TABLE I. Excretion, Retention and Thyroidal Uptake of I^{131} in Iodine Deficient Rats Following Injection of PTU and T4* or T3*.

Animal	Body wt, g	Thyroid wt, mg	Thyroid wt, % of body wt	Thyroid uptake, % of dose	Urine excretion, % of dose	Fecal excretion, % of dose
T4* + PTU						
I_2 deficient	436	*29.2 ± .70**	*6.7 ± .4*	*.25 ± .027*	16.0 ± 1.17	35.4 ± 5.3
Controls	455	18.1 ± 1.21	4.0 ± .15	.03 ± .007	22.9 ± 3.2	47.8 ± 2.6
T3* + PTU						
I_2 deficient	468	*30.1 ± 3.45*	*6.4 ± .55*	*.34 ± .06*	28.9 ± 1.5	46.5 ± 7.1
Controls	420	19.2 ± 1.01	4.6 ± .34	.07 ± .008	40.0 ± 4.5	35.5 ± 4.7

* ± stand. error of mean.

Italicized figures are significantly different from controls at 1% level or less.

TABLE II. Half-Life, Space of Distribution and Daily Degradation of Thyroidal Hormones in Iodine-Deficient and Control Rats.

Animal	Half-life hours	Daily turnover rate	Space of distribution, ml	Serum I^{127}, μ%	Daily degradation,* μ
T4* + PTU					
I_2 deficient	18.6	.89	76	2.7	1.46
Controls	18.2	.91	84	4.0	2.44
T3* + PTU					
I_2 deficient	10.3	1.65	300	1.5	1.49
Controls	11.0	1.50	389	2.2	2.57

* Assuming $\frac{4}{5}$ of serum PBI^{127} is T4 and $\frac{1}{5}$ is T3.

roidal hormone than its control, but metabolised whatever was available at the same rate as the control.

Summary. The peripheral metabolism of radiothyroxine (T4*) and radiotriiodothyronine (T3*) was studied in iodine deficient and control rats. The half-life and space of distribution of these hormones were similar in the 2 groups of animals. The quantity of hormone degraded daily, however, was less in iodine deficient than in its control rat because less was produced.

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Lipid Synthesis by Ascites Tumor Cells.* (25698)

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We have shown(1) that palmitic acid is readily absorbed by Ehrlich mouse ascites tumor cells *in vitro,* especially when presented as the albumin complex. Moreover, an average of about 40% of the absorbed palmitic acid was oxidized to CO_2 per hour under these conditions. However, it was not ascertained

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whether the remainder of the absorbed lipid was used for synthesis of cellular lipids or whether these could be absorbed from the medium. A preliminary experiment revealed that when tumor cells are exposed to labeled triglyceride present in the medium as low-density lipoprotein, oxidation of fatty acid does not occur, indicating that triglyceride is probably not absorbed.† Therefore, experiments were initiated to determine, first, if the various cellular lipids are in fact synthesized by these cells from absorbed fatty acids and at what rate, and second, under what conditions, if any, these newly synthesized lipids appear in the medium.

Methods. Treatment of tumor cells. Ehrlich ascites tumor cells and ascitic fluid were obtained as described previously(1). In each of the experiments, 4 mice were used, yielding a total of 45-50 ml of blood-free tumor. The tumor was separated by centrifugation into ascitic fluid (about 30 ml), which was retained for use as the incubation medium, and cells, which were washed twice with buffer. To obtain tumor cells containing palmitic-1-C^{14} acid and its metabolic products for incubation in a C^{14}-free medium, the cells were suspended in 30 ml of ascitic fluid in a 100 ml round-bottom flask and were incubated at 37° in air with 5 ml of an albumin-palmitate-1-C^{14} solution containing approximately 1.1 mg palmitic acid of specific activity 1.7 x 10^5 d.p.s. per mg for a total of 1.9 x 10^5 d.p.s. After 60 minutes, the cells were separated by centrifugation at 1300 x g and were washed twice with phosphate buffer. These washed cells, containing palmitic-1-C^{14} acid and its metabolic products were then reincubated with fresh ascitic fluid for 10, 20, 30, and 60 minute intervals, following which the cells were separated from the medium by centrifugation and lipids from both the cell and supernatant fractions were extracted as described previously(1). In an attempt to study a "0" time, the tagged cells were placed in 30 ml of fresh ascitic fluid, as in the case of the other timed experiments, but were immediately centrifuged to separate them from the medium, the entire procedure being carried out at 0°C. In one experiment, a portion of the cells, labeled with palmitate-1-C^{14}, were incubated with buffer and the remainder with ascitic fluid for 60 minutes to determine whether the nature of the medium might influence passage of lipids from the cells. *Separation of lipids.* Each sample of extracted lipid was separated by chromatography on silicic acid as described previously(1, 2). In view of the high activity of the added palmitate-1-C^{14} and the possibility of misinterpretation of small amounts of contamination of other lipid fractions, each fraction was further purified. Fraction 1, which was eluted with 1% ether in petroleum ether (normally the sterol ester fraction) was passed through a short alumina column in chloroform to ensure absence of any free fatty acid. Fraction 2, eluted with 5% ether in petroleum ether (normally the triglyceride fraction), fraction 4, eluted with 25% ether in petroleum ether (normally diglyceride) and fraction 5 eluted with ether (normally monoglyceride) were passed through short alumina columns with ether:ethanol (1:1).‡ Fraction 3, eluted with 10% ether in petroleum ether, (normally cholesterol and free fatty acids) was rechromatographed on silicic acid using 5%, 8% and 10% ether in petroleum ether as eluants. The last 2 fractions were then passed through a short alumina column in chloroform. The final fraction removed from silicic acid with methanol was purified by precipitation with acetone. Plating and counting procedures have been described(1). All counting was performed with a Nuclear Instruments and Chemical Corp. D-47 Micromil Flow Counter.

† One ml of ascitic fluid from a mouse inoculated with tumor 7-9 days previously, was emulsified with 10 mg of triolein transesterified with palmitic-1-C^{14} acid. The emulsion was reinjected intraperitoneally into the mouse and, after 5 minutes, the tumor was withdrawn and ascitic fluid separated by centrifugation into lipoprotein and albumin fractions. The low-density lipoprotein fraction was incubated 60 minutes with tumor cells from different mice as described previously(1). Results of 3 experiments revealed no significant activity in the center well, with activity distributed about equally between cells and medium.

‡ It is essential that the alumina columns are short and that glycerides are in contact with the alumina for as short a time as possible in order to avoid hydrolysis.

TABLE I. % of Recovered Activity in Cells and Supernatant Solution Following 60-Minute Incubation of Washed Cells Previously Labeled with Palmitic-1-C^{14} Acid.

Medium	% admin. activity in washed cells	% recovered activity in: Cells	Supernate
Buffer	17.8	93.6	6.4
Ascitic fluid	19.3	90.9	9.1

Results. Table I presents results of experiment to determine whether the nature of the medium influences passage of lipids out of cells previously incubated with palmitate-1-C^{14}. Although most of the activity remained in the cells, it is apparent that there is little or no difference in the amount appearing in the medium whether it be ascitic fluid or buffer. This result is quite different from that found by James, Lovelock and Webb(3) for the erythrocyte, in which synthesized lipid is readily exchanged with the medium only in presence of α-lipoproteins.

Table II summarizes mean relative specific activity and per cent total activities of the purified cell lipids at times from 0 to 60 minutes. After one hour's incubation with the palmitic acid, phospholipid synthesis is still progressing at the expense of free fatty acid, which declines steadily throughout the experimental period. Triglyceride synthesis is also taking place throughout the experiment, possibly at the expense of both free fatty acid and phospholipid. Synthesis of the other lipids is much slower, but apparently continues during the 60-minute period.

Table III summarizes mean relative specific activities and per cent total activities of the lipids from the incubation media at times from 0 to 60 minutes. Weights of the lipid fractions from the incubation media were so low that accurate determination was difficult, especially in the case of the di- and monoglycerides. Moreover, very little of the cell activity is released to the medium during 60 minutes (Table I).

Most of this is in the free fatty acid fraction, which may possibly have been bound loosely to the cell wall rather than within the cell, and therefore resisted the washing process and was slowly removed during incubation. The major synthesized lipid fraction, phospholipid, is also the major fraction released to the medium. Triglyceride is apparently retained within the cell.

Discussion. It has been shown(4,5,6) that neoplastic cells carry on an active, if not exclusive oxidation of fat as the normal energy source. Absorption of fatty acid may be, therefore, an important process for such cells, especially since it is possible that they may not be capable of fatty acid synthesis. The fatty acid absorbed by the cell is partially oxidized and partially converted into the various cell lipids (largely phospholipid with smaller amounts of triglyceride). The process of synthesis from fatty acid is apparent from its steady decline in the cells and the accompanying increase in the other fractions. The exact precursor-product relationships are, however, not revealed and are obviously too complex for a simple conversion mechanism. The decline of phospholipid and its possible participation in synthesis of other lipids are of interest.

Of the major lipids synthesized by the cell, only phospholipid is released into the medium

TABLE II. Activities of Lipid Fractions from Ascites Tumor Cells Following Incubation with Palmitic-1-C^{14} Acid.

Fraction	0 min.†	10 min.†	20 min.†	30 min.†	60 min.‡
Sterol ester	.1§(.3)‖	.3 (1.1)	1.2 (2.0)	1.0 (2.2)	8.5 (5.3)
Triglyceride	.4 (.8)	5.1 (13.0)	4.9 (14.6)	4.6 (15.4)	4.1 (19.7)
Fatty acid*	91.6 (51.7)	87.3 (34.8)	80.8 (24.8)	81.0 (20.3)	70.8 (12.5)
Sterol	.1 (.2)	.3 (1.0)	.4 (.8)	.8 (1.9)	.2 (.1)
Diglyceride	.2 (.3)	.8 (1.8)	1.2 (2.1)	1.6 (2.9)	1.9 (4.1)
Monoglyceride	.1 (.1)	.2 (.8)	.3 (1.2)	.5 (2.1)	7.0 (9.0)
Phospholipid	7.5 (46.7)	6.0 (47.5)	11.2 (54.4)	10.5 (55.2)	7.5 (49.4)

* Calculated from weight and activity retained on alumina column.
† Means of 3 determinations.
‡ " " 2 " .
§ Relative specific activity (specific activity of fraction/sum of specific activities).
‖ Values in parentheses represent % of total activity.

TABLE III. Activities of Lipid Fractions from Incubation Media of Ascites Tumor Cells Which Had Been Incubated with Palmitic-1-C^{14} Acid.

Fraction	0 min.†	10 min.†	20 min.†	30 min.†	60 min.‡
Sterol ester	.1§(.3)‖	— (.2)	.1 (.2)	.1 (.4)	.8 (1.9)
Triglyceride	.1 (.6)	.1 (.7)	— (.5)	.1 (.8)	.4 (2.0)
Fatty acid*	97.7 (85.1)	98.4 (86.3)	97.0 (78.3)	96.5 (78.7)	71.6 (30.5)
Sterol	.3 (3.2)	.1 (1.6)	.5 (3.3)	.2 (2.2)	2.8 (3.5)
Diglyceride	.1 (.8)	.1 (1.2)	.3 (1.5)	.2 (2.2)	8.4¶(19.9)
Monoglyceride	.2 (1.1)	.1 (1.4)	— (.4)	.1 (1.1)	9.6¶(10.4)
Phospholipid	1.5 (8.9)	1.1 (8.6)	2.2 (15.8)	2.9 (14.6)	6.4 (31.9)

* Calculated from weight and activity retained on alumina column.
† Means of 3 determinations.
‡ Only 1 determination.
§ Relative specific activity (specific activity of fraction/sum of specific activities).
‖ Values in parentheses represent % of total activity.
¶ Values are probably not reliable because of extremely small amount of material (less than 1 mg) in each case.

to any extent. Triglyceride does not appear to be either absorbed or released and sterol ester may behave similarly. Thus, it is possible that only the more polar lipids can readily pass through the cell wall.

Further studies with labeled lipids should serve to amplify these indications.

Summary. Ehrlich ascites tumor cells were incubated in ascitic fluid with albumin-palmitate-1-C^{14} complex and were then washed and incubated for varying time intervals with fresh media. Lipids of both cells and media were extracted, separated chromatographically and counted. Results show a decrease of cell free fatty acid with increases in all lipids, especially triglyceride. The major lipid synthesized is phospholipid, which, however declines after 30 minutes. Only phospholipid is released into the medium to any extent.

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Stimulation of Neurosecretion in Shark Embryos by Thyroid Hormones.* (25699)

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The principal physiological role of the hypothalamic neurosecretory system appears to be integration of pituitary gland with certain environmental changes(1). Since this integrative mechanism is involved in such phenomena as seasonal breeding it is not surprising that it is known to differentiate relatively late in the life cycle. In both rats(2) and teleost fishes(3) such differentiation, physiologically and morphologically, appears incomplete until post-embryonic life. Recently, while studying morphological features of the hypothalamic neurosecretory apparatus of shark (*Squalus suckleyi*) embryos, we found that its differentiation is hastened by treatment with thyroid hormones. Since nothing now is

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