In vivo Release of Radiocarbon from Labeled Ehrlich's Ascites Carcinoma Cells. (22613)

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The relationship between tumors and their host tissues, particularly with respect to protein metabolism, has been the subject of numerous investigations(1-4). Recently, isotopic experiments have been reported in which the administered isotope was confined to the transplanted tumors themselves(5,6). These studies have suggested that protein metabolism in tumors is essentially, though not strictly, a one-way passage. Babson and Winnick(5) transplanted Walker carcinoma tissue labeled with tyrosine or leucine-C^{14} into rats. A rapid initial loss of radioactivity was observed during the first day, resulting from cell breakdown during fragmentation and from initial autolysis. A small but significant amount of the remaining radioactivity was released in each of the following 5 days. Greenlees and LePage(6) injected TA 3 ascites cells, labeled *in vivo* with C^{14}-glycine, into the peritoneal cavity of normal mice. Radioactivity was released from the tumors with 9% appearing daily in various organs, urine and respiratory CO_2. The transfer of radioactivity from the tumor to the host tissues suggested that the loss of isotope from the tumor cells was continuous and regular. Since the tumor proteins were not separated and analyzed in these experiments (6) and radioglycine is known to contribute C^{14} to the lipides and nucleic acids, an exchange between the neoplasm and its host strictly with regard to amino acids cannot easily be interpreted.

In the present study, the release of radioactive protein from Ehrlich's mouse ascites carcinoma cells labeled with C^{14}-leucine was investigated. Radiocarbon from leucine is incorporated primarily into the proteins; however, since some of the radioactive products from leucine were taken up by the lipides and nucleic acids, analyses of these components are also presented.

Experimental. Ascites cells labeled with radiocarbon were prepared *in vivo* by intraperitoneal injections of leucine-2-C^{14} into mice bearing a 4-day-old ascites tumor. One mg of radioleucine with a specific activity of approximately 4.5 x 10^6 counts per minute (C.P.M.) per mg was injected per mouse. The radioactive cells were recovered 48 hours after injection of the isotope, separated from the suspending ascitic fluid by centrifugation, and resuspended in 10 volumes of Krebs-Ringer-Phosphate buffer solution, pH 7.4. Aliquots of 1 ml of the cell suspension were injected into the peritoneal cavity of normal mice. At the end of the experimental period the cells were recovered with the use of several 0.9% saline washes and separated by centrifugation.

The acid-soluble (TAS), lipide, nucleic acid and protein fractions were obtained by the procedure of Hurlburt and Potter(7) as modified by Tyner *et al.*(8). Duplicate aliquots from all samples were plated directly and counted with a gas-flow tube. Proteins were plated from a suspension in ethanol-water. A minimum of 4,000 counts were taken and the appropriate self-absorption corrections applied.

Results. The recovery of labeled transplanted tumors removed from the animal about 15 minutes after inoculation is presented in Table I. Approximately 20% of the administered radioactivity could not be recovered immediately after inoculation. Such losses, probably due to manipulation, experimental technic, etc., were consistent but greater than the 8% observed by Greenlees and LePage(6). In all of the following experiments, one animal was sacrificed immediately after inoculation and recoveries at subsequent times are expressed as per cent of the radioactivity recovered after 15 minutes.

Table II summarizes the results obtained with radioactive ascites cells maintained intraperitoneally for 24 and 48 hours. Consid-

TABLE I. Recovery of Radioactivity from Labeled Ascites Cell Transplants Withdrawn Immediately after Inoculation.*

Fraction	Exp. A			Exp. B		
	Original	Withdrawn in 15 min.	% recovered	Original	Withdrawn in 15 min.	% recovered
TAS	4,180	3,175	76	1,080	745	69
Lipides	940	1,020	—	216	200	93
Nucleic acids	2,140	1,750	82	459	354	77
Proteins	90,200	72,000	80	13,400	10,600	79

* Total C.P.M. per fraction.

erable tumor growth occurred as shown by the increase in the weight of tumor protein recovered. The lipides did not change consistently or significantly in any of the experiments. Twenty-four and 45% of the radioactivity in the nucleic acid and protein fractions respectively was lost from the tumor during the first day. Less than 10% of the remaining radioactivity in either of these 2 fractions was lost during the 2nd day. The release of isotope from the ascites cells is considerably slower and constant between the 2nd and the 5th day (Fig. 1). After a rapid initial loss during the first day, an average decrease of about 8% per day was observed in the nucleic acids and proteins recovered. The possibility that the marked loss of radioactivity during the first day may have been due to a reaction of the host against the inoculated tumor, resulting in a destruction of tumor cells, was tested by transplanting the labeled tumor into a mouse already bearing a tumor. Three days after inoculation of a mouse with 0.2 ml of an unlabeled tumor cell suspension, the isotopic ascites cells were injected. An aliquot of the labeled preparation was injected into a non-tumor-bearing mouse at the same time. The results are shown in Table III. No significant differences were found in the release of radioactivity from the 2 inocula. Over 30% of the C^{14} in the nucleic acids and proteins of both tumors was lost during the first 24 hours. This rapid initial release may

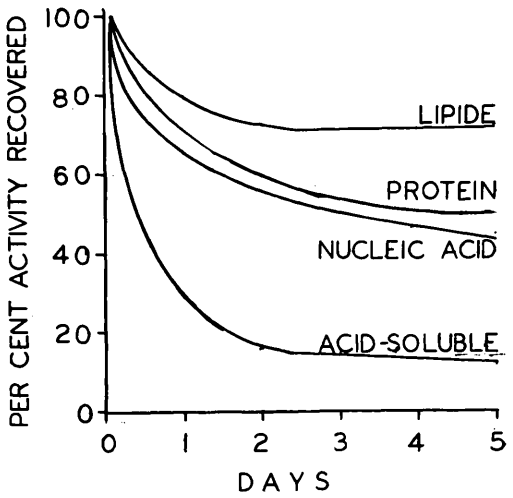

FIG. 1. Recovery of radioactivity in various fractions of labeled tumor transplants maintained intraperitoneally for 5 days. Values are expressed as % of activity recovered 15 min. after inoculation.

reflect the metabolic behavior of the transplanted cells. The possibility exists that a number of the cells in the preparation may be incapable of survival and growth, may autolyse, and are thus lost from the transplant soon after inoculation. Babson and Winnick (5) found that between the 2nd and the 5th day, the actively growing isotopic tumor transplants in their experiments released approximately 8% per day of the initial radioactivity administered. The authors cite as a possible criticism of their experiments that

TABLE II. Recovery of Radioactivity from Labeled Transplants Maintained for 2 Days.

Time	TAS		Lipides	Nucleic acids		Proteins		
	C.P.M.	% recovered	C.P.M.	C.P.M.	% recovered	C.P.M.	% recovered	Wt of protein (mg)
15 min.	3,175		1,020	1,750		72,000		23.9
24 hr	2,490	79	1,250	1,320	76	40,000	55	79.6
48 "	1,860	69	1,040	1,205	69	37,200	52	90.2

TABLE III. Influence of Pre-Existing Tumor on Release of Radioactivity from Labeled Transplants.*

	Normal mice			Tumor-bearing mice		
Time, hr	TAS	Nucleic acids	Proteins	TAS	Nucleic acids	Proteins
24	53	68	60	42	71	68
48	45	61	55	39	60	64

* Recoveries of radioactivity expressed as % of 15 min. values.

since the most rapid growth occurs at the periphery of the tumor, the original transplanted portion would probably be the first to become necrotic. Very little necrosis was observed however. Greenlees and LePage (6) found that labeled TA3 ascites cells released 10 to 20% daily of the radioactivity originally present. These workers present analyses of the combined lipide, nucleic acid and protein fractions from the recovered cells which showed a loss of 11% of the C^{14} on the first day and 60% in 2 days. The extent to which C^{14} is released from any of these components and thus contribute to the body pool cannot be estimated from this data. The present report indicates that actively growing Ehrlich's ascites cell proteins release about 8% of their radioactivity per day during the 2nd to the 5th day period.

The data presented here and that quoted above suggest that a small but significant exchange exists between the proteins of the tumor and its host. The possibility exists that the failure to recover all of the administered isotope may be due not to the metabolic release of labeled constituents from the ascites cells but to the loss of cells due to autolysis.

Summary. Ascites cells labeled with radiocarbon from leucine-2-C^{14} were injected into mice. The amounts of radioactivity remaining in the acid-soluble, lipides, proteins and nucleic acids of the transplants for periods up to 5 days was measured. A marked loss of C^{14} from all of these components occurred within the first day but did not appear to be due to a reaction of the host against the inoculated cells. Between the 2nd and the 5th day a small but significant amount of the C^{14} in the proteins and nucleic acids was lost per day. These results suggest that the transfer of labeled nitrogenous constituents from the tumor to the host tissues occurs to some extent. The possibility that these results may be due to the destruction of the cells must also be considered.

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Received July 6, 1956. P.S.E.B.M., 1956, v92.