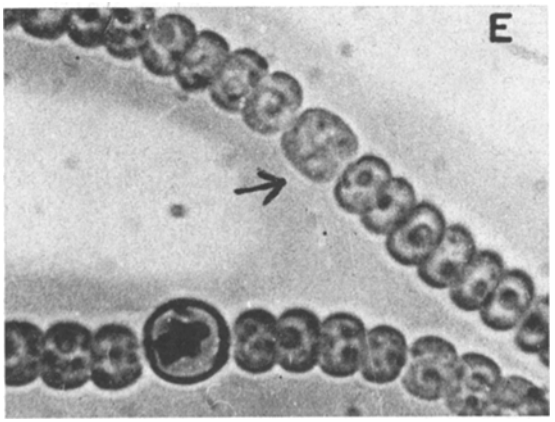
oxygen in light as they lack photosystem II[5,9,10], but need to be protected from the oxygen present in the surrounding medium.

Ammonia, inhibitor of heterocyst development specifically prevents the formation of cellulose around a proheterocyst[6,8]. It has been shown by STEWART et al.[11] that ammonia also inhibits the synthesis of the nitrogenase complex. The presence of a wall around heterocyst seems to be vital for the proper functioning of the enzymes in it. The chemical composition of the wall needs to be investigated.



Anabaena ambigua after treatment with TTC. Note the presence of formazon crystals in the heterocyst. The arrow indicates the proheterocyst devoid of wall and the absence of reduction.

Zusammenfassung. 2, 3, 5-Triphenyl-Tetrazolium-Chlorid (TTC) wird in den Heterocysten der Blaualge *Anabaena ambigua* nur bei einer gut ausgebildeten Zellwand in grösseren Mengen reduziert. In allen übrigen Zellen, auch in den Proheterocysten, ist höchstens eine schwache TTC-Reduktion zu beobachten.

SHANTA R. KALE, MAYA BAHAL and E. R. S. TALPASAYI[12]

Laboratory of Algal Physiology, Department of Botany, Banaras Hindu University, Varanasi-5 (India), 17 December 1969.

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Genetically Inactive DNA and Nuclear Lactate Dehydrogenase (1.1.1.27) Activity in the Erythrocyte of *Amphiuma tridactylum*

Amphibian red blood cells are particularly favourable objects in which to search for possible non-genetic functions of DNA. The dominant sequence of DNA transcription and translation is well known, and the characterization of the resultant haemoglobin in intact cells by microspectrophotometry at the region of the Soret band is simple[1-6]. The complications of DNA-replication and mitosis do not exist beyond the erythroblast stage. Since erythropoiesis in *Amphiuma* is completed only in the circulation, blood samples contain cells at all stages of maturation, from the basiphil erythroblast to the mature erythrocyte. In erythroblasts and early erythrocytes, DNA might reasonably be assumed to be actively transcribing the code for the synthesis of haemoglobin; in mature erythrocytes haemoglobin synthesis stops, and DNA no longer has a clear genetic function. *A. tridactylum* and other species of amphibians have been studied in sufficient detail[6,7] for the identification of the principal maturation stages of erythrocytes to be unequivocal.

The blood cells of amphibians are being examined in this laboratory by biophysical, cytochemical and ultrastructural methods. These include the histochemical demonstration of some 30 enzyme systems. The results will be described in detail elsewhere[1]. In this note, we wish to report the cytochemical identification of a metabolic function, *nuclear* lactate dehydrogenase activity, that was found to be correlated with the loss of genetic activity of DNA in erythrocytes of the giant newt, *Amphiuma tridactylum*.

Blood samples were taken from the auricles of heparinized *A. tridactylum* after anaesthesia in 3% w/v ethyl carbamate (urethane). Blood smears on coverslips were usually stabilized for 50 sec in ice-cold 0.02% v/v formaldehyde vapour, within 1 min of preparation. Air-dried unfixed smears and smears prepared after the blood had been collected in 100 vols. ice-cold balanced salt solution (BSS, modified from WOLF and QUIMBY[8]), containing 5% v/v polyvinylpyrrolidone, mol. wt. 160,000 (PVP), or 0.5% w/v crystallized bovine plasma albumin (BPA), buffered to pH 7.1, were also used. For the demonstration of lactate dehydrogenase activity (I.U.B. No. 1.1.1.27), the coverslips were incubated at 2–4 °C in a medium containing 0.03*M* sodium lactate, 0.03*M* NAD, 0.03*M* either tetranitroblue tetrazolium or nitroblue tetrazolium chlorides, in BSS buffered to pH 7.1 and sometimes containing 5% PVP or 0.5% BPA as adjuvants[9-11]. The coverslips were rapidly warmed to 25 °C after varying periods of incubation in the cold, further incubated at

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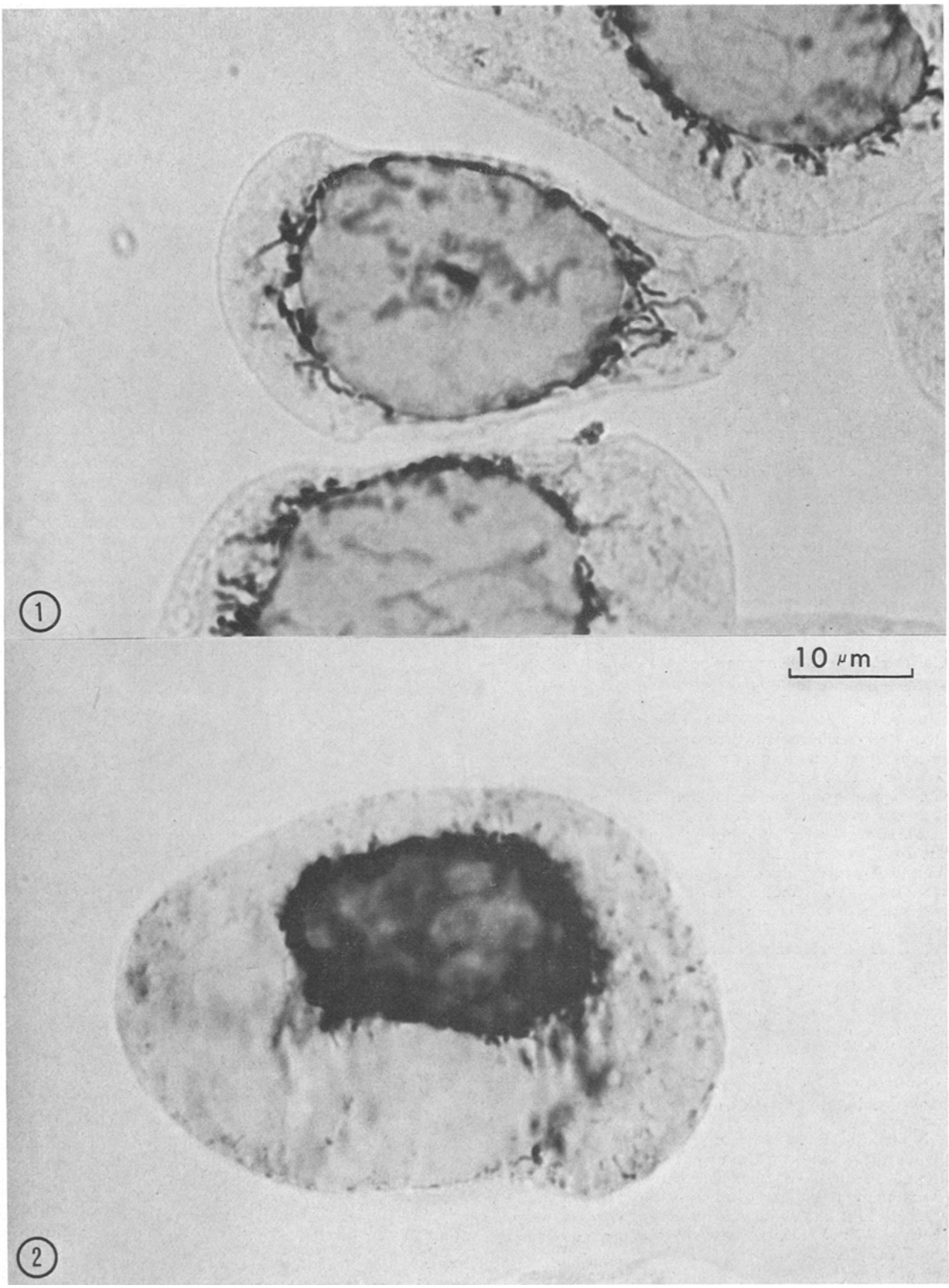

Fig. 1. Early erythrocyte from a blood smear of *Amphiuma tridactylum*, lactate dehydrogenase reaction, incubated on 0.03 *M* Na lactate, 0.03 *M* NAD, 0.03 *M* l.c. tetranitro blue tetrazolium, in WOLF and QUIMBY's[8] balanced salt solution with 5% PVP, photographed at 550 nm with a Zeiss Planapochromat 100/1.3 objective. Note the intense mitochondrial activity and compare with Figure 2.

Fig. 2. Mature erythrocyte of *Amphiuma tridactylum*, from the same smear as the cell illustrated in Figure 1. Note the intense nuclear lactate dehydrogenase activity.

25 °C as necessary, and examined before and after post-fixation in formaldehyde vapour. The preparative and examination procedures were essentially the same as those described in earlier papers[9-11], except for the ionic composition and osmolarity of the BSS. Since the observed differences between cells processed in different ways were trifling, a single description will suffice.

The level of lactate dehydrogenase activity in erythrocytes and their precursors was several orders of magnitude lower than that in leucocytes and thrombocytes in the same preparations, or in other types of cells[9-11]. In erythroblasts and early erythrocytes, demonstrable lactate dehydrogenase activity is largely confined to mitochondria. The mitochondria are numerous, filamentous, and often aligned end-to-end; most are found within a perinuclear region of the cytoplasm, sometimes forming a skein round the nucleus, more often sticking out radially toward the periphery of the cell. The remainder of the cell, including nucleus, endoplasmic reticulum, compound and simple globules, remains completely unstained. As erythrocytes begin to mature, and microspectrophotometrically detectable haemoglobin accumulates in the cytoplasm, the staining pattern gradually changes (Figure 1). The number of mitochondria decreases, and they become shorter; as haemoglobin accumulates, the mitochondria become packed into a narrow juxta-nuclear zone. Now, in addition to the mitochondria, a few cytoplasmic globules also show lactate dehydrogenase activity. Nuclear lactate dehydrogenase activity, both diffuse and associated with condensed chromatin, begins to appear. As maturation reaches completion, the nuclear lactate dehydrogenase activity increases (Figure 2). Often, not more than 2 or 3 active mitochondria remain in the cytoplasm. A few cytoplasmic globules are still positive, but the greater part of the total lactate dehydrogenase activity of the mature erythrocytes become nuclear. There are large variations in the amount and pattern of lactate dehydrogenase activity between individual erythrocytes.

In view of the generally low rate of enzymatic activity in erythrocytes, it was necessary to use suitable controls to exclude the possibility that the observed staining pattern might be due to unspecific effects rather than to the distribution pattern of lactate dehydrogenase activity. The staining was attributable to enzymatic activity, since the cells were completely unstained when lactate was omitted from the incubation medium. The tetrazolium salt captured electrons at the site of enzymatic activity, and not beyond the flavoprotein, since the localization pattern was quite different when the incubation medium contained $NADH_2$ and cytochrome *c* or menadione, instead of lactate and NAD[1]. Finally, lactate dehydrogenase and not some other NAD-linked glycolytic enzyme contributed the electrons, since added pyruvate substantially blocked the staining reaction[1].

We conclude from these experiments that there is an unequivocal negative correlation between the level of nuclear lactate dehydrogenase activity and the degree of clear genetic activity of DNA in *Amphiuma* red blood cells. Another cell in which this correlation seems to be valid is the mesocerebal neurone of the snail, *Helix pomatia*, in which nuclear lactate dehydrogenase activity had also been found[10-11]. Activation of lactate dehydrogenase by DNA had been noted briefly by MAZIA[12]. Several hypotheses arise out of the present findings: 1. lactate dehydrogenase may be involved in the regulation of DNA transcription; 2. in addition to any possible relationship between DNA and lactate dehydrogenase, the nucleus of the mature amphibian erythrocyte may show a form of 'adaptive metabolism', i.e., function in terms of intermediary metabolism as though it were a piece of cytoplasm. These hypotheses are being examined systematically in our laboratory.

Résumé. Dans les erythroblastes et jeunes hématies de la salamandre géante, *Amphiuma tridactylum*, l'activité de la deshydrogénase lactique n'est localisée que dans le chondriome. Pendant l'accumulation de l'hémoglobine et au cours de la croissance des hématies, l'activité devient de plus en plus nucléaire. On constate une correlation négative entre l'activité de la deshydrogénase lactique nucléaire et l'activité genétique de l'ADN.

G. B. DAVID and ALICE M. ACCOLA[13]

Department of Biological Sciences,
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Morphactins, Growth and Auxin Transport

Biological activity of morphactins – a new group of plant regulators[1] – was first reported by SCHNEIDER[2] on a few of them. These chemicals cause striking biological changes over an extraordinarily broad range[3]. Few works have been devoted before now to compare various synthesized morphactins[3]. In the present investigation, parallel action of 4 morphactins on plant cell growth and the in vitro auxin translocation[4] will be analyzed, using epicotyl segments of Lens culinaris.

Seeds were first soaked for 6 h in deionized water, washed and placed in terralite (dark; 22 °C). When used for experimental purposes, after 96 h, the length of the epicotyls was approximately 14 mm. For the *growth experiments*, 8.0 mm segments were placed (20 per 4 ml) in a buffered solution (pH 6.1) with 2% sucrose on a shaking

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