Carcinogenesis in the Hamster Cheek Pouch

II. Changes in Enzymes of Glucose-6-phosphate Oxidation

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SUMMARY

The activities of the enzymes, hexokinase, glucose-6-phosphate dehydrogenase, and phosphogluconate dehydrogenase were determined in extracts prepared from hamster cheek pouches which were being painted thrice weekly with 9,10-dimethyl-1,2-benzanthracene. Histology revealed five periods: I, no change, approximately 1 week; II, inflammation, 2d and 3d week; III, hyperplasia after the 3d week; IV, preneoplastic hyperplasia and appearance of papillomas after about 7 weeks; and V, appearance of malignant tumors.

The specific activities of all three enzymes were significantly higher in tumors than in unpainted cheek pouches or in controls painted with mineral oil. The patterns of change in enzyme activities in the cheek pouches during the course of carcinogenesis were different for each enzyme. These differences were especially evident in periods III to V. Hexokinase was low in period III and returned to the control range in period V. Glucose-6-phosphate dehydrogenase increased during periods IV and V. Phosphogluconate dehydrogenase was low during periods III and IV. A difference between the sexes was evident especially in the inflammatory reaction as well as in patterns of changes in enzyme activities.

It was concluded that changes of phosphogluconate show the greatest degree of correlation with the neoplastic state as compared with the hyperplastic state.

Aisenberg (4) has reviewed the metabolism of tumors and agrees with Greenstein (20) that "with regard to their energy metabolism, tumors tend to converge, enzymatically, to a common type of tissue." Aisenberg adds, "It is difficult to accept the view that the neoplastic process is in essence tumor energy metabolism... It is abnormal growth and division that best characterize the neoplastic process." It is evident that whatever the carcinogenetic stimulus, whether by a chemical, irradiation, or a virus, and whether a mutation is the primary event or not, there are secondary changes in the metabolism of the tissue of origin which lead to the type of metabolism which is common to most tumors. That is, the range of activities of each enzyme studied in tumors falls somewhere within the range for that enzyme in normal tissues. Some enzymes may be induced to increase or decrease in activity or amount during the transformation from normal to neoplastic tissue.

Previous studies with carcinogens used to produce tumors, especially of the skin and the liver, have often shown no change or a slight change in the activities of enzymes investigated during the period of preneoplastic hyperplasia and in the tumor-bearing tissue, whereas the tumor might have enzyme activities several-fold higher, greatly decreased, or reduced to zero.
Exceptions to this have been the increase in activity of cytochrome oxidase and the decrease in content of lipide (19) and of the cations, Ca++, Fe++, Cu+, and Zn++ in mouse skin painted with 0.6 per cent solution of methylcholanthrene as reported by Carruthers and Suntzeff (13–15). Glock and McLean (19) found that TPNH concentration was decreased during carcinogenesis with azo dye and was low in the tumor-bearing liver and very low in the tumors so produced as well as in all other tumors investigated.

We have studied enzyme systems and chemical substances which seemed especially related to growth and division of cells, rather than to energy relationships or special functions. The enzymes of hexose monophosphate oxidation and those dealing with TPNH generated by these enzymes have not been found to produce any high-energy phosphates, whereas they do produce ribosephosphate for the building of new nucleic acid, CO₂, for several essential anabolic steps and TPNH, for many biosynthetic reactions, such as fat and steroid synthesis, and for keeping GSH and proteins in the reduced state (27). Glucose-6-phosphate dehydrogenase has been reported to be high in certain tumors compared with that in normal liver (3, 18, 22, 36) and in Ehrlich ascites tumor (38).

We have chosen to determine whether there were changes in these parameters in the hamster cheek pouch during carcinogenesis by application of 9,10-dimethyl-1,2-benzanthracene (29). Cheek pouch tissue is simpler than skin, there being no hair follicles and sebaceous glands with their cyclic changes and portals of entry to complicate the picture. There is, however, a peculiar immunologic barrier in the basement membrane of this tissue, perhaps owing to lack of lymphatic drainage of the pouch (8). What effect this has on the course of tumor formation is not yet evident; it may be to confine the effect of the carcinogen.

The histological appearances and the changes in sulfhydryl content evident in hamster cheek pouches which were painted with the carcinogen 9,10-dimethyl-1,2-benzanthracene, were reported and discussed in a previous paper (25). Here we are reporting the changes in the activity of some enzymes of the hexose monophosphate oxidation series in the extracts of the same hamster cheek pouches.

MATERIALS AND METHODS

The care of albino Syrian hamsters, the method of painting the cheek pouches thrice weekly with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene (DMBA), and the preparation of specimens for histopathology and of extracts in 0.25 M sucrose have been described in the previous paper (25).

Glucose-6-phosphate and 6-phosphogluconate, as either the barium or sodium salts, TPN, TPNH, DPN, DPNH, and ATP were obtained from Sigma Chemical Co. of St. Louis, Missouri. Other chemicals were obtained from various commercial sources.

The activities of hexokinase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were determined as the rate of reduction of TPN measured at 340 μM in the Beckman DU spectrophotometer at 36° C., as reported previously (32).

All enzyme activities were determined in the range where the change in optical density at 340 μM was proportional to the amount of extract added to the reaction mixture which contained substrates, coenzymes, and ions in concentrations which were not limiting to the reaction. For measurement of glucose-6-phosphate dehydrogenase the range is wide. For phosphogluconate dehydrogenase a sample of extracts was chosen which gave an optical density increase of less than 0.040 per minute, and the values for the 2d and 3d minute were used. With more enzyme, or at later times, the rate is decreased by TPNH, which is inhibitory to the reaction. For determination of hexokinase, the range of enzyme concentration in which proportionality is maintained is rather narrow and the concentration high. At lower concentrations, an activator can be detected by addition of boiled extract. According to the method of determination of hexokinase activity by reaction of the glucose-6-phosphate formed with TPN, there is a lag phase during which the glucose-6-phosphate concentration is built up. If the sample of extract is too small, the lag phase is unduly prolonged. The glucose-6-phosphate dehydrogenase present in the extracts was always sufficient as not to be limiting. The advantage of the method is the removal of glucose-6-phosphate which inhibits hexokinase (16). The hexokinase activity determined in the extracts probably does not represent the total hexokinase of the tissue, since in other tissues hexokinase has been found in both soluble and particulate fractions, especially in the membrane of either cells or mitochondria and even in nuclei (17). By our method of grinding the tissue with alumina, the particulate fractions cannot be studied, and it is possible that some of the enzymes usually in these fractions may have been solubilized. The effect of different methods of extracting the tissue on the partition of hexokinase is being investigated.

Results are reported as μmoles TPN/min/gm
of protein of the extracts and are the means of two or more determinations.

Protein.—The concentration of protein in the extracts was determined by the dye method of Bonting and Jones (9).

Glucose concentration was determined by glucose oxidase by the “Glucostat” procedure obtained from Worthington Biochemical Corporation, Freehold, N.J.

RESULTS

Experimental design.—In total, 127 hamsters were used in six series of experiments. Of these 41 were controls, twenty treated with mineral oil, and 21 untreated. Fifty-eight females, 23 males, and five whose sex was not recorded were treated with the carcinogen. Fifteen large tumors were analyzed after separation from the residual pouch. In the first series, pouches from males and females were taken at alternate weeks, and an unexpected difference between the sexes was evident. Thereafter, the animals in a series were all of the same sex. The reaction of the hamster cheek pouches to 9,10-di-methyl-1,2-benzanthracene, with the concentration and schedule of painting employed in these experiments, seems to fall into five main sequences in time. In the first week, period I, there was no reaction. The first reaction was inflammation which occurred during the 2d and 3d week (period II). This reaction was not necessarily involved in the later neoplastic reaction, because with lower concentrations of the carcinogen or different schedules of application there might be no inflammatory reaction, and still tumors were produced, although the time of appearance was different (24). Period III came between subsidence of inflammation and the appearance of the first small papillomas. The tissue appeared normal grossly but microscopically revealed a uniform hyperplasia. Later, focal areas of atypical hyperplasia, followed by proliferative downgrowth into the connective tissue, could be observed. As adjacent proliferating strands coalesced and the overlying epithelium thickened, small papillomas formed at about 7 weeks (period IV). During period V the papillomas grossly increased in size and microscopically underwent alteration to frank malignancy (24). After approximately 12 weeks, one or more tumors measuring 1 cm. or more in diameter were present, and numerous malignant lesions of varying size were seen grossly and microscopically.

Pouch size and protein content of extracts.—Although it was not possible to excise all the pouch tissue, the ligature was made as closely as possible to the edges of the pouch while still leaving enough for proper healing and closure of the excision. The wet weights for the 44 control pouches, either untreated or painted with mineral oil alone, fell within the range of 200–575 mg. Only four, all from females, weighed more than 450 mg. The painted pouches were inflamed and enlarged in period II; all weighed more than 400 mg., and eight weighed between 700 and 960 mg. These last were all females, and the most acute inflammatory reactions occurred in one group of young females, in some of whom bleeding ulcers were present. During period III only three pouches weighed less than 300 mg.; all pouches of males had weights less than 480 mg., and pouches of females varied between 290 and 650 mg. In periods IV and V all pouches were enlarged, with females having higher ranges than the males.

The soluble protein content of the control pouches, either untreated or painted with mineral oil, ranged from 4.2 to 8.4 mg protein/ml extract. The concentrations of protein in extracts of pouches painted with carcinogen were increased during the inflammatory period, being as high as 14 mg/ml in the pouches showing severe inflammatory reactions. Values were decreased thereafter within the range of 5–9 mg/ml until the period of preneoplastic hyperplasia and tumor formation, when they again were high, 6–12 mg/ml. Here also the values for females tended to be higher than for males.

Enzymes of control pouches.—Enzyme activities were determined on pouches which were taken from the animals at different times, the left from 1 to 3 weeks after the right. With both sexes the cheek pouch enzymes were low in young and old animals whose weight was less than 50 gm. or greater than 160 gm. The greatest variation in enzyme activities was shown by females between 90 and 160 gm. The concentrations of enzyme in extracts of pouches painted with carcinogen were increased during the inflammatory period, being as high as 14 mg/ml in the pouches showing severe inflammatory reactions. Values were decreased thereafter within the range of 5–9 mg/ml until the period of preneoplastic hyperplasia and tumor formation, when they again were high, 6–12 mg/ml. Here also the values for females tended to be higher than for males.

Results as the means of duplicate or triplicate analyses of extracts of unpainted controls and of controls painted with mineral oil are in Table 2, giving the range, arithmetic mean, and the standard deviations. It may be noted that the mean control value of glucose-6-phosphate dehydrogen-
ase from female pouches had a higher standard deviation than that from male pouches. The mean enzyme activities of pouches painted with mineral oil were not significantly different from those of the controls, except that low enzyme activities were noted in extracts of three pouches which had been painted 3 times a week for 9 weeks, when the difference was perhaps significant.

**Enzyme of whole pouches painted with DMBA.** — In Chart 1 are plotted the specific activities of glucose-6-phosphate dehydrogenase as means of the three pouches taken at selected times after initiation of painting with DMBA, from all series. The pouches of males reacted with increasing activities of the enzyme throughout the course of the experiments. The initial reaction of the pouches of females was a drop in the specific activity. There was a rise in period IV to higher values than the males in period V.

The phosphogluconate dehydrogenase activities of the painted pouches of three series are shown in Chart 2. Here the pouches reacted with an early drop in activity, a rise during inflammation, and a fall thereafter, with low levels maintained in the hyperplastic periods.

The hexokinase activities of the pouch extracts fell between the two other enzymes with a pattern more like phosphogluconate dehydrogenase; but the extents of the early drop, the rise during inflammation, and the low values during hyperplasia were not as great as for phosphogluconate dehydrogenase. The hexokinase values after 50 days were nearly the same as those of the controls.

In Table 2 are listed the mean of specific activities of the enzymes during each period, the number of pouches from which the mean was derived, and the significance of differences from control values.

**TABLE 1**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Weight of animal</th>
<th>Pouch</th>
<th>Hexokinase*</th>
<th>Glucose-6-phosphate dehydrogenase*</th>
<th>Phosphogluconate dehydrogenase*</th>
</tr>
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<tbody>
<tr>
<td>♂</td>
<td>140</td>
<td>R</td>
<td>6.4</td>
<td>46</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>7.9</td>
<td>46</td>
<td>6.4</td>
</tr>
<tr>
<td>♂</td>
<td>128</td>
<td>R</td>
<td>11.7</td>
<td>79</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>12.2</td>
<td>84</td>
<td>14.1</td>
</tr>
<tr>
<td>♂</td>
<td>140</td>
<td>R</td>
<td>7.4</td>
<td>73</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>13.8</td>
<td>70</td>
<td>15.1</td>
</tr>
<tr>
<td>♂</td>
<td>35</td>
<td>R</td>
<td>6.1</td>
<td>26</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>6.4</td>
<td>34</td>
<td>5.8</td>
</tr>
<tr>
<td>♂</td>
<td>51</td>
<td>R</td>
<td>15.9</td>
<td>41</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>8.7</td>
<td>60</td>
<td>10.3</td>
</tr>
<tr>
<td>♂</td>
<td>90</td>
<td>R</td>
<td>11.7</td>
<td>56</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>9.0</td>
<td>48</td>
<td>5.5</td>
</tr>
<tr>
<td>♂</td>
<td>Between 90 and 140</td>
<td>R</td>
<td>16.7</td>
<td>140</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>9.0</td>
<td>40</td>
<td>11.9</td>
</tr>
<tr>
<td>♂</td>
<td>&quot;</td>
<td>R</td>
<td>16.9</td>
<td>71</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>5.1</td>
<td>49</td>
<td>9.2</td>
</tr>
<tr>
<td>♂</td>
<td>&quot;</td>
<td>R</td>
<td>17.8</td>
<td>70</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>12.8</td>
<td>58</td>
<td>9.5</td>
</tr>
</tbody>
</table>

*μmoles/min/gm protein.

For comparison of the patterns of change, Chart 3 shows the percentage difference from control values of the mean enzyme activities in each period. The difference between the glucose-6-phosphate dehydrogenase and the other two enzymes and the striking difference between the sexes is evident. The extent of change is greater for phosphogluconate dehydrogenase than for hexokinase.

When the total enzyme units per pouch were calculated, the increase in total pouch weight and the increase in soluble protein of the extracts during the inflammatory period and again in later periods led to values which were about 3 times control values in period II for all three enzymes.
**TABLE 2**

**ENZYME ACTIVITIES OF EXTRACTS OF HAMSTER CHEEK POUCHES***

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>PERIOD</th>
<th>NO. POUCHES</th>
<th>HEXOKINASE</th>
<th>GLUCOSE-6-PHOSPHATE DEHYDROGENASE</th>
<th>PHOSPHOGLUCONATE DEHYDROGENASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>DAYS</td>
<td>RANGE</td>
<td>MEAN</td>
<td>SD;</td>
</tr>
<tr>
<td>Controls, un-painted</td>
<td>29</td>
<td>1-5</td>
<td>4.2-15.0</td>
<td>9.6</td>
<td>±3.4</td>
</tr>
<tr>
<td>Controls, mineral oil</td>
<td>II</td>
<td>8-22</td>
<td>7.0-15.0</td>
<td>8.7</td>
<td>±3.5</td>
</tr>
<tr>
<td>DMBA</td>
<td>I</td>
<td>1-8</td>
<td>1.1-10</td>
<td>6.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5-15</td>
<td>5.7-17</td>
<td>9.5</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>22-45</td>
<td>7.6-15.0</td>
<td>7.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>48-57</td>
<td>4.3-8.6</td>
<td>8.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>57-85</td>
<td>9.3-13.8</td>
<td>8.7</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Activities as μmoles TPN reduced per gm. protein per minute at 36° C.

† SD = standard deviation; P = probability.

‡ Five pouches contained one or more small tumors.

§ Six pouches contained one or more tumors.

## CHART 1

Specific activities of glucose-6-phosphate dehydrogenase of hamster cheek pouches at the times indicated after initiation of painting with 0.5 per cent 9,10-dimethyl-1,2-benzanthracene. The filled symbols indicate males and the open symbols indicate females. Squares □ are for Experiment 1; circles ○ for Experiments 2 and 5; triangles △ for Experiment 3; and inverted triangles ▽ for Experiment 4. Each symbol represents the mean of values for three pouches. The means of activities of unpainted control pouches are indicated at the right margin.
and both sexes. The total phosphogluconate dehydrogenase values during periods III, IV, and V fell within the control range. The total hexokinase values in periods III and IV fell in the control range and were high in period V. All total glucose-6-phosphate dehydrogenase values were high after period I.

**Enzymes of tumors.**—In Table 3 are the enzyme activities of extracts of the tumors, the epithelial layers which were scraped away from the residual pouch tissue, and the residues of the pouches. In three of these experiments the extraction was performed by homogenizing both the tumor and the epithelial scrapings, by grinding the residue with alumina, and extracting each with sucrose or mannitol solution containing 0.1 M sodium versenate at pH 7.4. The greatest differences between these tissues were evident in the phosphogluconate dehydrogenase activities.

In Table 2 and Chart 3 the enzyme activities of

**Chart 2.**—Specific activities of phosphogluconate dehydrogenase in extracts of hamster cheek pouches at the times indicated after initiation of painting with carcinogen. The symbols are the same as for Chart 1. The filled symbols and solid lines indicate males, and the open symbols and broken lines indicate females.

**Chart 3.**—Specific activities of enzymes of extracts of hamster cheek pouches as percentage of mean control activities. Each symbol represents the mean of the activities of all extracts investigated during the period indicated. Period II is inflammation. Period III hyperplasia, Period IV preneoplastic hyperplasia and appearance of papillomas, and Period V appearance of malignant tumors. The symbols are: × = hexokinase, ○ = glucose-6-phosphate dehydrogenase; and • = phosphogluconate dehydrogenase.
whole pouches in which at least half of the tissue was neoplastic have been excluded from the means of period V. In these pouches the high activities for phosphogluconate dehydrogenase of the tumor and the low activities of the hyperplastic tissue together produced high values for the whole pouch. This effect was more evident for pouches of males than females and was less evident with the other enzymes. The mean enzyme activities are shown in Table 4.

Also listed in Table 4 are the enzyme activities of tumors and of residual pouch tissue from which the tumors were removed, the ratio between these activities, and comparison with control activities. There seemed to be only one class of tumors in male animals, whereas in females there was a difference between tumors taken from pouches which were still being painted and those taken after painting was discontinued. This difference was significant in lower hexokinase activities and perhaps in higher glucose-6-phosphate dehydrogenase activities 3–4 weeks post-painting, whereas there was no difference in the phosphogluconate dehydrogenase values. At still later times four pouches of females which had had large tumors were found to have no neoplastic areas or only small tumors at the end of the pouches. The enzyme activities of these pouches were low compared with those of control pouches. So far we have not noted regression of tumors in male hamsters.

### DISCUSSION

The specific activities of the three enzymes investigated were high in the tumors. During the course of carcinogenesis the direction of change in activity in the tumors might be the same as the residual pouch or even higher. The changes of the activity of phosphogluconate dehydrogenase during carcinogenesis fell into the third category. In period III, the specific activities dropped below the normal range and continued to be low in the nontumorous tissue after the appearance of frank tumors, whereas the tumors themselves were high in phosphogluconate dehydrogenase activity.

Although the changes of the enzymes in periods I and II, before and during inflammation, may not be significant in the neoplastic process, they are of interest to the general problem of the inflammatory reaction and will be discussed elsewhere.

Glucose-6-phosphate dehydrogenase has always been reported to be only in the soluble fraction of the cell except for some activity in the nuclei reported by Stern and Mirsky (35) and Werkheiser (37). It is probable that grinding with alumina

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weight of tissue (mg.)</th>
<th>Hexokinase units</th>
<th>Glucose-6-phosphate dehydrogenase units</th>
<th>Phosphogluconate dehydrogenase units</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Tumor</td>
<td>528</td>
<td>13.8</td>
<td>24.0</td>
<td>17.2</td>
</tr>
<tr>
<td>Epithelium</td>
<td>56</td>
<td>8.2</td>
<td>95.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Residue</td>
<td>1094</td>
<td>7.1</td>
<td>9.2</td>
<td>2.2</td>
</tr>
<tr>
<td>II Tumor</td>
<td>900</td>
<td>19.4</td>
<td>128</td>
<td>80.0</td>
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<tr>
<td>Epithelium</td>
<td>62</td>
<td>17.8</td>
<td>115</td>
<td>49.7</td>
</tr>
<tr>
<td>Residue</td>
<td>1111</td>
<td>13.3</td>
<td>88</td>
<td>22.4</td>
</tr>
<tr>
<td>III Tumor</td>
<td>980</td>
<td>21.2</td>
<td>229</td>
<td>63.0</td>
</tr>
<tr>
<td>Epithelium</td>
<td>45</td>
<td>18.6</td>
<td>196</td>
<td>50.4</td>
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<tr>
<td>Residue</td>
<td>1007</td>
<td>15.6</td>
<td>188</td>
<td>20.2</td>
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<tr>
<td>IV Tumor</td>
<td>1528</td>
<td>24.4</td>
<td>170</td>
<td>48.0</td>
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<tr>
<td>Epithelium</td>
<td>40</td>
<td>25.2</td>
<td>193</td>
<td>37.0</td>
</tr>
<tr>
<td>Residue</td>
<td>1043</td>
<td>26.5</td>
<td>180</td>
<td>26.0</td>
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</table>

* Units = μmoles/min/gm protein.
† Extracted with versene.
<table>
<thead>
<tr>
<th>Sex</th>
<th>Tissue</th>
<th>No.</th>
<th>Hexokinase</th>
<th>Glucose-6-phosphate dehydrogenase</th>
<th>Phosphogluconate dehydrogenase</th>
<th>Endogenous</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>M</td>
<td>Pouches containing large and/or multiple tumors</td>
<td>5</td>
<td>9.3-15.6</td>
<td>12.7 ± 2.0</td>
<td>160</td>
<td>160</td>
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<tr>
<td></td>
<td>Tumors</td>
<td>8</td>
<td>18.0-26.0</td>
<td>24.0 ± 6.7</td>
<td>250</td>
<td>92-210</td>
</tr>
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<td></td>
<td>Residue of pouch from which tumor was taken</td>
<td>8</td>
<td>8.7-26.0</td>
<td>15.2 ± 6.3</td>
<td>180</td>
<td>163 ± 38</td>
</tr>
<tr>
<td>F</td>
<td>Pouches containing large and/or multiple tumors</td>
<td>5</td>
<td>9.6-13.5</td>
<td>11.4 ± 2.1</td>
<td>104</td>
<td>104</td>
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<td>Tumors after 10-12 weeks painting</td>
<td>3</td>
<td>12.7-20.2</td>
<td>20.5 ± 6.7</td>
<td>186</td>
<td>158-204</td>
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<tr>
<td></td>
<td>Residue of pouch from which tumor was taken</td>
<td>3</td>
<td>8.0-15.0</td>
<td>10.9 ± 3.0</td>
<td>100</td>
<td>186 ± 20</td>
</tr>
<tr>
<td></td>
<td>Tumors, 3-4 weeks after cessation of painting</td>
<td>3</td>
<td>12.2-18.0</td>
<td>14.3 ± 2.6</td>
<td>130</td>
<td>156-210</td>
</tr>
<tr>
<td></td>
<td>Residue of pouch from which tumor was taken</td>
<td>3</td>
<td>6.1-11.5</td>
<td>9.1 ± 2.2</td>
<td>84</td>
<td>164 ± 38</td>
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<tr>
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<td>Pouches in which tumors had regressed</td>
<td>4</td>
<td>4.5-7.6</td>
<td>6.0 ± 1.5</td>
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<tr>
<td>M</td>
<td>Pouches containing large and/or multiple tumors</td>
<td>5</td>
<td>69-120</td>
<td>106 ± 17</td>
<td>170</td>
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<tr>
<td></td>
<td>Tumors</td>
<td>7</td>
<td>126-244</td>
<td>187 ± 40</td>
<td>200</td>
<td>186 ± 36</td>
</tr>
<tr>
<td></td>
<td>Residue of pouch from which tumor was taken</td>
<td>7</td>
<td>88-180</td>
<td>143 ± 24</td>
<td>230</td>
<td>95-200</td>
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<td>F</td>
<td>Pouches containing large and/or multiple tumors</td>
<td>5</td>
<td>106-173</td>
<td>188 ± 22</td>
<td>220</td>
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<tr>
<td></td>
<td>Tumors after 10-12 weeks painting</td>
<td>3</td>
<td>97-246</td>
<td>159 ± 63</td>
<td>260</td>
<td>99-195</td>
</tr>
<tr>
<td></td>
<td>Residue of pouch from which tumor was taken</td>
<td>3</td>
<td>98-126</td>
<td>109 ± 12</td>
<td>175</td>
<td>147 ± 40</td>
</tr>
<tr>
<td></td>
<td>Tumors, 3-4 weeks after cessation of painting</td>
<td>3</td>
<td>169-207</td>
<td>182 ± 18</td>
<td>295</td>
<td>86-227</td>
</tr>
<tr>
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<td>Residue of pouch from which tumor was taken</td>
<td>3</td>
<td>108-200</td>
<td>140 ± 43</td>
<td>230</td>
<td>166 ± 59</td>
</tr>
<tr>
<td></td>
<td>Pouches in which tumors had regressed</td>
<td>4</td>
<td>33-78</td>
<td>52 ± 16</td>
<td>84</td>
<td>84</td>
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<tr>
<td>M</td>
<td>Pouches containing large and/or multiple tumors</td>
<td>5</td>
<td>10.6-30.5</td>
<td>17.6 ± 8.8</td>
<td>160</td>
<td>160</td>
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<td>Tumors</td>
<td>7</td>
<td>33.0-80.0</td>
<td>50.0 ± 16.0</td>
<td>460</td>
<td>185-480</td>
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<td>Residue of pouch from which tumor was taken</td>
<td>7</td>
<td>8.2-26.0</td>
<td>18.0 ± 5.7</td>
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<td>290 ± 75</td>
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<td>18.2-18.3</td>
<td>14.8 ± 1.8</td>
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<td>16.8-30.2</td>
<td>21.4 ± 6.0</td>
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<td>198 ± 51</td>
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<td>Residue of pouch from which tumor was taken</td>
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<td>18.3-13.5</td>
<td>11.2 ± 2.2</td>
<td>94</td>
<td>129-254</td>
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<td>Tumors, 3-4 weeks after cessation of painting</td>
<td>3</td>
<td>16.5-28.0</td>
<td>21.2 ± 5.0</td>
<td>180</td>
<td>129-200</td>
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<td>Residue of pouch from which tumor was taken</td>
<td>3</td>
<td>12.0-14.0</td>
<td>12.9 ± 1.0</td>
<td>108</td>
<td>163 ± 29</td>
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<td>Pouches in which tumors had regressed</td>
<td>4</td>
<td>6.8-11.9</td>
<td>8.8 ± 1.9</td>
<td>74</td>
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<td>M</td>
<td>Tumors</td>
<td>5</td>
<td>0.6-9.6</td>
<td>5.0 ± 3.8</td>
<td>100</td>
<td>100</td>
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<td>Residues</td>
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<td>0.2-1.8</td>
<td>1.2 ± 0.6</td>
<td>100</td>
<td>100</td>
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<td>Tumors</td>
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<td>0.3-5.0</td>
<td>2.6 ± 1.4</td>
<td>100</td>
<td>100</td>
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<td>0-8.0</td>
<td>2.4 ± 2.6</td>
<td>100</td>
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* μmoles/min/gm extract protein.
† Each tumor compared with its own pouch.
released any enzyme within nuclei. It is evident from the work of Angeletti, Suntzeff, and Moore (6) that glucose-6-phosphate dehydrogenase consists of more than one species, because it is eluted from DEAE columns in two or three overlapping peaks. It is possible that one of these species may be induced to increase more than the others under certain conditions. There were statistically significant differences between the changes in enzyme activities of males and females which possibly also involved different glucose-6-phosphate dehydrogenases.

Phosphogluconate dehydrogenase has been found only in the soluble fraction of the cell. According to Angeletti, Suntzeff, and Moore (6), there was only one sharp peak on elution from DEAE columns of extracts of all tissues investigated.

Regarding the mechanism of induction of enzyme synthesis, some substrates or activators of enzymes may also act as inducers of enzyme synthesis. In E. coli both hexokinase and glucose-6-phosphate dehydrogenase were induced to increase by the presence of glucose in the medium (31, 33). The latter enzyme was also increased by certain substances which oxidize TPNH (indirectly), such as dinitrophenol (30). However, phosphogluconate dehydrogenase was not increased at the same time, nor was it increased by the presence of gluconate in the medium, which induced the formation of glucokinase (33) and the production of phosphogluconate.

In mammalian tissue the participation of the hexosemonophosphate oxidative enzymes in carbohydrate metabolism, as determined by isotopic studies, can be greatly increased in vivo by substances which increase glucose uptake, such as insulin (2), or which accept electrons from TPNH, such as phenazine methosulfate (23), methylene blue (7), or Synkavit, which is vitamin K3 phosphate (21). Continued high concentrations of TPN may be able to induce increased synthesis as well as activity of glucose-6-phosphate dehydrogenase. Tissues in which TPNH is used for biosynthetic reactions, such as lactating mammary gland or adrenal (18), have high glucose-6-phosphate dehydrogenase and may have high phosphogluconate dehydrogenase.

However, this is not always true for phosphogluconate dehydrogenase. It is possible that, for induction of synthesis of phosphogluconate dehydrogenase, increased phosphogluconate and TPN concentrations are not sufficient and that decreased TPNH and a low TPNH/TPN ratio are necessary. It has been found that phosphogluconate dehydrogenase activity is very sensitive to the TPNH/TPN ratio. With a ratio of 1 or higher, which is found in normal tissues and organisms (11), the rate of the phosphogluconate dehydrogenase is decreased to 25 per cent of maximal. Abdel-Latif and Alivisatos (1, 5) reported that DPNase catalyzes a reaction of pyridine nucleotides, especially TPN, with histamine, producing triphosphohistamine nucleotide. This reaction might be responsible for the low total TPN concentrations during carcinogenesis and the very low concentrations in tumors, reported by Glock and McLean (19).

Some natural substances which react with TPNH have been found to be low in tumors, such as FAD (28, 34) and glutathione (25). Price, Miller, Miller, and Weber (26) reported that the riboflavin content of rat livers was decreased during the course of feeding with diets containing azo dyes which had high but not low carcinogenic potency.

Thus it is possible the lowered concentration of some of the normal oxidants of TPNH and low levels of total TPN, caused by such reactions as the pyridine nucleotide histamine reactions (5), may be not sufficient to maintain a normal TPNH/TPN ratio in the presence of a carcinogen which oxidizes TPNH (10). A sufficiently low TPNH/TPN ratio may induce increases in phosphogluconate dehydrogenase, both in activity in vivo and in total amount of enzyme. Or it is possible that, if the activity of the enzyme in vivo is not inhibited by high TPNH concentration, the substrate, phosphogluconate, is then able to induce new enzyme formation—i.e., perhaps TPNH acts as a repressor.

In conclusion, it seems evident that, of the changes in enzyme activities reported here during carcinogenesis in the hamster cheek pouch, the one most closely correlated with the morphologic change from hyperplasia and benign papillomas to frankly malignant neoplasia was the increase in phosphogluconate dehydrogenase. In all probability the increase in phosphogluconate dehydrogenase was secondary to and perhaps induced by changes in the TPNH/TPN ratio as well as other reactions which are not yet evident.

REFERENCES
2. Abraham, S.; Cadby, P.; and Chaiikoff, I. L. Effect of Insulin in vitro on Pathways of Glucose Utilization, Other
3. D. B. M. Scott, The inhibition by TPNH of purified and crude phosphogluconate dehydrogenase from various sources, including the hamster cheek pouch tumors, will be reported elsewhere.
15. ———. Succinic Dehydrogenase and Cytochrome Oxidase in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice. Ibid., 7:14-19, 1947.
Carcinogenesis in the Hamster Cheek Pouch: II. Changes in Enzymes of Glucose-6-phosphate Oxidation

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