ual estrogens during pregnancy, the means of the total estrogens remain without significant change between 6 and 23 weeks of pregnancy. Total estrogens exhibit an overall 5-fold increase during pregnancy when compared to estrus. Of the individual estrogens, 16-epiestriol exhibits the most marked increase from a value of 0.2 μg/100 ml plasma during estrus to a mean of 0.47 μg to 6.93 μg/100 ml plasma during 6 to 23 weeks of pregnancy. The other estrogens exhibit a 2-fold to 3-fold increase during pregnancy.

The waning and waxing of individual estrogens during pregnancy has been noted by previous investigators, especially in the human(5). This variation may possibly reflect differential utilization and removal of these estrogens from circulation. However, a more comprehensive interpretation will be possible when plasma-bound estrogen analyses are completed for this species.

Summary. Isolation of free plasma estrogens in the white-tailed Texas deer, *Odocoilius virginianus texanus* led to identification of estradiol-17α, estradiol-17β, estrone, estriol, epiestriol and 16-ketoestradiol-17β. Because of extremely low quantities and poor recovery no measurements were made for estradiol-17α and estradiol-17β. However, in order of decreasing concentration during pregnancy, from 6 to 23 weeks, the 4 remaining estrogens were: 16-ketoestradiol-17β, estriol, estrone and 16-epiestriol. In general, there was a significant ($p<.001$) rise in total free plasma estrogens during pregnancy when compared to estrus. No significant variation was observed in total free estrogens during pregnancy from 6 to 23 weeks.

1. Preedy, J. R. K., Aitken, E. H., J. Biol. Chem., 1961, v236, 1300.

2. Roy, E. J., Brown, J. B., J. Endocrinol., 1960, v21, 9.

3. Roy, E. J., Mackay, R., J. Obstet. Gynec. Brit. Cwlth., 1962, v69, 13.

4. Roy, E. J., Harkness, R. A., Kerr, M. G., J. Endocrinol., 1965, v31, 177.

5. Slaunwhite, W. R., Jr., Sandberg, A. A., Proc. Soc. Exp. Biol. and Med., 1959, v101, 544.

6. Veenhuizen, E. L., Erb, R. E., Gorski, J., J. Dairy Sci., 1960, v43, 270.

7. Zaffaroni, A., Rec. Progr. Horm. Res., 1953, v8, 51.

8. McAnally, J. S., Hausman, E. R., J. Lab. Clin. Med., 1954, v44, 647.

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The Association of Murine Lymphoma with Reovirus Type 3 Infection.* (30705)

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We have recently reported on the nature of the acute and chronic disease established in mice following neonatal infection with reoviruses(1,2,3). The available evidence points to a chronic immunological injury which proceeds in the absence of infective virus but in the presence of antibody to the virus. In more detailed communications, we are reporting on the clinical picture and the nature of the lesions of a large number of these mice with the "late" chronic disease and on the effects of passage of their lymphoid cells into newly-born mice of the same strain. We report here the observations with only one mouse (2731/6/272) which is also the subject of the associated communication on electron microscopic findings(4).

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Materials and methods. These are identical with the procedures outlined in detail in previous communications(1,2,3).

Results. The mouse was one of a litter of eight infected by reovirus type 3 (HEV) by contact when 1 day old. The mouse showed

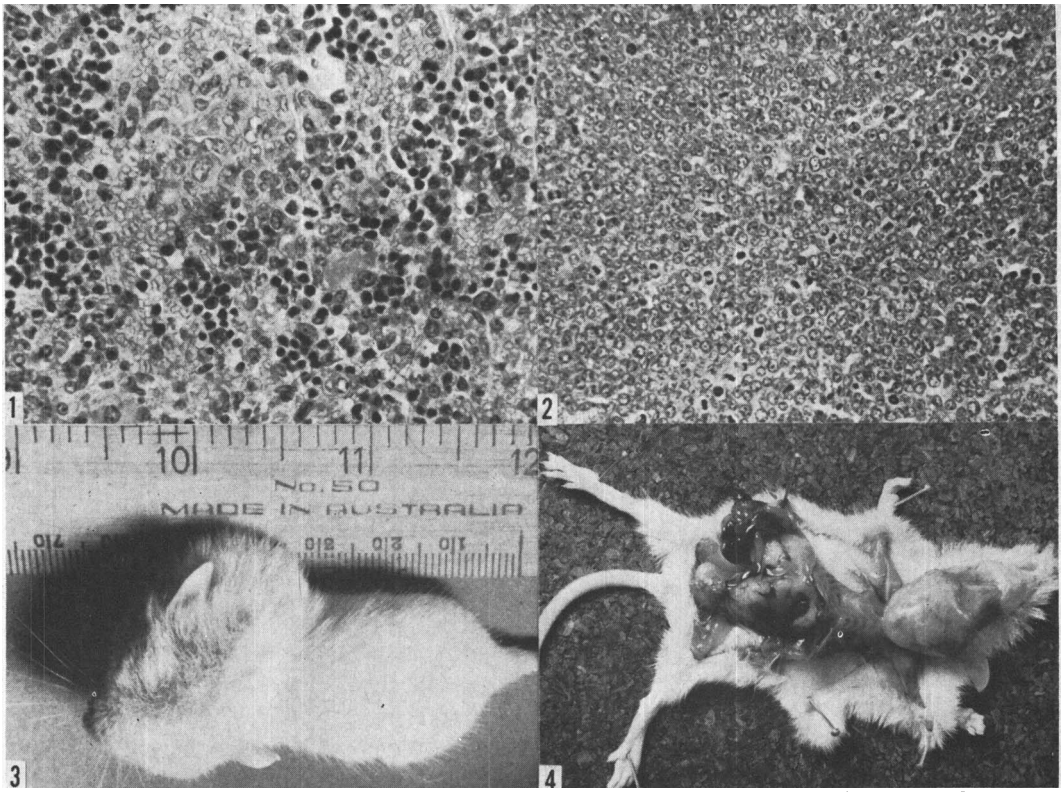

FIG. 1. Spleen. Large cells with vesicular nuclei and small hyperchromatic mononuclear cells fill the sinusoids. A mitotic figure is present near the center of the field. Haematoxylin and eosin stain. × 336.

FIG. 2. Tumor. Sheets of lymphoblastic cells showing numerous mitoses are present. Haematoxylin and eosin stain. × 266.

FIG. 3. A 34-day-old mouse, 13 days following administration of lymphoma cells.

FIG. 4. Post-mortem appearance of an 18-day-old mouse following inoculation of lymphoma cells intraperitoneally at birth. Note massive tumor in neck and mesentery.

oily hair effect from day 11 to day 22. On day 21, it became emaciated and behaved clinically as a runt until day 36. It was jaundiced from day 29 to day 38. It had severe conjunctivitis from day 59 to day 69 and remained ataxic and incoordinated until it was killed 272 days after infection. Apart from an enlarged spleen, post-mortem examination revealed nothing abnormal macroscopically. Complement-fixing antibody was present in the serum to a titer of 1/50. Virus could not be detected in liver, gut, brain or kidney, and fluorescent antibody studies did not reveal the presence of virus antigen in liver or brain.

Histopathological investigation revealed lesions in the spleen, lung, heart, liver and pancreas which fitted the pattern usually displayed in the late chronic disease.

The spleen was congested and infiltrated with two types of mononuclear cell, one being small and composed entirely of a dense hyperchromatic nucleus and the other large with a variable lobular and vesicular nucleus and pale foamy eosinophilic cytoplasm. Mitotic figures were plentiful (Fig. 1).

In the lung, a microscopic focus of alveolar cell hyperplasia and moderate chronic peribronchiolitis were seen.

Hepatic and pancreatic changes were those of small focal degenerations and necroses accompanied by mild chronic inflammation. The nuclei of hepatocytes showed frequent variations in their size and shape.

Early fatty degeneration of endocardial fibers was apparent in the heart.

This clinical and pathological picture follows closely the general pattern we have described for 50 other mice with the chronic disease(2).

A suspension of the spleen cells of mouse 2731/6/272 was given by the intraperitoneal route to infant mice of the same strain. Massive mesenteric lymph node tumors were developed by 31 days.

Transmission of the tumor cells by the I.P. route in newly-born mice for 10 passages results in a highly reproducible syndrome comprising a grey-white plaque of tumor stretching behind the stomach from the root of the mesentery to the tail of the pancreas, hepatomegaly (up to 4 times the normal size), bright yellow contents in the upper intestine and, quite often, atrophy of the spleen and thymus. Large tumors located in the jaw and neck are sometimes observed in addition to the mesenteric lymph node tumor (see Fig. 3 and 4).

The tumorous plaque is composed of infiltrating sheets of mononuclear cells resembling lymphoblasts. Their nuclei are vesicular and exhibit variability in size and shape.

Mitoses are plentiful and often abnormal; cells in mitotic division usually display a clear halo around the chromosomes (Fig. 2).

The tumor infiltrates and causes atrophy in most organs and structures, including lymph nodes, bone marrow, liver, pancreas, intestine, kidney, gonads, skeletal muscle, heart, lung and meninges.

The spleen loses its follicular architecture and, although neoplastic cells are present, no enlargement occurs. The thymus preserves its corticomedullary pattern and, although containing neoplastic cells, is not enlarged.

Discussion. The observations presented here together with our previous communications draw attention to two possible effects of neonatal murine infection with reovirus type 3. One response appears as immunological injury often associated with runting and the other as the production of lymphomas. It is possible that, as Schwartz and Beldotti (1965)(5) have suggested, runting in mice may escalate into malignant lymphomas. The reverse situation may also operate. On the other hand, there may be no relationship between the two. Stanley and Leak (1963)(6) drew attention to the association of a runting syndrome with murine infection with reovirus type 3. Since then, our studies of the reovirus chronic disease have confirmed the view that the chronic disease is an immunological one and not a persistent tolerant infection of the type illustrated by L.C.M. infection of mice. In another communication (Stanley *et al* 1965 (7)), we have shown that fatal runting is also a common syndrome developed by newly-born mice receiving homologous spleen cells from mice with the chronic disease between 200 and 600 days of age. No runting followed the administration of spleen cells from healthy adult mice of the same strain. The chronic disease may thus represent an experimental model of a virus-induced "autoimmune" disease, although this has not been firmly established.

In the same communication(7), we describe in detail the properties of the lymphoma derived from mice with chronic reovirus disease and showing premalignant changes in liver and spleen cells. Atrophy of thymus and spleen was frequently observed in these mice, as well as in the newly-born mice in which the lymphoma cells were passaged.

The production of tumors by reovirus type 3 is not unexpected in view of its very close relationship to Wound Tumor Virus in size, morphology, site of cytoplasmic replication and the double stranded RNA(8,9,10,11,12). Nevertheless, the possibility exists that the lymphoma was not induced by reovirus type 3 but arose spontaneously in the mice with age and was demonstrated by inoculations of spleen cells into newly-born mice. Evidence against this is presented elsewhere(7) where it is shown that:

(1) the mice used rarely develop leukemia, although a latent leukemia virus can be induced by X-radiation which produces a leukemia with properties quite distinct from the lymphoma described here;

(2) the tumor allegedly induced by reovirus is transmitted only by intact living cells and not by cell filtrates, and does not contain complete infectious virus;

(3) the malignant cell presumably contains the noninfectious reovirus type 3 viral genome; and

(4) there is a clear morphological and cultural relationship to the Burkitt lymphoma (13).

It is not the purpose of this communication to discuss the possible role of reovirus type 3 in the etiology of Burkitt's African lymphoma as this has been done elsewhere (14). We have recorded our preliminary observations on the association of a murine lymphoma with reovirus type 3 and suggest that this virus may induce two types of transformation in the cells of mice recovered from neonatal infection. One results in neoplasia and the other in chronic immunological injury (hepatitis, pancreatitis and C.N.S. involvement) (3,7). The relationship between the two is yet to be defined.

Summary. Suspensions of spleen cells from a mouse with late chronic infection (272 days) with reovirus type 3 produced a lymphomatous neoplasm; the tumor was easily passaged by the IP route to newly-born mice of the same strain.

1. Walters, M. N.-I., Joske, R. A., Leak, P. J., Stanley, N. F., Brit. J. Exp. Path., 1962, v44, 427.
2. Stanley, N. F., Leak, P. J., Walters, M. N.-I., Joske, R. A., ibid., 1964, v45, 152.
3. Walters, M. N.-I., Leak, P. J., Joske, R. A., Stanley, N. F., Perret, D. H., ibid., 1965, v46(2), 200.
4. Papadimitriou, J. M., Proc. Soc. Exp. Biol. and Med., 1966, v121, 93.
5. Schwartz, R. S., Beldotti, L. (Schwartz, personal communication).
6. Stanley, N. F., Leak, P. J., Nature, 1963, v199, 1309.
7. Stanley, N. F., Walters, M. N.-I., Joske, R. A., Papadimitriou, J. M., Keast, D., Leak, P. J., (1965), submitted for publication.
8. Gomatos, P. J., Tamm, I., Proc. Nat. Acad. Sci., 1963, v49, 707.
9. Gomatos, P. J., Tamm, I., Biochim. Biophys. Acta, 1963, v72, 651.
10. Dales, S., Proc. Nat. Acad. Sci., 1963, v50, 268.
11. Gomatos, P. J., Tamm, I., Proc. Nat. Acad. Sci., 1963, v50, 878.
12. Spendlove, R. S., Lennette, E. H., Chin, J. N., Knight, C. O., Cancer Res., 1964, v24(10), 1826.
13. Burkitt, D., Brit. Med. J., 1962, vii, 1019.
14. Stanley, N. F., 1965, submitted for publication.

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Electron Microscopic Findings of a Murine Lymphoma Associated with Reovirus Type 3 Infection.* (30706)

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As reported in the associated communication, spleen cells from a reovirus-infected mouse (2731/6/272) with the late chronic disease produced a highly malignant lymphoma of the mesenteric tissues of neonatal mice when passed intraperitoneally (1). The lymphoma could be reproduced by intraperitoneal passage of intact cells in newly-born mice.

Methods. Portions of lymphoma were immersed in osmic acid fixative, dehydrated, embedded in araldite and sectioned with a Huxley microtome using glass knives. The sections were examined on a JEM T6 60 KV electron microscope using a 50 μ objective aperture.

Results. The tumor consisted of lymphoblastic cells measuring 6-9 μ in diameter. Ribosomes were plentiful in the cytoplasm. Cytoplasmic organelles consisting of mitochondria, a few strands of endoplasmic reticulum and the lamellae and vesicles of the Golgi apparatus, were seen regularly in these malignant cells (Fig. 1). Centrosomes were often found in the paranuclear region.

Lipid droplets and granular osmiophilic membrane bounded inclusions probably representing phagosomes, were also in evidence in the cytoplasm of these cells. Fine fila-

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