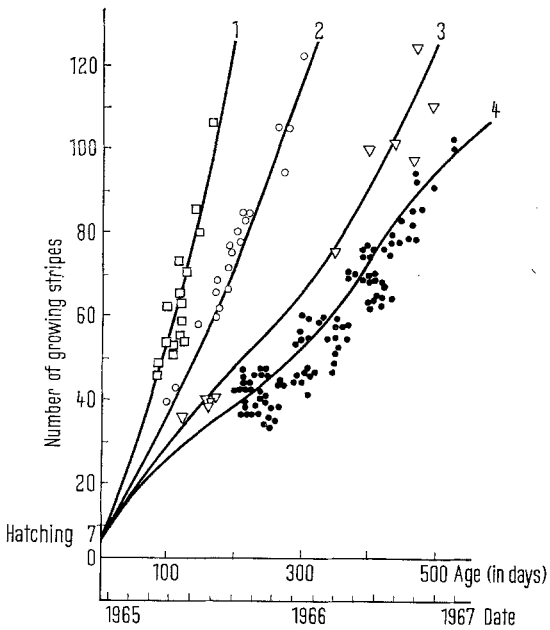
The graph lines corresponding to batches 1 and 2 are regular whereas those which stand for batches 3 and 4 reveal a variation in the rhythm of formation of shell stripe pattern which reflects the summer rise of temperature in the waters of the English Channel. The thermostatic system used was without cooling and the minimum experimental temperatures could not be maintained during the summer.

These observations may be further pursued if one estimates the rhythm of daily age markings. Allowing for thermic variations undergone by the cuttlefishes of batches 3 and 4, the true rhythm for these 15 and 13 °C temperatures has been worked out by measuring the curve on the graph for the winter period, when the thermostatic controls are effective (Table).

Influence of temperature on the rhythm of appearance of striped markings

| Experimental batch | 1 | 2 | 3 | 4 | Estimate | |
|---|---|---|---|---|---|---|
| Temperature (°C) (minimum control) | 25 | 20 | 15 | 13 | | |
| Temperature in fact (achieved) | 25 | 20 | varies between 19.5–15 | varies between 19.5–13 | 15 | 13 |
| Rhythm of appearance of striped markings (No. of days necessary for formation of each single stripe) | *1.6* | *2.6* | 4.3 | 5.4 | *6* | *8* |




Variation in the number of striped line according to the temperature in which the cuttlefish were reared. (1) Batch of cuttlefish reared at a temperature of 25 °C; (2) 20 °C temperature; (3) 15 °C temperature; (4) 13 °C temperature (this being the minimum control temperature).

As a result, recordings obtained by these different experimental rearings allow us to state that the rhythm of stripe formation depends on temperature; the number of markings on a shell are merely an approximate indication of age, scarcely more reliable than the length of the shell itself. The biological comparisons which one might be tempted to draw between living cephalopods of different oceans can only be true during those periods in which thermal conditions are similar.

By extrapolation of results it can, however, be estimated that the daily age markings observed by CHOE[2] would indeed be attained by *Sepia officinalis* L. in water the temperature of which was maintained at 30 °C, this being the maximum rearing temperature in Japan.

It may thus be supposed that the mineral metabolism, a function of the siphuncular epithelium responsible for age markings, is governed by a biological rhythm occasioned by external factors in the case of the different cuttlefish species.

*Résumé.* Le rythme de formation des stries d'accroissement de la coquille de seiche est fonction de la température. Il semble donc nécessaire d'en tenir compte avant de comparer divers Sepiidés (Age, Sous-espèces, etc.).

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## Proteinuria in the Rat: A Comparison of Tissue Components in the Voided and Renal Pelvic Urine

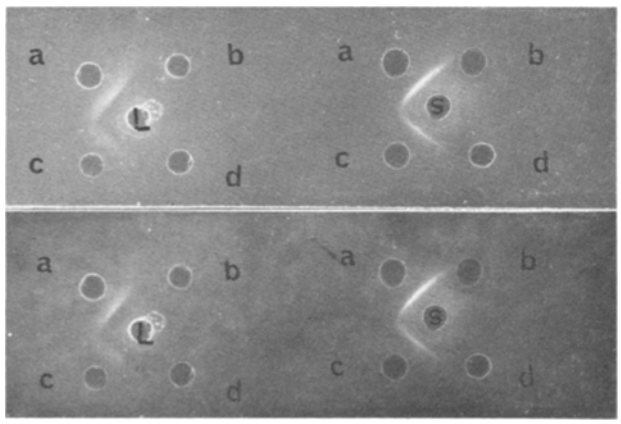
It is generally accepted that the proteinuria in the rat is the result of a discrepancy between glomerular filtration of blood serum proteins and tubular reabsorptive capacity[1]. BELL'S[2] assumption that the protein in the urine partly originates from the accessory genital glands has been recently reconsidered by PERRY[3] and ROSENMANN et al.[4] in the male rat and by BARNES et al.[5] in the human. The purpose of this communication is to present evidence that the urine of the male rat does contain tissue components derived solely from the accessory genital glands.

Antisera to organ-specific antigens of the accessory genital glands of the male rat were prepared in rabbits by an immunization schedule employing Freund's complete adjuvant as described previously[4]. 4 organ-specific antigens were detected by the double diffusion technique in agar gel using antisera directed against the sediment, soluble and lipid fractions of the glands. None of these antigens were found in the urine of female rats, whereas 1 or 2 antigens were demonstrated in the urine of male animals when reacted with anti-sediment and anti-lipid fraction sera; no precipitation bands developed with the anti-soluble fraction serum.

In order to prove that the antigens demonstrable in the urine of the male rat are not excreted or secreted by the kidneys, spontaneously voided and renal pelvic urine samples were compared. The left ureter of 8 adult male rats was exposed during laparotomy and ligated approximately 1 cm from the uretero-pelvic junction. 10–12 days after the operation, the animals were placed in individual metabolism cages over urine-faeces separators and urine was collected under a layer of liquid paraffin. The rats were killed 1 day later and the urine was aspirated from the hydronephrotic sac. The spontaneously

voided urine from the right kidney and the urine from the hydronephrotic left kidney were compared by the Ouchterlony technique with respect to the presence of the organ-specific antigens described above. No precipitation lines developed when urine from the hydronephrotic sac was reacted with antisera to the sediment and lipid fraction of the accessory genital glands. As expected, the spontaneously voided urine, passing through the urinary bladder and urethra, contained an antigen precipitating with the 2 antisera (figure). It is noteworthy that this reacting antigen is of high molecular weight, since it was excluded from a G-200 Sephadex column.

SELLERS[1] is of the opinion that proteinuria in the rat stems solely from the kidneys. THUNG[6] reports that in the mouse no proteins other than those of renal origin are demonstrable. RUEMKE and THUNG[7] believe that the mouse urine contains a protein produced in the liver, which is concentrated and excreted by the kidneys. The observations reported herein unequivocally demonstrate that the spontaneously voided urine of the male rat contains at least one proteinaceous component which is not excreted or secreted by the kidneys. This component has been found to be serologically organ-specific for the male accessory genital glands; its absence from the renal pelvic urine proves that non-renal tissue constituents are contributed to the proteinuria of the normal male rat[8].




Double immunodiffusion tests of antisera directed against the lipid fraction (L) and sediment (S) of the male accessory genital glands with the spontaneously voided urine (a and c) and renal pelvic urine (b and d). With the 2 urine specimens illustrated, a precipitation band developed only when the antisera were reacted with the voided urine.

*Zusammenfassung.* Es wird gezeigt, dass bei gesunden männlichen Ratten nur Urin, der die unteren Harnwege passiert, Bestandteile der Geschlechtsdrüsen enthält. Somit ist die normale Proteinurie zum Teil auf die Ausscheidung extrarenaler Gewebskomponenten zurückzuführen.

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## Comparison of the Effects of Atropine Sulfate and Procaine Hydrochloride on Nerve Conduction

In interpreting the central action of cholinomimetic agents, atropine sulfate is frequently used as a blocking agent to determine whether the action is mediated by a nicotinic or muscarinic mechanism. The question arises whether it is acting as a muscarinic blocking agent or as a nonspecific local anesthetic, since the chemical structure of atropine has certain similarities to cocaine. Many atropine analogues[1,2] have been reported to have local anesthetic activity. In 1960, CURTIS and PHILLIS[3] demonstrated that atropine sulfate behaved similarly to procaine HCl in depressing the spike potentials of spinal neurons evoked by chemicals (acetylcholine, glutamate ions) and nerve stimulation (ventral-root).

In reviewing the literature, the only paper that quantitatively describes the local anesthetic effect of atropine sulfate was published in 1948 by DE ELIO[4]. Atropine was reported to have half the potency of procaine by the method of i.c. injection in guinea-pigs. Although this is a useful screening method for local anesthetics[5], it is possible that atropine may appear active in this test because of interference with pain receptor mechanisms. The local anesthetic activity of atropine has therefore been reinvestigated on the isolated frog sciatic nerve in order to obtain a more direct assessment of its properties.

*Methods.* Frog Ringer: The following solution was used throughout the experiments: NaCl, 110 m$M$; KCl, 2.7 m$M$; $CaCl_2$, 1.8 m$M$; *Tris*-(hydroxymethyl)-aminomethane, 1.0 m$M$ and EDTA, 0.1 m$M$. The solution was adjusted to pH 7.4 with HCl. Each test solution was made up by dissolving the drug in the above solution and the pH was readjusted to 7.4 by adding dilute NaOH solution.

Nerve-bath: A small plastic rectangular tissue bath of $1.0 \times 2.0 \times 4.0$ cm$^3$ with a plastic cover was used. 9 platinum electrodes were arranged as shown in Figure 1, so that 2 of them (6 and 7) were immersed in the solution when it was partially filled (3 ml of the test solution). The distance between the platinum electrodes was 0.5 cm. During the experiment the nerve-bath was filled with 3 ml of the test solution. The nerve was threaded through the electrodes so that it lay above electrodes 1–5, below electrodes 6–7 and above electrodes 8–9, ensuring that a constant length of nerve was immersed in the test solution. The nerve was stimulated at its peripheral end through electrodes 1 and 2, by supramaximal square wave pulses (0.15 ms, 0.5/s) from a Grass S4B stimulator and General Radio 578A isolation transformer. Action potentials were recorded simultaneously between electrodes 4 and 5 and electrodes 8 and 9 using a Textronix 502 oscilloscope. Electrodes 3, 6 and 7 were connected to ground.