sealed. (6) No further enlargement in the opening in the shell is required for removal of the embryo.

Using this technic of inoculating eggs, we have succeeded in isolating influenza virus A from throat washings which had been preserved at a temperature of approximately —76° for nearly 2 years.

## 13961

## Influence of Estradiol Benzoate on Fat Storage.*

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In a recent study Loeb and Burr[1] showed that female rats store more body fat than males when maintained on a diet rich in saturated fat (hydrogenated coconut oil) but devoid of essential fatty acids. It then became a matter of interest to ascertain whether this finding could be attributed to the effect of estrogen on fat metabolism. Zondek and Marx[2] reported that estrogen not only induced a marked lipemia in fowl but also caused the accumulation of fat in the internal organs, particularly the liver. However, their findings with mammals were negative. Loeb[3] observed a definite, though moderate, lipemia in male rats fed a high fat diet lacking in essential fatty acids when estradiol benzoate was injected over a 4-week period. The present report is concerned with the storage of body fat under similar conditions.

*Methods.* Albino rats of the Wistar strain were taken at weaning time and placed on a low fat diet for 4 weeks to deplete the fat reserve. During the next 8 weeks, which comprised the experimental period, the animals were maintained on diet 580-B, a ration rich in saturated fat—71% hydrogenated coconut oil. One group received, subcutaneously, 3 large doses of estradiol benzoate‡ (100 μg per dose§), on alternate days, during the last week of the experimental period. Two other groups were given 24 more moderate doses (30 μg and 5 μg, respectively), daily except Sunday, throughout the last 4 weeks of the experimental period.[3] The animals were sacrificed by etherization and the carcasses† analyzed for total fat by digestion with 28% KOH in 50% alcohol for 4 hr followed by petroleum ether extraction of acidified aliquot portions. All analyses were done on duplicate aliquot portions which checked well within 1%.

Table I shows the mean values for total carcass lipid of the groups investigated. It will be seen that female controls (received no estradiol benzoate) had an appreciably higher carcass fat than the males. Rats which received 3 large doses of the hormone during the last week had a lower fat content than their male controls; but this effect was due to diminished food consumption under the influence of large doses of estrogen. Group C showed a higher content of body

* Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 265-1-71-236, Sub-project No. 382.

† Rockefeller Foundation Fellow.

1 Loeb, H. G., and Burr, G. O., unpublished results.

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3 Loeb, H. G., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 340.

4 Fisher, R. A., *Statistical Methods for Research Workers*, 7th Ed., Oliver and Boyd, London, 1938.

‡ The author wishes to thank Dr. R. G. Floody (Roche-Organon, Inc.) who kindly supplied the estradiol benzoate.

§ Each dose in every case was contained in 0.05 cc of olive oil.

† The carcass was prepared for analysis by removing the head, tail, and limbs.

TABLE I.
Carcass Fat of Rats Receiving Diet 580-B and Estradiol Benzoate.

| Group | No. of rats | Total estradiol benzoate ($\mu$g) | Total lipid, % | Groups compared | $t$* |
|---|---|---|---|---|---|
| A ♂ | 6 | — | 13.0 ± 0.93† | A vs. B | 4.32 |
| B ♀ | 6 | — | 20.1 ± 1.13 | | |
| C ♂ | 5 | 720 (24) | 16.1 ± 0.87 | A vs. C | 2.16 |
| D ♂ | 6 | 120 (24) | 12.7 ± 0.37 | | |

*The $t$ value was employed according to Fisher;[4] for the 5% level the value is 2.2, and 3.0 for the 1% level.
†Standard error of the mean.

TABLE II.
Food Consumption of Rats Receiving Estradiol Benzoate and Controls.

| Group* | Food intake (2nd to 7th wk., incl.) g/rat/day | Food intake (2nd to 7th wk., incl.) cal/rat/day | Food intake (2nd to 4th wk., incl.) g/rat/day | Food intake (5th to 7th wk., incl.) g/rat/day |
|---|---|---|---|---|
| 580-B Controls | | | | |
| A1 ♂ | 3.02 | 22.1 | 2.79 | 3.24 |
| A2 ♂ | 3.44 | 25.2 | 3.14 | 3.73 |
| B1 ♀ | 3.07 | 22.5 | 2.73 | 3.41 |
| B2 ♀ | 3.21 | 23.5 | 3.14 | 3.27 |
| 580-B M.E.† | | | | |
| C1 ♂ | 3.67 | 26.9 | 3.62 | 3.73 |
| C2 ♂ | 3.33 | 24.4 | 3.48 | 3.19 |
| 580-B L.E.† | | | | |
| D1 ♂ | 3.67 | 26.9 | 3.56 | 3.79 |
| D2 ♂ | 3.92 | 28.7 | 4.00 | 3.84 |

*Each of the 8 groups contained 3 rats except for Group C2 which had 2.
†M.E. = Medium estrogen dose (24 30-$\mu$g injections during 5th to 8th weeks, inclusive).
L.E. = Low estrogen dose (24 5-$\mu$g injections during 5th to 8th weeks, inclusive).

fat while no change was observed in Group D.

In evaluating the data it is necessary to take into account the effect of estrogen administration on appetite. Table II shows the average food consumption for 6 of the 8-week experimental period, and also for the 3-week period prior to hormone administration and the 3-week period during estrogen administration. Comparing Groups A and B it is found that the increased fat storage in females can not be ascribed to an increased food intake. The figures for Groups C and D show that administration of estradiol benzoate had no significant influence on appetite. The increased food consumption of rats in Group C as compared with the controls would not explain the increased fat storage since individuals of Group D which consumed as much or more food than those of Group C stored less fat. We are led to conclude that estradiol benzoate, *per se*, when given daily at a level of 30 $\mu$g over a 4-week period causes an increase in body fat.

From Table I it is apparent that the difference in fat storage of males and females when no estrogen is administered is highly significant. The increased body fat of rats receiving 30 $\mu$g doses of the estrogen can not be explained by increased food intake, and the $t$ value indicates that the difference is significant. The smallest dose administered (5 $\mu$g) did not influence the storage of body fat although, as shown in an earlier study, it did induce a moderate lipemia.

Turner and Mulliken[5] have recently reported that testosterone propionate administration accelerates the removal of corn oil injected subcutaneously in mice. With the large doses of estradiol benzoate which they

[5] Turner, J. C., and Mulliken, B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 585.

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.

## 13962 P

## 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.

* This work has been aided by grants from Lederle Laboratories, Inc., Pearl River, New York, and by the Dorothy Beard Research Fund.

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