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Influence of Setting-up Time, Temperature of Incubation and Age on the Transmural Potential Difference across Chicken Rectum

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The effects of different experimental conditions on the *in vitro* transmural potential difference (PD) have been studied in the chicken rectum by the Ussing and Zerahn technique. Results have been analyzed with a statistical method to reveal the contribution of different controlled variables to the response, as well as the possible contribution of interactions between them. The variables considered were: age, setting-up time, temperature of the incubation medium and time elapsed from the beginning of incubation. It can be concluded that *a)* PD increases when temperature rises from 31 °C to 37 °C and when age increases from 71 to 123 days; *b)* Changes in PD during incubation depend on the temperature of incubation medium; *c)* The influence of age on PD depends on the temperature of the incubation medium and on the time required to set-up the preparation; *d)* PD is inversely co-related with setting-up times ranging from 3.5 to 7.5 min.

Key words: Electrical parameters, Chicken rectum.

In *in vitro* transport studies, the time elapsed in setting up the preparation is critical since damage in the tissue may be a source of error in the measured variables (5). Other factors, such as the temperature of the incubation medium (4) and the animal age (7) have also been shown to contribute to the magnitude of the response. The influence of these factors in homogeneous experimental groups is not usually taken into account since they virtually affect all experimental groups in the same way. However, it is reasonable to think that more than one such factor may contribute to the response at the same time, and that they may be interrelated.

In the present work we have examined

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animal age, setting-up time, temperature of the incubation medium, and time elapsed after initiating the experiment (controlled variables) as factors which may contribute to the magnitude of the transmural potential difference (PD), an electrical parameter which is characteristic of the intestinal electrolyte transport function (PD will be also referred as the «dependent variable»). The effects of these factors on PD are difficult to detect by conventional methods since the contribution of each single variable is impossible to separate because they are part of the experiment itself. They can, however, be easily revealed by applying linear modelling approaches (1).

Materials an Methods

Male Leghorn chickens fed a commercial diet (Gordina, Purina), with ages varying between 71 and 123 days, were used. The animals were anaesthetized i.v. with sodium pentobarbital. Segments of the rectum were taken out, cut lengthwise along the mesenteric attachment and briefly washed in a Ringer solution. The mucosal layer was scrapped-pushed off of the underlying part of the gut wall and mounted in Ussing-type chambers. The tissues were immediately bathed with incubation medium and gassed with a mixture of 95 % O_2 and 5 % CO_2. The time elapsed from when the segment is excised to when the mucosa is fixed in the chambers (setting-up time) was measured in each experiment. This was comprised between 3.5 and 7.5 min. The experiments were carried out at different temperatures ranging from 29 to 39.9 °C.

The incubation medium was a Ringer of the following composition (in mmol/l): Na^+ 140, K^+ 5.4, Ca^{++} 1.2, Mg^{++} 1.2, Cl^- 124, $H_2PO_4^-$ 0.6, $HPO_4^=$ 2.4, HCO_3^- 21 and glucose 10 (pH 7.4). PD was measured similarly as described by Ussing and Zerahn (8).

The statistical analysis of PD was carried out using an ANOVA of the appropriate model (6). Calculations were made on a Sharp MZ-80B microcomputer (3). The variables considered in the initial model were: incubation time *(ti)*, setting-up time *(ts)*, temperature of the incubation medium *(T)* and age of animals (age), as well as the first-order interaction between them. To verify the initial model we used data from 38 tissues incubated for 100 min. Significant variables in the model were selected by a step-wise method (backwards), using a Bonferoni correction for the significance level (global $\alpha < 0.05$). On the basis of the final model, «populational» values and the significance of hypotheses were estimated for different experimental situations. No hypotheses were rejected at an α-risk level of 0.05. Confidence intervals of estimations were fixed at 95 %.

Results and Discussion

Results shown are values predicated by the final model. The variables *ts*, *ti*, *T*, and age as well as the first-order interactions between them are considered for the statistical analysis. That is, we studied if any of the four variables affect PD and whether PD is also affected by any of the other variables. The analysis indicates that the variables considered and some of the first-order interactions contribute to the response. They are the following: ti, $ti \cdot T$ and $ti \cdot T^2$ ($p < 10^{-3}$); T, age $\cdot T^2$, age^2, $T \cdot$ age, age$^2 \cdot T$ and $T^2 \cdot$ age^2 ($p < 10^{-4}$); ts, $ts \cdot$ age and $ts \cdot$ age^2 ($p < 10^{-6}$). This model explains a 79 % of the total variation. The meaning of squared variables contributing to PD is that when the controlled variables varies linearly the dependent variable (PD) varies nonlinearly. There is also interaction between different variables, indicating that the variation of one variable depends on the variation of another. For ex-

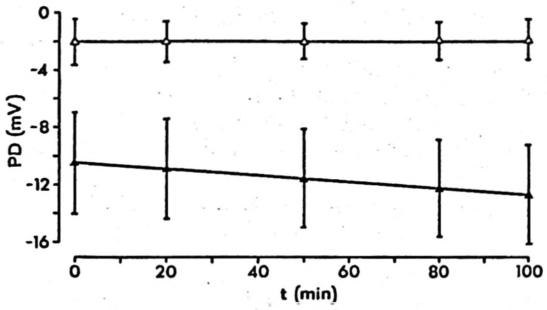

Fig. 1. *Influence of incubation time and temperature on PD across the chicken rectum.*
△, 37 °C; ▲, 31 °C. Results are populational values estimated from the final model. Age of chickens was fixed at 120 days and setting-up time at 7 min. Confidence intervals were fixed at 95 %.

ample, the interaction *ti* · T means that the variation of PD at different incubation times *(ti)* is affected by the temperature of incubation *(T)*. For the sake of simplicity, however, we are not going to discuss each mentioned first-order interactions. Rather, we will consider how each controlled variable influences the dependent variable as well as how their effect on PD is modified by the other variables.

The rectum of the chickens fed commercial diets has a reversed polarity (serosa-negative) indicating that the net flux of anions from the mucosal to the serosal compartment predominates over that of cations (2). The behaviour of PD with time depends on the temperature of the incubation medium. At 31 °C, there is a significant decrease in PD with time, 2.2 mV ± 1.6 ($p < 0.01$) after 100 min incubation. Above this temperature, the slope becomes progressively less pronounced so that at 37 °C PD does not change with time (fig. 1). This indicates that rectal PD is more stable during prolonged incubation at temperatures closer to the body temperature. When fixing *ti* at 50 min in the model, the difference in PD between tissues incubated at 31 °C and 37 °C attains 9.6 mV ± 3.6 ($p < 10^{-6}$), this being greater (less negative) at 37 °C than at 31 °C. In experiments performed in rats, EDMONDS and MARRIOTT (4) observed a similar effect, although they did not compare the rate of PD decrease with time at different temperatures. At 37 °C, the increase in ion conductivity and the greater activity of the Na/K pump, among other factors, counterbalance the higher net cation secretion observed at 31 °C. This results in lower PD values which are co-related to a less negative Isc.

The final model obtained shows that PD markedly depends on the age of animals and that it is also influenced by *ts* and *T*. This indicates that the observed changes in PD in relation to age are affected by the temperature of the incubation medium and by the setting up time (e.g. the change in PD with temperature is greater in younger animals than in the older ones. For *ts* effects see below). Table I compares the analysis of results from three age groups using both experimental data and data estimated from the model. We have chosen those experiments in which *ts* and *T* showed little variability since, as said before, both variables have a significant influence on PD. Both methods yield similar qualitative results, that is, a general tendency of PD to attain less negative values with increasing age, the change being steeper beginning from the 96-100 day-old age group. Discrepancies between experimental values and values estimated from the model are due to the impossibility of considering all the variables and interactions between them which contribute to the model.

The effect of *ts* on PD confirms GARDNER's paper (5) inasmuch as in short setting-up times the PD values obtained are greater (less negative) than at longer times. The PD/*ts* relationship is shown in fig. 2. The figure also shows that *ts* influence is more pronounced in the 70-90 day-old chickens than in the 100-120 day-old birds, that is, tissues from the younger an-

Table I. *Influence of age on PD across the chicken rectum.*
Estimated values have been obtained from the final model and, therefore, «number of experiments» cannot be assigned to each age group. PD values are accompanied by the SEM. The pre-set conditions in the model were: *ti*, 50 min; *T*, 36.5 °C; *ts*: 6 min. A negative PD indicates that the serosal compartment is negative vs. mucosal. Experimental values are given as means ± SEM from (n) experiments. They correspond to the PD after reaching a stable value. The average of temperature and setting-up time values of all the experiments considered was 36.5 °C and 6 min, respectively.

	Estimated values			Experimental values		
Age (days)	70	100	120	71-75	96-100	121-125
PD (mV)	—13.9 ± 2.4	—11.2 ± 0.6	2.5 ± 0.4	—7.6 ± 1.4	—10.2 ± 3.1	—5.0 ± 1.1
(n)	—	—	—	(6)	(3)	(9)

imals are more sensitive to temporary hypoxia than the older groups.

In conclusion, handling data by linear modelling analysis discloses more information about the sources of variability error in the responses of the experimental (dependent) variable than that obtained by traditional methods. It is very useful when a high number of experiments have been carried out in different conditions. The method enables estimation of the dependent variable (PD); however, when the controlled variables are outside the experimental intervals the response given by the model may be meaningless or even contradictory. The results point to the important contribution of setting-up time, age and temperature on the magnitude of the transmural potential difference. Such factors have to be either known, to be aware of their influence on results, or kept in very narrow margins to minimize their influence on the measured responses.

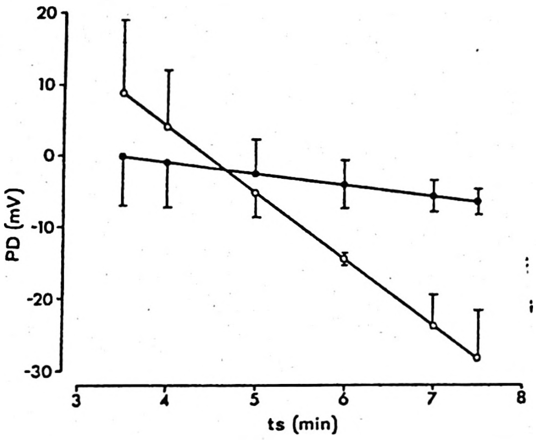

Fig. 2. *Influence of setting-up time on rectal PD at different ages.*
Results are the average (± standard error) of populational values estimated from the final model. Temperature was fixed at 37 °C and incubation time at 50 min. o, mean values obtained from 70, 80 and 90 day-old chickens; •, mean values obtained from 100, 110 and 120 day-old birds.

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Resumen

Se estudia el efecto de diferentes condiciones experimentales sobre la diferencia de potencial transmural (PD) del recto de pollo con la técnica *in vitro* de Ussing y Zerahn. Los resultados se analizan mediante un método estadístico que permite valorar la contribución a la respuesta de las distintas variables controladas así como sus interacciones. Las variables consideradas son: edad, tiempo de montaje, temperatura del medio de incubación y tiempo transcurrido desde su inicio. Se puede concluir que: *a)* La PD aumenta al incrementar la temperatura (de 31 a 37 °C) y la edad (de 71 a 123 días); *b)* Los cambios

en la PD durante la incubación dependen de la temperatura del medio; *c)* Los cambios en la PD con la edad dependen de la temperatura del medio de incubación así como del tiempo de montaje; *d)* La PD está inversamente relacionada con el tiempo de montaje (entre 3,5 y 7,5 min).

Palabras clave: Parámetros eléctricos, Recto de pollo.

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