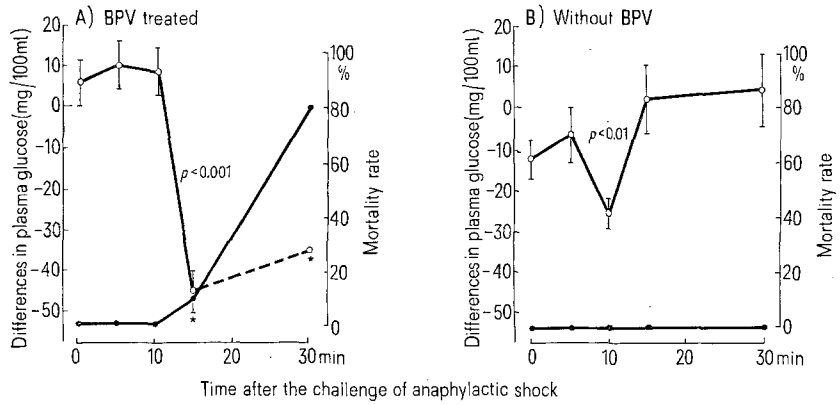

Effect of anaphylactic challenge on plasma glucose levels in rats sensibilized with (A) or wihtout (B) *Bordetella pertussis* vaccine. ● — ●, mortality rate (%) in groups of rats undergoing anaphylactic shock; ○—○, plasma glucose differences between unsensibilized controls and sensibilized groups, i.e. between groups 3 and 4 (A) or between groups 1 and 2 (B). Vertical lines represent the standard errors. Values of significance were related to the results obtained before anaphylactic challenge. *Results indicate the average of 9 and 2 animals corresponding to the mortality rate.

were submitted to anaphylactic shock and groups 1 and 3 served as their controls. 10 rats from each groups were killed before or at various intervals (see in the Figure) after the i.v. challenge if they did not die earlier. Plasma glucose levels were assayed by the glucose oxydase method of CAWLEY et al.[10]. Animals were fasted 18 h before killing them. Mortality ensued in various groups during anaphylaxis is shown in the Figure.

Challenge of anaphylaxis induced only a slight transitional decrease of blood glucose followed by a rapid recovery (Figure1B).This finding suggests that the increased peripheral glucose utilization of anaphylactized animals will be compensed by counterregulatory mechanisms such as the hyperglycemic action of released epinephrine, histamine and 5-hydroxytryptamine[7–9]. In these groups death did not occure and only moderate anaphylactic symptomps developed in the first 30 min of anaphylactic shock.

During the first 10 min blood glucose of BPV treated sensibilized and unsensibilized groups did not differ significantly from each other (Figure 1A). However, after this period, blood glucose dropped sharply and 10% of the anaphylactized rats died between the 10th and 15th min and another 70% from the 15th to 30th min.

In previous works, it has been shown that hyperglycemic effect of epinephrine[7,8] as well as that of histamine and 5-hydroxytryptamine[9] were considerably inihibited by BPV pretreatment. Thus, during anaphylaxis of BPV treated rats there is an inhibition in the above mentioned hyperglycemic counterregulation, and the prevailing hypoglycemic mechanisms results in a decrease of blood glucose, which may play an important role in the development of fatal anaphylactic symptomps. The latter hypothesis is supported by the fact that death had occured only after blood glucose dropped.

Zusammenfassung. Nachweis, dass der Blutzuckerspiegel von mit *Bordetella pertussis* vorbehandelten Ratten durch anaphylactischen Schock wesentlich vermindert ist, obwohl er in nicht vorbehandelten Tieren unverändert blieb.

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Androgen Receptor in the Thumb Pads of *Rana esculenta*

The thumb pad of *Rana esculenta* is a male secondary sexual character dependent upon gonadal hormones (LOFTS[1]). D'ISTRIA et al.[2] have demonstrated that the administration of small amounts of testosterone propionate to castrate adults induces hypertrophy in both the epidermal and glandular layers of the thumb pads and an increase in RNA and protein content. When labelled testosterone was injected into adult castrates (D'ISTRIA et al.[3]), the radioactivity showed a tendency to concentrate in the thumb pads. The study of in vitro metabolism indicated a conversion of testosterone into androstanolone and 11-Ketotestosterone. Both these metabolites stimulate the thumb pads when injected into castrate males.

In order to establish the presence of androgen receptor in thumb pads of *Rana esculenta*, the following experiments were done. Thumb pads, obtained from one month-castrates, were minced and homogenized in 1.5×10^{-3} *M* EDTA – 2×10^{-2} *M Tris*-HCl buffer pH 7.5, in an all-glass

Potter-Elvehjem homogenizer. The homogenate was centrifuged at $600 \times g$ for 10 min and the supernatant was centrifuged at $105{,}000 \times g$ for 60 min in an I.E.C. model B-60 centrifuge. All operations were carried out at 4 °C. The incubation media contained: 0.2 ml of supernatant at $105{,}000 \times g$ (about 0.3 mg of proteins), and 0.1 ml of buffer containing testosterone-H^3, S.A.87 Ci/mM, adjusted to different concentrations. Proteins were measured by the method of LOWRY et al.[4] The incubations were carried out for 2 h or overnight at 4 °C, and for 30 min at 37 °C. The charcoal adsorption method was used to measure the protein binding (0.25% Norit A and 0.025% Dextran T 70). Radioactivity was assayed by liquid scintillation counting in Insta-gel solution from Packard.

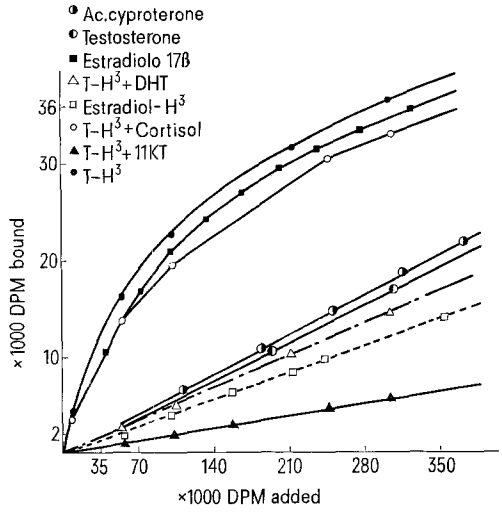

Fig. 1. Retention of radioactive steroids by thumb – pads $105{,}000 \times g$ supernatant during the incubation with 1,2,6,7-H^3-testosterone (3.38 μg per mCi) in 1.5×10^{-3} M EDTA – 2×10^{-2} M *Tris* – HCl puffer pH 7.5 at 4 °C overnight. The values are adjusted to correspond to a protein concentration of 1 mg/ml. DHT, dehydrotestosterone or androstanolone; 11-KT, 11-ketotestosterone.

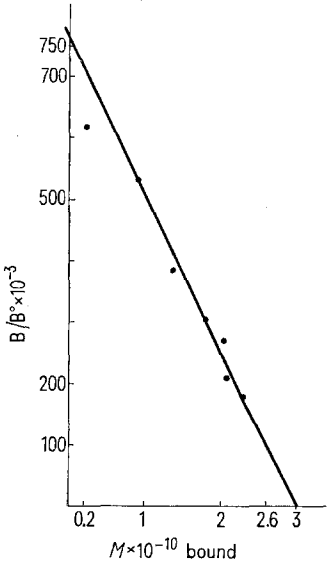

Fig. 2. Determination of the K_{ass} and the number of binding sites, using the Scatchard plot. B, amount of testosterone–H^3 bound; B°, total minus bound.

The retention of testosterone-H^3 by $105{,}000 \times g$ supernatant is shown in Figure 1. Increasing incubation time (from 2 h to overnight) causes an increase of protein-bound testosterone; the protein is capable of binding testosterone in considerable amounts also at 37 °C.

We have further studied the ability of various hormones to compete with tritiated testosterone for the receptor sites, adding 200 ng of hormone in the incubation medium. As shown in Figure 1, while there is a marked competition of testosterone, androstanolone, 11-keto-testosterone and cyproterone acetate (a powerful anti-androgen) with testosterone-H^3, no competition was shown by cortisol, estradiol-17β and free cyproterone. The receptors bound only a small quantity of estradiol-H^3 100Ci/mM.

The data of all experiments are adjusted to correspond to a protein concentration of 1 mg/ml. They can be analyzed in order to obtain both the concentration of binding sites and the equilibrium constant for the binding reaction. The Scatchard plots of various preparations of the $105{,}000 \times g$ supernatant from thumb pads were prepared and a K_{ass} ranging from 1.25 to 3.26×10^9 M and a concentration of binding sites varying from 1.15 to 3.01×10^{-10} M were obtained (Figure 2).

The values for K_{ass} are in reasonable agreement with the values reported by other investigators for mammals. KORENMAN and RAO[5] obtained a K_{ass} of 1.3×10^{11} M for the rabbit uterine cytosol; ALBERGA and BAULIEU[6] reported a value of 7×10^{11} M for rat endometrium; PUCA and BRESCIANI[7] obtained a value of 1.5×10^9 M for the K_{ass} of the nuclear receptor from calf uteri at 4 °C, whereas SHYMALA and GORSKI[8] reported a value of 1.1×10^9 M at 0 °C. It should be noted that we have used crude supernatant while the above-mentioned workers utilized purified material.

These observations indicate the presence of an androgen receptor in the thumb pad of *Rana esculenta*. The biochemical characterization of this receptor is under progress[9].

Riassunto. E' stato messo in evidenza un recettore per gli androgeni nel sopranatante a $105.000 \times g$ della callosità del pollice di *Rana esculenta*. Si é visto che competono fortemente col testosterone -H^3 il testosterone, il diidrotestosterone, l'11-chetotestosterone e l'acetato di ciproterone; mentre non competono l'estradiolo–17β, il cortisolo ed il ciproterone libero. La costante di associazione varia tra 1,25 e $3{,}26 \times 10^9$ M ed il numero di siti varia tra 1,15 e $3{,}01 \times 10^{-10}$ M.

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