tion triggered at the site of injury by heat-modified tissue proteins (*9*). Complement-mediated release of histamine from induced mast cells appears to follow enzymatic pathways for degranulation (*6*); heat-stimulated histamine release has not been proved to proceed enzymatically (*10*). Fredholm and Hagermark (*8*) have demonstrated a delayed histamine release with heating of rat peritoneal mast cells above 52°C. At higher temperatures the histamine release became more prompt and was almost instantaneous as the surrounding temperature exceeded 60°C. A similar pattern of delayed histamine release was observed by Horakova and Beaven (*11*) in animals subjected to mild thermal injury (53°C); however, increased histamine was measured almost immediately after more severe (56°C) thermal injury. Conversely, cold (5°C) has been shown to cause prompt, complete, and reversible inhibition of histamine release from rat peritoneal mast cells induced by antigen and compound 48/80 (Burroughs Wellcome Co.) (*10*). Histamine release caused by lytic agents (Triton X-100) was not inhibited by cold. It appears, therefore, that heat-stimulated mast cells not undergoing lysis, or complement-stimulated mast cells in burned tissues, can be "stablilized" by cold (CWT) and the resultant histamine release substantially inhibited. Our observations support this hypothesis.

Thus we conclude that the histamine released from a burn wound is the predominant vasoactive substance that mediates a delayed systemic permeability response and leads to remote edema formation after moderate scald injury, apparently by stimulation of the H_2-histamine receptor. Immediate CWT of scald burns may inhibit heat-stimulated and complement-induced degranulation of viable mast cells, preventing increased histamine release from burned tissues. Cold has also been demonstrated to inhibit kininogenase activator in human serum (*12*). This enzyme inactivation effectively inhibits kinin formation. It appears likely, then, that CWT properly applied to burned surfaces or, perhaps, to any traumatized or inflamed tissues, might effectively inhibit kinin formation as well as histamine release.

The clinical implications of our findings should signal the need for a reevaluation of the pathophysiology and treatment of burn shock. It is apparent that the use of cold-water therapy (or cooling) in the early treatment of burn victims should be reexamined. Aside from its use for pain reduction and burn edema suppresion, the potential of CWT as an effective means of promoting cardiovascular stability after burn injury appears promising. Such therapy in cases of major burns could prevent significant intravascular losses, hypovolemia, and "burn shock." The use of combined H_1- and H_2-histamine receptor antagonists in the early treatment of burns also appears to warrant investigation.

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Liver Tumors Induced in Rats by Oral Administration of the Antihistaminic Methapyrilene Hydrochloride

Abstract. *The antihistaminic over-the-counter drug methapyrilene hydrochloride, mixed with food at a concentration of 0.1 percent, was administered to 50 male and 50 female Fischer rats. A second group of 50 male and 50 female rats was given the same treatment together with 0.2 percent of sodium nitrite added to the food. Almost all of the rats in both groups developed liver neoplasms, mainly hepatocellular carcinomas and cholangiocarcinomas. The first rat died with a liver neoplasm at the 43rd week. Over 50 percent of the rats in both groups had metastases from the carcinomas of the liver to distant organs. Control rats treated with nitrite only, or untreated, did not develop liver neoplasms. There was no discernible effect of nitrite on the carcinogenicity of methapyrilene hydrochloride.*

In a previous study (*1*) the possibility was investigated that the common antihistaminic methapyrilene, used in many over-the-counter sleep aids in the United States, might form carcinogenic nitrosamines by reaction with nitrite in the mammalian stomach. Of 30 rats given methapyrilene hydrochloride in their drinking water together with sodium nitrite for 18 months, nine developed liver tumors (*1*). Although a nitrite-treated control group (which did not develop liver tumors) was included in the study, no group of animals was given methapyrilene alone. We have therefore repeated the study using groups of rats giv-

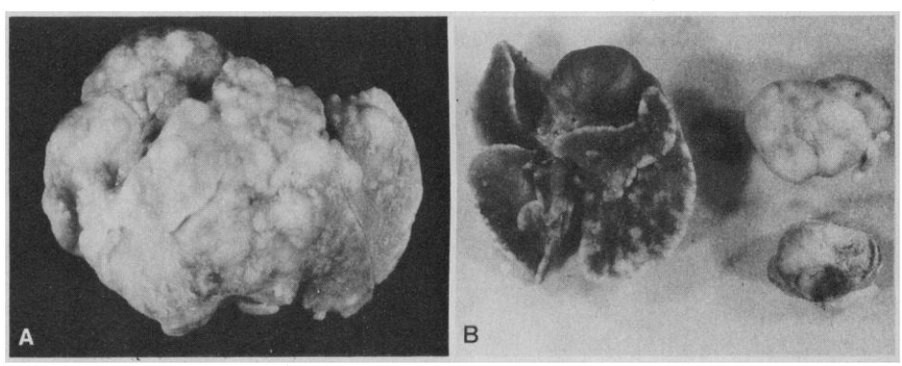

Fig. 1. (A) Carcinoma of the liver. The liver is replaced by firm, white carcinoma which is invading and replacing the adjacent liver tissue. (B) Carcinoma of the liver has metastasized to the lungs and portahepatic lymph nodes.

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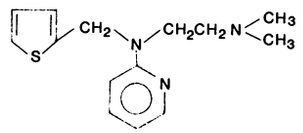
en methapyrilene plus nitrite or methapyrilene hydrochloride alone.



Methapyrilene

Fifty Fischer 344 rats of each sex (6 to 7 weeks old) from the colony of Charles River, Michigan, were fed a mixture of methapyrilene hydrochloride (*2*) in powdered food at a concentration of 0.1 percent (1000 parts per million). Another group of 50 male and 50 female Fischer 344 rats of the same age was fed a mixture of 1000 ppm methapyrilene hydrochloride plus 2000 ppm of sodium nitrite in powdered rat food. The rats were given free access to the diets and also received neutral tap water to drink. They were housed in polycarbonate cages with wire mesh bottom. A group of 20 male and 20 female Fischer 344 rats served as untreated controls. No concurrent group of nitrite-treated controls was used, although 6 months before we began this study we had started feeding a group of male and female rats on a powdered diet containing 2000 ppm of sodium nitrite.

At the beginning of this we gave the rats methapyrilene hydrochloride at a concentration of 2000 ppm in their food, but it was apparent within a few days that the animals found this unpalatable and were not eating much. We therefore lowered the concentration to 1000 ppm, and this seemed to be acceptable. Throughout the study, the average food consumption was approximately 30 g per day by the male rats and 20 g per day by the female rats, equating to 30 mg and 20 mg, respectively, per day of methapyrilene hydrochloride.

Treatment of both groups of rats continued until the 64th week, after which the animals received powdered food without the chemicals. The first animal died at the 43rd week of the experiment and had a large neoplasm of the liver. Thereafter animals of both groups and both sexes died with regularity, all with essentially the same type of neoplasm of the liver. A considerable number of animals had died by the 64th week and it was considered not worthwhile to continue treatment with the chemicals. Remaining animals in the treated groups were killed at the 83rd week of the experiment. Only two of the untreated control animals had died at that time, and neither had a liver neoplasm. Of the 40 rats given nitrite in powdered food, 21 had died by the 117th week of treatment; the remainder were killed at 130 weeks. Only one of these nitrite-treated rats had a liver neoplasm, an adenoma. The incidence of liver neoplasms in a large group of untreated Fischer rats kept for 2 years in several laboratories (including this one) was 1.7 percent in males and 3.1 percent in females (*3*).

The pattern of death of the rats receiving methapyrilene or nitrite plus methapyrilene is shown in Table 1. There appears to be little or no difference between the two groups, which suggests that formation of nitrosamine in vivo, if it occurs, does so at such a low level as to have no detectable influence on the carcinogenic effectiveness of methapyrilene, as measured in this experiment. Since virtually all of the treated animals of both groups developed liver neoplasms, no significant effect of the admixture of nitrite can be seen on tumor incidence.

On gross examination the livers were much enlarged and replaced by carcinomas (Fig. 1A). Carcinomas sometimes invaded the stomach, spleen, and pancreas. Metastatic carcinoma was present in the lungs, portahepatic lymph nodes, omentum and peritoneum, as well as other distant organs (Fig. 1B) (Table 2).

Histologically, the carcinomas were hepatocellular carcinomas and cholangiocellular carcinomas (Fig. 2, A and B) (Table 2). Hepatocellular carcinomas were made up of cells that varied from well to poorly differentiated to anaplastic, and grew in cords or sheets. Cholangiocellular carcinomas also were well or poorly differentiated or anaplastic.

Table 1. Survival of Fischer rats treated with methapyrilene hydrochloride (1000 ppm) with and without sodium nitrite (2000 ppm).

Sex of rats	Number of survivors at week:										Number of rats with liver tumors
	0	40	45	50	55	60	65	70	75	80*	
Methapyrilene hydrochloride											
Male	50	50	50	48	48	46	44	41	33	24	48
Female	50	50	49	49	48	48	45	39	33	27	48
Methapyrilene hydrochloride plus sodium nitrite											
Male	50	50	50	50	49	45	44	39	28	17	47
Female	50	50	50	50	50	47	45	41	33	26	49

*Survivors were killed between weeks 81 and 83.

Table 2. Incidence of tumors in rats treated with methapyrilene hydrochloride with and without sodium nitrite.

Sex of rats	Number of tumor-bearing animals	Number of rats with liver neoplasms			Number of rats with metastases
		Hepatocellular carcinomas	Cholangiocarcinomas	Hemangioendothelial sarcoma	
Methapyrilene hydrochloride					
Male	48	38	47	1	31
Female	48	36	45	1	26
Methapyrilene hydrochloride plus sodium nitrite					
Male	47	37	38	2	34
Female	49	45	45	2	25

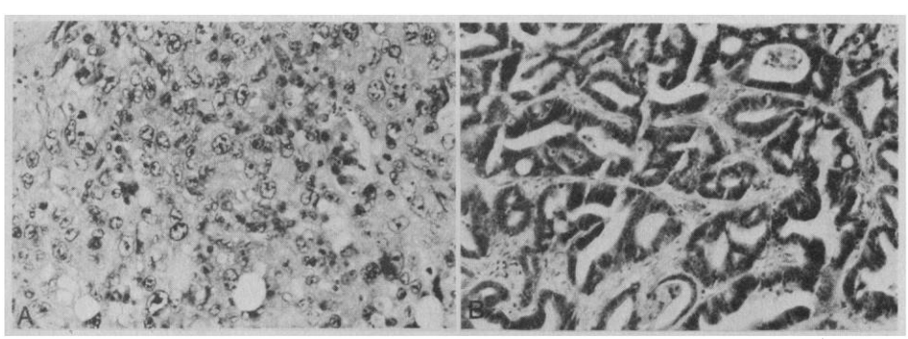


Fig. 2. (A) Anaplastic carcinoma of the liver. Cells vary greatly in size and shape and grow in solid sheets. Nuclei are huge. (Hematoxylin and eosin; ×330) (B) Cholangiocellular carcinoma of the liver. Columnar cells with oval nuclei and eosinophilic cytoplasm form ducts. There is a fibrous connective tissue stroma. (Hematoxylin and eosin; ×220)

Metastatic carcinoma was often seen in the lungs, portahepatic lymph nodes, and omentum and peritoneum; however, metastases were also found in such organs as brain, ovary, kidney, thymus, spleen, and parathymic and parapancreatic lymph nodes.

Our conclusion is that methapyrilene hydrochloride is strongly carcinogenic for the liver of Fischer 344 rats of both sexes, inducing almost a 100 percent incidence of liver neoplasms after 43 to 64 weeks of treatment, many with metastases. Sodium nitrite in this experiment did not appear to affect the incidence or type of methapyrilene-induced tumors. The total dose of methapyrilene hydrochloride received by the male rats was between 9 and 13.5 g (22 to 34 g per kilogram of body weight), and by the females, 7 to 9 g (30 to 40 g per kilogram of body weight). The dose of methapyrilene hydrochloride recommended for humans in several over-the-counter sleep aids was 50 mg. Several million people may have taken this compound, some for several years. After we presented preliminary reports of our findings, the manufacturers withdrew methapyrilene from the market. Some compounds of similar structure are in use.

Methapyrilene is a potent liver carcinogen in rats, yet its chemical structure does not resemble that of any known class of carcinogen. It is possible, therefore, that it represents a new type of carcinogen. It is also notable that in the *Salmonella* mutagenesis test devised by Ames (*4*), methapyrilene has not been mutagenic when activated by rat liver microsomal fractions. Neither has methapyrilene transformed hamster embryo cells in culture when activated by liver microsomes in the test described by Pienta *et al.* (*5*).

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Induction of Follicle Separation in the Mosquito by Physiological Amounts of Ecdysterone

Abstract. *Physiological quantities of the molting hormone, ecdysterone, injected into female* Aedes aegypti *prematurely induced separation of incipient follicles in the ovarioles, an event that normally occurs only in blood-fed females. It was possible to stimulate this morphological event with physiological amounts of hormone by mimicking, with two injections, the timing of normal increases in endogenous hormone of blood-fed females.*

The development of insects is regulated by the interplay of juvenile hormone (JH), ecdysteroids, and hormones from the cerebral neurosecretory system. These same hormones are also produced by the adult insect. Both in larval stages and in the adult, JH is synthesized and secreted by the corpora allata. In the larval stage ecdysone is produced by the prothoracic glands, which degenerate in most adult insects. However, the presence of ecdysteroids in the ovaries of adult or pharate adult insects is well documented (*1*-*6*), and its heterogeneity between male and female insects has been demonstrated (*4*, *5*, *7*). Nevertheless, the function of ecdysteroids in female insects is still not clear because only a very high concentration of exogenous ecdysterone will produce a response in vivo (*8*, *9*). We now report on the stimulation of a normal morphological event, the formation and separation of new follicles in the mosquito ovary, by physiological amounts of ecdysterone.

Each ovary of *Aedes aegypti* has about 75 ovarioles, each of which consists of a single primary follicle and germarium. These primary follicles grow in unison. A few days after emergence of the adult, and prior to the first blood meal, a secondary follicle becomes visible in each germarium (Fig. 1A). About 20 hours after the blood meal, as yolk deposition is about half completed in the primary follicles, each secondary follicle separates from its germarium (Fig. 1, B and C). By the time the primary follicle becomes a mature egg and is laid, each

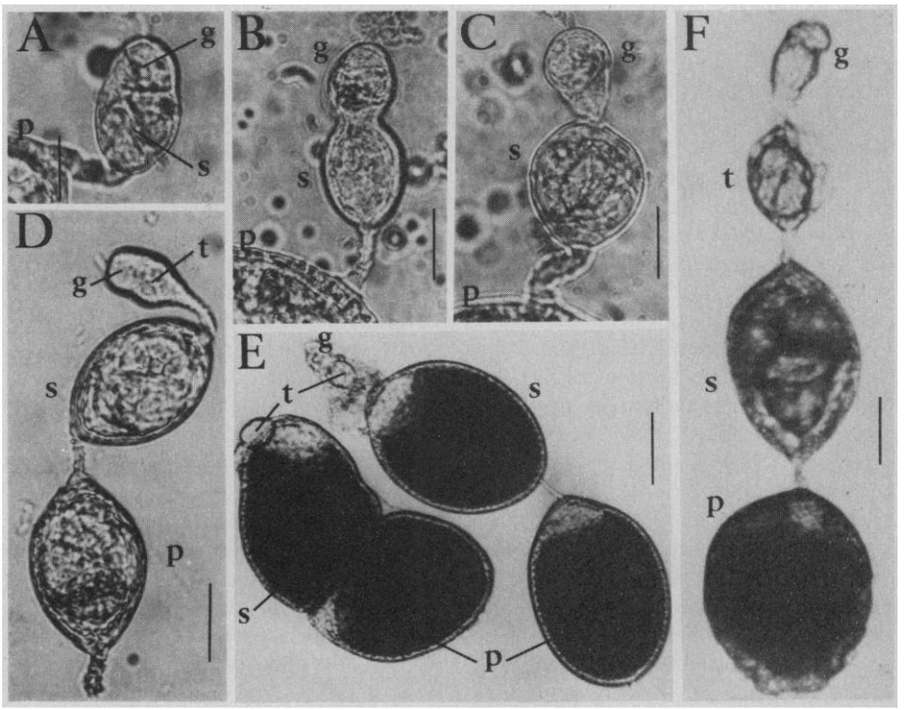

Fig. 1. Separation of the secondary follicle from the germarium in the ovary of *Aedes aegypti* (*19*). (A) Ovariole of unfed female. (B) Ovariole 17 hours after blood meal. (C) Separation complete 21 hours after blood meal. (D–F) Formation of additional follicles induced by ecdysterone: (D) Ovariole of unfed female after two injections of ecdysterone (as in Table 1, experiment A). (E) Same treatment as (D) but, in response to a blood meal taken 2 days after the first injection, yolk was deposited in both the primary and new secondary follicles. Small tertiary follicles had formed 24 hours after the meal. (F) Ovariole of female repeatedly injected with ecdysterone (*11*). A secondary and tertiary follicle have separated and another follicle is forming in the germarium. Vertical bars: 25 μm in (A) to (C), 50 μm in (D) and (F), and 100 μm in (E). Abbreviations: *g*, germarium; *p*, primary follicle; *s*, secondary follicle; and *t*, tertiary follicle.

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