in controls. Both lactate dehydrogenase and serum glutamic oxalacetic transaminase increased in treated subjects. It has been demonstrated repeatedly that anabolic steroids exert a positive effect on nitrogen retention and that the degree of nitrogen retention is partially dependent upon caloric and protein intake (*9*). The extent to which anabolic steroid treatment favorably influences protein synthesis has not been satisfactorily answered (*10*). In our study the combination of steroid treatment, high protein intake, and heavy muscular stress apparently accelerated protein synthesis in the muscle tissue, with this change being manifested by increased static and dynamic strength and body weight.

There were no consistent or apparently significant physiological side effects. A few individuals indicated an increase in urine production, and some felt a degree of tension which may have resulted from the strenuous training program. The near absence of normal muscle soreness and stiffness following the training sessions was noted by some. It appears possible to train at near maximum five or six times a week during the treatment. No attempt was made to analyze possible psychological implications. No reduction in sex drive was reported.

It appears that anabolic steroids can accelerate the acquisition of muscular strength and muscular power and simultaneously permit training at or near maximum capacity with greater frequency. Treatment should be used cautiously until more information is available on physiological effects on humans.

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Herpesvirus in Marek's Disease Tumors

Abstract. *Intranuclear and cytoplasmic virus particles of the herpes type were located in epithelial cells that line the kidney collecting tubules obtained from a chick with Marek's disease. The chick had contracted the disease by direct contact transmission. The virus was not observed in any of the invading tumor cells in the same kidney.*

The epizootiology of Marek's disease, a lymphoproliferative disease of chickens, suggests that it is of an infectious nature; and a cell-associated, herpes-type virus (HTV) has been demonstrated in cell cultures derived from tumors induced by various isolates (*1–3*). Although successful transmission of Marek's disease with a cell-free virus inoculum remains to be accomplished, circumstantial evidence has been presented that implicates HTV as the etiological agent (*4, 5*).

Although this virus replicates in cell cultures from kidneys of infected chicks and will also grow when transferred to fibroblast monolayers originating from normal chick or duck embryo (*1, 3, 4, 6*), HTV has not been shown to occur in Marek's disease tumors prior to cultivation. Our own efforts had failed to demonstrate the virus in tumors directly (*3*). Recently, however, we have observed typical HTV in the kidney tumor of a chick that had contracted Marek's disease by natural transmission, as a result of being caged with birds infected with the GA strain of this disease. We now describe the types of cells, as judged by electron microscopy, that harbor the virus particles before and during cultivation of Marek's disease tumors.

A group of 2-day-old Athens-Canadian chicks (*7*) was kept for 6 days with other chicks carrying Marek's disease. The exposed chicks were then separated and kept in isolation. Three chicks were killed weekly for diagnosis on the basis of gross and microscopic pathology, development of a characteristic cytopathic effect in chick kidney cell cultures, and the direct electron microscopic observation of gonads, liver, spleen, and kidney tissue. The tissues and cell cultures for electron microscopy were prepared as described (*3*). The observations reported here were made on chicks that had been held in isolation for 4 weeks after exposure. At autopsy, these birds showed enlargement of gonads and kidneys.

Figure 1 illustrates the massive enlargement of the kidneys due, as shown in Fig. 2, to infiltration with lymphoid cells. However, parenchymal elements with epithelial cells are easily found, and a tangential aspect of the wall of a distal uriniferous tubule still delineated by its basement membrane and surrounded by invading lymphoid cells can be seen (Fig. 2). The epithelial nature of the virus-bearing cells in the tumor is demonstrated by the presence of adhesion zones (Fig. 3) as well as their location with respect to the basement membrane and the lumen of the tubule (Fig. 2). The intercellular adhesion zones are of the intermediate junction type (*8*). Empty and nucleated HTV particles are observed in the nucleus and cytoplasm, but no enveloped virus particles are evident (Fig. 4). Small nuclear particles, as reported previously in virus-containing cultures originating from chicks with Marek's disease (*3*), are also apparent. The nuclear envelope does not show the "reduplication" phenomenon often seen in herpesvirus-infected cells; it appears normal except for a few unusual outpocketings, suggesting some activity perhaps related to the envelopment of virus particles (Fig. 4). It is of particular interest that no virus was seen in the lymphoid infiltrating cells of the same tumor.

Unexposed control chicks housed in another building did not develop Marek's disease, and HTV was not present in tissues or the cell cultures derived from their kidneys. The direct electron-microscopic examination of tissues or tumors of chicks killed 1, 2, 3, 5, and 6 weeks after exposure to the disease were also negative for HTV. However, HTV was recovered in cell cultures derived from kidneys of chicks held in isolation 2 weeks or more after exposure to the disease whether they developed gross tumors or not (*9*). All samples were screened by electron microscopy for an approximately equal time on the same number of sections.

Upon cultivation of the chick kidney tumor by conventional techniques, the epithelial and interstitial cells grew, formed a monolayer, and produced in 6 days a cytopathic effect consisting of clusters of refractile, rounded cells (Fig. 5). Each of these cells contained HTV, and the morphology was like that de-

scribed by Epstein *et al.* (*10*) and by us (*3*). Similarly, a typical cytopathic effect and HTV were produced when inoculated onto chick embryo fibroblast cultures. Most of the cells, before the appearance of the cytopathic effect, were slender and elongated with the ultrastructural characteristics previously described for mouse fibroblast (*11*) and chick embryo fibroblast cultures (*12*). The fibrils and microtubules were present throughout the cytoplasm and were often seen in large amounts near the plasma membrane. The chick kidney cell cultures also contained a few cells with characteristics of fibroblasts and were able to support the growth of HTV (Fig. 6).

In infected chick kidney and chick embryo fibroblast cultures, a lytic rather than proliferative response was evident by time-lapse photography. The cells that harbored HTV ceased to go through mitosis and were ultimately lysed (*9*).

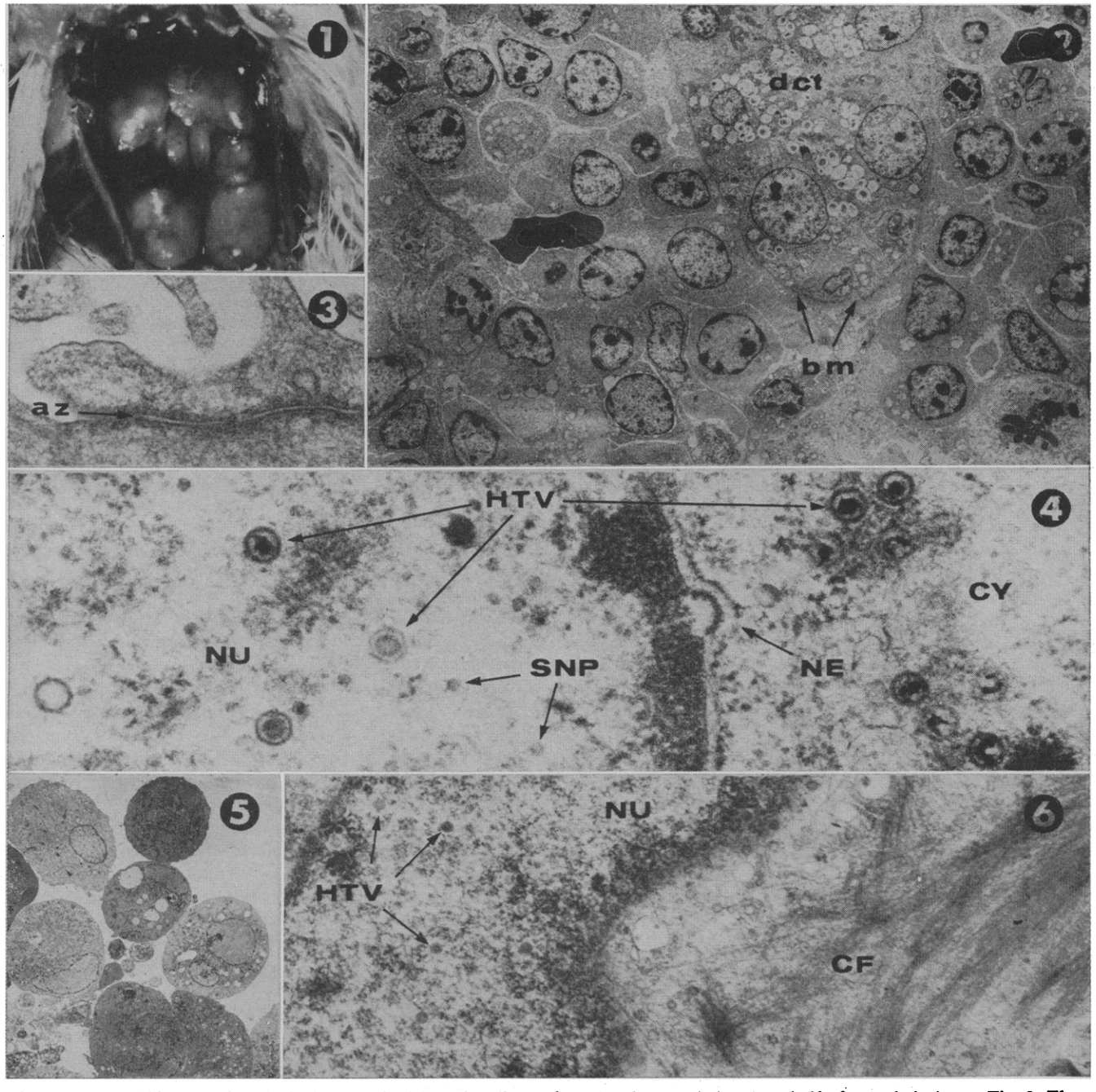

Fig. 1. Enlarged kidneys of a chick that acquired Marek's disease by natural transmission (one-half of actual size). Fig. 2. Electron micrograph of kidney tumor section; *dct*, wall of distal convoluted tubule with epithelial cells; *bm*, basement membrane. All cells surrounding the *dct*, except the two nucleated red blood cells, are invading lymphoid tumor cells (× 2,000). Fig. 3. Kidney epithelial cells within the tumor; *az*, adhesion zone between two epithelial cells (× 44,150). Fig. 4. Detail of virus-bearing epithelial cell in *dct; NU*, nucleus; *CY*, cytoplasm; *HTV*, herpes-type virus particles; *SNP*, small nuclear particles; *NE*, unusual "outpocketing" of nuclear envelope (× 53,950). Fig. 5. Electron micrograph of a group of round, refractile, virus-containing cells found within the cytopathic effect; from Marek's disease, kidney tumor cell culture (× 1,045). Fig. 6. Detail of fibroblast-like cell containing HTV in nucleus (*NU*) and randomly scattered fibrillar material within cytoplasm (*CF*), found in cultures such as that illustrated in Fig. 5 (× 16,470).

Although Marek's disease virus was located in the epithelial cells in the kidney before cultivation, the infection did not produce colonies of transformed epithelial cells. Conditions under which HTV can induce a proliferative rather than cytocidal response are unknown.

Herpes-type virus particles similar to those seen in Marek's disease have been described in many human cell cultures that originated from African Burkitt lymphoma (*13*), American lymphoma (*14*), human leukemia (*15*), and "normal" individuals (*16*). There is also serological evidence that the HTV found in Burkitt lymphoma cultures could be a cause of infectious mononucleosis in man (*17*). Generally, HTV cannot be visualized in tissue until after cultivation (*18*). However, HTV has been observed directly in the Lucké adenocarcinoma in the frog during hibernation (*19*); and it has also been observed in one case of Burkitt lymphoma (*20*).

Our positive observations have followed negative attempts by us (*3*) and others (*2*) to visualize virus particles directly in Marek's disease tumors; perhaps this indicates the low incidence of virus in such tissues. Because Marek's disease has a high degree of contagiousness and successful experimental passage has been reported with oral and nasal washings (*21*), the epithelial cells of oral and respiratory passages appear likely areas for discovering herpesvirus multiplicating sites in birds infected with Marek's disease. Another point is that our failure to observe microscopically the HTV particles in lymphoid tumor cells does not exclude the possibility that the virus may induce a neoplastic transformation in lymphoid cells. In such cells it may persist as a viral genome that normally fails to code for all elements of the viral particle when the cell is under the physiological influences operating within organized tissues of the intact chicken.

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Proteins Synthesized before and after Fertilization in Sea Urchin Eggs

Abstract. *Acrylamide-gel patterns of proteins made before and during the first 30 minutes after fertilization were the same. The patterns of gels containing proteins from eggs whose rate of protein synthesis was stimulated reversibly by prior anaerobiosis or removal of carbon dioxide were also the same as that of the mature unfertilized egg. Changes were detected in the pattern of proteins synthesized in gastrulas as compared to proteins made in unfertilized eggs or zygotes.*

Until recently it has been widely accepted that the unfertilized sea urchin egg is incapable of protein synthesis. However, several groups (*1*) have reported that unfertilized eggs are active in protein synthesis, and we (*2*) have evidence that the basal incorporation of protein precursors represents the rate of protein synthesis in mature eggs.

We reported evidence (*3*) suggesting that the chain-initiation step is limiting for protein synthesis in unfertilized sea urchin eggs. This view is in contrast to the "masked message" concepts proposed by others (*4*) as an explanation for the repressed state of unfertilized sea urchin eggs. A question central to this problem is whether fertilization initiates transcription of messenger RNA (mRNA) not previously in use.

The rate of synthesis in unfertilized eggs can be reversibly increased (but not in fertilized eggs) by lowering the partial pressure of CO_2 in the medium (*3, 5*). The rate of protein synthesis increases in eggs made anaerobic with N_2 and allowed to recover under aerobic conditions. Neither treatment prevents fertilization, and the combined treatments result in a rate of protein synthesis equal to that for which fertilization is responsible.

We now report results of compari-

Table 1. Extraction of protein for electrophoresis. Eggs were labeled for 3 hours with C^{14}-amino acids and homogenized in electrophoresis buffer containing urea as indicated. Equal volumes of homogenates and 150,000*g* supernatant were precipitated with trichloroacetic acid and counted to obtain the data for disintegrations per minute (dpm) in the supernatant and the homogenate. Data for disintegrations per minute in the lower gel was obtained by using a sample of supernatant for electrophoresis. The lower gel was digested and counted after electrophoresis. Efficiency is the ratio of disintegrations per minute in lower gel to disintegrations per minute in the homogenate, and represents the fraction of radioactivity in the homogenate which actually enters the lower (running) gel. Higher concentrations of Brij were not more effective in extraction of radioactivity.

Urea (*M*)	Detergent (1%)	Radioactivity (dpm)			
		Homogenate	Supernatant	Lower gel	Efficiency (%)
0		23850	2934	735	3
4		18954	5560	2553	14
8		4959	1873	1408	28
8	DOC Brij	4959	3123	2643	51
8	Brij	4959	3024	2623	50
8	NP40	4959	3206	2435	46
8	Triton X100	4959	2809	2196	44