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Independent Effects of Human Platelet Growth Factor and Hydrocortisone on Acetate Incorporation by GM-237 Human Fibroblasts (40191)

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Human platelet growth factor (HPGF) is a basic polypeptide (mw 13,000) isolated from human platelets (1, 2). Since its isolation, studies have been carried out focusing upon its mitogenic effect on cultured cells *in vitro*. These include Mouse 3T3 (1) and human diploid fibroblasts (3). The effect(s) of HPGF on lipid metabolism has not been investigated to date. In view of its strong mitogenic activity, it seems likely that lipid metabolism and in particular incorporation of lipid precursors such as acetate could be affected. This response may arise as a consequence of increased cell membrane biosynthesis during cell division where lipid serves as an integral component of its structure (4). As shown in this study, GM-237 human fibroblasts will double in number in the presence of HPGF plus a low serum level (0.5%) that normally by itself does not support cell multiplication (5). This system was chosen over the use of high serum levels in studying growth-related effects on acetate metabolism to avoid the appreciable lipid content of whole serum that has been demonstrated to inhibit lipid synthesis from acetate (6–8). Delipidized serum has often been used to circumvent the problem, but GM-237 human fibroblasts do not multiply in delipidized serum.

In the present study, the potentiation of acetate incorporation into lipids of human diploid skin fibroblasts (GM-237) by HPGF is shown. To determine whether the potentiating effect of HPGF on acetate incorporation could itself be modified by other metabolic modulators, the action of hydrocortisone (HC) was also studied. It was found that although HC decreases acetate incorporation by control cells, it has no effect on the enhancement associated with HPGF.

Materials and methods. Human diploid skin fibroblasts GM-237 (81-year female) were obtained from the General Mutant Cell Repository (Camden, NJ). Stock cells were grown as monolayer cultures in modified Eagle's MEM plus 10% fetal calf serum (FCS) (9). Only cells between the 20 and 25th generations in culture were used in the experiments.

Replicate cultures were plated at approximately 0.2×10^6 cells per 25 mm^2 flask with MEM plus 5% FCS. After 24 hr, the medium was changed into a FCS concentration of 0.5% and reincubated for 24 hours. Two microcuries of [1-^{14}C]acetate (55.7 Ci/mole; New England Nuclear, Boston, MA) in 4 ml of MEM supplemented with 0.5% FCS was then given to each cell culture in the presence or absence of human platelet growth factor and/or hydrocortisone. The flasks were equipped with CO_2 traps (10) and after 72 hr of incubation, the evolved $^{14}CO_2$ was absorbed onto filter paper moistened with 10% (wt/vol) potassium hydroxide. The radioactivity was counted in Scintiverse (Fisher Scientific, Medford, MA). Total cellular lipid was extracted quantitatively with a mixture of ethyl-acetate/acetone after trypsinization. Details of the extraction, thin-layer chromatography, and liquid scintillation counting have been described elsewhere (11).

Purified human platelet growth factor was generously provided by Dr. H. N. Antoniades of the Blood Research Institute, Boston, MA. The isolation and purification of HPGF has been described (2). The preparation of HPGF used in this study has been subjected to ion-exchange chromatography, gel filtration and isoelectric focusing, respectively, prior to additions to cell cultures. Hydrocortisone-21-sodium succinate was acquired from Sigma Chemical (St. Louis, MO).

Results. When GM-237 human skin fibroblasts were incubated for 72 hr in the presence of 0.5% FCS, no increase in cell number occurred either with control cells or those treated with HC at a concentration range of 1–200 μg/ml (Table I). However, HPGF-treated cells in the presence or absence of HC (1–200 μg/ml) doubled in number over the

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TABLE I. COMPARISON OF FINAL CELL NUMBERS REACHED AFTER 72-HR INCUBATIONS.[a]

Condition	Cells per flask ($\times 10^{-6}$)	Condition	Cells per flask ($\times 10^{-6}$)
Control	0.18	HPGF (18 ng/ml)	0.35
HC (1 μg/ml)	0.19	HPGF + HC (1 μg/ml)	0.35
HC (50 μg/ml)	0.17	HPGF + HC (50 μg/ml)	0.37
HC (100 μg/ml)	0.18	HPGF + HC (100 μg/ml)	0.34
HC (200 μg/ml)	0.19	HPGF + HC (200 μg/ml)	0.36

[a] Cells were plated at 0.2×10^{-6} per flask and treated according to conditions described in Materials and Methods. Stock solutions to hydrocortisone (HC) were prepared as 8 mg/ml^{-1} and human platelet growth factor (HPGF) as 720 ng/ml^{-1}. All values are means of determinations on three replicate flasks.

same period.

Figure 1 shows the total amount of [1-^{14}C]acetate incorporated into cells in the presence and absence of HPGF and/or HC. Relative to the control, HPGF at 18 ng/ml stimulated incorporation 2.4 times on a per cell basis. HC at 200 μg/ml caused a marked decrease to 24% of the control value in total intracellular incorporation of [1-^{14}C]acetate. When HPGF and HC were added simultaneously, the radioactivity which appeared in the cells was still elevated 1.6 times over the control values, and 6.9 times over that of HC-treated cells.

HPGF and HC could act by controlling the absolute uptake of acetate into the cells and/or by affecting directly lipid metabolism independent of substrate influx. These possibilities were examined by determining the distribution of radiolabel among all metabolic fractions of the cells, namely CO_2, cell pellet (including water soluble elements), and cell and medium lipid. Table II shows that HPGF caused the cells to divert a large proportion of intracellular [1-^{14}C]acetate into lipid (77.8% versus 46.9%). Conversely, HC decreased substantially the proportion of ^{14}C incorporated into cellular lipid (20.9% versus 46.9%). Addition of HPGF plus HC resulted in a cellular radioactivity profile very similar to that obtained with the treatment of HPGF alone. Thus HPGF and HC caused major metabolic alterations in intracellular ^{14}C distribution from acetate.

The distribution of radioactivity from [1-^{14}C]acetate within the lipid subclasses was also examined (Table III). Control fibroblasts in the resting state incorporated 65–70% of the radioactivity to phospholipid and 25–30% to cholesterol. The presence of HC caused little change in this distribution pattern. However, addition of HPGF caused a significant

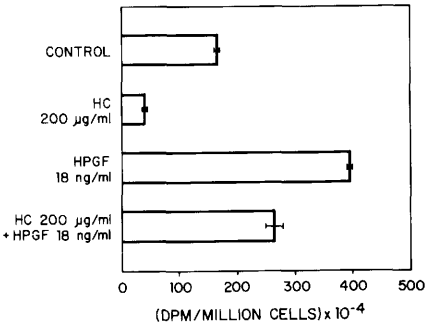

FIG. 1. Total incorporation of [1-^{14}C]acetate into human fibroblasts incubated for 72 hr in MEM with 0.5% FCS ± HPGF and/or HC. The bar represents the mean from three determinations ± SE (horizontal line).

increase of label into phospholipid while that of cholesterol alone decreased. This resulted in an increase in the phospholipid/cholesterol ratio (from 2.1 to 4.1; $P < 0.01$). Among the HPGF- and HC-treated cells, the radiolabel distribution pattern was similar to that in cells treated with HPGF alone.

Figure 2 shows the results obtained when the cell cultures were treated with various concentrations of HC (1–200 μg/ml) in the absence or presence of a fixed amount of HPGF (18 ng/ml). As before, the two agents were added simultaneously. In the absence of HPGF, as little as 1 μg/ml of HC caused a 58% reduction in acetate incorporation into lipid. Increasing the amount of HC caused further inhibition reaching 89% at a concentration of 200 μg/ml. With HPGF-treated cells such increases in the concentration of HC also produced a stepwise decrease in acetate incorporation into cell lipid. At an HC concentration of 1 μg/ml, a 13% suppression was seen, while 200 μg/ml caused a 32% suppression. It should be noted that the lowest concentration of HC (1 μg/ml) caused a much greater relative inhibition of acetate

TABLE II. AMOUNT AND PERCENT DISTRIBUTION OF INCORPORATED RADIOACTIVITY FROM ACETATE.[a]

	Radioactivity incorporated							
	Control		HPGF (18 ng/ml)		HC (200 μg/ml)		HPGF + HC	
Fractions	dpm	(%)	dpm	(%)	dpm	(%)	dpm	(%)
CO_2	34.5	20.7	19.5	4.9	13.4	33.7	13.3	4.8
Cell pellet[b]	43.5	26.2	41.9	10.6	15.0	37.8	37.8	13.7
Cell lipid	78.0	46.9	308.3	77.8	8.3	20.9	211.2	76.6
Medium lipid	10.3	6.2	26.7	6.7	3.0	7.6	13.5	4.9
Σ	166.3		396.4		39.7		275.8	

[a] The values are dpm × 10^{-4} per million cells and are the mean of $N = 3$.
[b] Includes water soluble constituents.

TABLE III. TOTAL AMOUNT AND DISTRIBUTION OF CELL LIPID RADIOACTIVITY FROM [1-^{14}C]ACETATE.[a]

	Control		HPGF		HC		HPGF + HC	
	dpm	(%)	dpm	(%)	dpm	(%)	dpm	(%)
Phospholipid	50.3	64.5	238.0	77.2	5.5	66.6	171.1	81.0
Cholesterol[b]	23.5	30.1	55.8	18.1	2.1	24.8	32.7	15.5
Free fatty acid	1.6	2.0	3.7	1.2	0.4	4.4	2.7	1.3
Triacylglycerol	2.5	3.2	10.5	3.4	0.3	3.4	4.2	2.0
Cholesteryl ester	0.2	0.2	0.3	0.1	0.1	0.8	0.4	0.2
Σ	78.1		308.3		8.4		211.1	
Phospholipid/cholesterol	2.1		4.1		2.7		5.2	

[a] Conditions as in Table II.
[b] Including trace amounts of 1,2-diacylglycerol.

incorporation than additional increments in concentration. Most data were based on a concentration of 200 μg/ml, however, because nearly complete inhibition was obtained. It was felt that if HC could influence the enhanced acetate incorporation associated with HPGF treatment it would be most obvious at this high level.

Discussion. It was previously demonstrated that HPGF markedly stimulated proliferation of two lines of fetal and two lines of adult human diploid fibroblasts grown in medium supplemented with 5% FCS (3). Of these GM-237 is a particularly good strain for studying the metabolic effects of HPGF since its growth response to HPGF was very sensitive to a wide range of concentrations. Upon lowering the serum concentration to a level which does not normally support cell multiplication, HPGF was still capable of stimulating GM-237 fibroblasts to double during the 3-day interval of this study. When the same strain of cells was cultured in medium supplemented with 5% FCS, cells underwent more than two doublings (3). Thus, the full mitogenic potential of HPGF was dependent upon the proper serum concentration in which the cells were maintained. Similar observations have been reported in the case of fibroblast growth factor (12) which has a similar molecular weight and isoelectric point as HPGF.

The effects of growth factors on macromolecular synthesis has been extensively studied (13) but information concerning their effects on lipid metabolism is relatively scarce. In the present study, the exogenous supply of lipid was limited due to the low serum concentration employed. If there was a demand for additional cellular lipid, it would have to be acquired via endogenous synthesis. The results obtained showed that HPGF greatly stimulated the incorporation of acetate and its conversion to lipids by strain GM-237 human fibroblasts. It is possible that this increased acetate incorporation arose from the need for additional lipid when new membranes were being formed as cells doubled (Table I). The importance of lipid synthesis in cell proliferation can be further realized by the results obtained in a recent study with WI-38 fibroblasts (14). When cells

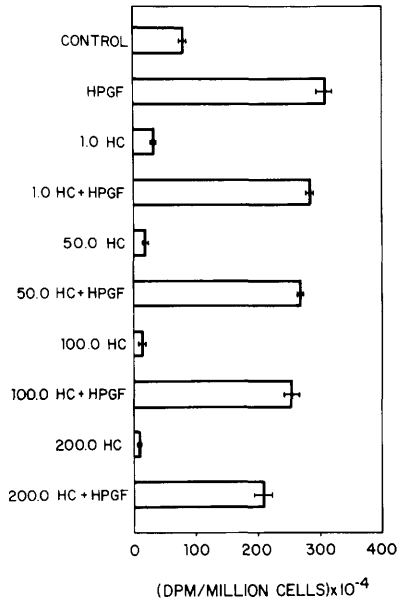

FIG. 2. Comparison of the stimulatory and inhibitory effects of HPGF and HC, respectively, on the transfer of [1-^{14}C]acetate into the lipid fraction of human fibroblasts. Cells were harvested and cell lipid extracted after a 72-hr incubation. The HPGF concentration was maintained at 18 ng/ml while the HC concentration was varied from 1 to 200 μg/ml. Each value represents the mean of $N = 3$.

were cultured in delipidized serum and synthesis of fatty acids, phospholipids and cholesterol were inhibited by various experimental means, division of these fibroblasts ceased and cells were arrested in the G1 segment of the cell cycle. Upon restoring lipid synthesis, cell proliferation resumed. The observation that there was a preferential diversion of acetate label into phospholipid when HPGF was present (Table III) also reflected the active synthesis of membranes since phospholipids are major components of cell membranes (15).

To investigate whether the HPGF-induced increase in acetate incorporation could be modulated by compounds such as hormones known to affect the metabolism of skin fibroblasts, HC was chosen. HC inhibits glucose uptake by cultured cells (16, 17) and it seemed possible that acetate metabolism could also be affected. Indeed, data in Table II demonstrated that total acetate incorporation was decreased substantially in the presence of HC. Although this could be due to inhibition of acetate entry into cells, the differential suppression of acetate into cellular lipid fraction suggests that additional mechanisms other than transport are involved. Growth inhibition could explain the observed effects if HC decreased growth of these fibroblasts. However, it has been observed that cortisol at pharmacological concentrations up to 3.1×10^{-4} *M* did not inhibit the growth of human diploid fibroblasts (18). Furthermore, Grove *et al.* (19) have reported that HC at a level of 14.0×10^{-6} *M* did not affect proliferation of GM-237 cells. This agrees with data in Table I showing HC at 2.1×10^{-6} *M* to 4.2×10^{-4} *M* did not affect cellular proliferation of these fibroblasts. Hong and Levine have demonstrated that HC inhibited prostaglandin synthesis in 3T3 cells by blocking the release of arachidonic acid from phospholipid (20). If this phenomenon applies to other fatty acyls in cellular lipid, then it is possible that HC inhibition of acetate incorporation into lipids is related to the increased biologic stability of these acyls. On the other hand, the decrease in acetate incorporation into cellular lipids due to HC could be ascribed to an increase in acetate pool size because of catabolism of lipids or other C_2 donors.

It has been generally accepted that the mechanism of action of glucocorticoids is mediated through the formation of a cytoplasmic complex by high affinity binding of the hormone to the receptor protein which is subsequently transferred into the nucleus to stimulate genetic transcription (21). Such a receptor system implies that saturation of receptor-sites exists well before a concentration of HC as high as 200 μg/ml is reached. In the present study, this concentration almost completely inhibited acetate incorporation into lipid. This raises questions concerning the mechanism responsible for the response which appeared to deviate from the classical steroid-receptor model. In studying cortisol metabolism in a strain of fibroblasts which was very resistant to the growth inhibitory action of cortisol, it was shown that cortisol was metabolized at least twice as fast as the cortisol susceptible strain (22). It is possible that the metabolism of HC by GM-237 human cells occurred at a much faster rate than other strains thus rendering some of the HC inactive. It is of interest to note

that glucocorticoids stimulated deposition of glycogen (23) and induced tyrosine aminotransferase (24, 25) in liver in the presence of inhibitors of RNA synthesis. Thus, it may also be possible that the effect of HC on acetate incorporation in GM-237 fibroblasts occurred without an obligatory dependence on nuclear processes and high affinity binding. These possibilities remain to be tested.

When human fibroblasts were treated with both HPGF and HC, cells divided to the same extent as those treated with HPGF alone (Table I) but differences in acetate incorporation were observed (Table II). Net total incorporation on a per cell basis was much greater than that obtained with control or HC-treated cells but lower than that found with HPGF supplementation. Since 78–80% of the label incorporated resided in cellular lipid, similar findings were observed in the cellular lipid fraction (Fig. 2). It should be noted that the percentage distribution of label among various fractions (Table II) of HPGF- and HC-treated cells resembled that of HPGF-treated cells instead of HC-treated cells. All of these observations indicate that the action of HPGF in terms of acetate incorporation could not be overridden by the presence of pharmacological doses of HC. Reexamination of the data in Table II reveals that HPGF and HC apparently acted independently of each other. This can be seen from a comparison of the percent differences between the total [1-^{14}C]acetate incorporation into the different experimental groups (Table IV). On a per cell basis, total cellular acetate incorporation was reduced 76.1% in the presence of 200 μg/ml HC. Cells in the presence of 18 ng/ml HPGF, on the other hand, increased total incorporation by 138.4%. If HPGF and HC acted independently of each other, their combined actions should be a simple algebraic sum of their individual effects. In other words, cells exposed to HPGF and HC should show a 62.3% increase in total acetate incorporation. Indeed, the observed value of 65.8% was not significantly different from this ($P < 0.05$). With the addition of 18 ng/ml HPGF and as little as 1 μg/ml of HC similar results were obtained. The apparent independent actions of HPGF and HC in these fibroblasts in terms of acetate incorporation imply that any changes in the size of the acetate pool(s) brought about by the presence of HPGF or HC alone were operating in a similar manner when HPGF and HC were present simultaneously.

TABLE IV. PERCENT DIFFERENCE IN TOTAL [1-^{14}C]-ACETATE INCORPORATION AMONG EXPERIMENTAL GROUPS.[a]

Group	Treatment	% Difference in radioactivity[b] incorporated normalized to control
A	HPGF	$\frac{396.4-166.3}{166.3} = +138.4$
B	HC	$\frac{39.7-166.3}{166.3} = -76.1$
C	HPGF + HC	$\frac{275.8-166.3}{166.3} = +65.8\ (+62.3)^*$

[a] Data derived from the Σ row of Table II.
[b] dpm $\times 10^{-4}/10^6$ cells.
* Expected value from A + B.

On a per culture basis, the absolute HC-induced suppression of total cellular acetate incorporation was greater among HPGF-treated cells than control cells. This was because HPGF-treated cells doubled in number, and, consequently, the total capacity to interact with HC increased accordingly. Hence, daughter cells arising from HPGF stimulation appeared to be normal with respect to their susceptibility to HC inhibition.

Obviously much remains to be done to elucidate the mechanism of these various effects. It is clear, however, that HPGF has a profound influence on the incorporation of acetate into lipid in GM-237 human fibroblasts, and that this occurs in a manner not subject to HC modulation.

Summary. In studying the metabolic effects of *human* platelet growth factor (HPGF), cellular [1-^{14}C]acetate incorporation was investigated among GM-237 *human* diploid fibroblasts. When cultured in 0.5% fetal calf serum, cells doubled in the presence of 18 ng/ml HPGF during 72 hours, and a 138.4% increase on a per cell basis in total [1-^{14}C]-acetate incorporation was found. Seventy-eight percent of the label was in cell lipid. Pharmacological doses of hydrocortisone (HC) at 1–200 μg/ml had no effect on cell division in the presence or absence of HPGF. However, a 40–76% reduction in total [1-^{14}C]-acetate incorporation was found when HC was present. When HPGF and HC were

added to cells simultaneously, analysis of the data revealed that HPGF and HC acted independently. Thus, HPGF can cause GM-237 human diploid fibroblasts to increase exogenous acetate incorporation and its conversion into lipids in a manner circumventing modulation by HC.

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