while the cells from stock cultures were unable, the variants were capable of converting sufficient ribose, xylose or lactate into glucose to meet the requirements of cell growth.

It should be pointed out that the basal carbohydrate-free medium is of unknown chemical composition, on account of the presence of dialyzed serum. However, since serum from one single horse or, in critical experiments, single batch of dialyzed serum from one single bleeding was used, this variability of nutrient media composition has been reduced.

I would like to emphasize that the described experiments were not designed to study quantitative differences. The apparent frequent appearance of d(+)xylose variants may or may not occur under other experimental conditions. The successful isolation, as of this date, of d-ribose and sodium lactate variants from HeLa and not conjunctival cells may or may not be due to difference between the two cell lines. Finally, the failure to isolate d-arabinose or sodium pyruvate variants does not imply that such variants cannot be eventually isolated.

Summary. A method used successfully in isolation of nutritional variants from the human conjunctival and HeLa cells has been described. Variants capable of continuous propagation, under prescribed experimental conditions, with d(+)xylose, d-ribose or sodium lactate as the sole source of added carbohydrate or intermediate have been isolated.

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Selective Accumulation of Cd^{115} by Cortex of Rat Kidney.* (23619)

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Up to the present time the only elements with sufficiently specific localization in an organ to warrant use of their radioactive isotopes as physiological research tools have been iodine, which is concentrated in the thyroid, and zinc, which is concentrated in the rat dorsolateral prostate(1). This study using radioisotopes reports another element, cadmium, which selectively accumulates in the cortex of rat kidney.

Procedure. For the study of Cd^{115} distribution in the tissues of the rat, 40 male and female Wistar rats, 12-16 weeks of age, were used. Cd^{115} (half-life 43 days) was administered by intracardiac injection to etherized rats in doses of 8 μc/kg.† The animals were sacrificed by chloroform and exsanguination at various intervals from 1 hour to 8 months after injection. The tissues to be studied were removed from the animal, dried, weighed, and

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† Cd-115-P processed, high specific activity, was purchased from Union Carbide Nuclear Co. as $Cd(NO_3)_2$ in HNO_3 solution. Dilutions were made with physiological saline.

the radioactivity of each organ was measured by an end-window Geiger-Müller counting tube according to technics described previously(2). Studies were undertaken to determine what factors, if any, would affect the selective accumulation of cadmium in the kidney cortex. 1) *Age.* Cd^{115} was administered intraperitoneally to a total of 53 male and female rats varying in ages from 1 week to 16 weeks.‡ Twenty-four hours after injection the Cd^{115} content of kidney and liver was determined. 2) *Unilateral nephrectomy.* Unilateral nephrectomy was performed in 8 rats of varying ages from 2 weeks to 16 weeks. Two weeks post-operative Cd^{115} was administered and 24 hours later the Cd^{115} uptake was measured in the remaining hypertrophied kidney. 3) *Testosterone administration.* Administration of testosterone propionate in doses of 100 μg daily was begun in five 2-week-old male and five 2-week-old female rats. After 7 days of hormone administration the animals were injected with Cd^{115} and 24 hours later the Cd^{115} uptake was measured in kidney and liver. 4) *Orchiectomy and hypophysectomy.* Castration was performed in three 16-week-old male rats and hypophysectomy in two 16-week-old males. Cd^{115} was administered at 10, 20 and 30 days post-castration and at 10 and 20 days post-hypophysectomy. Twenty-four hours later the Cd^{115} content was measured in the kidney.

Results. Tissue distribution studies. Fig. 1 shows the gradual accumulation of Cd^{115} in the kidney cortex of the rat. The initial high concentration of Cd^{115} in the liver fell at about 20 days post-injection and was subsequently lower than cortical levels. By 5 months the kidney cortex contained approximately 2.4 times as much Cd^{115} per mg dry wt of tissue as the liver. The differences between the mean values for kidney cortex and liver at 5 months were shown to be statistically significant ($P<.01$). By 8 months post-injection the kidney cortex had accumulated approximately 4-5 times as much radio-

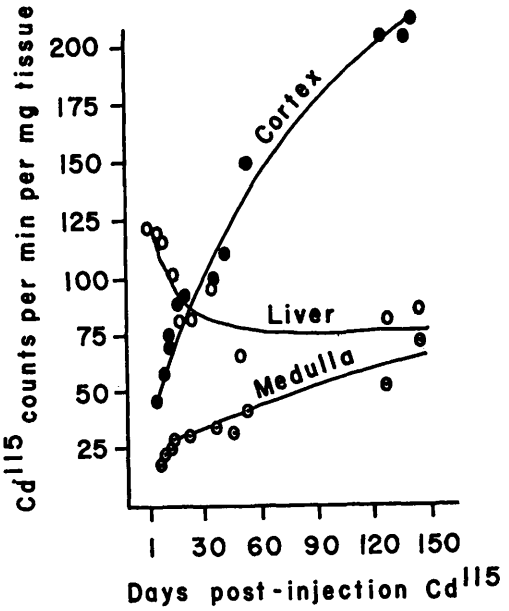

FIG. 1. Accumulation of Cd^{115} by kidney cortex of adult rat.

active cadmium per mg tissue as the liver (results not shown in the figure). Kidney medulla, though considerably lower in Cd^{115} uptake than kidney cortex, showed a gradual increase in the isotope content. By 8 months the Cd^{115} concentration in the medulla had exceeded that of the liver. The distribution of Cd^{115} in all body tissues was determined at 1, 3 and 5 hours; 1, 5, 7 and 9 days. At 1 hour the initial Cd^{115} uptake per mg of tissue was greatest in the liver. Pancreas, esophagus, ileum, large intestine and uterus contained from 30-60% as much Cd^{115} per mg as the liver. After 1 hour the Cd^{115} content of all tissues with the exception of kidney and liver, gradually fell or was maintained at low levels. By 9 days post-injection only the liver contained more Cd^{115} per mg than kidney cortex. The distribution of Cd^{115} in all other body tissues in relation to that in kidney cortex at 9 days was as follows: a) pancreas and uterus contained approximately 30% as much Cd^{115} per mg as the kidney cortex, b) seminal vesicles, coagulating gland, vas deferens, ventral prostate, dorsolateral prostate, ovary, submaxillary gland, lacrimal gland, pituitary and blood vessel contained 15-23% as much Cd^{115} per mg as the kidney cortex, c) esophagus,

‡ Studies had shown no difference in the 24-hour tissue distribution of Cd^{115} when the intraperitoneal rather than the intracardiac route of administration was employed.

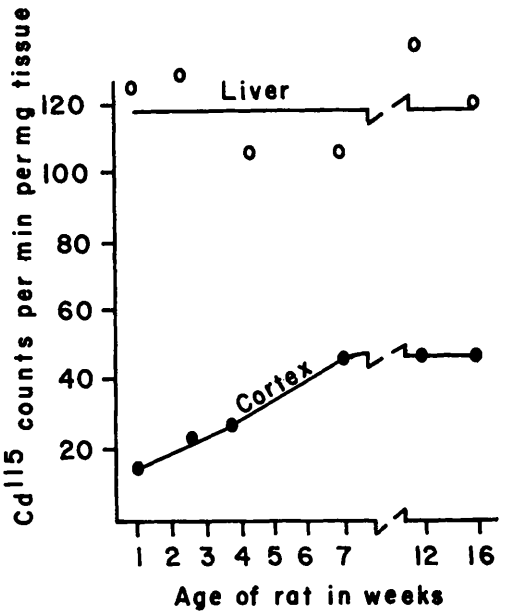

FIG. 2. Effect of age on 24-hour uptake of Cd^{115} by kidney cortex.

stomach, ileum, jejunum, large intestine, bladder, adrenal, thymus, lymph node and spleen contained 8-14% as much Cd^{115} per mg as the kidney cortex, and d) thyroid, testis, epididymis, lactating mammary gland, trachea, lung, heart, skeletal muscle, tongue, brain, spinal cord, eye, adipose tissue, skin, bone, tooth and blood contained 0-6% as much Cd^{115} per mg as the kidney cortex. Metabolic studies demonstrated Cd^{115} in the feces, the highest concentrations evident during the first 48-72 hours post-injection. Radioactivity in urine samples was absent. These metabolic findings are in accord with those recently reported by Decker and Byerrum(3).

Influence of age on Cd^{115} uptake in kidney cortex. Fig. 2 demonstrates relative inability of the young rat to concentrate Cd^{115} in the kidney cortex. With increasing age, up to about 7 weeks, there is a gradual increase in the Cd^{115} uptake per mg of tissue by the cortex. The differences between the mean values for 2½-week-old and 7-week-old rats were shown to be statistically significant ($P<.01$). The Cd^{115} uptake by the liver showed no significant differences from one age to another.

Influence of unilateral nephrectomy on Cd^{115} uptake of remaining kidney cortex. The 24-hour uptake of Cd^{115} per mg of tissue in the hypertrophied kidney following unilateral nephrectomy did not differ significantly from the intact control.

Influence of hormones on Cd^{115} uptake of kidney cortex. Neither orchiectomy, hypophysectomy, nor the administration of testosterone to the intact rat had any significant effect on the 24-hour uptake of Cd^{115} by the kidney cortex.

Discussion. One must consider whether the selective accumulation of Cd^{115} by the kidney cortex of the rat represents solely a toxic deposition of the element, or whether a part of this high concentration is a reflection of the natural amount of cadmium in the cortex which serves a definite physiological function. Evidence for a high natural cadmium content in the kidney as a whole is indicated in a report on trace elements in human tissue(4). Spectrographic analyses demonstrated that kidney contains higher concentrations of cadmium than any other tissue. The organs analyzed were from cases of accidental death, not necessarily from workers in industries using cadmium.

The studies reported in this paper revealed the inability of the renal cortex of the newborn rat to take up Cd^{115}, although age did not influence the concentration of the element in the liver. There was a gradual increase in Cd^{115} uptake by the cortex of the kidney with increasing age, reaching maximal adult levels at approximately 7 weeks of age. Kittelson (5) and Arataki(6) have reported that the cortex of the newborn rat has only one-third the number of glomeruli found in the adult, and that the full amount of glomeruli is not present until 3-8 weeks. Thus it appears that Cd^{115} uptake in the young rat kidney cortex parallels the number of nephron units present.

Studies in this paper have also shown that the 24-hour Cd^{115} uptake by the kidney cortex remains constant, in spite of unilateral nephrectomy and hormone imbalances known to affect other renal functions. This seems to answer the question raised by Shannon(7) and Taggart(8) who feel it is necessary to demonstrate unknown cellular elements available in constant but limited quantities in order to explain the known active transport of

large numbers of compounds across the tubular epithelium. Most recently Margoshes and Vallee(9) have reported the isolation of a cadmium-containing protein from the kidney cortex of the horse, thus furnishing more evidence that the presence of cadmium in the renal cortex reflects a biological need. It may be postulated that a possible function of cadmium in the kidney is concerned with water reabsorption, since mercury, a closely related element in physico-chemical properties, is known to inhibit water reabsorption in nontoxic doses.

Summary. Using radioisotopes another element, cadmium, has been found to selectively accumulate in one tissue, the kidney cortex of the rat, in concentrations sufficient to warrant its use as a research tool. The cortex of the young rat, which contains only one-third the number of nephron units found in the adult, is unable to concentrate Cd^{115} at the adult level. The studies reported indicate a possible physiological role of cadmium in renal function.

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Differentiation of Antiheparin and Thromboplastin-Generating Factors in Human Platelets.* (23620)

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Several authors(1,2,3,4) have suggested that platelets contain a factor which antagonized the effect of heparin in delaying blood coagulation. It has also been suggested that this factor is not the same as the thromboplastin-generating factor (Factor 3, of Seegers) on the basis of differential ultracentrifugation.

Materials. Platelet factors. Lyophilized human platelet material (LPM), prepared by the method of Klein *et al.*(5) was extracted with alcohol-ether (3:1 v/v) and the thromboplastin-generating material precipitated by adding three volumes of acetone in the cold. The precipitate was suspended in a small volume of water and lyophilized. This lyophilized platelet material (platelet lipid) was suspended in imidazole buffer pH 7.4 to a concentration of 1.0 mg/ml. This is the platelet lipid extract containing thromboplastin-generating activity(6). The residue of platelets after lipid extraction (lipid extracted platelet material) was freed of organic solvents and resuspended in imidazole buffer to a concentration of 3 mg/ml unless otherwise stated. *Brain lipid extract.* This was the alcohol-ether soluble acetone precipitated cephalin fraction of beef brain(7). Commercially available heparin sodium,‡ bovine fibrinogen (Fraction I§), and Thrombin Topical‖ were used.

Methods. The different materials were

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† Fulbright Research Scholar from Great Britain.

‡ Upjohn Co., Kalamazoo, Mich.

§ Armour and Co., Chicago, Ill.

‖ Parke Davis and Co., Detroit, Mich.