i.p.); 20 and 30 min after the injection, the rats were placed on a hot plate (56 °C). The morphine effect was measured by the response of the rat to the hot plate in terms of time taken (sec) to raise the hind paw. A 4th group of 10 rats was treated with thiopental (20 mg/kg i.v.), 2 further groups of 10 rats each with hexobarbital (50 mg/kg i.v.) and pentobarbital (25 mg/kg i.v.), respectively. The sleeping time was estimated as the time taken following injections for the animals to rise from a reclining to a standing position.

Results are given as mean±SEM ($\bar{x}\pm S_{\bar{x}}$). Statistical analysis of experimental values in comparison to controls was carried out using Student's t-test. Differences are denoted as significant when $p<0.05$.

Results. Feeding the rats for 25 weeks with the flavonoid-deficient diet increased their b.wt from 248±3 g (n=60) to 430±5 g (n=60), whereas the controls showed a significantly lower increase i.e. from 240±2 g (n=60) to only 391±5 g (n=60). In flavonoid-deficient rats, caffeine, harmine, morphine, hexobarbital and pentobarbital acted significantly longer than in normally fed animals. In contrast, thiopental was found to cause a decreased sleeping time (table).

Discussion. The prolonged action of metabolically inactivated barbiturates such as hexobarbital and pentobarbital[6] in flavonoid-deficient rats, as already demonstrated in animals of the Sprague-Dawley strain[3,4], has now been found to be a more general phenomenon, since, in the present experiments, similar results were obtained in Wistar rats. In contrast, thiopental, which is well known to be inactivated by redistribution phenomena[6], showed a decreased duration of action. The shortened action of thiopental may be related to a possible elevation of the lipid content in the deficient rats which, indeed, showed a greater increase of b.wt during the feeding period than did the controls.

Hexobarbital is also partially inactivated by redistribution. Nevertheless, it caused a prolonged sleeping time in the deficient animals. The prolonged effect of hexobarbital could be explained if an impairement in its metabolism counteracted the inactivation caused by redistribution. In rats, as in other species, caffeine, harmine and morphine are all eliminated mainly by metabolic degradation[7-9].

In order to explain the prolonged effects of these drugs in a flavonoid-deficiency state, the assumption is made that the metabolic inactivation of all these compounds in the liver is impaired, as seems to be the case with the metabolically eliminated barbiturates. Recently, it was shown that a flavonoid-free diet in rats caused distinct changes of the hepatic enzyme profile[10]. This may also be related to a general impairment of drug metabolism. These results support the concept that flavonoids have a vitamin-like character[2].

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Effect of non-steroidal anti-inflammatory drugs on Moloney sarcoma virus inoculated mice

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Summary. Chronic administration of phenylbutazone, flufenamic acid and a new, potent non-steroidal anti-inflammatory agent ITF (3-methyl-5-benzoyl-amino-isothiazole-4-carboxy-p-ethoxyphenylamine) to BALB/c mice inoculated with a Moloney sarcoma virus resulted in a stimulation of tumor growth and increased severity of disease. This treatment, however, had no effect on the spontaneous regression of tumors. Indomethacin in a dose of 5.0 or 2.5 mg per kg suppressed the MSV-induced tumor growth, but this effect was apparently connected with the high toxicity of this drug for mice.

The Moloney sarcoma virus (MSV) tumor in the mouse 'is at the same time a malignant disease and an infectious disease, with spreading of the virus from producer cells to surrounding normal cells'[1]. It has frequently been used as a model in tumor immunology, since large tumors develop rapidly at the site of virus inoculation, and in the mature animals the neoplasms regress in almost all instances. The regression process is immunologically mediated[1-3]. The tumor mass in the MSV-infected animals consists mainly of the neoplastic cells and infiltrating inflammatory cells, which in turn consist principally of T lymphocytes and macrophages[2,3].

Recently, Humes and Strausser et al.[4-7] reported that chronic administration of indomethacin, a potent prostaglandin synthetase inhibitor, to BALB/c mice inoculated with MSV, delayed the onset of the tumor and suppressed the tumor growth. The authors suggested that indomethacin may act by inhibiting prostaglandins synthesized in large amounts in tumors, and that this process may help to restore the depressed immune response of sarcoma-bearing mice[6,7].

Independently, Seifter et al.[8] showed that the feeding of laboratory chow containing 325 mg of aspirin per kg diet, to CBA mice inoculated with MSV, resulted in the decrease in tumor incidence and severity of disease. The authors characterized the action of aspirin as being anti-viral rather than antitumor. These observations and suggestions were intriguing, but at the same time they were difficult to reconcile with several facts. First of all, since the anti-inflammatory drugs inhibit influx as well as reactivity of

the inflammatory cells (determinant in tumor rejection), one would expect the drug-induced enhancement of tumor growth than its inhibition. The anti-inflammatory hormones, cortisone and ACTH, enhance virus production and suppress the capacity of mice to reject MSV-induced tumors[1].

On the other hand, it has been repeatedly demonstrated that prostaglandins E inhibit proliferation of tumor cells in vitro[10,11] and in vivo[11,12]. The inhibition of prostaglandin synthesis by indomethacin stimulated the rate of tumor cell proliferation[10].

In view of the great role of the non-steroidal anti-inflammatory drugs in human therapy, it would seem important to determine whether other agents than indomethacin or aspirin can affect the growth of an experimental tumor.

Materials and methods. Male BALB/c mice, 3–4 weeks old, derived from our own colony of the Institute of Immunology and Experimental Therapy in Wrocław (breeders were originally supplied by Dr E.A. Boyse, Memorial Sloan Kettering Cancer Center, New York, USA). The MSV used derived from the preparation provided by Dr J.P. Lévy, Hôpital Cochin, Paris, France. The virus was passed in 3–4-day-old BALB/c mice. The 20% (w/v) extract of tumors was prepared in Eagle's Minimal Essential Medium MEM and it was stored in liquid nitrogen. Shortly before use MSV was diluted 1:4 or 1:5 in MEM and 0.15 ml of the preparation was injected into the right hind leg of the experimental mice.

For the experiments we used: phenylbutazone (Polfa), indomethacin (Polfa), flufenamic acid (Parke, Davis and Co.) and a new potent anti-inflammatory compound, ITF (3-methyl-5-benzoyl-amino-isothiazole-4-carboxy-p-ethoxy-phenylamine) synthetized by Prof. Z. Machoń[13,14]. The suspensions of the drugs were prepared in 1.25% methylcellulose (Loba Chemie, Austria) in phosphate buffered saline. There were 9 groups of mice, each containing 10 animals. Beginning at the day of virus inoculation, 0.2 ml of each drug was injected i.p. every other day for 2 weeks. The doses in the treatment series were: indomethacin 2.5 and 5.0 mg/kg, phenylbuthazone 17 mg/kg, flufenamic acid 17 mg/kg and 8.5 mg/kg, and ITF 29.5 mg/kg. The control mice received no treatment. The growth pattern of tumor was determined by daily measurements of MSV-infected hind legs. Results were analysed statistically by Student's t-test.

Results and discussion. Results of the experiments (figures 1 and 2) showed that chronic administration of phenylbutazone, flufenamic acid or compound ITF had a marked potentiating effect on the growth of MSV-induced tumors. In the drug-treated mice, tumors appeared earlier and were larger than in the control, untreated mice. Statistical significance of the difference among drug-treated and control groups of mice were $p < 0.005$ or $p < 0.001$ 9–11 days after infection with MSV. The spontaneous regression of tumor occurred in all mice, drug-treated and untreated (figures 1 and 2) and no deaths due to tumors were observed.

In contrast, indomethacin at the dose of 5.0 mg/kg or 2.5 mg/kg suppressed the growth of MSV-induced tumors ($p < 0.005$ 9 days after infection).

However, at the dosage used for the experiments, indomethacin was toxic for BALB/c mice. The indomethacin-treated animals developed suppression of weight gain dur-

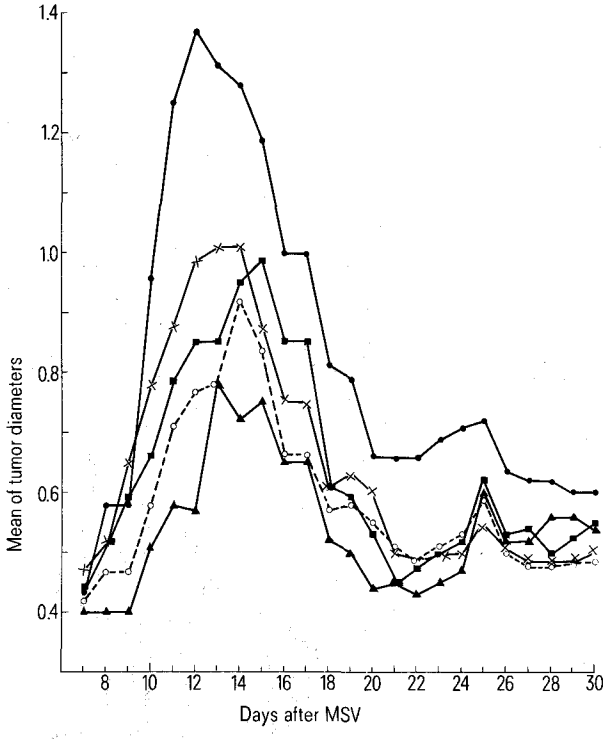

Fig. 1. Effect of non-steroidal anti-inflammatory drugs on infection of mice with Moloney sarcoma virus: O - - - O, infected untreated; ×——×, treated with phenylbutazone (17 mg/kg); ●——●, treated with ITF (29,5 mg/kg); ▲——▲, treated with indomethacin (5 mg/kg); ■——■, treated with indomethacin (2.5 mg/kg).

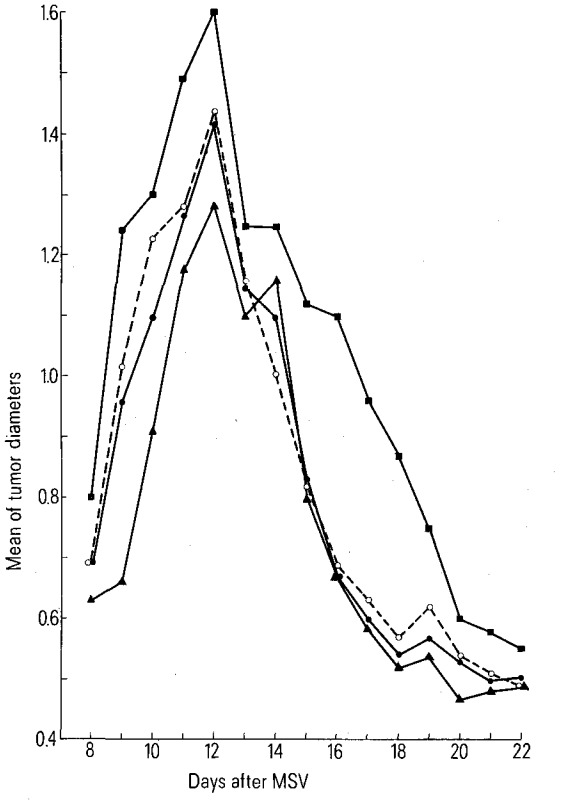

Fig. 2. Effect of non-steroidal anti-inflammatory drugs on infection of mice with Moloney sarcoma virus: O - - - O, infected untreated; ■——■, treated with phenylbutazone (17 mg/kg); ●——●, treated with flufenamic acid (8.5 mg/kg); ▲——▲, treated with indomethacin (2.5 mg/kg).

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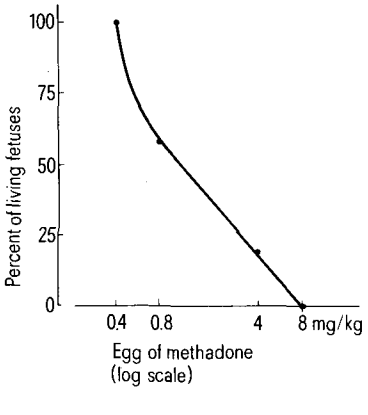
Summary. Injections of methadone into the air space of fertile chicken eggs affected development of the embryo. Both methadone and morphine caused decreases in liver weight and brain protein, and morphine increased liver protein levels.

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.