A Comparison of the Ratios of Metaphase to Prophase in Normal and Neoplastic Tissues

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Since one of the major objectives in cancer research is to discover those characteristics of cellular morphology and physiology which distinguish the normal from the neoplastic cell, any evidence presented which indicates such differentiation is worthy of further investigation. Thus, the observation made by Timonen and Therman (14, 15) that the relative duration of the different stages of mitosis in cancer cells as expressed in the ratio of metaphases to prophase was markedly different from that of embryonic and normal proliferative tissue cells induced us (since many prepared sections of normal and malignant tissues were available) to make some observations in an effort to determine if such ratios could be relied upon as a criterion differentiating the normal from the malignant cell in division.

In a comparison of 35 cases of human normal proliferative endometrium and 174 cases of carcinoma of the female genital tract, Therman and Timonen (14) found that the ratio of metaphases to prophase (M/P) of the carcinomas was greater than the ratio in the normal endometrium. The M/P ratio for the malignant cells, which they illustrated with bar-graphs (converted into M/P ratios from their numerical data) were: 1.9, 8.2, 3.7, 1.7, and 8.1 (av., 4.7). The M/P ratios for the normal endometrium were: 1.2, 0.95, 0.73, 0.94, and 1.4 (av., 0.84). This difference they interpreted as indicating a shorter duration of the prophase stage in the neoplastic cells, and they attributed it to the more rapid formation of the division spindle.

PROCEDURE

The specimens examined were fixed in Bouin's fluid, sectioned at a thickness of 8 μ, and stained with hematoxylin. Cell counts were made with the aid of a Whipple disc at a magnification of 1,455 X. All mitotic counts were made by one observer in order to minimize variations as much as possible. Only metaphases and mid- and late prophyases were counted in the various specimens. Sufficient nuclei were examined so that from 50 to 100 mitotic figures were counted per section. Some sections were recounted as a test for consistency, while in others, where it was possible, sections taken from various levels of the tissue were studied and counted in order to determine the variations which might be expected in a single specimen.

EXPERIMENTAL

Tumor tissue.—Nine different tumors of the mouse, one of the rabbit, and three from human subjects were investigated.

The various tumors (Table 1) show a rather consistent degree of uniformity in the M/P ratio. Exceptions to this uniformity were found in tumors which revealed extensive areas of necrosis. Thus, No. 16, a Sarcoma 180, with a fairly large central area of necrosis, gave an M/P ratio of 6.8. A dbrB carcinoma which had been completely removed surgically 16 days post-transplant, reinserted immediately, and removed 15 days later for sectioning and staining, showed a very large center of necrosis with an exceptionally high M/P of 12.3. In another autoimplantation of a dbrB carcinoma, the periphery of viable malignant cells adjacent to the large necrotic center showed practically nothing but metaphase stages, but these showed such a large proportion of abnormalities such as stickiness, lagging, fragmentation, and overcondensation of chromosomes, it was thought advisable to attempt mitotic counts.

Normal tissue.—The normal proliferative tissues used for a basis of comparison were the endometrium of the monkey, the epithelial cells of the crypts of Lieberkühn of the mouse and cat, and the cells of the stratum germinativum of the esophagus of the mouse and man.

Although it appeared at the outset of these investigations that the findings of Therman and Timonen (14) were to be confirmed, it became evident as more tissues and sections were subjected to scrutiny, that the M/P ratio for normally pro-
liferating tissues was not in all preparations smaller than that found for neoplastic tissue. The M/P ratio in dividing cells of normal tissue was often equal to that of the malignant cell and was also at times considerably smaller.

An inspection of Table 2 indicates that there exists more variation in the M/P ratios for normal than for neoplastic cells.

A particularly interesting observation was made in sections No. 37, 38, and 39. In specimen No. 37, which was removed from the middle of the human esophagus, more prophases than metaphases were observed among the mitotic figures. In another section (No. 38), taken from near the cardiac sphincter, the metaphases were predominant. In comparing this to another section (No. 39) taken from the same region, it was found that the average proportion of prophases to metaphases was again encountered. With these appreciable variations found in the same tissue, it was decided to inspect malignant and normal tissues taken from the same strain of animals at different times of the day.

Comparison of M/P for dbrB carcinoma and normal esophagus in DBA mice.—The dbrB carcinomas (av. vol., 2.8 c.mm.) and esophagi were removed from DBA mice (kept in individual cages) at 2:30 A.M., 4:00 A.M., 5:00 A.M., 8:00 A.M., 11:00 A.M., 2:00 P.M., 5:00 P.M., 9:30 P.M., and 11:00 P.M. Tissue size, fixation time, sectioning and staining were kept constant for all specimens. Only nine animals were available for this experiment; however, it will be noted from an inspection of Table 3 that there is no indication of a significant difference in the M/P for normal and malignant tissue when these specimens are obtained from a highly inbred strain of animals.

DISCUSSION

While it appeared at first that the number of prophases in dividing neoplastic cells were fewer than those in dividing normal cells, it became evi-
dent upon continued observation that the latter can also exhibit relatively few cells in the prophase stage. Since it is generally conceded that cancer is not altogether influenced by the host’s regulatory mechanism, it is perhaps to be expected that greater variations would be encountered in normal cells than in malignant cells. Although it has been found, for example, that a diurnal mitotic rhythm occurred in plants (10), in animals (1, 3, 4, 5, 19), in man (7), and in tissues cultivated in vitro (9), no such phenomenon has as yet been detected in malignant tissues of animals (1) and of man (8).

The variations in the relative number of prophase and metaphases in normal proliferative cells found in our histologic studies have also been observed by others. Although data given by Kaufmann et al. (11) showed that they observed an M/P ratio of 0.5 for both rabbit and rat corneal epithelium, Buschke et al. (6) found that half of the mitotic figures in the corneal epithelium of the rat consisted of metaphases. Furthermore, whereas Kaufmann observed considerable variability in the distribution of dividing cells of a single cornea and between the two corneas of the same animal, Buschke found only minor variations from cornea to cornea of the same animal.

Brues and Marble (9) observed wide variations in the percentage of mitotic figures in the liver undergoing hyperplasia, which demonstrated that single mitotic counts could not be relied upon to determine the growth rate of the organ. The M/P ratios calculated from the data of these authors from 24 hours after hepatectomy to 49 hours were: 2.3, 3.4, 2.5, 6.3, 3.5, 5.6, and 12.5.

From the available data studied concerning the relationship of prophases to metaphases, it appears that the most conspicuous feature distinguishing dividing malignant from normal cells is not so much the difference in the M/P ratios as it is the relative uniformity of these ratios for malignant cells as compared to normal cells. Although a sufficient number of determinations has not been made, this does not appear to hold for normal and malignant tissue taken from the same inbred strain of animals. This uniformity may, however, apply only to the M/P ratio, for, according to Lambert (12), the time required for the complete mitotic cycle is more variable for cancer cells than for normal cells in vitro; being an average of 38 minutes (range 21–50 min.) for rat fibroblasts and 56 minutes (range, 24–97 min.) for the cells of a rat sarcoma.

SUMMARY

1. The metaphase to prophase ratios of neoplastic cells showed less variation than those of normal proliferating cells.

2. That the ratio of metaphases to prophases in normal tissue cells in division is smaller than in malignant cells has not been conclusively verified by our observations.

3. There appears to be an increase in the metaphase to prophase ratio in malignant cells of tumors which contain a substantial volume of necrotic material.

REFERENCES


4. —. Age and Mitotic Activity in the Male Mouse, Mus Musculus L. Ibid., pp. 86–96.


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