## Hypersalemia and Tubular Azotemia

The mechanism of azotemia, renal as well as prerenal, is generally connected with one or both of two causes: nitrogen retention and excessive protein catabolism. The first possibility is characterized by the decreased glomerular filtration rate (GFR), i.e. the decrease in the clearance of some substances which are neither secreted nor reabsorbed by the renal tubules (endogeneous creatinin, mannitol, inulin, thiosulphate). The drop of GFR may be due to renal damage, as in nephritis, or to prerenal azotemia with anatomically normal kidney structure. In case of an excessive protein catabolism, the otherwise normal kidney is not capable of getting rid of the large amount of urea to be excreted.

It is well known that a part of the filtered urea is excreted, while another part is reabsorbed. The ratio of filtered urea to the excreted amount is expressed by the ratio: urea clearance/endogeneous creatinin or inulin clearance, which normally is about 0·6, i.e. 40% of the filtered amount of urea is reabsorbed during its way through the tubuli. VAN SLYKE *et al.*[1] found that under certain experimental conditions the ratio mentioned above decreases markedly, in which case the tubulary wall seems to have lost its normal capacity of resisting excessive urea reabsorption.

Theoretically it is quite conceivable that an azotemia may develop with normal GFR, without excessive protein catabolism, purely through excessive reabsorption of the normally filtered urea. It is known from RICHARDS'[2] experiments on the frog kidney that in mercury bichloride intoxication, in spite of the normal quantity of glomerular filtrate, as confirmed by glomerular puncture, an anuria develops. In this case all filtered material is reabsorbed by the tubules.

FERRO-LUZZI[3] reports a case of tertiary syphilis with azotemia, accompanied by normal creatinin and decreased urea clearance. PHILLIPS and HAMILTON[1] and FÖLDI, RUSZNYÁK, and SZABÓ[2] found that, after clamping the renal artery for two hours, an excessive reabsorption of creatinin, inulin, mannitol, para-aminohippuric acid, and urea takes place. In spite of the fact that histological examination revealed no pathologic changes in the glomeruli, the clearances were depressed by the reabsorption, and thus no direct biochemical proof was available of the normal function of the glomerular apparatus.

As far as we know, no significant decrease of the ratio urea clearance/creatinin clearance has been observed under azotemic conditions, thus no direct experimental proof of an exclusively tubular azotemia is at hand. In a series of experiments, by injecting large doses of a hypertonic (3·6 resp. 7·2%) NaCl solution subcutaneously, we were able to demonstrate that during hypersalemia, i.e. serum sodium values above about 170 maeq. for a period of 24 hours, an azotemia developed.

In our experiments we used rabbits of about 2–3 kg. After a control period of 3 to 4 hours, the fasting and thirsting rabbits were given subcutaneously a NaCl solution of 3·6 resp. 7·2%, amounting to 2 to 6% of their body weight. This procedure was continued daily until the animal's death, which occured generally on the 3rd to 6th day of the experiment. Urine was collected and the collection completed each morning by catheterization. A blood sample was drawn from the marginal vein of the ear each morning. Serum and urine were analysed for sodium, NPN and protein (BÁLINT and KABDEBÓ[3]), chloride (RUSZNYÁK[4]), potassium (RAPPAPORT[5]), urea (BÁLINT, KINCSES, and ZSIGA[6]), and creatinin (FOLIN[7]).

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| No. | Interval min. | Urine flow ml/min | Serum | | | | Urine urea N mg% | Creatinin | | Urea clear. | Urea clearance / Creat. clearance | N production mg *pro die*/kg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | sodium maeq. | chloride maeq. | urea N mg% | NPN mg% | | U/P | clear. | | | | |
| 510/1 | 2860 | 0·025 | 137 | 99 | 21 | 28 | 1920 | 185 | 4·6 | 2·3 | 0·50 | 480 | without salt |
| 2 | 2900 | 0·024 | 134 | 107 | 26 | 40 | 3120 | 209 | 5·0 | 2·8 | 0·56 | 665 | without salt |
| 3 | 2880 | 0·028 | 137 | 111 | 32 | 42 | 2560 | 177 | 5·0 | 2·5 | 0·50 | 510 | without salt |
| 4 | 2910 | 0·026 | 138 | 113 | 42 | 54 | 2720 | 173 | 4·5 | 1·9 | 0·42 | 590 | without salt |
| 5 | 2680 | 0·040 | 145 | 112 | 54 | 74 | 3280 | 107 | 4·3 | 2·7 | 0·63 | 1020 | without salt |
| 6 | 3060 | 0·046 | 148 | 105 | 53 | 74 | 2960 | 64 | 3·0 | 2·5 | 0·84 | 865 | without salt |
| 619/1 | 230 | 0·065 | 150 | 108 | 29 | 46 | 2480 | 120 | 7·8 | 5·6 | 0·71 | | without salt |
| 2 | 1210 | 0·055 | 168 | 132 | 42 | 50 | 1200 | 128 | 7·0 | 1·9 | 0·27 | 870 | *a*) |
| 3 | 1365 | 0·051 | 178 | 122 | 67 | 78 | 1800 | 100 | 5·1 | 1·7 | 0·33 | 890 | *a*) |
| 629/1 | 250 | 0·100 | 138 | 109 | 14 | 26 | 520 | 53 | 5·3 | 3·7 | 0·70 | | without salt |
| 2 | 1180 | 0·082 | 150 | 128 | 18 | 29 | 894 | 59 | 4·8 | 3·9 | 0·82 | 300 | *b*) |
| 3 | 1450 | 0·081 | 175 | 143 | 40 | 46 | 1085 | 73 | 5·9 | 3·0 | 0·51 | 650 | *b*) |
| 4 | 1295 | 0·084 | 190 | 152 | 72 | 98 | 1320 | 82 | 6·9 | 2·1 | 0·30 | 760 | *b*) |
| 68/1 | 240 | 0·025 | 146 | 108 | 17 | 36 | 2160 | 163 | 4·1 | 3·3 | 0·81 | | without salt |
| 2 | 1365 | 0·096 | 155 | 125 | 22 | 33 | 1160 | 56 | 5·4 | 5·3 | 0·98 | 770 | *c*) |
| 3 | 1375 | 0·037 | 175 | 141 | 63 | 72 | 760 | 89 | 3·3 | 0·7 | 0·21 | 490 | *c*) |

*a*) 29 ml/kg body weight of a 3·6% NaCl solution subcutaneously
*b*) 20 ml/kg body weight of a 7·2% NaCl solution subcutaneously
*c*) 62 ml/kg body weight of a 3·6% NaCl solution subcutaneously

Urea and creatinin clearances were calculated according to the formula $UV/P$. We are aware that there was much discussion about the value of the endogeneous creatinin clearance determinations, but considering the use of BARRET and ADDIS'[1] modifications for the determination of the serum creatinin .and KAPLAN and SMITH'S[2] report about the identity of creatinin and inulin clearances in the rabbit, we thought the procedure sufficient for determining rough changes in the GFR values. With the more usual exogeneous infusion technics it would have been impossible to extend the duration of one period to 24 hours.

We found that in all cases where we succeeded in sustaining the elevated serum sodium values above 170 maeq. for a period of at least 18 hours an azotemia developed. This effect was reversible, as, when giving water *ad libitum*, we sometimes succeeded in restoring the normal chemical composition of the serum. There was much difference between the animals in their reaction to the enormous quantities of NaCl injected. Some of them excreted the injected quantity in the form of 1,6 osmolar urine. In these cases there was no hypersalemia and no azotemia. In other cases we gave less salt, and nevertheless the hypersalemia and consequent azotemia developed.

In the table we are giving the data of one control (fasting and thirsting rabbit, with no salt given) and three typical experiments. Comparison of the data with the control reveals that the three sera have an increased sodium content and a marked azotemia. The endogeneous creatinin clearances do not show significant changes (compared with the control period of each animal), and so there is no sign of a retention azotemia. The N production, i.e. N excreted + N retained, calculated in mg/day/kg, is not higher than in cases with excessive protein catabolism without azotemia. Comparing the ratio urea clearance/GFR, a significant drop can be seen, the reabsorption of urea being 67, 79, and 70% respectively of the filtered quantity. The drop in urea clearance is well known in connection with decreased urine flow, but in our cases the rate of diuresis was fairly large throughout the experiment.

The histological examination of the kidney (Prof. G. Romhányi) revealed a fatty infiltration in the stroma of the loop of Henle and on the basal membrane of the collecting ducts as well. The nuclei in the ascending loop of Henle are pyknotic. Cortex and glomeruli were perfectly normal.

According to FISHBERG[3] "it has not been demonstrated that structural changes in the kidney cause impairment of tubular reabsorption while glomerular filtration is unimpaired". By giving large quantities of hypertonic NaCl solution subcutaneously to fasting and thirsting rabbits we succeeded in developing an enduring hypersalemic condition, always connected with azotemia. GFR (measured by endogeneous creatinin clearance) remained unaltered and the ratio urea clearance/GFR decreased. Thus the greater part of the filtered urea was reabsorbed through the damaged tubulary wall, which seems to have lost its capacity to withstand the enhanced urea reabsorption (or rediffusion). As the quantity of the filtered urea and N production of the body remained normal, the azotemia was exclusively due to tubular reabsorption. The histological picture of the kidney is an additional proof of the exclusivity of tubular damage.

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*Zusammenfassung*

Durch subkutane Verabreichung großer Mengen einer hypertonischen Kochsalzlösung an hungernde und durstende Kaninchen gelang es uns, einen hypersalämischen Dauerzustand mit anschließender Azotämie hervorzurufen. Die Bestimmung der glomerulären Filtration ergab normale Werte, das Verhältnis Harnstoff-Clearance/Kreatinin-Clearance sank jedoch erheblich. Dies kann nur so gedeutet werden, daß von der normalen Menge filtrierten Harnstoffes ein pathologisch hoher Prozentsatz resorbiert wird. Es gelang also, eine Azotämie ausschließlich tubulären Ursprungs hervorzurufen. Das histologische Bild der Niere mit ausschließlich tubulären Veränderungen bekräftigt obige Annahme.

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[2] B. I. KAPLAN and H. W. SMITH, Amer. J. Physiol. *113*, 354 (1935).

[3] A. M. FISHBERG, Hypertension and Nephritis (Lea and Febiger, Philadelphia, 1947), p. 32.

## PRO LABORATORIO

### Einige Verbesserungen in der Haltung und Aufzucht von *Xenopus laevis*

Die Grundlinien der Zucht von *Xenopus* sind schon seit einigen Jahren bekannt und auch in der Literatur zugänglich[1]. Trotzdem bereitet die Gewinnung regelmäßiger und gut entwicklungsfähiger Laichablagen in manchen Instituten Schwierigkeiten. Dem Wunsche verschiedener Kollegen entsprechend teilen wir unsere Erfahrungen mit, wobei wir hauptsächlich die von uns erprobten Verbesserungen (*), die u.W. noch nicht bekannt sind, anführen. In den übrigen Einzelheiten entspricht unsere Zuchtmethode weitgehend der von GASCHE[2] und OCHSÉ[3], erfordert jedoch viel weniger Arbeit.

1. *Adulte Tiere.* Die numerierten erwachsenen Tiere (vgl. OCHSÉ, 1948) werden, ♀ und ♂ getrennt, in großen Aquarien gehalten, im * Sommer womöglich im Freien in gutbelichteten, aber nicht direkt besonnten Zuchttrögen (Hebung der Fortpflanzungsleistungen und des Gesundheitszustandes). Adulte Spornfrösche ertragen größere Temperaturschwankungen ohne Schaden, doch sollte die Wassertemperatur nicht unter 10–12° sinken. Das Optimum liegt bei etwa 23°.

2. *Gewinnung des Laiches.* Die Spornfrösche sind im Sommer (Mai–August) gegenüber der Hormonbehandlung empfindlicher (sogar spontanes Laichen wurde im Freien beobachtet), während sie vom November bis Februar nur auf stärkere Hormondosen reagieren (siehe unten). Zur Behandlung wird gonadotropes Chorionhormon aus Schwangerenharn (Präparat der Ciba) verwendet (200 IE/mg). Der Extrakt wird in Frosch-Ringer-Lösung gelöst (Konzentration: 5–6 mg auf 2 $cm^3$ im Sommer, 8 mg auf 2 $cm^3$ im Winter) und in den Rückenlymphsack injiziert (Abb. bei OCHSÉ, 1948). Die Dosis richtet sich nach Jahreszeit und Zustand des Tieres. Bei den ♂, oft auch bei den ♀, hat sich eine * *Vorbehandlung* sehr gut bewährt.

*Behandlung der ♂:* * Vorbehandlung: Innert 4–7 Tagen 1–2 Injektionen mit 0,6 $cm^3$. Im Winter ist meist die stärkere Behandlung nötig. Im übrigen muß man

[1] Eine Zusammenstellung der über *Xenopus* erschienenen Arbeiten findet sich in: H. ZWARENSTEIN, N. SAPEIKA, and H. A. SHAPIRO, *Xenopus laevis*, a bibliography (The African Bookman, Cape Town, 1946).

[2] P. GASCHE, Rev. suisse de Zool. *50*, 262 (1943).

[3] W. OCHSÉ, Gynaecologia *126* (Fasc. 2), 57 (1948).