THE TUBULAR FACTOR IN RENAL HEMOGLOBIN EXCRETION*

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In 1932 Whipple and his associates (2) demonstrated that the renal threshold for hemoglobin in dogs could be markedly lowered by multiple daily injections large enough to cause hemoglobinuria. On histological examination the epithelium of the convoluted tubules was always found to contain dense deposits of iron-staining pigment following repeated injections of hemoglobin (5) and the lowered thresholds observed were attributed to a cessation of tubular reabsorption.

It was felt then that further information regarding the rôle of the tubular epithelium in renal hemoglobin elimination might be gained if this phenomenon and any associated changes were studied with the precise quantitative methods recently used to determine the renal clearance of hemoglobin (4). Consequently, renal threshold values for hemoglobin in relation to plasma concentration were obtained, initially and following repeated daily hemoglobin injections sufficient to cause a fairly marked hemoglobinuria. The diminution in the threshold was found to be even greater than the previously observed average of 46 per cent and amounted to over 60 per cent in the small series of animals studied.

Further interest was aroused by the finding that above the new low threshold the rate of hemoglobin excretion paralleled that observed initially. According to the concept of renal hemoglobin excretion recently proposed by Monke and Yuile (4), this observation implies that a diminution in the maximum rate of, but not cessation of, tubular reabsorption is responsible for lowering the threshold.

In order to test the accuracy of estimated tubular reabsorption rates in dogs under normal conditions and when the threshold had been lowered, injections of hemoglobin containing radio-active iron were given to animals of each type. The actual amounts of radio-active iron retained by the kidneys were then de-

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termined and the actual and estimated rates of tubular reabsorption were compared.

Methods

All methods used to determine the renal threshold for hemoglobin were essentially similar to those described in a previous publication (4). Simultaneous creatinine excretion was not studied, however, and the colorimetric determinations were made on a Klett photoelectric colorimeter.

Preceding each daily injection of approximately 1.5 gm. of hemoglobin prepared from normal dog blood, 300 cc. of water containing a few grams of sodium bicarbonate were given by stomach tube.

Hemoglobin containing radio-active iron was made in the usual manner (4) from the red cells of dogs which had received injections of radio-active iron over considerable periods of time (1).

Twenty-four hours after injecting the radio-active material, the animals were subjected to viviperfusion (6) to free the organs of blood. The kidneys were weighed and after wet digestion the radio-activity was determined on a Geiger counter (1).

Blocks of tissue were fixed in a mixture of 3 per cent potassium ferricyanide and 10 per cent formalin and sections were stained for iron and for hemoglobin (3). The staining method used for the latter pigment, described by Lison (3) is based on the oxidation of a leuco-form of patent blue to its original color, in the presence of hemoglobin.

EXPERIMENTAL OBSERVATIONS

Table I summarizes the data relating to changes in the renal threshold for hemoglobin following repeated daily injections. The value given in column 3 for dog 39-104 represents the recovery threshold level 6 weeks after the seventeenth consecutive daily injection. This is probably below the true initial threshold which was not determined in this instance. The other values in column 3 are the threshold levels obtained with the first of the daily injections of hemoglobin and all values in column 6 are the levels existing at the time of the last injection. The total number of injections and the total amount of hemoglobin received by each animal are listed in columns 4 and 5, respectively. Column 7 indicates the percentage drop below the higher threshold level in each experiment. During each experiment the plasma, tested at intervals of 2 or 3 days, never contained any residual hemoglobin 24 hours after an injection.

Table II records the results obtained following the injection of hemoglobin containing radio-iron in two normal animals (39-307 and 40-310) and in two with thresholds previously lowered by repeated daily injections of hemoglobin (39-225 and 40-79). In columns 5 and 6 it will be noted that a somewhat smaller percentage of radio-active iron is retained by the kidneys of the normal animals than those in the low threshold group. When these actually determined values are compared with the estimated total amounts of hemoglobin reabsorbed
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by the tubules, listed in Table II, column 9, as percentages of the initial injection, it is found that, although there is a very close approximation between the actual and estimated figures for dogs with lowered thresholds, the actual amounts retained by the kidneys of the normal animals are far below the estimated values.

Fig. 1 graphically illustrates the data of one comparable experiment in each group. Graphs A and C show the rates of disappearance of hemoglobin from

**TABLE I**

Renal Thresholds for Hemoglobin Lowered by Multiple Injections

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight</th>
<th>Initial threshold</th>
<th>Total No. of injections</th>
<th>Total hemoglobin injected</th>
<th>Final threshold</th>
<th>Drop in threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nos.</td>
<td>kg.</td>
<td>mg./100 cc.</td>
<td>gm.</td>
<td></td>
<td>gm./100 cc.</td>
<td>per cent</td>
</tr>
<tr>
<td>39-104</td>
<td>9.4</td>
<td>100*</td>
<td>17</td>
<td>24.2</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>39-225</td>
<td>17.0</td>
<td>200</td>
<td>17</td>
<td>28.5</td>
<td>68</td>
<td>66</td>
</tr>
<tr>
<td>40-79</td>
<td>11.8</td>
<td>160</td>
<td>21</td>
<td>32.1</td>
<td>53</td>
<td>67</td>
</tr>
</tbody>
</table>

* Recovery threshold 6 weeks after cessation of hemoglobin injections.

**TABLE II**

Results Following Injection of Hemoglobin Containing Radio-Active Iron

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight of dog</th>
<th>Hemoglobin injected</th>
<th>Threshold Hemoglobin injected</th>
<th>Radio-iron in kidneys</th>
<th>Per cent of injection</th>
<th>Weight of kidneys</th>
<th>Tubular reabsorption (estimated)</th>
<th>Hemoglobin in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nos.</td>
<td>kg.</td>
<td>gm.</td>
<td>mg./100 cc.</td>
<td>Normal Low threshold animals</td>
<td>gm.</td>
<td>Rate mg./min. Total per cent of injection per cent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>39-307</td>
<td>17.0</td>
<td>4.8</td>
<td>147</td>
<td>15.3</td>
<td>—</td>
<td>67.3</td>
<td>3.36</td>
<td>45.0</td>
</tr>
<tr>
<td>40-310</td>
<td>20.0</td>
<td>5.1</td>
<td>125</td>
<td>17.3</td>
<td>—</td>
<td>104.2</td>
<td>3.38</td>
<td>53.0</td>
</tr>
<tr>
<td>39-225</td>
<td>17.0</td>
<td>5.0</td>
<td>68</td>
<td>—</td>
<td>23.0</td>
<td>63.0</td>
<td>1.66</td>
<td>20.0</td>
</tr>
<tr>
<td>40-79</td>
<td>11.8</td>
<td>4.6</td>
<td>53</td>
<td>—</td>
<td>19.5</td>
<td>54.3</td>
<td>1.05</td>
<td>17.5</td>
</tr>
</tbody>
</table>

It is noteworthy that the plasma of the animal which had received seventeen previous injections was cleared of hemoglobin more rapidly than the plasma of the normal animal. This difference was equally distinct in the two experiments not shown graphically. In graphs B and D the rates of hemoglobin excretion are plotted in relation to plasma concentration and the method of deriving the rates of tubular reabsorption, listed in Table II, column 8, is illustrated. This method of analysis is based on the assumption that hemoglobin filters through the glomerulus at a constant rate and that the threshold phenomenon results from tubular reabsorption which reaches a maximum at the threshold and remains constant at higher plasma con-
centrations (4). The estimated total amount of hemoglobin reabsorbed (Table II, column 9) is calculated from rates of tubular reabsorption so obtained, both above and below the threshold, and the time required to clear the plasma of hemoglobin. Graph D shows the excretion rate curves for dog 39-225, both initially and after repeated hemoglobin injections. Both curves are parallel originating at the respective threshold levels and it is apparent that a marked diminution in the theoretical reabsorption rate accompanies the lowering of the threshold.

Histologically, there were dense iron-staining deposits of pigment in the epithelium of the convoluted tubules in the animals subjected to repeated hemoglobin injections but no traces of iron-staining pigment were seen in the normal group 24 hours after the initial injection. The hemoglobin stain employed showed a complete absence of red blood cells in the kidneys and in both groups of animals there was a delicate blue, finely granular haze visible in the

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**Fig. 1.** A graphic illustration of the results obtained following the injection of hemoglobin containing radio-active iron, in one normal dog and in one with a lowered threshold.

Graphs A and C represent the rates of disappearance of hemoglobin from the plasma, and Graphs B and D show the curves relating the rates of renal excretion to plasma concentration.
cytoplasm of the epithelium cells of the convoluted tubules. Iron-staining pigment was not stained by this method.

The fact that no iron-staining material was observed after the initial injection of hemoglobin in the normal dogs indicates that the pigment remaining in the kidneys is not markedly altered within 24 hours.

**DISCUSSION**

The earlier observations on the lowering of renal thresholds for hemoglobin by repeated injections have been confirmed and variations in the threshold have been related to plasma concentration. From the foregoing experimental data, it is apparent that this phenomenon is not associated with a cessation of tubular reabsorption as previously suggested by Newman and Whipple (5). Since in the early experiments diminishing doses of hemoglobin were given, it is possible that the relatively constant low threshold levels attained were due to a lowered rate of tubular reabsorption representing a state of equilibrium between the amounts of hemoglobin picked up by and removed from the tubular epithelium each day.

If the concept of a constant rate of glomerular filtration and a rate of tubular reabsorption which reaches a maximum at the threshold (4) is correct, the parallelism between hemoglobin excretion rate curves, obtained initially and after lowering the threshold, indicates that a diminution in the rate of tubular reabsorption is responsible for the changes observed in the threshold level. Under these conditions it is reasonable to suppose that a cessation of tubular reabsorption would coincide with a complete elimination of the threshold. However, when hemoglobin containing radio-active iron is injected, the kidneys of animals with lowered thresholds are found to have retained more hemoglobin products in 24 hours than the kidneys of normal animals.

As a tentative explanation to account for the discrepancy between the estimated total tubular reabsorption and the actual amount of radio-active iron retained by the kidneys, it is suggested that in normal animals hemoglobin is rapidly removed from the renal epithelium. This would imply that when the threshold has been lowered by repeated daily injections, the rates of both reabsorption from the tubular lumina and removal from the tubular epithelium are diminished, since under these conditions the estimated and actual amounts of hemoglobin picked up are approximately equal.

It is conceivable that the phenomenon results from some modification of glomerular permeability without alteration of the rate of tubular reabsorption, but definite proof one way or the other must await further investigations.

**SUMMARY**

A drop has been observed in the renal threshold for hemoglobin in dogs, of over 60 per cent, following repeated injections daily. It was not associated with a cessation of tubular reabsorption.
Hemoglobin excretion rate curves, obtained initially and after lowering the threshold, have proved to be parallel lines originating at the respective levels.

Hemoglobin containing radio-active iron has been used to determine the amount of iron retained by the kidneys 24 hours after injection.

The kidneys of normal animals retain slightly less iron than those of animals with lowered thresholds, despite the fact that the former group has a much higher estimated rate of tubular reabsorption.

It is suggested that hemoglobin products are more rapidly removed from the kidneys of normal animals, following reabsorption, than from those of animals which have received multiple injections of hemoglobin.

BIBLIOGRAPHY