The Growth and Transplantability of the Carcinosarcoma of Walker 256 in the Ascitic Form

There are many problems on the dissemination of cancer cells in the blood which could be further investigated in laboratory animals by using the proper tumor and proper techniques. For example, problems such as the relationship of the presence and persistence of cancer cells in the circulating blood to the production of metastases, chemotherapy, transplantation by means of single cells, chromosome cytology and histocompatibility and multiplication of tumor cells.

In recent years cancer cells in the blood have been demonstrated in experimental animals[1-3]; and in humans[4-6]. The nature of these cancer cells is not clear, some of them are thought to be bone marrow cells such as megacaryocytes[7-9].

For these and many other reasons in 1956 we transferred the carcinosarcoma of Walker 256 from the solid to the ascitic form; and since then it has been maintained in our Institute by serial intraperitoneal passages.

This tumor developed spontaneously in the region of the mammary gland in a pregnant albino rat in 1928 in the laboratory of George Walker at the Johns Hopkins University School of Medicine. The mass was a size of a pecan. On sections, a portion was composed of a firm, granular, greyish-red tissue with a small foci of necrosis. There was no metastatic growth[10].

A 15-day-old subcutaneous transplant is described as firm, round, lobulated, and partially encapsulated. There is invasion of the surrounding connective tissue and muscle and occasionally of the vertebral and spinal cord. On sections, a central area of semisolid, yellowish white, necrotic material surrounded by a zone of firm, white, glistening, viable tumor growth is generally present. Metastatic growth of the tumor is regularly found in the regional lymph nodes and occasionally in the lungs[10].

Material and methods. The Walker 256 carcinosarcoma in the solid form was obtained from Dr. K. SUGIURA of our Institute[11]. This tumor was composed of 3 types of cells; 2 of the 3 types form an independent pattern. The 2 architectural patterns, carcinoma and sarcoma may stand alone or may form a mixed type of growth, a carcinosarcoma.

As a subcutaneous solid transplant the tumor became of a palpable size 1 week following s.c. inoculation and killed the animal within 6 weeks, at which time it measured 4–5 cm in diameter.

The tumor was removed from a donor rat using aseptic technique and placed in a Petri dish with 0.85% NaCl (Figure 1). The excess necrotic material was then discarded and about 10 g of healthy tumor tissue was cut into small pieces, and dropped into a sterile 35 ml glass homogenizer (No. 3 Klopp, New York). 10 ml of sterile saline was then added to the tumor tissue and it was homogenized for 3–4 min. After this procedure the suspension was returned to a sterile bottle, a cell count was made in a white blood cell pipette by making a 1:20 dilution with 0.85% NaCl to which acetocarmine solution was added to stain the cells. A Neubauer hemacytometer was used to obtain the cell counts. At least 2 determinations were made each time.

Mitotic chromosome counts of the tumor cells were made after the cells had been fixed and stained with aqueous acetic acid and aceto-orcein. The mitotic index was determined after counting 500 or more cells. Photomicrographs of the stained cells were taken.

One ml of the homogenate containing about 5 million cells was inoculated i.p. into each of 10 young rats (C. River) weighing about 80–100 g. The i.p. inoculation of fluid and cells never exceeded 2 ml. The tumor in the ascitic form became apparent in all animals after 6–7 days and killed the host within 2 weeks. The fluid was of a dark red color, it contained 6–8 million cancer cells/ml. Ten young rats were inoculated i.p. with this fluid; and about 10 days later all the animals developed ascites.

1 D. AGOSTINO, C. E. GROSSI and E. E. CLIFFTON, J. natn. Cancer Inst. *27*, 17 (1961).
2 C. E. GROSSI, D. AGOSTINO and E. E. CLIFFTON, Cancer Res. *20*, 605 (1960).
3 E. E. CLIFFTON and D. AGOSTINO, Surgery *50*, 395 (1961).
4 W. H. COLE, G. O. MCDONALD and S. S. ROBERTS, Jl. Coll. Surg. Edinb. *4*, 218 (1959).
5 S. S. ROBERTS, O. JONASSON, L. LONG, E. A. MCGREW, R. MCGRATH and W. H. COLE, Cancer *15*, 232 (1962).
6 A. A. SANDEMBERG and G. E. MOORE, J. natn. Cancer Inst. *19*, 1 (1957).
7 M. MELAMED, E. E. CLIFFTON and S. H. SEAL, Am. J. clin. Path. *37*, 381 (1962).
8 V. TRAYKOVICH and M. R. RIVIERE, Cancer *19*, 1717 (1966).
9 J. F. JACKSON, Cancer *15*, 259 (1962).
10 H. L. STUART, K. C. SNELL, L. J. DUNJAM and S. M. SCHLEYN, *American Registry of Pathology* (Armed Forces Institute of Pathology, Washington D.C. 1959).
11 K. SURIURA, in *Method of Animal Experimentation* (Ed. W.I. GRAY; Academic Press, New York 1965), vol. 2, p. 171.

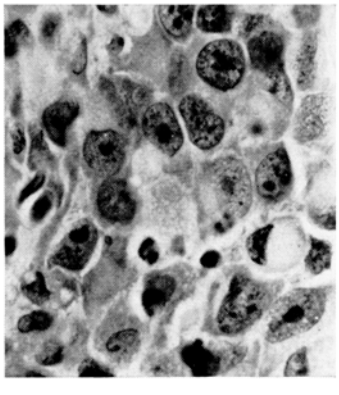

Fig. 1. Microphotograph of solid form of transplant tumor. This area is composed chiefly of principal cells. Interspersed among these are several undifferentiated cells. Hematoxylin and eosin. × 100.

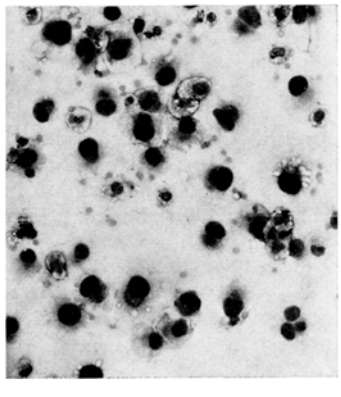

Fig. 2. Microphotograph of a stained smear of the peritoneal fluid of a rat bearing a transplant of ascites tumor. Viable and degenerating cells are seen. Giemsa stain. × 800.

However, the tumor cell count dropped significantly on the third passage. At this time it was felt that for the ascites transplant, some should have also been inoculated s.c. with the same fluid. Consequently 10 rats were inoculated i.p. with 2 ml of ascitic fluid, and 10 more were inoculated s.c. with 1 ml of ascitic fluid. The tumor did not grow in any of the animals inoculated i.p. It did however grow s.c. in all animals. After about 2 weeks, 1 animal bearing the solid tumor was sacrificed and the mass was resected and placed in a Petri dish with saline. The tumor mass appeared to be composed of blood red material. Again about 10 g of this tumor was homogenized in the same manner as previously reported, and 2 ml of the suspension was inoculated i.p. to each of 10 young rats. The ascites grew in all the animals, and since then it has been carried by serial transplants in our Institute.

The ascitic form of the Walker 256 resembles the carcinomatous variant in that it is composed mainly of so-called 'principal cells'. The principal cell is polygonal, round or oval, and has a distinct cell membrane. The cytoplasm is granular, basophilic, and often vacuolated. There is usually 1 centrally located nucleus that may be round or oval and sometimes indented. Occasionally 2 or 3 nuclei are present. The deeply stained nuclear membrane encloses a fine mesh and 1–4 small clumps of chromatin. There is generally 1 large eosinophilic nucleus. Three or 4 mitotic figures, often tripolar, may be found in nearly every field. The individual principal cells are often found to be in various stages of degeneration, necrosis and resorption (Figure 2).

Discussion and conclusions. A method of transplanting the carcinosarcoma Walker 256 to the ascitic form has been described. This tumor grows in about 90–100% of the animals, and kills the host in about 2 weeks. The fluid in the peritoneal cavity becomes apparent in 5–6 days, and at 10 days may reach the amount of 20–30 ml or more. Tumor cell counts vary, but the normal limits are 5–6 million/ml.

It is felt that this tumor may be a useful tool, especially for the investigation and study of the circulating cancer cells in the blood and metastases[12].

Riassunto. Una serie di ricerche per ottenere tumori in forma ascitica usando materiale cellulare di tumori solidi sono state esequite. Un omogenato del carcinosarcoma di Walker 256 è stato iniettato in cavità peritoneale e si è avuto lo sviluppo di forme tumorali ascitiche che è stato possibile trapiantare nelle successive generazioni di ratti.

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Die DNS-Synthese im Rattenherzen als Funktion des Lebensalters. Autoradiographische Untersuchungen mit ^{3}H-Thymidin

Im hypertrophierenden Herzmuskel[1] und in der Randzone von Herzinfarkten[2] konnten tierexperimentell DNS-synthetisierende Herzmuskelkerne nachgewiesen werden. Für das unversehrte Herz bestehen einheitliche Aussagen nur, soweit sie sich auf das wachsende Organ verschiedener Tierarten beziehen[3–12]. Jenseits des 2. Lebensmonats sind synthetisierende Herzmuskelkerne zwar gelegentlich beobachtet[6–8,11], aber nicht quantitativ erfasst worden.

Deshalb injizierten wir Sprague-Dawley-Ratten im Alter von 7, 12, 18, 24 und 30 Tagen, 2, 4, 8, 12, 18, 24 und 27 Monaten 1 h vor Tötung durch Dekapitation im Ätherrausch jeweils zwischen 09.00 und 10.00 Uhr i.p. 500 μCi ^{3}H-Thymidin, spez. Akt. 6,0 Ci/m*M* (Schwarz Bio Research, N.Y.). Fixation der Herzen für 48 h in neutralem Formalin unter Zusatz von inaktivem Thymidin. Waschen (1 h) der entparaffinierten 5 μ dicken Schnitte in hochkonzentrierter Lösung inaktiven Vorläufers zum Austausch freien ^{3}H-Thymidins. Exposition der Autoradiogramme (stripping film, Kodak AR 10) für 8–12 Tage. Nachfärben mit Hämalaun. Für den 7.–18. Lebenstag standen je 4 Tiere, für die späteren Zeitpunkte 2 Tiere zur Verfügung; lediglich der 24. Tag war mit nur 1 Tier besetzt. Ausgewertet wurden pro Tier 60000 bis 80000 Herzmuskelkerne und 70000 bis 95000 Bindegewebskerne, unabhängig von ihrer Zugehörigkeit zu Kapillaren oder Insterstitium. Die Mittelwerte markierter und in Teilung begriffener Kerne sind – wegen der geringen Ausbeute – in ‰ kurvenmässig dargestellt. Stärkere Abweichungen ergaben sich im abfallenden Kurvenschenkel.

Die Zahl DNS-synthetisierender Herzmuskelkerne beträgt am 7. Lebenstag der weissen Ratte etwa 90‰ (Figur a). Dieser Wert stimmt überein mit den Ergebnissen von Rumyantsev[9], der überdies zum Zeitpunkt der Geburt noch etwa doppelt soviel markierte Herzmuskelkerne ermittelt hat. Bis zum 30. Lebenstag fällt die Zahl markierter Kerne in unserer Versuchsreihe auf 10‰ aller Zellen stark ab und sinkt auch im weiteren Verlauf noch deutlich über 1,2‰ zu Beginn des 2. Lebensmonats auf Minimalwerte zwischen 0,2 und 0,6‰, die für den Rest der geprüften Versuchszeit aufrechterhalten bleiben. Es besteht also bei der unvorbehandelten Ratte zu allen

[1] G. Wegner und E. Mölbert, Virchows Arch. path. Anat. Physiol. *341*, 54 (1966).
[2] O. Klinge, Z. Zellforsch. mikrosk. Anat. *80*, 488 (1967).
[3] R. A. MacDonald and G. K. Mallory, Lab. Invest. *8*, 1547 (1959).
[4] B. Messier and C. P. Leblond, Am. J. Anat. *106*, 247 (1960).
[5] H. R. Overy and R. E. Priest, Lab. Invest. *15*, 1100 (1966).
[6] S. R. Pelc, Expl Cell Res. *29*, 194 (1963).
[7] S. R. Pelc, J. Cell Biol. *22*, 21 (1964).
[8] R. O. Peterson and R. Baserga, Expl Cell. Res. *40*, 340 (1965).
[9] P. P. Rumyantsev, Folia histochem. cytochem. *1*, 463 (1963).
[10] P. P. Rumyantsev, Archs Anat. Histol. Embryol. *47*, 59 (1964), zit. nach: Ber. Biol. *257*, 14 (1966).
[11] R. G. Shorter and J. L. Titus, Proc. Staff Meet. Mayo Clin. *37*, 669 (1962).
[12] K. Wegener, S. Hollweg und W. Maurer, Z. Zellforsch. mikrosk. Anat. *63*, 309 (1964).