week. During the following weeks both Mn and Cu retention returned to normal proportions. Continued administration of vitamin B_1 failed to influence Cu metabolism as long as the Mn intake remained at the normal level of 100 γ. The Cu intake of about 70 to 80 γ daily remained unchanged throughout the experiment.

10909

Relation Between Latent Period and Growth Rate in Chemically Induced Tumors.

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It has been customary to rate the potency of carcinogenic action according to the shortness of the latent period or the percentage of animals developing tumors (Boyland and Warren,[1] Cook, *et al.*[2]). It is of interest to know whether malignancy of the tumors produced is correlated with the potency of carcinogenic action as measured by the latent period. Of the various criteria of malignancy, it has seemed to us most appropriate to investigate growth rate as measured directly *in vivo*, or indirectly by means of mitosis counts.

The mice in these experiments, representing various inbred strains, were injected in the subcutaneous tissues of the right axilla with carcinogenic agents dissolved in lard or in cholesterol pellets. The mice were examined biweekly and the latent period was reckoned from the time of injection to the time when tumors were first noted by palpation. Tumors were then measured biweekly in 2 dimensions by a single observer, who recorded the diameters to the nearest millimeter, using calipers with approximately constant pressure. Growth rate was estimated in terms of the increase in the mean of these diameters per week of observation. Only those tumors were included in the series in which at least 4 observations covering a period of 10 days or more were made. Most mice were killed after tumors had reached considerable size and paraffin sections were made. Mitoses were enumerated in groups of 1000 or more counted tumor cells. The findings are recorded in Table I.

1 Boyland, E., and Warren, F. L., *J. Path. Bact.*, 1937, **45**, 171.

2 Cook, J. W., Hieger, I., Kennaway, E. L., and Mayneord, W. L., *Proc. Roy. Soc. London*, 1932, **3**, 455.

TABLE I.
Mean Latent Period, Growth Rate, and Mitosis Rate.

Strain of mice	No. of mice	Agent	Vehicle	Mean latent period, wk	Growth rate, mm per wk	Mitoses. % of tumor cells
C3H	37	2 mg MCA	lard	8½	4.68±.25	.96±.35
A	15	" "	"	10½	4.42±.58	
RPE*	22	" "	"	15	3.00±.11	.70±.36
I	20	" "	"	16	2.82±.50	.70±.26
C3H	37	" "	"	8½	4.68±.25	
"	15	1 " "	"	9½	3.78±.27	
"	36	0.5 " DBA	"	20	3.02±.30	
C57— black	22	0.45 " MCA	Cholesterol	12	3.01±.31	
"	6	0.45 " DBA	"	26	1.06±.71	

* Roberts Pink-Eye.

It will be seen that with the same carcinogenic technic, those strains of mice which produce tumors slowly, develop slowly-growing tumors. When the dosage or agent applied to a single strain is varied, we see the same correlation between speed of response and growth rate. In fact, under the various conditions of these experiments, there is in all cases a high direct correlation between growth rate and shortness of the latent period. No significant differences in growth rate were seen between the early and late appearing tumors of a single series, due perhaps to the small numbers of animals in the series.

In the 3 groups in which mitosis counts were done, they were seen to show a good degree of correlation with growth rate. Here, again, it was not possible to demonstrate such correlations in individual instances, but only by groups.

The question naturally arises as to what extent the latent period is determined by the growth of a tumor which has not yet reached visible size. A rough idea of the magnitude of the period of tumor development can be obtained by the help of a few simplifying assumptions. The mean duration of mitosis in a tumor cell has been estimated as at least one hour (Lewis and Lewis,[3] Lewis[4]), or slightly more than in normal tissues (Brues and Marble[5]). On this basis the proportion of cells in mitosis represents approximately the hourly percentage increase in tumor size. If we make the reasonable assumption that growth rate remains constant without retarding influences such as are seen in embryos and regenerating tissues (Brues

[3] Lewis, W. H., and Lewis, M. R., *Am. J. Cancer*, 1932, **16**, 1153.

[4] Lewis, W. H., *Am. J. Cancer*, 1939, **35**, 408.

[5] Brues, A. M., and Marble, B. B., *J. Exp. Med.*, 1937, **65**, 15.

TABLE II.

% cells in mitosis	Days required for tumor to double in size (intermitotic period)	Days required for tumor to increase from 10^{-9} to 0.1 cm^3	Days required for tumor to increase from 10^{-6} to 0.1 cm^3	Actual latent period (days)
0.70	4.1	109	70	105
0.96	3.0	80	50	60

and Marble[5]) the growth should follow an exponential curve ($v = v_o \times e^{kt}$) when v and v_o are final and initial tumor volumes after a lapse of time t, and k is a constant determined by instantaneous growth rate and reflected in the mitotic index. On this basis Table II has been constructed to show the "latent periods" in the development of a tumor of 0.1 cm^3 from a single cell and from 1,000 cells (estimated as 10^{-9} and 10^{-6} cm^3, respectively), with growth rates represented by 0.96 and 0.70% per hour. The latent periods actually seen in the development of tumors with similar mitotic rates (C3H and RPE strain) are shown alongside those so calculated.

Thus, if we suppose that neoplasia begins with a relatively small number of malignant cells and follows a constant growth rate, a great part of the latent period is involved in the growth of a microscopic tumor to palpable size. This supposition is confirmed by our observations that the great majority of these tumors are made up of homogeneous cells, although cell type varies widely from one tumor to the next.

Similarly, when the development of spontaneous mammary tumors is inhibited, palpable tumors continue to appear for 4 months (Nathanson and Andervont[6]). The median growth rate of a series of mammary tumors in mice has been shown to be less than 3 mm per week (Haddow[7]). It seems significant, therefore, that those induced tumors in our series which show a parallel growth rate (I strain mice) have a latent period of 16 weeks. In any case, it is probable that the latent period is greatly influenced by the growth rate.

When a constant dose of carcinogen is used on various strains of mice, the parallel variations in growth rate and latent period may well be due to quantitative differences in response of the individual cells, reflected in different tumor growth rates. It has been observed that these several strains of mice respond also with a tendency to develop different types of tumor (Brues and Andervont, unpublished data). When various types and dosages of carcinogen are used,

6 Nathanson, I. T., and Andervont, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 421.

7 Haddow, A., *J. Path. Bact.*, 1938, **47**, 553.

however, variations in latent period might be due to differences in the period before initial carcinogenesis, or in the number of malignant cells originally produced.* But the correlation between latent period and growth rate suggests that here, too, growth rate is an important factor. The short latent period in the induction of tumors by virus would necessarily be explained by the fact that large numbers of malignant cells are produced simultaneously. These observations are consistent with those of Mottram[9] that latent periods and growth rates of tar warts and epitheliomata extrapolate in the same way.

Summary. There is a high degree of correlation between the malignancy of chemically induced tumors, as measured by growth rate and mitotic index, and shortness of the latent period before appearance of palpable tumors. This relation holds true in the various responses of different strains of mice to the same agent, and in responses to different agents and modes of administration.

It is suggested that the growth rate of a tumor in the microscopic stage is important and may be the main factor determining the latent period in carcinogenesis.

10910 P

Observations on the Specific Cause and the Nature of "Quail Disease" or Ulcerative Enteritis in Quail.*

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Ulcerative enteritis of quail (so-called quail disease) constitutes the most important disease problem in propagation of quail in captivity. Many game breeders have had their entire stock decimated or practically wiped out by an epizootic of this disease. Once introduced on a game bird farm, the infection is extremely difficult to eradicate. Heavy losses from this disease have occurred in wild trapped quail

* Similarly, it is well known[8] that the latent period after transplantation of tumors depends on the number of tumor cells inoculated.

8 Schrek, R., *Am. J. Cancer*, 1936, **28**, 364.

9 Mottram, J. C., *J. Path. and Bact.*, 1935, **40**, 407.

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