suggests that whatever causes amyloid formation may also cause terminal degeneration. It is possible that amyloid precursors leak from the bloodstream into the neuropil and cause both neurite degeneration and local amyloid formation. Most proposed mechanisms of amyloidosis involve the immune system in the production of precursor proteins, and recently Glenner *et al.* (*12*) have suggested that amyloid may be formed by phagocytes from the lysosomal degradation of antigen-antibody complexes. Even though the involvement of antigen-antibody complexes forms an attractive hypothesis for understanding the pathogenesis of plaque formation, we are not entitled to assume that this applies in scrapie since no specific humoral antibody responses to scrapie agents have been identified. Direct evidence on the chemical composition of the amyloid is therefore essential to understanding its origin. However, the absence of conventional immunological responses in scrapie is founded on work with only two agents, ME7 and Chandler (*13*), and neither produces a high frequency of plaques. Work is therefore needed on conventional immune responses with high plaque-producing agents.

This model offers possibilities for fundamental studies of the pathogenesis of senile plaques. Our findings should also encourage others to search for possible infectious agents in patients with presenile and senile dementia.

HENRYK M. WIŚNIEWSKI
MRC Demyelinating Diseases Unit, Newcastle General Hospital, Newcastle upon Tyne, NE4 6BE, England
MOIRA E. BRUCE, HUGH FRASER
ARC Animal Breeding Research Organisation and *Moredun Research Institute, Edinburgh, Scotland*

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Adenosine 3′,5′-Monophosphate in Reproducing and Differentiated Trypanosomes

Abstract. Trypanosoma lewisi, *a blood protozoan of rats, undergoes differentiation from a rapidly reproducing form to a nonreproducing form in response to the host antibody ablastin. Intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP), which has been implicated in controlling reproduction in cultured mammalian cells, was measured in the two developmental forms of* T. lewisi. *The concentrations were significantly different, and the results support a hypothesis under which ablastin stimulates an increase in intracellular cyclic AMP.*

Trypanosomes are protozoan parasites of the bloodstream and tissues of a large number of vertebrates including man. The pathogenic trypanosomes of the *brucei* group, responsible for nagana in cattle or African sleeping sickness in man, and the nonpathogenic rat parasite *Trypanosoma lewisi* have been widely studied in laboratory rodents. During residence in mammalian hosts and insect vectors, they undergo the equivalent of differentiation. The transformation of *T. lewisi* in the rat progresses from a rapidly reproducing population to a nonreproducing population. However, in pleomorphic strains of *T. brucei* subspecies, differentiation in the mammalian host is incomplete, and a pool of reproducing forms is always retained. These reproducing forms vary antigenically and cause relapsing infections and severe disease in the host. Although the antigenic variants arise in response to humoral antibody (*1*), the factors that induce differentiation of part of the population to nonreproducing forms are not known, but apparently they are not a result of the host immune system (*2*). The transformation in *T. lewisi*, in contrast, is induced by an antibody called ablastin. This antibody inhibits reproduction of the parasites without otherwise damaging them (*3*). The above-mentioned transformation of *T. lewisi* is complete in the presence of ablastin, but is reversed on its removal (*4, 5*). Therefore, the host's immune system first controls this infection by inhibiting parasite reproduction and eventually terminates it by mounting a trypanocidal response. In the *brucei* complex and in *T. lewisi* the reproducing and nonreproducing forms have distinguishable morphological, biochemical, and physiological features (*6*).

Because of its benign nature and the relative simplicity of its blood phase, *T. lewisi* has been studied as a model of controlled trypanosomiasis. Ablastin halts reproduction in *T. lewisi*, and morphological changes in the parasites appear (*3*). The reproducing forms are a heterogeneous population, varying in length and width (Fig. 1A). The nonreproducing (Fig. 1B) or adult forms are uniformly long and slender (*3, 7*). The coefficient of variation (CV) is a statistical measure that can be used to distinguish the populations; a reproducing population has a CV of about 25 percent while an adult population has a CV of about 3 to 5 percent (*7*). In adults, the morphological alterations are accompanied by decreased glucose and increased oxygen consumption with more oxygen consumption per mole of glucose (*7, 8*). Protein synthesis (*9*) and nucleic acid synthesis (*9, 10*) are virtually halted in adult forms. In addi-

Table 1. Cyclic AMP content of reproducing and adult forms of *Trypanosoma lewisi*. After separation from blood cells and platelets, trypanosomes were placed in a mixture of serum and Hanks balanced salt solution with glucose (50 mg/100 ml). The organisms were removed from the serum by centrifugation at 12,000*g* for 5 minutes. The supernatant was discarded, and cells were resuspended in 5 ml of HBSS. The cells were again centrifuged at 12,000*g*, and the supernatant was removed. A portion of this final supernatant washing was assayed for exogenous cyclic AMP. The cells were homogenized by high-speed vortexing in 5 percent trichloroacetic acid (0°C) (4×10^9 cell/ml). The insoluble material was removed by centrifugation at 27,000*g* for 10 minutes, and the supernatant was extracted with three volumes of water-saturated diethyl ether. The aqueous layer was removed and diluted with three volumes of distilled water; the *p*H of the water extract was adjusted to 7.1 to 7.4. The extract was then stored at −20°C until assayed. The data represent 95 percent confidence intervals around the pooled results for two extracts, each assayed in sextuplicate. The values for exogenous cyclic AMP represent the largest obtained in a given set of extracts and therefore are maximum values. Generally two-thirds of these values were negative. The limit of error of the assay was 0.2 pmole. The coefficient of variation (CV) was calculated by the formula $(\hat{s}/\bar{x}) \times 100$, where $\bar{x}$ is the mean length of a population and $\hat{s}$ is the standard deviation (*23*).

Cyclic AMP	Developmental form	
	Reproducing CV = 30.7%	Nonreproducing CV = 8.4%
Intracellular (pmole/10^9 cell)	13.2 ± 2.47	25.9 ± 1.91
Intracellular (μM)	0.167 ± 0.031	0.328 ± 0.024
Last washing supernatant (μM)	0.0024	0.0034

tion to the intracellular alterations, there are changes in membrane functions. Glucose and thymidine transport characteristics are affected by serum containing ablastin. Thymidine uptake is inhibited. The rate of efflux of labeled glucose analogs in the presence of glucose and the maximum velocity (V_{max}) of glucose uptake are reduced (*11*).

The exact intracellular events that occur in response to ablastin and bring about the major changes in *T. lewisi* are obscure. However, the intracellular agents that control reproduction of mammalian cells in vitro have been investigated, including an examination of the effects of adenosine 3′,5′-monophosphate (cyclic AMP). This agent or its analogs partially restore transformed cells to contact inhibited growth (*12, 13*), reduce the response of transformed cells to plant lectins to levels associated with nontransformed cells, and induce a morphological alteration to a cell shape resembling the nontransformed cell (*13*). Intracellular cyclic AMP is lower in transformed cells than in nontransformed cells (*14, 15*); it increases at high cell density, while in transformation or growth stimulation it decreases (*16*). Cyclic AMP (or its analogs), when applied exogenously to primary cultures in the growth phase, inhibits DNA synthesis and arrests cells in the G1 phase (*17*). As indicated by the lectin-binding studies, there are membrane changes concomitant with transformation, and these are accompanied by changes in membrane transport (*18*). In addition, caffeine raises the intracellular cyclic AMP concentration and blocks DNA synthesis in *Tetrahymena pyriformis* (*19*). Because of these results, we have examined *T. lewisi* to determine whether it had measurable cyclic AMP and whether intracellular cyclic AMP concentrations were the same or different in reproducing and adult forms.

Adult, female Sprague-Dawley rats were subjected to 900 rads, central-axis, whole-body x-irradiation and infected with 1×10^6 *T. lewisi*. This treatment impairs ablastin production and allows isolation of large numbers of reproducing forms (*20*). Trypanosomes were harvested by cardiac puncture 5 days after the rats were infected. Intact, adult female rats were infected with 5×10^5 trypanosomes, and the parasites were harvested on the day after the first number crisis (a decrease in parasitemia that usually takes place 9 days after infection) to provide adult populations of *T. lewisi*. Smears of the organisms were routinely scanned as a check on population type. Usually 1×10^9 to 5×10^9 trypanosomes were isolated from each rat. Blood from two infected rats was pooled for each extract, and trypanosomes were separated from blood cells by differential centrifugation (*21*) (Table 1). Cyclic AMP was measured by the method of Brown *et al.* (*22*).

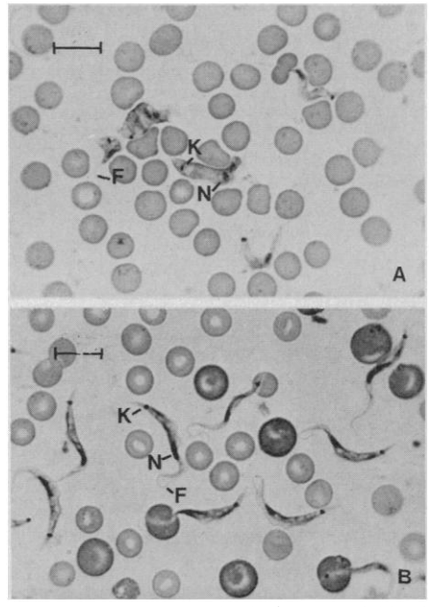

Fig. 1. Photographs of Giemsa-stained blood films taken from rats infected with *T. lewisi*. (A) Reproducing forms. (B) Nonreproducing forms. The nucleus (*N*), kinetoplast (*K*), and flagellum (*F*) are the main cytological features visualized by light microscopy (bar, 10 μm).

Trypanosoma lewisi does have cyclic AMP (Table 1), and the levels are in the range of those found in other cellular systems when expressed as molar concentrations (*14*). The contribution from any possible exogenous cyclic AMP was minor as indicated by the data for the supernatant of the last washing. We also measured cyclic AMP in a highly virulent, monomorphic, laboratory strain of *T. brucei brucei* selected for rapid reproduction; this strain does not show differentiation. Although we found measurable cyclic AMP, there were large deviations between the different extracts. We did not control the age of the infections from which the populations were isolated, and it is possible that this is an important variable. These deviations may also be due in part to the length of time it took to isolate the trypanosomes from the blood. The isolation procedure was complete within an hour, and during this time the organisms were kept cold and in host serum. The procedure works well when isolating consistent populations of organisms with respect to transport functions (*11*). It is conceivable that the intracellular cyclic AMP may change during the isolation procedure since *T. b. brucei* is especially sensitive to changes in environment.

In *T. lewisi* we did control the age of the infection from which the populations were isolated and did not find large deviations between extracts from similar populations. Furthermore, there is a significant difference between the amount of cyclic AMP in reproducing and adult forms. While these data are not extensive, they are consistent with a hypothesis under which ablastin acts to stimulate an increase in intracellular cyclic AMP concentration. Since *T. lewisi* can be grown axenically (*4*), it should be possible to test the ability of cyclic AMP analogs to mimic ablastin in vitro, using the various criteria of (i) inhibition of reproduction, (ii) morphological alterations, and (iii) changes in membrane transport. It should also be possible to isolate, from immune rat serum, a protein with ablastic properties and measure its in vitro effect on intracellular cyclic AMP concentration and on adenylate cyclase of *T. lewisi*.

JAMES E. STRICKLER
Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut 06510
CURTIS L. PATTON
Department of Epidemiology and Public Health, Yale University School of Medicine

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Axonal Projections of Medial Preoptic and Anterior Hypothalamic Neurons

Abstract. *Projections from medial preoptic area (mPOA) and medial anterior hypothalamic area (mAHA) neurons were investigated in albino rats with the use of tritiated amino acid autoradiography. Both the mPOA and the mAHA gave long-axon projections to structures in limbic forebrain and midbrain as well as short-axon projections to other hypothalamic regions. Differences between mPOA and mAHA neurons were observed in projections to the mid-septal region, ventromedial hypothalamus, premammillary region, and central gray. Further, while axons from the mPOA traveled within the medial forebrain bundle, those from the mAHA remained in a band ventromedial to the fornix. These anatomical differences may underlie functional differences between the mPOA and mAHA which have been demonstrated with other experimental techniques.*

The involvement of the preoptic area (POA) and anterior hypothalamus in a variety of physiological, endocrine, and behavioral functions is well known and intensively studied. For example, in regard to endocrine function, medial preoptic area (mPOA) and medial anterior hypothalamic area (mAHA) neurons are known to concentrate estradiol and to be involved both in production of leutinizing hormone releasing factor and in its effect on reproductive behavior (*1*). Attempts have been made to study the anatomical connections of the mPOA and mAHA (*2*), but stains for degenerating fibers, useful elsewhere in the brain, have not given satisfactory results when applied to the hypothalamus. Difficulties in staining the small-calibered axons involved and uncertainties in interpreting lesions which damage fibers-of-passage have hindered investigation of the projections from the hypothalamus and preoptic area (*2*).

With the use of tritiated amino acid autoradiography in tracing axonal connections (*3*), these problems can be circumvented. In this technique, locally injected tritiated amino acids are taken up by neuronal cell bodies, which incorporate the amino acids into protein and transport the labeled protein down their axons in an autoradiographically visible form (*3*). Using autoradiographic techniques (*3, 4*), we examined the projections from the medial preoptic area and anterior hypothalamus in a series of 35 brains from adult albino rats of both sexes with injections in these sites. Injections of tritiated leucine or proline (10 to 50 nl; 200 μc/μl) in physiological saline were made stereotaxically into mPOA (N = 13) or mAHA (N = 22); the animals survived 48 hours postoperatively. After perfusion and paraffin embedding, brains were sectioned (6-μm serial sections), and Kodak NTB-3 nuclear emulsion was applied to the mounted sections. The autoradiograms thus prepared were developed after 21 to 30 days exposure, then systematically scanned and charted. For details of the autoradiographic method, see (*3, 4*). Labeled axons were recognized by patterns of silver grains (usually above Luxol–Fast Blue stained fibers) arranged in linear (longitudinal section of fibers) or clumped fashion (cross section of fibers). This report will concentrate on a comparison of the results from mPOA and mAHA brains; a more complete analysis of mPOA and mAHA projections, including results from injection sites in the nuclei of the diagonal bands, the bed nucleus of the stria terminals, the periventricular POA and mAHA, and the dorsal and lateral mPOA and mAHA, is in preparation (*5*).

In Fig. 1, a series of charts through two brains, one with an injection site in the mPOA (case 39) and one with an mAHA site (case 34) are compared. The injection volumes (10 nl), survival times (48 hours), and exposure times (30 days) were identical in these two animals. Axons of mPOA and mAHA neurons are not restricted to short, local projections (see Fig. 1, A, E, and F) as has often been suggested. In a number of regions, we found the projections of mPOA and mAHA neurons to be similar. Both the mPOA and the mAHA sent fibers to the very anterior lateral septum under the genu of the corpus callosum. Also, both mPOA and mAHA projected to the nuclei of the diagonal bands and to the bed nucleus of the stria terminalis (Fig. 1A). Clearly labeled fibers were observed leading into the periventricular thalamus (Fig. 1, B to D); labeled fibers were also followed into the stria terminalis and the stria medullaris at the diencephalic-telencephalic junction (Fig. 1, B and C). In both mPOA and mAHA cases, the labeled fibers in the stria medullaris distributed to the habenula as diffuse grains within the lateral nucleus (Fig. 1, C and D). Labeled fibers from the mPOA and mAHA in the stria terminalis distributed in the amygdala to the anterior area and medial nucleus (Fig. 1, C and D). The stria terminalis projection to the amygdala was supplemented by labeled fibers spreading laterally into the anterior area and by labeled fibers coursing over the optic tract into the medial nucleus (Fig. 1, C and D).

From both mPOA and mAHA, labeled fiber bundles were followed into the median eminence, and label was seen in the arcuate nucleus (Fig. 1, C and D). More posteriorly, we observed a bilateral projection to the supramammillary nucleus (Fig. 1E) and labeled fibers running through the lateral mammillary nucleus into the ventral tegmental area of Tsai (Fig. 1, E and F). Fibers from this region coursed posterolaterally into the midbrain reticular formation and dorsally into the midbrain raphe nuclei (Fig. 1F).

We also noted differences in axonal projections between mPOA and mAHA neu-

Table 1. Quantitative evaluation of projections to medial and ventrolateral subdivisions of the ventromedial nucleus of the hypothalamus (VM) and the region just posterior to the VM, in matched anatomical positions. The values are mean numbers of grains per reticule square (10 μm per side) in a 100-square reticule, ± standard deviation.

Brain No. (and injection site)	Anterior-posterior level	Mean grains per reticule square	
		Medial VM	Ventrolateral VM
No. 34 (mAHA)	In VM	51.82 ± 9.49	30.66 ± 15.65
	Posterior to VM	14.50 ± 6.25*	30.32 ± 14.63
No. 39 (mPOA)	In VM	1.43 ± 1.37	8.05 ± 3.61
	Posterior to VM	3.61 ± 2.75*	5.69 ± 3.16*

*Indicates a significant difference between VM and posterior to VM at the $P < .001$ level, calculated by a two-tailed Student's t-test.