thalamus, as well as the absence of response in those implanted in the anterior amygdaloid area and posterior hypothalamus suggests that the drug has a specific and short-lasting effect by blocking the cholinergic mechanisms related to the release of the ovulatory quota of gonadotrophins. The decrease of the ovarian weight as well as the inhibition of compensatory hypertrophy after unilateral castration by hypothalamic implants of atropine (unpublished observations) would suggest the blockade of the folliculotrophic hormones as a whole (FSH-LH).

Résumé. Des implantations d'atropine dans l'hypothalamus antérieur et latéral de la rate provoquent un diestrus prolongé et une diminution du poids de l'ovaire sans réponse utérine au déciduome traumatique. On conclut que l'atropine agit sur des voies colinérgiques en relation avec la sécrétion des gonadotrophines.

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Do the Products of Messenger RNA Hydrolysis Cause 'Cloudy Swelling'?

The accumulation of intra-cellular water by cells subjected to toxic stresses was first noted by Virchow[1] who termed the process 'cloudy swelling' and believed it to be one of the primary causes of cell death. Its pathogenesis is still not fully understood[2]. However, it is clear that it involves a failure of the cellular osmotic control mechanisms[3]. During recent experiments on RNA stability in *Tetrahymena* we noticed that these ciliated protozoons were subject to slight hydropic changes during periods of increased RNA hydrolysis caused by synchronizing heat-shifts. These cells are quite viable at synchronizing temperatures provided the duration of exposure is limited[4]. If Actinomycin D (ActD) is added to the medium during these temperature shifts a more perceptible increase in cell size is consistently noted. We have examined this swelling phenomenon in some detail. The results suggest that it is a result of *net* hydrolysis of unstable RNA. If the hydrolysis is allowed to proceed at a rapid rate and to a sufficient degree the cells will die of severe hydropic degeneration. The cytological changes seen in such cells are virtually identical to those found in classical 'cloudy swelling'. Since the effects of ActD and temperature are synergistic and both are known to induce net RNA degradation it appears that the edema may be initiated by the RNA hydrolysis products.

Material and method. To follow the rate of degradation of unstable RNA, the cells were synchronized and resuspended in inorganic medium. This facilitates rapid RNA labeling and has no effect on either cell viability or cell division[5]. At the beginning of the experiment 100 µc of H^3 uridine was added to the medium and the cells were labeled for 20 min. ActD (50 µg/ml) was then added and the culture divided into 3 parts. One portion was incubated at 29 °C (the optimum growth temperature), one at 34 °C, and one at 37 °C[6]. The rate of decay of prelabeled RNA was followed using the direct filter paper disc procedure[7]. After 60 min a small sample was removed from each culture, fixed with saturated $HgCl_2$, and photographed. Control samples lacking ActD were also incubated at each of the 3 temperatures. Previous experiments have shown that the uptake of exogenous uridine ceases at 34 °C[5]. This inhibition of uridine uptake is presumably due to the expansion of intra-cellular nucleotide pools[8].

Results and discussion. The effect of adding ActD to the medium after pulse labeling with uridine is seen in Figure 1. At 29 °C the uptake of label slows and decay begins after a short delay. At 34 and 37 °C decay begins almost immediately and proceeds at rates roughly proportional to the degree of temperature elevation. The effects on cellular morphology are seen in Figure 2. These

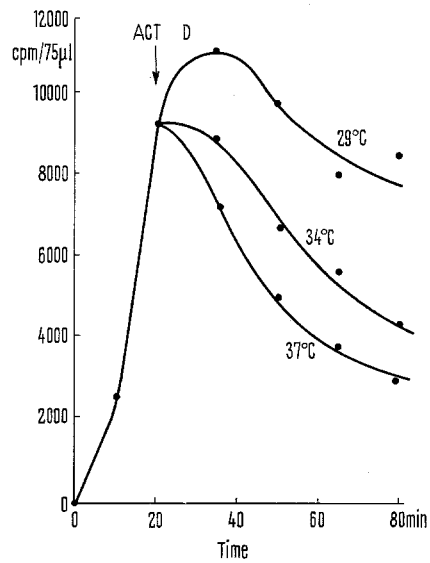

Fig. 1. Effect of temperature variation of the rate of decay of prelabeled RNA. Synchronized *Tetrahymena pyriformis* GL were labeled with H^3 uridine for 20 min as described in the text. Actinomycin D was then added (50 µg/ml) and the culture divided into 3 parts and incubated at the temperatures indicated. The rate of decay is roughly proportional to the temperature for values above the optimum growth temperature (29 °C).

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cells are normally ellipsoids with a marked indentation towards the anterior end where the oral apparatus is located. After exposure to ActD at 29 °C for 60 min there is a barely perceptible cellular swelling most marked anteriorly (Figure 2a). When the cells are exposed to 34 °C in the presence of the antibiotic a more marked fullness is seen (Figure 2b), and observation of the living cells shows an increased water vacuole activity. Viability (as determined by active swimming) is maintained for at least 60 min at 34 °C under these circumstances, followed by cell death. If the temperature elevation is raised to 37 °C water vacuole formation becomes striking. In addition, non-vacuolar edema becomes apparent and steadily progresses to cell death with extreme water inhibition (Figure 2d). Observable cell swelling is also seen in cells exposed to 37 °C without the antibiotic but it lags significantly behind that found when both RNA depolymerizing stimuli are present (Figure 2c). The large intra-cellular vacuoles and granular cytoplasmic precipitates which occur closely resemble VIRCHOW's[1] original description of 'cloudy swelling' made on unstained human necropsy material.

The exact mechanism of this swelling has not as yet been elucidated. Several possibilities seem to be excluded on a circumstantial basis. Since it is not reproduced by cycloheximide, turnover of a labile protein needed for membrane integrity is unlikely. Similarly, the possibility that a specific non-template RNA fraction is involved seems unlikely since ancillary studies have failed to note any variation in the rate of decay of unstable RNA fractions[9] and there is no evidence as yet for a structural role of RNA in membrane stability. Direct inhibition of the water excretory mechanism by both heat and ActD is possible but the water vacuoles are initially highly active suggesting that these mechanisms are intact. The immense vacuoles found in dead and dying cells therefore probably represent terminal failure. Elsewhere we have presented evidence that the primary effect of heat and/or ActD in these forms is not at the energy level[9,10].

Recently there have been several other reports in the literature which in aggregate imply that a significant link between the metabolism of unstable RNA and the maintenance of water and electrolyte balance may exist.

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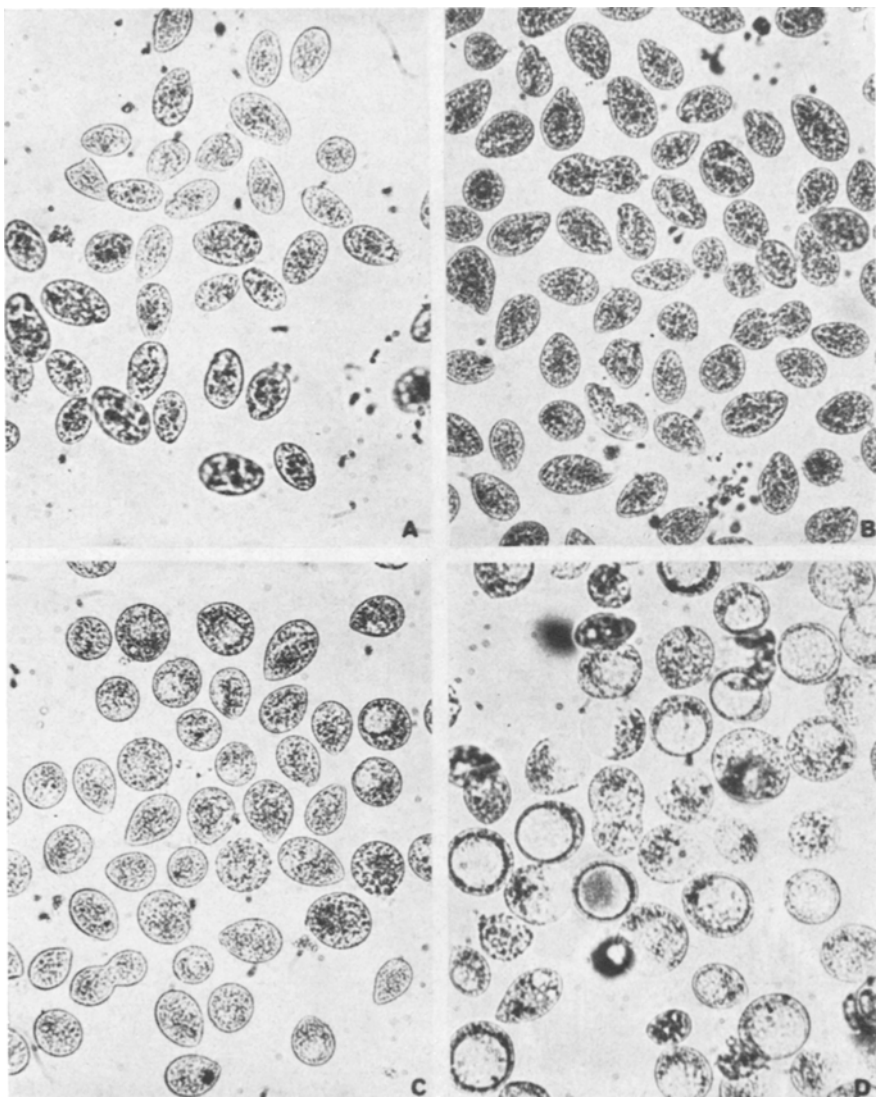

Fig. 2. Cellular morphology during template hydrolysis. These photographs (except c) were taken of cells treated with Actinomycin D for 60 min as indicated in Figure 1. Plate (a) shows cells treated for 60 min at 29 °C, plate (b) at 34 °C, and plates (c) and (d) at 37 °C. No Actinomycin D was added in the case of (c). A progressive increase in cell swelling and vacuole formation is seen as the temperature rises above 29 °C. This swelling is considerably less when the antibiotic is not present (Figure 2c). Both factors (Actinomycin D and temperatures above the growth optimum) serve to initiate net template RNA hydrolysis. All magnifications × 240.

Both enucleation and UV-irradiation have been shown to cause increased water vacuole activity in *Ameba proteus*[11,12]. The osmolar content of such vacuoles is normal, verifying increased net water transport[13]. Either procedure might be expected to cause net hydrolysis of unstable RNA through physical removal or inhibition of RNA polymerase. Exposure of mammalian cells to amphotericin B causes reduced protein and nucleic acid synthesis in a manner that has been related to cation transport[14]. The addition of glucose to the medium will partially protect mammalian cells against the action of ActD[15], perhaps on an osmotic basis. However, it remains to be proven that any of these phenomena are directly related to the osmotic effects of accumulating intra-cellular nucleotides. Hydrolysis of large quantities of nucleic acids almost certainly exposes the cells to significant acid-base changes. *Tetrahymena*, like many cells, contain significant quantities of bound K^+[16]. Release of K^+ or other cations may also play a role in perturbation of the water controlling mechanisms. The elucidation of these effects remains an intriguing problem for future investigations.

Résumé. L'actinomycin D et les températures d'incubation dépassant l'optimum de croissance causent indépendamment une dégradation de l'ARN instable chez *Tetrahymena pyriformis* vivant. L'œdème cellulaire se produit simultanément et il est proportionnel au degré de dépolymérisation. Puisque les effets de l'antibiotique et les changements de température sont additif, ces observations suggèrent que les produits de hydrolyse peuvent aboutir à l'accumulation excessive de l'eau dans la cellule.

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Interactions Between Amphetamine and Reserpine in vitro

The in vivo interactions between amphetamine and reserpine have been investigated by a number of workers[1–5]. Contrary to the expected response, administration of reserpine in animals pretreated with amphetamine elicits a marked and prolonged presor response. This effect appears only once. Repetition of reserpine fails to show any response[3]. The blood pressure increase is blocked and/or prevented by alpha-adrenergic blocking agents; however, it is not modified by bilateral adrenalectomy, descentralization, ganglion blocking agents, antihistaminics and antiserotoninics[4].

These results seem to indicate that the blood pressure response elicited by reserpine is mainly due to a peripheral adrenergic mechanism. In order to get some more information concerning the mechanism involved in this phenomenon, we tried to reproduce it in vitro using the isolated rat vas deferens preparation, which shows a dense sympathetic innervation[6] and a high noradrenaline content[7]. The results obtained are reported in this paper.

Material and methods. Male Wistar rats (180–220 g) have been used throughout all experiments. Animals were killed by a blow on the head and both vasa deferentia were removed and suspended in Krebs solution aireated with a mixture of 95% O_2 and 5% CO_2. Bath capacity: 10 ml. Temperature was kept constant at 31 °C. The contractions were registered on a smoked drum by means of a frontal isotonic lever with an 8-fold magnification. The following substances were used: DL-amphetamine sulphate, which in our experimental conditions shows the same activity as the optical isomers, and reserpine as a commercial preparation (Serpasol® Ciba). Solutions were made in isotonic saline 0.9%. Before adding any drug the preparation was allowed to stabilize for 30 min. The sequence of each experiment was as follows: (1) Amphetamine was added to the bath and the response to the concentration used was registered. (2) After washing the preparation, reserpine was added. The time between both drugs differed from one experiment to another, since relaxation after amphetamine is variable; however, it never exceeded 15 min. Actually, this is not a critical point, since we have observed that even 1 h after addition of amphetamine, reserpine shows the same pattern of activity.

Both drugs were used only once in each preparation and no other substance was employed before the sequence described. The height of the contraction was measured in mm.

Results and discussion. Reserpine, in concentrations up to 3×10^{-4} g/ml, does not elicit any response; however, when added after amphetamine, under our experimental conditions, it produces a sustained contraction of similar character to that determined by amphetamine, i.e. slow, crenated and irregular. The results are shown in the Figure.

Columns on the left side represent the mean height of the contractions obtained with the 3 concentrations of amphetamine used (2.5×10^{-7}, 5×10^{-7} and 1×10^{-6} g/ml, expressed as a base), and the vertical bars are the standard error of the mean. Each column represents the mean of at least 40 experiments.

On the right, the height of the contractions is shown determined by various doses of reserpine following 1 of

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