Increased radiation mortality by lead acetate[1]

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Summary. The mortality of X-irradiated rats and mice was increased if a nontoxic dose of lead acetate was given i.v. 3 and 7 days following radiation. The increase was equivalent to 100 R for X-ray doses between 790 and 540 R.

A bacterial endotoxin originating from intestinal E. coli has been found in the blood of irradiated mice that may contribute to the radiation syndrome[4]. Considering these findings and the concept of Fine et al.[5,6], it has been suggested that bacterial endotoxins from the injured intestine are causally connected both with the mortality and the increased phagocytic activity of the reticuloendothelial system[7,8]. The presence of bacterial endotoxins in the blood would also explain the increased susceptibility of irradiated animals to parenterally administered bacterial endotoxins[9].

Selye et al.[10] have shown with non-irradiated animals that the lethal effect of bacterial endotoxins was highly increased by a single, normally well-tolerated nontoxic dose of lead acetate. With respect to the presence of such endotoxins in the blood of the irradiated animals, the question arose whether the lethal effect of whole body X-irradiation would be equally increased by lead acetate. In order to test this hypothesis, mice and rats were exposed to various X-ray doses and a nontoxic dose of lead acetate was given a few days later during the intestinal phase of the radiation disease. The mortality rate was determined 30 days post radiation; in a few experiments, it was determined on the day when 50% of the irradiated control animals had died. A marked increase in radiation mortality by nonlethal doses of lead acetate was found.

Methods. Animals: Adult male mice of an inbred strain, 9–12 weeks old, weighing 18–23 g. Female rats (Wistar), 8–10 weeks old, weighing 121–150 g. Nutrition: Standard pellets (Altromin R), tap water ad libitum.

Whole body irradiation: X-ray exposure in special plastic boxes. Radiation factors: 200 kV, 15 mV, 0.43 mm Cu filter; h.v.l. 0.87 mm Cu, SSD 40 cm; exposure rate 80 R per min. In order to administer various X-ray doses, the exposure time was changed accordingly.

Lead acetate: A neutralized solution of lead (II) acetate-3-hydrate (pro analysi, Merck-Darmstadt, No. 7375) containing 5 g/l aqua bidest. was used, 50 mg/kg b.wt were injected i.v.

Results. The results of table 1 show that the mortality of whole-body-irradiated mice and rats was enhanced if lead acetate had been injected a few days following radiation. Due to the strong lethal effect of 820 R (mice) and 1000 R (rats), the sensitizing effect of lead acetate could not be demonstrated 30 days after radiation. In these cases, however, it could be clearly established if the 50% mortality of the irradiated controls was used as point of reference (table 1, values for 820 R, x and 1000 R, x).

The question arose whether the increasing effect of lead acetate on radiation mortality would also refer to moderately lethal and sublethal doses of X-rays. In appropri-

Table 1. Increase in radiation mortality by a nontoxic dose of lead acetate

Species	X-ray dose (R)	Rate of mortality Radiation only (control)			Radiation plus lead acetate		
		Dead	Total	%	Dead	Total	%
Mice	650	17	67	25	67	88	76***
	750	20	35	61	26	27	96***
	820	24	26	92	26	26	100
	820 X	13	26	50	21	26	81**
Rats	750	4	30	13	25	30	84***
	1000	37	38	98	28	28	100
	1000 X	19	38	50	21	28	75**

Whole body X-irradiation. Mortality rate 30 days post radiation except for 820 R x and 1000 R x. Mortality rate for 820 R x 12 days and for 1000 R x 6 days post radiation. – Lead acetate 50 mg/kg b.wt; mice were injected 7 days and rats 3 days post radiation. $\chi 2$ test. Significantly different from control value: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 2. Effect of lead acetate applying different radiation doses

X-ray dose (R)	Rate of mortality Radiation only (control)			Radiation plus lead acetate		
	Dead	Total	%	Dead	Total	%
490	0	20	0	0	20	0
540	0	30	0	6	30	20*
580	2	26	7	11	16	69***
640	15	36	42	27	35	77**
690	16	24	67	22	24	92*
730	14	20	70	20	20	100
790	31	33	94	20	20	100

Male mice. Mortality rate 30 days post radiation. Otherwise see legend of table 1.

1 The basic experiments have been carried out 1967/1968 in the Heiligenberg-Institut.

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ate experiments, an increased susceptibility to the lethal effect of radiation was established for a broad series of doses ranging from 790 R to 490 R, i.e. also for moderately lethal and sublethal doses of 580 and 540 R respectively (table 2). For the whole range of doses, the increase in mortality caused by lead acetate was equivalent to an additional X-ray dose of about 100 R. The threshold between partially lethal and nonlethal doses appeared to be decreased to the same extent.

Discussion. It appears that the increased susceptibility to radiation caused by lead acetate can easily be explained in the light of the references cited in the introduction. According to those data, the blood of the irradiated animals would contain bacterial endotoxins[4] and the noxious effects of such endotoxins are enhanced by lead acetate[9,10]. This means that the lead acetate administered a few days following radiation would equally sensitize the animals against the lethal effect of intrinsic bacterial endotoxins originating from the intestine, as it sensitizes them against the lethal effect of extrinsic endotoxins parenterally administered. In accordance with this hypothesis, lead acetate proved to be ineffective in preliminary experiments if applied immediately before or after irradiation, i.e. when no intestinal damage existed.

5-Hydroxytryptamine binding to butanol extracts from myelin fragments

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Summary. The myelin fraction of rat brain stem was treated with butanol-water mixtures, and the extracted proteolipids were separated by Sephadex LH_{20} column chromatography. 2 peaks of proteolipids eluted in chloroform-methanol 4/1 showed the binding capacity for C^{14} · 5-HT. This finding suggests the necessity of the more careful investigations for the probability of proteolipids as receptor proteins in the central nervous system.

Proteolipids are a specialized group of hydrophobic proteins described by Folch-Pi and Lees[1]. They occur in brain and other tissues and have the unusual property of being soluble in chloroform-methanol[2] or butanol-water mixtures[3] but insoluble in water. In the nervous tissues, the highest concentrations of proteolipids occur in white matter, lower concentrations in grey matter and lowest in peripheral nerve[4].

Recently, several workes[5–8] have implicated the proteolipids in the binding of neurotransmitters and drugs to lipid extracts of nervous tissue. Godwin and Sneddon[9] suggested that butanol extracts from rat brain stem have a physiological function as a receptor protein to 5-HT. However, morphological examination of the specimens used there is insufficient, and thus it is uncertain whether the isolated proteolipids originate from the nerve endings. The object of the present paper is to examine the morphological features of the particulate fraction described by Godwin and Sneddon[9] and, moreover, 5-HT binding properties of the proteolipids extracted from myelin fragments will be presented.

Materials and methods. Male Wistar rats (150–200 g) were decapitated and the brains were removed in the cold. The brain stems including hypothalamus, midbrain and medulla oblongata + pons were taken and homogenized in 0.32 M sucrose (10%) with a Teflon-glass homogenizer. The myelin fraction was isolated from the homogenate by the method of Whittaker et al.[10] and examined by electron microscopy. Preparation of the particulate fraction was based on the method of Godwin and Sneddon[9], i.e., the brain stems were homogenized in 20 vol. of water and the homogenate was centrifuged at $30,000 \times g$ for 15 min. Both pellets of the myelin and particulate fraction were resuspended in 50% sucrose (2 ml/g brain stem) and extracted with 10 vol. of water-saturated butanol for 2 h at room temperature. The extraction mixture was centrifuged at $1000 \times g$ for 20 min and the butanol phase isolated. This was concentrated under N_2 at 38 °C to about one-third of its original volume (TE). Aliquots of each TE (4 ml) were treated with water (14%, v/v) to dissolve the insoluble materials, and a 3 ml sample was incubated at room temperature for 20 min with 5×10^{-7} M of C^{14} · 5-HT (48.54 mCi/mmol). After incubation, the mixtures were loaded onto a Sephadex LH_{20} column (2×30 cm). Stepwise elution was carried out with solvents of increasing polarity: 100 ml chloroform, 50 ml each of chloroform-methanol (CM) 15/1, 10/1 and 6/1, and then 120 ml of CM 4/1. Protein contents of the TE and collected fractions were assayed using the method of Lees and Paxman[11]. Lipid phosphorus was assayed by the method of Chen et al.[12]. Radioactivity of the collected fractions was counted in a tT-21 emulsion phosphor[13]. Morphological examination of the myelin and particulate fraction was performed by a Hitachi HU-12A electron microscope. The details of electron microscopy have been reported previously[14].

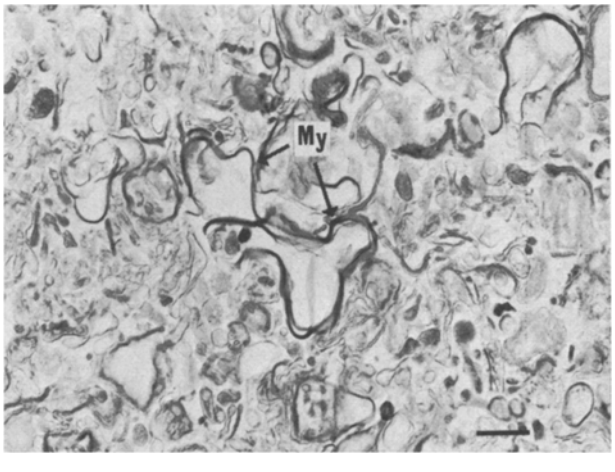

Fig. 1. Electronmicrograph of particulate fraction showing the presence of several myelin fragments (My). Bar equals 0.5 μm.