TABLE I. Mean Incidence of Terminal Symptoms.

| Treatment | No. animals | No. of animals affected: No symptoms | Paralysis | Urin. incont. | Fec. impac. | Wt loss |
|---|---|---|---|---|---|---|
| Antigen + 6-MP | 18 | 1/18 | 6/18 | 3/18 | 3/18 | 3/18 |
| " only | 17 | 1/17 | 4/17 | 1/17 | 3/17 | 1/17 |

TABLE II. Mean Day of Onset of Symptoms.

| Treatment | No. animals | Paralysis | Urin. incont. | Fec. impac. | Wt loss |
|---|---|---|---|---|---|
| Antigen + 6-MP | 18 | 24.5 | 23.7 | 28.0 | 23.3 |
| " only | 17 | 30.5 | 30.0 | 27.3 | 27.5 |
| Antigen + 6-MP *vs* antigen only (*p* values) | | .35 | .47 | .78 | .77 |

times more frequent in animals receiving both 6-MP and antigen than in those receiving antigen alone; frequency of paralysis and fecal impaction was about the same in both groups. Table II presents the mean days of onset of symptoms, together with a statistical analysis (*p* values) of the differences between the means of the 2 groups. Although animals receiving antigen and 6-MP showed slightly earlier onset of paralysis, urinary incontinence and weight loss, these differences were not statistically significant.

*Summary.* Guinea pigs injected with both antigen (homologous brain plus Freund's complete adjuvant) and 6-MP showed a slightly higher incidence of urinary incontinence and rapid weight loss but no definite difference in incidence of paralysis than did animals given only antigen; no change in mean day of onset of symptoms was evident. While antigen alone increased the number of circulating neutrophils (but not of lymphocytes), antigen and 6-MP together and 6-MP alone produced similar leukocyte patterns: delayed but marked neutropenia, and a marked rapid decline in lymphocytes. In animals given both 6-MP and antigen, a left shift in the Arneth Index of neutrophils occurred, in spite of the reduced level of circulating neutrophils.

1. Hoyer, L. W., Condie, R. M., Good, R. N., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 205.
2. Field, E. J., *Proc. Roy. Soc. Med.*, 1961, v54, 15.
3. Alvord, E. C., Jr., Kies, M. W., *J. Neuropathol. and Exp. Neurol.*, 1959, v18, 447.

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## Mechanism of Excretion of Urea by the Kidney in the Rabbit.* (27721)

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Urea, according to the traditional view, is excreted by the kidney as a result of glomerular filtration and partial passive reabsorption in the tubules. Schmidt-Nielsen(1) in a recent review cites observations which necessitate modification of this theory. One such observation is the steep concentration gradient of urea in the medulla of rats and other rodents, increasing from the cortical border up to the papilla. Gottschalk(2), following the theory of Wirz, Hargitay and Kuhn(3), proposes that sodium chloride is actively transported in the ascending limb of the loop and that urea also may be, although he considers it more likely that the urea gradient re-

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sults from passive diffusion secondary to the sodium chloride gradient. Recently evidence from Gottschalk and his group supports the view of Klümper *et al.*(4) that the concentration gradient of urea in the medulla is the result of back diffusion of urea from the fluid of the collecting ducts(5).

If urea is diffusible in the kidney medulla it would also move from blood to tubule fluid and because of the close association of the limbs with the vasa recta this might occur rapidly. Labeled urea, injected into the blood stream should appear in the urine before a simultaneously injected glomerular indicator such as ferrocyanide.

We planned to study this question by injecting rapidly a small volume of labeled urea and ferrocyanide intravenously and following their appearance in the urine by free flow technic. In other experiments we followed their excretion by stop-flow technic.

*Method.* Rabbits were anesthetized with ether and after laparotomy polyethylene tubes were inserted in one or both ureters as close to the renal pelvis as feasible. The ends of the tubes were led out through the abdominal wall and the abdominal incision was closed. Another tube was inserted into the jugular vein for injections. On recovery from the ether the animal was infused with 10% sucrose to produce an osmotic diuresis. The animals sat quietly without restraint. When a flow of 3-4 ml/min was attained the experiment proper was started. For the free flow type a solution containing $C^{14}$ urea and ferrocyanide was quickly injected and serial sampling of urine was started at once. Periods of collection were 5 seconds. For stop flow tests a pressure of 80 mm Hg was imposed in the ureter. The urea-ferrocyanide injection was then given and the pressure maintained for 2 minutes. The ureter was then released and serial urine sampling was carried out as for free flow.

One-tenth ml was taken from each sample and tested for ferrocyanide with ferric ion. The comparative concentration of ferrocyanide in the samples containing it was determined by diluting the samples until they all had the same depth of color as the weakest sample.

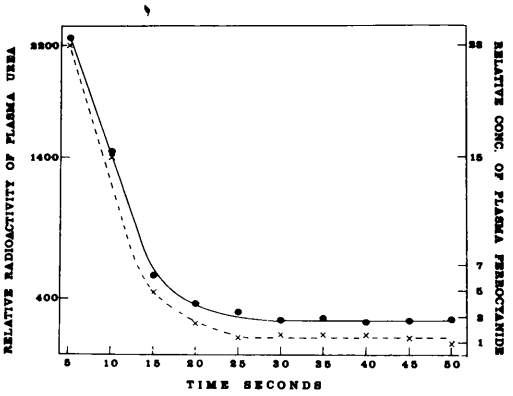


FIG. 1. Changes in concentration of urea (interrupted line curve) and ferrocyanide (solid line curve) show that these concentrations parallel each other closely. The drop in concentration after 50 seconds is so gradual that the blood to tubular gradient would be fairly constant.

We converted the carbon of the remainder of the sample to $CO_2$ by wet oxidation (the urine contained enough sucrose to give enough carbon for a saturated planchet). The carbon was counted as $BaCO_3$.

*Results.* In one animal we followed the arterial plasma concentrations of $C^{14}$ urea and ferrocyanide after I.V. injection. After cannulating the carotid artery we injected the usual solution I.V. in the same manner as used in all experiments. Arterial blood was sampled every 5 seconds and concentration of ferrocyanide was determined in the plasmas colorimetrically. Urea radioactivity was estimated by wet oxidation of the plasma and the carbon counted as $BaCO_3$.

Changes in concentration of urea and ferrocyanide in the arterial plasma after intravenous injection are given in Fig. 1. The concentrations parallel each other fairly closely. After a sharp decline during the first 15 seconds the drop becomes less and less and after one minute is quite gradual. This indicates that the 2 substances should be filtered in the glomerulus in the same relative proportions although at first both would be diluted similarly in passage between the transverse aorta and the afferent arteriole of the glomerulus.

The results of a free flow experiment are given in Table I. Obviously, some urea molecules get into the urine that were not filtered in the glomerulus. However, the sharp rise

TABLE I. Results in Free Flow and Stop Flow Urine after I.V. Injection of $C^{14}$ Labeled Urea and Ferrocyanide.

| Free flow | | | Stop flow | | | Stop flow | | |
|---|---|---|---|---|---|---|---|---|
| Sample No. | Urea $C^{14}$ activity | FeCN, rel. color | Sample No. | Urea $C^{14}$ activity | FeCN, rel. color | Sample No. | Urea $C^{14}$ activity | FeCN |
| 1–3 | negative | | 1 | 393 | 0 | 1 | 33 | 0 |
| 4 | 26 | 0 | 2 | 118 | 0 | 2 | 240 | 0 |
| 5 | 52 | 0 | 3 | 202 | 0 | 3 | 490 | 0 |
| 6 | 72 | 0 | 4 | 448 | 0 | 4 | 986 | 0 |
| 7 | 250 | 0 | 5 | 1012 | 0 | 5 | 376 | 0 |
| 8 | 1290 | faint tr. | 6 | 28 | 0 | 6 | 730 | 0 |
| 9 | 4670 | 26 | 7 | 624 | 15 | 7 | 611 | faint tr. |
| 10 | 7600 | 35 | 8 | 947 | 40 | 8 | 993 | tr. |
| 11 | 8060 | 32 | 9 | 712 | 50 | 9 | 1175 | + |
| 12 | 6200 | 26 | 10 | 1369 | 50 | 10 | 1650 | + |
| 13 | 6600 | 24 | | | | | | |
| 14 | 4830 | 20 | | | | | | |
| 15 | 3970 | 18 | | | | | | |
| 16 | 3850 | 13 | | | | | | |
| 17 | 2280 | 12 | | | | | | |

Urea act. = Counts/ml urine. FeCN, rel. color = Ferrocyanide, relative color after addition of ferric ion.

in labeled urea occurring at the time of appearance of ferrocyanide indicates that most of the urea of the urine has been filtered.

The stop flow results are given in Table I. Here more time has been allowed for exchange of urea between blood and tubular fluid, so that much more labeled urea appears in the urine before the ferrocyanide and the rise of the isotope is less abrupt with the appearance of filtered fluid.

*Discussion.* The rapid appearance of the labeled urea in the free flow urine in low concentration is in accord with the view that urea diffuses to some extent from blood into tubular fluid. It is much slower than $H_2O$ (6). The marked rise in activity at time of appearance of the ferrocyanide indicates that most of the urea molecules excreted in the urine had been actually filtered in the glomerulus.

In stop-flow experiments considerable radioactivity appears in the pre-glomerular samples. This suggests that the time of the stop-flow interval, 2 minutes, is long enough to allow considerable transfer of urea between blood and tubular fluid, presumably by diffusion, although other processes could be involved.

Schmidt-Nielsen(1) assumes that diffusion can take place rather freely in the medulla between tubules and vasa recta. Our results indicate that diffusion takes place but that it is not rapid, at least in the rabbit during osmotic diuresis.

1. Schmidt-Nielsen, B., *Physiol. Revs.*, 1958, v38, 139.

2. Gottschalk, C. W., Mylle, M., *Am. J. Physiol.*, 1959, v196, 927.

3. Wirz, H., Hargitay, B., Kuhn, W., *Helvet. Physiol. et Pharm.*, 1951, v9, 196.

4. Klümper, J. D., Ullrich, K. J., Hilger, H. H., *Arch. ges. Physiol.*, Pflüger's, 1958, v267, 238.

5. Lassiter, W. E., Gottschalk, C. W., Mylle, M., *Am. J. Physiol.*, 1961, v200, 1139.

6. White, H. L., Rolf, D., Tosteson, D. C., *ibid.*, 1961, v200, 591.

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