brain and spinal cord of E.A.E. rabbits. It is believed that further elucidation of these changes and their possible underlying metabolic-enzymatic cause merit continued investigation.

Summary. Determination of the ratios of sphingomyelin : lipid-galactose in brain and spinal cord of a group of E.A.E. rabbits and a group of normal rabbits showed remarkable similarity of the ratios within each of the groups of rabbits. Comparison of the values of the two groups with one another, however, showed that the sphingomyelin : lipid-galactose ratios of both brain and spinal cord from the E.A.E. rabbits were significantly lower than those from the normal ones. The metabolic-enzymatic cause of this change is subject of further investigation.

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Monolayer Cultures of Brown Fat Cells.* (30094)

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Classical techniques of explant(1,2) and organ(3) cultures of the brown fat body have been used to study the differentiation of fat cells and the growth of rabies virus in adipose tissue. This paper and a previous abstract(4) report on the growth and hormonal responses of brown fat cells *in vitro* using a technique of trypsin dissociation and reaggregation, to produce monolayer cell cultures.

Interscapular fat pads from one- to three-day-old rats were diced and dissociated by vigorous pipetting in 0.25% trypsin (1:300) in a Ca-Mg-free Hanks' solution(5). Pads from older animals were not used since they have accumulated too much fat and their cells will not settle on glass. Repeated trypsinization of larger fragments was usually necessary. The cellular residues were spun at 1000 rpm in a refrigerated centrifuge at 10°C, and the cell pellets resuspended in Eagle's Medium(6) supplemented with bovine fetal serum. Cell viability was checked by trypan blue ingestion. The cell number was adjusted by dilution to 500,000/ml of medium, and the cells allowed to settle on coverslip surfaces in Leighton tubes. Cultures were incubated at 37°C, and coverslips with attached cells were withdrawn at 12 hour intervals over a 3 day period, fixed in 10% formalin, and stained with Sudan Black in 70% ethanol, and Wright-Giemsa stain. Insulin was prepared as a stock solution of 5 mg/ml by dissolving the crystalline hor-

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mone in Hanks' solution adjusted to pH 8.5 with additional sodium bicarbonate. Penicillin was added routinely to cultures (500 units/ml).

Results. The 12, 36, 72 hour cultures best illustrate the developmental potentialities of these cells. Early monolayer cultures show sparse rounded cells with relatively fine, sudan-positive granulation. These cellular elements are clearly differentiated from spindle-shaped ones which have already begun to flatten and spread (Fig. 1). By 36 hours (Fig. 2), the cells are enlarged and have acquired more sudanophilic granulation. Their rounded, vesicular nuclei usually contain a single prominent nucleolus. The spindle-

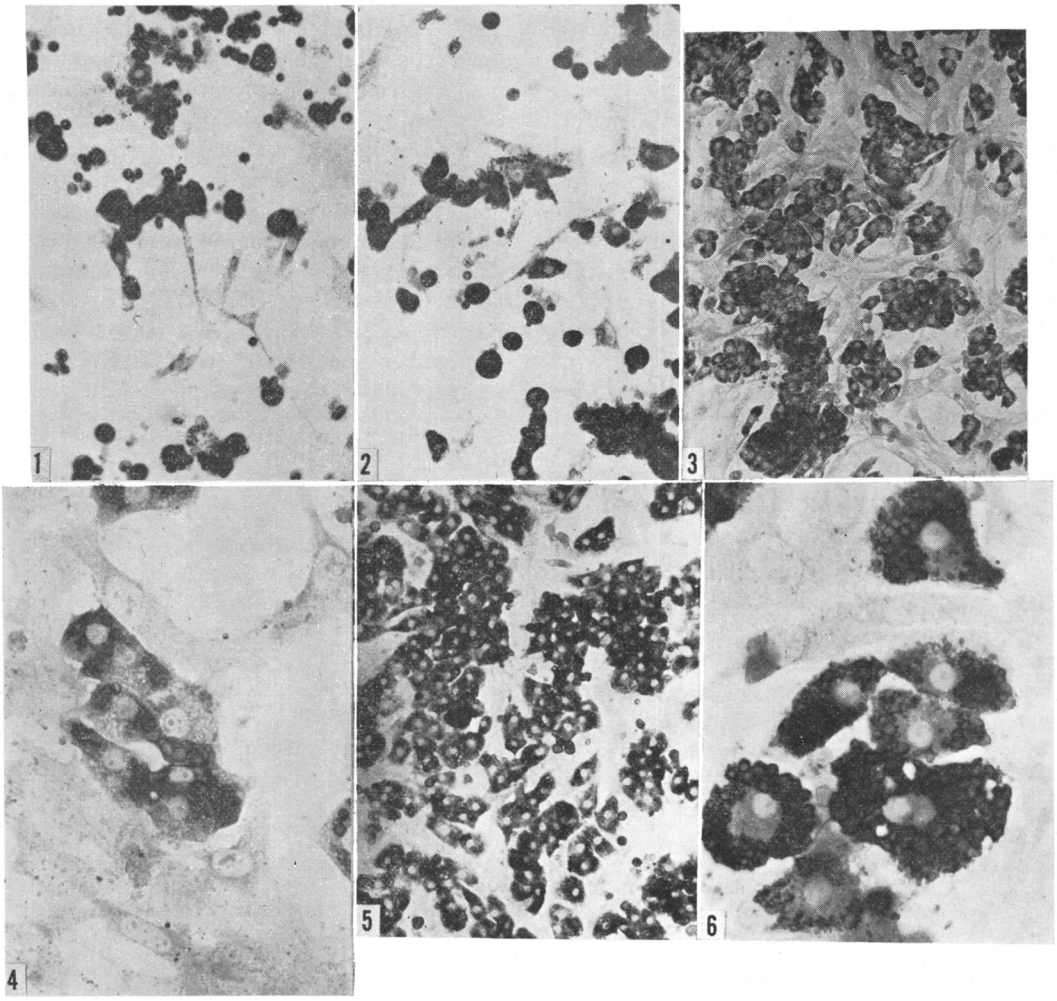

FIG. 1. 12 hour culture. Fibroblasts are elongated and flattened; fat cells are still condensed and show little histological detail. Small round elements are erythrocytes. 120 ×.

FIG. 2. 36 hours. Epithelioid fat cells, with their sudanophilic droplets are now clearly distinguishable. 120 ×.

FIG. 3. 72 hours. This is a densely populated area of coverslip showing reaggregation of fat cells into islands surrounded by fibroblasts. 120 ×.

FIG. 4. 72 hours. An island of fat cells showing sparse droplets and clear areas in the cytoplasm. Some scattered sudan positive material overlies the fibroblasts. Nucleolar differentiation can be seen. 400 ×.

FIG. 5. 72 hours. Insulin added at 48 hr. An increase in size of the fat islands, almost obliterating the interstitial fibroblasts. 120 ×.

FIG. 6. 72 hours. Insulin added at 48 hr. Size of the cells, and number and size of fat droplets are markedly increased over that shown in Fig. 4. 400 ×.

shaped cells are now clearly distinguishable as fibroblasts, with oval nuclei and several nucleoli. There is a tendency at this time for the fibroblasts to circumscribe clusters of fat cells. At 72 hours (Fig. 3), reaggregation of fat cells into islands surrounded by fibroblasts is well established. The lobulation is similar to that of the mature parent tissue. Numerous sudanophilic droplets interspersed with clear vacuolated areas are visible in the cytoplasm of the epithelioid cells (Fig. 4).

Addition of insulin to the culture medium (20 μg/ml at 48 hours) produced a marked change in organization of the adipose cell islands. The fat cells enlarged, almost obliterating the fibroblastic septa, (Fig. 5). The cytoplasm became more basophilic and the fat droplets increased in size and number (Fig. 6). Aside from mechanical compression, no obvious cytoplasmic changes were seen in the fibroblasts.

Discussion. These preliminary observations show that the technique of monolayer cell culture lends itself to the study of adipose cells and their response to insulin. Cells of the brown fat body show the same ability to reaggregate after dissociation as Moscona(7) and Steinberg(8) described in other tissues. No conversion of multilocular to unilocular fat cells was observed, although this occurs normally in the interscapular fat pad *in vivo*. Transformation of fibroblasts to fat cells *in vivo* has been a controversial topic. In this study, fibroblasts were not converted to fat cells regardless of insulin level. Mitotic figures were found in early cultures, but could not be assigned with certainty to either cell category. The increase in total cell numbers, however, suggests that fat cells do divide, at least before they have acquired a full complement of fat droplets.

The addition of insulin to cultures markedly enhanced lipogenesis in these cells. The low level of fat formation in control cultures could have been due either to the presence of some insulin adsorbed on the parent cells or introduced in the serum, or may represent a low intrinsic activity.

Summary. Dissociated interscapular fat pads of 1-3-day-old rats were studied in monolayer cultures. The fat cells were observed to form islands of epithelioid cells which were clearly distinguishable from fibroblasts. Addition of insulin to cultures induced hypertrophy of adipose cells and increased the number and size of their fatty droplets. The fibroblasts were unaffected.

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Prevention of Tuberculosis and Reversal of Tuberculin Reaction in Guinea Pigs.* (30095)

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A significant number of infected guinea pigs showed a complete reversal of tuberculin reaction following treatment with isoniazid (INH). In addition, the minimal infective dose of *Mycobacterium tuberculosis* employed in this study failed to produce disease in animals treated before inoculation. The design of this experiment differs from other studies in several respects(1-3). Each animal was followed individually with periodic tuber-

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