Pharmacological activities in IU per mg

| Compound | Oxytocin-like activities | | | | Vasopressin-like activities | |
|---|---|---|---|---|---|---|
| | Rat uterus (in vitro) | Cat uterus (in situ) | Rabbit mammary gland (in situ) | Chicken blood pressure | Rat blood pressure | Rat antidiuresis |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| (A) Oxytocin-dimers ($\alpha+\beta$) | 3.0 ± 0.15 | 15.4[a] | 3.7[a] | 3.1[a] | 0.044 ± 0.003 | 0.02 ± 0.0025 |
| Oxytocin-dimer[b] | | | | | | |
| $\alpha$ = parallel | 1 | — | — | — | — | — |
| $\beta$ = antiparallel | 1 | — | — | — | — | — |
| (B) Oxytocin[c] | 450 ± 30 | 450 | 450 ± 30 | 450 ± 30 | 5 ± 1 | 5 ± 1 |
| A:B | 0.007 | 0.034 | 0.008 | 0.007 | 0.009 | 0.004 |

[a] Approximations calculated from dose-response-lines as substance interferes with usual test procedure. [b] According to YAMASHIRO, HOPE and DU VIGNEAUD[3]. [c] According to BERDE and BOISSONNAS[6]. 1. HOLTON[7]; 2. BERDE, DOEPFER and KONZETT[8]; 3. BERDE and CERLETTI[9]; 4. U.S.P. XIV[10]; 5. British Pharmacopeia[11]; 6. BERDE and CERLETTI[12].

The biological activity of the oxytocin-dimers ($\alpha + \beta$) was determined in the usual tests, and the results are summarized in the Table. For comparison, the published activities of the oxytocin-dimers $\alpha$ and $\beta$ and corresponding potencies of oxytocin are also included.

The oxytocin-dimers ($\alpha + \beta$) showed very low but definite oxytocin- and vasopressin-like activities. The result on the isolated rat uterus is similar to previous findings. Compared with oxytocin, the relative activity (A:B) is of the order of 1% on a weight basis and 2% on a molar-basis respectively with one exception: the cat uterus in situ where it reaches 3.4% (w/w) and 6.8% (mol/mol) respectively. In the rat preparations there were practically no qualitative differences between the pharmacological effects of equipotent doses of oxytocin and oxytocin-dimers ($\alpha + \beta$). However, in the experiments using cats, rabbits and chickens, differences in time course and moreover influences on the reactions to subsequent injections of oxytocin have been observed. Oxytocin-dimers ($\alpha + \beta$) are less toxic than oxytocin when administered i.v. to rats. The acute $LD_{50}$ is 43 mg/kg (oxytocin: 25 mg/kg).

*Zusammenfassung.* Oxytocin-Dimer ($\alpha + \beta$), ein Nebenprodukt der Oxytocin-Synthese, zeigt in 6 Versuchsanordnungen schwache, aber messbare neurohypophysäre Aktivitäten.

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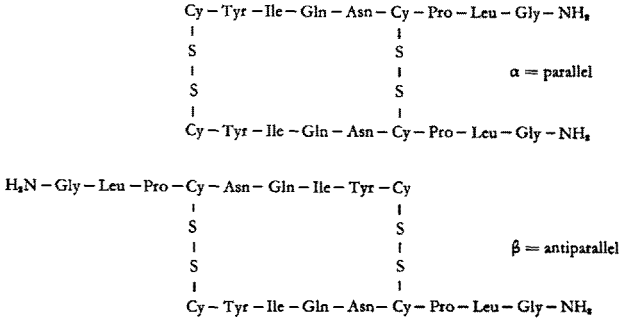
Oxytocin-dimers

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## A Comparative Study of Inulin and Sodium Sulfanilate Clearances in Sheep and Goats

Considerable research has been conducted on the problem of glomerular filtration rate (GFR)[1,2] and the mechanism of tubular transport of several substances present in the glomerular filtration or in the blood plasma which perfuses the tubules[3–5]. The concept of 'clearance' has been generalized for all aspects of renal excretion and includes filtration, tubular secretion and absorption. During the decade of 1925–1935 several investigators found different substances that have the ability of being transported[6–8] and concentrated by kidney in a larger amount than their plasma concentration; creatinine for instance shows that property in some species.

The introduction of inulin for the measurement of glomerular filtration rate made it possible to study the physiology of the tubules. Tubular activity can easily be shown by comparison of the clearances of substances which are secreted or reabsorbed by the tubules with that of inulin[9,10]. If a substance has a higher clearance than inulin, the substance must have been added to the filtrate by secretion of the tubules; conversely if substances which have been filtered do not appear in the urine or have clear ance rates less than that of inulin the substances must have been removed from the filtrate by the tubules. Examples of the former mechanism are the clearance of

creatinine in man, phenol red in the dog[11], and iodophyracet[12]. Glucose and urea clearances are examples of the latter. In ruminants, creatinine has been shown to have a certain degree of tubular transport[13].

SMITH et al.[14] also proved that the kidneys cannot excrete more of any substance per unit of time than is carried to them by the blood in that interval; that is, the upper limit of renal clearance values will be that of a substance which is completely cleared from the blood, and this clearance will be identical with the rate at which the blood or plasma is circulating through the kidneys.

It is also important to note that the clearance of a substance which is secreted decreases proportionally as the plasma concentration increases because of progressive saturation of the secretory mechanism which occurs at relatively low plasma levels[14].

*Materials and methods.* 5 healthy mature ewes and 2 female goats weighing 36 to 50 kg were selected. Each animal was held under laboratory conditions for 24 to 72 h prior to the beginning of an experiment. 1 gallon of water was given by means of a stomach tube 2 h before the clearance studies began, insuring adequate urine flow.

Urine was collected by bladder catherization with a No. 16 Bard Foley (C. R. Bard, Inc.) retention catheter. Both jugular veins were cannulated with plastic tubing. Infusions were made in one cannula and samples collected from the other. Inulin and sodium sulfanilate clearances were determined by the methods described by RUIZ[15].

To show that inulin and sodium sulfanilate are removed only by the kidney, the same procedure was performed on a sheep in which the renal blood vessels were tied 15 min after zero time.

Inulin concentrations in plasma and urine and the measurement of the glomerular filtration rate (GFR) by means of sodium sulfanilate were determined by the methods of DAVIDSON et al.[16], and BRATTON and MARSHALL[17] respectively.

All determinations were done in duplicate and a set of standards was included in each determination. The calculation for the GFR can be determined as suggested by PEOPLES[18].

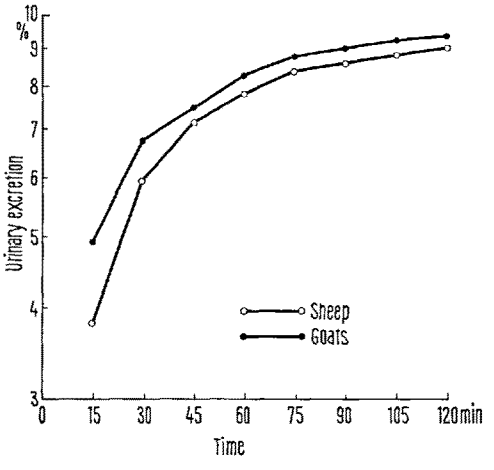


Fig. 1. Cumulative concentration of sodium sulfanilate recovered in urine 2 h following a single i.v. injection (10 mg/kg of body weight).

Table I. Clearance values for sodium sulfanilate after a single i.v. injection in normal animals

| Goats (cm³/kg/min) | Sheep (cm³/kg/min) |
|---|---|
| 10.70 | 8.45 |
| 10.20 | 7.89 |
| 9.14 | 6.89 |
| 8.00 | 6.72 |
| 7.85 | 6.65 |
| 8.00 | 5.50 |
| 7.85 | 5.22 |
| 7.11 | 4.42 |
| 6.20 | 4.35 |
| | 3.95 |
| Mean 9.00 | Mean 6.20 |

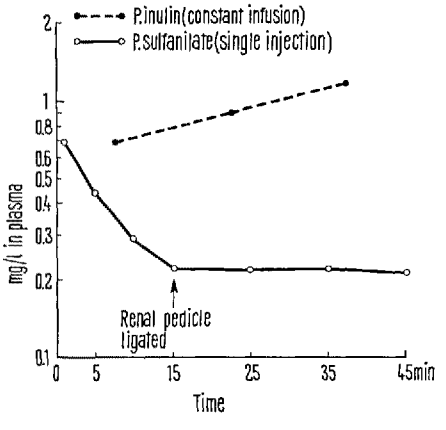


Fig. 2. Effect of the ligature of the renal pedicle on the plasma concentration of inulin and sodium sulfanilate in the blood.


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Table II. Simultaneous sodium sulfanilate and inulin clearances in sheep and goats

| Sheep | | | | Goats | | | |
|---|---|---|---|---|---|---|---|
| Animal No. | $C_I$ cm³/min/kg | $C_s$[b] cm³/min/kg | $C_s/C_I$ | Animal No. | $C_I$[a] cm³/min/kg | $C_s$ cm/min/kg | $C_s/C_I$ |
| 225 | 1.63 | 4.90 | 3.00 | 291 | 2.22 | 6.30 | 2.75 |
| 227 | 1.46 | 3.00 | 2.05 | 293 | 2.40 | 7.90 | 3.30 |
| 248 | 1.44 | 5.03 | 3.50 | 291[c] | 1.80 | 4.95 | 2.75 |
| | | | | 293[c] | 2.80 | 3.78 | 1.36 |
| | | | Mean 2.85 | | | | Mean 2.54 |

[a] Priming dose of inulin 15 mg/kg followed by a constant infusion of 0.08 mg/min. After 60 min, the equilibration period, sulfanilate was injected. The blood and urine samples were also collected after this period. [b] The values for sodium sulfanilate clearances are corrected against the hematocrit. [c] The experiment was repeated after 1 month on the same animals.

*Results.* The mean values obtained for sulfanilate clearance ($C_S$) after a single injection of this substance in the animals studied were respectively 6.20 cm³/kg/min (S.D ± 0.39) in sheep and 9.00 cm³/kg/min (S.D ± 2.16) in goats (Table I). The mean values for inulin clearance ($C_I$) in the same animals were 2.30 cm³/kg/min (S.D ± 0.24) in goats and 1.51 cm³/kg/min (S.D ± 0.14) in sheep. The simultaneons clearances of sulfanilate and inulin were compared by means of the ratio of their clearances $C_S/C_I$. The mean values obtained were 2.85 for sheep (S.D ± 0.36) and 2.54 for goats (S.D ± 1.53) (Table II). 90% of the sulfanilate dose given per kilogram for the sulfanilate clearance determinations was recovered in urine within a 2 h period (Figure 1).

The bilateral occlusion of the renal pedicle which was performed 15 min after zero time for the $C_S$ and $C_I$ determinations produced an increase in the plasma concentration of inulin and a steady plasma value for sodium sulfanilate (Figure 2). The hematocrit (PCV) values in the animals studied showed a range of 30 to 32% for sheep and 30 to 34% in goats.

*Discussion.* The single injection method showed that sodium sulfanilate requires a period of less than 15 min to be in equilibrium with the extracellular fluid. This is evident since the slope of the disappearance curve stabilizes and remains unchanged after the first 10 min[18]. Inulin has a low diffusibility coefficient[19] and all the trials for clearances in comparative studies with inulin must allow an equilibrium period of 1 h, the shortest time for inulin equilibrium with the extracellular fluid.

The $C_S$ values change according to the material employed for its determination. The figures obtained by using whole blood were about 10% lower than those obtained by the employment of plasma. The reason for this is the cell volume; since it has been proved[15] that the cells are impermeable to sulfanilate, inulin, and many other substances employed for kidney function tests, the estimation of the effective plasma volume perfusing the kidney is low when whole blood is used for these determinations, and consequently the hematocrit correction is necessary.

If several substances suitable for estimation of the glomerular filtration rate are present simultaneously in the blood, their excretion is equally simultaneous and their clearances remain equal under all conditions, even under those which may be presumed to change the filtration rate[19,20], and the clearance ratio is always one or close to one.

In the two species studied, 90% of the sodium sulfanilate was recovered after 2 h. In these experiments the rate at which sodium sulfanilate decreases in the plasma is an index of the kidney function responsible for clearing the blood of that substance.

However, the use of this substance for the measurement of the glomerular filtration rate in sheep and goats is questionable since the mean values of $C_s/C_I$ obtained by simultaneous determinations in this study were 2.85 in sheep and 2.54 in goats (Table II). This suggests tubular addition of sulfanilate to the glomerular filtrate. If sulfanilate had the same mechanism of excretion as inulin (filtration only), the ratio would be one or close to one.

These experiments indicate that both the GFR and a certain degree of tubular removal of sodium sulfanilate from the blood proportional to its concentration in the plasma, similar to the removal of PAH by the tubules[19]. Further work needs to be done to elucidate this tubular mechanism. That the type of elimination of inulin and sodium sulfanilate is predominantly renal is proved by the bilateral ligation of the renal pedicles which produced an increase of the inulin concentration in the blood while inulin is being continuously infused (Figure 2). Constant values for sulfanilate concentrations in plasma are obtained under these conditions.

The average $C_I$ values found in sheep and goats in terms of body weight are 2.30 cm³/kg/min for goats, and 1.50 cm³ kg/min for sheep; these figures agree with the values of 1.9 cm³/kg/min reported by SHANNON[21].

These data show again the value of inulin for the determination of the filtration rate and also suggest that it is the preferred substance to be used as a reference to measure GFR in comparative studies on kidney function[22,23].

*Zusammenfassung.* Natrium-Sulfanilat-Clearance kann bei Schafen und Ziegen nicht als Mass für die Glomerulumfiltration verwendet werden, da Natrium-Sulfanilat glomerulär filtriert und tubulär sezerniert wird.

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