mine A manque d'antigénécité, elle possède donc une adjuvanticité extrinsèque en présence de la globue line γ dont elle intensifie la réponse immunitaire.

Summary. In this work, we have studied the role of different active forms of vitamin A considered as adjuvant of the immune mechanism in the rat. We also pointed out the adjuvant effect of retinol and retinal in comparison with the other active components of that vitamin with the bovine γ-globulin which is not an immunogenic antigen in itself in the control rat. Despite the fact that vitamin A has no antigenecity, it still possess an extrinsic adjuvanticity in presence of bovine γ-globulin. The degree of the immune response is increased.

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Immunochemical Characterization of the Multimolecular Forms of Squid Ink Gland Tyrosinase

The primary function of the ink gland of squid (*Loligo pealii*) is the continuous production of the enzyme tyrosinase (E.C. 1.10.3.1) which mediates the conversion of tyrosine and L-Dopa (3,4-dihydroxyphenylalanine) to melanin. SZABO[1] reported that the squid ink gland contains folds of connective tissue which are lined with simple columnar epithelial cells, and these cells secrete melanin which is the major component of the squid ink. Numerous small vacuoles which may be filled with melanin (ink) granules are found at the apical regions of these cells[1]. The melanin granules are secreted into the lumen of the ink gland and then transferred to the ink sac until the ink is expelled from the organism as a defense mechanism. The present communication is concerned with the immunochemical characterization of the molecular forms of squid ink gland tyrosinase.

Methods and materials. Ink glands employed in this study were excised from squid collected off the coast of Falmouth, Mass. Tyrosinase extracts were prepared by homogenization of washed ink glands in a 0.25 *M* sucrose solution. The homogenate was then centrifuged at $600 \times g$ for 10 min to remove cellular debris. This was followed by further sedimentation at $105{,}000 \times g$ for 60 min and the resulting supernatant was used in all investigations cited. Hardy-Passey mouse melanoma and human melanoma tyrosinase were extracted by the methods of BURNETT[2,3].

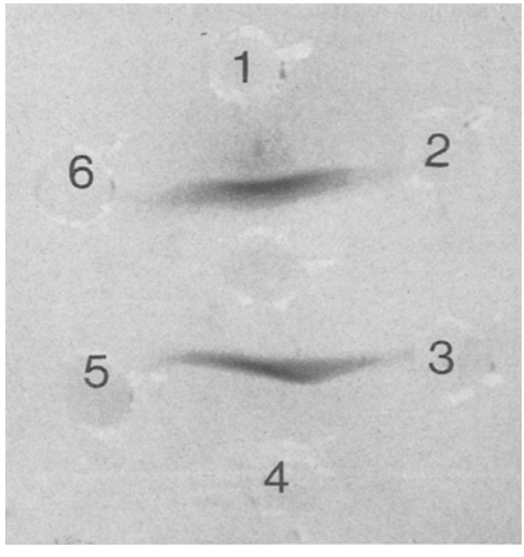

Fig. 1. Immunodiffusion plate containing 5 µl squid tyrosinase (peripheral wells 1 and 4), 5 µl human melanoma tyrosinase (wells 2 and 5), and 5 µl Hardy-Passey mouse melanoma tyrosinase (wells 3 and 6). The central well is filled with 5 µl anti-squid tyrosinase serum.

Rabbit antiserum to squid tyrosinase was obtained by the method reported by CAMPBELL[4]. Immunodiffusion plates were prepared by a modification of the technique of CLAUSEN[5]. Immunelectrophoresis was performed on Agarose Universal Electrophoretic Films (Anayltical Chemists, Inc., Palo Alto, Calif.) which were manufactured according to the formulations of ELEVITCH[6]. After the formation of precipitin lines in the immunodiffusion and immunoelectrophoresis plates, the presence of specific tyrosinase-antibody complexes was detected by the method of BURNETT[7].

Results. It can be seen in Figure 1 that only squid tyrosinase combined with the antiserum to squid tyrosinase. Two precipitin lines were detectable by the L-Dopa detection technique, however, the sensitivity of this technique was not of the same standard as immunoelectrophoresis described below. No L-Dopa-positive lines were discerned with the Hardy-Passey mouse melanoma and the human melanoma tyrosinase contained in wells 3 and 6 and wells 2 and 5 respectively.

Upon immunoelectrophoresis, 3 Dopa-positive precipitin lines were resolved for the squid tyrosinase. Two of these lines, designated T1 and T2, possessed similar electrophoretic mobilities but differed immunochemically (Figure 2). The slowest migrating precipitin line, which was termed T3, displayed antigenic similarity to T2. The immunochemical relationship of T1 and T2 and T3 could not be elucidated because of the distance between the precipited lines in the immunoelectrophoresis plates.

The fact that the Dopa-positive precipitin lines represented the presence of tyrosinase and not a non enzymatic darkening was acertained by heat inactivation studies. Squid tyrosinase is completely inactivative by

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heating at 60 °C for 10 min[8]. In all experiments parallel studies were performed during which the immunodiffusion and immunoelectrophoresis plates were heated for 10 min at 60 °C after the formation of precipitin lines but before the administration of the L-Dopa detection assay. This treatment completely inhibited the formation of Dopa-positive lines.

Discussion. It can be seen from the immunodiffusion studies that the antiserum to the squid tyrosinase possessed a complete lack of immunochemical reactivity with the enzyme found in mouse Hardy-Passey melanoma and Human melanoma tyrosinase. This is not surprizing in view of the phylogenetic distance between the squid tyrosinase and that of the mouse and human melanomas.

It was also obvious that the tyrosinase combining with its antibody retained its enzymatic activity. Thus the enzymatic active site of tyrosinase is not affected by the immunochemical reaction. This same finding was pointed out by OHTAKI[9] for the tyrosinase-antiserum complex of Hardy-Passey mouse melanoma.

Although 2 forms of squid tyrosinase could be discerned by means of agar gel diffusion, 3 forms were observed by the use of immunoelectrophoresis. T 2 and T 3 were seen to be immunochemically related which suggest that the two forms of the enzyme contain similar antigenic groups. The immunochemical relationship of T1 with T 2 and T 3 could not be elucidated by the present techniques utilized. An investigation into this relationship is presently under way.

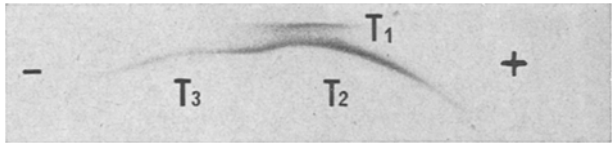

Fig. 2. Immunoelectrophoresis plate of squid tyrosinase isozymes.

Résumé. Sur le plan immunochimique, la tyrosinase de la poche à encre du Calmar *Loligo pealii* n'est pas apparentée à la tyrosinase isolée des mêlanomes humains ou des mélanomes de la souris. Trois formes moléculaires de cet enzyme ont été séparées par immunoéléctrophorèse.

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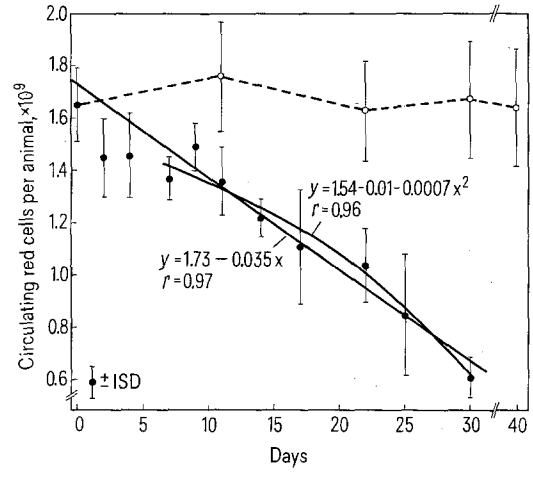
Red Cell Survival: Finite Life Span versus Random Destruction

Erythrocyte life span measurements, using cohort labelling techniques, indicate two ways of cell disappearance: A fraction of cells is apparently randomly removed from circulation irrespective of age, while the rest reaches a constant life span limited by senescence. Random destruction does not affect more than 10–20% of cells in man but is considerably greater in certain mammals, e.g. in pigs, rabbits, rats and mice[1,2]. The commonly used isotope techniques however to not discriminate between loss of cells and cell material. The search for a method to separate these processes was stimulated by the finding that rat erythrocytes ageing in vivo undergo both a reduction in cell volume and haemoglobin content[3]. For this purpose erythropoiesis was suppressed in 150 18-week-old ICR male mice by daily i.p. injections of actinomycin D[4]. Circulating reticulocytes dropped from 4.3% in normal animals to 2.6 and 0.3% after 2 and 4 days of treatment respectively and were completely absent thereafter. At short intervals the erythrocyte volume (EV) and the mean corpuscular volume (MCV) were determined in groups of 5–7 animals, so as to calculate the number of red cells remaining in circulation (Figure). Cell disappearance in presence of unequal survival expectancy would be characterized by a changing slope with an initial rapid phase. In contrast, cell loss proved to be linearely correlated with time; quadratic regression analysis in fact revealed slight curvature of the regression line in the opposite direction to that expected on the basis of different life expectancy populations. By extrapolation 46 days of mean mouse



Rate of removal of red cells from the circulation of mice during daily i.p. injections of actinomycin D, 0.06 µg/g. The number of circulating erythrocytes was obtained by dividing the erythrocyte volume (EV, ml) by the mean corpuscular volume (µm³). The EV was measured by means of weighed amounts of ^{59}Fe-labelled red cells injected i.v. (0.002 ml approximately, diluted with human albumin, 1:30). Cell donor mice received 50 µC ^{59}Fe-citrate 3–10 days prior to the studies. Reticulocytes dissappeared linearly with time over 4.5 days, creating a quadratic term in the decrease of circulating cells; for this reason the first 3 points had to be excluded from mathematical analysis. The dotted line stands for the untreated control animals.

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