spread of virus to associates of individuals fed attenuated virus in an institution. Although virus was being excreted, very little spread to those individuals that had naturally acquired antibody; however, most of the vaccinated associates became infected as evidenced by both virus excretion and antibody rise.

In the present study virus was recovered from 11 of the 20 contact monkeys, but ten of these remained non-paralytic and no evidence of antibody formation could be demonstrated, suggesting strongly that virus had merely passed through the animals without multiplying or stimulating the production of antibodies. Of the nine contact monkeys not excreting virus, one developed paralysis and no antibodies were detected in the others. In fact, the only contact monkeys shown to develop antibodies were those which became paralyzed. Unless virus excretion alone in the absence of subsequent antibody rise is accepted as evidence of sub-clinical disease, an unlikely conclusion, the infection rate in the contact animals was only 2 of 20 (10%). In contrast to these findings, 17 of the 20 monkeys which had been fed virus were shown to excrete the agent; 14 of these became paralyzed and the other three developed antibodies. Of the 3 which did not excrete virus all became paralyzed; therefore, the extent of infection in these animals was 100%.

Virus appears to spread among monkeys almost as effectively as among familial associates of a human case of poliomyelitis. However, in contrast to the expected event in the human, sub-clinical infection resulting from contact exposure in simians could not be demonstrated in the present study.

*Summary.* 1) Poliomyelitis virus was fed to 4 groups of 5 monkeys each which were subsequently caged with an equal number of normal animals. 2) All of those fed virus became infected as evidenced by either the development of paralysis or virus excretion accompanied by an antibody rise. 3) Two of the contact monkeys became paralyzed and although one of these and 10 others excreted virus briefly, antibodies developed and were demonstrated only in the 2 paralyzed animals suggesting that sub-clinical infection did not occur.

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## Antimitotic Action of Maleuric Acid.* (24340)

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Since the first report of the antimitotic effects of maleic acid(1), there has been considerable interest in this and related substances such as maleimide(2), N-ethylmaleimide(2), and maleic hydrazide(3). The antimitotic activity of these compounds has been attributed largely to their ability to form –SH adducts(2). Indeed, the ultraviolet spectral changes accompanying the interaction of –SH compounds with N-ethylmalei-

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mide have been utilized in establishing a rapid spectrophotometric procedure for the quantitative determination of –SH groups(4). It was of interest to study the effects of maleuric acid (monomaleylurea) on living cells,, since this compound combines the structural features of maleic acid with some of those of glutamine (as seen from molecular models). a substance of special interest in the study of metabolism of tumor cells [see(5) for pertinent references].

*Methods.* A sample of maleuric acid (MA) was obtained from the Naugatuck Chemical Division of the U. S. Rubber Company, Naugatuck, Connecticut. Bulbs of the common onion, *Allium cepa,* were placed on top of bottles containing tap water at 20°C, which was replaced at 24 hour intervals until 48 hours, at which time the solution was changed to tap water containing 0.09% MA ($5.69 \times 10^{-3}$ M). Single roots were taken at random from each of the treated bulbs at 2, 4, 8, 10, 24, and 48 hours. The roots were fixed in 3:1 alcohol-acetic acid, squash preparations made from the meristem, and staining performed with acetic dahlia. Roots from control bulbs kept in tap water alone were treated in a similar manner and counts of 3,000 cells were made at the beginning and end of the experiment. At least 3,000 cells were counted for each experimental sample and the numbers of cells in each phase of mitosis recorded. The cytological effects of MA on Ehrlich ascites tumor cells also were studied. MA (2-6 mg) contained in 0.5 ml of physiological saline was injected intraperitoneally into C57 black mice approximately 20 g in weight which had been injected 5 days previously with an amount of ascitic fluid containing approximately $5 \times 10^6$ tumor cells. At various intervals commencing with 30 minutes samples of tumor ascites were removed by peritoneal puncture, squashed with acetic dahlia and examined cytologically.

*Results. Effects of MA on onion root-tip cells.* MA arrested the mitotic processes of the root-tip cells (Table I). Even within 2 hours after exposure to the compound the number of detectable mitoses decreased from the initial value of 5% to 3.3% and at all times observed remained below the control level. The minimal mitotic rate was observed at 10 hours, at which time the maximal metaphase index also was attained. The increase in proportion of metaphases was accompanied by decreases both in prophases and the sum of anaphase and telophase figures up to 10 hours, but only the proportion of anaphases and telophases was below normal at 24 and 48 hours. After 12 hours the roots gave a flaccid appearance and showed no recovery of growth when they were placed in fresh tap water. No irregular mitotic cells were observed despite the mitotic arrest. The effects, thus, do not resemble those of many of the known mitotic poisons(6).

TABLE I. Effect of Maleuric Acid (0.09%) on Mitosis of Onion Root-Tip Cells.

| Hr of exposure | Total mitosis, % | % of mitotic cells | | |
|---|---|---|---|---|
| | | Metaphase | Prophase | Ana-telophase |
| Control | 5.0 | 19.7 | 43.4 | 36.9 |
| 2 | 3.3 | 50.9 | 29.4 | 19.7 |
| 4 | 3.3 | 49 | 36 | 15 |
| 8 | 2.1 | 61.5 | 24.6 | 13.9 |
| 10 | 1.4 | 61.3 | 25 | 13.7 |
| 24 | 2.0 | 41.2 | 50.7 | 8.1 |
| 48 | 2.0 | 54.2 | 37.1 | 8.7 |
| 48 control | 5.1 | 19.5 | 39.2 | 41.3 |

*Effect of maleuric acid on the Ehrlich ascites tumor.* Serial cytological studies have been made of tumor cells obtained from mice after single injections of MA on the 6th day after implantation of the tumor. The following numbers of mice were employed at each dose level: 2 mg, 6; 3 mg, 24; 4 mg, 4; 5 mg, 3; and 6 mg, 3. The life spans of the animals receiving 2 and 3 mg were the same as those of the controls, 10-12 days after transplantation. The animals receiving the larger doses died sooner, suggesting a toxic effect of the compound. Results for animals receiving multiple treatments of various doses of MA alone and in combination with other agents will be reported elsewhere.

Typical quantitative results are shown in Fig. 1. Within 1 hour after injection of MA no normal mitotic figures were observable, a condition which usually persisted for from 6 to 12 hours with the 3 mg dose and for longer periods at the higher dosage levels. Larger

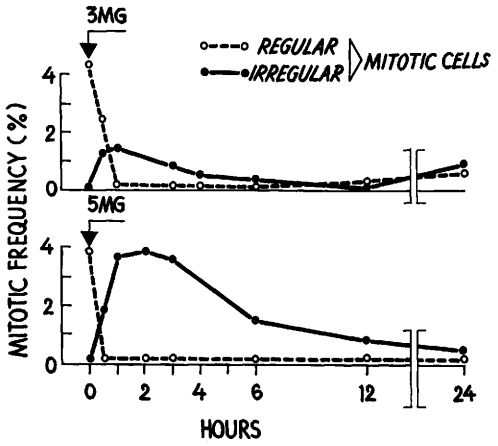
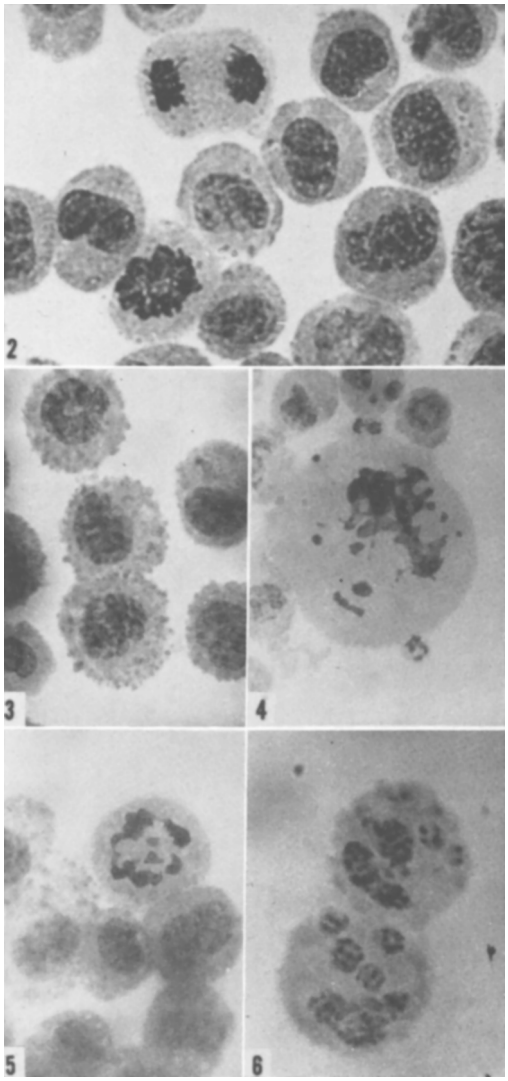



FIG. 1. Distribution of normal and abnormal mitotic figures in Ehrlich ascites tumor cells at various times after injection of 3 and 5 mg of MA.

numbers of abnormal mitotic figures were observed at higher doses.

Typical tumor cells from a control animal are shown in Fig. 2. The following description of sequential cytological changes will be based upon observations made on cells from mice receiving a single dose of 3 mg. Both resting and dividing cells showed visible signs of damage within 30 minutes. There was severe distortion of the cytoplasm due to blebbing (Fig. 3). A striking decrease occurred in the number of normal-appearing dividing cells, agglutination at metaphase being observed (Fig. 4). Marked disintegration of tumor cells was observed at 1 hour, almost all of the mitotic cells showing stickiness and clumping of chromosomes or other degenerative changes which appeared to increase progressively with time. At the 2-3 hour interval clumping was observed between the sticky chromosomes at anaphase, while the clumped chromosomes showed a ring-like arrangement at metaphase (Fig. 5). In addition to chromosome aberrations, numerous micronuclei were observed at anaphase and telophase (Fig. 6). The impression was gained that some of the mitotic cells were able to complete cytoplasmic cleavage despite the abnormalities. At 4-6 hours the total number of mitotic cells observed was very small and the typical cytoplasmic abnormalities still were observed. Subsequently, there was a gradual increase in the number of dividing cells, both normal and abnormal. After 12 hours the resting cells showed a normal appearance.

*Some chemical observations.* When solutions of N-ethylmaleimide (NEM) are mixed with solutions of reduced glutathione or other sulfhydryl compounds in 0.1 M phosphate buffer, pH 6.0, remarkable spectral changes occur coincidentally with the virtually instantaneous reaction which takes place(4,7). However, mixture of solutions of various concentrations of glutathione with solutions of




FIGS. 2-6. Appearance of untreated Ehrlich ascites tumor cells (Fig. 2) and various types of abnormalities induced by injection of 3 mg of MA (Figs. 3-6) as described in text.

MA gave no spectral evidence of interaction, the spectra being additive at all wavelengths between 210 and 340 mμ. In addition, experiments in which prior mixture of MA and glutathione solutions was followed by the addition of NEM showed that the amount of –SH available for reaction with NEM was not reduced by the presence of MA. The above results do not preclude the possibility that at least some of the biological effects of MA are attributable to interaction with –SH compounds, but do serve to indicate that the affinity of MA for soluble –SH compounds under the above conditions is much less than that of NEM.

In one experiment samples of 6-day tumor ascites were removed from mice before and at 30 minutes and 1 and 2 hour intervals after the intraperitoneal administration of 4 mg of MA. Although the marked cytoplasmic and nuclear abnormalities described above were found in these samples, paper chromatographic determinations of free amino acids in alcoholic extracts of the tumor cells and ascitic fluids showed no detectable differences between experimental and control samples, no free glutamine being detected at any time. This is in marked contrast to the effect on this tumor of sarkomycin, which caused an early increase in free glutamine in cells and fluid as well as other changes in amino acid distribution(8).

*Discussion.* Although maleic acid, maleimide, N-ethylmaleimide, and maleic hydrazide have been shown to have antimitotic properties in various test systems(1-3), none of these compounds has been found to have antitumor effects as judged by prolongation of life of tumor bearing animals or gross retardation of growth of various types of experimental animal tumors(9,10). In the present experiments single doses of MA which produced marked visible damage in the tumor cells also did not prolong the life of the animals. Maleic hydrazide, an extremely effective agent in breaking chromosomes in plants (3) which has found commercial use as a selective weed killer and an antisprouting agent for potatoes, had no significant effects on growth of normal mammals or on the gross or microscopic features of several *in vitro* test systems employing mammalian cells(10), nor did it show any carcinogenic potency(10,11). MA, on the other hand, produced far greater cytoplasmic and nuclear damage in Ehrlich ascites tumor than in onion root-tips and appears to be a substance worthy of further study, both from the point of view of the mechanism of action as well as a potential adjunct to existing modes of cancer chemotherapy.

*Summary.* 1. Maleuric acid produced mitotic inhibition in onion root-tip cells and an increase in proportion of mitotic cells in metaphase, but little or no cytoplasmic or nuclear abnormality was observed. 2. A single intraperitoneal injection of maleuric acid into mice bearing the Ehrlich ascites tumor resulted in marked cytoplasmic blebbing of the tumor cells. Mitotic abnormalities produced by the treatment persisted for prolonged periods. 3. The affected tumor cells showed essentially normal patterns of free amino acids.

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