cell functions. Perhaps the ketone group of acetone and the S = O group of DMSO cause such an effect. Our data, however, in no way imply a site of action or differences between actions for either DMSO, acetone or methanol.

It is quite clear from these data that these effects on the fibroblasts are not unique for DMSO. Whatever the reactions taking place, the end results were equally well achieved with acetone and to a certain extent with methanol as well.

Summary. Human fibroblast cultures grown in 1% DMSO have demonstrated a lag in generation time, but an increase in total hydroxyproline concentration when compared with control cultures. An inverse relationship between growth rate and hydroxyproline concentration for each culture was noted. Somewhat similar findings were obtained in cultures treated with acetone and methanol.

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Enzyme Activities of Some Cultured Human Cells.* (32421)

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Previous studies of the enzymatic activities of cells in culture have shown that, although a number of specific differences exist, the overall pattern of enzyme activity is similar in such cells, irrespective of origin or karyotype(1).

During our studies of the enzyme activity of human diploid cells in culture, we have measured the activity of 3 enzymes for a number of human cells *in vitro* under similar conditions of culture, growth phase, and homogenization techniques. For purposes of comparison, we also include in this report data for several cultures which are being published elsewhere(2).

We describe below the results of this study based upon observations of the following enzymes: acid and alkaline phosphatase (orthophosphoric monoester phosphohydrolases E.C. 3.1 3.2: 3.1 3.1, respectively) and lactic dehydrogenase (L lactate; NAD oxidoreductase EC 1.1.1.27).

Materials and methods. Cell cultures used in this study (Table I) include 5 fibroblastic cell strains cultured from normal tissues(3), 5 epithelial-like cell lines derived from fibroblast cultures by SV_{40} virus transformation (4,5,6,7), and 2 cell lines of malignant origin (8,9).

Diploid human cell cultures derived from normal fetal tissue have a limited life span *in vitro* and can be grown for only about 50 generations. Their life cycle has been divided arbitrarily into 3 phases. Phase I represents the period when the cells are freed from intact tissue and are establishing themselves on the glass surface. Phase II represents the period of rapid proliferation in culture. During phase III, the cells show a decreased capacity for proliferation which ultimately results in the death of the culture(3).

Methods for the derivation of the SV_{40}-transformed cell lines have been described(7). Cultures WI-38VA13 (A and C) were derived

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TABLE I. Tissue Cultures Studied. These cultures were prepared and handled according to the methods described previously(2,16). Culture A-11 L was obtained from Dr. A. Fogel of the Wistar Institute; culture 14-S was obtained from Dr. William Mellman, University of Pennsylvania School of Medicine; cultures of HeLa and H. Ep. #2 were obtained from Microbiological Associates, Bethesda, Md.

Cell designation	Origin (human)	Karyotype	Morphology	Reference
A-11 L	Fetal lung	Undetermined	Fibroblastic	Unpublished
WI-38	" ♀	Diploid	"	(10)
WI-26	" ♂	"	"	(10)
WI-1006	Adult lung	"	"	(10)
14-S	Infant skin	Undetermined	"	Unpublished
WI-38VA13A*	Fetal lung	Heteroploid	Epithelial-like	(7)
WI-38VA13C*	"	"	"	(7)
WI-26VA4*	"	"	"	(7)
W-18VA2†	Buccal mucosa	Subdiploid	"	(6)
W-98VA1H†	Adult skin	Heteroploid	"	(7)
HeLa	Cervical carcinoma	"	"	(8)
H. Ep. #2	Epidermoid laryngeal carcinoma	"	"	(9)

* Derived from normal human diploid fibroblast strains WI-38 and WI-26 by transformation with SV_{40} virus.

† Derived from cultures of fibroblasts by transformation with SV_{40} virus.

from human fetal lung fibroblasts (strain WI-38). Culture WI-26VA4 was derived from human fetal lung fibroblast (strain WI-26). Cultures W18VA2 and W98VA1H were originally cultured from apparently normal tissues of terminal cancer patients and subsequently transformed with SV_{40} virus.

The fibroblastic cell strains showed typical phase III phenomena(3,10), while all 7 epithelial-like cultures grew as permanent cell lines.

All cultures were subcultivated and handled in the same way. Methods of subcultivation and harvesting used in this laboratory have been described(2,11).

For studies with human fetal lung, fresh tissue was excised from fetuses of 2-4 month gestation, cut into small pieces and carefully washed free of blood with a cold phosphate-buffered balanced salt solution. The tissue was then minced with sharp-pointed scissors and homogenized.

The details of the methods used in preparing the cell culture homogenates have been described(2).

The phosphatases were determined by an adaptation of the method of Bessey, Lowry and Brock(12), which depends on the hydrolysis of p-nitrophenylphosphate. Appropriate aliquots of cell homogenate were incubated with buffered substrate (6.1 mM) for 30 minutes at 37°. The reaction was stopped by the addition of alkali, and the p-nitrophenol was measured colorimetrically at 410 mμ. Acid phosphatase activity was measured in 0.05 M citrate buffer at pH 5.0 and alkaline phosphatase activity at pH 10.2 in 0.05 M glycine buffer containing 1 mM $MgCl_2$.

Lactic dehydrogenase activity was estimated at pH 7.6 by following the rate of oxidation of NADH spectrophotometrically at 340 mμ and 25°, according to the method of Kornberg(13).

Samples of the variously diluted homogenates were frozen and later assayed for protein content according to the method of Lowry *et al*(14). Phosphatase specific activity is reported per mg of total cell protein extracted, while lactate dehydrogenase specific activity is reported per mg of supernatant (non-sedimentable at 105,000 × *g* for 30 minutes) protein.

For the conditions of each assay, the velocity of the reaction was always proportional to the amount of enzyme added. Cells were continually monitored and were consistently found to be free of mycoplasmas.

Results and discussion. Table II summarizes the activity of acid and alkaline phosphatase and lactate dehydrogenase in a series of human cell cultures. The results reported for diploid cell strains WI-38, A-11-L and 14-S were obtained from young, actively pro-

TABLE II. Enzyme Activity in Human Tissue Cultures. Assays were carried out according to methods described in text. Figures in the table designate either mean ± standard error of the mean or the range followed by number of determinations in parentheses.

Cell type	Enzyme activity in mμmoles/min/mg protein		
	Acid phosphatase	Alkaline phosphatase	Lactate dehydrogenase
WI-38	35 ± 3.2 (8)	3.02 ± .65 (8)	2152 ± 254 (7)
WI-26	47.7 ± .7 (4)	3.40 ± .93 (4)	1690 ± 330 (4)
A-11 L	37.5–40.4 (2)	2.29–3.20 (2)	1900–2068 (2)
WI-1006	51.8 ± 4.6 (3)	2.21 ± .36 (3)	2060 ± 166 (3)
14-S	53.3 ± 3.2 (12)	1.31 ± .08 (12)	2252 ± 180 (12)
WI-38VA13A	40.8 ± 2.9 (13)	0.88 ± .10 (13)	2352 ± 156 (13)
WI-38VA13C	44.2 ± 5.3 (6)	1.43 ± .20 (6)	1836 ± 318 (6)
WI-26VA-4	33.4–40.2 (2)	0.69– 1.17 (2)	1380–1840 (2)
W-18VA-2	25.6–32.8 (2)	0.93– 1.12 (2)	1380–1900 (2)
W-98VA-1H	36.3–42.5 (2)	1.02– 1.68 (2)	1730–1960 (2)
HeLa	40.7–53.5 (2)	24.1 –29.7 (2)	4320– 570 (2)
H. Ep. #2	26.2–36.6 (2)	21.5 –25.2 (2)	3520–4000 (2)
Human fetal lung	68.5 ± 8.6 (4)	4.11 ± 1.24 (4)	1652 ± 58 (4)

liferating cultures (fewer than 33 passages), while strains WI-26 and WI-1006 were measured during late phase II or early phase III. Data for strain WI-26 were obtained from cultures between passages 33 and 46. Culture WI-1006, although studied between passages 13 and 15, was derived from adult lung and had a doubling potential in culture of less than 20 passages.

The acid phosphatase activity of fibroblasts derived from human adult lung (WI-1006) was significantly higher than that of human fetal lung strain WI-38 ($P<0.02$). In their acid phosphatase activity, human infant skin fibroblasts, as well as early phase III cultures of human fetal lung (strain WI-26) resembled cultures of adult lung more than of young fetal lung. All of the SV_{40} virus-transformed cells exhibited acid phosphatase activities within the range of the diploid fetal and adult cultures and did not differ significantly from either. HeLa cells showed a range somewhat higher than the human fetal lung cultures, while HEp#2 showed a slightly lower range. Human fetal lung tissue homogenates, shown in Table II for comparison, had a mean acid phosphatase activity higher than that of any of the values obtained with cell cultures.

Alkaline phosphatase activity (Table II) in all of the lung fibroblast strains and in human fetal lung tissue was low. Tissue cultures of malignant origin (HeLa and Hep#2), grown and measured under the same conditions, showed activities approximately 10 times higher than the normal lung fibroblast strains. In contrast to the malignant cells, the entire series of SV_{40}-transformed permanent cell lines demonstrated lower alkaline phosphatase activities than did the lung fibroblasts. For example, alkaline phosphatase activity in WI-38VA13A was significantly lower than the activity in strain WI-38 from which it was derived ($P<.001$).

Lactate dehydrogenase activities were, for the most part, similar in all of the tissue cultures studied, except for the two cell lines of malignant origin which showed activities approximately 2-fold higher than the normal or the SV_{40}-transformed cells.

In general, the enzyme activities of these different cell cultures were similar to each other and closely resembled the values reported in the literature for other cell cultures, *e.g.*, see Lieberman and Ove(1). The most plausible explanation for this similarity is that it reflects an adaptation by populations of cells which best suits them to the artificial environment of *in vitro* growth. Despite this general similarity, some reproducible differences did occur.

The somewhat higher acid phosphatase activity of cultures WI-1006 and WI-26 can be considered to reflect the increased relative age of these cultures(2). The higher activity of culture 14-S is more difficult to interpret. This culture showed a typical phase III phenomenon, but were assayed during the

period of rapid growth (phase II). However, since culture 14-S is derived from human infant skin, this difference may be the result of either a specific tissue variation, or a variation reflecting the time at which the original tissue is placed into culture.

Striking differences were evident in alkaline phosphatase activities. In our experiments, cultures of HeLa and HEp#2 showed alkaline phosphatase activities approximately 10-fold higher than those of the diploid lung fibroblasts, thus supporting the association observed among epithelial-like morphology, heteroploid karyotype and a high alkaline phosphatase activity(15,16). Other data presented here allow a further examination of this association by comparing the diploid fibroblasts with cultures of heteroploid cells of epithelial-like morphology derived directly from diploid fibroblasts by viral transformation, including 3 matched pairs (WI-38 *vs* WI-38VA13 (A and C); WI-26 *vs* WI-26VA4). In every case, the transformed cells showed activities lower than that of the normals. These findings indicate that the heteroploidy and epithelial-like morphology evident in these transformed cultures is not associated with an increased alkaline phosphatase activity.

There was some variation in the lactate dehydrogenase activities of the cultures studied; *e.g.*, the cultures of malignant origin (HeLa and HEp#2) had activities 1.5- to 2-fold higher than cultures of normal origin.

Summary. The data presented here allow a comparison of the activity of 3 enzymes in diploid and heteroploid human cell cultures derived from both normal and neoplastic tissues. In general, acid phosphatase activity is similar in all of these cultures, although there is evidence that diploid cultures approaching or in phase III (decreased capacity for proliferation) have higher activities of this enzyme. Alkaline phosphatase activity was high in the cultures derived from neoplastic tissue, and low in the diploid cultures derived from normal tissues. Cultures derived from normal tissues and transformed *in vitro* by an oncogenic virus show activities lower than any of the diploid cultures.

Cultures derived from neoplastic tissues show lactate dehydrogenase activities 1.5- to 2-fold higher than the normal diploid or transformed cultures.

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