Our results are of practical as well as theoretical interest since they are at variance with the widely held belief that arteriosclerotic lesions in the human generally occur more frequently in males than in females. Complex pathologic processes in human disease are often difficult to interpret because one rarely has the opportunity systematically to isolate and study the precise effect of such crucial conditioning factors as sex, diet, age, genetics, stress, and others. It remains to be seen whether the various parameters of aging can be clarified through the use of the experimental progeria-like model and to that end various conditioning factors other than sex are now under investigation in this laboratory.

Summary. Intact male and female rats of the same age, same strain, and approximately the same initial body weight were subjected to a relatively low dose of dihydrotachysterol (DHT) over a period of 28 consecutive days. All the females developed a severe progeria-like syndrome (medial calcification of the coronary arteries and aorta, kyphosis, dental abnormalities, severe body weight loss, osteosclerosis, loss of hair, and atrophy of the skin, adipose tissue, skeletal musculature, and large parenchymatous organs). The males, on the other hand, were protected against this senile-like syndrome with the exception of a moderate degree of osteosclerosis and a minimal calcific reaction in the interstitial tissue of the renal medulla. The significance of these observations are discussed.

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Modification of the Basal Architecture of Renal Tubule Cells in Aged Rats.* (29645)

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Thickening of the peritubular basement membrane has been observed in the kidneys of humans and rats in association with the nephropathies of disease and aging. Farquhar, Vernier and Good(1) reported this change in the kidneys of patients having glomerulonephritis. Gellman *et al*(2) and Freeman and Roberts(3) described a similar observation in cases of diabetic nephropathy and renal glycosuria. Scott and Foltz(4) noted that thickening of the peritubular basement membrane was a consistently occurring feature in normal aged rats.

While investigating the histological changes associated with single essential amino acid deficiencies in aged rats, we observed a definitive morphological alteration in the appearance of the thickened peritubular basement membrane in the kidneys of all rats included in the experiment. Since it was not possible to determine the nature of this modification with light microscopy alone, kidneys of normal aged male rats were examined by electron microscopy.

Method. Kidney sections of 30 aged male

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rats (over 600 days of age) from a previous study of valine deficiency were examined by light microscopy. They comprised 3 groups of animals: normal-control (10 rats); valine-deficient (10 rats); and pair-fed-control (10 rats). The normal-control rats were fed a purified diet consisting of a mixture of 19 crystalline amino acids, vitamins, sucrose, cottonseed oil and minerals as described by Rose, Oesterling and Womack(5). The deficient rats received the same diet except for the total omission of valine. The caloric value of the missing amino acid was supplied by additional sucrose. The pair-fed rats were fed the complete diet in such amount as was consumed by each deficient partner on the previous day. All animals had free access to water and, with the exception of the pair-fed group, were fed *ad libitum*. At the end of a 70-day experimental period, the animals were killed by exsanguination and a portion of one kidney from each was fixed in Bouin's solution, paraffin embedded and prepared for light microscopy by staining with hematoxylin and eosin and by the periodic acid-Schiff (PAS) method for demonstration of the basement membrane.

In addition, for study by electron microscopy, one kidney from each of 7 aged male rats (over 600 days of age) which had been fed laboratory chow and had free access to water, was prepared by fixation in 2% buffered osmium tetroxide and embedment in Araldite 502. Ultrathin sections were stained with lead citrate(6) and thick sections were stained by the PAS method for examination by light microscopy.

For comparison, using the methods described above, one kidney from each of 15 normal weanling and 15 normal young-adult male rats were examined by light microscopy. One kidney from each of 4 normal young-adult male rats was prepared for electron microscopy.

Results. Since thickening of the peritubular basement membrane and modification of the basal architecture of the adjacent tubule cells was observed in the kidneys of all aged rats regardless of the type, quality or quantity of their diet, the observations described here are presented on the basis of a single group of aged male rats.

Kidney sections of all rats, whether prepared for light or electron microscopy, showed remarkable thickening of the peritubular basement membrane. This change was present throughout the cortical area, but did not occur in all tubules and was more frequently associated with the proximal convoluted tubules than with other regions of the nephron. In electron micrographs, the point of contact between the widened peritubular basement membrane and that of the adjacent capillary endothelium was frequently distinguishable as an area of less density (Fig. 3).

In the PAS-stained sections of both paraffin and epoxy embedded kidneys, the clearly defined basement membrane of the proximal and distal tubules appeared to have indentations or notching at regular intervals on the tubule side which gave the basement membrane a serrated appearance (Fig. 1, 2). The indentations penetrated the basement membrane for a considerable distance, but were never observed to penetrate completely through. In PAS-stained sections having tubules sectioned favorably in transverse and tangential planes, the indentations apparent in the transverse sections were continuous with parallel light areas of the tangential sections (Fig. 1, 2). Since light microscopy did not reveal details of the nature of the indentations or of the relationship between the tubule cells and the basement membrane in areas where indentations were present, electron microscopy was employed for further study.

The electron microscope showed that the indentations were caused by protrusions from the base of the tubule epithelial cells. These protrusions were regularly spaced and their depth of penetration into the basement membrane varied (Fig. 3, 4). In no instance were they seen to penetrate into the basement membrane of an adjacent tubule or capillary. Each protrusion was continuous with the cell of origin and included a number of cytoplasmic compartments and infoldings of the cell membrane. Neither vesicles nor organelles were present in the protrusions. Where 2

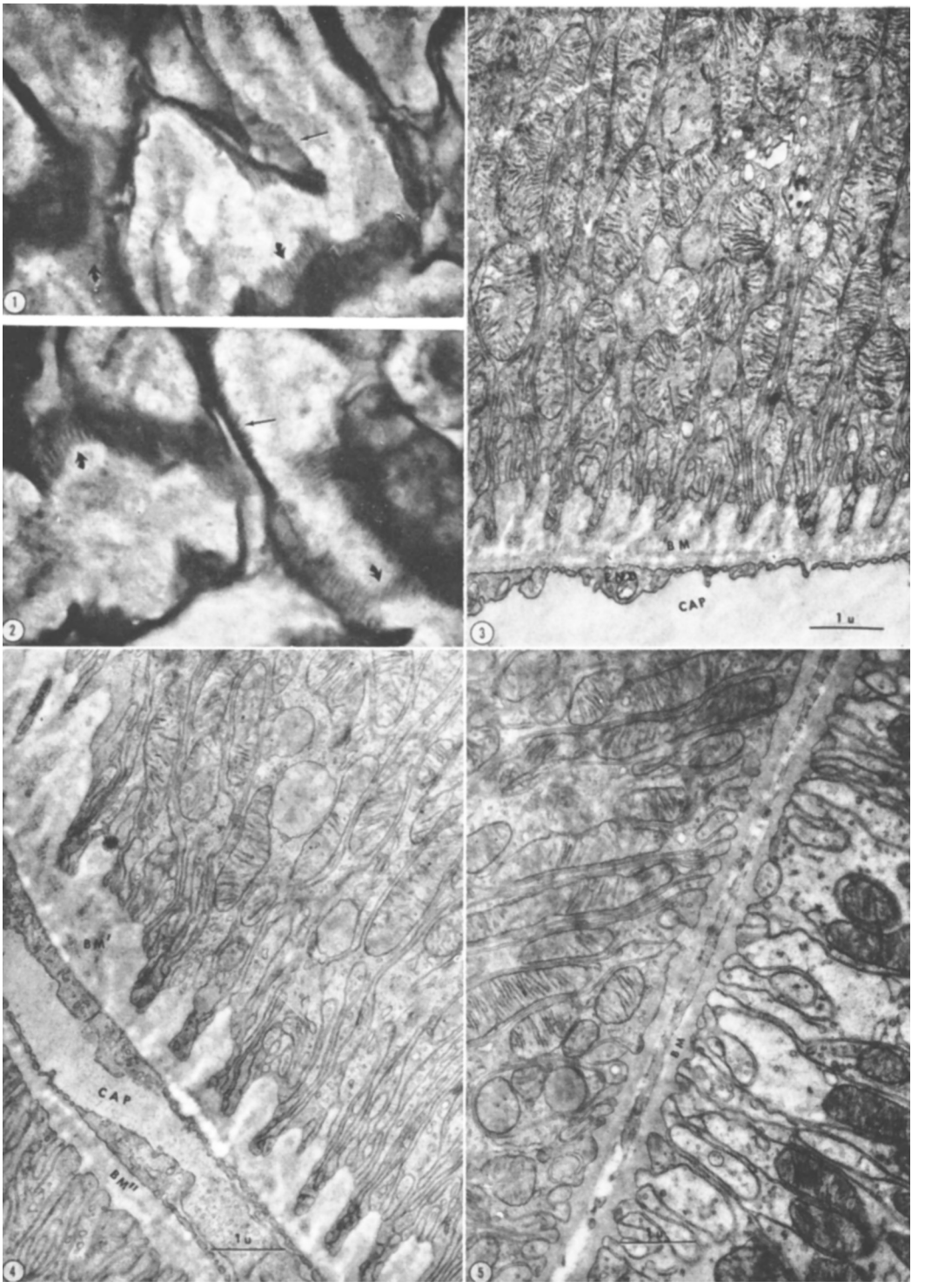

FIG. 1, 2. Sections of renal tubules of aged rat showing indentations or notchings in thickened peritubular basement membrane. Note that indentations indicated by the thin arrows merge into long parallel bands, indicated by broad arrows. PAS stain. 464 ×.

FIG. 3. Electron micrograph of base of a proximal tubule cell showing penetration of basal protrusions into thickened peritubular basement membrane. The point of fusion of the peritubular and capillary basement membranes is distinguishable. BM, basement membrane; END, endothelium; CAP, capillary. Lead citrate stain. 10,500 ×.

FIG. 4. Electron micrograph of basal areas of 2 adjacent tubules in aged rat kidney. The cell resting upon the thickened basement membrane (BM′) has numerous protrusions; that resting upon a thin basement membrane (BM″) is normal. CAP, capillary. Lead citrate stain. 10,500 ×.

FIG. 5. Electron micrograph of the basal areas of 2 adjacent tubule cells of a young-adult rat kidney. Width of the peritubular basement membrane (BM) and basal architecture of the cells are normal. Lead citrate stain. 10,500 ×.

adjacent tubules were present in the same field, one having a thick basement membrane, the other a basement membrane of normal dimension, the cells resting on the thick basement membrane usually had basal protrusions, while those cells abutting the thin basement membrane were normal (Fig. 4).

Ultrathin tangential sections of tubules were few and electron micrographs of transverse sections gave the impression that the protrusions were villiform. However, since in the PAS-stained sections the indentations of transverse sections were continuous with the light areas of the tangential sections (Fig. 1, 2), it was apparent that the protrusions were not villiform but consisted of long, parallel outfoldings of the base of the cells.

Kidney sections of weanling and young-adult male rats which were examined by light and electron microscopy showed no thickening of the peritubular basement membrane or alteration of the basal area of the tubule epithelium (Fig. 5).

Discussion. The underlying cause of an increase in the thickness of renal peritubular basement membrane in disease and aging is not understood. It has been suggested that such an increase is related to the interstitial connective tissue(1) and this change has also been described as a "cuffing" and the strongly PAS-positive material has been considered to be "outside, but on, the peritubular basement membrane"(2,3).

In an excellent study of the formation of the glomerular basement membrane, Kurtz and Feldman(7) demonstrated that new basement membrane is derived from the epithelial cells and that the newly deposited material is identical with the original basement membrane. They concluded that the growth and thickening of basement membrane is achieved in a similar manner in both normal and pathologic conditions. On this basis, it is highly likely that thickening of the peritubular basement membrane also results from tubule epithelium as a result of a stimulus as yet unidentified.

Since the basal architecture of the tubule cells was usually altered when the cells rested upon a thickened basement membrane, it is reasonable to conclude that the protrusions were directly related to this change in the basement membrane. Under normal circumstances, the resistance to transport offered by the peritubular basement membrane is considered minimal, but with an increase in thickness, resistance to transport would rise. We suggest, therefore, that the protrusions are formed by the cells to compensate for this increased resistance, in an attempt to shorten the distance of transport across the peritubular basement membrane.

Summary. Modification, in the form of basal protrusions, occurred in renal tubule cells of aged rats in areas where the peritubular basement membrane had become thickened. These changes were present in all aged rats regardless of the type, quality or quantity of their diet. It is suggested that this alteration is an attempt by the cell to compensate for an increase in resistance to transport presented by thickening of the basement membrane.

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Related Effects of Acute Exposure to Cold and Fasting on Free Fatty Acid Release by Adipose Tissue.* (29646)

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The exposure of animals to a low environmental temperature causes an increase in oxygen consumption of isolated tissues(1-3). The increase has been associated with high rates of turnover and oxidation of reserve metabolic substrates(4). The accelerated rate of fatty acid synthesis from acetate by adipose tissue from cold-acclimated rats has been postulated by Patkin and Masoro(5) as one source of substrate to meet the energy requirements of these animals. Mallov and Witt(6) demonstrated an increase of plasma free fatty acid (FFA)† to result from cold stress and Mallov(7) observed a stimulation of output of fatty acid by adipose tissue *in vitro* resulting from exposure of the donor animal to cold.

A number of hormones have been shown to increase the rate of release of FFA from adipose tissue(8). Prominent among these are the catecholamines(9,10). These have been found to stimulate heat production in cold-adapted rats(11,12) and urinary excretion of both epinephrine and norepinephrine has been reported to increase rapidly as the result of exposure to cold(13). The existence of mechanisms which link epinephrine and norepinephrine to tissue oxidation and also to the overall response to cold and FFA release from adipose tissue seem to be established. It, therefore, was of interest to compare the effects of fasting and of cold exposure on the production of FFA by adipose tissue, and to determine whether the tissue becomes more sensitive to the stimulating effect of catecholamines as the result of cold stress. To this end, the following experiments were performed.

Methods. Male albino rats of the Holtzman strain, body weight 200-250 g, were fed Purina laboratory chow *ad libitum*. Control animals were housed at a room temperature of 24-25°C. Animals used for exposure to cold were selected at random and were exposed at a temperature of 3-5°C in individual cages in a cold room. At the end of the exposure time, they were sacrificed by an occipital blow and the epididymal fat pad was removed and prepared for incubation in a manner similar to that described by Winegrad and Renold(15). In all experiments the thin distal portion of the fat body was used. Portions of approximately 100 mg were weighed on a Roller-Smith balance and placed in Krebs-Ringer phosphate buffer, pH 7.4, which contained Armour's crystalline bovine serum albumin at a concentration of 5 g/100 ml. Three ml of medium were used in a 25-ml Erlenmeyer flask. The flasks were gassed with oxygen and incubated for 2 hours at 37°C in a Dubnoff metabolic shaker. Samples of the medium before and after incubation were analyzed for FFA by the method of Dole and Meinertz(16). Both right and left epididymal fat pads from each animal were examined. The results obtained in the 2 flasks from each animal were averaged, and this value was used in the final interpretation of the data. The results are reported as

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† The letters FFA will be used to refer to free (unesterified) fatty acids released from adipose tissue.