pears in the medium, growth occurs or growth inhibition is observed. However, chromatographic examination of the 6-MP indicated less than 1% contamination with hypoxanthine. The trough of the response curve disappears after 48 hours incubation, at which time growth in concentrations up to 150 γ/ml is comparable to the control. Inhibition of the riboside is also overcome within a 120-hour incubation period. An alternative explanation for the 6-MP results is that 6-MP over a narrow concentration range serves as a purine source (after conversion to hypoxanthine) and thus stimulates growth and overcomes to a degree unchanged antagonist at certain concentrations. Inhibition by low levels in the early phase of growth may be due in some way to interference with the *de novo* formation of coenzymes or precursors necessary for biosynthesis of nucleic acids.

The present results require considerable extension prior to any final conclusion regarding the possible value of 6-mercaptopurine riboside as a chemotherapeutic agent; however, to date no evidence has been obtained which would suggest any advantage over the much more easily prepared 6-mercaptopurine.

Observation of cross-resistance of 6-mercaptopurine-resistant bacterial and neoplastic cells to 6-MP-riboside deletes one theoretical explanation of the mechanism of 6-MP-resistance—that is, that such resistant cells have lost the ability to ribosidate 6-MP.

*Summary.* (1) 6-Mercaptopurine riboside has been assayed for inhibitory activity against Adenocarcinoma 755 in mice and has been found to be of approximately the same activity as 6-mercaptopurine. (2) Lines of *S. faecalis* resistant to 6-mercaptopurine have been found to be cross-resistant to 6-mercaptopurine riboside. (3) A line of Adenocarcinoma 755 selected for resistance to 6-mercaptopurine showed cross-resistance to 6-mercaptopurine riboside.

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## Influence of Thiourea on Growth of Cells of Midbrain in Frogs.* (23145)

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Recent studies demonstrate that thyroid hormone affects the differentiation of particular cells of the amphibian central nervous system. Following treatment with thyroxine, certain cells of the medulla oblongata grow (1,2). Cells of the lateral motor column increase their nuclear size in response to both direct and indirect thyroxine stimulation(3). Several independent lines of investigation have shown that the level of differentiation attained by cells of the mesencephalic nucleus of the trigeminal nerve in tadpoles of *Rana pipiens* is *directly* dependent upon concentration of thyroid hormone available to those cells(4). From these studies it is inferred that blockage of normal metamorphosis by treatment of tadpoles with thiourea might result in termination of growth of the cells of the mesencephalic V nucleus, and perhaps even in their involution. Moreover, treatment of the metamorphosed frog with

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thiourea might reveal the extent to which these specialized midbrain cells are under thyroid hormone control in the postmetamorphic period. The present study reports the testing of these inferences.

*Material and methods.* All animals were reared from eggs laid in the laboratory (*Xenopus laevis*) or in ponds near Iowa City (*Rana pipiens*). Stock animals were kept in groups in aquaria, while experimental and control tadpoles were kept in individual bowls in 125 ml of water. Metamorphosed Xenopus were kept individually in covered dishes with 300-500 ml of water. Thiourea was added to the water of the bowls to attain concentrations of either 0.033%(5) or 0.05-0.06% for Rana, and 0.25-0.30% for Xenopus larvae and adults. In order to permit the initial spurt of rapid mesencephalic V nucleus cell growth, which occurs late in the larval period(6), treatment was deferred until the tadpoles were within 5-10 days of the time of expected emergence of the forelimbs(7,8). Control animals were selected to be about the same age, stage and size as the experimental animals at completion of the thiourea treatment. On the average the control animals were very slightly shorter than the matching thiourea treated partners. This discrepancy could bias the results only in the sense of decreasing the anticipated differences in cell size, since there is a positive correlation between body size and mesencephalic V nucleus cell size(6). Fixation of the animals, preparation of the slides, and selection and measurement of at least 75 cells on one side of each animal were carried out as before(4,6). The significance of differences between treated and control animals was determined by the method of paired comparisons (related measures).

*Results. Rana.* Treatment of 25 tadpoles with 0.033% thiourea failed to inhibit metamorphosis. These animals were discarded. A similar group immersed in 0.05-0.06% thiourea yielded 4 animals in which metamorphosis was completely blocked after 8 or more days, and 4 others in which metamorphosis was significantly slowed. In the 4 animals showing metamorphic stasis, and in 2 of the others, the sizes of cells and nuclei of the mesencephalic V nucleus were smaller than in control tadpoles (Table I).

TABLE I. Thiourea Treatment Time of *Rana pipiens*, and Stage at Fixation, with Average Sizes of Cells and Nuclei, in $\mu^2$, Compared to Those of Non-Treated Animals.

| Days in thiourea | Fixation stage (7) | Experimental | | Control | |
|---|---|---|---|---|---|
| | | Cell* | Nucleus* | Cell* | Nucleus* |
| 8 | XXIII– | 184 | 84 | 226 | 105 |
| 13 | XXII | 185 | 85 | 219 | 100 |
| 17 | XXI | 235 | 100 | 215 | 97 |
| 17 | " | 232 | 104 | 207 | 95 |
| 23† | XVII+ | 94 | 67 | 168 | 89 |
| 26† | " | 123 | 78 | 172 | 93 |
| 27† | " | 151 | 86 | 185 | 96 |
| 32† | XVI+ | 120 | 73 | 156 | 81 |
| Avg 20 | XIX+ | 166 | 85 | 194 | 96 |

* P of cells = .05; of nuclei = .04.
† Metamorphic stasis obtained.

*Xenopus.* Eight tadpoles were kept in 0.25-0.30% thiourea for 14-35 days. The rate of metamorphosis became progressively slower, and finally metamorphic stasis was achieved in each case, but only after the forelimbs had emerged. The mesencephalic V nucleus cells of experimental and control groups were compared with respect to maximum and average cell sizes, maximum and average nuclear sizes, nucleo-cytoplasmic ratios, and total cell numbers. In only 4 of the 48 comparisons were the anticipated relationships between experimental and control groups unrealized. Of the 6 sets of paired comparisons, only that dealing with cell numbers failed to show significance. Table II shows these relationships.

Fourteen metamorphosed Xenopus were kept in 0.25-0.30% thiourea for 23-32 days (avg 29). As in the larval study, 6 sets of comparisons with 14 matched control animals were made. Maximum and average nuclear sizes were significantly reduced. Maximum cell sizes were slightly lowered (5% level of confidence). The other 3 comparisons failed to show significant differences (Table II). Postmetamorphic age may be an important factor in thyroid hormone control of cell size, since the 7 small juveniles (24-34 mm) showed average cell size reductions 4 times as great

TABLE II. Comparisons of 8 Pairs of *Xenopus* Tadpoles (Upper Group), and of 14 Pairs* of Juvenile Metamorphosed *Xenopus*.

| | Max cell size ($\mu^2$) | | Avg cell size ($\mu^2$) | | Max nuclear size ($\mu^2$) | | Avg nuclear size ($\mu^2$) | | N/C† ratio | | Cell count | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Exp. | Control | Exp. | Control | Exp. | Control | Exp. | Control | Exp. | Control | Exp. | Control |
| | 314 | 337 | 113 | 180 | 85 | 107 | 53 | 68 | 46 | 37 | 151 | 132 |
| | 279 | 252 | 138 | 156 | 83 | 96 | 59 | 65 | 43 | 42 | 85 | 92 |
| | 246 | 299 | 124 | 172 | 110 | 103 | 63 | 68 | 51 | 39 | 86 | 94 |
| | 215 | 316 | 120 | 203 | 88 | 99 | 58 | 68 | 47 | 33 | 108 | 118 |
| | 228 | 292 | 119 | 187 | 78 | 100 | 53 | 71 | 44 | 38 | 79 | 127 |
| | 246 | 349 | 140 | 192 | 91 | 92 | 64 | 68 | 45 | 36 | 89 | 123 |
| | 232 | 369 | 115 | 190 | 76 | 95 | 54 | 67 | 46 | 35 | 116 | 108 |
| | 197 | 337 | 82 | 198 | 90 | 97 | 48 | 72 | 58 | 36 | 86 | 99 |
| Avg | 245 | 319 | 119 | 185 | 89 | 99 | 57 | 68 | 48 | 37 | 100 | 112 |
| p | <.008 | | <.001 | | <.03 | | <.002 | | <.001 | | <.25 | |
| | 419 | 493 | 263 | 284 | 104 | 116 | 68 | 79 | 26 | 27 | 94 | 97 |
| | 491 | 428 | 339 | 299 | 114 | 119 | 88 | 80 | 26 | 26 | 75 | 83 |
| | 357 | 525 | 272 | 318 | 107 | 113 | 76 | 81 | 27 | 25 | 85 | 75 |
| | 372 | 421 | 269 | 279 | 103 | 131 | 75 | 80 | 30 | 28 | 90 | 101 |
| | 478 | 550 | 303 | 310 | 118 | 124 | 88 | 87 | 29 | 28 | 104 | 105 |
| | 484 | 460 | 259 | 260 | 116 | 109 | 74 | 80 | 29 | 30 | 94 | 77 |
| | 400 | 392 | 256 | 236 | 108 | 108 | 68 | 68 | 26 | 28 | 77 | 105 |
| | 282 | 319 | 157 | 183 | 81 | 109 | 55 | 61 | 35 | 33 | 76 | 106 |
| | 320 | 356 | 194 | 174 | 97 | 96 | 61 | 60 | 32 | 34 | 97 | 102 |
| | 388 | 424 | 219 | 253 | 94 | 108 | 65 | 73 | 29 | 29 | 90 | 102 |
| | 361 | 384 | 213 | 239 | 104 | 104 | 64 | 73 | 30 | 30 | 105 | 97 |
| | 345 | 352 | 193 | 208 | 86 | 95 | 59 | 66 | 31 | 32 | 104 | 83 |
| | 283 | 280 | 163 | 183 | 76 | 94 | 54 | 62 | 33 | 34 | 106 | 103 |
| | 276 | 329 | 188 | 198 | 80 | 91 | 59 | 66 | 31 | 33 | 81 | 79 |
| Avg | 375 | 408 | 235 | 244 | 99 | 108 | 68 | 73 | 30 | 30 | 91 | 94 |
| p | <.05 | | <.15 | | <.01 | | <.01 | | <.5 | | <.25 | |

* Arranged in order of decreasing body length (65-20 mm).

† $\frac{\text{Avg nuclear size}}{\text{Avg cell size}} \times 100$.

as those shown by the large ones (43-68 mm). Nuclear differences also varied correspondingly.

*Discussion.* The treatment of *Rana pipiens* larvae with thiourea during the rapid growth phase of the cells of the mesencephalic V nucleus resulted in reduction of the sizes of these cells and their nuclei. This has been even more convincingly shown in the more completely inhibited series with Xenopus larvae, and extended, in part, into the early postmetamorphic period. These results for the larval studies were predicted on the basis of earlier work on the cells of this nucleus(4,6). The realization of the prediction gives added support to the conclusion that level of differentiation of cells of the mesencephalic V nucleus is continuously influenced by the concentration of thyroid hormone in the circulation, in the same sense that cells of the mammalian prostate gland are under the control of androgens(9). A few additional neural cells in amphibians have been shown to respond by either growth or involution(1,2,10) to the addition of thyroid hormone (Mauthner's cell, other cells in the medulla oblongata, lateral motor column cells of the posterior trunk segments of the spinal cord), but thus far only the mesencephalic V nucleus cells have been shown to respond directly to the hormone (see references 3, 4, for discussions and review). That other hormones influence brain cells in other vertebrates is also clearly demonstrated by the influence of hormones upon behavior(11).

*Summary.* 1. Tadpoles of *Rana pipiens* and *Xenopus laevis* in premetamorphic stages were placed in solutions of thiourea up to 35 days. After 8 days or longer significant slowing of metamorphosis was achieved. Commonly complete arrest of metamorphosis occurred. 2. Compared to values in control animals, the sizes of cells and nuclei of the mesencephalic V nucleus were significantly

reduced, and the nucleo-cytoplasmic ratio was increased, *i.e.*, the changes were toward values characterizing younger larval stages. 3. Similar treatment of juvenile postmetamorphic Xenopus gave comparable but less marked changes in cell and nuclear sizes. The changes were greatest in smaller animals.

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## Collagen Digestion by Dog Pancreatic Juice.* (23146)

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Although collagen has long been recognized to be resistant to enzymic hydrolysis, Ssasdikow(1) reported that trypsin-free fractions of pancreatic extract partially degrade collagen. Weak collagenolytic activity was also found in dog pancreatic juice obtained by Pavlov fistula. Recently Ziffren and Hosie(2) reported degradation of collagen by commercial trypsin preparations and pancreatic juice, and postulated the presence of a heat labile enzyme, collagenase, in pancreatic juice. Banga and Balo(3) noted liberation of carbohydrate from collagen during incubation with an enzyme from a pancreatic extract they called collagenmucoproteinase. Certain bacteria, notably *Clostridia*, produce collagenase as an exoenzyme.

Because of the uncertainty of the identity of the enzyme responsible for collagen degradation in dog pancreatic juice, the present study was undertaken in an attempt to define the nature of "collagenase."

*Methods.* Collagen was prepared from beef achilles tendon by the method of Buechler(4). Electron microscopy† of the preparation revealed that the great majority of particles showed the characteristic structure of native collagen. Crystalline trypsin, chymotrypsin, and soy-bean inhibitor were obtained from Worthington Biochemical Corp., Freehold, N. J. Carboxypeptidase was obtained from Pentex, Inc., Kankakee, Ill. Bacterial collagenase from *Clostridium histolyticum* was generously supplied by Dr. R. E. Kallio of the Department of Bacteriology. Pancreatic juice was obtained from mongrel dogs by the procedures of Ziffren and Hosie(2). Collagen (50 mg) was suspended in 3 ml of pancreatic juice, enzyme solutions, or buffer solutions, each at pH 8.0. After incubation at 37° for 24 hours with occasional shaking, 7 ml of water was added, the suspension was shaken well and centrifuged. The residuum was saved for further study and a portion of supernatant was autoclaved for 3 hours in 6 N HCl at 15 lb/sq. in. An aliquot of the hydrolysate was neutralized to pH 4-5 with 6 N NaOH and the free hydroxyproline was determined by the method of Neuman and Logan(5). Undigested collagen obtained by

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