employed (300 μg in 2 doses) about half of their animals died and the effect on injected oil was inconclusive in the remaining individuals. Since estradiol benzoate at a 30 μg level (24 injections) brought about an increase in carcass fat it is possible that the ratio of androgens to estrogens determines the net effect of these hormones on body fat.

Summary. Estradiol benzoate, *per se*, causes an increased storage of body fat in male rats when they are maintained on a diet rich in saturated fat but devoid of essential fatty acids. The effect depends on the size of the dose and the length of the period in which it is administered.

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Electron Micrography of the Eastern Strain Equine Encephalomyelitis Virus.

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In recent reports from this laboratory, there have been described the results of preliminary studies with the electron microscope on two small animal viruses, namely, the agent of rabbit papillomatosis[1] and that of the Western strain equine encephalomyelitis.[2] The present paper is concerned with the findings in a similar examination of the Eastern strain equine encephalomyelitis virus.

The virus was propagated in chick embryos and purified by ultracentrifugation as previously described.[3] The final product was dissolved in Ringer solution[4] pH 8.5. Examinations have been made of the virus obtained from 5 different batches of diseased embryos at various intervals after purification. The dried films for electron micrography were prepared as previously described.[1,2]

A micrograph of the Eastern strain equine encephalomyelitis virus is shown in Fig. 1. For preparation of the film a solution containing 2.2 mg virus per cc in Ringer fluid was diluted fourfold with distilled water. As shown, the predominant images are circular in shape and appear as individuals or small irregular agglomerates dispersed at random over the field. There is present also a small amount of ill-defined amorphous extraneous material. The circular images are, like those of the papilloma and Western strain viruses, uniform in size, shape and appearance. The central dark area of the images indicates, through greater scattering of electrons, a relatively high density of the corresponding region of the virus particle. Extending peripherally from the central area, the enveloping substance appears to be progressively less dense and finally fades indefinitely into the film background. This appearance of the generality of the images gives the impression to be expected of a spherical particle made up of a loose structure of regularly arranged diffraction centers, the density in the thickest dimension diminishing toward the outer limits. Evidence of structure and differentiation internally was not generally observed in fresh preparations.

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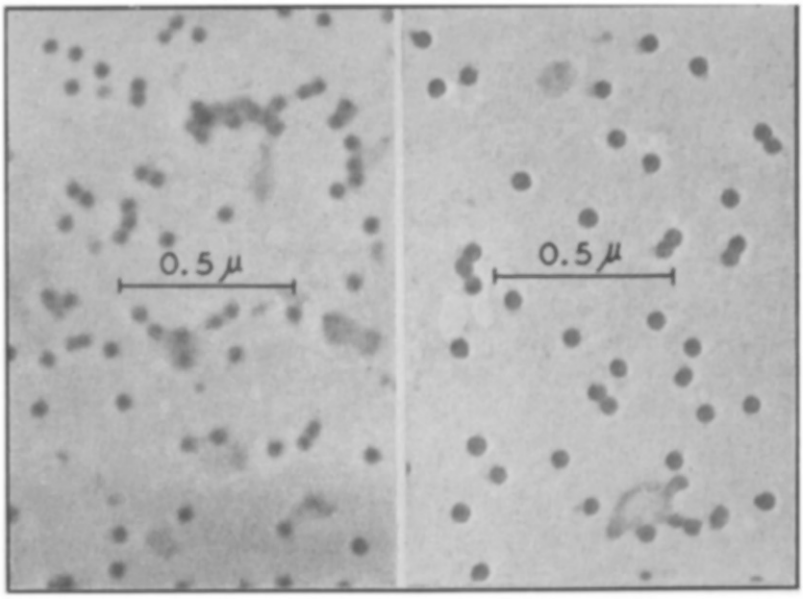

Equine Encephalomyelitis Virus, Eastern Strain.

FIG. 1. Virus particles placed on collodion film in Ringer solution diluted 1:4 with water.

FIG. 2. Virus particles in Ringer solution diluted 1:3 with 0.25% $CaCl_2$ solution and kept at room temperature for 1 hour before preparation of film. Photographs were taken with 55 kilovolts electrons. The magnification is 46,000 ×, calculated from measurements on the width of tobacco mosaic virus rods which has been reported to be 15 mμ.[5]

In some of the images, however, there appeared to be a definite central rounded area of increased density approximately two-thirds of the total image diameter.

The vagueness of external outline of the images seen in Fig. 1 has been a regular observation with the Eastern strain virus, and, too, with the papilloma virus[1] and with fresh preparations of the Western strain equine encephalomyelitis virus.[2] In the case of the Western strain virus, the images were invariably more clearly defined after the virus had stood some days in Ringer solution at 2 to 8°C. On the hypothesis that the particle density with respect to electron scattering might be associated with salt factors, studies were made to learn the effect of changing the concentration and proportion of the salts of Ringer fluid. No definite effect was observed with NaCl or KCl. On the other hand, the character of the images was markedly altered with changes in $CaCl_2$ concentration. In Fig. 2 is shown the micrograph of a virus preparation in Ringer fluid diluted threefold with 0.25% $CaCl_2$ and kept for 1 hr at room temperature before preparation of the film. No attempt was made to wash the prepared film. The images of highly uniform size now show clearly defined limits with a reversal of the regions of relative density. In the central area, the density appears less than that toward the surface, revealing a relatively light (in the micrograph) structure corresponding in position to the rather indefinite dense internal region of the images of Fig. 1. This increase in density related to $CaCl_2$ was reversible, at least in great part, since washing with water of a film prepared as for Fig. 2 resulted in a micrograph like that of Fig. 1. Treatment of the virus with $MgCl_2$ produced an effect somewhat similar to that seen with $CaCl_2$.

The average diameter of the particles of Fig. 1, including the outermost vague limits was 40.2 mμ as compared with 47.5 mμ for the particles of Fig. 2. The mechanism responsible for the alteration in the appearance

and size of the images of the virus particles is not clear. There is the possibility that the calcium salt may have entered into a loose combination with the peripheral substance, which, by this means, became sufficiently dense for demonstration. The apparently greater size of the particles represented in Fig. 2, however, was not paralleled by change in the sedimentation rate which was not altered by treatment for 1 hr with $CaCl_2$. It is unlikely that the change was due wholly to drying effects occurring at the particle surface while on the film, since the appearance of the image was altered only when the virus was in contact with the $CaCl_2$ for some time in solution.

The present results indicate that the Eastern strain equine encephalomyelitis virus is probably spherical in shape, constituted peripherally of a substance the limits of which were ill-defined in the micrographs in the absence of special treatment. The average diameter of the particles of vague outline of Fig. 1 was 40.2 mμ. If the true particle limits are those portrayed after treatment with $CaCl_2$, the diameter was 47.5 mμ.

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Further Experiments Dealing with Embryonic Enucleation in Amblystoma.

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In a previous communication[1] we described the results of embryonic enucleation in *Amblystoma punctatum* upon growth, metamorphosis and pigmentary changes. Our results upon growth did not conform with those of Browman[2] who found that rats enucleated at birth, regardless of environment, had a growth rate consistently lower than their litter mate controls of the same sex.

Browman[3] found that rats enucleated at birth suffered retarded development of the genital tracts and the gonads. We investigated this matter in *Amblystoma punctatum,* but could find no evidence at metamorphosis because of insufficient development of the gonads. Because of the difficulties of raising *A. punctatum* through metamorphosis, we have repeated the work on *A. tigrinum* and *A. mexicanum* (axolotl).

Twenty embryos (tail bud stage) of *A. tigrinum* were enucleated. Ten eyeless and 5 normal controls were reared in darkness, and 10 eyeless with 5 controls were kept under ordinary daylight illumination. Ten unilateral and 10 bilateral enucleations were done on axolotl embryos (tail bud stage). These along with 10 normal embryos were reared in aquaria under normal conditions of illumination.

In *A. tigrinum* we dissected the gonads of 5 females without eyes and 2 with eyes which had been raised in the dark; also 3 males without eyes and 3 with eyes reared in the light. The animals studied ranged from 109 to 170 days of age. All had metamorphosed except one. We found considerable variation in the size of the ovaries and the testes irrespective of the presence or absence of eyes or the conditions of illumination. Although the gonads were well differentiated, they had not reached maturity in either the experimental or the control animals. Because of our failure to obtain evidence of any effects of eyelessness upon gonadal development in *A. tigrinum* up to 170 days of age, we reared the axolotls *(A. mexicanum)* for two years. At the end of this period, we dissected 2 control and 5 eyeless axolotls and found no significant size difference between the gonads of the two groups.

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