

Zusammenfassung. Mit Hilfe des synthetischen Kollagenasesubstrates PZ-Pro-Leu-Gly-Pro-D-Arg wurden im humanen Serum kollagenolytische Enzyme nachgewiesen, deren Spezifität der Clostridiopeptidase A entspricht. Die Spaltungsgeschwindigkeit zeigt in Abhängigkeit von pH ein Optimum bei pH 7,2 und bei pH 8,0. Das spricht für die Existenz zweier Enzyme. Zur quantitativen Bestimmung der Enzymaktivitäten wurde eine Methode

ausgearbeitet, mit der eine Reihe von normalen humanen Seren analysiert wurde.

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A Sensitive Bioassay for Corticosteroids

Several authors¹⁻⁴ reported that the duodenum of the chick embryo is a target organ of adrenocortical steroids.

In the present paper, a bioassay for the quantitative estimation of corticoids in submicrogram amounts has been designed on the basis of the histological changes already described by HAYES⁵ on the duodenal mucosa of 16-day-old chick embryos cultivated with cortisone. The results indicate that this method can be employed with advantage in a tissue culture laboratory, for it combines specificity, simplicity, a good precision and simple equipment.

Material and methods. The duodenum of chick Hy-line embryos at 16 days of development was aseptically dissected, and cut longitudinal and transversally in order to obtain pieces of 0.5 mm². They were explanted according to the WOLFF and HAFEN's technique⁶, placing the muscular face down on the medium. The culture was obtained by mixing 1 vol. of embryonic extract (from 9-day-old chick embryos), 1 vol. of Eagle's medium with the addition of lactalbumin hydrolysate (0.61% w/v), and 2 vol. of agar (1% w/v) in Hank's solution. The explants were cultivated during 48 h at 37 °C, with air as the gas phase.

The following compounds were assayed: corticosterone, cortisol, 11-desoxy corticosterone (DOC), aldosterone, 17-hydroxy 11-desoxy corticosterone (compound 'S'), estradiol, testosterone, adrenaline hydrochloride and nor-adrenaline bitartrate.

At the end of incubation period, the explants were fixed in Bouin's fluid for 24 h, paraffin embedded, serially cut at 6 µ and stained with hematoxylin-eosin, for the histological study. A series of sections of each explant was performed and one showing a cut perpendicular to the mucosal surface was selected. In this section, the height of the mucosa, taken from the limit with the muscular layer to the free border of the villi, was measured in 4 different zones, with the aid of a reticulated eyepiece; the average reading was computed as the final height of the duodenal mucosa (Figure 1).

Standard statistical methods were employed for the evaluation of the data^{7,8}.

Results. The duodenum explants treated with corticosterone and cortisol exhibited edema in the connective tissue of the mucosa and dilated 'vascular channels' in the deepest layer of the mucosa near the limit of the muscular layer (Figure 1). Aldosterone and DOC produce edema, but not very evidently the aforementioned 'vascular channels'. In both cases the histological modifications were registered as an increase in the height of the mucosa. Figure 2 shows the linear response of duodenal height to corticosterone in doses ranging from 0.015 to 8.0 µg/ml. (F for linearity: 1.77; $p > 0.05$.) An index of precision (λ) equal to 0.22 was calculated with the means of 35 explants for each dose. Cortisol (Figure 2), aldosterone and DOC (Figure 3) also exhibited linear responses in the dosage

range studied. The slopes obtained for these compounds did not differ significantly from that obtained with corticosterone (F: 1.27; $p > 0.05$). Each figure shows also the dose which produces the maximal effect; 0.5 µg/ml for cortisol aldosterone and D.O.C. and 2 µg/ml for cortisol. Corticosterone was still active at 8.0 µg/ml. Within the range of linear response, corticosterone, aldosterone and cortisol are equally active, while DOC is 4 times less potent than any one of the other compounds.

In 9 different experiments the slopes (b) of the dosage-response curves for corticosterone ranged between 0.07 and 0.11.



Fig. 1. Section of a chick embryo duodenum at 16 days of development cultivated in a medium containing corticosterone (4 µg/ml) for 48 h. Hematoxylin-eosin. $\times 150$.

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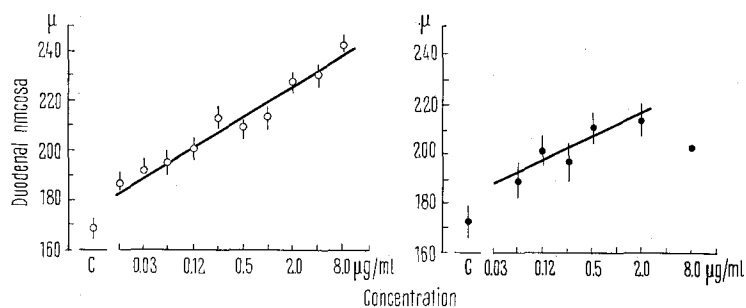


Fig. 2. Effect of corticosterone (○) and cortisol (●) on the height of the duodenal mucosa. Abscissa: concentration of each compound in the culture medium. Ordinates: height of the duodenal mucosa measured in μ . C: control values. Each point indicates the mean of 35 explants. Bars represent once the standard error of the means.

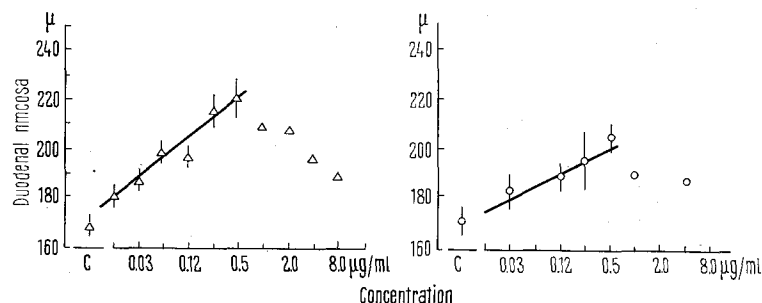


Fig. 3. Effect of aldosterone (Δ) and DOC (○) on the height of the duodenal mucosa. C: control values. Each point represents the mean of 25 explants. Bars represent once the standard error of the means.

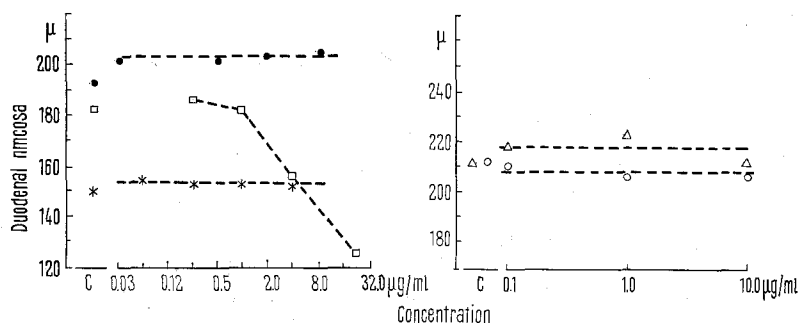


Fig. 4. Effect of compound 'S' (●), estradiol (*), testosterone (□), adrenaline (Δ) and noradrenaline (○) on the height of the duodenal mucosa. C: control values. Each point represents the mean of 20 explants.

Compound 'S', estradiol, adrenaline and noradrenaline (Figure 4) were without effect. Testosterone (Figure 4) had no effect at a low dose and exhibited toxic effects at high doses.

Discussion. The reported histological changes of the duodenal mucosa produced by the incubation of explants with several hormones, confirm the findings of HAYES⁵ on the appearance of enlarged 'vascular channels' under similar conditions. Further, this phenomenon is produced by corticosterone and cortisol and not by aldosterone and DOC. The phenomenon of edema described in this paper was not observed by HAYES, but this may be due to a difference in culture methods.

The height increasing effect, appears to be a specific response to biologically active corticoids. Compound of closely related structure with low or no corticoid-like activity, as well as catecholamines were found to be without effect.

The chick embryo duodenal mucosa appears to be a very sensitive target organ because it reacts significantly to corticosterone concentrations of the order of $1/100 \mu\text{g/ml}$. The method has a good precision index (λ : 0.22), working with 35 explants per dose. About 60 explants can be obtained in 30 min of dissection from a single duodenum.

It should be remarked that wide dosage range in which corticosterone produces a linear response.

The present bioassay has been employed successfully in the study of corticosteroid secretion of chick embryo adrenal tissue in vitro (unpublished results).

Resumen. Se describe un ensayo biológico para corticosteroides basado en la capacidad de estas hormonas de aumentar la altura de la mucosa duodenal embrionaria cultivada in vitro.

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