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.

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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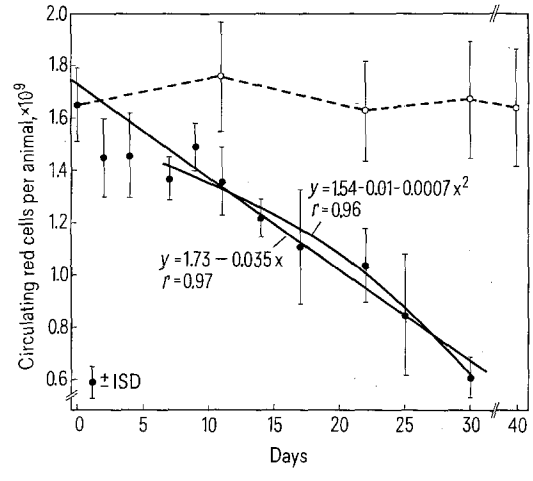
Fig. 2. Immunoelectrophoresis plate of squid tyrosinase isozymes.

[8] J. B. BURNETT, unpublished results.
[9] N. OHTAKI and K. MIYAZAKI, J. Invest. Dermat. *58*, 238 (1972).

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.

[1] R. L. EVANS, Nature, Lond. *173*, 129 (1954).
[2] N. I. BERLIN, T. A. WALDMANN and S. M. WEISSMANN, Physiol. Rev. *39*, 577 (1959).
[3] A. M. GANZONI, R. OAKES and R. S. HILLMAN, J. clin. Invest., *50*, 1373 (1971).
[4] K. R. REISSMANN and K. ITO, Blood *28*, 201 (1966).

erythrocyte life span were obtained, in good agreement with published data[5,6].

It is important to note that the assay animals were in a haematologically stable state between week 10 and 18 as evident from constant EV's: 1.02 ± 0.11 (SD), 0.98 ± 0.10, 1.00 ± 0.15, 0.96 ± 0.13, and 0.98 ± 0.09 ml at the age of 10, 12, 14, 16 and 18 weeks respectively.

During 30 days of continued marrow suppression, the mean erythrocyte age bacame more uniform and shifted from 23 to 38 days. At the same time the MCV dropped from 58.8 μm^3 by 15%, and the mean corpuscular haemoglobin from 19.5 μg by 12%, while the mean corpuscular haemoglobin concentration increased by 5% from an initial value of 33.4 g/100 ml. Loss of cell material permits us to reconcile our finding of a constant red cell life span with the seemingly random destruction suggested by single age population labeling techniques. Red cell fragmentation represents a well-known phenomenon in states of accelerated haemoysis[7,8]. It might also be a mechanism operating under physiological circumstances, possibly contributing to final removal of aged and rigid cells from circulation.

Zusammenfassung. In Actinomycin-behandelten Mäusen nahm die Zahl der zirkulierenden Erythrozyten linear ab. Zellulärer Hämoglobinverlust erklärt die scheinbar altersunabhängige Komponente der Erythrozytendestruktion welche Isotopenstudien vermuten lassen.

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5 September 1972.

[5] E. L. BURWELL, B. A. BRICKLEY and C. A. FINCH, Am. J. Physiol. *172*, 718 (1953).
[6] L. M. VAN PUTTEN, Blood *13*, 789 (1958).
[7] R. I. WEED, Semin. Hemat. *7*, 249 (1970).
[8] P. L. LA CELLE, Semin. Hemat. *7*, 355 (1970).
[9] We thank Dr. H. VOELLMY for the mathematical analysis. This investigation was supported by the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung, Grant No. 3.393.70.

Pyrrolidone Carboxylic Acid (Pyroglutamic Acid) in Normal Plasma

Pyrrolidone carboxylic acid (PCA) is a major soluble nitrogen compound of mammalian epidermis[1,2]. The presence of free PCA in tissues and body fluids, other than urine[3], has only very recently been reported[4,5]. This 'ninhydrin negative' lactam of glutamic acid is not retained on negatively-charged ion exchange resins and thus is not detected by the usual methods of amino acid analysis. PCA is quantitatively hydrolyzed to glutamic acid by heating in 1 *M* HCl. STEIN and MOORE[6] reported an 8-fold increase in glutamic acid concentration following acid hydrolysis of protein-free plasma from normal males, but they attributed this to deamination of glutamine. Using gas chromatography JELLUM et al.[4] reported finding 45 µmole PCA/100 ml serum from a patient with pyroglutamic aciduria, but they stated that this compound is not present in normal sera. We here report the finding of PCA in normal human and guinea-pig plasma.

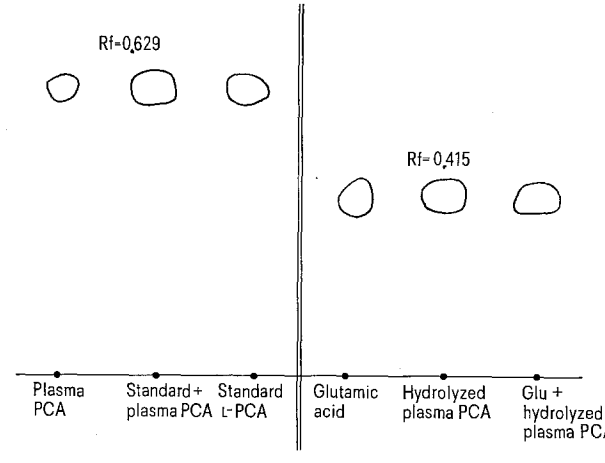
Fifty ml portions of pooled guinea-pig or normal male human plasma were deproteinized. Ultrafiltration was found to be most convenient method of deproteinization since no precipitant need be added to the plasma. Perchloric acid precipitation was a satisfactory method of deproteinization since the major portion of the perchlorate can be removed before subsequent procedures by treatment with KOH. Trichloroacetic acid precipitation was unsatisfactory because of the difficulty in extracting the precipitant without serious loss of PCA. The protein-free plasma solutions were concentrated 5-fold by freeze-drying. Aliquots of these concentrated plasma extracts were analyzed for total free amino acids[7] (Table). The remainder of these solutions were applied to 2.5 × 32 cm

[1] G. PASCHER, Arch. klin. exp. Derm. *203*, 234 (1956).
[2] J. TABACHNICK and J. LABADIE, J. Invest. Derm. *54*, 24 (1970).
[3] R. THAM, L. NYSTRÖM and B. HOLMSTEDT, Biochem. Pharmac. *17*, 1735 (1968).
[4] E. JELLUM, T. KLUGE, H. C. BÖRRESEN, O. STOKKE and L. ELDJARN, Scand. J. clin. Lab. Invest. *26*, 327 (1970).
[5] M. G. WOLFERSBERGER, B. S. FINKELSTEIN and J. TABACHNICK, Fedn. Proc. *31*, 642 (1972).
[6] W. H. STEIN and S. MOORE, J. biol. Chem. *211*, 915 (1954).
[7] E. C. COCKING and E. W. YEMM, Biochem. J. *58*, 12 (1954).

Total free pyrrolidone carboxylic acid (PCA) and amino acids of human and guinea-pig plasma

	Amino acids (µmole/100 ml plasma)	PCA (µmole/100 ml plasma)
Human	282.2[a] ± 25.8	21.6[a] ± 4.4
Guinea-pig	223.6[b] ± 41.8	32.6[b] ± 11.1

[a] Mean ± S.E. for duplicate determinations on samples from 1 normal 33-year-old male and 1 normal 44-year-old male. [b] Mean ± S.E. for 6 samples of pooled guinea-pig plasma.



Chromatography of PCA and glutamic acid. An ascending chromatogram was developed for 16 h with 1-butanol:water:acetic acid (40:25:15). After air drying, the portion containing PCA was sprayed with chlorine-starch-iodine and the other portion with ninhydrin.