TABLE III. Desalting of Various Iodinated Compounds.

Original compound	R_F before desalting* 1	2	3	R_F after desalting* 1	2	3	Resulting compound
O-Methyl thyroxine	.53	.61		.42	.48		Deiodination
Thio-ether analogue of thyroxine	.35	.75		.33	.71		"
Thyroxamine	.85	.79		.90	.76		"
3′, 5′-Dichloro-3,5-diiodothyronine	.30	.73		.18	.48		"
3′, 5′-Dibromo-3,5-diiodothyronine	.19	.79		.25	.38		Deiodination (thyronine?)
2,6-Diiodophenoxyacetic					.10		NaI
3,5- "					.10		"
Tetraiodophenolphthalein	.56	.94		.55	.94		No change

* Same solvent systems as Table I.

eral other iodinated compounds, some derivatives of thyroxine and others quite different in structure. Since none of the deiodinated products is available, it is only possible to consider that loss of iodine and change of R_F demonstrate at least deiodination. Other alterations may be produced, similar to those found in 3,5-diiodothyronine (Table I).

O-Methyl thyroxine, the thio-ether analogue of thyroxine and thyroxamine were all deiodinated, the last much more slowly. Since 3′,5′-dichloro- and 3′,5′dibromo-3,5-diiodothyronines were deiodinated, it is probable that dehalogenation was complete, but the R_F's obtained after treatment are not truly the R_F of thyronine. Tetraiodophenolphthalein was completely stable. Two iodophenoxyacetic acids did not react with the ceric-arsenious acid reagent before treatment, but did show the presence of some inorganic iodide afterwards.

Conclusions. 1) Use of electrolytic desalter to remove inorganic ions from solution, results in removal of iodine from aromatic linkage, including thyroxine, triiodothyronine, diiodotyrosine and several of the fatty acid side-chain analogues of thyroxine. 2) This prevents use of apparatus for preparation of salt-free solutions in thyroid studies. The procedure might be useful in preparation of various dehalogenated derivatives where the parent compounds are readily available.

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Lactic Dehydrogenase Production in Tissue Culture of Normal, Transformed, and Malignant Human Cell Lines.*† (24185)

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The lactic dehydrogenase activity (LD) of serous effusions containing and/or bathing proliferating malignant neoplastic cells has been found greater than LD activity of blood plasma from which the serous effusion is derived. It was postulated that the greater enzyme activity might be due to its accumulation in serous fluid as a result of either multi-

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plication or disintegration of malignant cells (1). Since many strains of malignant cells are well established in tissue culture, it seemed that information relative to the contribution of LD activity to the fluid bathing them might be gained by a study of their enzymatic behavior in tissue culture. In addition, these same methods could be used to characterize enzymatic behavior of established cell lines known to have been isolated from carcinomas as well as those which were isolated from normal tissue, but which during their serial passage in tissue culture had changed their characteristics(2). Normal cell lines derived from first generation amnion cells or from recently isolated fibroblastic cell lines were used for comparison.

Materials and methods. Cell lines. The following human cell lines have been used: from known malignant sources—HEP #1, HEP #2(3), Skiff(4), and J-111(5); from normal tissues which changed in tissue culture —Chang's liver and conjunctiva(6) and FL amnion(7); from normal sources—normal amnions prepared and planted in tissue culture according to standard methods(8) and human fibroblasts, derived either from adult or embryonic sources, which had had less than 7 trypsinizations and which morphologically and chromosomally appeared unchanged. All cell lines were grown in Eagle's synthetic media(9) to which had been added 15% human serum to promote growth. Whenever determinations were made, a sample of the media without cells was included. *Chemical determinations* for LD, glutamic oxaloacetic transaminase (GO-T), and glutamic pyruvic transaminase (GP-T) activity were done spectrophotometrically(10,11,12). After clarification by centrifugation, determinations were done on culture media in which the cells had grown, and on cells themselves after they had been broken up by alternate freezing in a dry ice-CO_2 bath (−76°C) and thawing in a 37°C water bath 5 times. The frozen and thawed specimens were then centrifuged so that the determinations could be made on the clear supernatant fluid.

Results. Alteration in LD activity in supernatant fluid of tissue cultures. Approximately equal numbers of cells from the above mentioned sources were placed in 10 cc of media, inoculated into milk dilution bottles, and placed at 37°C where they settled on the glass surface and began to multiply. One ml of fluid was removed at one- to 2-day intervals, and LD, GO-T, and GP-T activity determinations were made. The same amounts of cells were also set up anaerobically in flat bottomed Erlenmeyer flasks which were then layered with heavy mineral oil to insure preservation of anaerobic conditions. Observation of amount of cell growth was made daily by examining the cells microscopically for the aerobic bottle cultures, and by noting the pH change which occurred in anaerobic cultures. Fig. 1 and 2 show amounts (average of 6 experiments) of LD activity which appeared in the tissue culture fluid following incubation at 37°C up to 26 days. It appears that for the first week of incubation, the fluid bathing the cells showed about the same amount of LD activity, but that in the next 10 days a marked increase of enzymatic activity was noted in the media bathing the malignant cell lines, J-111 and HEP #2, somewhat less in the cell line HEP #1 and the Chang liver, while the first generation amnion and the conjunctiva showed relatively little increase in LD activity, and that of the control media without cells was the lowest. Following the high values, the amount of LD drops quite precipitously at a time when the cells are in a poor nutritional state as evidenced by granularity and sloughing off the glass surface. The behavior of cells and enzymes was the same under anaerobic conditions except that the conjunctival cell line showed a significant increase in LD activity. The GO-T and GP-T determinations done on these same samples failed to vary significantly from day to day, indicating that of the 3 enzyme systems the LD activity was the one reflecting the changes which occurred in the fluid bathing malignant cells.

Amount of LD in cells and media during growth of cells. In these experiments a known number of cells was implanted on the side of a test tube, 1.5 cc of culture media was added, and at different points during their growth 2 tubes of each cell line were sacrificed, and the

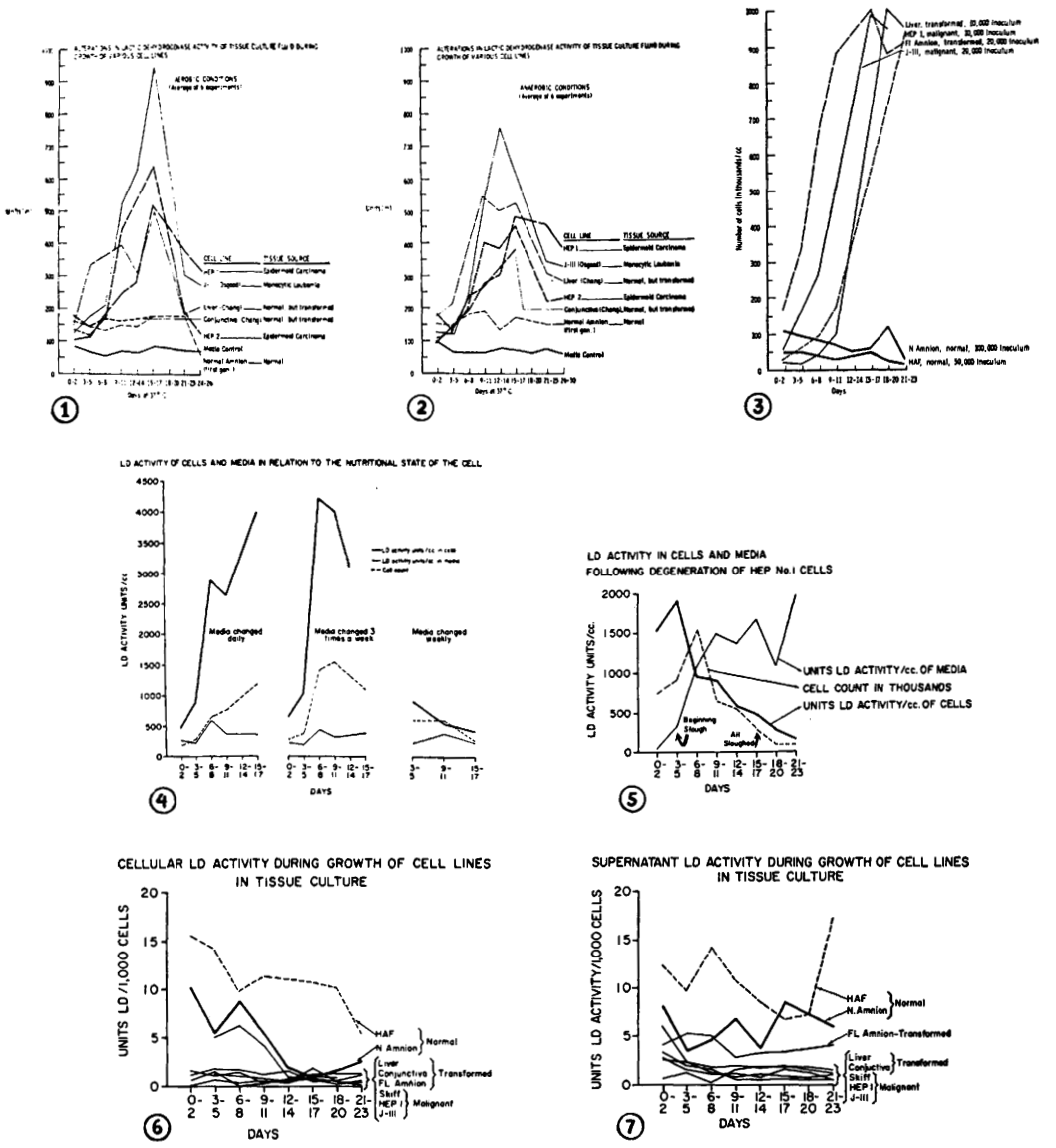

FIG. 1-7.

media in which they had grown was sent for LD activity study. The cells were then detached from the glass surface by trypsin, pooled, sedimented, resuspended in 1 cc of PO_4 buffer, and after removal of 0.1 cc for counting, the remaining cells were frozen and thawed and LD activity determined after centrifugation. It was thus possible to compare enzyme activity in the cells with that of the fluid in which they had grown. In one set of experiments the fluid was completely changed in the remaining tubes twice weekly. The following conclusions seem warranted:

1) When cells were implanted in each tube and counts made at frequent intervals (Fig. 3), it can be seen that the amniotic and human fibroblasts failed to multiply to any significant extent, whereas the transformed lines as represented by the FL amnion and the Chang liver, and the known malignant cell lines (J-111 and HEP #1) grew very well even though much smaller inocula had been used. In these experiments the media had been changed twice a week, thus providing

TABLE I. Average Units LD Activity/1,000 Cells in Disrupted Cells and Media during Experimental Periods.

		Days of growth								
		0-8			9-17			18-26		
Cell line	Source	*	Cells	Media	*	Cells	Media	*	Cells	Media
HAF	Normal	9	13.32	12.17	8	11.34	7.89	5	7.7	13.17
Amnion	"	15	7.16	4.8	17	6.4	6.14	13	3.97	3.72
FL amnion	Transformed	8	.92	5.45	9	1.31	3.15	7	1.73	4.08
Liver	"	12	3.47	2.49	14	2.55	.92	10	2.29	.60
Conjunctiva	"	4	3.15	2.78	7	2.58	1.67	3	1.31	1.43
HEP #1	Malignant	17	2.73	1.79	16	1.71	1.22	12	1.32	1.03
Skiff	"	6	2.18	.93	6	2.52	1.79	4	2.52	1.67
J-111	"	7	5.81	1.89	9	2.76	.67	4	1.08	1.0

* No. of determinations.

good conditions for growth.

2) When LD activity in the frozen and thawed cells and the respective media was compared with the cell count, the results depended to a great extent on whether the media had been changed or not, suggesting that the state of nutrition of the cells played a significant role. Fig. 4 illustrates LD activity observed in the frozen and thawed cells and respective media when 20,000 HEP #1 cells were planted in each tube and the media completely removed and replaced (1.5 cc) from one set daily, from another set 3 times a week, and from still a third set, once a week. The results presented in Fig. 4 show that in the 2 groups with more frequent media change the cells multiplied very well and that sizable activity of LD was found in the cells. Comparatively little LD activity was found in the media, probably because it had been removed and had not had the chance to accumulate. This explanation is suggested by the data obtained from a study of a culture of this same tumor cell in which the media was not changed, and the cells, after a week of vigorous growth, showed large quantities of LD with increasing amounts in the media. As more and more cells sloughed off the glass surface and degenerated, less LD activity was found in the cells themselves, while large activities appeared in the media (Fig. 5). Comparison of the normal, transformed, and malignant cell lines in relation to LD activity in the cells and in the media showed this same effect. When the cells were in a state of good nutrition, significantly more LD activity was present in the cells, no matter from what source they were derived.

3) *Activity of LD/cell in media and cells.* When the amounts of LD per cell are calculated, it appears that the cells from normal sources contain more enzyme activity per cell than do the malignant and transformed cell lines. Table I gives average activity of LD/1,000 cells found in the media and cells during the different periods of growth. This is shown graphically at shorter time intervals in Fig. 6 and 7. In the cells (Fig. 6) human adult fibroblasts, normal amnion, and the J-111 cell lines had more enzyme activity for the first 2 weeks, and thereafter the latter two resemble the other cell lines. In the media (Fig. 7) enzymatic activity per cell is highest throughout for the normal cell lines, while the FL amnion occupies a midway position between them and the others.

In a more direct experiment, growing cells were trypsinized from the glass surface of bottles, counted, and made up to 100,000 in Gey's balanced saline. After being broken up by freezing and thawing, LD activity was determined on the centrifugate. Table II gives the results and confirms the impression that the normal cells contain more of the enzyme activity.

Discussion. The observation that LD activity accumulates in fluid bathing malignant cells when grown in tissue culture provides an explanation for the elevated LD activity found in serous effusions. Observations of the condition of the cells have shown that healthy proliferating cells contain large amounts of LD activity, but then they begin to degenerate, less enzyme activity is found

TABLE II. LD Activity Units Released when 100,000 Cells Were Disrupted.

Cell line	Type of cell	LD activity, units/cc
HAF	Normal	1400
Amnion	"	768
Conjunctiva	Transformed	480
FL amnion	"	230
Liver	"	68
HEP #1	Malignant	132
HEP #2	"	142
J-111	"	140
Skiff	"	38

in the cells themselves and a comparable increase is found in the medium surrounding them. This would seem to be a condition analogous to that found clinically when fluids containing exfoliated cells are found to be the richest in the enzyme activity.

When LD activity for the cell lines derived from normal, transformed, and cancer sources, and grown in tissue culture, are compared, large amounts of activity are found in the known cancer cell lines and in the transformed lines, but little in the normal cell lines. This appears to be a reflection of the rapid growth rate, however, rather than a characteristic of the cell itself, since LD activity per cell is found to be greater in both the normal fibroblasts and amnion cells than in the other cells. How the large amount of LD activity in the fluid bathing the malignant cells accumulates is unknown, but one may speculate that it could diffuse out through the cell wall, or it may escape into the media at the time of cell division.

Summary. Normal, malignant, and transformed cell lines have been studied for variations in 3 different enzyme activities, GO-T, GP-T, and LD. Only LD activity changed during the growth period. The enzyme activity both in the cells and in the fluid bathing them was greatly increased in the malignant cell lines and in 2 transformed lines, in contrast to the normal cell lines and the conjunctiva cell line where large quantities did not appear in the media. The increase in LD activity was found to be related to the cellular growth rate rather than to be a characteristic of the cell, since the normal cells in most instances were found to contain more enzyme activity per cell than the malignant ones.

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Interactions Between Phosphate and Nystatin in *Candida stellatoidea*.* (24186)

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Nystatin, an antibiotic produced by *Streptomyces noursei*, inhibits growth of fungi but does not affect growth of bacteria and viruses. It is therefore widely used clinically against a variety of mycotic infections, particularly candidiasis(1). Neither the structure nor mode

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