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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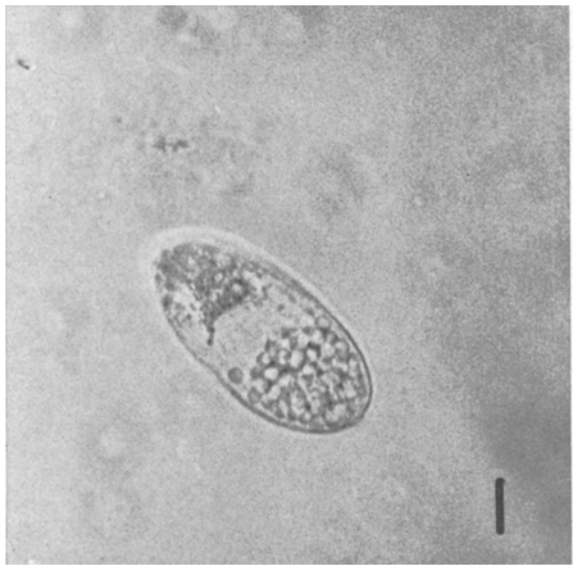

Fig. 1. Living *T. pyriformis* showing bipolarization induced by mouse ascites fluid, ciliates and fluid mixed 72 h earlier. Small bodies accumulate anteriorly, large ones, posteriorly. Phase microscopy. ×400.

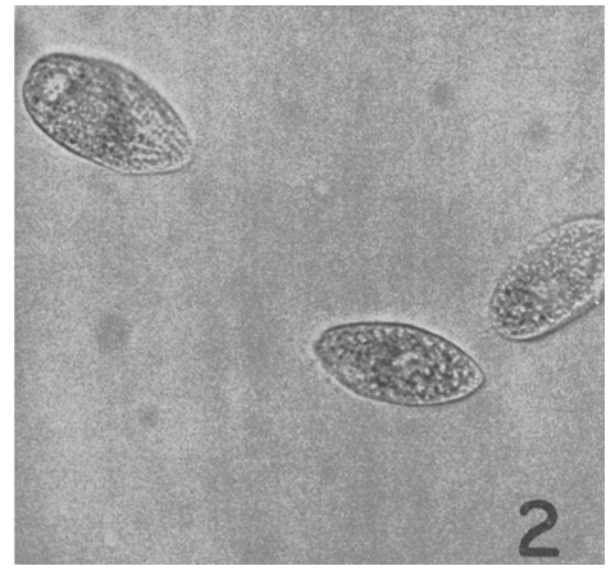

Fig. 2. *T. pyriformis* after 48 h in 0.15% NaCl solution. Fixed with 0.1% glutaraldehyde. Polaroid photograph. ×450.

ed in Petri dishes) reversion to normal-appearing TP occurred. Bipolar animals transferred into broth medium on another cover glass, which was promptly sealed onto a slide, became normal in appearance within 15 min. The slide, from which these animals were taken, had been immediately resealed to its cover glass. Most of the ciliates on this slide again were normal in appearance within 15 min. Loss of the bipolar appearance began with the mixing of the large and small bodies along the periphery of the ciliate about 6 min after transfer. Bipolarization did not reappear on any slide on which reversion had occurred, even after 24 h.

Nothing has been learned through phase microscopy about the nature of the small and large bodies. The rapidity of bipolarization on exposure to air has presented the greatest obstacle to further study of the phenomenon. Introduction of fixatives under the cover glass has generally given unsatisfactory results, the ciliates usually rounding up and failing to show bipolarization. The control TP in Figure 2 were photographed within min after introducing glutaraldehyde under the cover glass. Staining with Janus Green B has not been successful when the dye has been introduced under the cover glass or was present in the culture medium.

Additional control experiments in summary were: Slide preparations of both 3-day-old TP and washed 3-day-old TP mixed with the broth always showed normal-appearing TP even after 70 h. Bovine serum albumin diluted from 30% to about 7.5% reduced mobility and killed most of the TP by 24 h and all by 45 h, effects presumably due to traces of globulins in the albumin. At 0.75% concentration most of the TP appeared normal. A few tended toward bipolarization at 24 h and 43 h but definite bipolarization was never seen. Osmotic effects of NaCl solutions on these fresh water animals were never apparent in concentrations of 0.5% and 0.15%, but, within 30 min at 0.9% the TP were strikingly immobilized and the contractile vacuole was often nonfunctional, while at 1.5%, immobilization, death and disintegration were marked. 4 h later granulation, vacuolization and death were widespread at 0.9% and death and disintegration complete at 1.5%.

The conditions under which bipolarization occurred (flat slide-cover glass preparations, tubes with little air space, hanging-drop preparations) and under wich reversion has been observed (admittance of air, attempst to subculture) tentatively suggest reduced O_2 tension and/or increased CO_2 tension as requirements for bipolarization.

The external effects of specific anti-TP serum (immobilization, agglutination, etc.) were paralleled by the fluids of nonimmunized animals in my experiments. Thus the latter fluids behave like the specific antibody. It is not yet clear if this antibody-like substance is also responsible for the internal event, bipolarization. Apart from this point, one might infer that this antibody-like substance must enter the animal by either ingestion, pinocytosis, or by both processes, if the intracellular events are to occur. The provocative question then remains: Does the antibody-like substance resist digestion (and loss of activity) or does it trigger events, prior to its digestion, which give rise to bipolarization and giant cell formation?[5]

Résumé. Dans le *Tetrahymena pyriformis*, les sérums des lapins et humains et les ascites fluides des souris ont une apparence bipolaire. Les petit «corps» s'accumulent antérieurement, les grands «corps» postérieurement. On peut présumer que la réduction de la tension du O_2 et/ou l'augmentation de la tension du CO_2 sont requis, puisque l'état bipolaire ne s'est produit que dans des conditions limitées d'exposition à l'air. La bipolarisation est reversible. Les animaux bipolaires qui ont été exposés brièvement à l'air ressemblent aux animaux normaux.

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