exposed to 23,000 feet for a similar period of time. The splenic CFU began to increase within 24 hours after the beginning of treatment and reached levels of 5-fold those of controls between days 8 and 16. The extent to which migration of CFU from the marrow and/or replication of CFU *in situ* is responsible for the increase in splenic CFU is discussed. It is concluded that the stem cell participates in the physiologic response to erythropoietin but without depletion of this compartment. The possibility that the previously reported decrease in CFU in hypoxic animals represents a secondary perhaps "toxic" effect of hypoxia unrelated to erythropoietin is considered.

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Reabsorption of Albumin-Evans Blue Via Membrane Vesicles of Proximal Kidney Tubule Cells* (32798)

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Small amounts of foreign and natural blood plasma protein filter into proximal tubule of kidney. Tissue electron micrographs of labeled protein followed in time after injection reveal the following path of reabsorption into tubule cells. Label becomes lodged inside invaginations of tubular plasma membrane from its surface between the base of microvilli of the brush border. Small apical vacuoles are presumed to bud off the invaginations. These fuse into large apical vacuoles slightly deeper in the tubule cell. Ultimately protein label becomes enclosed as cytoplasmic bodies or granules which lie surrounding the nucleus as though related to the Golgi apparatus, which may supply proteolytic enzymes. Probably proteins are split to constituent amino acids and are absorbed as such rather than as intact macromolecules into peritubular blood. This pathway is followed by all proteins (1) that can be visualized by electron microscope or histochemistry (hemoglobin, ferritin, horse radish peroxidase, albumin-^{125}I by electron autoradiograph). Evans blue T1824, a firmly bound label for blood albumin, may follow a similar pathway into the tubular cell (2). This interpretation is strengthened by our present freeze-dry histology, which is far superior to that previously published. We can immobilize and detect albumin–Evans blue in free solution. Travel across tubular epithelium is not as free molecules but inside the vesicle system. Recent literature provides more complete interpretation than possible when earlier publication on T1824 appeared.

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Method. Male Syrian golden hamsters, weighing about 130 gm, received intraperitoneal Na pentobarbital (6.6 mg/100 gm of body wt.). The Evans blue (12.6 mg/100 gm of body wt. as 3.5% solution in saline) was injected through a 26-gauge syringe needle into the continuation of a left foreleg vein where it crosses chest muscles superficially near their anterior border. The vein was exposed through a short skin incision but not cleaned of connective tissue so that it did not bleed when the needle was withdrawn. The incision was closed by stitches. Animals survived and were active up to 6 days.

The left kidney was quick frozen *in situ* after chosen time with second anesthetization. We accomplished this by suddenly placing a prechilled (liquid N_2) brass cylinder (2.6 cm diameter) about the exposed kidney and flooding the cylinder with cold isopentane (—140°C). Small 2-mm bits of tissue cut from cortex surface were excellently preserved. All other details of freeze-drying, embedding (epoxy resin) and sectioning were as in Wilde and Vorburger (3).

Results. Because of our extensive experience with histology of blood plasma in frozen-dried tissue sections (3), we learned to identify albumin–dye complex in free solution as distinct to forms seen here in cellular cytoplasm. During freezing of free solutions, as an ice crystal forms, free protein dye is pushed out ahead at the ice interface. In microtome sections the resulting blue "screen" is readily recognized.

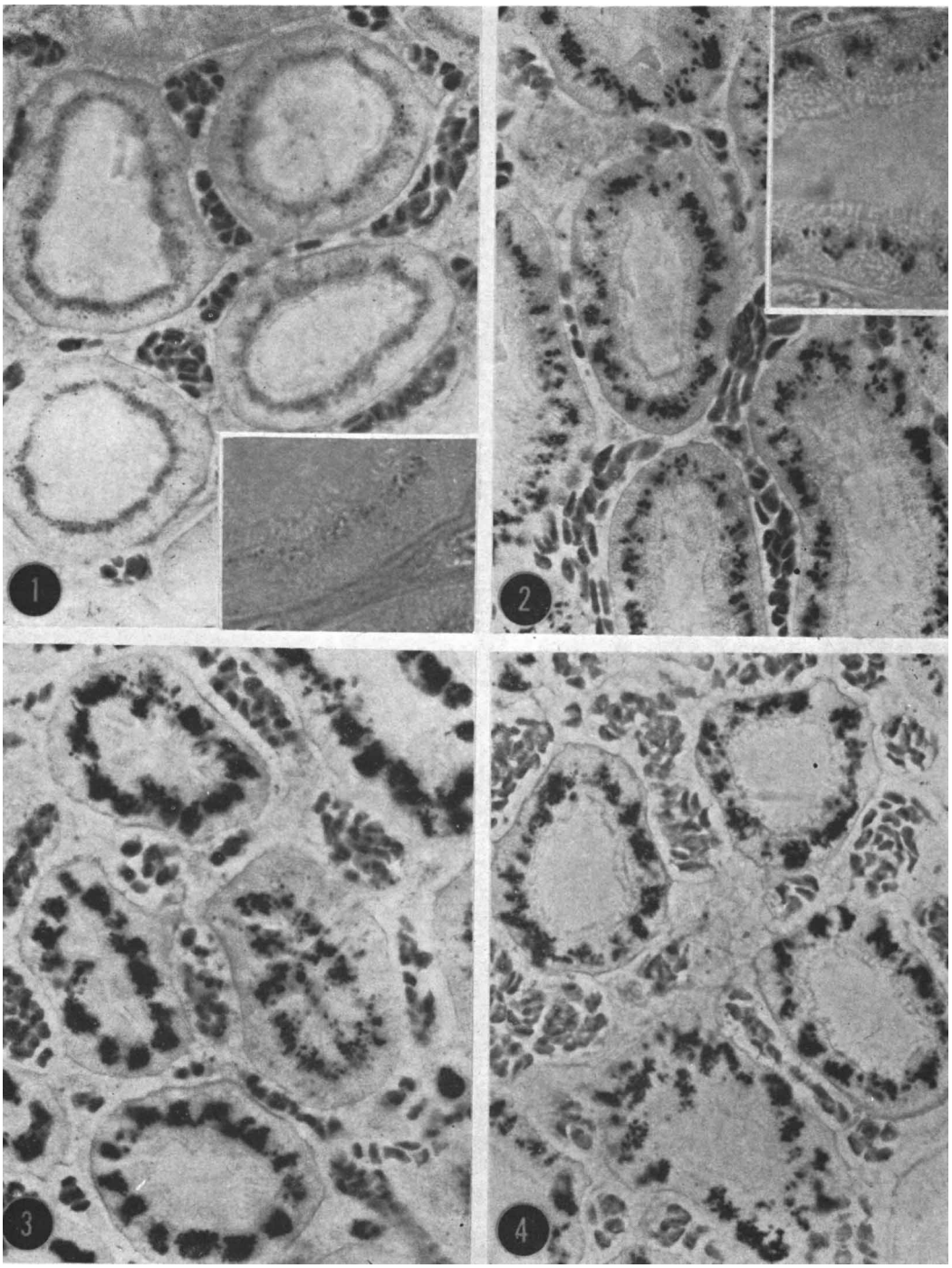
The cytoplasm of tubular cells in our frozen-dried preparations presents a very fine screen of dark material taken to be uncolored cell protein. Any free albumin-Evans blue would appear as a blue screen. Instead all cellular blue seen was in fine or coarse dots. We believe these represented protein–dye that is bound or contained in the various membranous vesicles described above as involved in protein reabsorption (1) and which are not disrupted during freezing. Liver cells in the hamsters contained such blue dots. Interstitial tissue contained blue dots in cellular elements and blue screen in the interspace but the latter, which indicates free albumin–dye appeared only early in time during the first hour.

Cellular uptake of dye during first hour. After 5 min of mixing in the circulation no visible dye entered proximal epithelium. At 1 hour small dark blue granules were scattered evenly just under the brush border (BB) (Fig. 1; inset shows higher magnification with excellent preservation of BB facing lumen at upper left). Some of these granules are so small as to appear dust-like. A very few (larger) granules have reached the middle or base of tubule cells. No tubular segments below the proximal show dye deposition in cytoplasm at any time. At no time, early or late, is dye seen in tubular passageway until the collecting ducts are reached. Here dye is mostly smeared irregular in shape in the lumen or occasionally forms a uniform screen in the cross-section as in the plasma of blood vessels (3). Blood vessels still contain dye at this time.

First day. After 1 day blue granules were larger and migrated completely away from BB, mostly to the middle of the cells (Fig. 2, with inset ×1000). Clusters of blue granules were already forming in special segment S1 of the proximal tubule (See next section) and granules in segment S2 were more scattered.

At this time blood plasma and interstitial space, even in the protein-rich papilla (3), were free of albumin–dye visible under the microscope. Special interstitial cells in cortex contained dye granules. Faint blue was visible in centrifuged plasma. Urine was now clear.

First 3 days. At this time near the beginning of proximal tubule blue granules clumped into special clusters. Proximal tubule was specialized along its length into 3 or 4 segments most recently described by Maunsbach (4). Epithelial cells of segment S1, which began at the glomerulus, stood on conspicuous cytoplasmic extensions or lamellae, which were tallest at the edge where they interdigitated with lamellae of neighboring cells (5). Very elongate mitochondria stood as vertical columns in the lamellae. Under the light microscope appropriately stained sections showed tall mitochondria (rodlets of Heidenhain) nearer the vague cell borders and hence between consecutive cell nuclei. Thus in segment S1, the intermediate zone of the cells, which exclusively houses cytoplasmic



FIGS. 1-4. Reabsorption of iv albumin-Evans blue by membrane vesicles in cytoplasm of proximal tubule cells of hamster kidney. Frozen-dried, 7 μ epoxy sections of tubules. Frozen while blood flow continued. Lumens mostly are open and interspace capilliaries are engorged with erythrocytes.

FIG. 1. One hour after iv dye. × 540. Blue albumin–dye photographs as black granules just

granules (presumably containing Evans blue) was crowded above cytoplasmic lamellae (mitochondria) and nuclei into a peculiar shape, deep at the side of central nuclei and shallower between cells where mitochondria are tallest. This special shape of the intermediate zone must determine the cluster arrangement of blue granules. However thus confined the Evans blue granules in S1 at 3 days crowded into distinct clusters (four tubules at left, above and below in Fig. 3). As best we can interpret, the cluster lies above and around the nucleus to the side, near the Golgi. In thin 3 μ sections, a nucleus indents the cluster. At this time (3 days) blue granules were the largest seen in S1. Granules were so densely packed that the cluster appeared as a blotch. The zone just below the BB was now clear. Compared to S2 this segment had higher epithelium, taller BB, and larger apical vacuoles, and as emphasized taller basally located mitochondria.

In segment S2, next down the tubule, cytoplasmic lamellae and mitochondria were shorter. Consequently the intermediate zone occupied a larger space and cytoplasmic granules were scattered without pattern (4). Correspondingly Evans blue granules were not clustered but scattered all through the cytoplasm (Fig. 3; tubule right of center). When as often S2 was collapsed the lumen filled with folds or arches of BB. The apical cytoplasm under the arches then filled with blue granules, close to the BB and extended toward cell base except when interrupted by a nucleus. Maunsbach found a similar distribution of large cytoplasmic granules (4) in S2, particularly in male rats, whether granules were identified by phase contrast, autofluorescence, or toluidine blue. The difference in distribution of blue granules in S1 and S2 is easily seen even in collapsed tubules (S1 and S2 are oriented left and right across the middle of Fig. 3). Tissue sections under low power contain easily identifiable patches of S1 and S2. We judge by comparing our Evans blue sections with v. Mollendorff's exhaustive and early study (6) with trypan blue, that both dyes, which differ only in the position of sulfonate groups, behave identically. From v. Mollendorff's dissected tubules we infer that S1 is a very short segment and that S1 and S2 specializations occur in all tubules, whatever the position in the cortex of the glomerulus of origin. The blue clusters are so distinct that they could be used in live exposed kidneys to identify tubules for micropuncture.

First 6 days. The S1 contained considerably less but still enormous amounts of dye. Clusters of granules seemed smaller, disrupted into strands and faded (Fig. 4). More granules separated off the cluster and often lay closer to the BB than at 3 days. One tubule section had clusters on one side and a row of granules just under the BB on the other side. If the latter granules were in S2 they must have been migrating toward the lumen. Since the plasma and filtrate were clear of dye, these granules could not represent dye still moving into the cells. Dye which had been ingested earlier must be moving out to the lumen. The faded appearance of dye in the clusters may indicate that dye is no longer bound to the albumin which had accompanied it into the cells. We judge from v. Mollendorff's observation with trypan blue that dye leaves the S2 segments much more quickly than from S1 clusters. We still saw scattered blue granules in S2. We have never seen dye in BB or in lumen at this level in our best preparations either in 1-hour or 6-day animals.

Discussion. Because we identified freely dissolved dye–protein complex by its 'screen' (3) we can state categorically that the complex does not traverse the proximal tubule

under brush border. Inset: × 1000. Brush border faces lumen at upper right.

FIG. 2. One day after iv dye. × 540. Dye moved to form cluster of granules in center of cells. Only segment type S1 shown. Inset: × 1000. Central lumen bordered by brush border.

FIG. 3. Three days after iv dye. × 540. Tubule right of center contains scattered dye granules typical of a second segment S2 of proximal tubule. Other 4 tubules contain clusters of granules, even more dense than at 1 day and typical of first segment S1 of proximal tubule.

FIG. 4. Six days after iv dye. × 540. Only S1 proximal segments shown. Granule clusters disrupted and faded with some granules lying close to BB.

cytoplasm in free form. Migration must be via the membranous vesicles described. Light microscopy cannot visualize membranes around blue granules but the path followed by blue granules is like that described for vesicles known to contain other tagged protein (1). More specifically Trump (7) examined cytoplasmic bodies in rats under electron microscope (EM) after injecting trypan blue, which differs from Evans blue only in position of the 4 sulfonate groups. Evidence that the EM can identify dye was inferred from intensified densities developed when dye was present and from similar size and distribution of densities and of blue color. The inference is that Evans blue like trypan blue is always contained in vesicles bounded by single layer membranes. Very early after injection (12 min) trypan blue lies dispersed in small vesicles just under BB. After 2 hours dye migrated deeper into apical cytoplasm where it lay very dense and uniform in larger vesicles. Possibly several apical vesicles coalesced to form a denser mass of dye, which, considering its density, may still be albumin bound. After 1 or more days dye lay dispersed inside even larger vesicles in the midregion of the cell. These vesicles often lie near Golgi. Trump believes these vesicles also contain phosphatase and hydrolyzing enzymes. Dye, now appeared less compact in irregular patches within the vesicle, may be separated off protein with which it entered the cell. Evans blue probably behaves the same way. Separated or segregated dye corresponds to faded dye in our 6-day hamster. Unfortunately Trump did not consider the different behavior in segments S1 and S2.

The initial suggestion in 'pinocytosis' was that protein is engulfed proportionately with water and other solutes by invaginating vesicles. Polysaccharides are indeed found in cytoplasmic granules (1). However, preliminary micropuncture data suggest that whereas inulin concentration rises along proximal tubule, albumin concentration remains stationary (8). Inulin is not being reabsorbed in proportion to protein. Protein must adsorb specifically onto the inside wall of invaginating vesicles. Subsequent coalescence of vesicles might produce dense condensations seen deeper in the cells by Trump (7).

Prolonged sojourn of Evans blue in proximal tubule cell suggests that it does not traverse the entire cell combined with intact albumin molecules. Since protein is in dynamic equilibrium it must move out of the tubule cell as fast as it moves in, whether entry and exit are both as combined protein–dye or whether exit is as amino acid residues, dye moving or being segregated separately. Sellers *et al.* (2) determined tissue content of dye and precursor values for filtering plasma dye. Plasma dye has nearly disappeared just as tissue is maximally loaded. In a simple turnover or traversal of dye–protein through the cell, dye would be expected to leave as quickly as it entered. In their rats maximum dye accumulated in 24 hours but still lingered in maximum amounts in tissue at 50 hours. Separated dye must segregate in cytoplasmic bodies.

How dye ultimately leaves the epithelium is unknown. At 6 days plasma is clear so that no dye is filtering to reach BB. Yet now some blue granules moved just under BB. Is this dye on its way to tubular lumen?

Summary. Reabsorption of filtered blood albumin–Evans blue across proximal tubule cells was not of free molecules through cytoplasm. The blue "screen" we use to identify free dye-protein was absent. The path and timing of migration in tubular epithelium of the special Evans blue granules seen was the same as that shown by other authors for labeled proteins and for trypan blue (close chemical relative to Evans blue), which are known through electron microscopy to migrate by enclosure inside single membrane vesicles. In reabsorption, albumin–Evans blue must migrate in this same vesicle system.

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Effect of Low Molecular Weight Dextran on Gastric Ulceration and Gastric Secretion in Pylorus-Ligated Rats* (32799)

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There is recurrent interest in the possible role of vascular factors in the genesis of peptic ulceration. Low molecular weight dextran (LMWD) has been advised in a wide variety of circulatory disorders in which tissue perfusion may be impaired. In view of its biorheological properties, the present study was designed to ascertain the effects of LMWD on gastric ulceration and acid secretion in rats.

Methods and Materials. A. *Effects on gastric ulceration.* Adult male Sprague-Dawley rats ranging in weight from 180–220 gm were deprived of food for 48 hours and water for 12 hours before surgery. The animals were lightly anesthetized with ether, and pylorus ligation (1) was performed through a midline incision by placing a 4-0 silk ligature around the pyloroduodenal junction, taking care not to occlude any blood vessels. Randomized test rats received 150 mg/100 gm of body weight of intravenous 10% isoosmotic LMWD (Rheomacrodex 40,000, from Pharmacia Laboratories, Piscataway, New Market, New Jersey) and control rats received an equal volume of normal saline. Following recovery from anesthesia the rats were kept under constant laboratory conditions until they were sacrificed after 6, 9, 12, and 16 hours, respectively. Each animal was housed individually in a cage with a raised bottom of wide wire mesh to insure immediate passage of feces from the cage and did not have access to food or water during this time.

At the end of the period the surviving rats were lightly reanesthetized with ether, venous blood samples were taken for hematocrit determination, and rectal temperatures were recorded; the stomachs were removed and each animal was sacrificed by bilateral thoracotomy. The stomachs were opened along the greater curvature and examined with a dissecting binocular microscope with 10× magnification for the presence of ulceration. Rats which were found dead before reoperation were included in the study only if there was definite evidence of perforation, and of the presence of gastric contents in the pleural or peritoneal cavity at autopsy. The severity of ulceration was determined by the methods of Pauls *et al.* (2), in which the grade of ulceration signifies the number of ulcers from grade 1-plus to 4-plus. The effects of LMWD on ulcer activity was evaluated by comparing the Ulcer Index (average plus grade of ulceration in a given group × percent of animals showing ulceration in that group) in the test and control groups.

B. *Effect on gastric secretion.* Effects of LMWD on gastric secretion was studied in identically performed experiments in which the rats were sacrificed after 4 hours, thus avoiding the contamination of secretion by blood when longer periods are used. In all 69 rats, gastric contents were centrifuged and analyzed individually for volume, acid, and pepsin. Titratable acid was determined to pH 7.0 with 0.01 *N* NaOH. Pepsin concentration was determined by the method of

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