was found to inhibit the respiration of the cells. The amount of oligomycin sufficient to inhibit a wet weight of the cells of 110 mg was $7.5 \cdot 10^{-10}$ moles. 110 mg of the cells contain $7.7 \cdot 10^{-10}$ moles of cytochrome *a*, based on the assumption that the concentration of cytochrome *a* in ascites hepatoma cells AH 49 is the same as in Ehrlich ascites tumour cells ($7 \cdot 10^{-9}$ moles/g of cells) as determined by CHANCE AND HESS[6]. Although the calculation of the number of oligomycin-sensitive sites requires both accurate standardization of oligomycin and determination of the concentration of energy-yielding systems in the cells, it may be assumed from this titration that oligomycin combines with energy-yielding systems preferentially even if it is applied to intact cell suspensions. Oligomycin has been found to be a very useful reagent for studying energy-yielding reactions not only in mitochondrial suspension[2], but also in intact cells and tissues.

Similar inhibition has been observed in the metabolic control of rabbit polymorphonuclear granulocytes[8], the details of which will be discussed elsewhere.

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A synthetic polypeptide antigen devoid of charge

Studies carried out in recent years in several laboratories have shown that synthetic polypeptides may be good immunogens of high antigenic specificity[1–6]. The availability of synthetic models permitted a systematic approach to the elucidation of the minimal requirements necessary to confer antigenic properties upon a molecule. In this respect, information was obtained concerning the role of composition, size[1,3–5], shape, accessibility of the immunologically important area to the biosynthetic site[1], configuration[7], etc. We report here recent experiments showing that the presence of electric charge on the molecule is not necessary to render it immunogenic.

The synthetic multichain polypeptide antigen, p(Tyr, Glu)–pAla–pLys[1,8] (for nomenclature, see ref. 1) contains both negatively charged carboxylate ions of the glutamate residues, and positively charged ammonium ions at the termini of the

polymeric side chains. In order to obtain an uncharged water-soluble analogue of the above antigen, multichain poly-DL-alanine[8] (pAla—pLys) was reacted as usual with the *N*-carboxyanhydrides of L-tyrosine and benzyl-L-glutamate, but instead of removing the benzyl groups with anhydrous hydrogen bromide to yield free carboxylate ions, the benzylglutamate residues in the product were reacted with propanolamine to yield N^5-(3-hydroxypropyl)-glutaminyl residues[9], according to the following scheme:

$$\begin{matrix}-NH\\-CO\end{matrix}\!\!>\!CH(CH_2)_2COOCH_2C_6H_5 + NH_2(CH_2)_3OH \rightarrow \begin{matrix}-NH\\-CO\end{matrix}\!\!>\!CH(CH_2)_2CONH(CH_2)_3OH + C_6H_5CH_2OH$$

The resulting p(Tyr, Hydroxypropylglutaminyl)–pAla—pLys (1 g) was desaminated with nitrous acid (500 ml 0.0625 N sodium nitrite and 120 ml 2.5 M acetic acid) for 1 h at 37°. The dialysed and lyophilized product contained no amino groups (Van Slyke analysis).

The uncharged desaminated multichain polypeptide elicited antibodies (300 μg/ml serum) in rabbits immunized in Freund's adjuvant according to previously described procedures[1], as checked by the homologous precipitin reaction (Fig. 1). The antiserum cross-reacted to a smaller extent with the non-desaminated polymer (which carries some positive charges), and gave only a poor cross-reaction with the highly charged p(Tyr, Glu)–pAla—pLys (Fig. 1). The last finding suggests that no conversion of hydroxypropylglutaminyl to glutamyl residues had occurred *in vivo* between the time of injection and the time of the "imprint" at the biosynthetic site.

Charged groups have been shown to be of decisive importance in conferring upon a molecule its antigenic specificity[10,11,2]. It was suggested recently that the presence of charged groups is important also in making a molecule immunogenic[4]. The experiments described here show that a completely uncharged synthetic polypeptide pos-

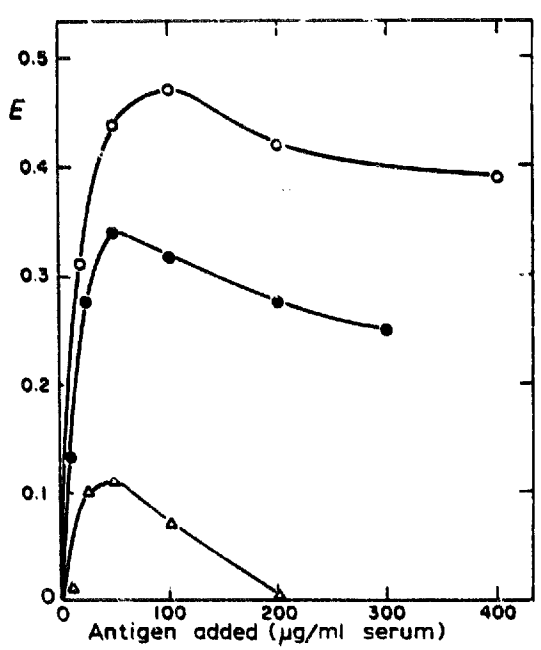

Fig. 1. Extinction at 2800 Å of solutions in 0.1 N sodium hydroxide of precipitates obtained by the addition to an antiserum to the uncharged polypeptide (desaminated p(Tyr, Hydroxypropylglutaminyl)-pAla–pLys), of: O—O, desaminated p(Tyr, Hydroxypropylglutaminyl)-pAla–pLys; ●—●, p(Tyr, Hydroxypropylglutaminyl)-pAla–pLys; Δ--Δ, p(Tyr, Glu)-pAla–pLys.

sessing the necessary immunogenic features (in this case tyrosine) is capable of eliciting antibodies in rabbits and, therefore, that the presence of charged groups in the molecule is not always essential for immunogenicity.

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