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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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

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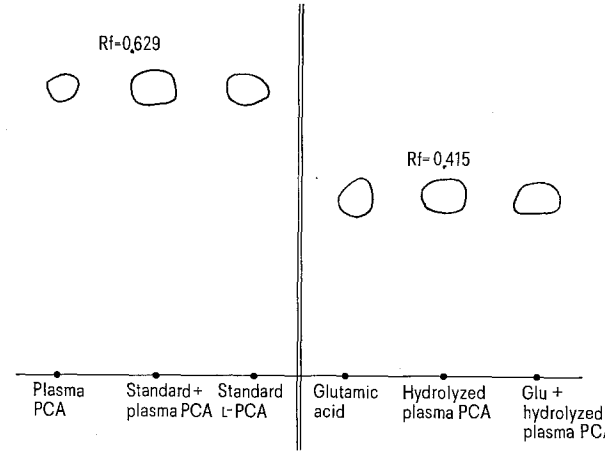
Total free pyrrolidone carboxylic acid (PCA) and amino acids of human and guinea-pig plasma

	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.

Dowex 50 (H^+) columns, the PCA was eluted with 1 volume (160 ml) of deionized water, and concentrated 10-fold by freeze-drying. The PCA content (Table) of these concentrates was determined after hydrolysis (1 h at 100 °C in 1 *M* HCl) by ninhydrin assay of the glutamic acid formed[2]. Care must be taken to avoid hemolysis in the preparation of plasma since we find guinea-pig red blood cells contain more than half the total free amino acids and PCA (5.8 and 0.8 µmole/g wet wt, respectively) of whole blood.

After water elution from Dowex 50 the plasma PCA concentrates were further purified by chromatography on 1.5 × 23 cm Dowex 1 (formate) columns. The columns were sequentially eluted with 200 ml portions of water (I), 1 *N* formic acid (II) and 4 *N* formic acid (III). These fractions were concentrated by freeze-drying which also removed formic acid. Following paper chromatography of aliquots of these fractions on Whatman No. 1, the chlorine-starch-iodide peptide bond reagent of RYDON and SMITH[8] revealed a single spot in fraction II with the same Rf as pure L-PCA (Mann Research Laboratories, Orangeburg, New York). A mixture of the purified compound in fraction II and pure L-PCA migrated as a single spot (Figure). The identification of this purified compound from normal plasma as PCA was confirmed by its complete conversion to glutamic acid by 1 *M* HCl hydrolysis at 100 °C for 1 h (Figure).

These experiments establish PCA as a component of normal plasma. Its concentration is similar to that of free amino acids of intermediate abundance such as lysine, proline and valine[2].

Zusammenfassung. Wir isolierten und identifizierten Pyrrolidoncarbonsäure als einen Bestandteil des normalen Plasmas. Im menschlichen und Meerschweinchenplasma war die Konzentration 0,22 bezw. 0,33 µmole/ml.

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Intracellular Response by *Tetrahymena pyriformis* to Fluids from Unimmunized Animals

Among the effects on the ciliate, *Tetrahymena pyriformis* (TP), of specific antiserum whose complement (C) activity has been removed by heating (56 °C., 30 min) are agglutination, immobilization, exudate formation on the cilia and around the animal, and formation of chains, multinucleated giant cells, or monsters[1–4]. When C was present, killing and lysis could also be observed. C-active normal sera behaved essentially like C-active specific antisera. However, C-inactive (heated) normal sera failed to cause any of these reactions, except for a transitory immobilization[4]. This paper describes a new phenomenon elicited in TP by C-inactive fluids. Normal rabbit and human sera and mouse ascites fluids induce a visually detectable intracellular response within TP. Ciliates so exposed and placed on a flat slide to which a cover glass is then affixed assume a 'bipolar' appearance about 24 h later: Small 'bodies' (granules, vesicles or vacuoles?) accumulate anteriorly, large ones posteriorly.

Log phase TP (3 day culture) of the A.M. Elliott strain WH_6 (Wh_1) syngen 1, mating type I, cultivated in 1% proteose-peptone and tryptone broth, pH 7.2, were sedimented and washed twice with 0.5% NaCl solution by gentle centrifugation and suspended in the 0.5% saline. Aseptic precautions were not observed following cultivation.

Ascites fluid was induced in female CFW mice after 5 i.p. injections of a 1 : 1 mixture of 0.85% NaCl solution and Freund's Complete Adjuvant. All ascites fluids and human and rabbit sera were heated for 30 min at 56 °C (complement inactivation) before storing at -60 °C. Most of the work was done with ascites fluids, since these readily induced the bipolar state.

Equal parts of a fluid and TP were mixed in test tubes. One drop of the mixture was placed on a cover glass which was then affixed to a flat microscope slide with petrolatum. When not being observed the slides were kept at 25 °C. Each slide also contained a mixture of TP and 0.5% saline as a control.

Immobilization to various degrees and exudate formation nearly always were evident, agglutination was fairly common, giant or monster cell formation occasional. The bipolar phenomenon (Figure 1) appeared as early as 7 h, was widespread 16 to 48 h after mixing fluid and TP and persisted for several more days. Bipolarization could be observed at 40 × magnification and was generally studied at 450 ×. Variations possibly representing different stages of bipolarization (absence of 'clear' area between large and small bodies, occurrence only of many large bodies either dispersed or at one end, bipolarization of small bodies) were also seen at times. Saline controls (Figure 2) never displayed frank bipolarization, even though, very rarely, bipolarization of small bodies occurred. In the bipolar state cyclosis of the large and small bodies was not evident, although these bodies did not appear to be immobile. They exhibited Brownian movement. Bipolarization has been observed not only on flat slides but also in hanging drop preparations, in stoppered small tubes having very little air space and in clay-sealed capillary tubes, also having very little air space. Ciliates from TP-fluid mixtures kept 24 to 48 h in test tubes with a large air space (5 to 10 times the volume of the mixture) never were in a bipolar state when observed in a fresh slide preparation. Attempts to culture bipolar animals or monsters failed to yield either form. In single cell culture (well slides stor-

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