THE *IN VIVO* REUTILIZATION OF LYMPHOCYTIC AND SARCOMA DNA BY CELLS GROWING IN THE PERITONEAL CAVITY

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ABSTRACT

An ascites tumor, Sarcoma I, was transplanted to isologous and homologous mice which had been labeled with tritiated thymidine from 1 to 24 hours previously. Radioautographic preparations revealed labeled host lymphocytes emerging to mingle with the transplanted tumor and the subsequent appearance of nuclear radioactivity in the sarcoma. Sarcoma cells cultured subcutaneously or in Millipore diffusion chambers in previously labeled mice did not demonstrate significant radioactivity. Transplantation of washed, H^3-thymidine-labeled lymphocytes to non-radioactive, sarcoma-bearing mice was followed by the gradual appearance of nuclear radioactivity in the sarcoma. The label in the sarcoma was entirely removed by deoxyribonuclease but not by ribonuclease treatment prior to radioautography. Intraperitoneal injections of purified, H^3-thymidine-labeled sarcoma or lymphoid DNA in normal or tumor-bearing mice were followed by radioactivity appearing in sarcoma or normal peritoneal mononuclear cells. It was concluded that reutilization of DNA and its metabolites may occur *in vivo*, and the conditions under which reutilization may be detected are discussed.

INTRODUCTION

Little is known of the fate of deoxyribonucleic acid (DNA) from dying mammalian cells. This is true despite the importance of such information for genetics and also for the proper interpretation of tracer studies of DNA metabolism.

In 1954 Dancis and Balis (1) studied reutilization of nucleic acid metabolites *in vivo*. Using animals labeled with C^{14}-adenine, these workers were unable to demonstrate reutilization of radioactive metabolites by transplanted tumors, by parabiotic rats, or by the offspring of the labeled animals. The belief that DNA and its metabolites were not passed from one cell to another was expressed in 1959 by Cronkite and coworkers (2). Subsequently Rubini and coworkers (3) mentioned the possibility of DNA reutilization but felt that in tracer studies it was ". . . not a serious problem because of great dilution." That DNA and its metabolites are not reutilized at a detectable level has been the tacit assumption in many other excellent studies of DNA synthesis and cellular kinetics in various systems (4–7).

However, Ottesen (8) in 1954 reported that either human lymphocytes are very long lived or that DNA reutilization does occur in this cell series. Emphasis was subsequently placed on DNA reutilization within lymphocytic tissues by the biochemical studies of Hamilton (9) and *in vitro* morphologic work of Trowell (10). The possibility of the passage of DNA between lymphocytes was suggested again in a 1960 publication of Alpen and coworkers (11). Perhaps the most direct evidence for DNA reutilization *in vivo* comes from the very recent work of Hill and Drásil (12) and Hill (13). These studies report that radioactivity may be detected in the DNA of several cell types from

irradiated mice given isologous P^{32}-labeled thymocytes. However, more than half of the radioactivity in the injected thymocytes was in acid-soluble compounds and not in fully polymerized DNA. It, therefore, may be that the label found in the DNA of the recipient animal's cells originated from compounds other than DNA itself.

That at least certain cell types have the ability to utilize labeled nucleic acids *in vitro* has been reported by numerous investigators (14–17). Whether these findings represented incorporation of intact nucleic acid or some degradation product thereof was not determined. Bensch and King (18) have shown that strain L tissue culture cells can phagocytose and utilize high molecular weight DNA complexed to protein, but they were unable to demonstrate significant uptake of soluble DNA. Borenfreund and Bendich (19) have recently produced convincing evidence for the incorporation of purified polymerized DNA from bacteria or human leucocytes into the DNA of growing HeLa cells. Similarly, the very recent work of Kay (20) indicates that Ehrlich-Littre ascites cells utilize DNA presented to them *in vitro*.

The most interesting question arising from the above studies is whether reutilization of DNA or its metabolites can occur at a significant level *in vivo*, and, if so, what is the sort and size of molecule transferred. To answer this question experiments were performed in which DNA-synthesizing tissues were grown *in vivo* in the presence of DNA previously tagged with radioactive thymidine.

MATERIALS AND METHODS

Three general classes of experiments utilizing an ascites tumor, Sarcoma I, and adult mice of either sex from the Ajax or C_{57}Bl strain were performed. The sarcoma was isologous to the Ajax strain and was supplied to this laboratory through the generosity of Professor R. Weiser, Department of Microbiology, University of Washington, Seattle.

1. *Transplantation of Sarcoma Cells to Hosts Previously Labeled with H^3-Thymidine*

Twelve mice (six from each strain) received a subcutaneous or intraperitoneal injection of 1 to 1.5 μc/gm body weight tritiated thymidine (TTH, Schwarz Laboratories, Mt. Vernon, New York, specific activity either 0.36 or 1.9 c/mM). Of these animals, four groups of two received intraperitoneal injections of 0.3 cc ascites sarcoma fluid (approximately 1.5×10^7 cells to each animal) at 1, 4, 18, or 24 hours after isotope administration. Smears were made of the sarcoma fluid at the time of transplantation and of peritoneal aspirates drawn from recipient animals at intervals of 1 to 54 hours after tumor introduction. Control smears corresponding in intervals to the experimental series were taken from an animal which received an injection of saline instead of isotope 18 hours before sarcoma transplantation.

One of the initial twelve labeled mice received 0.05 cc sarcoma fluid subcutaneously 3 hours after isotope administration. The tumor was allowed to grow for 24 hours before it was excised and teased for smear preparations.

Each of the final three mice had a Millipore diffusion chamber (21) containing 0.15 cc sarcoma fluid implanted in its peritoneal cavity 12 hours after isotope injection. These animals were sacrificed at 12, 24, and 48 hours after chamber implantation so that smears of chamber contents and of the inner surfaces of the diffusion membranes could be made.

Control and experimental smears were processed together for radioautography utilizing the liquid emulsion technique (21, 22). The coated slides were exposed 2 to 5 weeks and, after developing and drying, were stained 15 to 30 minutes at 5°C in 1 per cent Leishmann-Giemsa (1:3) pH 6.7. Finished radioautographs were evaluated microscopically and in some instances subjected to grain counts. Counts were accomplished by enumerating silver grains over one to two hundred sarcoma cells and then subtracting the number of background grains found in areas adjacent to and approximately the same size as the areas of the cells counted. The difference figure thus obtained was divided by the number of cells counted to yield an estimate of average net grains per cell (Fig. 5). Before counting the preparations, they were coded and mixed so that the author was unaware of whether an experimental or control smear was to be examined.

2. *Transplantation of H^3-Thymidine-Labeled Lymphocytes to Sarcoma-Bearing Mice*

One Ajax and one C_{57}Bl mouse received daily injections of 0.5 to 1 μc/gm body weight TTH amounting in total to 76 and 100 μc respectively. Two hours after their last injection they were sacrificed and cell suspensions were prepared by mincing the pooled spleen, thymus, and mesenteric lymph nodes of each animal. The cells were twice washed in cold Ringer's solution and then were suspended in homologous serum for injection directly into the ascites fluid of non-radioactive, sarcoma-bearing Ajax mice. One mouse received 9.3×10^7 and another received 1.6×10^8 nucleated cells. Smears were made of the transplanted lymphoid cells and of fluid obtained by peritoneal paracentesis from the recipient animals at 1, 3, 5, 7, 9, 11, and 24 hours after lymphocyte transplantation. These smears were

processed for radioautography as above except that two smears of each interval were saved for specific nuclease treatment prior to liquid emulsion coating. One smear of each interval was digested for 1 hour at 37°C with ribonuclease (Mann Research Laboratories, New York) in a concentration of 0.5 mg/cc in phosphate buffer pH 6.7. Another smear of each interval was treated for 1 hour at 37°C with deoxyribonuclease (Worthington Biochemical Corporation, Freehold, New Jersey) in a concentration of 0.5 mg/cc in phosphate buffer pH 6.7 which was 10^{-3} M in Mg^{++}. After enzyme digestion, the slides were washed in distilled water, dried, and radioautographed with untreated slides.

3. *Injection of H^3-Thymidine-Labeled Sarcoma or Lymphoid DNA into Tumor-Bearing and Normal Mice*

The biochemical methods of Kirby (23) were employed to isolate radioactive sarcoma and lymphoid DNA from tumor-bearing and normal TTH-injected mice. For this purpose a sarcoma-bearing mouse received two intraperitoneal injections totaling 40 μc TTH, and a normal mouse similarly received two injections totalling 33 μc. After isolation of the DNA from the sarcoma, or from the pooled spleen, thymus, and mesenteric lymph nodes of the normal animal, the DNA was freed of RNA by ribonuclease digestion (1 hour at 35°C in phosphate buffer pH 6.7 with RNase in final concentration of 0.3 mg/cc). Colorimetric assays (24, 25) were then employed to determine DNA content and confirm the absence of RNA. Half of each DNA sample was treated with deoxyribonuclease (conditions as described) until colorimetric assays showed that at least 95 per cent of the original polymerized DNA had been degraded. For injection the DNA and the DNase-digested samples were suspended in saline and made 10^{-3} M in Mg^{++}. Approximations of the amount of radioactivity in the purified DNA were made by assaying, 1, 5, and 10 μg aliquots in a windowless, gas-flow counter. The specific activity of the sarcoma DNA was found to be about sixty times greater than that of the lymphoid DNA.

Incorporation of the sarcoma DNA preparations was tested by making intraperitoneal injections of 100 μg of the purified DNA or an equivalent amount of the DNase-digested material twice daily in normal and tumor-bearing Ajax mice. Similarly, injections of 50 μg amounts of lymphoid DNA and digested DNA were made in normal and tumor-bearing animals. Smears of peritoneal aspirates were made for radioautography at daily intervals from all animals after the beginning of the injections.

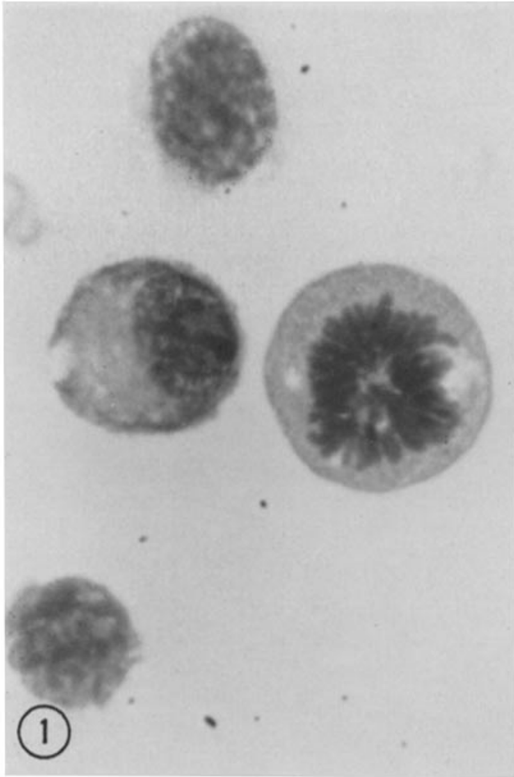
RESULTS AND DISCUSSION

Appearance of Radioactivity in Sarcoma I Transplanted to Hosts Previously Labeled with H^3-Thymidine

Figs. 1 to 4 depict the temporal appearance of nuclear radioactivity in sarcoma cells cultured in a mouse which was injected with TTH 18 hours before tumor transplantation. It is to be noted that labeled host lymphoid cells emerged to mingle with the tumor cells (Fig. 2) and that their appearance was followed by the gradual development of radioactivity in the sarcoma. Fig. 5 expresses graphically the rate, degree, and duration of labeling of tumor cells in this same experimental animal compared to a saline-injected control. The slope and area under the curve in Fig. 5 were found to be a function of the interval which separated the injection of the host with isotope and the transplantation of the sarcoma. Short intervals of 1 to 2 hours produced maximally steep slopes and large areas under the curve. Twenty-four hour intervals flattened the curve and shifted it to the right. The curve did not seem to be influenced by either the specific activity or the route of administration of the isotope.

In the most general sense, the appearance of radioactivity localized discretely in the nuclei of sarcoma cells was taken to mean that either the sarcoma had used host radioactive materials in synthesizing DNA or that previously labeled host cells had transformed to sarcoma cells. A selection between these alternatives was suggested by the marked difference in silver grain density in radioautographs of tumor cells compared to lymphocytes. In no case was a sarcoma cell observed with a grain density even approaching that which would have been expected if it had arisen by transformation of the usual well labeled host lymphocyte. That transformation was not occurring was further argued by other experiments (26) which demonstrated that the percentage of directly labeled sarcoma cells cultured in a non-labeled host did not decline in the first 48 hours. Such a decrease would have been expected if non-labeled host cells were transforming to sarcoma cells.

The evidence that the sarcoma was utilizing labeled host materials in synthesizing its DNA raised the question of the source of these substances. It did not seem that persistent unbound host TTH could have caused the labeling, for tumor transplantation was not undertaken until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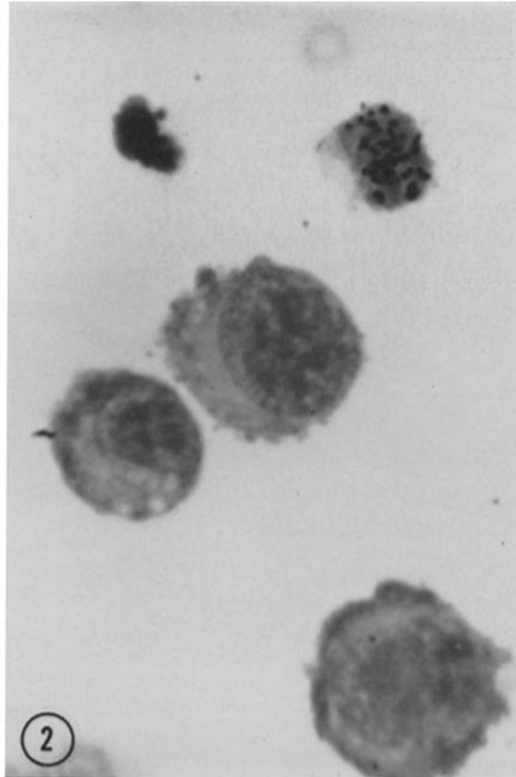
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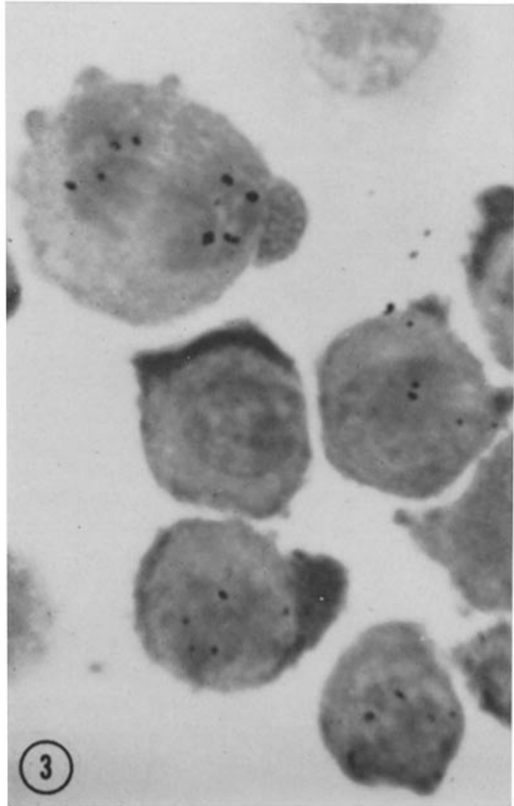
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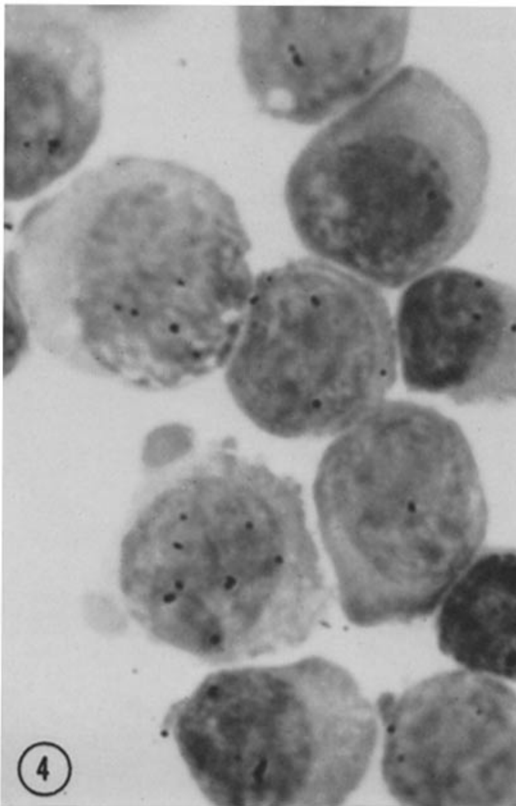
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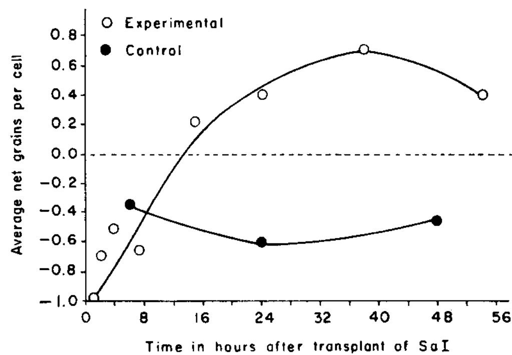


FIGURE 5

Appearance of radioactivity in Sarcoma I cells transplanted to a previously labeled mouse compared to sarcoma cells transplanted to a control animal. The experimental animal was injected with 1 μc/gm H^3-thymidine 18 hours before tumor introduction while the control received saline rather than isotope. See text (Materials and Methods) for description of grain counts represented here. The fact that the control curve remains negative rather than zero is a reflection of the fact that background reduction in radioautography is usually greater in areas where there are no cells than in areas directly over cells.

the availability time of TTH in the host had been exceeded by ½ to 24 hours (2, 3). The gradual increase in radioactivity in the sarcoma (Fig. 5) also argued against prolonged availability of TTH unless it were slowly being released from some unknown body pool. Furthermore, neither a subcutaneously implanted sarcoma (Fig. 6) nor one grown intraperitoneally in a Millipore diffusion chamber (Fig. 7) took up significant radioactivity from a previously labeled host. Tumors grown in these sites provided strongest evidence that neither persistent TTH nor, indeed, any freely circulating metabolite of it was the labeling substance. The capability of these latter tumors to utilize a circulating metabolite if it had been present seemed certain. The subcutaneous tumor grew rapidly, and intraperitoneal chamber tumors were easily labeled by TTH given intravenously as much as 40 hours after chamber implantation (Fig. 8).

As noted above, radioactivity was detected in sarcoma cells growing freely in the peritoneal cavity only after labeled host lymphoid cells had appeared and mixed with the tumor. Significant radioactivity was not found in a subcutaneous tumor but neither were any number of labeled host cells found. Similarly, label of any extent was not found in diffusion chamber sarcoma cells which were prevented from contacting radioactive host cells by the presence of the diffusion membrane. These findings strongly suggested that the substance effecting the labeling in the sarcoma was lymphocyte bound.

FIGURE 1

Radioautographic preparation of Sarcoma I cells 1 hour after transplantation to a mouse which had been injected with H^3-thymidine 18 hours before tumor introduction. The sarcoma contains no radioactivity although it is growing well (note metaphase). × 1440.

FIGURE 2

Radioautograph of Sarcoma I cells from the same experiment as Fig. 1, now 3 hours after transplantation. The sarcoma still has no detectable radioactivity but a host lymphocyte which has emerged is well labeled. Note unlabeled pyknotic host cell at upper left. × 1440.

FIGURE 3

Radioautograph of Sarcoma I cells from the same experiment as Figs. 1 and 2, but now 12 hours after transplantation. Note definite nuclear label on several sarcoma cells including the anaphase figure at upper left. × 1440.

FIGURE 4

Radioautograph of Sarcoma I cells from the same experiment as above, now 24 hours after transplantation. Many sarcoma cells now show distinct nuclear lable. × 1440.

Appearance of Radioactivity in Sarcoma I which Received Transplanted H^3-Thymidine-Labeled Lymphocytes

Transplantation of washed pooled thymus, spleen, and mesenteric node lymphocytes was undertaken from either isologous or homologous TTH-labeled donors to non-labeled, sarcoma-bearing recipients. These experiments proved to be critical in the demonstration of the cell-fixed character of the substance causing sarcoma labeling. The results (Figs. 9 to 11) were strikingly similar to those obtained with transplantation of sarcoma cells to previously labeled hosts (Figs. 1 to 4). In all cases the sarcoma gradually became radioactive. Specific nuclease treatment of the sarcoma was then employed to demonstrate that the labeling truly represented incorporation of material from lymphocytes into sarcoma DNA. Ribonuclease digestion did not remove the label in the tumor cells (Fig. 12), but deoxyribonuclease treatment did (Fig. 13). Lymphocytes undergoing pyknosis and karyorrhexis were frequently observed in these experiments as well as in those involving transplantation of the sarcoma to previously labeled hosts. The evidence of lymphocytolysis suggested that released radioactive DNA was the cell-fixed product responsible for the labeling observed on the sarcoma. Accordingly the capacity of biochemically purified, radioactive DNA to label DNA-synthesizing cells was investigated.

Appearance of Radioactivity in Normal and Malignant Cells after Injections of H^3-Thymidine-Labeled DNA

When biosynthetically labeled, purified, lymphoid DNA was injected into non-radioactive, sarcoma-bearing mice, the tumor cells incorpo-

FIGURE 6

Radioautographic preparation of Sarcoma I cells transplanted subcutaneously to a mouse which had been injected with H^3-thymidine 3 hours previously. This preparation was made after the sarcoma had grown for 24 hours and it may be seen that the cells do not contain radioactivity. $\times$ 1200.

FIGURE 7

Radioautographic preparation of Sarcoma I cells cultured 24 hours in a Millipore diffusion chamber which was implanted intraperitoneally 12 hours after the host was labeled with H^3-thymidine. The cells grew well, but did not become radioactive. $\times$ 1200.

FIGURE 8

Radioautograph of Sarcoma I cells cultured 40 hours in an intraperitoneal Millipore diffusion chamber. To test the viability of these cells, the host mouse was labeled for the first time by the intravenous injection of H^3-thymidine 30 minutes before this preparation was made. The incorporation of thymidine is marked. $\times$ 1440.

FIGURE 9

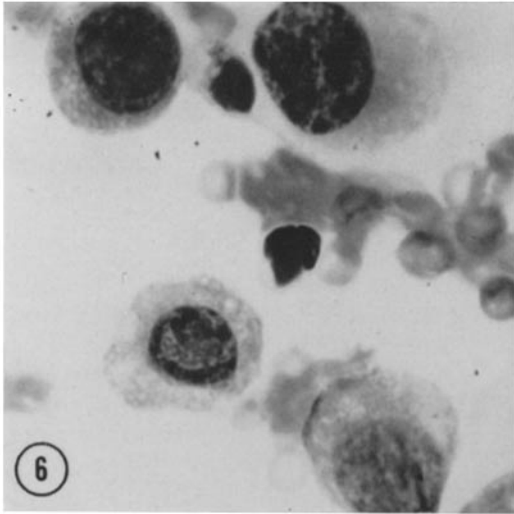
Radioautograph of Sarcoma I cells from a non-radioactive, tumor-bearing mouse which 1 hour previously had been injected with washed, H^3-thymidine-tagged lymphocytes. Note labeled lymphocytes, but absence of radioactivity on the sarcoma. $\times$ 1440.

FIGURE 10

Radioautograph of Sarcoma I cells from the same experiment as Fig. 9, now 5 hours after lymphocyte introduction. Note the labeled transplanted lymphocyte (upper right) and the beginning appearance of radioactivity in the sarcoma. $\times$ 1440.

FIGURE 11

Radioautograph of Sarcoma I cells from the same experiment as in Figs. 9 and 10, but now 24 hours after radioactive lymphocyte transplantation. Sarcoma cells now frequently show distinct nuclear label. $\times$ 1440.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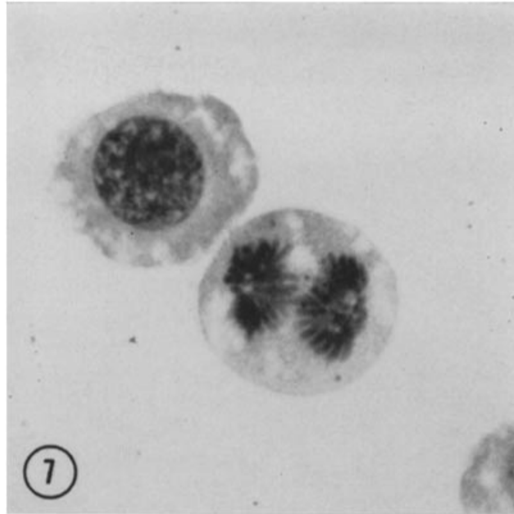
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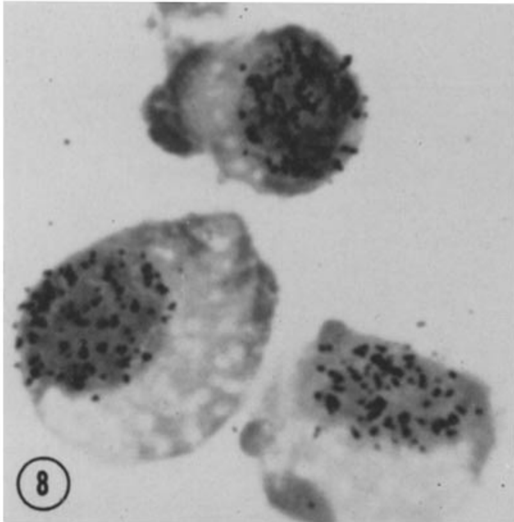
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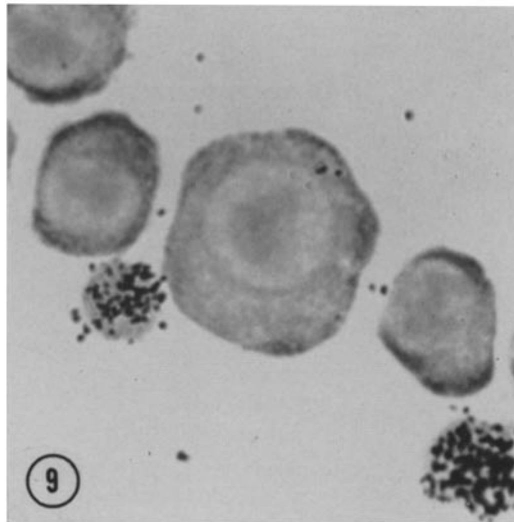
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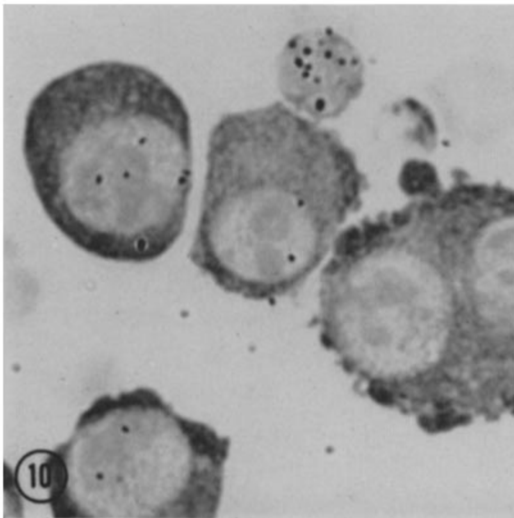
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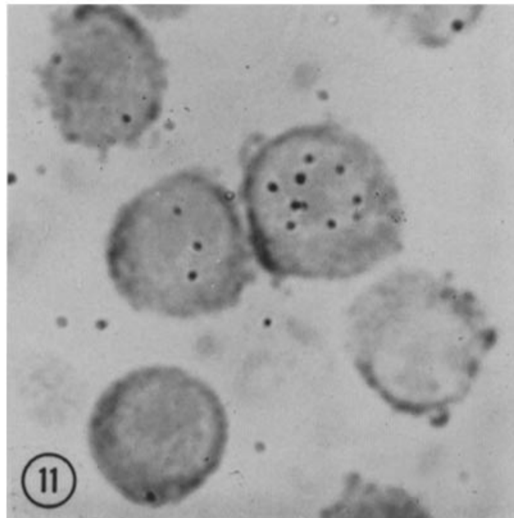
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rated the label into their DNA (Fig. 14). Similar results were obtained with labeled sarcoma DNA presented to sarcoma cells (Fig. 15). The appearance time, degree, and duration of radioactivity in these sarcoma cells was very similar to that observed in the earlier experiments (Fig. 5).

Whether normal as well as neoplastic cells would utilize DNA was then studied in peritoneal mononuclear cells recovered from normal mice which had received radioactive DNA intraperitoneally. TTH-labeled, isologous lymphoid DNA caused radioactivity to appear in several cell types, particularly in cells resembling large undifferentiated blasts and in lymphocytes (Fig. 16). Labeled sarcoma DNA also was utilized by many normal cells, but apparently especially by macrophages (Fig. 17). Approximately 1 per cent of the lymphocytes and macrophages were labeled by the injected DNA, but the differing specific activities of the lymphoid and sarcoma DNA made it impossible to compare the degree of uptake of each. The demonstration of incorporation of injected DNA is in good correlation with the earlier studies of Hudnik-Plevnik and coworkers (27) who followed the fate of P^{32}-labeled spleen DNA injected intraperitoneally.

A striking observation worthy of emphasis was that polymerized DNA from any source was much more effective in labeling normal or neoplastic cells than was a DNase digest with the same radioactive and nucleotide content. Intact DNA molecules labeled more cells and labeled them more heavily than did the same quantity of DNA which had been hydrolyzed by specific nuclease treatment.

The presentation of equal quantities of radio-

FIGURE 12

Radioautograph of Sarcoma I cells from the same experiment and interval as Fig. 11, but digested with ribonuclease before radioautographic processing. Note from the cell in the upper right that RNase did not remove the nuclear label. × 1440.

FIGURE 13

Radioautographic preparation of Sarcoma I cells from the same experiment and interval as Fig. 11, but digested with deoxyribonuclease before radioautographic processing. Note that nuclear radioactivity is entirely removed. × 1440.

FIGURE 14

Radioautograph of Sarcoma I cells from a mouse which had been injected with H^3-thymidine-tagged isologous lymphoid DNA 22 hours previously. Note uptake of radioactive DNA into the nucleus of the sarcoma cell in the center. × 1440.

FIGURE 15

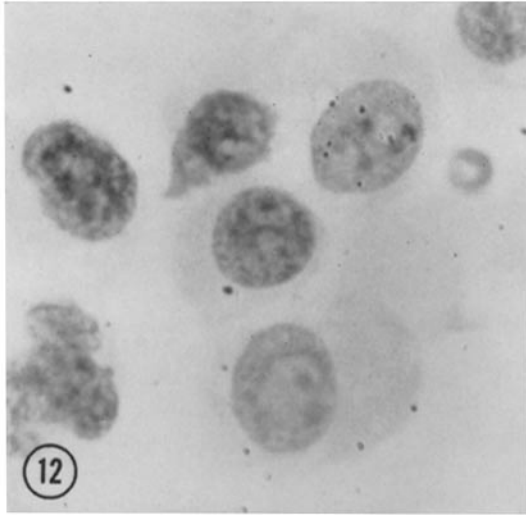
Radioautograph of Sarcoma I cells from a mouse which had been injected with H^3-thymidine-tagged sarcoma DNA 4 hours previously. Note uptake of radioactive DNA into the nuclei of the tumor cells. × 1440.

FIGURE 16

Radioautograph of a peritoneal mononuclear cell (right) and lymphocyte (left) taken from the peritoneal cavity of a normal mouse which had been injected with H^3-thymidine-tagged, isologous lymphoid DNA 24 hours previously. Note uptake of radioactive DNA into the nuclei of these cells taken from different fields on the same slide. × 1440.

FIGURE 17

Radioautograph of peritoneal macrophages taken from a normal mouse which had been injected with H^3-thymidine-tagged sarcoma DNA 67 hours previously. Note uptake of radioactive DNA into the nucleus of the cell on the right. × 1440.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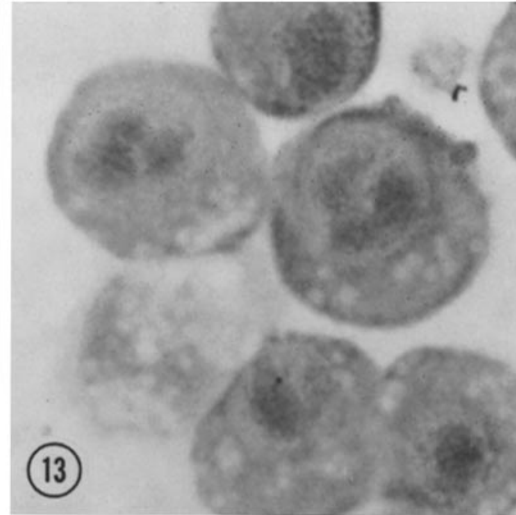
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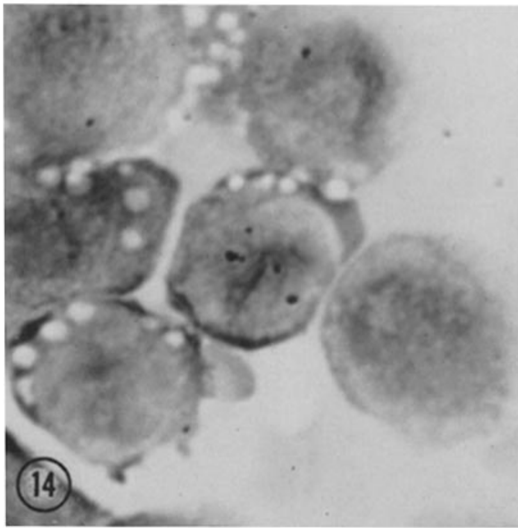
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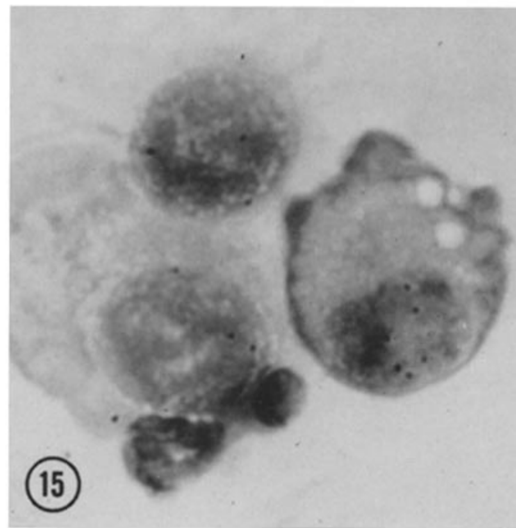
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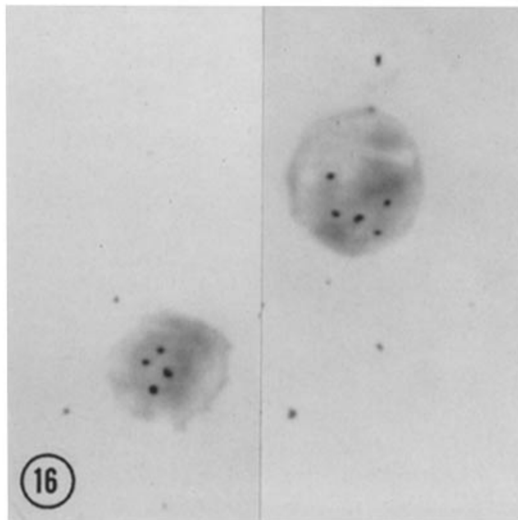
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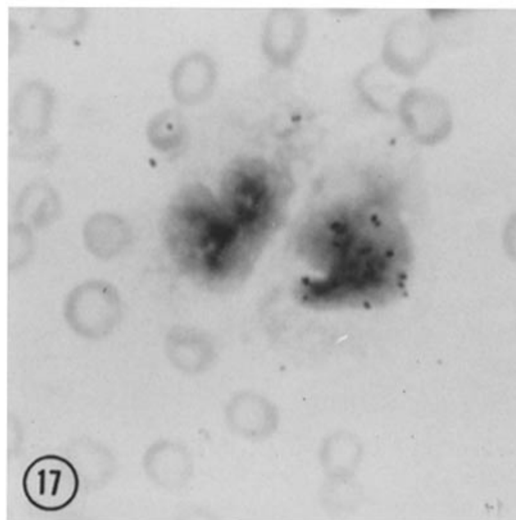
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activity in such different molecular forms provided direct evidence that labeled cells resulted from actual incorporation of labeled materials and not from exchange of tritium atoms between labeled and unlabeled molecules. The stability of tritium on thymidine in DNA has not been seriously challenged, but has been little more than a well based assumption (2).

Conditions and Significance of Reutilization of DNA and Its Metabolites

The observations above left little doubt that both neoplastic and normal cells were capable of significant reutilization of DNA and its metabolites. Relatively precise identification of the conditions under which reutilization occurred seemed, then, of obvious importance. Accordingly, the following four characteristics of reutilization were developed from the experimental data.

In the first place, reutilization was noted only when labeled host cells (mainly lymphocytes) had direct access to unlabeled (sarcoma) cells. Only after direct cellular interplay was extensive lymphocytolysis with release and subsequent incorporation of labeled DNA observed. The incorporation of the released DNA probably was achieved by pinocytosis. Incorporation did not seem to occur by phagocytosis as reported in lymphoid reticulum cells by Trowell (10) and in strain L cells by Bensch and King (18), for phagocytosis of intact cells or gross amounts of DNA was only very rarely observed. Furthermore, the minimal labeling observed in sarcoma cells growing among very heavily labeled lymphocytes argued against frequent phagocytosis of large bits of radioactive debris. Why lymphocytes were induced to die and release their nucleoproteins so soon after transplantation of an isologous tumor was unclear. Medawar (28), of course, has postulated that lymphocytes may function as vectors of nucleic acids.

In the second place, only cells which were actually synthesizing DNA incorporated labeled DNA or its metabolites. All of the cell types labeled by reutilization in this study (*viz.* sarcoma cells, macrophages, large mononuclear cells, and lymphocytes) were known to be capable of DNA synthesis as judged by the ability to use TTH (21, 26). Hill and Drásil (12), however, reported finding labeled mature neutrophils in lethally irradiated mice injected with P^{32}-tagged thymocytes. The neutrophil in their study probably was not capable of DNA synthesis, which does not occur in this cell series after the last myelocyte division (2, 29). It may be that labeled nuclear debris from injected thymocytes was incorporated just prior to this division, but the fact that at least some of the label was cytoplasmic instead of nuclear makes it more probable that incorporation was by phagocytosis. The phagocytic properties of neutrophils are well known, and the radiation given the recipient animals possibly enhanced this process.

Thirdly, reutilization of labeled DNA materials was most easily detected when short intervals separated the injection of an animal with TTH and the introduction of an unlabeled, DNA-synthesizing tissue. Although it was clear that radioactive, polymerized DNA was reutilized in some of the above experiments, it was probable that labeled DNA precursors were also reutilized. When the sarcoma was transplanted to hosts which had been labeled only a few hours previously, a high percentage of labeled lymphocytes emerged to meet the sarcoma. These lymphocytes probably contained large amounts of labeled DNA precursor substances (anabolites). Indeed, Crathorn and Shooter (30) have shown that 1 hour after the presentation of TTH to (ascites) cells *in vitro* the majority of cell-fixed radioactivity is not in DNA, but is in DNA precursors (mono-, di-, and triphosphonucleotides). The release of such anabolites by lysis of recently labeled lymphocytes probably provided a readily available pool of radioactive DNA precursors.

In the fourth place, reutilization was best demonstrated when it was the consequence of acutely increased metabolic demand placed on a labeled host. For example, transplantation of the usual inoculum of tumor cells (1.5×10^7) increased the number of cells to be supported by a 20 gm mouse in a manner roughly equivalent to adding abruptly 7 per cent to the total number of bone marrow cells synthesizing DNA (31). That the effect of transplanting the sarcoma was metabolic rather than "toxic" was concluded from the observation of nearly equivalent reutilization of DNA by tumors transplanted to either isologous or homologous hosts. In fact, even in previously immunized, homologous hosts, the sarcoma incorporated radioactivity as long as it lived.

The data suggested that the molecules reutilized under the conditions above included both DNA and its metabolites. Probably the degree of incorporation of each was dependent on the type of

experiment and the chemical form in which labeled materials were presented. Evidence for the uptake of anabolic DNA substances has been cited above, and that catabolites similarly were available was considered probable from reports that ascites cells contain DNase (32) and release at least certain of their enzymes into their growth medium (33).

The evidence for incorporation of relatively in tact DNA derived from the observation that polymerized, radioactive DNA effected greater labeling than did equal quantities of DNase-digested material. The indication that intact DNA was incorporated in some experiments gained support from the studies of Borenfreund and Bendich (19) who employed an antibody against DNase to demonstrate the incorporation of polymerized DNA by HeLa cells *in vitro*. Kay (20) has reported that once DNA is incorporated it may be utilized without intracellular degradation. If large molecules were incorporated and utilized intact in some of the above experiments, genetic change should eventually be detectable. Such a change has been reported by Kurita and coworkers (34) who transferred alanine-nitrogen mustard resistance to Yoshida sarcoma cells in a DNase, gene-specific reaction. Furthermore, the very recent studies of Kraus (35) have shown that altered hemoglobin synthesis may be induced in human bone marrow by incubating it with homologous DNA *in vitro*.

The significant conclusion drawn from this work when considered with earlier studies (8–11) is that reutilization of DNA and its metabolites definitely occurred *in vivo*, and that the kind of labeled lymphocyte here observed played a central role in the process. The ease with which reutilization was demonstrated was a function of the amount and specific activity of available radioactive DNA (or DNA products), the cell type studied, and the metabolic demands placed on the labeled host. The failure of earlier workers (1) to demonstrate reutilization most likely was a consequence of the low specific activity of the tracer employed.

It is a pleasure to thank Mrs. Shirley Weber for her competent technical help in many phases of this work and Mrs. Ruth Caffrey for her advice and assistance in biochemical techniques.

This study was supported by funds from an institutional cancer research grant of the American Cancer Society and from the National Institutes of Health, United States Public Health Service, Grant RG-6309.

Received for publication, October 16, 1961.

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