

DETERMINATION OF MELATONIN IN BOVINE CEREBROSPINAL FLUID

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Melatonin is considered to be a specific hormone of the pineal gland [1]. Since the pineal gland is bathed by the cerebrospinal fluid, it is logical to expect the presence of melatonin in this fluid. In actual fact, it has been possible to determine melatonin in the human fluid by a fluorometric method [2]. The present investigation was directed to identifying melatonin in bovine cerebrospinal fluid obtained by biopsy.

To 100 ml of cerebrospinal fluid was added 25 ml of a 40% solution of trichloroacetic acid, and the mixture was centrifuged at 5000 rpm for 10 min. The supernatant liquid was transferred to a separatory funnel with a ground-in stopper containing 150 ml of chloroform, CHCl_3 . The funnel was shaken for 5 min. After this, the chloroform layer was separated off and was centrifuged at 5000 rpm for 10 min. Another 150 ml of CHCl_3 was added to the aqueous phase in a funnel, and the preceding operation was repeated. The first and second chloroform extracts were mixed and transferred into another separatory funnel containing 15 ml of 3 N HCl. The funnel was shaken for 5 min, after which the aqueous layer was removed. Another 15 ml of 3 N HCl was added to the funnel and the preceding operation was repeated. The aqueous phases obtained were mixed and brought to pH 7.0 with ammonia. The melatonin was extracted from the aqueous phase fractionally with CHCl_3 (3×10 ml). Then the CHCl_3 was distilled off in vacuum. The melatonin remained on the walls of the vessel. A few drops of ethanol was added to dissolve the melatonin, and it was subjected to chromatographic analysis by the method of N. T. Raikhlin and I. M. Dvetnoi [3, 4] using thin-layer chromatography of Silufol I in the isopropanol-benzene-ammonia (10:5:1) system. The spots were revealed with Ehrlich's reagent: a 2% solution of p-dimethylbenzaldehyde in 96% ethanol-hydrochloric acid (10:2). For comparison we used synthetic melatonin. Thin-layer chromatography permitted the identification of melatonin (R_f 0.74) in bovine cerebrospinal fluid.

The quantitative determination of melatonin in bovine fluid has been carried out in Prof. A. Yu. Makarov's laboratory. The results of the investigation of five samples of the fluid gave a mean melatonin content of 0.015 $\mu\text{g/ml}$.

Thus, the presence of melatonin in bovine cerebrospinal fluid has been definitely established.

LITERATURE CITED

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