already described elsewhere[6]. The tubules are enlarged and filled with secretory granules; the excretory ducts are also enlarged and rich in granules. No significant changes are detectable in the acinar portion of the gland. In the actinomycin-treated animals, some signs of cellular damage were visible both in the acinar and in the tubular portions; the cells appeared pale, with some vacuolation in the cytoplasm, in addition to a loss of secretory granules within the tubules. A similar picture was observed in the glands of animals treated with testosterone + actinomycin-D. In agreement with previous observations, testosterone injections into female mice produced a sharp increase of the protease activity and of the NGF concentration in the submaxillary gland (Table II). No significant changes though were observed in the amylase activity, which remained at normal levels after 5 days of treatment. In the presence of actinomycin-D, the testosterone effect on protease and on NGF activity was completely inhibited. Actinomycin alone causes only a moderate fall of activity of both the protease and α-amylase; the NGF concentration appeared reduced to about 50% of the control female glands.

Table II. Effect of testosterone and of actinomycin-D on the specific activities of protease, amylase and NGF in the female submaxillary glands (5 days of treatment). Each value represents the average from 5 separate determinations ± SD

	Protease U/mg	α-Amylase U/mg	NGF[a] μg/ml
Control	6.06 ± 0.32	5.2 ± 0.7	15–30
Testosterone	10.5 ± 0.85	5.4 ± 1.1	1.5– 3
Actinomycin-D	5.7 ± 0.45	5.05 ± 0.62	30–60
Actinomycin-D + testosterone	6.7 ± 0.71	5.8 ± 0.45	15–30

[a] The NGF activity is expressed as the minimum protein concentration required to give a 3+ response in tissue culture (14).

From the results of our experiments, it appears that the action of the male hormone is well localized in only one component of the gland and seems to be exerted on the synthesis or turnover of certain cell constituents. The relative increase of the soluble proteins in the testosterone-treated glands shows that net protein synthesis has taken place under the hormonal stimulation. So far as is known, the complete inhibition of the testosterone effect on the submaxillary gland by actinomycin can be accounted for to a blockage at the level of *m*-RNA synthesis[12]. More definite evidence, however, should come from *in vitro* experiments on cell-free preparations. They should also provide an answer as to whether the NGF protein is actually produced in the salivary gland rather than stored there and simply activated by the testosterone treatment. Experiments along this line are now in progress[13].

Riassunto. La somministrazione di testosterone in topi femmina produce notevoli modificazioni della ghiandola sottomascellare, che acquista i caratteri di tipo maschile. Actinomicina-D, somministrata contemporaneamente all'ormone maschile, inibisce tale effetto sulla ghiandola sottomascellare. I risultati suggeriscono che l'azione del testosterone si svolga al livello della sintesi di RNA nucleare.

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[12] J. R. Tata, Nature *197*, 1167 (1963).

[13] This work was supported by NIH Grant B-3777 and by the Merck-Sharp and Dohme Lab., Rahway (N.J.). – Actinomycin was kindly supplied by Dr. Muschett of Merck-Sharp and Dohme Lab., Rahway (N.J.).

Electrical Properties and Glucose Transfer in the Goldfish Intestine

Transmural potential differences (PD) have now been recorded from many *in vitro* intestinal preparations[1–4]. In all cases the serosa shows a positive potential with respect to the mucosa and the magnitude of the current needed to short circuit the PD correlates approximately with the net transfer of sodium from mucosa to serosa. The intestine of the marine teleost *Cottus scorpius* is unusual in that it transports sodium from the mucosa to serosa in the absence of a recordable PD[5]. The following work was undertaken to test whether this property was common to the fresh-water teleost, *Carassius auratus*, whose salt requirements are very different from those of its marine counterpart.

The preparation used was a 1.5–3 cm sac of everted goldfish intestine consisting of the intestinal bulb and part of the anterior intestine. The sac was suspended in 60 ml of Krebs-Henseleit[6] medium containing 27.7 m*M* glucose and gassed with 95% O_2 + 5% CO_2. 0.1 ml of the same medium was placed within the sac at the start of the experiment and the transmural PD then recorded over a 60 min period with a Vibron electrometer using calomel electrodes and agar bridges made with 0.9% w/v NaCl. The PD was corrected for the junction potential. At the end of incubation the medium within the sac was weighed and the amount of glucose present determined by the method of Hansen[7]. In every case a definite

[1] R. J. C. Barry, D. H. Smyth, and E. M. Wright, J. Physiol. *168*, 50P (1963).
[2] S. G. Schultz and R. Zalusky, J. gen. Physiol. *47*, 567 (1964).
[3] H. H. Ussing and B. Anderson, Proc. 3rd Internat. Cong. Biochem. (Brussels, 1955), p. 434.
[4] I. L. Cooperstein and C. A. M. Hogben, J. gen. Physiol. *42*, 461 (1959).
[5] C. R. House and K. Green, Nature (Lond.) *199*, 1293 (1963).
[6] H. A. Krebs and K. Henseleit, Hoppe-Seylers Z. *210*, 33 (1932).
[7] O. Hansen, Scand. J. clin. lab. Invest. *14*, 651 (1962).

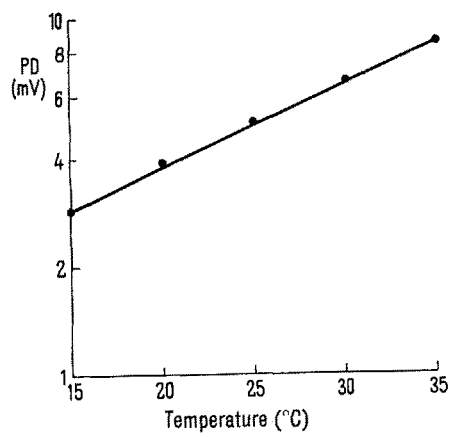
transmural PD was recorded with the serosa positive to the mucosa. The Figure shows the relation between PD and the temperature of incubation.

The PD measurements are those obtained after 60 min incubation, except where the temperature of incubation was 35°C. At this temperature the initial high PD fell after 10 min incubation and the maximum value has been used in this case. At lower temperatures the PD rose slightly throughout the incubation period. Plotted on a semi-logarithmic scale the PD is shown to be linearly related to temperature with a Q_{10} of 1.75. The small intestine of the rat has been shown to behave in a similar way with a Q_{10} of 1.7[8]. The transfer of glucose during incubation of the everted sacs at different temperatures is shown in the Table. At or above 25°C glucose moved against its concentration gradient. Below 25°C some transfer took place but the final glucose concentration fell below that present at the start of incubation owing partly to fluid transfer from mucosa to serosa. This fluid transfer was about 15 μl and was not changed noticeably by changes in the temperature of incubation. The total amount of glucose which appeared in the serosal fluid during incubation was dependent on temperature.

An everted sac of goldfish intestine, incubated at 30°C for 35 min in the normal medium maintained a steady PD, but this fell rapidly to a new steady level after transfer to glucose free medium. This new PD could be maintained at the same level for at least 30 min and was half that found in the presence of glucose. The initial PD was restored within 3 sec of replacing the sac in medium containing glucose. Similar rapid changes have been shown for the rat intestine[9]. The rapidity of these changes points to an action at the luminal membrane of the mucosa; glucose and sodium transport are probably linked in a direct way. The evidence for this view is the correlation between the net glucose transport and PD and the rapidity of changes in PD caused by removal or addition of glucose, which seems to rule out an indirect metabolic effect for glucose.

Ouabain, 50 μM, in contact with the serosa of the everted sac in the presence of glucose reduced the PD to below 1 mV after a short lag phase but had little or no effect when placed in contact with the mucosa. It has been shown[10] that ouabain blocks glucose transfer when applied to the serosal surface of the intestine, but not when applied to the mucosa, and it is thought that ouabain acts in the vicinity of the basal membranes where a substrate specific ATPase can be shown to exist[11]. But it is not known whether the PD postulated for the luminal border of the mucosa falls as a consequence of ouabain action at the base of the mucosa, where the glucose-independent PD is possibly situated, or because of direct inhibition of ATPase in the membranes of the microvilli.

Whatever the mechanism for the maintenance of a transmural PD may be in the goldfish intestine there is no doubt that it exists and can be changed in a similar fashion to that found in mammalian intestines. The partial dependence of PD on glucose, the absolute PD measured at 35°C, the inhibition by ouabain and the rate of change of PD with temperature are all properties common to goldfish and mammals. Both need to conserve sodium and probably use the same enzymatic mechanism for sodium transport in the intestine. The marine teleost absorbs large amounts of salt to obtain its water, the unwanted salt then being extruded from the gills, and it would appear that the mechanism for intestinal sodium transport in this case is qualitatively different from that of the fresh-water teleost.



Transmural PD of the goldfish intestine measured at different temperatures. Each point is the mean of 5 experiments.

Transfer of glucose by everted sacs of goldfish intestine. The sacs contained initially 0.1 ml Krebs-Henseleit medium with 5 mg/ml glucose and were incubated at different temperatures for 60 min in 60 ml of the same medium. Each value is the average of 5 experiments

Temperature (°C)	Glucose concentration (mg/100 ml)		Net transfer of glucose (μg)
	Initial	Final	
15	500	461	30
20	500	498	73
25	500	509	85
30	500	539	120
35	500	596	185

Zusammenfassung. Mit *in vitro* Dünndarmpräparat vom Goldfisch wird Glukose gegen ein Konzentrationsgefälle bei Temperaturen über 25°C transportiert. Es tritt eine temperaturabhängige elektrische Potentialdifferenz auf der Serosaseite positiv in Erscheinung. Bei Entfernung der Glukose verkleinerte sich die Potentialdifferenz um die Hälfte und verschwand völlig, wenn Ouabain der Serosaseite zugesetzt wurde. Die Befunde werden mit Experimenten an marinen Teleostiern verglichen.

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Subdepartment of Chemical Physiology, A.R.C. Institute of Animal Physiology, Babraham, Cambridge (England), June 18, 1964.

[8] M. SAWADA and T. ASANO, Am. J. Physiol. *204*, 105 (1963).
[9] T. W. CLARKSON, A. C. CROSS, and S. TOOLE, Nature (Lond.) *191*, 501 (1961).
[10] M. W. SMITH, in press.
[11] B. C. S. HOLLANDS and M. W. SMITH, in press.