Metabolism of the DBA Mouse Ascites Thymoma

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INTRODUCTION

In preparation for an investigation of protein synthesis by the cells of a mouse ascites thymoma and the energetics related to such synthesis, we wished to establish the metabolic characteristics of this tumor as a basis for the projected work. The tumor we employed was a transplantable lymphoma which arose originally as a tumor of the thymus in a DBA 212 mouse at the National Cancer Institute.1 Previous study of this tumor has been primarily concerned with the cultural growth characteristics of free tumor cells in the peritoneal fluid (4) and with their average nucleic acid content per cell as correlated with their capacity for growth (3).

In conjunction with the establishment of the metabolic quotients of the lymphoma (QO2, QCO2, Qlact, QGU, QEU, and R.Q.), we investigated the relative amounts of pentosenucleic acid (PNA) and desoxypentosenucleic acid (DNA) of the tumor cells at various age levels of the tumor. The most convenient way to have tumors of similar "age" is to use tumor cell suspensions after the same number of days' incubation of comparable inocula in the peritoneal cavities of the host mice maintained under standardized conditions. In our experimental work, groups of about 25 animals were inoculated uniformly with an ascites thymoma cell suspension, and at certain times covering the period 4—14 days after inoculation, four to six animals were sacrificed, the ascites tumors pooled, and studied. While our experience with many generations of this tumor has indicated that a given chronological age does not necessarily assure a definite "biological age," we have observed in appropriate experiments a consistent variation in some metabolic activities of the tumor with its increasing chronological age. That such variation does occur is of considerable interest in itself and of importance for contemplated biochemical and physiological studies with this tumor.

Tiedemann (14) determined several metabolic quotients of washed Ehrlich ascites tumor cells in salt solutions. He found that the cells had high glycolytic capacities and that glucose had an inhibitory effect on the respiration. Warburg and Hiepler (16) found that the metabolic quotients of the Ehrlich tumor when measured in ascitic fluid were even higher than those obtained by Tiedemann in salt solution. Kun, Talalay, and Williams-Ashman (7) have reported on the metabolism of the Ehrlich ascites tumor. They used washed tumor cells of unspecified age, freed of the ascitic fluid, in their determinations. We have employed both washed and unwashed cells of the DBA thymoma for comparative purposes. Because we believe the metabolism of the unwashed cells in ascitic fluid represents more nearly that of cells present in the peritoneal cavity of the host, most of our work has been done with such cells. A preliminary report of some of this work has been presented (8).

MATERIALS AND METHODS

The DBA thymoma was maintained by repeated transfers on an ascites tumor in the DBA/2 male mouse. All attempts to carry the tumor in the DBA/1 failed, while in line 2 all transfers were successful. Ordinarily, the inoculum was 0.2 ml. of an ascitic cell suspension obtained from a 7-10-day-old tumor. The number of cells thus inoculated into the peritoneal cavity of the mouse varied between 35 and 50 million. The mice were kept at 27° C. ± 2° C. and were fed a regular diet of laboratory chow (Derwood Mills) and water ad libitum.

For the manometric tests, the ascites cell suspension was used directly in ascitic fluid, in Krebs-
Ringer phosphate (K-R.p.), or in Krebs-Ringer bicarbonate (K-R.c.) media (15). To each milliliter of the cell suspension was added 30 µg of heparin. As short a time interval as possible was allowed to elapse between the time of removal from the peritoneum of the cell suspension and the first manometric reading of its metabolic activity. This time interval seldom exceeded 30 minutes. When washed cells were used, they were prepared by diluting the ascites cell suspension in about 5 times its volume of 0.9 per cent NaCl solution (saline), centrifuging at 2°-4° C. in a Servall SS-1 centrifuge at 55 v. (approx. 2,200 X g) for 5 minutes, and resuspending the cells in a volume of saline roughly equal to 6 times the original volume of cell suspension. After recentrifuging under similar conditions, the loosely packed cells were diluted to a given concentration in the desired medium and used immediately.

The dry weight of cells in a tumor suspension was obtained by washing a known volume of cells in the manner described above, making the cells up to a known volume with saline, drying an aliquot of the washed cell suspension to constant weight at 105° C., and correcting for the weight of NaCl in the suspending liquid. For the dry weight determination, aliquots of cells were taken off prior to, rather than after, incubation.

For the determination of cell number, a measured volume of the ascites tumor cell suspension was diluted in a suitable volume of physiological saline and lightly stained with gentian violet. All counts were then made on the diluted suspension in a hemocytometer.

The volume of cells/ml of cell suspension was obtained by centrifuging 1 ml of the suspension in a centrifuge tube with a graduated capillary tip, in an International No. 1 centrifuge at 1,600 X g for 15 minutes. The cell volume/ml divided by the number of cells/ml gave the average volume per cell.

The turbidity of a cell suspension was determined as follows: 0.1 ml of cell suspension was diluted with 15 ml of saline and read in a Pfaltz and Bauer fluorophotometer which had been set to give a galvanometer reading of 50.0 with a Pfaltz R.R.p. buffer (pH 7.4) plus glucose. The turbidity determinations were usually made in the conventional one- or two-armed Warburg respirometer vessels with KOH in the center well and K-R.p. buffer (pH 7.4) or ascitic plasma plus glucose (which was decarbonated and adjusted to pH 7.4 by careful addition of H3PO4) as diluents of the tumor cell suspension in the main compartment. To assess the effect on the QO2 of a CO2 pressure more nearly representative of the conditions obtaining in the peritoneum itself than those established by the KOH in the experimental vessel, we determined the QO2 with Pardee's solution (11) in the center well. The CO2 in the gas phase was maintained at approximately 2 per cent. The Pardee solution was stabilized against autoxidation by addition of thiourea, as recommended by Krebs (6). The control thermobarometer was set up with Pardee solution in its center well to correct for the residual autoxidation which did take place.

The QO2 values were ordinarily determined by Warburg's direct method in which the gas phase was 95 per cent N2 and 5 per cent CO2 and the suspending fluid was either the ascitic plasma or bicarbonate buffer (K-R.c.) plus glucose. When ascitic plasma was used, correction was made for CO2 retention, usually by multiplying the observed manometric change in millimeters by four-thirds.

The QO2 and QCO2 values were determined by means of the Barker and Summerson lactic acid method as outlined in Umbreit (15). Incubation of the tumor cell suspension with glucose was carried out in undiluted ascitic plasma or in ascitic plasma diluted with 4 times its volume of Krebs-Ringer carbonate. The gas phase was 5 per cent CO2 plus 95 per cent O2 or 95 per cent N2, and the duration of incubation was 40-60 minutes. These quotients were also determined for washed cells in K-R.c. plus glucose.

R.Q. and QO2 values for the thymoma suspension in ascitic fluid diluted 1:1 with K-R.c. plus glucose were obtained through use of Summerson differential manometers (13). Concurrent QO2 determinations were also made.

The nucleic acids were extracted from the cells with hot trichloroacetic acid, after washing with saline and thorough extraction with cold trichloroacetic acid to remove acid-soluble nucleotides (18). Pentosenucleic acid (PNA) was determined colorimetrically through the use of von Euler's phloroglucinol reaction (9) and desoxypentosenucleic acid (DNA) by Duchè's diphenylamine procedure (1). The total nucleic acids so obtained agreed well with the total nucleic acid concentration obtained through measurement of the optical absorption at 260 mµ.

RESULTS

PHYSICAL AND CULTURAL CHARACTERISTICS OF THE CELL SUSPENSION

Examination of the course of the development of the ascites tumor with respect to cell weight, cell number, and cell volume, all per milliliter of suspension, gave the results expressed in Chart 1.

It will be seen that the cell dry weight/ml of suspension reached a maximum about 6 days after inoculation, and then declined. The number of cells/ml simultaneously reached a maximum and
then declined, but the changes were not so great as those in dry weight. As a consequence, the weight per cell declined with time after reaching a maximum. The volume per cell changed in a manner similar to the weight per cell. The cell suspension was either free of blood or slightly hemorrhagic.

Within about 16 days after inoculation, most of the animals were dead, although some lived as long as 3 weeks. It is of interest that intraperitoneal inoculation of a small amount of blood from the heart or tail vein of an infected animal gave rise to a regular ascites thymoma within about 2 weeks.

METABOLIC QUOTIENTS

Anaerobic glycolysis (Q_{Glu}^o and Q_{lactic}^o).—In the absence of added glucose, an ascitic tumor cell suspension diluted 1.5 with K-R.c. in equilibrium with 95 per cent N₂ and 5 per cent CO₂ produced little CO₂ (Q_{Glu}^o = 1–2). With 1 per cent glucose, however, the glycolytic rate of such tumor cells was high in a similar medium and in 100 per cent ascitic plasma. Comparisons between the glycolytic rates of the same tumor suspensions in K-R.c.-diluted and 100 per cent ascitic plasma showed no significant or consistent differences attributable to the different media. The Q_{Glu}^o values found with cell suspensions from tumors 4–14 days old varied from 33 to 83.

In any given series in which tumors were studied over a period of days, the glycolytic Q values increased with increasing age of tumor. For example, in three different series the following values were found: 39 and 73 for a 4- and 7-day-old tumor, respectively; 33 and 56 for a 9- and 13-day-old tumor, respectively; and 49 and 81 for a 6- and 11-day-old tumor, respectively. It is clear that the absolute Q values for any given age of tumor from different series vary considerably. Chemical lactic acid determinations gave Q_{lactic}^o values which were in accord with the manometric Q_{Glu}^o values.

A few experiments using washed cells suspended in K-R.c. gave Q_{Glu}^o and Q_{lactic}^o values similar to those in the unwashed cells in K-R.c. and ascitic plasma.

Aerobic glycolysis (Q_{Glu}^{ab} and Q_{lactic}^{ab}).—Aerobic glycolysis of 1 per cent glucose by the ascites thymoma was determined in ascites plasma and in ascitic plasma diluted 1:5 with K-R.c. The gas phase was 95 per cent O₂ and 5 per cent CO₂. The Q_{Glu}^{ab} values observed varied from 8 to 28 and were found to increase with increasing age of the tumor, as did the anaerobic Q_{lactic}^o values. A typical series gave figures of 8.4, 16, 21.3, and 27.9 for the Q_{Glu}^{ab} of ascitic cell suspensions 6, 8, 11, and 13 days after inoculation of the host mice. In the experiments where Q_{Glu}^{ab} was determined manometrically by Summerson differential manometers, there was correspondence with the Q_{Glu}^{man} so that all the acid produced under aerobic conditions by these thymoma cells was lactic acid. The aerobic glycolysis of any given tumor cell suspension was only 30–50 per cent of the anaerobic glycolysis of the same cells.

Washed thymoma cells in K-R.c. plus 1 per cent glucose showed aerobic glycolytic activity similar to those of unwashed cells and of a value 30 per cent of their anaerobic glycolytic activity.

Respiration (Q_{o_2}).—The Q_{o_2} of unwashed tumor cells suspended in ascitic plasma and in ascitic plasma diluted fivefold with K-R.c. or K-R.p., in the presence or absence of glucose, was found to vary between 10 and 16 in air and in oxygen. The average value was 13. No consistent difference was observed in the Q_{o_2} values determined in the presence of physiological concentrations of CO₂ or in its virtual absence. Furthermore, in contrast to the anaerobic and aerobic glycolysis of the ascites thymoma, the respiration did not increase or change consistently with increasing age of the tumor.

As a consequence of the fact that the absolute values of Q_{Glu}^{ab} and Q_{Glu}^{man} rose as the age of tumor increased, with the ratio between them remaining essentially constant, the absolute Pasteur effect increased. Since the Q_{o_2} did not rise proportionately, the Meyerhof quotient (Q_{man}^o - Q_{man}^{ab}/Q_{o_2}) also increased with advancing age of tumor. For example, a 6-day-old tumor showed a Meyerhof quotient of 27.7 - 8.4/12.1 or 1.6, while the quotient of the 11-day-old tumor was 64.3 - 21.3/18.2 or 3.26.

Kun et al. (7), working with washed cells of the Ehrlich ascites carcinoma, found that the rate of oxygen uptake by those cells in 100 per cent oxygen was invariably depressed 40–60 per cent by glucose over a concentration range of 0.4–1 per cent. Using washed cells of the ascites thymoma suspended in K-R.p. in an atmosphere of oxygen, we tested the effect of 0.25 per cent, 0.5 per cent, 1.0 per cent, and 2.0 per cent glucose on the Q_{o_2}. No inhibitory effect of glucose on the oxygen uptake by these tumor cells was observed.

The respiratory quotients (R.Q.) of thymoma cell suspensions were determined in ascitic plasma diluted 1:1 with K-R.c. in an atmosphere of 95 per cent O₂ and 5 per cent CO₂ with Summerson differential manometers. The results show that the R.Q.'s of young tumors (4–6 days) range from 0.82 to 0.85, while those of old tumors (10–14 days) rose to values varying from 0.88 to 0.93.
NUCLEIC ACID

Since the preceding data have indicated that there is a variation with age of some metabolic activities of the ascites thymoma, it was of interest to examine the tumor cells for a possible corresponding variation in their nucleic acid content. To this end, the concentrations of PNA and DNA in these cells as a function of the time after inoculation were determined. Chart 2 illustrates the variation of PNA concentration of the ascites cells with time after inoculation of the tumor, the PNA content being expressed as μg PNA/cell and as percentage of the dry weight of the cells.

It will be seen that the percentage PNA reached a maximum at about the same time as, or just slightly later than, the time of maximum cell population (6th–7th day, as shown in Chart 1), and then declined by about 25 percent. As a consequence of the decline in the percentage of PNA and the concurrent decrease in cell size and weight (cf. Chart 1), the PNA per cell dropped even more markedly.

The percentage of DNA also changed with the age of the tumor. In one case the percentage of DNA rose from an initial value of 10 per cent to 12 per cent at the 6th day and then declined to about 10 per cent after 13 days. During a comparable period of time, the tumor cells used to obtain the data of Chart 3 showed a steady rise from 11 to 15 per cent. It is likely that longer observation would have revealed a decline in this case also. However, in all cases the cyclic increase and decrease in cell size resulted in a corresponding rise and fall in DNA per cell. In the case illustrated in Chart 3 the maximum was $11.3 \times 10^{-4}$ μg/cell, at 6 days, while at 13 days the value had fallen to $8.8 \times 10^{-4}$ μg/cell.

* For the purpose of enabling direct comparison, the series of experiments chosen for presentation of the nucleic acid data is the same series that was used for the data of Chart 1.

DISCUSSION

The regular variation in DNA per cell found here is not necessarily inconsistent with the rather widely held view that the DNA content per nucleus is constant for a given species, because that hypothesis was developed primarily for resting tissues, while we are here dealing with a growing tissue. If the period of time required by the cell in preparation for and completion of mitosis—during which period the DNA concentration increases—were of suitable length (2–4 hours), then a changing percentage of cells preparing to divide or in division over the period of observed tumor growth could account for our cyclic changes in average DNA per cell. On this basis, of course, one would expect maximum mitotic activity when the DNA per cell is maximal, i.e., 6–8 days after inoculation (Chart 3). That the cells are undergoing maximum protein synthesis at this time is suggested by the fact that the PNA per cell is highest then (Chart 2).

Klein (5) determined the nucleic acid concentration of a group of ascites tumors without particular attention to the length of time after inoculation. He found that his ascites thymoma cells did not differ in size among themselves nor from normal lymphocytes. He also found no deviation in DNA per cell from the value usually given for normal mouse somatic cells. Our lymphoma ascites cells, on the other hand, varied in size during their growth cycle (Chart 1), and during at least part of the cycle had higher than normal DNA per cell. Our lymphoma cells, therefore, are more like Klein's nonlymphoma ascites tumors, in which he also found increased cell size and high DNA per cell.
Since the changes in dry weight of cell substance/ml of cell suspension (Chart 1) are largely attributable to the host's production of ascitic fluid, one cannot assume that the time when one finds the maximum cell substance/ml is the time of maximum cell reproductive rate. The difficulty of removing all the cells from the peritoneal cavity makes hard the determination of total cell weight as a function of time after inoculation. However, the combination of circumstantial evidence presented in Charts 1, 2, and 3 makes it plausible that the ascites tumor cell suspension undergoes a period of rapid growth followed by a decline in growth rate, not unlike that found in microorganisms. (For a comparison of nucleic acid concentration and cell growth rate in micro-organisms see [9].)

A number of differences between the DBA thymoma and the Ehrlich carcinoma are worth noting. Tiedemann (14) and Kun et al. (7) reported that glucose inhibits the respiration of washed Ehrlich ascites carcinoma cells. We have repeated and confirmed this with both washed and unwashed Ehrlich carcinoma cells. We found, however, no such effect with the thymoma. Further, Tiedemann found, with the Ehrlich tumor, no change in the glycolytic activities as a function of the number of days after inoculation. With the thymoma, we observed very definite increases in glycolysis as the tumor grew older. Marked differences in the aldolase activity of the blood plasma and ascitic fluids of animals bearing these tumors have been reported elsewhere (10). Whether or not these differences are reflections of any fundamental metabolic differences between these two tumors remains to be determined.

SUMMARY

Some metabolic characteristics of an ascites thymoma in DBA mice, as a function of the number of days after inoculation of the tumor into the host, have been determined. The weight/cell, volume/cell, and cell dry weight/ml of cell suspension rose to a maximum 6–8 days after inoculation and then declined. The amount of PNA/cell and of DNA/cell also showed a cyclic rise and fall. The R.Q., Q20, and Q25 increased with age, the latter reaching values as high as 83. The Qo remained essentially constant during the course of the growth of the tumor. It is suggested that the ascites tumor undergoes a period of rapid proliferation, followed by a decline in growth rate.

REFERENCES


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