyrate at different times and for various periods.

Concentrations of $1 \mu\text{g}$ of cycloheximide/ml and $0.5 \mu\text{g}$ of actinomycin D/ml were used, since these concentrations almost completely inhibited protein and RNA syntheses (97%) without affecting cell viability for at least 15 h. Three parallel cultures without inhibitors were set up and after 15, 19, 20, 25 and 30 h, three portions were withdrawn, treated respectively with actinomycin D, cycloheximide or vehicle only, and incubated further, and their cell number and prostaglandin synthase activity were measured at intervals. The results are shown in Fig. 4.

After addition of cycloheximide at all times tested the enzyme activity remained at the original value, i.e. inhibition of newly synthesized protein did not cause further induction of the enzyme activity. On the other hand although after addition of actinomycin D for up to 24 h the enzyme activity remained at the original value, its addition after 30 h was not inhibitory. This suggests that the synthesis of new RNA that is required for the induction of prostaglandin synthase was completed within 30 h. Thus the time of half-maximum induction of new RNA, presumably mRNA, was estimated as less than 15 h. Fig. 4 also shows that both mRNA and the newly induced enzymes for prostaglandin synthesis were very stable, because in some experiments, the original activity was maintained for at least 15 h after addition of actinomycin D.

Conversion of prostaglandin H_2 in cell homogenates

To elucidate which enzyme(s) concerned with prostaglandin synthesis was induced by n-butyrate treatment, [^{14}C]prostaglandin H_2 was used as a precursor instead of [^{14}C]arachidonic acid. As shown in Table 2, homogenates derived from both treated and untreated cells converted [^{14}C]prostaglandin H_2 into prostaglandins D_2 , E_2 and $F_{2\alpha}$ respectively to similar extents. This result suggested that n-butyrate-untreated cells cannot produce any prostaglandin, owing to lack of fatty acid cyclo-oxygenase, in spite of originally having prostaglandin endoperoxide prostaglandin D_2 isomerase, prostaglandin E_2 isomerase and prostaglandin $F_{2\alpha}$ reductase in the cells.

Incorporation of [^3H]acetylsalicylic acid into 75 000-mol.wt. protein

To investigate selective induction of fatty acid cyclo-oxygenase by n-butyrate treatment, [^3H]acetylsalicylic acid incorporation was investigated.

Fatty acid cyclo-oxygenase, which is located in the microsomal fraction, is specifically acetylated by acetylsalicylic acid at low concentration irreversibly (Smith & Lands, 1971; Hamberg & Samuelsson, 1974). Cells with or without treatment with 1 mM-n-butyrate for 40h were labelled with [^3H]-

acetylsalicylic acid for 2h and the particulate fraction was isolated by centrifugation at 40 000 rev./min for 60 min as described elsewhere in the text. The solubilized cell particulate fraction was subjected to electrophoresis on 5% polyacrylamide/0.1% sodium dodecyl sulphate gel to isolate the specific fraction. The radioactivity of the 75 000-mol.wt. peak, the reported molecular weight of the cyclo-oxygenase (Hemler & Lands, 1976; Roth *et al.*, 1980), was compared for treated and untreated cells. The radioactivity in treated cells was twice that in untreated cells, and it amounted to approx. 1–2% of that in labelled particulate fractions (Table 3). This result suggests that n-butyrate induced the cyclo-oxygenase, as concluded from other results described above.

Discussion

In the present work we found that enhancement of prostaglandin synthase activity by n-butyrate treatment involved new synthesis of RNA, probably mRNA. n-Butyrate caused hyperacetylation of histones H3 and H4 in mastocytoma cells (results not shown), as reported to occur in HeLa cells (Sealy & Chalkley, 1978; Cousens *et al.*, 1979). Some correlation between increased histone acetylation and gene activation for RNA synthesis has been

Table 2. Prostaglandin synthesis with [^{14}C]prostaglandin H_2 as a substrate

Randomly growing 2-E-6 cells were treated with or without 1 mM-n-butyrate for 40h. Cells (1×10^7) were assayed for prostaglandin synthesis with [^{14}C]prostaglandin H_2 as a substrate as described in the text. The radioactivities of prostaglandin synthesized were expressed as percentage of total radioactivity on the t.l.c. plate.

Prostaglandin	Prostaglandin synthesized (%)			Ratio of synthesis for treated/untreated cells
	[^{14}C]Prostaglandin H_2 without cells	Untreated cells	n-Butyrate-treated cells	
H_2	90.57	23.09	29.70	1.29
D_2	2.78	16.57	13.40	0.81
E_2	2.66	19.69	15.91	0.81
$F_{2\alpha}$	0.65	24.25	23.45	0.97

Table 3. [^3H]Acetylsalicylic acid incorporation into 75 000-mol.wt. protein

Cells (1×10^7) treated with or without 1 mM-n-butyrate for 40h were incubated with 33.5 μCi of [^3H]acetylsalicylic acid at 37°C for 2h. The cells were fractionated as described in the text.

Fraction	Radioactivity (c.p.m.)		Ratio of radioactivity for treated/untreated cells
	Untreated cells	n-Butyrate-treated cells	
Total uptake (cell homogenate)	239 450	341 350	1.43
Particulate fraction (40 000 rev./min precipitate)	52 200	59 863	1.15
75 000-mol. wt.-protein fraction	847	1721	2.03

reported (Allfrey *et al.*, 1964; Allfrey, 1970). Therefore hyperacetylation of histones H3 and H4 in mastocytoma cells may result in new RNA synthesis. Actually, RNA synthesis in 2-E-6 cells was increased to about three times the control value after treatment for 40h with n-butyrate (results not shown). This RNA synthesis must include synthesis of mRNA for prostaglandin synthase.

Microfluorimetric analysis of the DNA content showed that 1 mM-n-butyrate arrested cells in the G₁ phase and inhibited cell growth (Fig. 2). However, growth inhibition only caused slight induction or enhancement of prostaglandin-synthesizing activity in mastocytoma cells, as obtained in experiments with hydroxyurea (Fig. 3), dimethyl sulphoxide, cyclic AMP, cyclic GMP, dibutyryl cyclic AMP, dibutyryl cyclic GMP and monobutyryl cyclic AMP (results not shown). Moreover, untreated cells did not have any significant prostaglandin synthase activity either in the randomly growing phase or in the stationary phase (Fig. 1). These observations suggest that growth inhibition is not primarily responsible for the induction of prostaglandin synthase activity.

Of the other short-chain fatty acids tested, only sodium propionate, especially at high concentration, caused induction of prostaglandin synthase activity (Table 1). These results are different from those on hyperacetylation reported by Sealy & Chalkley (1978). They found that sodium propionate, isobutyrate and acetate at the same concentration as sodium n-butyrate also caused hyperacetylation of histones H3 and H4, sodium propionate having the most effect. On analysis of the prostaglandin content of the culture medium by radioimmunoassay, prostaglandins E₂ and F_{2α} were detected only in sodium n-butyrate-treated cultures (results not shown). Thus n-butyrate-treated cells actually produce significant amounts of prostaglandins E₂ and F_{2α}. The prostaglandin D₂ content of the medium could not be measured because antibody against prostaglandin D₂ was not available.

The experiments with [¹⁴C]prostaglandin H₂ (Table 2) and [³H]acetylsalicylic acid (Table 3) strongly suggest selective induction of fatty acid cyclo-oxygenase by n-butyrate treatment. However, further evidence that confirms an increase in cyclo-oxygenase itself by immunoreaction with the antibody of cyclo-oxygenase is required.

The present paper shows that n-butyrate is a useful tool for analysis of prostaglandin synthesis at a molecular level. By using suitable inhibitors of protein and RNA syntheses the life span of mRNA for prostaglandin synthases and the mechanisms of processing the enzymes can be studied. Fig. 4 shows preliminary results on these problems, but further studies are required.

References

- Allfrey, V. G. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1447–1460
- Allfrey, V. G., Faulkner, R. & Mirsky, A. E. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **51**, 786–794
- Bombik, B. M. & Burger, M. (1973) *Exp. Cell Res.* **80**, 88–94
- Cousens, L. S., Gallwitz, D. & Albert, B. M. (1979) *J. Biol. Chem.* **254**, 1716–1723
- Dunn, T. B. & Potter, M. (1957) *J. Natl. Cancer Inst.* **18**, 587–601
- Fishman, P. H. & Atikkan, E. E. (1979) *J. Biol. Chem.* **254**, 4342–4344
- Hagopian, H. K., Riggs, M. G., Swartz, L. A. & Ingram, V. M. (1977) *Cell* **12**, 855–860
- Hamberg, M. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3400–3404
- Hemler, M. & Lands, W. E. (1976) *J. Biol. Chem.* **251**, 5575–5579
- Herzenberg, L. A., Sweet, R. G. & Herzenberg, L. A. (1976) *Sci. Am.* **234**, 108–117
- Horan, P. K. & Wheelless, L. L., Jr. (1977) *Science* **198**, 149–157
- Koshihara, Y., Senshu, T., Kawamura, M. & Murota, S. (1980) *Biochim. Biophys. Acta* **617**, 536–539
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Murota, S., Kawamura, M. & Morita, I. (1978) *Biochim. Biophys. Acta* **528**, 507–511
- Ogino, N., Miyamoto, T., Yamamoto, S. & Hayaishi, O. (1977) *J. Biol. Chem.* **252**, 890–895
- Plasad, K. N. & Sinha, P. K. (1976) *In Vitro* **12**, 125–132
- Reeves, R. & Cserjesi, P. (1979) *J. Biol. Chem.* **254**, 4283–4290
- Roth, G. J., Siok, C. J. & Ozols, J. (1980) *J. Biol. Chem.* **255**, 1301–1304
- Schneider, F. H. (1976) *Biochem. Pharmacol.* **25**, 2309–2317
- Sealy, L. & Chalkley, R. (1978) *Cell* **14**, 115–121
- Smith, W. L. & Lands, W. E. M. (1971) *J. Biol. Chem.* **246**, 6700–6704