The Effect of the Carcinogenesis and the presence of Neoplastic Cells on the Mitotic Activity of Oral Epithelia in Rats and Mice

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INTRODUCTION

It is well known that the mitotic rate of the epithelial cells of digestive tract is fairly constant under normal conditions and hence it is possible to speak of the mitotic homeostasis of the organs.[3)17)19)] On the other hand, it is easily affected by the change of external and internal environmental conditions with considerable reversibility (general nutritional state,[14)15)30)] hormone,[9)18)29)] radiation,[40)] karyoklastic agents,[37)] and physiological variation such as diurnal rhythm,[3)6)39)] for example).

There is little information on the mitotic activity of digestive tract in pathological conditions, especially on the possible competition for growth between tumor tissue and actively growing tissue in an otherwise normal organism. Suzuki[34)35)] observed that gastric and buccal mucosa cells, which normally undergo a rapid renewal, showed a significant depression in mitotic frequency in cancer patients. A question arises whether the depression of mitotic activity of body tissues in cancer-bearing individuals has already existed in the precancerous stage.

In an attempt to learn the effect of the presence of neoplastic cells on the mitotic activity of oral epithelia in rats and mice, the present investigation was undertaken from two points of view; in one group of experiments the effect of a liver carcinogen DAB on the mitotic activity of buccal mucosa in rats was studied at various stages of carcinogenesis, in another the mice were transplanted with Ehrlich ascites tumor intraperitoneally and subsequent effects on the mitotic activity of tongue surface were observed. Furthermore, the effect of an anabolic steroid hormone on mitotic activity of oral mucosa and growth of tumor tissues was also examined.

MATERIALS AND METHODS

The experiment was carried out in the following two groups.

Expeiment A:

Male and female rats of Wister strain weighing 140 to 240 gm. were used in

this investigation. The animals were fed a diet containing the potent carcinogen, p-dimethyl-aminoazobenzen (DAB), for 150 days from May to September. The diet used was composed of 75 per cent rice powder, 10 per cent casein, 4 per cent salt mixture, 1 per cent vitamin mixture, 1 per cent plant oil and 0.09 per cent DAB.[25] The animals were given free access to this diet and fresh water. Cages housing the animals were kept at natural temperature without access to direct sunlight.

Thirty rats were given 0.35mg./100gm. body wt. of anabolic steroid hormone (Durabolin Sankyo) into the thigh muscles once a week. Forty rats without it were also examined as a control.

Beforehand, as preliminary experiment, it has been proved that anabolic steroid hormone stimulates the mitotic activity of the epithelium of buccal mucosa and superior aspect of the tongue (Fig. 1 and 2).

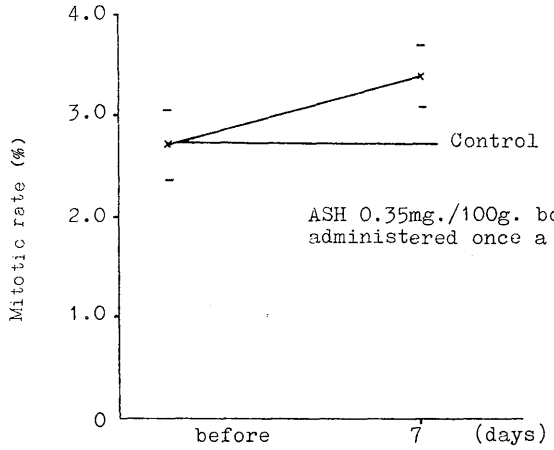

Fig. 1. Changes of mitotic counts of the buccal mucosa in male rats after administration of anabolic steroid hormone (ASH)

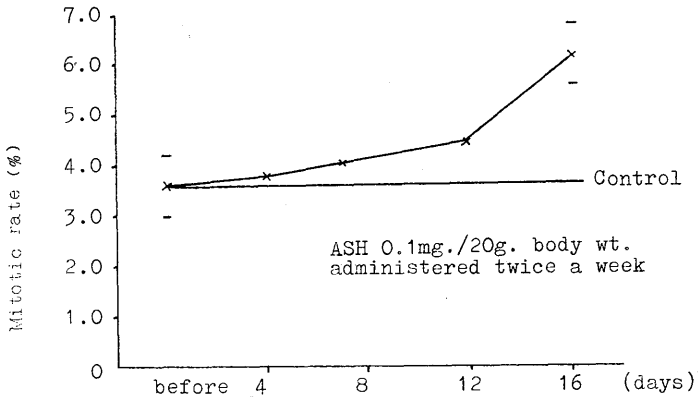

Fig. 2. Changes of mitotic counts of tongue surface in male mice after administration of anabolic steroid hormone (ASH)

To obtain high mitotic values, the colchicine method was used. Colchicine arrests the mitotic process at metaphase stage.[4)36)] The rats were given 0.15 mg./100 gm. body wt. of colchicine subcutaneously at 10:00 A. M. and the specimens of buccal mucosa were taken at 4:00 P. M.. It is said that a frequency peak of colchicine mitoses appears approximately six hours after colchicine injection.

The specimens of buccal mucosa were taken before, and once every 30 day after giving the toxic diet. After 150 days, all animals were killed with ether and their livers were observed.

The specimens obtained from buccal mucosa were fixed in 10 per cent Formalin and blocked in paraffin. The specimens were cut into sections, 5 μ. in thickness, and stained with Hematoxylin and Eosin.

In the estimation of the mitotic activity, all figures observed under a magnification of 600 were counted in the basal layer of the section where the mucosa was cut vertically to the base and any area obliquely cut was omitted. At least 2000 nuclei of resting and dividing cells were counted in each specimen.

Expeiment B:

Adult male of dd strain mice weighing 18 to 22 gm. at the beginning of the experimental period, were used in this investigation. The animals were maintained on Oriental Chow with free access to food and water during the experimental period.

The strain of Ehrlich ascites tumor cells was originally obtained from the 1st Pathological Dept. of Yamaguchi University School of Medicine, and maintained for 12 days in our laboratory. The mice were transplanted 2 million cells of Ehrlich ascites tumor intraperitoneally. The mice were injected 0.1mg./20 gm. body wt. of anabolic steroid hormone into the thigh muscles twice a week. Each animal was injected 0.2mg./100gm. body wt. of colchicine subcutaneously at 10:00 A. M. and the specimens of tongue and ascites tumor cells were taken at 4:00 P.M. under deep anesthesia with ether 4, 7, 12 and 16 days after the transplantation of Ehrlich ascites tumor. The specimens were prepared in the same manner as described above. The ascites tumor cells taken from the abdominal cavity were smeared on the object-glasses and after fixation in Methanol they were stained with Giemsa's method.

RESULTS

Mortality was lower in the male rats given the anabolic steroid hormone than in the control. But it was higher in the female rats given anabolic steroid hormone than in the control. As a whole, male rats showed higher mortality than female rats during the course of carcinogenesis (Fig. 3).

The incidence of hepatoma was strictly lower in the male rats given anabolic steroid hormone than in the control rats (Fig. 3). The hepatoma was not seen

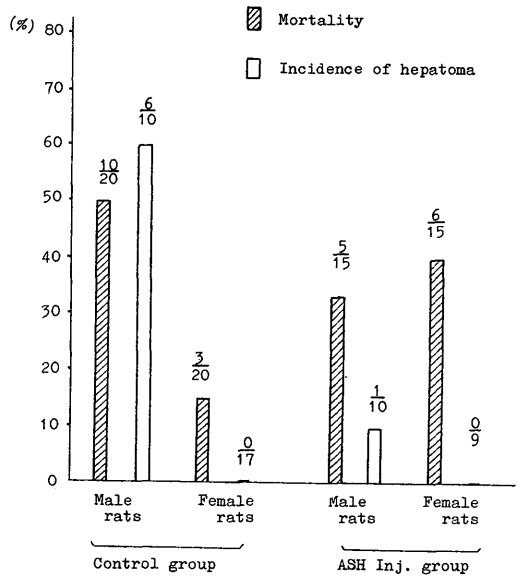

Fig. 3. Mortality and incidence of hepatoma

in the female rats.

Table 1. Mitotic rate in the epithelial cells of buccal mucosa in male rats during the course of carcinogenesis
(percentage ± standard deviation)

Days		before	30	60	90	120	150
Hepatoma-bearing rats	6 animals	3.0±0.49	2.8±0.33	2.2±0.36	1.8±0.40	1.7±0.24	1.7±0.31
Non-hepatoma-bearing rats	4 animals	3.0±0.59	2.9±0.59	2.2±0.17	2.1±0.24	2.2±0.20	2.3±0.28

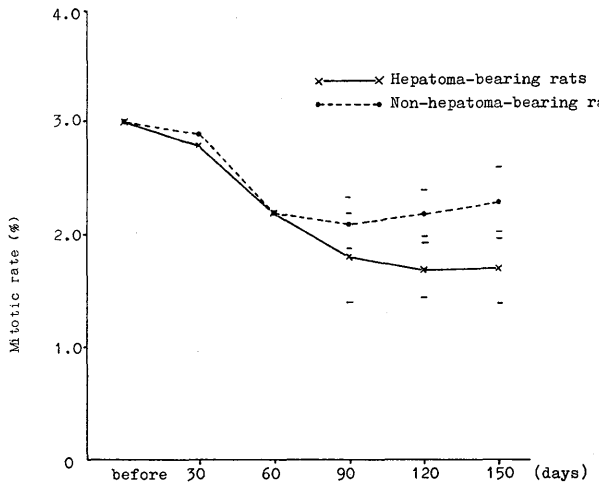

Fig. 4. Mitotic rate in the epithelial cells of buccal mucosa in male rats during the course of carcinogenesis

The results of mitotic count are summarized in Table 1 and Fig. 4. In the control group, the hepatoma-bearing rats showed lower mitotic values than the rats without tumors placed on administration of DAB for 90 days. In the group treated with anabolic steroid hormone, the values showed nearly the same as in the control group (Table 2, and Fig. 5).

Table 2. Mitotic rate of the buccal mucosa in male rats treated with anabolic steroid hormone (ASH)

(percentage ± standard deviation)

Days		before	30	60	90	120	150
Hepatoma-bearing rat	1 animal	3.2	2.6	2.0	1.6	1.6	1.5
Non-hepatoma-bearing rats	9 animals	3.1±0.44	2.8±0.28	2.4±0.36	2.1±0.26	2.0±0.28	1.9±0.17

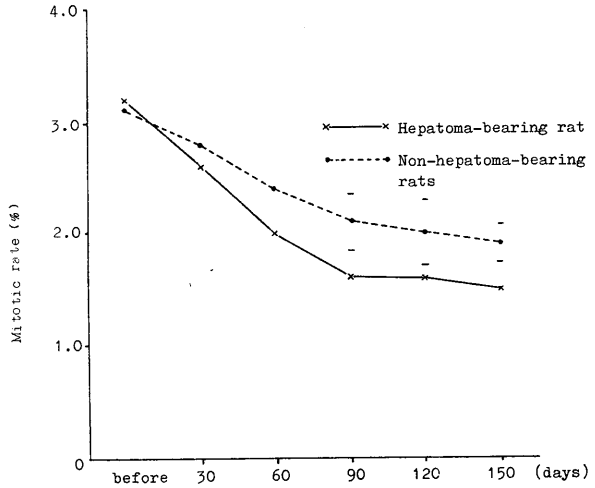

Fig. 5. Mitotic rate of the buccal mucosa in male rats treated with anabolic steroid hormone (ASH)

In the female rats, though hepatic cancer was not seen, the mitotic rate began to decrease gradually approximately 30 days after DAB feeding (Table 3, and Fig. 6). As a whole, the rate in the cancer group appeared lower than that in the non-cancer group, and each of them was strictly divided by a borderline of 2.0 per cent level.

As shown in Table 4 and Fig. 7, in the mice transplanted with Ehrlich ascites carcinoma, the mitotic rate in the superior aspect of tongue increased slightly on the 7th day and then decreased gradually. On the 16th day it fell to 56 per cent in control value. On the other hand, in the mice treated with anabolic steroid hormone, the high mitotic values were obtained; 172 per cent of control value on the 7th day and almost the same as control on the 16th day.

Table 3. Mitotic rate of the buccal mucosa in female rats
(percentage ± standard deviation)

Days		before	30	60	90	120	150
ASH non-treated group	17 animals	3.1±0.35	2.6±0.46	2.3±0.39	2.2±0.41	2.2±0.50	2.3±0.46
ASH treated group	9 animals	3.1±0.26	2.8±0.28	2.3±0.32	2.3±0.46	2.4±0.40	2.4±0.47

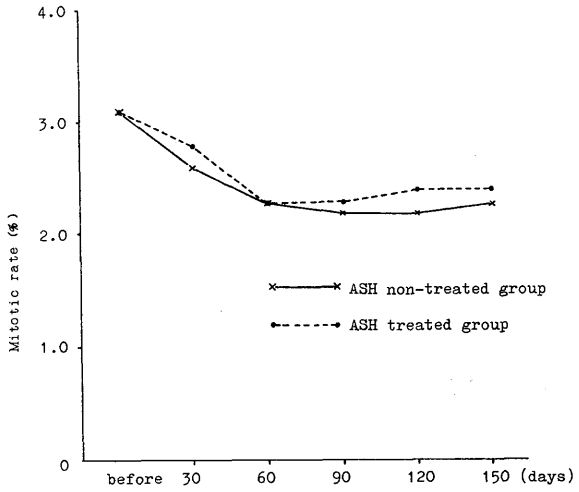

Fig. 6. Mitotic rate of the buccal mucosa in female rats

The mitotic rate of Ehrlich ascites tumor cells was summarized in Table 5 and Fig. 8.

DISCUSSON

Many effects of tumors on the physiology of the tumor-bearing host have been reported.[2)7)8)11)13)24)] One of these reported changes is an increase in the mitotic activity of the liver in mice and rats with spontaneous and transplanted tumors.[1)22)] The same phenomenon has been demonstrated also in non-tumor-bearing mice treated with a presumably cell-free supernatant fraction of tumor tissue.[22)] From these results, Malmgren et al.[23)] suggested that a circulating factor, liver mitotic stimulant, was released from the tumor. On the other hand, Suzuki[34)35)] observed a decrease in the mitotic activity of the digestive tract in cancer patients, and speculated that a mitotic inhibitor might be released from the cancer tissue. Furthermore, Holmberg[12)] observed that a polypeptide obtained from human ascites tumor fluid inhibited normal cell growth by interfering with the DNA synthesis or with processes closely linked to this. And also he speculated that the diffusible polypeptide, if presented as a large amount of free substance in body fluids, might affect tissues distant from the tumor site.

Table 4. Mitotic rate of the tongue surface of mice after transplantation of Ehrlich ascites tumor

(percentage ± standard deviation)

Days		4	7	12	16
ASH non-treated group	5 animals	3.3±0.35	3.9±0.47	3.0±0.49	2.0±0.58
ASH treated group	5 animals	5.8±0.66	6.2±0.89	4.3±0.65	3.5±0.57
Control	10 animals	3.6±0.57			

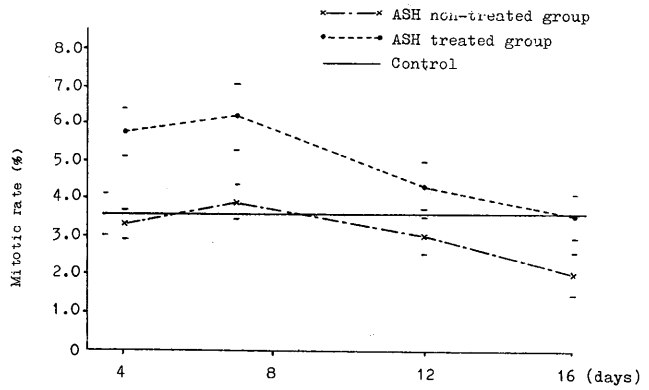

Fig. 7. Mitotic rate of the tongue surface of mice after transplantation of Ehrlich ascites tumor

Table 5. Mitotic rate in Ehrlich ascites tumor cells of mice

(percentage ± standard deviation)

Days		4	7	12	16
ASH non-treated group	5 animals	7.7±0.80	4.4±0.58	2.1±0.57	1.1±0.44
ASH treated group	5 animals	7.2±0.53	3.1±0.44	1.5±0.51	0.6±0.28

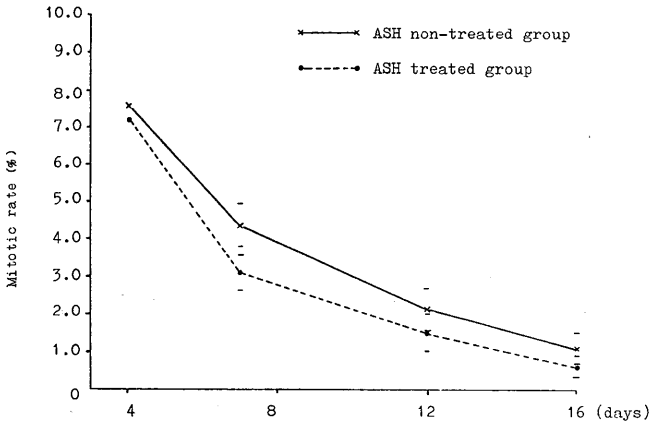

Fig. 8. Mitotic rate in Ehrlich ascites tumor cells of mice

To observe the effect of the presence of neoplastic cells on the mitotic activity of body tissues, buccal mucosa and superior surface of the tongue which are located at the uppermost part of the digestive tract were used as a material because of their easy accessibility.

Mitotic rate of epithelial cells of buccal mucosa of rats decreased gradually during the first 60 days of DAB administration. In this stage, there is no significant difference in mitotic rate between hepatoma-rats and DAB admimistered rats without liver tumors. In the rats fed more 90 days, however, the rate was significantly different between these two groups; the rate in hepatoma-bearing rats was less than that in non-hepatoma-bearing rats, and each of them was divided by a borderline of 2.0 per cent level.

Liver carcinogens have ability to damage the chromosomes and induce proliferation.[20)33)] Furthermore, the developement of unresponsiveness to growth regulations can be a third factor involved in the process of carcinogenesis.[21)] Each of the three phenomena, chromosome damage, proliferation and unresponsiveness appears to be insufficient individually to lead to the formation of neoplastic cells.[21)]

To observe the effect of mitosis stimulating agent on the mitotic activity of oral mucosa cells and incidence of hepatoma in rats during the course of carcinogenesis, an anabolic steroid hormone (ASH) was used. As shown in Fig. 1 and 2, the ASH has a little mitogenic effect in normal rats and mice. The buccal mucosa cells of rats fed DAB for long period ceased to respond to this mitosis stimulating agent. No increase in mitotic rate after ASH injection was found in either early or later stage of hepatomas produced by DAB feeding. On the other hand, only slight decrease was found in the DAB administered rats without tumors.

From these results, it is reasonable to presume that the liver carcinogen DAB induces mitotic irregularities not only in hepatic tissue but in general body tissues, and it leads to unresponsiveness to growth stimulating substance. In this connection, Maini and Stich's observation[21)] is interesting; the rats fed the liver carcinogen 3'Me-DAB ceased to respond to the mitotic stimulus of partial hepatectomy and injection of serum from partially hepatectomized rats, which stimulated mitosis in the regenerating liver lobe of normal rats, in the course of carcinogenesis. This unresponsiveness is reversible in the precancerous liver, and apparently depends on the presence of the carcinogen. While, that of hepatoma is irreversible and independent of the presence of the carcinogen and persists over many cell generations. From this observation, they have postulated that the parenchymal cells developed an unresponsiveness during the course of carcinogenesis and the mitosis stimulating factor in the injected serum became inactive before reaching the hepatic tissue.

For many years it has been known that, after partial hepatectomy in animals, the remaining liver cells multiply and rapidly restore the amount of tissue removed. Using a parabiotic animals, Bucher et al.[5)] reported that a humoral agent was

liberated by the regenerating liver which stimulated growth of the intact liver. Paschkis et al.[27][28] has speculated that the action of this humoral growth-promoting agent is not limited to the liver, inasmuch as it also enhances the growth of certain malignant tumors. As described previously, however, this humoral growth-promoting agent may be inactivated in animals fed DAB for long period.

To study the effect of the presence of transplantable neoplastic cells on the mitotic activity of tongue surface epithelia of mice, Ehrlich ascites tumor was transplanted in mice. The effect of ASH on the mitotic activity of tongue epithelial cells and that of Ehrlich ascites tumor cells was also examined. In mice transplanted with Ehrlich ascites carcinoma, the mitotic rate of tongue surface was slightly increased for the first 7 days (109 per cent) after transplantation, and it decreased gradually. On the 16th day it was fallen to 56 per cent of the control level. According to Kitamura,[16] the mitotic rate of the tongue surface was decreased gradually after transplantation of Ehrlich ascites carcinoma, and it became to 55 per cent of the control level on the 12th day.

In the group treated with ASH, the mitotic activity of the tongue surface epithelia showed a peak on the 7th day and fell to control level on the 16th day after transplantation of Ehrlich ascites tumor. In this group, it showed clearly higher mitotic activity than in the non-treated group. Conversely, the mitotic rate of Ehrlich ascites tumor cells was inhibited in the mice treated with ASH.

Paschkis et al.[26] and Trotter[38] studied the effect of partial hepatectomy on transplantable tumors in rats and mice, and found that some tumors grew more rapidly in partially hepatectomized rats than in intact rats. Some hepatomas in their early transplanted generations were considered responsive. They also found that, after partial hepatectomy the growth of the regenerating livers of rats bearing these tumors, was greater than the growth of regenerating livers of rats without tumors.[27] While, in present investigation, the mitotic rate of Ehrlich ascites tumor cells was inhibited by administration of ASH. This discrepancy can be explained in the following reason; Ehrlich ascites carcinoma is an undifferentiated tumor originated spontaneously as a carcinoma of the mammary gland of a stock mouse.[31] Anabolic steroid hormone may peculiarly control the Ehrlich ascites tumor by reason that it is a conductor of androgen.

In any way, from the result of present investigation, it is speculated that the tongue surface cells in cancer-bearing mice are responsible to ASH. While, Ehrlich ascites tumor cells are unresponsible. Furthermore, ASH has an ability to exhibit the mitotic activity of body tissues in rats during the course of DAB administration, and to inhibit the incidence of DAB hepatomas.

Finally, one may also speculate that the activation of mitotic inhibitors or inactivation of mitotic stimulators would occur in rats fed DAB for long period, and in mice transplanted with Ehrlich ascites tumor. While, ASH may inactivate the mitotic inhibitors or activate the mitotic stimulators. These two factors have

been said to be controlling the rate of growth of mammalian tissues.[10)32)]

SUMMARY

Using the rats fed DAB diet and the mice transplanted with Ehrlich ascites tumor, the relation between mitotic activity of the epithelium of the oral cavity and the presence of neoplastic cells was observed with colchicine technique. The effect of anabolic steroid hormone was also examined.

In the male rats received the ASH, the incidence of hepatoma was strictly lower than that of control rats 150 days after administration of DAB.

Mitotic rate of epithelial cells of buccal mucosa from hepatoma-bearing rats was inhibited more than that from non-hepatoma-bearing rats, and each of them was divided by a borderline of 2.0 per cent level.

The tongue surface of mice transplanted with Ehrlich ascites tumor showed clearly higher mitotic activity in the group treated with ASH than in the non-treated group. Conversely, mitotic rate of Ehrlich ascites tumor cells was inhibited in the mice treated with ASH.

From these results, it was discussed on mitosis stimulating agent in the process of carcinogenesis.

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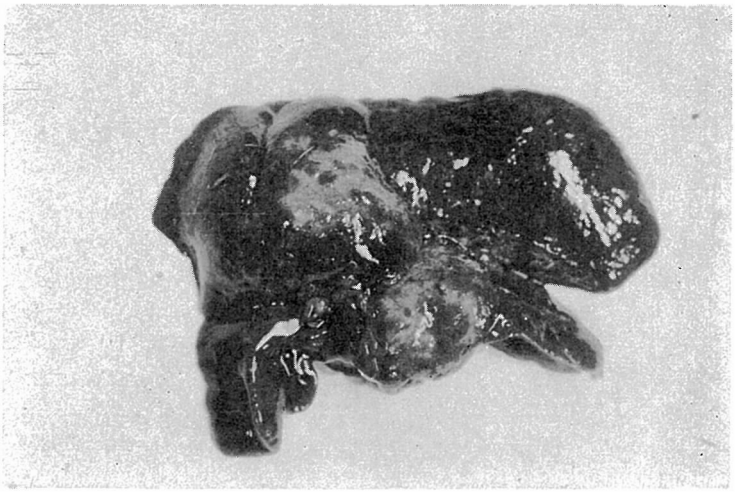
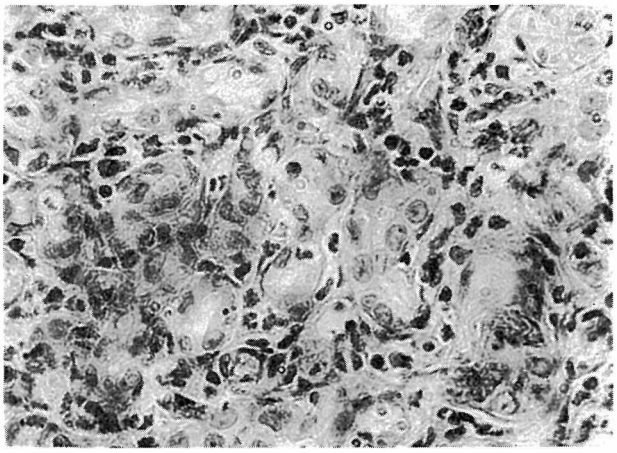


Photo. 1. A case of hepatoma of rat fed DAB diet for 150 days.



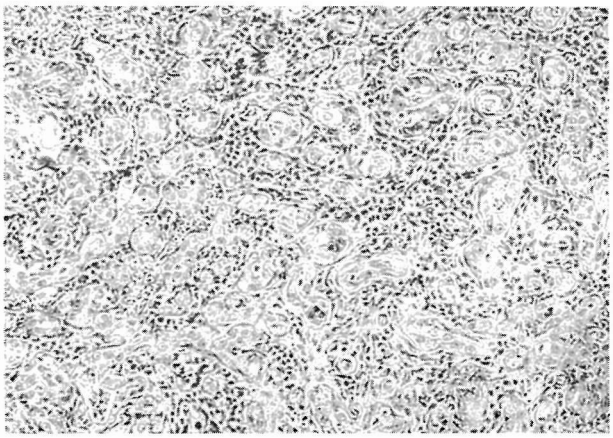


Photo. 2.
A segment of cholangioma of liver of a rat fed DAB diet for 150 days.

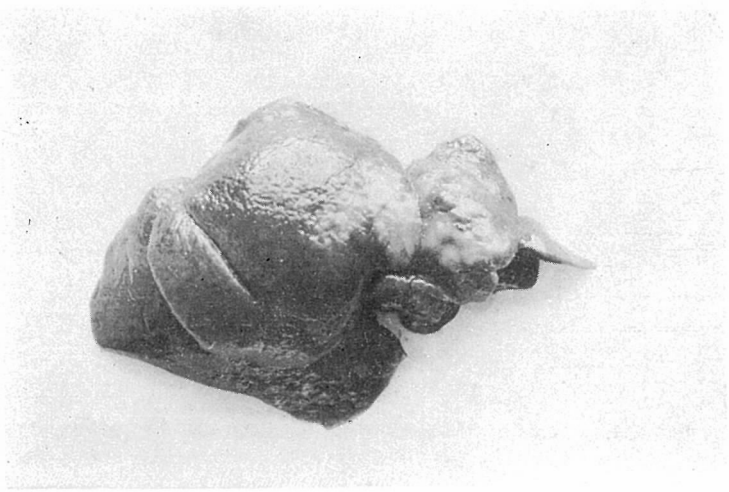


Photo. 3. A case of hepatoma of rat fed DAB diet for 90 days.

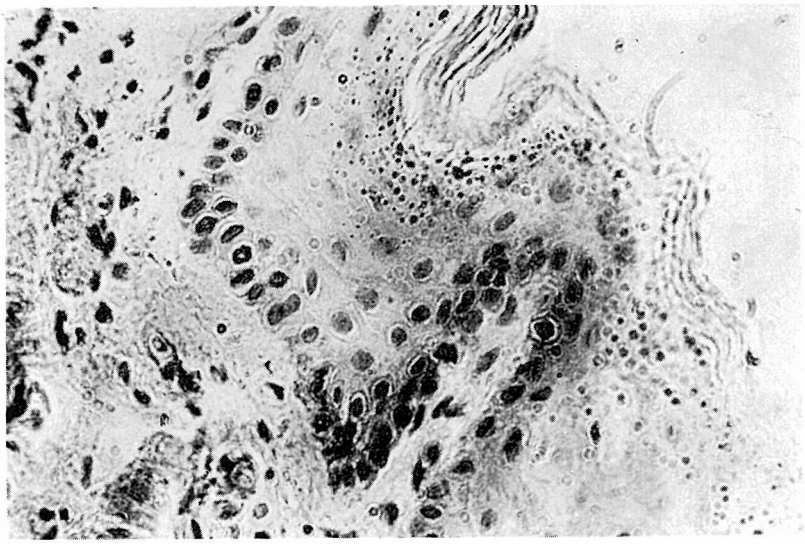


Photo. 4. Mitotic figure in buccai mucosa from a non-hepatoma-bearing rat fed DAB diet for 150 days.

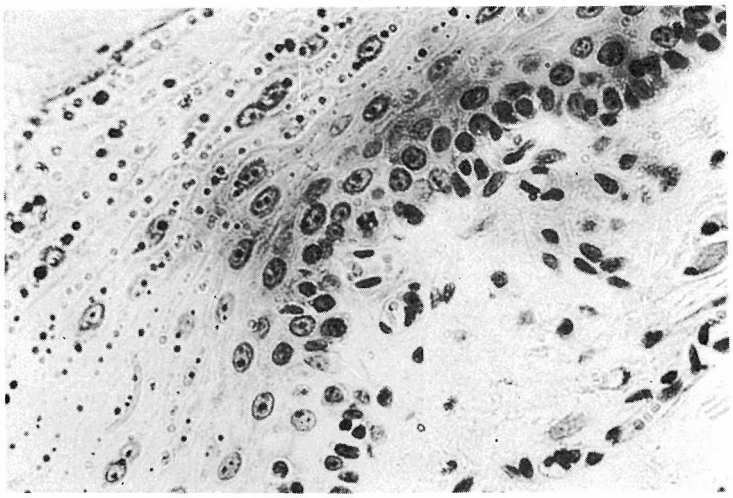


Photo. 5. Decreased mitotic activity in buccal mucosa from a hepatoma-bearing rat.

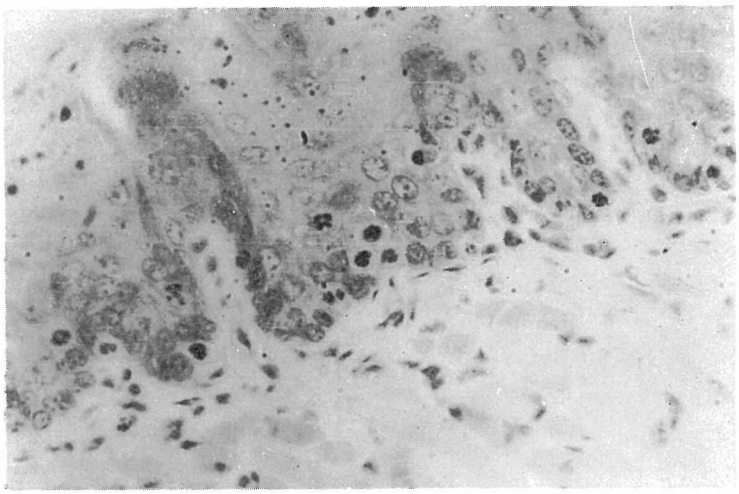


Photo. 6. Numerous mitoses in tongue surface of a mouse treated with ASH 7 days after Ehrlich ascites tumor transplantation.

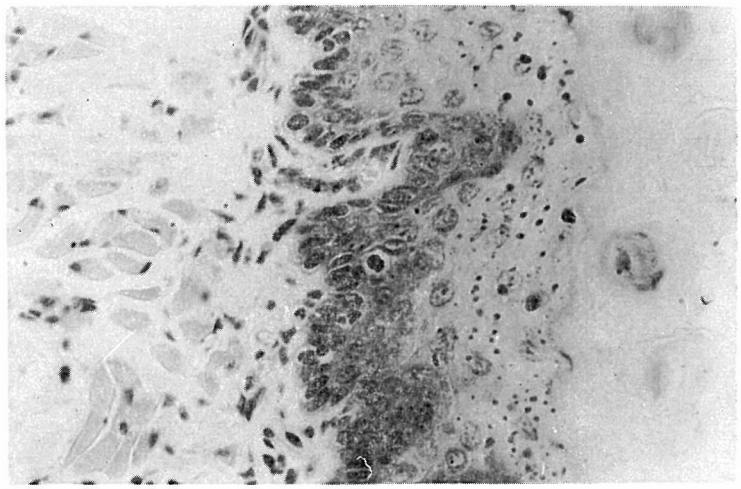


Photo. 7. Decreased mitotic activity in tongue surface from a mouse 16 days after transplantation of Ehrlich ascites tumor.

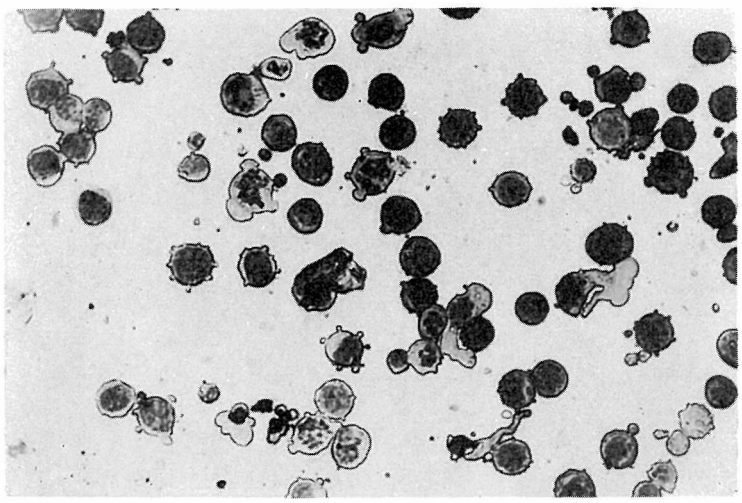


Photo. 8. Numerous mitoses in Ehrlich ascites tumor cells of a mouse 4 days after transplantation.

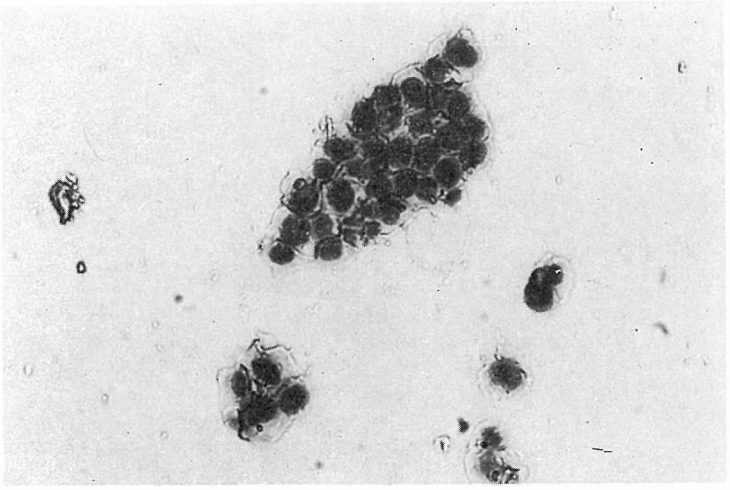


Photo. 9. Decreased mitoses in Ehrlich ascites tumor cells of a mouse 16 days after transplantation.