Toxic Effect of Cadmium Stearate on Rat Cerebellum in Culture

by MINORU KASUYA, NAOKI SUGAWARA, and AKIRA OKADA[1]

Department of Public Health
Sapporo Medical College
Sapporo, Japan

Cadmium stearate is used in poly vinyl chloride factories as a stabilizer. The compound will pollute not only work rooms, but will also spread throughout our environment through products and waste.

Although many investigators (SAKABE and USHIO,1960; SUZUKI et al. 1965)have reported that the dust of cadmium stearate was absorbed by workers and produced poisoning, whose symptoms were nausea, vomiting, loss of appetite, epigastralgia, and proteinuria, toxicological studies on the compound are limited. Many investigators have reported that cadmium chloride produced toxic effects on gasserian and sensory spinal ganglia (GABBIANI et al., 1967a), sensory spinal ganglia (SCHLAEPFER, 1971), and central nervous tissues (GABBIANI et al., 1967b). Furthermore, tissue culture studies on dorsal root ganglia (TISCHNER and SCHRÖDER, 1972), and on cerebellum (KASUYA and OKADA, 1971) from rats showed that cadmium chloride can produce pathological changes in these tissues directly, apart from damaging the endothelium of blood vessels.

This paper reports the results of the effect of cadmium stearate on cerebellar tissue from newborn rats in tissue culture.

METHODS

Cerebella from newborn rats (5 to 9 days old) were sliced into small particles of 1-2 mm^3, and cultured on collagen-coated plastic dishes (Falcon dishes for cell cultures, 35 X 10 mm) at 35.0° C for 4 days in an incubator. The culture medium contained calf serum and Eagle MEM (1:1)(but did not contain L-glutamine).

---

[1]Present address: Department of Public Health, Faculty of Medicine, University of Kanazawa, Japan.

Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 5 © 1974 by Springer-Verlag New York Inc.

Glucose was added to the medium at a final concentration of 550 mg/100 ml.

Cadmium stearate was dissolved in alkaline water or in calf serum directly, and was filtered through a Millipore Filter (0.45 μ). Cadmium stearate was dissolved in calf serum as follows: 0.0015-0.01 g of cadmium stearate was suspended in 2 ml of ethyl ether or chloroform in a polyethylen tube and allowed to stand several hours until the solvent evaporated. 4.5 ml of calf serum was added to the tube and sonicated for 5 min (Insonator Model 200 M, Kubota, 60 W, 1 A). Cadmium stearate could be dissolved only to the extent of 0.3 ppm in alkaline water, but dissolved in calf serum to the extent of 1.55 to 13.1 ppm. Concentrations of cadmium stearate in alkaline water and in serum were measured by an Atomic Absorption Spectophotometer (Hitachi, Model 203, wave length, 2286 Å).

The degree of outgrowth was expressed as a function of the outgrowth of nerve fibers and fibroblasts considering the degenerative changes as follows.

VI: Normal outgrowth of nerve fibers and fibroblasts.

V : Normal outgrowth of fibers and depressed outgrowth of fibroblasts, or depressed outgrowth of fibers and normal outgrowth of fibroblasts.

IV: Depressed outgrowth of fibers and fibroblasts.

III: No outgrowth of fibers and normal outgrowth of fibroblasts, or normal outgrowth of fibers and no outgrowth of fibroblasts.

II: Depressed outgrowth of fibers and no outgrowth of fibroblasts, or no outgrowth of fibers and depressed outgrowth of fibroblasts.

I : No outgrowth of fibers and fibroblasts.

RESULTS AND DISCUSSION

In the control culture, nerve fibers developed forming networks, and well dispersed glial cells accompanied the fibers. Fibroblasts developed forming a sheet (Fig. 1,A).

At low concentrations of cadmium stearate (0.003-0.06 X 10^{-6} M) no detectable changes were found in the cultures (Table 1,A).

As shown in Fig. 1,B, the outgrowth of nerve fibers

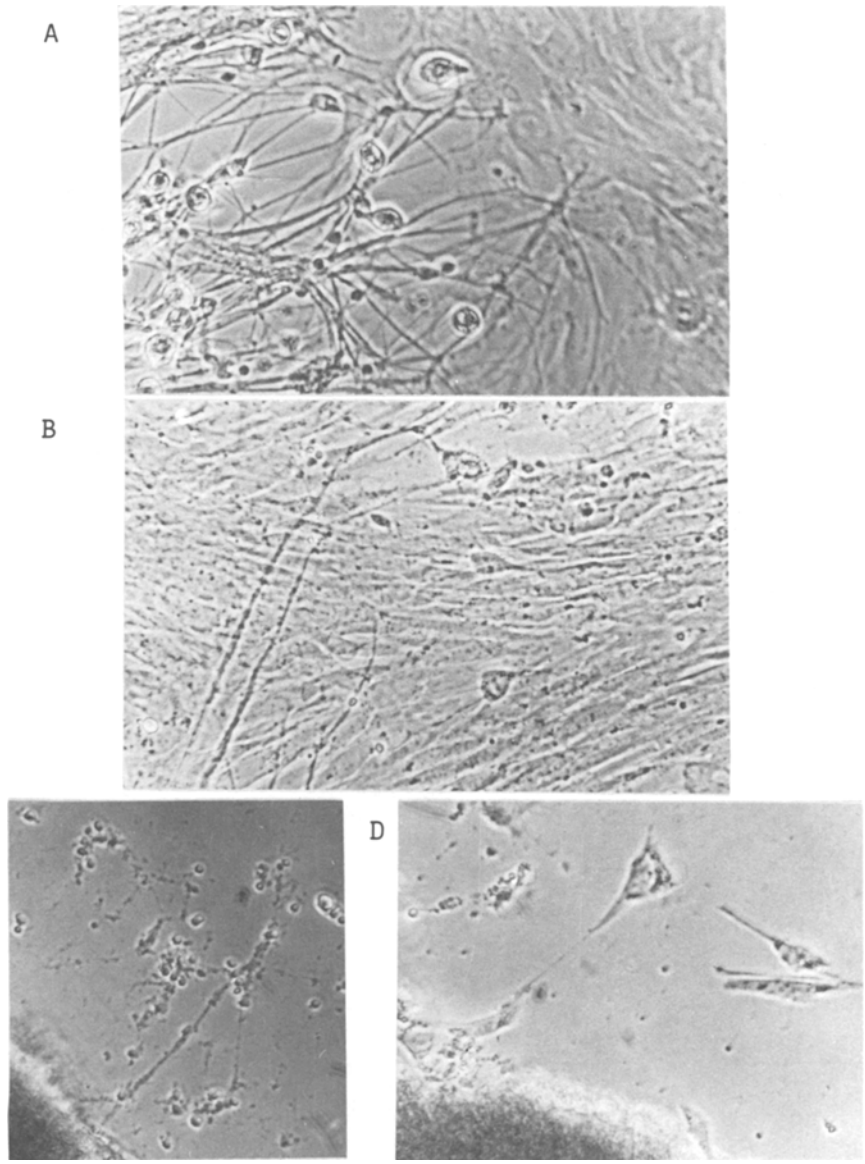

Fig.1. The effect of cadmium stearate on nerve fibers, glial cells, and fibroblasts from newborn rat cerebellum in tissue culture. (4 days in vitro). Phase contrast microscope, 10 X 10.

A. Control culture.
B. 0.58 X 10^{-6} M cadmium stearate.
C,D. 1.2 X 10^{-6} M cadmium stearate.

TABLE 1

Neurotoxic effect of cadmium stearate

A. At low concentration
(Cadmium stearate was dissolved in alkaline water.)

		Cadmium stearate ($\times 10^{-6}$ M)					
		0	0.003	0.008	0.015	0.03	0.06
Degree of outgrowth	VI	8	7	8	6	6	8
	V	1	2	2	3	3	2
	IV				1	1	
	III						
	II						
	I	1	1				
U-values[a]			45.5[b]	51[b]	41.5[b]	41.5[b]	51[b]

B. At high concentration
(Cadmium stearate was dissolved in serum directly.)

		Cadmium stearate ($\times 10^{-6}$ M)							
		0	0.58	1.2	2.3	4.6	6.9	0	58.4
Degree of outgrowth	VI	10						8	
	V		5					2	
	IV		3	1					
	III								
	II		2	5	2				3
	I			4	8	10	10		7
U-values[a]			0	0	0	0	0		0

[a]The Mann-Whitney U test. Critical value of U for a two-tailed test at α =0.05 is 23.

[b]Not significantly different from control.

Numerals in the Table show the number of explants of cerebellum. On the degree of the outgrowth, see text.

was depressed at 0.58 X 10^{-6} M cadmium stearate; i.e. network formation of nerve fibers was depressed and granules were observed in the fibers. Glial cells showed depressed outgrowth at this concentration. No or depressed sheet formation of fibroblasts was observed in some explants and granules were formed in the fibroblast cells.

At 1.2 X 10^{-6} M cadmium stearate, the development of nerve fibers was strongly depressed showing degenerative changes such as granules and lytic change, and no development of the fibers was found on some explants (Fig. 1,C). Development of glial cells was also strongly depressed. Fibroblasts showed no sheet formation and only a few fibroblasts developed dispersedly (Fig. 1,D).

The development of nerve fibers, glial cells, and fibroblasts was scarcely observed at concentrations of cadmium stearate higher than 2.3 X 10^{-6} M.

These results are shown in Table 1. It is probable that the minimal dose for injurious effect of cadmium stearate on cerebellum is less than 0.58 X 10^{-6} M and the minimal dose for total inhibition is about 2.3 X 10^{-6} M.

We reported previously (KASUYA and OKADA, 1971) that cadmium chloride completely inhibited the outgrowth of cerebellar cells from rats in culture at the concentration of 4 X 10^{-5} M, although the degenerative effect of cadmium chloride on nerve fibers and fibroblasts was not apparent. Since the concentration of cadmium stearate required to inhibit completely the outgrowth of cerebellar cells is around 2.3 X 10^{-6} M, and it produced degenerative changes, cadmium stearate may be more toxic than cadmium chloride. It seems to be probable that the strong toxicity of cadmium stearate is due to the hydrophobic property, because cadmium stearate is insoluble in water while cadmium chloride is very soluble.

The low solubility in water of cadmium stearate may make it inappropriate for infiltration into a living body. However, since cadmium stearate is soluble in serum to some extent (see Methods), the compound, if it pollutes our environment, can accumulate in tissues and produce adverse effects on nervous tissues directly.

SUMMARY

Cadmium stearate inhibited the outgrowth of cerebellar cells from newborn rat in tissue culture and produced degenerative changes at a concentration of 0.58 X 10^{-6} M, and the minimal dose for total inhibition of nerve fibers, glial cells, and fibroblasts was at about 2.3 X 10^{-6} M.

ACKNOWLEDGEMENT

The authors express their thanks to Professor H.Miyake for valuable discussion.

REFERENCES

GABBIANI,G., GREGORY,A., and BAIC,D.: J.Neuropath.Exp. Neurol. 26, 498 (1967a).

GABBIANI,G., BAIC,D., and DÉZIEL,C.: Exp.Neurol. 18, 154 (1967b).

KASUYA,M. and OKADA,A.: Igaku no Ayumi 79, 178 (1971) (in Japanese).

SAKABE,H. and USHIO,K.: Bull.Nat.Inst.Ind.Health 3, 56 (1960).

SCHLAEPFER,W.W.: Lab.Invest. 25, 556 (1971).

SUZUKI,S., SUZUKI,T., and ASHIZAWA,M.: Ind.Health 3, 73 (1965).

TISCHNER,K.H. and SCHRÖDER,J.M.: J.neurol.Sci. 16, 383 (1972).