Effect of methadone and morphine on developing chick embryo

	Dose (mg/kg) egg[a]	Number of eggs	Percent developing	Wet weight[b]) Body (g)	Brain (mg)	Liver (mg)	µg protein/mg wet weight[b]) Brain	Liver
dl-Metha-	0.081	11	100	10.0±1.3	228±43	165±24	52.3± 6.4**	123±23
done	0.405	12	83	10.8±0.8	222±47	153±18*	51.7± 4.0*	113± 5
Morphine	0.074	11	100	10.5±0.7	252±29	154±28	52.2±10.6	109±18
	0.370	11	100	10.2±0.7	237±23	150±29***	42.5± 6.8*	141±28*
Control	-	11	100	10.8±0.5	235±42	174±17	59.6± 5.6	113± 5

[a] Equimolar concentrations of both drugs dissolved in 25 µl of 0.9% NaCl were injected daily on days 2 through 12 with sacrifice on day 13. [b] Means±SD. * $p<0.01$; ** $p<0.02$; *** $p<0.05$.

drugs, the liver protein concentration was increased by morphine (table).

Discussion. The data indicate that both methadone and morphine can interfere directly with the development of the embryo, without indirect maternal-fetal interaction. These results are in agreement with some observations from animal experiments. In pregnant rats, for example, methadone has been shown to lead, in a dose-related fashion, to a decreased number of live offspring per litter, an increased resorption and stillbirth rate, and lowered birth weights[5-8]. Recently, methadone has been shown to inhibit cellular protein and nuclei acid synthesis[22]. The morphine-induced increase of liver protein concentration (table) may be related to a morphine-induced increase of liver microsomal enzymes[14].

Some of the doses of methadone and morphine which caused developmental and biochemical effects in the chick embryos are much lower than those used therapeutically. Further and more detailed studies of the effects of these drugs at various stages of embryogenesis may therefore have important clinical implications.

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Autophagy in mouse hepatocytes induced by lysine acetylsalicylate

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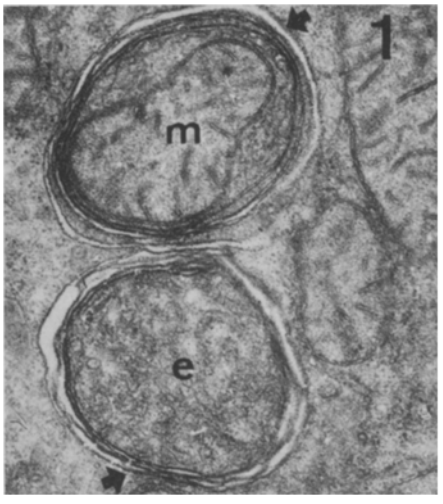
Summary. I.v. administration of lysine acetylsalicylate inces autophagy in mouse liver cells. Single and multiple membrane-bounded vacuoles were found. The latter seems to be an unusual morphological form of the sequestration process. These findings could express a transitory sublethal liver cell injury induced by the drug.

Salicylates are widely used anti-inflammatory drugs, for treating acute and chronic polyarthropaties. Hepatic ultrastructural alterations have been described in animals receiving oral salicylates in a high-dose intake[2-4]. Toxic effects have also been reported in the liver of patients on chronic oral aspirin therapy[5-10]. The purpose of this paper is to describe autophagic vacuoles in the mouse hepatocytes after i.v. administration of lysine acetylsalicylate (LAS), a new pharmacological form introduced to overcome gastric toxicity and allowing higher dosage schedules.

Material and methods. 5 groups of 5 female white Swiss mice, weighing 25 g, were used. Groups I, II, and III were injected i.v. with LAS in a single dose of 350 mg/kg (LD_{50}= 1425 mg/kg) and sacrificed, by cervical dislocation, 30 min, 90 min and 3 h after the injection, respectively. Groups IV and V were used as controls. The mice from the first of these 2 groups were injected i.v. with 0.25 ml of distilled water (the solvent used for LAS administration) and those from the latter group received no treatment. Fragments from the left lateral lobe of the liver, were fixed in cacodylate-buffered 3% glutaraldehyde pH 7.3, and postfixed in veronal-acetate buffered 1% osmium tetroxide.



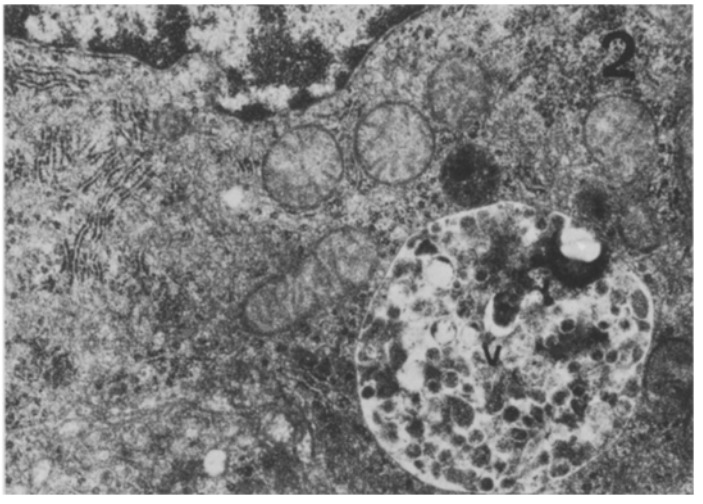


Fig. 1. Section of the cytoplasm of a hepatocyte from a lysine acetylsalicylate-treated mouse, 90 min after the injection. 2 multiple membrane-bounded bodies, lined by pairs of membranes (arrows), contain sequestrated hyaloplasm, one mitochondrion (m), and smooth endoplasmic reticulum (e). × 28,000.

Fig. 2. Section of a hepatocyte from a lysine acetylsalicylate-treated mouse, 90 min after the injection. Large single membrane-bounded autophagic vacuole (v) containing membrane fragments and cell debris. × 16,500.

Following dehydration in graded ethanols, they were embedded in Epon 812. The ultrathin sections were stained with uranyl acetate and lead citrate and examined on a Philips EM 300 electron microscope.

Results and discussion. An increased number of membrane-sequestrated cytoplasmic areas were observed in the hepatic cells of all the LAS-treated animals. 2 different morphological types of vacuoles were found. The predominant one consisted of a single membrane-bounded body, containing membrane fragments and cell debris, and was frequently placed near the bile canaliculus. The other type of autophagic vacuole was a multiple membrane-bounded body lined by a variable number of pairs of membranes, usually 2–3; they presented a concentric parallel arrangement, with hyaloplasmic material or clear spaces between the adjacent pairs of membranes. Well preserved organelles and hyaloplasm were contained by the multiple membrane-bounded bodies.

The sequestration bodies of both types predominate in group II, their incidence being lower in group I. In group III only single membrane-bounded vacuoles were observed, slightly more frequently than in groups IV and V (controls). No multiple membrane-bounded bodies were seen in group IV and V animals.

2 different types of autophagic vacuoles are commonly described in the literature: the single membrane-bounded and the double membrane-bounded bodies[11-14]. After LAS administration we have found 2 different sequestration bodies in the mouse hepatocytes: those similar to the previously reported single membrane-bounded vacuoles and the multiple membrane-bounded vacuoles. The single membrane-bounded bodies are supposed to be late stages in cell autophagy, their inner membranes being previously digested by lysosomal enzymes[11-13]. The multiple membrane-bounded vacuoles are an unusual morphological finding in autophagic mechanism. Their sequestrating membranes could be due to the bending of parallel cisternae of endoplasmic reticulum, with accidental entrapment of cytoplasmic elements. The multiple membrane-bounded vacuole may correspond to early stages of autophagy, since they contain well preserved cytoplasmic material and also they were not observed 3 h after the drug injection, when single membrane-bounded bodies were still present.

Autophagy has also been reported in the hepatic cells of a patient on chronic salicylate therapy with a different morphology of the sequestrating bodies from that observed in the LAS-treated mouse liver[17,18]. It is believed that an increased number of autophagic vacuoles is due to a sublethal intracellular focal injury caused by noxious agents[11,14,16]. In our experimental conditions, the LAS administration (in less than $\frac{1}{4}$ of the lethal dose) has produced a transitory mouse liver injury. This effect seems to be reversible because of the slightly more frequent number of autophagic vacuoles in the group sacrificed 3 h after the injection than in the control groups. Although our study suggests a transitory character of the lesion, it is reasonable to assume the possibility of toxic effects in the human liver induced by LAS administration, as it is reported after oral acute and chronic intake of salicylates.

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