# MECHANISM OF UREA EXCRETION.

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PLATES 8 AND 9.

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Since the time of modern physiological investigation, two theories have dominated our conception of the mechanism of renal excretion. Bowman in 1842, basing his deductions on the anatomical structure of the renal unit, suggested that the glomerulus furnished the water of the urine, and that the solid substances were added to it by the activity of the renal cells which line the tubule. The experimental evidence for this view was furnished by Heidenhain[1] and his pupils in a long series of investigations.

2 years later, Ludwig[2] advanced his theory that all the urinary constituents were derived by filtration through the glomerulus, the ultimate concentration of the urine being arrived at by a process of absorption of water by the renal tubule. Both processes were held to be purely physical.

The various modifications which have been offered by the pupils of these two schools and by other observers are innumerable. The most recent concept is that of Cushny,[3] and is termed by him the modern theory. While neither of the original theories can explain the mass of physiological and morphological data that has accumulated since their origin, the modern theory covers in a much more satisfactory manner our present knowledge.

According to Cushny, the formation of the urine may be explained by two processes: first, a purely physical filtration through the glomerulus of all the constituents of the plasma except the colloids; and, second, a resorption from this filtrate by the vital activity of the epithelium as it passes down the tubule. The former process furnishes the urinary constituents, the latter modifies their amounts so that they correspond to those of the completed urine.

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[1] Heidenhain, R., in Hermann, L., Handbuch der Physiologie, Leipsic, 1883, v, 279.

[2] Ludwig, C., Lehrbuch der Physiologie des Menschen, Leipsic and Heidelberg, 1861, ii, 373.

[3] Cushny, A. R., The secretion of the urine, London and New York, 1917.

Water is obviously the substance which will be handled in greatest amount by both filtration and absorption, and the conception of such a double process with this substance furnishes little difficulty. A complication arises, however, when the solid constituents are considered, as a uniform rate of absorption will not explain the varying levels of concentration that the different solids show in the completed urine. These are therefore divided into "threshold" and "non-threshold" substances. The former appear in the urine only as they exceed a certain value below which they are completely absorbed. Sugar and the chlorides are examples. The non-threshold substances, on the other hand, are found in the urine in direct proportion to their absolute amounts in the plasma, as they are not absorbed. Of these, urea and sulfates are the most important. The urine therefore contains all the urea of the glomerular filtrate and none of the sugar.

TABLE I.

*Amount of Filtrate and Absorbed Fluid Required to Form 1 Liter of Urine.*
Blood urea 70 mg. per 100 cc.; urine urea 5.5 per cent; urine 1,000 cc.

| | Urea. | Water. |
|---|---|---|
| | *gm.* | *cc.* |
| Blood | 0.07 | 100 |
| Glomerular filtrate | 55.0 | 78,500 |
| Amount absorbed by tubule | 0.0 | 77,500 |
| Urine | 55.0 | 1,000 |

Table I shows the amount of filtrate and absorbed fluid which would be required to form 1 liter of urine in an actually observed case of high urea excretion. The figures in the table hold only in case the entire urea content of the blood is available for filtration. In a recent article Cushny[4] claims that as the urea is distributed in about equal amount between the plasma and the corpuscles, only that half free in the plasma can pass through the glomerular filter. In this case, to obtain the 55 gm. found in the urine, twice as much filtrate would be needed as is shown in the table; 157,000 cc. of filtrate would be formed, of which 156,000 cc. would be absorbed.

Such a mechanism, though perhaps indirect, will nevertheless account for those alterations which would be required to convert the

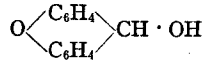
[4] Cushny, A. R., *J. Physiol.*, 1919–20, liii, 391.

blood plasma to urine without the intervention of a secretory factor. The completeness with which the various experimental and clinical facts are covered by this theory is well shown by Cushny.[3] Even changes in the mitochondria and the appearance of dyes in the tubule cells, which Heidenhain considers morphological evidence of a secretory process, may be equally well accounted for by the assumption of a vital absorption from the lumen of the tubule. This also applies to the demonstration of uric acid in the tubule cells, as this substance is one of the threshold group, and, according to the modern theory, is absorbed from the lumen of the tubule during concentration of the urine.

Urea, however, is a non-threshold substance and, if demonstrated in the renal cells, cannot be explained by a process of absorption from the lumen. The concentration of urea in the glomerular filtrate must be raised to that of the urine, and this could be done slowly, or not at all, if urea was absorbed along with the water.

Urea has been demonstrated in the proximal convoluted tubule by Leschke[5] and his experiments have been confirmed by the writer.[6] The method, which depends on the formation of an insoluble compound between urea and mercuric nitrate, may be criticized, as the resulting reaction is not so distinctive as might be desired. The protoplasm of all cells reacts more or less, though always less than that of cells of the proximal convoluted tubules. Whether this is due to the delicacy of the reaction, for Marshall and Davis[7] have shown the widespread distribution of urea in all tissues, or to a lack of specificity, is impossible to say. The quantitative variation in the degree of the reaction in the cells of the proximal convoluted tubule depending on the concentration of urea in the secreted urine has been taken as evidence of its specificity.[6]

Subsequently a new qualitative reagent for urea has been described by Fosse.[8] This is xanthydrol



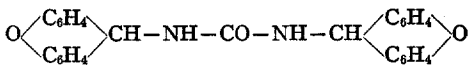
[5] Leschke, E., *Z. klin. Med.*, 1915, lxxxi, 14.

[6] Oliver, J., *J. Exp. Med.*, 1916, xxiii, 301.

[7] Marshall, E. K., Jr., and Davis, D. M., *J. Biol. Chem.*, 1914, xviii, 53.

[8] Fosse, R., *Bull. sc. pharmacol.*, 1914, xxi, 74, 502.

which forms with urea a characteristic crystalline product



The crystals are insoluble in acetic acid and differ in this way from the compounds which xanthydrol forms with other substances.

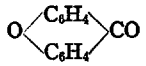
Two attempts have been made to demonstrate urea in the tissues with this reagent. Policard[9] was unable to find the crystals in any of the injected kidneys, though they were present in the lumina of the collecting tubules. He therefore concluded that urea does not exist in a free form in the kidney cells, but that it is bound in some intimate combination with the protoplasm. Chevallier and Chabanier,[10] however, describe the typical crystals of the urea compound in the cells of the convoluted tubules, in all the vessels of the kidney, and in the lumina of the ducts of Bellini.

The importance of these findings with regard to the mechanism of urea excretion, as well as the need for the accurate localization of the urea in the various parts of the kidney, is obvious. For these reasons the experiments have been repeated with certain modifications.

## *Technique.*

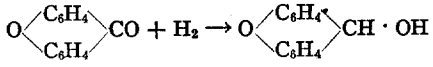
*Reagents.*—As it is impossible to obtain xanthydrol in the market, it was prepared in the following manner.

100 gm. of salol are heated to boiling in a distillation flask and the first fluid fraction, consisting largely of phenol, of 35 to 40 gm., is discarded. The remaining fraction, xanthone,



comes over and condenses in the form of long needles. These are collected and heated with NaOH, washed free of alkali, and purified by recrystallization from alcohol.[11] The xanthone gives a bright yellow color with a light blue fluorescence if treated with concentrated $H_2SO_4$.

The next step consists in the reduction of xanthone to xanthydrol.



[9] Policard, A., *Compt. rend. Soc. biol.*, 1915, lxxviii, 32.

[10] Chevallier, P., and Chabanier, H., *Compt. rend. Soc. biol.*, 1915, lxxviii, 689.

[11] Vanino, L., Handbuch der präparativen Chemie, Stuttgart, 1914, ii, 512.

This is accomplished by boiling in a reflux condenser 10 gm. of xanthone, 40 gm. of NaOH, and 400 cc. of alcohol, adding from time to time a pinch of zinc dust so that there is always a small amount present. The process is continued for from 6 to 8 hours, and the fluid then poured into cold water. The xanthydrol comes down in the form of fine crystals which are washed and dissolved in boiling alcohol and recrystallized by pouring again into an excess of water.[12] The xanthydrol gives with concentrated $H_2SO_4$ a bright yellow color with a light green fluorescence.

The mixture used for the injection of the kidneys was made up fresh for each experiment, as follows: 2 gm. of xanthydrol were triturated with 10 to 15 cc. of methyl alcohol, and 20 cc. of glacial acetic acid were added. The turbid fluid was filtered and was then a clear light yellow. If added to water, the xanthydrol comes out of solution, and the same precipitation occurs to some degree in the tissues. These crystals, however, are soluble in alcohol, and their disappearance may be easily followed as the specimen passes through the alcohols during embedding and staining.

*Animals Used.*—Rats were used for the experiments as their kidneys require less reagent than those of larger animals. In order to get a satisfactory concentration of urea in the urine, some animals were either fed a mixture of lard and corn meal containing urea, or a dilute solution of urea was injected into the peritoneal cavity. Others were injected after having lived on a normal diet. The first procedure was preferred as more nearly approaching physiological conditions. The animal was killed, the thorax quickly opened, and the reagent injected by way of the aorta. The injection was continued until the kidney was completely fixed. Thin slices were made and placed over night in 95 per cent alcohol, the tissue was embedded in paraffin, and sections were stained with hematoxylin.

## EXPERIMENTAL.

If a rat whose kidney is excreting urine high in urea concentration is injected with the xanthydrol reagent, the organ swells somewhat and turns a light opaque yellow. On the cut surface one can see

[12] Meyer, R., and Saul, E., *Ber. chem. Ges.*, 1893, xxvi, 1276.

with a hand lens that this discoloration is due to the presence of innumerable minute crystals scattered throughout the cortex and appearing in the medulla in long thin stripes.

Microscopic examination of the sections shows the exact location of these crystals. They lie in three places: in the blood vessels, in the cells of certain tubules, and in the lumina of the tubules (Figs. 1 to 5).

*The Intravascular Crystals.*

In all parts of the kidney and in the surrounding tissues (perirenal fat), all blood vessels contain typical crystals (Figs. 1, 3, and 5). These are in the form of fine, pointed needles, varying in size from 4 to 50 $\mu$ in length, lying either singly or arranged in the form of rosettes. On account of the arrangement of the vessels, the crystals appear in the medulla in long rows between the straight tubules, while in the tortuous capillaries of the cortex they are more irregularly scattered. They are also seen in the capillaries of the glomerular loop (Fig. 1).

The number of crystals in the individual cross-sections varies greatly; some are empty, others completely filled. This irregular distribution is evidently due to the current of the injection fluid which carries the crystals with it.

*The Intracellular Crystals.*

Crystals contained in cells are found only in the cortex, where the thick epithelium of the proximal convoluted tubule is filled with them (Fig. 1). They lie in all parts of the cell, near the membrana propria, in the region of the nucleus, or just beneath the brush border (Fig. 2). They are smaller than those seen in the blood, and when arranged in rosettes are more or less deformed. Their characteristic structure is nevertheless evident.

The number of crystals in the tubule cells gradually decreases so that the terminal spiral portions of the proximal tubule show definitely fewer crystals than the cross-sections which lie in the proximity of the glomerulus.

The other divisions of the renal tubule practically never show intracellular crystals. This contrast is not so evident in the cortex, where the great majority of the cross-sections are of the proximal convoluted

tubule, but at the junction of the outer and inner stripes of the outer zone of the medulla it is striking. Here the large spiral terminal portions of the proximal convoluted tubules, situated in the outer stripe, end suddenly to form the line of demarcation from the inner stripe, which contains the broad ascending limbs of Henle's loop. The former contain the crystals in moderate number, while the latter do not show them (Fig. 3).

### *The Crystals in the Lumen of the Tubule.*

The lumen of the entire renal unit, from the capsular space to the large ducts of Bellini, contain the crystals, which resemble in their long needle shape those seen in the blood. The increase in number and in the size of the rosettes toward the end of the tubule is striking.

In Bowman's space the crystals are comparatively small and infrequent (Fig. 1). Beginning in the lumen of the proximal convoluted tubule, however, there is a definite increase in their number, which becomes even greater in the narrow descending limb of Henle's loop (Fig. 4). The sections of the small collecting tubules in the cortex show an added increase (Figs. 1 and 3), while in the terminal ducts of Bellini relatively large rosettes are seen (Fig. 5).

As in the case of the blood vessels, not every cross-section of a tubule shows an equal amount of crystals. In those regions where the urine is comparatively dilute, as in the proximal convoluted tubules, many are empty. This may be explained by the fact that a volume of urine produces a relatively much smaller volume of crystalline product, so that as the urea is condensed in crystalline form, it leaves the surounding areas free. A single rosette of crystals may thus represent the urea from a considerable length of tubule.

### *Demonstration of Urea Crystals in Other Tissues.*

As a control, the livers of certain of the animals were injected with the reagent. Here the hepatic vessels contained the crystals in the same amount as those of the kidney; the hepatic cells, however, did not show them.

## DISCUSSION.

The interpretation of the above findings is greatly aided by our accurate knowledge of the distribution of urea in the body. It has been shown that all the tissues except the fat contain urea in the same concentration as that found in the blood, the kidney alone exceeding this amount (Marshall and Davis[7]).

From this it follows that the concentration of urea in the cells of the proximal convoluted tubules, which show the crystals of urea, must be higher than that of the blood, as in the liver and the other tubules of the kidney, where the concentration is equal to that of the blood, there is no reaction.

There is therefore a certain threshold below which the reaction does not take place in the protoplasm. The deformity and small size of the intracellular crystals is further evidence of this embarrassment to the reaction. This threshold lies somewhere above the concentration of urea in the blood, and is only reached in the proximal convoluted tubule.

The question now arises as to whether the source of this excess urea is the lumen of the tubule (absorption) or the blood (excretion).

Any theory concerning the mechanism of urea excretion should explain the great rise in urea concentration of the urine as contrasted with that of the blood. By a process of absorption this can only be accomplished if the other constitutents of the urine (water) are absorbed and the urea is left behind, and such an assumption is made by the modern theory. Urea would not, therefore, be found in a higher concentration in the cells of the tubule than in the blood.

The excretion of urea, on the other hand, will raise the concentration in the urine, and the high concentration in the cells would be expected. Our demonstration of such a high concentration can therefore only be explained by the assumption of an excretion of urea from the blood into the lumen of the tubules.

That absorption of water without absorption of urea takes place, however, is shown by the increased concentration of urea in the urine as the tubule is descended so that large rosettes of crystals are seen in the ducts of Bellini but none in the epithelium (Fig. 5).

The mechanism of urea excretion may be summarized as follows: Urea passes through the glomerular capsule in the same concentration as that found in the plasma. A certain amount is added to this filtrate by excretion through the cells of the proximal convoluted tubule, and the ultimate concentration is reached by an absorption of water in the remainder of the tubule.

Such a modification will not affect the fundaments of the modern theory, as Cushny states that "it may be necessary to supplement what I have termed the modern view with an active secretion in the tubules."[18]

## CONCLUSIONS.

1. Urea is present in the cells of the proximal convoluted tubule in a concentration higher than that of the blood or than that of the cells of any of the other kidney tubules.

2. Such a condition can only be reconciled to an assumption of an active secretion (excretion) on the part of these cells.

3. Urea also passes through the glomerular filter with the other crystalloids of the blood plasma.

4. The final concentration of urea is due to the above mentioned secretion by the proximal convoluted tubule, and to the absorption of water in other parts of the tubule.

## EXPLANATION OF PLATES.

Drawings made with the aid of a camera lucida. Bausch and Lomb ocular 1, objective $\frac{1}{6}$.

### PLATE 8.

FIG. 1. Rat 1. 2 cc. of 5 per cent urea intraperitoneally; killed 1 hour later. A glomerulus is shown with several sections of the proximal convoluted tubule and one of a collecting tubule. Crystals of urea-xanthydrol are seen in the vessels near the glomerulus, in the loops of the capillary tuft, in the space of Bowman's capsule, and in greater number in the cells of the proximal convoluted tubule. The lumen of the collecting tubule also contains some rosettes of crystals.

FIG. 2. Rat 1. Higher magnification of one of the proximal convoluted tubules. Small rosettes of crystals are seen scattered throughout the epithelial cells. There are some crystals in the vessels. Bausch and Lomb ocular 1, objective $\frac{1}{12}$.

[18] Cushny,[3] p. 52.

FIG. 3. Rat 2. Same as Rat 1. The junction of the inner and outer stripes of the outer zone of the medulla is shown. Above, the larger terminal ends of the proximal convoluted tubules containing crystals in their cells, and in one case a rosette of crystals in the lumen. Below, the smaller ascending limbs of Henle's loop which contain no crystals. Scattered crystals and rosettes are seen in the intertubular capillaries and in the collecting tubule at the left.

## PLATE 9.

FIG. 4. Rat 3. Fed a mixture of urea (10 per cent), corn meal, and lard for 3 days; killed and injected. The tubules represented were situated in the inner stripe of the outer zone of the medulla. Four ascending limbs of Henle's loop are shown surrounding the loop proper which is formed by the narrow limb of the tubule in this case. Its lumen contains many crystals.

The loop shown in this figure is one of the short type described by Peter,[14] in which the bend lies close to the end of the proximal convoluted tubule. There could have been, therefore, little opportunity for the absorption of water to produce the relatively high concentration of urea as indicated by the mass of crystals.

FIG. 5. Rat 4. Rat on normal diet, no urea given. Margin of the papilla of the medulla. Three large ducts of Bellini are shown, their lumina filled with huge rosettes of crystals. Scattered rosettes are also seen in the pelvis, and a few in the intertubular capillaries.

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[14] Peter, K., Untersuchungen über Bau und Entwickelung der Niere, Jena, 1909.

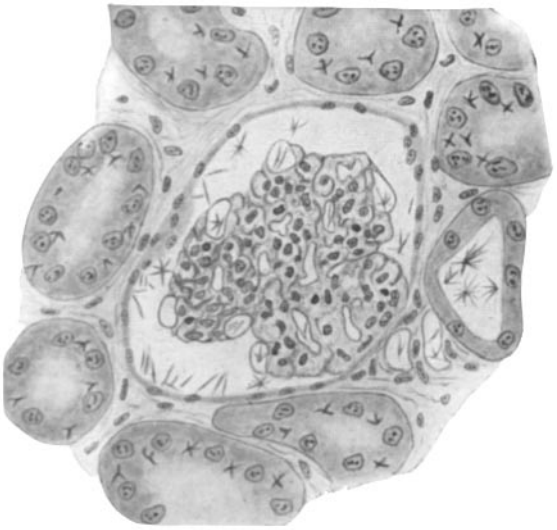

FIG. 1.

FIG. 2.

FIG. 3.

(Oliver: Mechanism of urea excretion.)

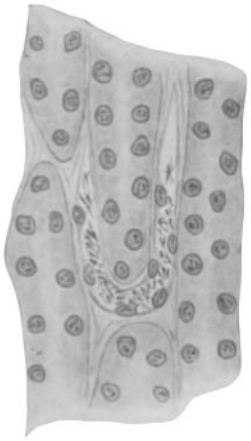

FIG. 4.

FIG. 5.