3. Hydrogenolysis with Raney nickel: A sample of the presumed selenocystathionine was dissolved in 3 ml of water and refluxed for 2 h with 30 mg Raney nickel. The Raney nickel was removed by filtration and the filtrate was extracted with an equal volume of 1 % 8-hydroxyquinoline in $CHCl_3$ (w/v) and twice with $CHCl_3$ alone. The aqueous solution was evaporated to a small volume and aliquots were applied to Whatman No. 1 for chromatography in Solvent 1. Two prominent ninhydrin spots of equal intensity appeared on the chromatogram in the positions of alanine and α-aminobutyric acid[6].

These experiments suggest that the compound under investigation is selenocystathionine. Its optical configuration could not be determined because of the small amounts isolated.

Selenocystathionine accounted for about 10% of the radioactivity in the trichloroacetic acid extract of *Stanleya pinnata*. The other organic selenium compounds in the extract are under investigation.

Cystathionine is a key intermediate in the metabolic conversion of methionine to cysteine and also in the synthesis of methionine from cysteine in microorganisms[7]. Whether selenocystathionine has a similar role in the synthesis of seleno-amino acids in *Stanleya pinnata* is not known. It should be pointed out that under our experimental conditions selenocystathionine was not formed in detectable amounts by the selenium accumulators, *Astragalus crotalariae*, *A. bisulcatus*, and *Oonopsis condensata*. These differences among the selenium-accumulators may indicate a special role for selenocystathionine in *Stanleya pinnata*.

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PN 1283

The effect of oligomycin on the phosphorylating respiration of ascites hepatoma cell

After the first experimental use of oligomycin by LARDY, JOHNSON AND MCMURRAY[1], it was found to be useful as a "true inhibitor of oxidative phosphorylation"[2]. The effect of the antibiotic on isolated mitochondria has been studied extensively[1–4]. However, few studies, except those using rat-liver slices[5], have been reported con-

cerning its effect on intact cells and tissues. The present report deals with the effect of oligomycin on Yoshida ascites hepatoma cells AH 49, which show a pattern of respiratory control similar to Ehrlich ascites tumour cells, studied extensively by CHANCE AND HESS[6].

The cells* were washed with isotonic saline, by centrifuging for 1 min at 1000 rev./min, and were then suspended in calcium-free Krebs–Ringer phosphate buffer at pH 7.4. The respiratory rate was measured polarographically using a Clark oxygen electrode (Yellow Springs Instrument Co.) in a closed vessel** which was immersed in a water bath at 38°. 1–10 μl of oligomycin***, dissolved in ethanol, were added directly to the cell suspension (2.5 ml).

As shown in Fig. 1, the addition of oligomycin to the endogenously respiring cells caused inhibition of the respiration (up to 85 %). The addition of glucose at this state induced no change in the respiration, whereas in the absence of the antibiotic, typical "Chance-Hess" responses (*i.e.* glucose-induced, temporary acceleration of respiration followed by intense inhibition) were observed in the cell suspension

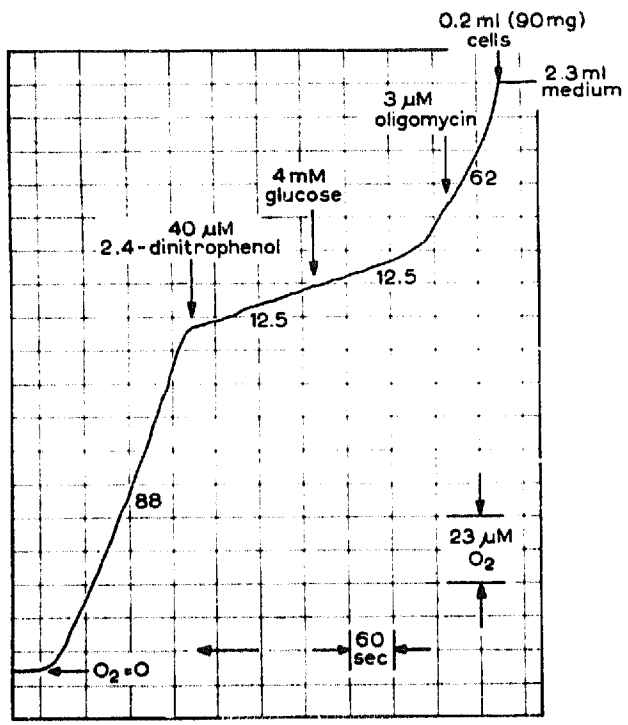

Fig. 1. Oxygen-electrode trace of the respiration of ascites hepatoma cells AH 49 suspended in air-saturated Krebs–Ringer phosphate medium (2.5 ml) at 38°. Oligomycin effected the inhibition of respiration after a lag of about 60 sec. Glucose caused no change in the respiration. Dinitrophenol reversed the inhibition. The reagents were added at the point indicated and the final concentrations were marked. The rates of oxygen utilization marked on the trace are given in mμmoles per min. Q_{O_2} of the dinitrophenol-stimulated respiration was −6.5.

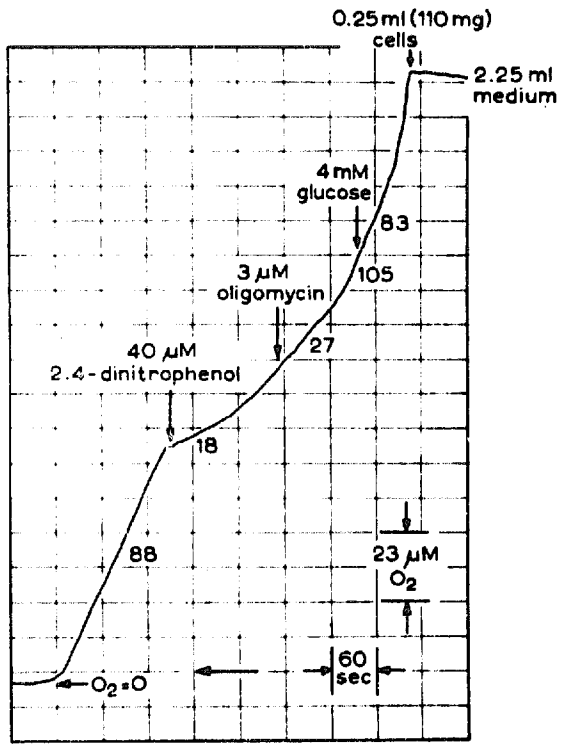

Fig. 2. Oxygen-electrode trace. The conditions are the same as in Fig. 1. Glucose caused acceleration which continued for only 30 sec. This was followed by inhibition. Oligomycin lowered the respiration further. Dinitrophenol reversed the inhibition.

* The cells were kindly supplied by Prof. H. HIRAI and Miss H. TAGA, to whom thanks are due.

** Designed by Dr. R. W. ESTABROOK, University of Pennsylvania.

*** Rutamycin, Eli Lilly & Co. Generous gift of Dr. V. P. MANN. Rutamycin belongs to the oligomycin family.

as shown in Fig. 2. The oligomycin-inhibited respiration was completely relieved by uncouplers such as dicoumarol and 2,4-dinitrophenol (Figs. 1 and 2).

These findings can be explained by reported observations on the effect of oligomycin in mitochondrial suspension[2] and by the detailed studies of CHANCE AND HESS[6] on the metabolic control mechanism in ascites tumour cells. The inhibition of the endogenous respiration of the cells by oligomycin can be attributed to the inhibitory effect of the antibiotic on the oxidative phosphorylation of mitochondria. The inhibited state corresponds to the omission of phosphate, or of adenosine diphosphate, from the mitochondrial suspension. The respiratory rate in the presence of oligomycin is roughly equal to (or lower than) the respiratory rate of the cells in the state of intense respiratory inhibition induced by glucose. This state was characterized as exhaustion of adenosine diphosphate due to the compartmentalization of adenosine triphosphate[6]. The addition of glucose to the oligomycin-inhibited cells did not cause any acceleration of the respiratory rate, as adenosine diphosphate produced by phosphorylation of glucose[6] could not be utilized by the oligomycin-inhibited mitochondria inside the cells. The inhibition of the respiration was relieved by uncouplers, as in the case of mitochondrial suspension. These observations imply that the effect of oligomycin on cell suspensions is almost identical to that on mitochondrial suspensions. The respiratory control ratio of the intact cells as calculated from (rate in presence of uncouplers per rate in presence of oligomycin) was found to vary between 5 and 7.

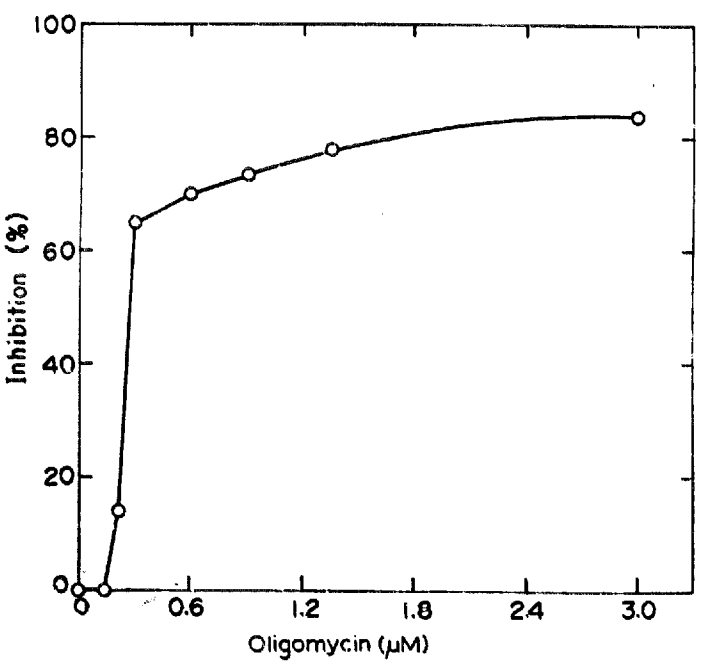

Fig. 3. The titration of ascites hepatoma cells with oligomycin. The cells (110 mg, wet wt.) were suspended in 2.5 ml of medium at 38°; endogenous substrate. With a low concentration of oligomycin (lower than 0.2 μM), the inhibition started after a long lag period (5 min or longer), so that the inhibition of the respiration could not be determined by the oxygen-electrode measurements. The end-point (0.3 μM) corresponds to $7.5 \cdot 10^{-10}$ moles of oligomycin.

The titration curve of the ascites hepatoma cells by oligomycin is shown in Fig. 3. The concentration of oligomycin was calculated assuming the molecular weight to be 333 according to LARDY *et al.*[1]*. A concentration of oligomycin as low as 0.25 μg/ml

* This value was too low according to HUIJING AND SLATER[2].

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