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Pathology

C-type virus particles in placenta of normal healthy Sprague–Dawley rats

(vertical transmission/rat tumors/rat leukemia)

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ABSTRACT **C-type virus particles were found on electron-microscopic examination in placentas from two out of four young healthy Sprague–Dawley rats. One of these specimens contained virus particles budding from the plasma membranes of cells in the junctional zone of the placenta, i.e., the region where the fetal and maternal cell layers meet. In the other placenta, immature and mature C-type virus particles were found among cell debris also in the junctional region. This observation adds another species of animals to those recently reported, such as rhesus and baboon monkeys, as well as humans, in which C-type virus particles were found in the placenta. The presence of C-type virus particles in the placenta of Sprague–Dawley rats is particularly significant in view of the fact that a considerable number of these animals develop spontaneously a variety of malignant tumors, occasionally also leukemia and malignant lymphomas; however, none of these spontaneous tumors reveals the presence of virus particles on electron-microscopic examination. The nature of virus particles detected in rat placenta remains to be determined. As a working hypothesis, it is possible to assume that they may represent the passage of latent, presumably oncogenic, viruses transmitted "vertically" from parents to offspring. In the course of this passage some of them may be formed, emerging temporarily from their latency, before losing their identity and being again incorporated into the cell genetic components.**

C-type virus particles can be found readily in primary, spontaneous, or in transplanted leukemias, lymphomas, sarcomas, or fibrosarcomas in certain animal species, such as mice, cats, or chickens, and B-type virus particles can be found in spontaneous mammary tumors in several strains of mice (1). On the other hand, in certain other species, such as rats or dogs, it is usually not possible to find C-type virus particles in primary spontaneous leukemia, or in malignant lymphomas, or in other spontaneously occurring tumors, such as fibrosarcomas or mammary carcinomas (refs. 1 and 2; also†). Similarly, except for a few isolated reports, it is, for all practical purposes, impossible to find virus particles in human leukemia or lymphomas, or in human sarcomas, carcinomas, or other malignant tumors (1). These observations appeared to favor the attitude of those investigators who felt that not all malignant tumors are caused by viruses, and that under certain conditions malignant tumors and lymphomas may develop in the absence of viruses.

The situation has changed in the past several years, since characteristic C-type virus particles have been found in the placenta of baboon and rhesus monkeys in two independent and concurrent studies by Kalter (3), Schidlovsky (4), and their associates, and most interesting of all, shortly thereafter, by Kalter and his colleagues (5), also in human placenta. These unexpected and rather startling observations were promptly confirmed in several laboratories (6–8). Similar C-type virus particles were found recently also in one of two near-term placentas of cottontop marmoset monkeys (9). The virus particles observed in monkey and human placentas were budding or extracellular, with an average diameter of approximately 100 nm. With some reservations concerning comparison with particles observed in human placenta, those found in rhesus and baboon monkey placentas were quite similar to the type-C virus particles commonly observed in mouse or cat leukemias or sarcomas. In addition, smaller particles, about 30 nm in diameter, were observed by Feldman in rhesus and baboon placentas (8). In a somewhat related observation, intracisternal A-type particles were recently described in the placenta of normal strain-2 guinea pigs (10).

It appeared surprising to us that, under these circumstances, no virus particles have been observed thus far in the placenta of rats. We have examined in the electron microscope ultrathin sections of several specimens of placentas removed from Sprague–Dawley and Long–Evans rats prior to the positive observations reported in monkeys and humans; we found no virus particles in these preliminary studies.

These negative findings were difficult to reconcile with the fact that both Sprague–Dawley and Long–Evans rats, as well as rats of other inbred strains, develop a high incidence of spontaneous malignant tumors, such as mammary fibroadenomas and carcinomas, ovarian tumors, subcutaneous fibrosarcomas, etc., and occasionally also spontaneous leukemia and malignant lymphomas (11–18). Assuming, as a working hypothesis, that these neoplasms, including leukemia and lymphomas, are caused by oncogenic viruses, it would be possible to speculate that such viruses are transmitted "vertically" from one generation to another (19), that they may be formed in the placenta in the course of transmission from parents to offspring, and that at least some of them may become at that point detectable in the electron microscope. Spontaneous rat tumors and spontaneous rat leukemias and malignant lymphomas, as well as malignant tumors induced in rats with carcinogenic chemicals, do not usually contain virus particles (refs. 1, 2, and 20; also†). There are several reports of virus particles observed in rat leukemias or rat tumors; however, with only few exceptions (21) they refer to rat leukemia (22–24) or to rat tumors (25),

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† D. Feldman and L. Gross, unpublished data.

including hepatomas (26, 27), that have been transplanted, by cell graft, for a number of generations, serially, from one animal to another. It is presumed, probably with good reason, that virus particles observed in such tumors or leukemias have been related to the original neoplastic tumor cells. The possibility cannot be excluded, however, that in some instances at least, extraneous virus particles unrelated to the primary tumors or leukemia might have been "picked up" in the course of successive transplantations from some of the rat donors serving for tumor cell passage, and that such particles might have been carried in the grafted tumor cells.

The many spontaneous mammary or ovarian adenomas or carcinomas, or the subcutaneous fibrosarcomas, so common in rats of the Sprague–Dawley or Long–Evans strains, as well as the occasionally observed spontaneous leukemias or malignant lymphomas, do not seem to contain detectable virus particles. We have examined in the electron microscope 14 tumors and four leukemias from both groups of animals, but we have found no evidence of the presence of virus particles in either tumors or in leukemias or malignant lymphomas which developed spontaneously in our rats†. The apparent absence of virus particles in spontaneously developing rat leukemia remains in striking contrast to the abundance of typical C-type virus particles observed in leukemias and malignant lymphomas, including reticulum-cell sarcomas and Hodgkin's-like lesions, induced in rats with the passage A mouse leukemia virus (Gross). In every single leukemia or lymphoma induced in Sprague–Dawley or Long–Evans rats with the mouse leukemia (Gross) virus that we have examined, the leukemic cells and frequently also a variety of other cells, either related or unrelated to the hematopoietic system, contained C-type virus particles, indistinguishable from those observed in leukemic mice (ref. 28; also†). No virus particles were found on electron-microscopic examination of organs such as thymus, lymph nodes, spleen, liver, or bone marrow removed from 10 normal healthy Sprague–Dawley or Long–Evans rats (28).

After we have learned that C-type virus particles could be detected in the placenta of several species in which spontaneous tumors or lymphomas do not usually reveal the presence of virus particles, we thought that it would be of interest to re-examine placenta of rats more carefully, assuming that virus particles may not be uniformly distributed and may possibly be found more readily in certain parts of the rat placenta.

MATERIALS AND METHODS

The rat donors used in the present study were from our own colony of Sprague–Dawley rats raised in our laboratory since 1960 by brother-to-sister mating. The incidence of spontaneous tumors and leukemia in a recent sample consisting of 94 females and 95 males of our colony of breeders was as follows: 24 of the 94 females developed a variety of tumors, such as ovarian and mammary fibroadenomas or carcinomas, or subcutaneous fibrosarcomas, and three animals developed leukemia. Among the 95 males, five developed tumors, mostly subcutaneous fibrosarcomas, and two developed leukemia. The tumors or leukemia developed in both groups at ages varying from 9 to 27 months.

Preparation of Specimens for Electron Microscopy. Placentas were removed aseptically from four young, untreated, healthy, pregnant Sprague–Dawley female rats from our colony of these animals. One was an early placenta, another was close to mid-term, and the remaining two placentas were between mid- and full-terms.

The rat placentas, as well as fragments of leukemic rat organs also used in this study, were provided by the Bronx V.A. Hospital's Cancer Research Unit. The baboon, rhesus, and human placentas were provided by the John L. Smith Memorial for Cancer Research, Pfizer Inc. However, all tissues were processed by the same method, as described in this paper, and were examined in the same electron microscope, and by the same investigator (G.S.).

Tissues were fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer (2 hr at room temperature), rinsed in the same buffer, post-fixed in chrome-osmium for 2 hr at room temperature, rinsed in distilled water, exposed to 2% uranyl acetate in 50% ethanol overnight, then rapidly dehydrated through increasing concentrations of ethanol, and embedded in Epon. Ultrathin sections were stained with lead citrate.

RESULTS

Presence of C-type virus particles in two of the placentas examined

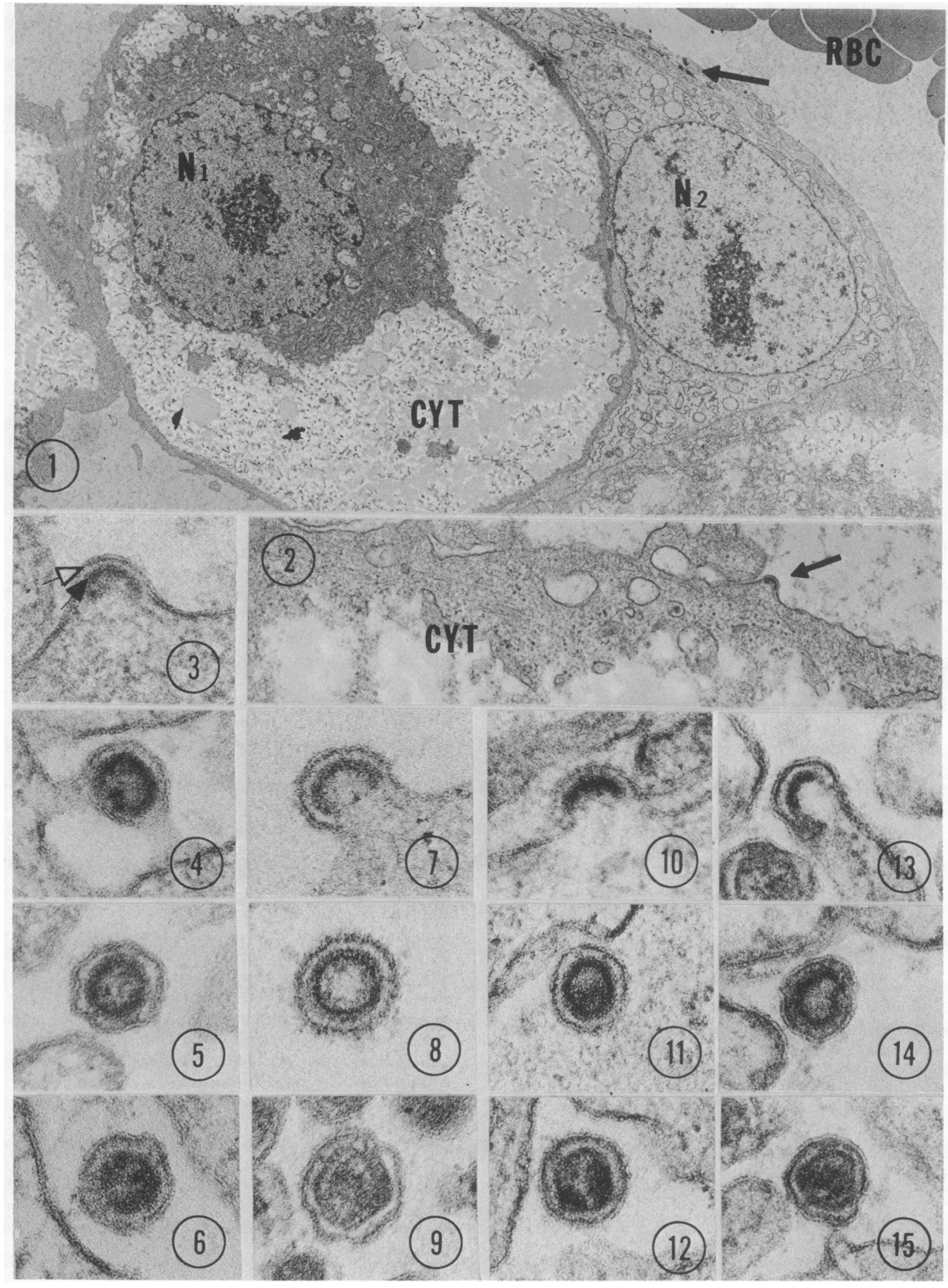
The placental barrier of the rat consists of three layers of trophoblast cells which on one side are in direct contact with maternal blood and on the other side are resting on a basement membrane that separates them from the endothelial cells of embryonic capillaries and other mesenchymal tissues (29, 30). Such areas did not show any virus particles on electron-microscopic examination.

However, when sections were examined from the junctional zone, i.e., that region of the placenta where maternal and fetal tissues meet, virus particles were found in specimens of two of the four placentas examined, namely, in fragments of the early and of the near mid-term placentas. In one of these two placentas, some sections within the junctional zone revealed the presence of generally spherical cells with smooth surfaces, which appeared to be interconnected with each other without desmosomes, forming rather loose and random chain-like networks. A major part of the cytoplasm of such cells was filled with amorphous, relatively electron-lucent matter, and also contained patches of rough-surfaced endoplasmic reticulum. The nuclei of these cells were circular and rather small (Fig. 1). Several of these cells showed, at the edge of the plasma membrane, typical budding virus particles (Figs. 2 and 3). Viral budding was not observed in other cells; however, because of the very small number of budding virus particles found in the tissues which we have examined, this negative observation may not be significant. In addition, extracellular spherical virus particles, about 100 nm in diameter, either immature with electron-lucent centers, or mature with electron-dense nucleoids, were found in sections prepared from the placentas of the same and also that of another rat donor (Figs. 4 and 5). The extracellular C-type virus particles were found again in the junctional zone, among large areas of cellular debris or fibrillar matrix.

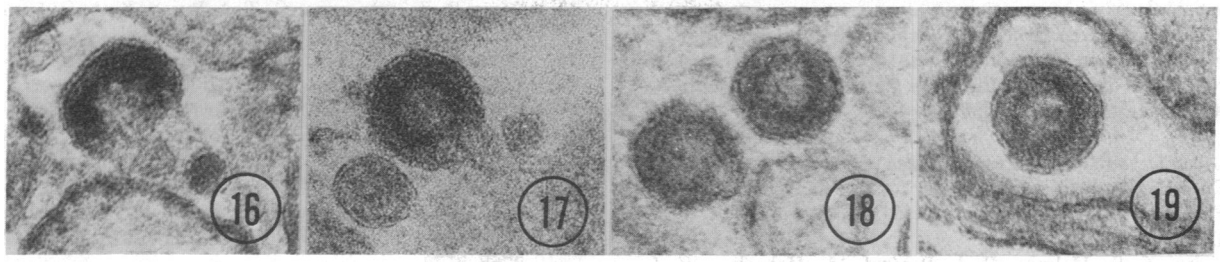
No virus particles were found in fragments of the remaining two rat placentas; however, the number of virus particles found in the two positive placentas was so small that they might have been missed in the remaining two placentas, in spite of an apparently thorough electron microscopic examination.

DISCUSSION

Ultrathin sections prepared from placentas removed from four young, healthy Sprague–Dawley rats were examined in the electron microscope. In two of them, budding typical



FIGS. 1–15. *(Legend appears at the bottom of the next page.)*



FIGS. 16–19. *Type-C-like virus particles in human placenta. Fig. 16* is a rare and rather questionable budding form. Fully formed extracellular particles (*Figs. 17–19*) do not show a clear separation between the more electron-dense circular internal components and the envelope, which is a characteristic feature of type-C virus particles. Furthermore, at least on the electron micrographs reproduced here, which are representative samples of those examined, there is no clear evidence of the presence of an electron-dense, centrally located nucleoid. ×165,000.

C-type virus particles were found, therefore placing the rat placenta in the same group as those of several other species, such as rhesus, baboon, and marmoset monkeys, as well as humans, in which C-type virus particles were recently detected on electron microscopic examination (3–9). Only very few virus particles were present, and these were found with considerable difficulty, after prolonged examination, and in a certain area only, namely, in the junctional zone of the placenta, either budding from cell plasma membrane (Figs. 2 and 3), or among debris in extracellular spaces (Figs. 4–6).

It may appear at the present time, that the junctional zone of the rat placenta may be a region favoring C-type virus formation. On the other hand, the fact that particles were not found budding from other types of cells and that they were found only in the junctional zone may not necessarily be significant because only a few small fragments from each placenta were examined and no attempt has yet been made in this preliminary study to survey all parts of placenta methodically. Furthermore, since the number of virus particles found in the tissues examined was very small, it is possible that some of them might have been missed in other areas examined.

The virus particles detected in the rat placenta (Figs. 2–6) appear to be similar to the particles observed in the leukemic organs of Sprague–Dawley rats in which leukemia was induced with the mouse leukemia (Gross) virus (Figs. 7–9). They are also quite similar to virus particles recently observed in the placenta of the baboon (Figs. 10–12) and rhesus (Figs. 13–15) monkeys, as well as those described in humans (Figs. 16–19). There are some differences, however; a characteristic feature of type-C virus particles is the distinct separation of the viral nucleoid from the viral envelope by a more electron-lucent space (Figs. 3–15). This feature distinguishes the typical type-C virus particles seen in placentas of rats, baboon, and rhesus monkeys (Figs. 3–6 and 10–15), as well as the rat leukemia (Gross) virus (Figs. 7–9), from particles observed in human placentas (Figs. 16–19).

It is of considerable interest that typical C-type virus particles have been detected in the placenta of several species in which spontaneous tumors, leukemias, and malignant lymphomas in most instances do not reveal the presence of virus particles. However, there is no information available at this time which would implicate a possible oncogenic potential of the particles detected in the placenta.

At the present time we can only register the fact that virus particles can be found in the placenta of normal healthy hosts of such species and evaluate the possible significance of the presence of these virus particles on the basis of the similarity of their morphology to that of other virus particles found in sarcomas, leukemias, or lymphomas known to be caused by filterable viruses and occurring in other animal species, such as chickens, mice, and cats (1).

It would be tempting to speculate that the virus particles detected in the placenta of Sprague–Dawley rats as well as those observed in monkeys and humans have a causal relationship to the development of spontaneous tumors, leukemias, or lymphomas in these species. They may represent latent, presumably oncogenic viruses, transmitted "vertically" from one generation to another (19). In the course of this passage some of them may be formed, emerging temporarily from their latency, before losing their identity and being again incorporated into the cell genetic components. However, such an assumption represents at best only a working hypothesis. Accordingly, the possible significance of the presence of virus particles detected in the placenta of Sprague–Dawley rats remains unknown at the present time, but encourages further, more extensive studies.

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FIGS. 1–15 (on preceding page).

FIG. 1. *Rat placenta.* Low magnification of junctional zone of a Sprague–Dawley rat placenta. The large cell with nucleus (N_1) and cytoplasm (CYT), consisting of an amorphous relatively clear area and also containing dark patches of endoplasmic reticulum, is representative of the type of cells that were found to show budding of virus particles at the plasma membrane. Cell with nucleus N_2 appears to be a trophoblastic cell joined by desmosomes (arrow) to adjacent trophoblastic cells, lining a blood sinusoid with presumably maternal red blood cells (RBC). ×3,750.

FIGS. 2–6. *Type-C virus particles in rat placenta. Fig. 2.* Higher magnification of periphery of a cell showing budding of a virus particle (arrow) ×30,000. *Fig. 3.* Further enlargement of virus budding (shown in Fig. 2). The separation of the virus envelope (clear arrow) from the viral nucleoid (dark arrow) is characteristic of type-C virus particles. *Figs. 4 and 5.* Stages of maturation of extracellular immature virus particles. *Fig. 6.* Mature virus particle. ×15,000 (Figs. 3–6).

FIGS. 7–9. *Type-C virus particles in rat leukemia induced with the mouse leukemia (Gross) virus.* Budding particle (*Fig. 7*) and extracellular immature particle (*Fig. 8*) both in the leukemic rat spleen. *Fig. 9.* Extracellular mature virus particle in the bone marrow of the same leukemic rat. ×150,000.

FIGS. 10–12. *Type-C virus particles in baboon placenta.* Budding (*Fig. 10*) and extracellular, immature (*Fig. 11*) and mature (*Fig. 12*) virus particles. ×150,000.

FIGS. 13–15. *Type-C virus particles in rhesus monkey placenta.* Budding (*Fig. 13*) and extracellular immature (*Fig. 14*) and mature (*Fig. 15*) virus particles. ×150,000.

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