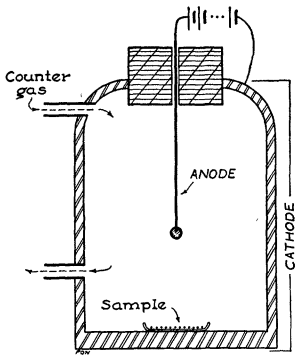

FIG. 1. Diagram of gas-flow counting chamber.

standard was erratic. Where quantitative answers were needed, much of the advantage gained by the high efficiency of counting was thereby lost. It seemed possible that this lack of reproducibility was caused by changes in the electrical properties of the counting chamber. Fig. 1 shows diagrammatically the physical arrangement of a conventional flow counter. The sample at the bottom may be introduced by the rotation of a turntable which provides a gas-tight seal. On examination it is seen that the electric field above the sample depends on the effective charge density on the surface of the sample, and that this charge density could vary from time to time if the sample had a high dielectric constant, but would remain quite constant if the sample were a good conductor. Since many of the samples are preparations which present a film with a high dielectric constant, it seemed possible that elimination of this might resolve the difficulty. Colloidal graphite, which has a high conductance per unit weight, was introduced into the samples for this purpose.

Data on typical samples illustrative of the results of incorporation of colloidal graphite are given in Table 1. In the preparation of each sample 1 ml of a slightly basic aqueous solution containing I^{131} was evaporated to dryness in a shallow aluminum sample container 2.5 cm in diameter. Where graphite was incorporated, 0.05 ml of a colloidal graphite solution containing 5.5 mg of colloidal graphite was added to the solution before evaporation. The counter was operated using a helium-isobutane mixture and a 1450-v anode potential. Each of the samples was counted at two different times at least 20 hr apart under otherwise identical conditions; the values were normalized (for radioactive decay) to the same time. The values for the graphitized samples reproduced within statistical limits; the values for the nongraphitized samples did not. All other samples graphitized and tested as described above have shown counting reproducibility. A thin sheet of aluminum was mounted over the C^{14}-polystyrene standard; following this its erratic counting behavior disappeared immediately.

It is evident that presentation of a conducting surface by a sample improves its counting reproducibility in a flow counter. This means an over-all increase in accuracy of counting determinations which may mean, as it has for some projects in our laboratories, the difference between using and not using the gas-flow counting technique. The colloidal graphite concentrations used in the examples are not necessarily optimum for all purposes and may be reduced for greater sensitivity in counting very weak β-rays.

TABLE 1

REPRODUCIBILITY IN THE GAS-FLOW COUNTER OF SAMPLES WITH AND WITHOUT COLLOIDAL GRAPHITE

Sample			Without graphite		With graphite (1.1 mg/cm²)	
Main constituent	Mg/cm² without graphite	Day	Total counts	Counts/min ± P.E. (corrected)	Total counts	Counts/min ± P.E. (corrected)
Sodium chloride	0.10	1	27,069	4980 ± 20	25,023	4616 ± 20
		2	23,888	4790 21	23,032	4620 20
Serum solids	0.30	1	6,918	467 4	8,869	313 2
		2	2,994	325 4	6,754	314 3
Serum solids	0.30	1	8,873	481 3	16,326	628 3
		2	5,600	650 6	6,754	631 5

Reference

1. THOMAS, H. C., and UNDERWOOD, N. *Rev. Sci. Instruments*, **19**, 637 (1948).

Manuscript received February 18, 1952.

A Method for Quantitative Evaluation of the Effects of Ionizing Radiations on Growth of Adenocarcinoma *in vivo*[1]

T. P. Ting

Department of Physiology, University of Saskatchewan,* and the *Cancer Commission of Saskatchewan

In estimating the inhibiting effects of ionizing radiations on the growth of tumors *in vivo,* one customary practice is to observe the fraction of tumors completely regressed at a given period following the irradiation. When the radioresistance of the tumor is high, this fraction is small, and any quantitative as-

[1] Supported by grants-in-aid from the National Cancer Institute of Canada.

sessment of effects based on such data is subject to a high degree of statistical uncertainty. An alternative method, often used in the assay of the effect of drugs, is to make the daily growth rate the criterion. Since this quantity depends on the existing size of the tumor, this method also suffers from large sampling error unless the number of animals used is quite substantial. This latter practice is not always feasible under ordinary laboratory conditions.

Recently, in the course of investigating the biological effectiveness of the x-radiation from the betatron on the regression of mouse tumors, it was noted that adenocarcinoma E0771 transplanted by the usual trocar method and measured externally by calipers, grew at a constant rate according to a simple expression originally derived by Blum (*1*). As seen in Fig. 1, the control tumors grew at a constant rate

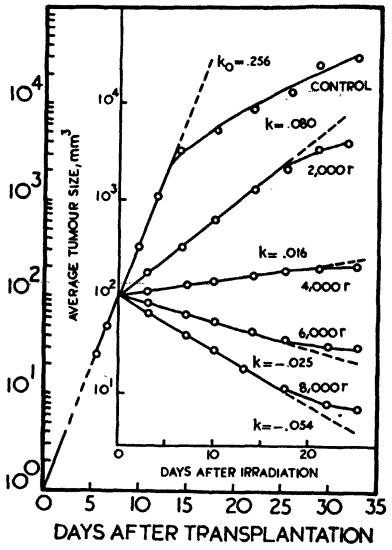

FIG. 1. Growth curves of mouse adenocarcinoma E0771. Each group, consisting of 20 tumors, was given a single dose of 200 kv x-radiation. Note the linear portion of each curve fits the growth function, $\log V/V_o = kt$, where V is the volume of tumor at time t, V_o is the initial volume, and k is the growth constant.

k_o from the time of transplantation until reaching a size of about 5 cm³, beyond which there was a gradual drift from the predicted course. Morphological examination revealed that this latter effect was caused by cyst formation, necrosis, and peripheral ulceration. Irradiated tumors grew at a constant but reduced growth rate, and the reduction in growth rate (decreased slope) increased with increasing dosage of radiation. With larger dosages, the slope was negative (the tumor decreased in size). At $k = o$, the growth rate of the treated tumor remained stationary, the result of an exact replacement of those cells destroyed by radiation with those formed by the few survivors able to maintain their normal carcinogenic activities. With the irradiated tumors also, following the initial period, there were significant departures from the theoretical curves. This may be attributed mainly to the fact that a few cells which escaped injury because of the statistical spatial distribution of ionization were able to multiply by normal processes of proliferation. This phenomenon is usually known as "recovery" in radiotherapy.

In order to relate quantitatively the degree of biological effect with the radiation dose, one may define a ratio $K = (k_o - k)/k_o$ as the regression index and plot this quantity against the dose. As shown in Fig. 2, the dose-effect curve in this particular case was

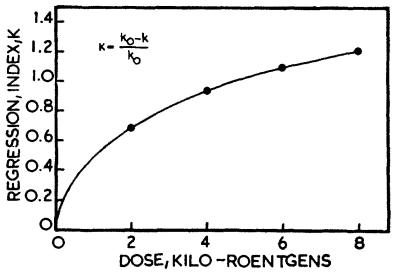

FIG. 2. Dose-effect curve fits the empirical equation $D = aK^b$, where D = dose in kilo-roentgens, K = regression index, and a and b are constants. Mouse adenocarcinoma E0771, 200 kv x-radiation.

found to follow the empirical equation $D = aK^b$, where b is a function of the biological material used and a is a constant depending on the type of radiation alone, being numerically equal to D at $K = 1$. If the same tumor is used to compare the biological effects produced by two types of x-radiation, indicated as 1 and 2, respectively, then the relative effectiveness of the former with reference to the latter is as follows:

$$\eta = \frac{D_2}{D_1} = \frac{a_2 K_2^b}{a_1 K_1^b}.$$

If, then, the two types of radiation are compared for the same growth rate ($K_1 = K_2$), $\eta = \frac{a_2}{a_1}$, and its value is independent of the degree of biological effect chosen for this comparison, and consequently may be evaluated at very low dosage range. This does not follow if b, dependent on the biological material, is found to vary with the type of radiation. In this case, it would be necessary to limit the comparison to the unique value of K at $k = o$.

With mouse adenocarcinoma E0771 as the test object, the feasibility of this method has been demonstrated in the evaluation of the biological effectiveness of the 23.5-mev x-radiation as compared with the conventional 200-kv x-radiation. A detailed report of the results obtained with this method will appear elsewhere (*2*). Suffice it to say that the value of η obtained in this way was found to agree within the accuracy of the experiments with that obtained by using lethal regression as the criterion, where a much higher dosage range was required to produce any observable effect.

The above method is preferable to existing methods for several related reasons. One major advantage is the measurability of the effect produced by a dose far below that required for a lethal effect. This is par-

ticularly valuable where the tumors are so resistant that administration of a massive dose of sufficient magnitude to produce complete regression is not practical. Elaborate measurements are reduced to a minimum because of the shorter period of observation required. Sampling errors arising from variation in tumor size are avoided, since such variations have little effect on the slope of the growth curve as long as the observation is confined within the interval where the growth rate remains constant. This difference is obviously due to the fact that, theoretically at least, lethal regression will not occur until all the cells in the tumor are affected lethally, whereas relative reduction in growth rate as in the present method is sufficient to indicate an effect. Frequently, resumption of growth at an accelerated rate takes place after a latent period following the administration of a sublethal dose. An example of this nature has also been observed when treatment of this tumor with guanazolo is discontinued (*3*). Such a phenomenon, when it occurs, renders both lethal regression and daily growth rate useless as criteria for the quantitative appraisal of the effect of the therapeutic agent.

As a prerequisite to the applicability of this method, both the control and irradiated tumors must grow at a constant rate for a sufficient period immediately after the irradiation. Under these circumstances, it is noted that change of slope k with dose is independent of the time interval, even though the growth of the irradiated tumor relative to that of the control tumor at any subsequent time may decrease appreciably with this interval.

References

1. BLUM, H. F. *J. Natl. Cancer Inst.*, **4**, 21 (1943).
2. TING, T. P., and JOHNS, H. E. *Can. J. Med. Sci.* (in press).
3. KIDDER, G. W., *et al. Science*, **109**, 511 (1949).

Manuscript received March 3, 1952.

The Effect of Anesthesia upon Adrenergic Blockade[1]

W. Glen Moss

Department of Physiology, College of Medicine, University of Illinois, Chicago

In a number of instances the author has observed effects of adrenergic blocking agents in trained unanesthetized dogs which did not seem to be in accord with the pharmacodynamic effects reported for such drugs (*1*). Since the usual procedure for determining the effects of such drugs is to measure changes effected in the responses of anesthetized animals, it was deemed necessary that a comparative study be made on anesthetized and unanesthetized animals using identical techniques in both.

Epinephrine and nor-epinephrine were used as constricting agents. These were injected into the carotid artery so that only the constricting effect on blood vessels was measured. A dose of 0.1 μg/kg was used, since it causes a constriction in the blood vessels of the ear equivalent to that produced by a standard intravenous dose of 2 μg/kg. Section of the sternocleidomastoid muscle and suturing it beneath the common carotid artery make intra-arterial injection a simple procedure in trained unanesthetized dogs. Vascular volume changes in a section of the ear were measured, using a photometric technique employing a photomultiplier tube (RCA 931A) and recording the output from this tube with a string galvanometer. Mean blood pressure was recorded by a membrane manometer with its lever suspended in the light beam beside the shadow of the galvanometer string. The photomultiplier tube was activated by a white light which passed through an area of the ear measuring 5×15 mm. The light intensity was adjusted so that the control output from the tube was between 15 and 20 mv. Changes in caliber of the blood vessels in this area are recorded in arbitrary units representing 0.1-mv change in the output of the tube.

An attempt was made to select the same area of the ear for each assay. However, there were day-to-day variations in the amount of light required to produce the same activity of the phototube. This probably indicates that the volume of blood in the vessels of this area of ear varied from day to day. Moderate asphyxia produces only minimal changes in the light transmission when this technique is used and does not influence the results.

The degree of constriction produced by epinephrine and nor-epinephrine in control experiments was relatively constant. In the trained dogs after control values were established, the degree of constriction was measured after adrenergic blockade, using a β-chloroethyl amine (SY 28, 2 mg/kg) and an ergot (D.H.O. 180, 0.2 mg/kg).

The results of these procedures are shown in Table 1. Each figure represents the average of 8–10 experiments. It is quite evident that, when an animal is anesthetized, either SY 28 or D.H.O. 180 is effective in reducing the degree of constriction produced by either test compound. However, if animals are not anesthetized, adrenergic blockade has little effect on the constrictor action. The slight difference between average control responses of anesthetized and unanesthetized dogs is not significant. In this study D.H.O. 180 seems somewhat more effective in blocking constrictor action than SY 28. As little as 0.006 μg/kg of epinephrine caused a measurable constriction when injected into the carotid artery.

The results on anesthetized dogs agree with those of Folkow *et al.* (*2*), but differ from those of Bülbring and Burn (*3, 4*). I also agree with Folkow that on rare occasions one finds a dilator response following the intra-arterial injection of epinephrine. One more commonly finds dilatation in the unanesthetized dog without adrenergic blockade. Such a response may be reversed in less than ½ hr, for no apparent reason

[1] Most of the research here reported was done in the Department of Pharmacology, Temple University, aided by a grant from the Smith, Kline, & French Laboratories.