opment of the 2-cell mouse ovum when cultured *in vitro*(6), and certain mammalian spermatazoa are capable of utilizing lactate for energy under aerobic conditions(7).

Summary. The oviducts of non-pregnant intact rabbits, castrates and mated rabbits 16-72 hours after coitus were studied in the Warburg apparatus. In the intact, non-pregnant group, O_2 uptake was essentially the same in Krebs-Ringer phosphate solution (K.R.p.) alone, K.R.p. with glucose and K.R.p. with fructose. It was increased 2-fold by addition of succinate. Lactic acid production was substantially higher in glucose than in fructose or in K.R.p. alone. At the end of 2 hours, glucose uptake was 50.7 ± S.E. 4.3 μg/mg dry weight of tissue and lactic acid production was 24.9 ± S.E. 1.4 μg mg dry weight of tissue. Under anaerobic conditions virtually all of the glucose taken up could be accounted for as lactic acid. In castrates, O_2 uptake in various media and glucose uptake were not significantly different from those observed in intact, non-pregnant animals, but lactic acid output in glucose was higher. Among mated animals O_2 uptake was similar, and it did not vary significantly among specimens studied from 16-72 hours after mating. Lactic acid production in glucose was increased in the third 24-hour period following mating ($p<0.01$), indicating an increased utilization of anaerobic pathways during this interval.

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1. Mastroianni, L., Winternitz, W. W., Lowi, N. P., *Fertil. and Steril.*, 1958, v9, 500.
2. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
3. Barker, S. B., Summerson, W. M., *ibid.*, 1941, v138, 535.
4. Bishop, D. W., *Internat. J. Fertil.*, 1957, v2, 11.
5. Mastroianni, L., Wallach, R. C., *Am. J. Physiol.*, 1961, in press.
6. Whitten, W. K., *Nature*, 1957, v179, 1081.
7. Mann, T., *Biochemistry of Semen.* London Methuen and Co., London, 1957, 53.

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Inoculation of Chicken Sarcoma Virus into Chicken Thymus. An Electron Microscopy. (26544)

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It has been reported that in the chicken sarcoma, when the tumor is transplanted subcutaneously(1,2,3,4) or intraperitoneally(5), the virus particles identifiable by electron microscope are about 50 or less per single cell section. This number has been somewhat increased by X-ray irradiation(6). Bernhard's recent report deals chiefly with extracellular viruses of Rous sarcoma(7).

We found recently that a significantly large number of virus particles was attached to the tumor cell when virus suspension was injected into the thymus.

The sarcoma of Chiba strain(8), which is 100% transplantable, was used in this experiment. A 10 g piece of tissue taken from the tumor, implanted subcutaneously 15 days before, was homogenized with a glass homogenizer in 50 cc of physiological saline and centrifuged at 4,500 rpm for 20 minutes. One ml of the supernatant virus suspension was injected into the 2nd or the 3rd thymus of 120- to 150-day-old male chickens. A tumor of myxosarcomatous pattern developed in the interlobular stroma of the thymus at site of injection, reached a macroscopic size 4 days after injection, and increased in size subsequently.

Virus particles were first detected in the tumor 4 days after injection. They were round or ovoid, 70 to 85 mμ in diameter, and consisted of a central nucleoid body, 30 to 40 mμ in diameter, surrounded with a single or double membrane (Fig. 2).

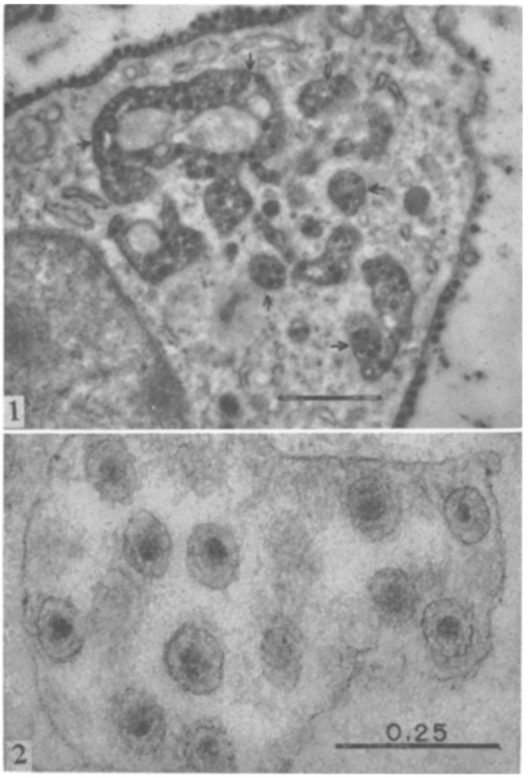

FIG. 1. Virus particles on the surface and in the peculiar intracellular spaces (arrow) of a tumor cell 6 days after intra-thymic inoculation of virus.

FIG. 2. High power view of viruses found in the peculiar intracellular space of a tumor cell. Some with double membranes. 6 days after intra-thymic inoculation.

The number of virus particles reached a maximum 6 or 7 days after inoculation, when one in 2 to 5 cells contained viruses. In some parts of the tumor, almost all tumor cells contained virus particles. Occasionally, as many as 200 to 500 virus particles were found attached to a tumor cell in a single section.

One of the conspicuous sites of virus in this period was the cell surface. Here, the virus particles were attached to the cell surface at irregular intervals or, more frequently, were arranged closely to one another in a single row (Fig. 1). Not infrequently, nearly a half or the entire circumference of the cell surface was occupied by virus particles. The virus particles on the cell surface were found embedded in a thin layer of electron dense amorphous material, which separated the particles from each other.

In the cytoplasmic vacuoles, the virus particles tended to gather in small groups. In 3 out of 6 chicks used in the winter experiment series (kept at a room temperature of 5° to 15°C and killed 6 or 7 days after inoculation), a considerable number of the particles were found in a peculiar intracellular (ergastoplasmic ?) space (Fig. 1). The virus particles were packed closely in some spaces and arranged rather loosely in others. Some of these spaces were vacuole-like and others appeared to be irregular anastomosing canaliculi. The spaces contained as a rule diffusely electron dense material in which the virus particles were embedded. A small number of viruses was present outside the cells. No virus particles were found free in the cytoplasm.

Eight days after inoculation, a sudden sharp decrease in number of virus particles detectable in the tumor was noticed.

Inoculation experiments with the same strain of virus into the subcutaneous tissue of the right wing, the testes and the kidneys showed that the number of virus particles detectable electronmicroscopically on the surface or in the cytoplasmic vacuoles of the tumor cell did not exceed 10 to 20 per single cell section. No such peculiar intracellular spaces as above described with or without virus particles were observable in tumor cells.

Although the causal factors of vigorous multiplication of Rous virus in the thymus and details of virus-to-cell relationship are subject of future studies, intra-thymic injection is worthy of trial with other tumor viruses.

Summary. Intra-thymic inoculation of virus suspension is introduced as a method for electron microscopic study of chicken sarcoma of Chiba strain. In the tumor produced in the thymus, a significantly larger number of virus particles attached to the tumor cell was observed than in those produced at other sites. The virus particles were detected chiefly on the cell surface, in cytoplasmic vacuoles and in peculiar intracellular spaces.

1. Bernhard, W., Oberling, Ch., Vigier, Ph., *Bull. du Cancer,* 1956, v43, 407.
2. Fuse, Y., *Gann,* 1956, v47 Suppl., 530.
3. Mannweiler, Kl., Bernhard, W., *Bull. du Cancer,* 1958, v45, 223.
4. Okano, H., Nishiumi, Y., Imai, T., *Gann,* 1959, v50 Suppl., 219.
5. Epstein, M. A., *Brit. J. Cancer,* 1956, v10, 33.
6. Shimpo, K., Narimatsu, E., Nishida, K., Higashi, H., Moroya, K., *Gann,* 1959, v50 Suppl., 223.
7. Bernhard, W., *Cancer Res.,* 1960, v20, 712.
8. Chiba, T., *Gann,* 1951, v42, 2.

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"Vertical" Transmission of Passage A Leukemic Virus from Inoculated C3H Mice to their Untreated Offspring.* (26545)

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When the initial experiments on cell-free transmission of mouse leukemia were reported (1,2), the problem of natural transmission of the virus was also considered. It was known at that time that foster-nursing of mice born to mothers of high-leukemic strains C58(3) or Ak(4,5) by female mice of low-leukemic lines, did not prevent development of leukemia in the foster-nursed animals. Incidence of leukemia in descendants of high-leukemic strains was essentially undiminished even though such mice had been removed from the womb of their Ak mother by Caesarean section(6) and were then nursed by a low-leukemic-line foster mother. It was apparent that the leukemic agent was not transmitted through the milk, unlike the mouse mammary carcinoma virus(7). Transmission occurred most probably directly through the embryos(1).

The natural transmission of the leukemic virus from injected parents to their non-treated offspring was demonstrated in a series of experiments in which newborn C3H mice were inoculated with cell-free Ak leukemic extracts; after these mice had reached sexual maturity, they were mated and their untreated offspring observed for development of leukemia. Incidence of leukemia in the F_1 generation was significant in some experiments(2), but relatively low in others(8), since the leukemic filtrates initially employed had a low content of infective virus. After a highly potent strain of leukemic virus (passage A) was developed(9) it appeared of interest to repeat these experiments. Preliminary results of this study are reported here.

Materials and Methods. The 23rd to 26th cell-free serial passage A of the leukemic virus strain, initially derived from spontaneous Ak leukemia, was employed in this study. At this passage level, the potency of this virus is sufficiently high to induce an incidence of leukemia of over 95%, after a latency of less than 3 months, following inoculation into newborn or suckling mice of either C3H/Bi, or C57 Brown/cd inbred lines. Filtrates (Selas 02) of 20% concentration prepared in the usual manner(9) from C3H donor mice with passage A virus-induced leukemia, were inoculated intraperitoneally (0.2 to 0.3 ml). All animals were either of strain C3H(f)/Bi (free from the mammary carcinoma virus), or of the C57 Brown/cd inbred line. Incidence of spontaneous leukemia in our laboratory in mice of either strain has not exceeded, during the past several years, 0.5 to 1% in animals beyond 12 months of age.

Experimental. Development of leukemia in non-treated offspring of both virus-injected C3H(f) parents. In this series of experiments, both males and females of the C3H strain were inoculated, within a few days after birth, with passage A leukemic filtrate. After these mice reached sexual maturity, they were mated; litters were born and

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