ing of [$^3$H]QNB during seizures induced by the anticholinesterase poison. It can be tentatively suggested that the differences thus revealed may be due both to dysfunction of cholinergic structures and to the membranotropic effects of DFP.

## REFERENCES


1. M. B. Predtechenskii, N. M. Elaeva, and Z. N. Smirnova, Byull. Éksp. Biol. Med., **108**, No. 10, 459 (1989).
2. R. S. Aronstam, M. D. Smith, and J. J. Buccafusco, Neurosci. Lett., **78**, No. 1, 107 (1987).
3. G. L. Ellman, K. D. Courtney, V. Anders, et al., Biochem. Pharmacol., **7**, No. 2, 88 (1961).
4. M. Hasan and S. F. Ali, Neurotoxicology, **2**, No. 1, 43 (1981).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., **193**, No. 1, 265 (1951).
6. G. L. Petersen, Neurosci. Res., **22**, No. 4, 488 (1989).
7. G. D. Schiller, Life Sci., **24**, 1159 (1979).
8. A. Stalc, M. Sentjurc, and B. Klinar, Pharmacol. Toxicol., **64**, No. 1, 144 (1989).
9. Zhao De-Lu, S. Pei, and C. Liu, Acta Pharmacol. Sin., **6**, No. 4, 227 (1985).


# ULTRASTRUCTURE OF NORMAL HUMAN BLOOD LYMPHOCYTES INCUBATED WITH DEATH-HEAD (*Amanita phalloides*) TOXIN


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**KEY WORDS:** Amanita phalloides toxin; lymphocyte; electron microscopy


Functional characteristics of lymphocytes, which are responsible for the main types of immunologic reaction, including antibody production and accumulation of sensitized lymphocytes, capable of recognizing and eliminating foreign substances [4], have now been well studied [5, 9]. Investigations have shown that lymphocytes constitute a quite heterogeneous population.

There is much evidence in the literature of the effect of immunocompetent cells on tumor growth [1, 8], and on diseases of the hepatobiliary system [2, 3, 6]. An important group of diseases is formed by those produced by exogenous poisons, including poisoning by the most deadly of the Hymenomycetes, the death-head (*Amanita phalloides*) [7]. The problem of the effect of death-head toxin (DHT) on immunocompetent human blood cells has not been discussed in the literature.

The aim of this investigation was to study the action of DHT on human blood lymphocytes in relation to dose and duration of exposure.

## EXPERIMENTAL METHOD

Heparinized (12 IU/ml) blood was obtained from healthy donors and incubated at 37°C with DHT in a dose of 0.05 and 0.5 $LD_{100}$ for 1 and 3 h. The lymphocytes were sedimented by centrifugation, fixed with a 1% solution of glutaraldehyde in 0.1 M phosphate buffer, postfixed in a 1% solution of osmium, dehydrated in alcohols, and embed-


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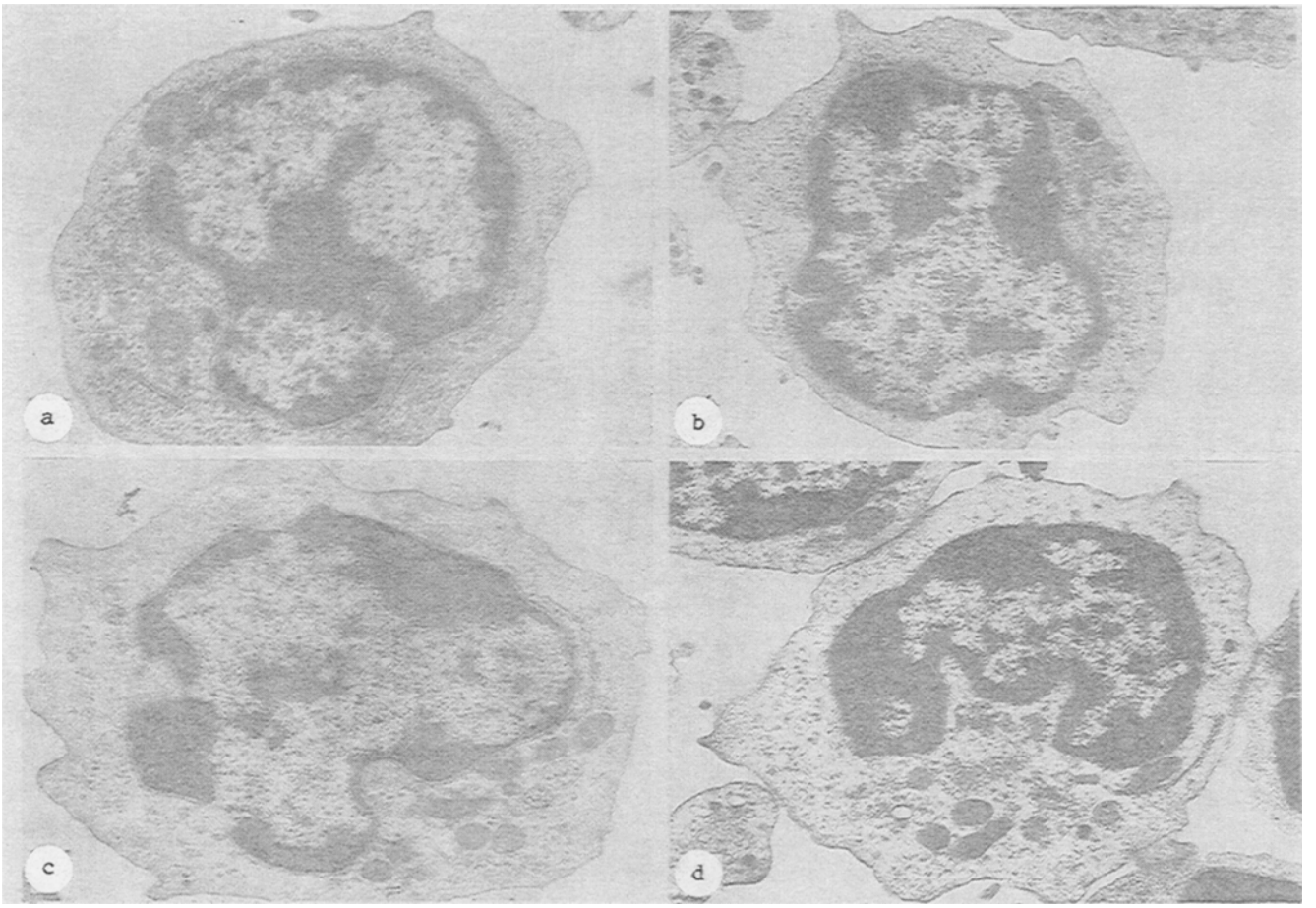


Fig. 1. Changes in lymphocyte ultrastructure 1 h (a, c) and 3 h (b, d) after addition of DHT: a) homogenization of mitochondrial matrix with reduction of cristae (16,000×); b) degranulation of cytoplasm (13,000×); c) reduction of nuclear heterochromatin and partial destruction of karyolemma (15,000×); d) foci of destruction of cytoplasm (13,000×).

ded in Araldite. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined in the ÉVM-100 LM electron microscope.

## EXPERIMENTAL RESULTS

After incubation for 1 h with 0.05 $LD_{100}$ of DHT most lymphocytes contained a round or bean-shaped nucleus, situated eccentrically. Euchromatin was located around the periphery of the karyolemma. The nucleolus was clearly outlined. The perinuclear space was widened. Mitochondria were round, with a predominantly granular matrix, with partially destroyed cristae. The Golgi complex was ill defined and consisted of solitary vacuolated cisterns. The cell cytoplasm contained traces of a partly destroyed rough endoplasmic reticulum. A few ribosomes and polysomes were present. Secondary lysosomes appeared (Fig. 1a).

The lymphocytes preserved their circular shape 3 h after addition of DHT to the blood. The nucleus was irregular in shape, and less heterochromatin was present. The karyolemma was destroyed in places and only a few ribosomes were present on the surface of the outer membrane. Solitary traces of the rough endoplasmic reticulum were located in the cytoplasm, and there were fewer ribosomes and polysomes. Marked changes were present in the mitochondria: they were reduced in number, the integrity of their membranes was disturbed, their matrix homogenized, and their cristae reduced. The Golgi complex was present in the form of single lamellae and vacuoles in a few lymphocytes. The number of lysosomes was increased (Fig. 1b).

Most lymphocytes 1 h after addition of DHT in a dose of $0.5LD_{100}$ were somewhat elongated in shape. The nucleus was irregular, with a reduced heterochromatin content, and with areas of translucency of the karyoplasm. The outlines of the nucleolus had signs of erosion. The karyolemma was destroyed in some places, the perinuclear space was widened, and solitary ribosomes remained on the outer membrane. The cytoplasm of the lymphocytes was translucent, and the number of organoids was much reduced. Mitochondria were oval or elongated in shape, with areas of disturbance of integrity of the membranes and homogenization of the contents. Some mitochondria still preserved their cristae. Destruction of the rough endoplasmic reticulum was present. Areas of autolysis, microvesicles, and vacuoles appeared. The number of ribosomes and polysomes and the glycogen content in the cytoplasm of the lymphocytes were greatly reduced (Fig. 1c).

Increased disorganization of the lymphocytes was observed after 3 h, and they had lost their usual shape. The nucleus was located eccentrically and reduced in size, as also was the nucleolus. The karyolemma was vacuolated and partly destroyed in some places, with release of heterochromatin into the cytoplasm. The cytoplasm of the lymphocytes was translucent, with areas of autolysis, and with total absence of organoids. There were fewer ribosomes and polysomes. The mitochondria were now electron-dense, their matrix was homogenized, and the integrity of their membranes disturbed. The rough endoplasmic reticulum was represented by separate half-destroyed tubules with few ribosomes. The number of lysosomes was increased. Some cells were seen to be completely destroyed (Fig. 1d).

The toxin of *Amanita phalloides* thus has a destructive effect on human lymphocytes, and its severity increases with an increase in dose of the toxin and the duration of exposure to it.

## REFERENCES


1. M. M. Berezhnaya and V. G. Pinchuk, Biochemistry of Animals and Man [in Russian], No. 6, 49 (1984).
2. K. P. Zak, M. A. Gruzov, B. M. Khomenko, et al., Byull. Éksp. Biol. Med., No. 10, 494 (1984).
3. K. A. Zufarov and A. F. Sadriddinov, Byull. Éksp. Biol. Med., No. 9, 372 (1989).
4. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
5. Yu. A. Umanskii and V. G. Pinchuk, Lymphocytes and Tumor Growth [in Russian], Kiev (1982).
6. P. Shima, I. Miler, V. Vetvichka, et al., Immunologiya, No. 3, 40 (1985).
7. R. Cholewa, S. Pach, M. Bogusz, et al., Przegl. Lek., **40**, No. 6, 503 (1983).
8. R. Prehn, Science, **176**, 170 (1972).
9. T. Teranichi, T. Hirano, N. Arima, et al., J. Immunol., **128**, No. 4, 1903 (1982).