BBA 65580

GENETIC CONTROL OF LACTATE DEHYDROGENASE AND MALATE DEHYDROGENASE ISOZYMES IN CULTURES OF LYMPHOCYTES AND GRANULOCYTES: EFFECT OF ADDITION OF PHYTOHEMAGGLUTININ, ACTINOMYCIN D OR PUROMYCIN

YALE RABINOWITZ AND ALBERT DIETZ

Research Service, Veterans Administration Hospital, Hines, Ill. and the Department of Medicine, Loyola Stritch School of Medicine, Hines, Ill. (U.S.A.)

(Received October 19th, 1966)

SUMMARY

1. The effect of the addition of phytohemagglutinin to cultures of purified lymphocytes and granulocytes upon the isozymes of lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) and malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37) was studied.

2. Increase in the proportion of muscle-type (M-type) lactate dehydrogenase was demonstrable with acrylamide-gel disc electrophoresis in 4 h, while a significant increase ($P < 0.001$) occurred within 24 h. Actinomycin D or puromycin prevented this change. Granulocytes, which had a high proportion of M-type lactate dehydrogenase initially, showed no alteration in the isozyme pattern in cultures with or without phytohemagglutinin.

3. Two malate dehydrogenase isozyme bands were found in lymphocytes and granulocytes. The slow-moving Band 2 represented 44.8% of the total malate dehydrogenase in lymphocytes and only 13.4% in granulocytes. In cultures, with or without phytohemagglutinin, little change was seen in lymphocytes. Granulocytes, however, showed a marked increase in Band 2 ($P < 0.001$) in 24 h, but this occurred with or without the addition of phytohemagglutinin. Actinomycin D or puromycin blocked this change.

4. The results indicate that in lymphocytes phytohemagglutinin acts at the two genetic loci controlling synthesis of muscle- and heart-type (M- and H-)lactate dehydrogenase polypeptide subunits. The synthesis of the two isozymes of malate dehydrogenase also may be under the control of separate genes which in granulocytes are influenced by conditions of cell culture, rather than by phytohemagglutinin.

Abbreviations: LDH, lactate dehydrogenase; MDH, malate dehydrogenase; H, heart-type LDH polypeptide subunit; M, muscle-type LDH polypeptide subunit.

INTRODUCTION

NOWELL[1] showed that phytohemagglutinin initiated mitosis and the formation of blast-like cells in normal human leukocytes cultured 48–72 h by the method of HUNGERFORD *et al.*[2]. Lymphocytes appear to be the source of the altered cells[3,4]. Morphological changes in lymphocytes cultured with phytohemagglutinin are barely discernable until the end of the first day of culture when some increase in cell size and prominence of nucleoli may be seen. In the next 24–48 h progressive stages in the formation of blast-like cells are evident[5,6].

Increased RNA synthesis may be detected during the first hours in lymphocytes cultured with phytohemagglutinin. An increase in protein synthesis follows, but rise in DNA synthesis is delayed until the end of the first day of culture[7–9]. Since HIRSCHHORN *et al.*[10] noted that actinomycin D can block the stimulation of γ-globulin synthesis by phytohemagglutinin, MUELLER AND LEMAHIEU[9] suggested that phytohemagglutinin may act primarily to counteract normal repressor mechanisms regulating RNA synthesis.

Our objective in the present study was to investigate a specific protein whose synthesis was either stimulated or inhibited by phytohemagglutinin action. Since phytohemagglutinin causes a marked increase in glucose consumption and lactic acid production in lymphocyte cultures[11,12], two enzymes which might be involved in this process, namely lactate dehydrogenase (LDH) and malate dehydrogenase (MDH), were chosen for study. Since both LDH and MDH exist in multiple molecular forms and since, in the case of LDH at least, the proportions of the isozymes were believed to be under genetic control[13], these enzymes seemed to be particularly suitable for study of possible phytohemagglutinin action at the gene level.

Results of the present study are compatible with the theory that phytohemagglutinin action in the case of LDH may be the result of binding of allosteric[14] sites on protein repressors or inducers of operator genes. In the case of MDH, alterations in isozyme patterns in granulocytes occurred in cultures with or without phytohemagglutinin, apparently the result of unknown factors. The changes in MDH isozyme pattern in cultured granulocytes also appeared to be the result of action at the gene level.

MATERIALS AND METHODS

Leukocyte collection, separation and storage

Normal blood was obtained from students and laboratory workers. Heparin without preservative (Abbott 6945), 4–5 units/ml of blood, was used as anticoagulant. Erythrocytes were sedimented with 6% dextran (Pharmachem, Bethlehem, Pa.). The procedure for separation of cell types from leukocyte-rich supernatant plasma on glass bead columns was previously described in detail[3]. Normal lymphocytes were collected almost completely free of platelets, granulocytes, and monocytes, but were contaminated with 1–3 erythrocytes per lymphocyte.

Contaminating erythrocytes were removed by hemolysis with 0.83% NH_4Cl (ref. 15). If any erythrocytes remained intact, they were hemolyzed by a 15-sec exposure to distilled water instead of the 30 sec recommended by FALLON *et al.*[16].

Cells were then washed with Hanks balanced salt solution and the pellet quick-

frozen with solid CO_2 and alcohol and stored at −98°. Cells were thawed rapidly for use. Cells treated in this way were shown to retain their activity for LDH and MDH[17], as well as a number of other enzymes[18], for many months.

Preparation of enzyme

Cells were suspended in 0.02 M Tris–HCl (pH 8.0) and sonicated for 5 min in a Ratheon S102A sonicator. Supernatants, after centrifugation at 10 000 × *g* for 20 min at 4°, were used as the enzyme preparation.

Assay procedures

Total LDH and MDH enzyme activities were assayed by procedures adapted from the fluorimetric methods of BURCH *et al.*[19]. These procedures have been previously described in detail[18].

Acrylamide-gel (disc) electrophoresis

The isozymes of LDH and MDH were separated with a Canalco Model 12 electrophoresis cell by a procedure modified from the method of DAVIS[20]. A 7.5% gel solution was prepared, diluted with water to 5.5%, and added to 7 mm × 63 mm tubes to a depth of 42 mm. After polymerization, the sample was applied to the top of the gel with a 10-μl syringe. Extract from $0.5 \cdot 10^6$ cells was used for LDH and from $1–2 \cdot 10^6$ cells for MDH. Sucrose, 15 μl of a 40% solution, was added to the homogenate on the gel. A fresh 5.5% gel solution was then polymerized above the sample. Electrophoresis at 5° in Tris–glycine buffer (pH 8.3) at 2.5 mA and 30 V per tube was continued until the dye front[20] had moved 35 mm into the lower gel (40–45 min). The gels were removed to 10 mm × 75 mm tubes and covered with substrate and staining solution. The reagents of VAN DER HELM[21] were used for LDH, but incubation time was reduced to 30 min at 37°. The conditions for MDH were identical except that 0.1 M L-malic acid at pH 6.5 was substituted for the lactic acid and incubation was for 45 min. Stained gels were washed with water and stored in 7.5% acetic acid in the dark at 4°. Densitometry was done with a Canalco Microdensitometer, Model E, with a Wratten X-1 filter in the light path.

Percentages of heart- and muscle-type (H- and M-)LDH were calculated from the densitometry in accordance with the tetramer theory[22,23]: LDH-1 = 100% H-type; LDH-2 = 75% H- and 25% M-type; LDH-3 = 50% H- and 50% M-type; LDH-4 = 25% H- and 75% M-type; and LDH-5 = 100% M-type.

Cell culture

Cells were cultured in 16 mm × 125 mm screw-cap test tubes in a medium of 20% autologous serum, 30% Hanks balanced salt solution, and 50% Parker medium 199 (ref. 3). Initial cell concentration was $1–2 \cdot 10^6$ per ml, with 7 ml per tube. Cultures were gassed with 5% CO_2 in air.

Phytohemagglutinin-P (Difco) was added at concentrations of 5 μl/ml of culture. Actinomycin D was a gift of Merck, Sharpe and Dohme, West Point, Pa. Puromycin was purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

Morphological observations

Morphology of the cells was studied both in the living state with phase microscopy and with May–Gruenwald–Giemsa stained smears[3].

RESULTS

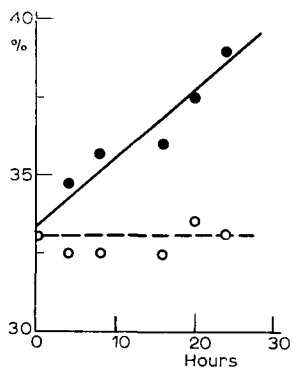
Morphological changes in cell culture

Lymphocytes after 3 days in culture showed no evidence of morphological transformation. Lymphocytes cultured with phytohemagglutinin showed 65–75% transformation to blast-like cells after 2–3 days incubation at 37°. Addition of 5 μg of actinomycin D per ml of culture medium or 50 μg/ml puromycin 10 min prior to the addition of phytohemagglutinin prevented blast-cell formation.

Granulocytes showed no morphological transformation when cultured with or without the addition of phytohemagglutinin.

LDH of lymphocytes in culture: effect of addition of phytohemagglutinin, actinomycin D or puromycin

Results of LDH assays and isozyme studies are given in Table I and Figs. 1–3. Little change in total enzyme activity or in LDH isozyme pattern occurred in lymphocytes during 3 days of culture. When, however, lymphocytes were cultured with phytohemagglutinin added to the medium alteration of the LDH isozyme pattern



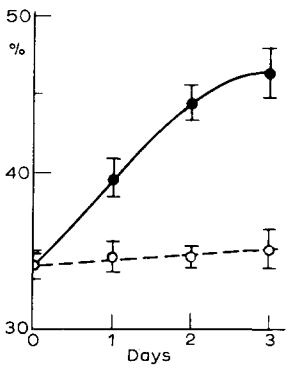


Fig. 1. Time course of increase in proportion of M-type LDH as expressed by % of total activity in lymphocytes during first day of culture with phytohemagglutinin added to the medium (●—●) and without phytohemagglutinin (○- - -○). Procedures used are described in text (MATERIALS AND METHODS). The results shown represent a single typical run.

Fig. 2. Increase in proportion of M-type LDH as expressed by % of total activity in lymphocytes during 3 days of culture with phytohemagglutinin added to the medium (●—●) and without phytohemagglutinin (○- - -○). Procedures used are described in text (MATERIALS AND METHODS). The results shown represent the mean ± S.E. of 7 complete runs.

was discernable as early as 4 h after phytohemagglutinin addition as shown by the increase in M-type LDH in Fig. 1. A relative rise in LDH-4 and LDH-5 and decrease in LDH-1 and LDH-2 was visible on the gels at this time. Increase of M-type LDH was linear (Figs. 1 and 2) for 2 days and then the rate decreased. Increases in total enzyme activity, percentages of M-type LDH, LDH-4 and LDH-5, as well as decreases in percentages of LDH-1 and LDH-2 were all significant ($P < 0.001$) after 1 day in culture with phytohemagglutinin (Table I). The rise in total LDH represented an absolute increase in both H- and M- subunits, but the proportions of newly formed M-subunits was greater.

TABLE I

LDH AND MDH ENZYME ACTIVITIES AND ISOZYME PATTERNS OF LYMPHOCYTES IN CULTURE

Cell culture, acrylamide-gel electrophoresis, densitometry and calculation of M-type LDH subunits were performed as described in the text (MATERIALS AND METHODS). Additions were made so that final concentrations per ml of culture medium were: phytohemagglutinin, 5 μl; actinomycin, 5 μg; puromycin, 50 μg. With combined additions, phytohemagglutinin was added 10 min after actinomycin or puromycin. N represents the number of different bloods studied.

Time of culture	*Additions to medium*	*N*	*Total LDH**	*% in each LDH band*					*M-type LDH (%)*	*Total MDH**	*% MDH Band 2*
				1	*2*	*3*	*4*	*5*			
0 time	—	7	247 ± 23	22.6 ± 2.6	34.6 ± 1.6	29.5 ± 2.2	10.3 ± 1.3	3.0 ± 1.1	34.0 ± 2.0	292 ± 50	44.8 ± 4.7
1 day	None	7	263 ± 20	21.9 ± 2.5	34.3 ± 2.9	30.3 ± 2.3	10.1 ± 2.4	3.4 ± 1.5	34.6 ± 2.7	276 ± 39	47.8 ± 5.6
	Phytohemagglutinin	7	338 ± 39	18.8 ± 3.0	31.9 ± 3.4	28.3 ± 2.5	13.3 ± 2.9	7.6 ± 1.4	39.7 ± 3.2	296 ± 47	49.4 ± 7.9
	Actinomycin	4	163 ± 18	22.8 ± 3.8	38.0 ± 3.2	30.3 ± 3.9	7.3 ± 2.4	1.5 ± 1.3	32.8 ± 2.8	164 ± 20	38.8 ± 4.3
	Actinomycin *plus* phytohemagglutinin	4	171 ± 29	20.7 ± 4.0	41.5 ± 2.7	30.8 ± 2.2	6.0 ± 2.6	0.9 ± 1.0	32.1 ± 1.3	155 ± 56	39.1 ± 3.5
	Puromycin	3	110 ± 8	22.0 ± 4.2	36.6 ± 2.9	30.4 ± 3.6	8.7 ± 2.4	2.3 ± 1.0	33.3 ± 2.9	155 ± 35	32.6 ± 1.3
	Puromycin *plus* phytohemagglutinin	3	121 ± 39	21.7 ± 3 2	38.7 ± 1.9	30.5 ± 1.7	7.3 ± 2.9	1.8 ± 0.9	32.1 ± 3.0	113 ± 25	38.2 ± 4.6
2 day	None	7	257 ± 32	22.3 ± 2.4	34.3 ± 2.4	28.8 ± 2.4	10.0 ± 1.8	4.5 ± 2.1	34.7 ± 1.9	275 ± 36	48.5 ± 6.2
	Phytohemagglutinin	7	393 ± 40	15.9 ± 2.4	28.2 ± 3.0	28.5 ± 2.2	16.8 ± 2.7	10.6 ± 1.6	44.5 ± 3.0	315 ± 46	50.7 ± 5.8
3 day	None	7	257 ± 36	21.3 ± 4.2	34.6 ± 2.0	30.4 ± 3.4	9.4 ± 2.3	4.1 ± 1.1	35.2 ± 3.3	261 ± 56	47.6 ± 6.7
	Phytohemagglutinin	7	427 ± 41	15.0 ± 3.1	27.1 ± 3.4	27.5 ± 2.4	19.1 ± 4.0	11.5 ± 2.5	46.4 ± 4.2	360 ± 25	51.6 ± 4.6

* μmoles/10^{10} cells per min ± S.D.

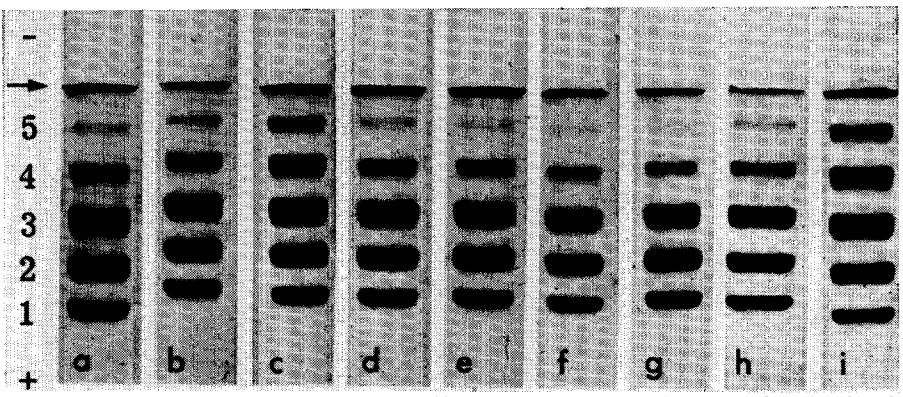


Fig. 3 LDH isozyme patterns from cultures of lymphocytes. a. Zero time. b–g. One-day incubation: b. No additions to complete medium; c. Addition of 5 μl/ml phytohemagglutinin; d. Addition of 5 μg/ml actinomycin D; e. Addition of actinomycin D followed in 10 min by phytohemagglutinin; f. Addition of 50 μg/ml puromycin; g. Addition of puromycin followed in 10 min by phytohemagglutinin. h, i. Two-day incubation. h. No additions; i. Addition of phytohemagglutinin. Procedures used are described in text (MATERIALS AND METHODS).

Actinomycin D or puromycin added to lymphocytic cultures gave similar results in the presence or absence of phytohemagglutinin (Table I and Fig. 3). A fall in total enzyme activity occurred, with a slight decrease in M-type LDH which was associated with small reductions in LDH-4 and LDH-5.

LDH of granulocytes in culture: effect of addition of phytohemagglutinin, actinomycin D or puromycin

Results of LDH studies of cultured granulocytes are given in Table II. Granulocytes, which are rich in M-type LDH (64%) initially, showed only a slight rise in M-type LDH units in culture. Total LDH enzyme activity, however, fell greatly during 3 days of culture. There was little difference in LDH isozyme pattern or total enzyme activity whether phytohemagglutinin was added or not. Addition of actinomycin D or puromycin to granulocyte cultures also had little effect on the isozyme patterns but there was a greater fall in total enzyme activity.

MDH of lymphocytes in culture: effect of addition of phytohemagglutinin, actinomycin D or puromycin

Lymphocytes (Table I) and granulocytes (Table II and Fig. 4) showed two MDH isozyme bands with acrylamide-gel electrophoresis. Lymphocytes contained a greater proportion of slow moving MDH-2 (44.8%) than did granulocytes (13.4%).

During the first day of culture the lymphocytes showed a slight rise in MDH-2 ($P < 0.05$), but no further change was seen during two additional days of culture. In lymphocytes cultured with phytohemagglutinin, the increase in MDH-2 was a little greater but was not significantly so. Total enzyme activity diminished a little (not significant) in 3 days of culture without phytohemagglutinin, but increased moderately ($P < 0.05$) after 3 days of culture with phytohemagglutinin. Addition of actinomycin D or puromycin to cultures with or without phytohemagglutinin resulted in a significant fall in total enzyme activity and a significant decrease in the proportions of MDH-2.

TABLE II

LDH AND MDH ENZYME ACTIVITIES AND ISOZYME PATTERNS OF GRANULOCYTES IN CULTURE

Procedures as for lymphocyte cultures (Table I). Results represent mean of 3 sets of observations ± S.D. except MDH electrophoresis is a single complete run.

Time of culture	*Additions to medium*	*Total LDH**	*% in each LDH band*					*M-type LDH (%)*	*Total MDH**	*% MDH Band 2*
			1	*2*	*3*	*4*	*5*			
0 time	—	606 ± 32	1.6 ± 1.0	18.0 ± 2.1	28.3 ± 1.9	25.9 ± 1.5	26.1 ± 2.6	64.2 ± 2.6	400 ± 45	13.4
1 day	None	567 ± 62	1.0 ± 0.9	16.3 ± 1.3	21.8 ± 2.0	24.9 ± 0.2	32.6 ± 4.0	68.0 ± 2.7	412 ± 43	27.6
	Phytohemagglutinin	547 ± 49	1.5 ± 1.4	16.6 ± 3.1	25.0 ± 2.6	22.6 ± 0.4	34.4 ± 3.1	67.9 ± 3.0	383 ± 22	30.2
	Actinomycin	432 ± 43	1.3 ± 1.2	18.8 ± 2.8	26.1 ± 1.7	23.9 ± 1.2	30.3 ± 4.1	65.8 ± 3.5	285 ± 13	17.9
	Actinomycin *plus* phytohemagglutinin	351 ± 37	0.7 ± 1.2	16.3 ± 2.5	26.3 ± 2.5	23.0 ± 1.2	31.7 ± 3.6	66.5 ± 3.1	208 ± 47	13.8
	Puromycin	495 ± 17	1.1 ± 1.6	17.4 ± 2.0	26.2 ± 0.6	24.3 ± 0.1	31.0 ± 2.1	66.6 ± 2.8	280 ± 13	13.8
	Puromycin *plus* phytohemagglutinin	425 ± 22	0	17.4 ± 2.8	27.1 ± 2.5	23.8 ± 0.1	31.9 ± 3.2	67.6 ± 3.5	210 ± 42	8.2
2 day	None	349 ± 51	1.6 ± 1.1	16.9 ± 3.1	26.2 ± 2.9	21.1 ± 2.4	31.9 ± 4.9	66.8 ± 3.4	390 ± 27	32.0
	Phytohemagglutinin	362 ± 75	1.4 ± 1.4	16.1 ± 2.8	25.2 ± 0.8	22.2 ± 2.7	33.0 ± 3.6	67.9 ± 3.4	349 ± 34	33.3
	Actinomycin	213 ± 30	2.7 ± 1.8	19.7 ± 3.7	26.2 ± 2.9	23.4 ± 2.8	30.3 ± 3.1	64.2 ± 3.9	115 ± 36	21.1
	Actinomycin *plus* phytohemagglutinin	165 ± 47	2.7 ± 1.9	19.7 ± 3.9	26.7 ± 2.4	24.3 ± 1.3	28.6 ± 3.8	63.5 ± 3.3	135 ± 14	21.9
	Puromycin	180 ± 42	2.9 ± 1.8	20.0 ± 2.7	28.2 ± 2.1	25.1 ± 1.1	28.3 ± 2.7	62.8 ± 3.9	140 ± 39	25.7
	Puromycin *plus* phytohemagglutinin	205 ± 42	3.7 ± 2.2	18.0 ± 3.3	27.0 ± 3.5	22.3 ± 0.8	28.5 ± 3.0	63.6 ± 3.5	121 ± 41	17.4

* μmoles/10^{10} cells per min.

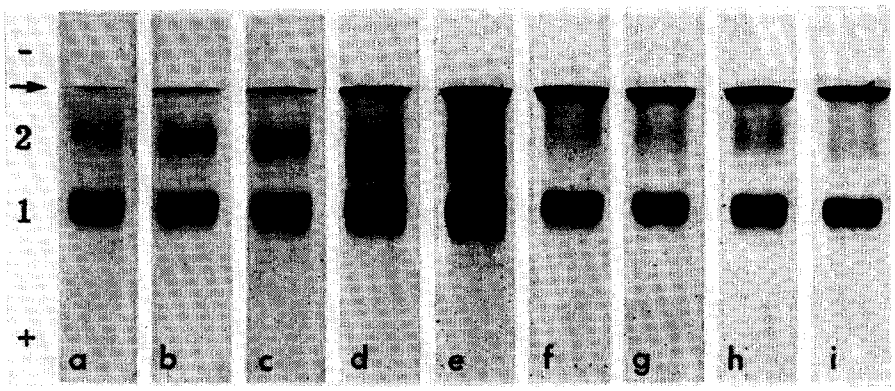


Fig. 4. MDH isozyme patterns from cultures of granulocytes. a. Zero time. b, c. One-day incubation. b. No additions to medium; c. Addition of 5 μl/ml phytohemagglutinin. d–i. Two-day incubation. d. No additions to complete medium; e. Addition of phytohemagglutinin; f. Addition of 5 μg/ml actinomycin D ;g. Addition of actinomycin D followed in 10 min by phytohemagglutinin; h. Addition of 50 μg/ml puromycin; i. Addition of puromycin followed in 10 min by phytohemagglutinin. Procedures used are described in text (MATERIALS AND METHODS).

MDH of granulocytes in culture: effect of addition of phytohemagglutinin, actinomycin D or puromycin

Granulocytes in culture showed little change in total MDH enzyme activity during 2 days of culture with or without phytohemagglutinin (Table II). Both relative and absolute amounts of MDH-2, however, were markedly increased after 24 h of culture (Table II and Fig. 4). This increase in MDH-2 occurred whether phytohemagglutinin was added or not. Addition of actinomycin D or puromycin blocked the rise in MDH-2 and also caused a fall in total MDH enzyme activity.

DISCUSSION

The changes in isozyme patterns found perhaps provide some metabolic advantage to the cells in culture. In the case of lymphocytes there is a very marked increase in glucose consumption and lactic acid production with phytohemagglutinin added[24,25]. The increase in M-type LDH may promote this increase in glycolysis[26–29]. It has been suggested that glycolysis may be an important energy source for the synthesizing activities of the transforming proliferating lymphocytes[30]. The implication is that increased glycolysis is necessary for blast-like cells to form.

The changes in MDH pattern in cultured granulocytes perhaps also produce some advantage for the cells in culture. There is evidence that oxalacetate causes substrate inhibition of mitochondrial MDH[31] while the cytoplasmic variety of MDH does not exhibit this phenomenon[32]. The advantages accruing to the cultured cells by the change in MDH pattern are, however, obscure.

In primary cultures a variety of tissues from the same organism developed uniform LDH isozyme patterns[33] with diminution or disappearance of anodal bands and increase in cathodal bands. Established cell lines, on the other hand, had varied LDH isozyme patterns[34]. Development of common LDH isozyme patterns in primary (diploid) cultures from different tissues may be due to overgrowth of a cell common to all the tissues (*e.g.* fibroblast) or to dedifferentiation in specialized cells with

emergence of a uniform tissue culture adapted cell[34]. With the former possibility the need for a genetic explanation is eliminated. In either case a number of cell divisions and serial passages are required to produce the changes seen. In the present study, on the other hand, alterations in LDH isozyme pattern in lymphocyte-phytohemagglutinin cultures were demonstrated in a few hours, while alterations of MDH in granulocytes were found within 24 h.

The changes in isozyme patterns observed occurred in the same cells that were originally introduced into the cultures. Frequency of mitosis is negligible in lymphocytes cultured without phytohemagglutinin. With phytohemagglutinin added increased mitosis in lymphocytes begins about the third day of culture. Since significant alterations in LDH pattern in lymphocyte-phytohemagglutinin cultures were found during the first day the results were not due to the appearance of a new population of cells. Since granulocytes do not divide in culture, with or without phytohemagglutinin, the changes in MDH were likewise within the original cell population. The change in LDH isozyme pattern in lymphocytes cultured with phytohemagglutinin is in the direction of the granulocytic pattern, while the change in MDH isozymes of granulocytes in culture is in the direction of the lymphocytic pattern. It is important to note that highly purified lymphocyte (99%) and granulocyte (> 99%) preparations were used in these studies. Thus, the results obtained are not due to a decrease of one cell type in the cultures with a relative increase of the other.

Actinomycin D acts to block reactions affecting genetic control by inhibiting DNA-directed synthesis of messenger RNA[35–39]. Puromycin, on the other hand, inhibits the synthesis of protein by causing the release of nascent peptides from the ribosomes[40–42].

Results in the present study show that phytohemagglutinin can cause a change in the proportion of H- and M-type LDH subunits synthesized by lymphocytes. That new synthesis occurred was indicated by a rise in total LDH activity in conjunction with the increase in proportion of M-type polypeptide subunits. Puromycin block of these changes provides additional evidence that they are the result of new protein synthesis.

Synthesis of H- and M-type LDH polypeptide units are believed to be under the control of two genetic loci[13]. Phytohemagglutinin may control these genes directly, or by initiating processes which result in altered gene activity. Block of the LDH changes in lymphocytes by actinomycin indicates that phytohemagglutinin action must be at or before the gene level. Phytohemagglutinin must somehow alter the activities of the genetic loci for H- and M-type LDH subunits. Phytohemagglutinin may act at allosteric sites on protein repressors or inducers of operator genes for the H- and M-type polypeptides in accordance with the theories of MONOD, CHANGEUX AND JACOB[14]. There seems little doubt that phytohemagglutinin also acts at many other sites to produce its effects in lymphocytes. MONOD, CHANGEUX AND JACOB[14] pointed out that "it seems difficult to imagine any biochemical mechanism other than allosteric which would allow a single chemical signal to be understood and interpreted simultaneously in different ways in different systems." The action of phytohemagglutinin in many respects parallels that of hormones. SÜDI[43] commenting on the multiple effect of plant hormones at many sites also suggested that similar allosteric sites on a variety of functionally different proteins made up auxin receptor sites.

Hormonal control of the synthesis of a variety of enzymes in plants and animals was the subject of a recent symposium[44]. It might be noted particularly that GOODFRIEND AND KAPLAN[45] found that estradiol administered to immature rats and rabbits caused an increase in uterine M-type LDH subunits. The increase in MDH-2 in cultured granulocytes very likely is also the result of altered genetic activity. Actinomycin D and puromycin again blocked the change in isozyme pattern. It seems probable that in this case there are also separate genetic loci for the two MDH isozymes. Although these loci are not affected by phytohemagglutinin, they are responsive to the altered environmental conditions present in culture.

ACKNOWLEDGEMENTS

We wish to thank Misses B. A. WILHITE, T. LUBRANO, I. SCHIMO, M. KLODNYCKY and Mrs. E. GOETZ for their excellent technical assistance. This investigation was supported in part by U.S. Public Health Service Research Grant CA 06504-05, from the National Cancer Institute.

REFERENCES

1 P. C. NOWELL, *Cancer Res.*, 20 (1960) 462.
2 D. A. HUNGERFORD, A. J. DONNELLY, P. C. NOWELL AND S. BECK, *Am. J. Human Genet.*, 11 (1959) 215.
3 Y. RABINOWITZ, *Blood*, 23 (1964) 811.
4 J. H. ROBBINS, *Science*, 146 (1964) 1648.
5 R. SCHREK AND Y. RABINOWITZ, *Proc. Soc. Exptl. Biol. Med.*, 113 (1963) 191.
6 M. W. ELVES AND J. F. WILKINSON, *Exptl. Cell Res.*, 30 (1963) 200.
7 O. R. MCINTYRE AND F. G. EBAUGH, JR., *Blood*, 19 (1962) 443.
8 H. L. COOPER AND A. D. RUBIN, *Science*, 152 (1966) 517.
9 G. C. MUELLER AND M. LEMAHIEU, *Biochim. Biophys. Acta*, 114 (1966) 100.
10 K. HIRSCHHORN, F. BACH, R. L. KOLODNY, I. L. FIRSCHEIN AND N. HASHEM, *Science*, 142 (1963) 1185.
11 E. H. COOPER AND P. BARKHAN, *Brit. J. Haematol.*, 9 (1963) 101.
12 F. PARENTI, P. FRANCESCHINI, G. FORTI AND R. CEPELLINI, *Biochim. Biophys. Acta*, 123 (1966) 181.
13 E. S. VESELL, in A. G. STEINBERG AND A. G. BEARN, *Progress in Medical Genetics*, Vol. 4, Grune and Stratton, New York, 1965, p. 128.
14 J. MONOD, J.-P. CHANGEUX AND F. JACOB, *J. Mol. Biol.*, 6 (1963) 306.
15 N. DIOGUARDI, A. AGOSTONI, G. FIORELLI AND B. LOMANTO, *J. Lab. Clin. Med.*, 61 (1963) 713.
16 H. J. FALLON, E. FREI, III, J. D. DAVIDSON, J. S. TRIER AND D. BURK, *J. Lab. Clin. Med.*, 59 (1962) 779.
17 Y. RABINOWITZ AND A. DIETZ, *Blood*, 29 (1967) 182.
18 Y. RABINOWITZ, *Blood*, 27 (1966) 470.
19 H. B. BURCH, O. H. LOWRY, A. M. KUHLMAN, J. SKERJANCE, E. J. DIAMANT, J. R. LOWRY AND P. VON DIPPE, *J. Biol. Chem.*, 238 (1963) 2267.
20 B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
21 H. J. VAN DER HELM, *Lancet*, 2 (1961) 108.
22 E. APPELLA AND C. L. MARKERT, *Biochem. Biophys. Res. Commun.*, 6 (1961) 171.
23 C. L. MARKERT, *Science*, 140 (1963) 1329.
24 D. QUAGLINO, F. G. J. HAYHOE AND R. J. FLEMANS, *Nature*, 196 (1962) 338.
25 E. H. COOPER, P. BARKHAN AND A. J. HALE, *Brit. J. Haematol.*, 9 (1963) 101.
26 P. G. W. PLAGEMANN, K. F. GREGORY AND F. J. WROBLESWKI, *J. Biol. Chem.*, 235 (1960) 2288.
27 G. PFLEIDERER AND E. D. WACHSMUTH, *Klin. Wochschr.*, 39 (1961) 352.
28 E. S. VESELL AND A. G. BEARN, *J. Clin. Invest.*, 40 (1961) 586.
29 D. M. DAWSON, T. L. GOODFRIEND AND N. O. KAPLAN, *Science*, 143 (1964) 929.
30 J. W. PARKER, H. WAKASA AND R. J. LUKES, *Lab. Invest.*, 14 (1965) 1736.

31 D. D. DAVIES AND E. KUN, *Biochem. J.*, 66 (1957) 307.
32 A. DELBRÜCK, A. E. ZEBE AND T. BÜCHER, *Biochem. Z.*, 331 (1959) 273.
33 E. S. VESELL, J. PHILIP AND A. G. BEARN, *J. Exptl. Med.*, 116 (1962) 797.
34 H. M. NITKOWSKY AND D. D. SODERMAN, *Exptl. Cell Res.*, 33 (1964) 562.
35 E. REICH, R. M. FRANKLIN, A. J. SHATKIN AND E. L. TATUM, *Science*, 134 (1961) 556.
36 I. H. GOLDBERG AND M. RABINOWITZ, *Science*, 136 (1962) 315.
37 E. REICH, I. H. GOLDBERG AND M. RABINOWITZ, *Nature*, 196 (1962) 743.
38 J. HURWITZ, J. J. FURTH, M. MALAMY AND M. ALEXANDER, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1222.
39 I. H. GOLDBERG, M. RABINOWITZ AND E. REICH, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 2094.
40 M. B. YARMOLINSKY AND G. L. DELA HABA, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1721.
41 D. NATHANS AND F. LIPMANN, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 497.
42 D. NATHANS, *Federation Proc.*, 23 (1964) 984.
43 J. SÜDI, *New Phytologist*, 65 (1966) 9.
44 A. HOLLAENDER, *Symp. Hormonal Control Protein Biosynthesis, Oak Ridge National Laboratory, Gatlinburg, Tenn., 1965, J. Cell. Comp. Physiol.*, 66, Suppl. 1 (1965).
45 T. L. GOODFRIEND AND N. O. KAPLAN, *J. Biol. Chem.*, 239 (1964) 130.