ing the 2nd week of treatment. Some animals had diarrhea, and after cessation of the drug-regimen, they failed to regain their weight losses completely.

The mortality due to indomethacin toxicity was 40% and 20% in the animals dosed at 5.0 mg/kg and 2.5 mg/kg, respectively.

We conclude, therefore, that the reduction or blocking of the inflammatory response against MSV by the non-steroidal anti-inflammatory drugs stimulates growth of tumor. However, the treatment with the drugs is not able to stop the rejection of MSV-induced tumors. The apparent inhibition of MSV-tumor development by indomethacin may be explained in terms of high toxicity of this drug for mice. Moreover, indomethacin as well as aspirin, under some conditions, may act as a weak antiviral agent[8,15].

All the drugs investigated in the present experiments were found to be inhibitors of the prostaglandin synthesis[16] (unpublished experiments). Whether the described enhancement of tumor growth by the non-steroidal anti-inflammatory drugs is connected with the inhibition of prostaglandin synthesis[12,17] or to other effects e.g. related to interferon remains to be determined.


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## Effects of dl-methadone and morphine on developing chick embryo


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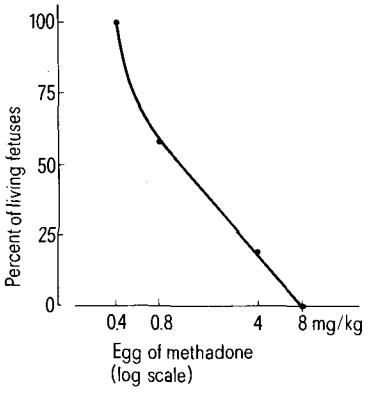
*Summary.* Injections of methadone into the air space of fertile chicken eggs affected development of the embryo. Both methadone and morphine caused decreases in liver weight and brain protein, and morphine increased liver protein levels.


A number of recent reports have presented evidence that developing and growing organisms may be severely and adversely affected by methadone, heroin or morphine[2-9]. In humans as well, it has been reported that heroin-addicted and methadone-treated mothers have babies of low birth weight despite full term gestation[10-15]. Methadone easily crosses the placenta and enters fetal circulation and may alter maternal-fetal interactions[16]. Since the avian embryo avoids the possibility of mother-fetus interactions, it is often used to study the toxicity and teratogenicity of chemical compounds[17-20]. The present paper reports some results indicating effects of methadone and morphine on the developing chick embryo.

*Methods.* White leghorn eggs (55-60 g) were incubated in a Janesway 252 incubator. dl-Methadone hydrochloride or morphine sulfate solution in 0.9% NaCl was injected into the air sac over the inner shell membrane. Control eggs received the same amount of vehicle. 2 arbitrary schedules as indicated in the figure and the table were used for repeated administration of various doses of the drugs. After the gestation was terminated, the dead embryos were weighed. The brain and liver were removed and weighed before homogenizing in 10% TCA[18]. Total protein was estimated in each sample[21].

*Results.* 3 doses of methadone at 0.8 mg/kg egg or more had a dose-related effect on the percentage of viable embryos with none developing when the dose was 8 mg/kg egg (figure). The wet b. wt of the living embryos was normal at the 2 lowest doses used but was 65±5% of control in the 4 mg/kg egg group ($p < 0.001$).

Daily injections of fertilized eggs with very low doses of methadone or morphine for 10 days did not affect the total body and brain wet weights of developed embryos at 13 days gestation (table). The only embryos which failed to develop were in the group receiving 0.4 mg/kg egg of methadone. The concentration of protein in the brain was significantly decreased with either dose of methadone, as well as with the higher dose of morphine. Even though the liver wet weight was decreased with the higher dose of both




Semi-log plot of the percentage of embryos surviving to day 15 after injections on days 3, 5 and 7 with the indicated doses of dl-methadone in 50 µl of 0.9% NaCl. All controls survived (b. wt 13.9±0.7 g). 11-12 eggs/group.

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.