The Effect of 8-Azaguanine on Physiologic Growth Measured by the Rate of Eruption of the Incisor of the Mouse*

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Following his demonstration that guanine is a metabolic requirement in Tetrahymena, Kidder (2) showed that guanine deficiency, brought about through competitive inhibition by the structural analogue 8-azaguanine, resulted in inhibition of the growth of Tetrahymena colonies. It had previously been shown by Brown et al. (1) that guanine is not a nutritive requirement for rats. Guanine fed to rats is not used as raw material for the synthesis of nucleic acids but is broken down and excreted (1). Kidder reasoned that, if tumor metabolism resembled that of ciliates rather than that of normal mammalian tissues, then guanine might be a dietary requirement for tumor growth and 8-azaguanine might inhibit the growth of tumors by competitive inhibition, while not affecting normal tissues.

Tests in mice (2) showed that the subcutaneous or intraperitoneal administration of 8-azaguanine had a very pronounced action in retarding the growth of adenocarcinoma E 0771 and of several other tumors, while not seeming to affect the health of control animals.

It seemed interesting, therefore, to test Kidder's hypothesis that the growth-inhibiting action of 8-azaguanine is limited to tumor tissue. For this purpose, a more sensitive method was required than the commonly used procedures of following weight curves or of determining "therapeutic ratios" (8). It was hoped that a study of the rate of incisor eruption might prove to be a better tool. As is known, the incisors in rodents are continuously erupting teeth. While the peripheral end shears off, the same over-all length is maintained through continuous growth at the open basal end and through eruption of the tooth. The rate of eruption, therefore, is a direct measure of rate of growth of the tooth. It was felt that the rate at which growth of the tooth proceeds is likely to be indicative of the physiological state and the general conditions for growth in the animal. An appraisal of the method will be presented in some detail below.

MATERIALS AND METHODS

Seventy-eight mice of strain C57 black, obtained from the Roscoe B. Jackson Laboratories in Bar Harbor, Maine, were used. The animals were kept on a diet of Ralston Purina Fox Chow checkers and water ad libitum. The rate of incisor eruption was observed in the following groups of animals:

a) Control groups V and VI, consisting of ten and eleven animals, were left untreated. Control group VII, ten animals, was given Locke's solution in two daily injections of 0.5 cc. each. All control animals were 7—8 weeks old at the beginning of observation.

b) Experimental group III consisted of eighteen animals 7—8 weeks of age at the beginning of the experiment, and Group O of nine animals, aged 10—11 weeks. Experimental group II contained nine animals 7—8 weeks of age, into which adenocarcinoma E 0771 had been transplanted. 8-Azaguanine, obtained by courtesy of the Lederle Laboratories Division of the American Cynamide Company, was dissolved and administered to the experimental animals as suggested by Kidder, i.e., twice daily, either subcutaneously or intraperitoneally, in quantities of 0.5 mg. in 0.5 cc. of solution per injection.

The rate of eruption was measured in one or both lower incisors of control and experimental animals. A shallow scratch was applied to the tooth with a carborundum disc rotating in a dental engine handpiece. The distance between the mark and the gingival margin was measured once a week with calipers calibrated in tenths of millimeters and a fresh mark applied when necessary.

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The animals did not have to be anesthetized for this procedure.

RESULTS

APPRAISAL OF METHOD

Error of measurement.—To evaluate the accuracy with which the weekly rate of eruption could be measured in mice, double determinations, on right and left incisors, respectively, were carried out during a 4-week period. The averages for right incisor eruption and left incisor eruption were worked out during a 4-week period. The averages for right incisor eruption and left incisor eruption were found to differ by 0.08, 0.02, 0.10, and 0.05 mm. in right incisor eruption and left incisor eruption were worked out during a 4-week period. The averages for right incisor eruption and left incisor eruption were found to differ by 0.08, 0.02, 0.10, and 0.05 mm.

Variation in time, in the same animal.—Two groups of control animals were observed for a period of 4 weeks (one untreated, the other receiving twice daily an injection of 0.5 cc. of Locke’s solution). There were, in a total of 88 measurements of the rate of incisor eruption, seven instances of an upward shift of 0.7 mm. or more, six instances of a downward shift of 0.7 mm. or more, and twelve instances of a downward shift of 0.4—0.7 mm., and twelve instances of a downward shift of 0.4—0.7 mm. The weekly averages for the three groups were 1.71, 1.72, and 1.79, respectively, and the standard deviations 0.59, 0.41, and 0.36. The standard error of the mean for the same three groups was found to be 0.12, 0.13, and 0.12, respectively. Thus, fairly small to moderate effects on the rate of eruption can be tested for, without necessitating very large groups of animals.

TABLE 1

RATATION OF ERIUPTION OF INCISORS

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Av. eruption (mm.)</th>
<th>Std. error</th>
<th>Mean weight (g.)</th>
<th>Deaths in week</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>10</td>
<td>1.79</td>
<td>0.12</td>
<td>17.6</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>1.73</td>
<td>0.13</td>
<td>17.0</td>
<td>None</td>
</tr>
<tr>
<td>VI (Right)</td>
<td>11</td>
<td>1.67</td>
<td>0.13</td>
<td>18.5</td>
<td>None</td>
</tr>
<tr>
<td>VI (Left)</td>
<td>11</td>
<td>1.75</td>
<td>0.11</td>
<td>18.5</td>
<td>None</td>
</tr>
<tr>
<td>III</td>
<td>18</td>
<td>1.04</td>
<td>0.07</td>
<td>19.3</td>
<td>None</td>
</tr>
<tr>
<td>O</td>
<td>9</td>
<td>0.16</td>
<td>0.05</td>
<td>19.3</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>1.21</td>
<td>0.12</td>
<td>17.0</td>
<td>None</td>
</tr>
<tr>
<td>VII</td>
<td>10</td>
<td>1.66</td>
<td>0.12</td>
<td>17.0</td>
<td>None</td>
</tr>
<tr>
<td>VI (Right)</td>
<td>11</td>
<td>1.60</td>
<td>0.15</td>
<td>18.9</td>
<td>None</td>
</tr>
<tr>
<td>VI (Left)</td>
<td>11</td>
<td>1.60</td>
<td>0.15</td>
<td>18.9</td>
<td>None</td>
</tr>
<tr>
<td>III</td>
<td>18</td>
<td>1.02</td>
<td>0.09</td>
<td>17.0</td>
<td>None</td>
</tr>
<tr>
<td>O</td>
<td>9</td>
<td>1.23</td>
<td>0.17</td>
<td>17.0</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>1.05</td>
<td>0.14</td>
<td>17.0</td>
<td>None</td>
</tr>
</tbody>
</table>

The error of measurement, therefore, is small enough to permit reliable averages in fairly small groups of animals. As regards the reliability of individual measurements, the standard deviation for the series of discrepancies between double measurements is 0.280 mm.; and for an individual measurement, 0.20 mm. The odds, therefore, are as 22:1 against an individual rate being more than 0.4 mm. distant from the true value.

Variation between individuals of the same age.—In the three control groups summarized in Table 1, there was found to be a rather close agreement with respect to upper and lower extremes of rate, weekly average for each group, and variability (standard deviation). In the first week, e.g., the extremes in Group VI (including right and left measurements) were 1.2 mm. and 2.4 mm.; in Group V, 1.1 and 2.3; in Group VII, 1.4 and 2.1 mm. The weekly averages for the three groups were 1.71, 1.72, and 1.79, respectively, and the standard deviations 0.59, 0.41, and 0.36. The standard error of the mean for the same three groups was found to be 0.12, 0.13, and 0.12, respectively. Thus, fairly small to moderate effects on the rate of eruption can be tested for, without necessitating very large groups of animals.

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ble 1 and Chart 1) show that there was a slight downward trend in the rate of eruption of our control animals (probably caused by somewhat unfavorable conditions occurring in our animal hospital at the time of the experiment), which in the case of Group VI was pronounced enough to be statistically significant.

In using the rate of incisor eruption as a test object, it is therefore preferable to carry out control measurements in a group of untreated animals kept under identical conditions rather than to establish "before and after" values in the test animals.

THE EFFECT OF 8-AGAQUANINE ON THE RATE OF ERUPTION

The effect of 8-azaguanine treatment was studied in three groups of animals: a small trial group, a larger group of somewhat younger animals, and a group of animals into which tumor transplants had been made (Groups O, III, and II of Table 1). This latter group is included for discussion in this paper because the values found for incisor eruption during the first 4 weeks of the administration of 8-azaguanine were very similar to those found in the other two groups, despite the presence of the tumor.

Effect during fourth to eleventh day of treatment.—A strongly depressing effect of 8-azaguanine during the first week of its administration was unmistakable. The average rate of eruption found in the three groups was 1.04, 0.96, and 1.21 mm., respectively, as compared to 1.71, 1.72, and 1.79 for the three control groups or 1.74 for all control animals taken together. The difference between the average for all control animals and the average for Group III was $0.70 \pm 0.089$, or 7.8 times its standard error; for Group O, $0.78 \pm 0.144$, or 5.4 times its standard error; for Group II, $0.58 \pm 0.136$, or 3.9 times its standard error. Chart 2 shows the percentage distribution of the animals over the range of rates of eruption. The distribution curves for the three groups of animals receiving 8-azaguanine are shifted toward the lower rates, showing that the averages were depressed because the rate of eruption was depressed in most, if not all, animals. In fact, of the 36 animals receiving 8-azaguanine, there was only one (with a rate of 1.7 mm.) in the region of the average of the controls (1.74). There was one animal with a rate of 1.6 mm. and five with rates of 1.5 mm. Of the seven animals just mentioned, five showed very depressed rates in the second week of observation, and one showed by its higher later readings that 1.5 mm. was a depressed rate for this animal. Only one animal with an eruption rate of 1.5 gave no evidence of being affected by 8-azaguanine administration.

The length of time for which 8-azaguanine had to be administered before its full inhibitory effect could be seen varied in different animals. Thus, not only in the five animals just mentioned but in eight additional ones with rates already low in the first week, there was a further slowing of eruption in the second week, whereas in eleven animals maximal inhibition seemed reached during the first week of observation, and faster eruption occurred in the week thereafter. The averages shown in the table conceal these shifts, since gains and losses very nearly balanced in each of the three groups.
Later effects: recovery from 8-azaguanine.—While it can thus be stated that 8-azaguanine depressed the rate of tooth eruption in nearly all animals observed, a certain degree of "escape" from its effects while administration is continued seemed to be an equally universal occurrence. This recovery phenomenon was somewhat variable in time and degree and was, in a majority of the animals, transient in character. Among the animals surviving for 3 weeks or more, there were only two in which the rate of eruption in later weeks did not rise beyond that measured in the first and/or second week. One of these was the animal unaffected by 8-azaguanine mentioned in the preceding section, the other one was the only animal with a consistently low rate which we have observed.

a) Timing: Most commonly, the "escape" occurred in the third week of administration. (For this reason, the group averages for this week show an increase in all three groups; see Table 1.) Of the 32 animals observed for this length of time, 21 showed higher readings in the third week than in the second week and in the fourth week, 7 had a peak rate in the second week already, 2 in the fourth week, and 2 in the fifth week.

b) Degree: As regards degree of recovery, values well within the control range are reached by all animals of Group O (a group of somewhat older animals), and values very close to it by all but two of the surviving animals of Groups III and II.

c) Duration: Recovery from the effects of 8-azaguanine administration was transient, lasting usually for 1, in some cases for 2 weeks. In all but one case it was followed by renewed depression, usually at least as marked as the initial one. However, most commonly this depression was again followed by recovery, very often to rates higher than those of the initial recovery phase. Although the number of animals observed for this length of time is small and the timing of the ups and downs varied in the different animals, the peculiar cyclical reaction to the continuous administration of 8-azaguanine was pronounced enough to be reflected in the weekly averages given in Chart 1.

This merely qualitative description of the effects of prolonged treatment is motivated by the small number of animals and the lack of control readings covering the same period. It was not possible to include Group II in this analysis, since animals with tumor E 0771, whether or not they are treated with 8-azaguanine, rarely survive for this length of time. However, even in the animals without tumors, particularly in the younger group (III), there was a rather high rate of mortality: one animal died in the third week, two in the fourth, five in the fifth, five in the sixth, one in the seventh week; the remainder survived. Among the older animals, only one died after 7 weeks of 8-azaguanine administration. There were no fatalities in the two control groups in the 4 weeks during which they were measured, nor in the following 8 weeks of observation.

DISCUSSION

Influence of weight changes on rate averages.—As the figures in Table 1 show, a pronounced tendency to lose weight was one of the effects of 8-azaguanine administration. Since this loss of weight may represent a diminished food intake, the decreased rates of eruption in the 8-azaguanine-treated animals might be ascribed to this factor rather than to a direct action of 8-azaguanine on the growth process in the tooth. The relationship between the rate of eruption and the weight of the animal was therefore analyzed. There was no correlation between rate and weight, nor was there (Chart 3) any relation between change in rate and change in rate. Rates lower than those of the preceding week were as frequently associated with a gain as with a loss in weight, and the same held for increased rates of eruption.

In the treated animals, there was also a random association of rate and weight. As regards weight change, small to moderate losses or gains showed random association with the large shifts in rate found in these animals (Chart 4). Thus, it was reported above that the rates of eruption observed during the second week of 8-azaguanine administration were partly higher, partly lower, and partly unchanged, as compared to those of the first week. Yet, during the same period, all except one stationary animal showed losses of weight ranging from 1 to 4 gm.

Only for the extreme weight changes observed was there a tendency for gains to be associated with gains and losses with losses in rate. Since even in this range of weight shifts this held only for the majority, and not for all the cases, loss of weight can at most be a factor which further depresses the rate of tooth eruption in the treated animals.

Influence of moribund animals on rate averages.—As was remarked before, there was a high mortality rate in the 8-azaguanine-treated animals. Many of the animals appeared to be sick many days before death (or recovery) occurred. The last rates of tooth eruption measured in these animals, however, showed as many instances of increase as of decrease over the preceding rate (10 decreases, 10 increases, and 2 stationary, in 22 animals dying during the period of 8-azaguanine administration). In only 2 of the 22 cases were ex-
The following comments and conclusions seem permissible:

The method of following the rate of incisor eruption in mice or rats is useful in testing for the presence or absence and the severity of the effects of drugs on physiological growth. It seems of particular usefulness in the field of tumor chemotherapy where the goal is inhibition of a local growth process with—ideally—no effect on systemic growth processes.

The inhibitory effect of 8-azaguanine is not limited to tumor tissue. The fact that a drug also inhibits the growth of incisors and, therefore, presumably of other growing tissues, need not contra-indicate its use in tumor therapy. However, since the tumor-inhibiting action of a substance like 8-azaguanine does not outlast the period of administration, the concomitant inhibition of systemic growth might be of more serious concern.

**SUMMARY**

The rate of eruption of the lower incisors was studied in 78 mice of strain C57 black.

1. It was found that weekly rates of eruption can be measured with a degree of accuracy which permits reliable averages to be computed from fairly small groups of animals.

2. The rates of eruption found in different groups of untreated animals were found very similar in averages and range.

3. One ml. of Locke's solution per day, injected in two doses, had no significant effect on the rate of eruption.

4. 8-Azaguanine, in the dosage effective for pronounced inhibition of tumor growth in mice, markedly depressed the rate of incisor eruption in two groups of mice without tumors and in one group with transplanted adenocarcinoma E 0771.

5. Loss of weight was only partially responsible for this effect. It was concluded that the growth inhibiting effect of 8-azaguanine is not limited to tumor tissue.

**REFERENCES**


1 Unpublished data of the Department of Dental Histology make it apparent that the rate of incisor eruption in rats is an equally reliable test object.
The Effect of 8-Azaguanine on Physiologic Growth Measured by the Rate of Eruption of the Incisor of the Mouse

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