The teratogenic effects of sugars on the chick embryo

By A. F. HUGHES, R. B. FREEMAN AND T. FADEM

From Anatomy Department, Case Western Reserve University

SUMMARY

The effect of treatment of chick embryos during the first day of incubation with a number of sugars is described. To some embryos solid sugars were applied in opened eggs; for others the substances were injected in solution. By both methods, all sugars tested were found to be teratogenic, but no apparent general differences between mono-, di-, and trisaccharides were found. Nor were there any correlations between those which can be metabolised at these stages and their teratogenicity.

The range of defects produced is similar to those found when embryos of this age are treated with other substances. In embryos treated with [14C]sucrose, some of the label is retained within the tissues in a bound, insoluble form. Possible implications of this finding are suggested.

INTRODUCTION

Over 20 years ago, experiments were reported by Hunt (1951), in which a variety of embryonic malformations was obtained by the injection of sucrose into the albumen of the hen’s egg. He treated eggs before incubation with 1 ml of 2 M sucrose, and examined the embryos after periods of incubation from 72 to 120 h. More recently, the present authors have observed abnormalities resulting from placing small quantities, around 0.5 mg, of the solid near embryos at 20 h, and continuing incubation by means of the Romanoff technique (Romanoff, 1931) whereby the opened egg is maintained in a closed space at a high humidity.

The types of abnormality of the neural tube, trunk, and tail region which we have thus obtained, and the proportion of malformed embryos are similar to those which we have found in corresponding experiments with a number of steroidal substances, which included alkaloids of the Veratrum group (Keeler & Binns, 1968; Keeler, 1969). Among these observations those with cyclopamine (Freeman & Hughes, 1973) have already been briefly reported. Others still remain to be described.

Meanwhile, it seemed worth while to examine whether other sugars could also act as teratogens on the chick embryo under these conditions, with the aim of seeking factors common to the action of these agents.

1 Authors’ address: Anatomy Department, Case Western Reserve University, 2119 Abington, Cleveland, Ohio 44106.
METHODS

The effects of ten sugars were tested by two methods, first by treatment of opened eggs with small quantities of the solid (Romanoff series), and secondly by the injection of solutions into the albumen. Several dozen eggs were used in each separate test. Each was repeated at least twice, and was accompanied by controls, namely opened ones for the Romanoff series, and untreated and unopened eggs for both groups. In some instances two sugars were tested in a single experiment, with common controls. A total of 1656 eggs was used in this stage of the work. They were all of the Babcock hybrid strain of White Leghorn. On opening, the blastoderm was visible at the time of treatment, and one could reject infertile eggs, the proportion of which, however, was very low. The stage of development of the embryo could be judged from the external diameter of the area opaca, which at 20 h of incubation varied from 15–20 mm, corresponding to stages 4–5 of the Hamburger–Hamilton series (1951) for embryos of the definitive streak and head process stages. Solid sugars in quantities ranging from about 0·3 to 1·0 mg were placed near the edge of the blastoderm.

For the injection of sugars in solution, the shell was pierced at two points, one near the equator of the egg to admit a hypodermic needle. Another over the air space allowed air to escape as the volume of the albumen was increased by the injected solution. We found that no more than 0·3 ml could be introduced in this way. For all sugars, a 0·5 M solution was used. The weight of sugar thus applied varied from 27 mg for anhydrous monosaccharides to 89 mg for raffinose pentahydrate.

Most injections were performed at 20 h of incubation, though a few were at earlier stages. No differences were seen in the results for injections between 0 and 20 h. As a first enquiry into the mode of teratogenic action of sugars, Romanoff cultures were then treated with $[^{14}C]$sucrose. The eggs were opened at 25 h, and solid fragments of the labeled sugar placed near the blastoderm. Twenty-eight mg of specific activity 6·4 mCi/mm were divided among as many eggs, so that each received approximately one mg of activity 3·6 μCi.

After a total incubation of 72 h, the embryos were examined in saline and then fixed in Bouin's fluid. Subsequently they were embedded and sectioned at 4 μm. Immediately after deparaffinizing the slides, they were coated with Kodak NTB-2 emulsion, incubated for 21 days at −5 °C, and then developed and fixed at 18 °C. The slides were examined under the phase microscope unstained.

In parts of selected sections, overlying silver grains were counted under the phase microscope using a ×40 water-immersion objective within a test area of 218 μm² as defined by an eyepiece micrometer. Each estimation consisted of five such counts, for which the mean and the standard deviation were calculated.

Background levels of grain density were measured in groups of five counts within test areas of a slide adjacent to a section in which labeling was assessed.
As before, the mean and the standard deviation were calculated for each group. The modal values of all the background numbers for silver grains per standard area were between four and six. In Fig. 7 the data shown above the zero line are the mean label density minus the corresponding background mean.

RESULTS

In scoring our results we have included under malformations only those embryos in which the heart was operating effectively at the time of fixation. This procedure excluded from the abnormals some embryos which had recently died, and yet which could be recognized as grossly malformed.

It was otherwise impossible to draw any clear line between 'malformations' and 'deaths', which could occur, as far as one can judge, at any time between treatment and examination. In some dead embryos there was an area vasculosa, but in others, development seemed to have ceased before the migration of lateral mesoderm from the primitive streak. Where vitelline arteries were present, circulation must have begun several hours earlier (Hughes, 1936).

In Table 1, the results with each sugar are recorded under the three headings 'Number used', 'Dead' and 'Malformed'. Malformed embryos are recorded both as percentages of survivors and also of all treated embryos. In Table 2 the various abnormalities in each region of the embryo are recorded separately.

The removal of part of the shell during incubation has itself considerable effects both on mortality and the proportions of abnormalities, as has been observed by several authors. Romanoff (1931) found that when part of the shell was removed after a day's incubation, over 40% of the embryos were dead by the fourth day. Ancel (1956), in an exhaustive study, found that by the third day the mortality after opening at 20 h was 17-2%, while the percentage of dead embryos which could be recognized as abnormal was 32%. McCallion & Clarke (1959) found that after removal of 1 cm² of the shell together with the underlying shell membranes at 24 h, 59% of the embryos were abnormal after a further day of incubation. Attention has again been drawn to the teratological consequences of opening eggs during incubation by Mann, Moore & Persaud (1973).

The figures for mortality among control embryos in our Romanoff series ranged from 22-6 to 40% (Table 1), while those for major abnormalities of the neural tube among survivors were much lower than those reported by McCallion & Clarke (1959).

Such consequences of opening eggs after a day of incubation would seem to limit the use of the early chick embryo for testing added substances for teratological effects. Nevertheless, by working with eggs in sufficient numbers, and repeating each test at least twice for each sugar, we have observed clear differences between experimental and control series. Paired t-tests were worked for the percentages of malformed survivors between treated and control embryos, both for the injection series (columns A and B, Table 1) and for the corresponding
<table>
<thead>
<tr>
<th>Sugar</th>
<th>Time of treatment (h)</th>
<th>No. used</th>
<th>Dead</th>
<th>Of all</th>
<th>Malformed</th>
<th>No. used</th>
<th>Dead</th>
<th>Of all</th>
<th>Malformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>20</td>
<td>82</td>
<td>24-4</td>
<td>11-0</td>
<td>14-5</td>
<td>55</td>
<td>5-4</td>
<td>3-7</td>
<td>3-8</td>
</tr>
<tr>
<td>L-Glucose</td>
<td>20</td>
<td>45</td>
<td>33-3</td>
<td>17-8</td>
<td>26-7</td>
<td>26</td>
<td>11-6</td>
<td>7-7</td>
<td>8-7</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>20</td>
<td>50</td>
<td>32-0</td>
<td>18-0</td>
<td>26-5</td>
<td>26</td>
<td>11-6</td>
<td>7-7</td>
<td>8-7</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>20</td>
<td>51</td>
<td>33-4</td>
<td>11-8</td>
<td>17-7</td>
<td>30</td>
<td>6-7</td>
<td>10-0</td>
<td>10-7</td>
</tr>
<tr>
<td>Fructose</td>
<td>20</td>
<td>51</td>
<td>25-5</td>
<td>17-6</td>
<td>23-7</td>
<td>30</td>
<td>6-7</td>
<td>10-0</td>
<td>10-7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0-20</td>
<td>81</td>
<td>27-2</td>
<td>14-8</td>
<td>20-3</td>
<td>34</td>
<td>2-9</td>
<td>8-8</td>
<td>9-1</td>
</tr>
<tr>
<td>Maltose</td>
<td>20</td>
<td>47</td>
<td>27-7</td>
<td>8-5</td>
<td>11-8</td>
<td>31</td>
<td>12-9</td>
<td>6-5</td>
<td>7-4</td>
</tr>
<tr>
<td>Lactose</td>
<td>20</td>
<td>82</td>
<td>40-2</td>
<td>18-3</td>
<td>30-6</td>
<td>55</td>
<td>7-3</td>
<td>5-5</td>
<td>5-9</td>
</tr>
<tr>
<td>Trehalose</td>
<td>10-20</td>
<td>50</td>
<td>22-0</td>
<td>12-0</td>
<td>15-4</td>
<td>17</td>
<td>0</td>
<td>5-9</td>
<td>5-9</td>
</tr>
<tr>
<td>Raffinose</td>
<td>20</td>
<td>58</td>
<td>32-8</td>
<td>17-2</td>
<td>25-7</td>
<td>17</td>
<td>0</td>
<td>5-9</td>
<td>5-9</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Time of Treatment (h)</th>
<th>No. used</th>
<th>Dead</th>
<th>Of all</th>
<th>Malformed</th>
<th>No. used</th>
<th>Dead</th>
<th>Of all</th>
<th>Malformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>20</td>
<td>39</td>
<td>15-4</td>
<td>15-4</td>
<td>18-2</td>
<td>31</td>
<td>22-6</td>
<td>9-7</td>
<td>12-5</td>
</tr>
<tr>
<td>L-Glucose</td>
<td>20</td>
<td>39</td>
<td>15-4</td>
<td>30-8</td>
<td>36-4</td>
<td>33</td>
<td>30-4</td>
<td>18-2</td>
<td>26-1</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>20</td>
<td>56</td>
<td>25-0</td>
<td>35-7</td>
<td>47-5</td>
<td>37</td>
<td>27-0</td>
<td>21-6</td>
<td>29-7</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>20</td>
<td>86</td>
<td>18-6</td>
<td>39-4</td>
<td>48-5</td>
<td>75</td>
<td>37-3</td>
<td>18-7</td>
<td>29-8</td>
</tr>
<tr>
<td>Fructose</td>
<td>20</td>
<td>51</td>
<td>19-6</td>
<td>31-4</td>
<td>39-0</td>
<td>37</td>
<td>27-0</td>
<td>21-6</td>
<td>29-7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20</td>
<td>56</td>
<td>19-6</td>
<td>44-6</td>
<td>55-5</td>
<td>25</td>
<td>36-0</td>
<td>16-0</td>
<td>25-0</td>
</tr>
<tr>
<td>Maltose</td>
<td>20</td>
<td>64</td>
<td>36-0</td>
<td>37-5</td>
<td>58-4</td>
<td>32</td>
<td>37-5</td>
<td>18-8</td>
<td>30-0</td>
</tr>
<tr>
<td>Lactose</td>
<td>20</td>
<td>69</td>
<td>20-3</td>
<td>37-6</td>
<td>47-3</td>
<td>39</td>
<td>35-9</td>
<td>10-3</td>
<td>16-0</td>
</tr>
<tr>
<td>Trehalose</td>
<td>20</td>
<td>72</td>
<td>29-2</td>
<td>30-6</td>
<td>43-1</td>
<td>45</td>
<td>40-0</td>
<td>24-4</td>
<td>40-7</td>
</tr>
<tr>
<td>Raffinose</td>
<td>20</td>
<td>65</td>
<td>33-8</td>
<td>29-3</td>
<td>44-2</td>
<td>36</td>
<td>33-3</td>
<td>30-6</td>
<td>46-0</td>
</tr>
</tbody>
</table>

data in the Romanoff series (columns C and D, Table 1). In both, results of high significance were obtained, with $P$ values less than 0.01.

There were, however, some general differences between the results for the treatment of opened and injected eggs. With the latter, where about one hundred times the amount of each sugar was applied as in the Romanoff series, deaths among treated embryos were usually higher. Injection of a third the usual volume of 0·5 M sucrose resulted in a decrease in mortalities to 13·8 %, though with a less marked reduction in the proportion of malformations. With opened eggs, a
consistent but unexplained finding was that the mortality for treated embryos was less than that for the controls.

Table 1 indicates that the teratogenicity of the various sugars is maximal for lactose, and least for D-glucose, of which 150 mg is normally present in the albumen of an egg (Needham, 1931). A Rank-Difference Coefficient of Correlation (Handbook of Chemistry and Physics, 1949) was calculated for the apparent order of teratogenicity for each sugar, indicated by the differences in percentages of malformation between treated and control embryos both for the Romanoff series (figures in column A minus those in column B, Table 1) and also for the injection series (C minus D, Table 1). The value obtained was −0·03, which indicates that random variations in the experiments obscure what differences there may be in teratogenicity between the various sugars.

This result may be compared with those obtained by Spratt (1949) in a comparative study of the utilization of various sugars by the explanted chick embryo at 2–3 days. This author has shown in previous papers (Spratt, 1947, 1948) that the embryo at the primitive streak stage is unable to survive on a simple saline-agar medium. When, however, dextrose or several other hexoses (with the exception of L-sorbose) were incorporated in the medium, development continued, as also in the presence of the disaccharide D-maltose. The embryo was unable to utilize any other disaccharide, or either of the trisaccharides raffinose and melezitose, or any pentose sugar. The assumption that the label seen within the tissues of the embryo after treatment with [14C]sucrose is associated with sucrose and not with a metabolite rests at present on Spratt’s finding that this sugar cannot be utilized at this period of development.

The ability of the embryo to grow on a saline-agar medium in the presence of D-glucose was confirmed by Taylor and Schechtman (1949). In a later review, Spratt (1958) listed a ranking order for the facility with which sugars could promote the growth of the explanted embryo, namely dextrose > mannose > fructose > galactose. It thus seems that the varying ability of the embryo to metabolise these sugars at this stage bears little relation to their teratogenicity when applied under the present conditions.

The varying mortalities and incidences of abnormality seen in control embryos are due to the operation of unknown and intangible factors which must determine the reaction of the embryo to the conditions of experiment. It is possible that in the treated embryo, these factors act synergistically with the influence of the added sugars. Such heightened effects of two teratogenic influences acting simultaneously are well known. This possibility was tested by Rank-Difference Correlation Coefficients between percentages of abnormalities in control and treated series for each method of treatment. The values obtained between the percentages in columns A and B (Table 1) for the injection series, and between columns C and D were respectively 0·146 and 0·3, indicating that only with the Romanoff Cultures was there any appreciable synergistic effect.

In Table 2 the major abnormalities recorded are listed separately. Where an
embryo shows several malformations, each of them is scored. We have included all failures of the neural tube to close at rostral levels under the term 'exencephaly', though this term belongs more especially to neonatal pathology, and other names have been proposed for embryonic brain malformations (Ancel, 1955, 1956). This is the commonest single abnormality with our Romanoff cultures, in which head and trunk defects are nearly equal in number, while the latter are more common in the injected series. Examination of treated embryos after longer periods of development may show a different proportion of defects. Thus at 4–5 days there is a high proportion of caudal abnormalities among the survivors of embryos treated with lactose.

Some of the various defects of head and tail regions are illustrated in Figs. 1–3 in order to show that the abnormalities which we have obtained are similar to those obtained by other workers and by various means. Failure of the neural plate to close may result in a flat plate (Fig. 1A–C) projecting forwards over a space corresponding to the buccal cavity. Beneath is an apparently normal fore-gut. At early somite stages the neural plate of such an abnormal embryo may remain flat. Mitoses on the upper surface are frequent (Fig. 4).

In other examples (Fig. 2A–D) the forwardly projecting neural brain is apparently a collapsed forebrain with dorsal and ventral surfaces in apposition. It leads to a midbrain roofed over by a membrane of necrotic cells (Figs. 2B, 5) apparently in course of resorption, behind which is an open hind brain (Fig. 2C)

---

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>% dead</th>
<th>Full anencephaly</th>
<th>Suppression of forebrain</th>
<th>Exencephaly</th>
<th>Dilated brain ventricles</th>
<th>All embryos, with only head abnormalities</th>
<th>Open spinal cord</th>
<th>Multiple lumen</th>
<th>Trunk and tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Unopened controls</td>
<td>195</td>
<td>10-1</td>
<td>—</td>
<td>—</td>
<td>2-3</td>
<td>—</td>
<td>2-9</td>
<td>2-9</td>
<td>1-7</td>
<td>—</td>
</tr>
<tr>
<td>(B) Sugars injected 0–20 h</td>
<td>597</td>
<td>22-3</td>
<td>1-4</td>
<td>0-7</td>
<td>4-8</td>
<td>1-4</td>
<td>5-3</td>
<td>7-2</td>
<td>7-4</td>
<td>1-0</td>
</tr>
<tr>
<td>(C) Opened controls 20 h</td>
<td>267</td>
<td>34-0</td>
<td>0-6</td>
<td>2-3</td>
<td>10-4</td>
<td>2-3</td>
<td>10-4</td>
<td>7-9</td>
<td>4-0</td>
<td>1-7</td>
</tr>
<tr>
<td>(D) Opened and treated with sugars at 20 h</td>
<td>597</td>
<td>30-0</td>
<td>1-4</td>
<td>2-4</td>
<td>20-5</td>
<td>3-0</td>
<td>13-0</td>
<td>16-6</td>
<td>3-9</td>
<td>2-4</td>
</tr>
</tbody>
</table>
Defects in chick embryos caused by sugars

and spinal cord (Fig. 2D). Such conditions suggest that even in such early abnormal embryos, an open neural tube may be of secondary origin.

Another variant involves a partial anencephaly with the suppression of forebrain and midbrain. The heart projects forward beyond a truncated neural plate (Fig. 3A), which is greatly contorted into irregular grooves (Fig. 3D). These suggest a locally abnormal proliferation of the neural epithelium. In this embryo the neural tube is normal in appearance at thoracic levels (Fig. 3G). However, in more caudal regions, where in all embryos at this stage there is an axial zone with no distinction between ectoderm and mesoderm, and where the neural tube is formed by cavitation (Criley, 1969), a further malformation is seen in this example with the formation of an irregular diverticulum (Figs. 3H, I, 6) leading from the external surface towards the endoderm beneath. This condition was first recognized by Rabaud (1900) under the name of ourentérie and was further described by Moseley (1947) in a study of the effects of insulin injected into the unincubated egg. Her abnormal embryos developed caudal malformations similar to the inherited condition known as ‘rumplessness’, particularly of the recessive variety. In the present study, two other caudal anomalies have been seen in whole embryos, namely suppression of sacral and caudal somites, and the appearance of irregular protuberances from this region which we have termed ‘tail papillae’. One such is shown in Fig. 3J. We have not yet determined how many of such embryos in our material are ourenteric, for section series are needed to reveal this abnormality.

Results after treatment with radio-sucrose were similar to those obtained with the unlabeled sugar. Of 28 treated eggs, there were 22 survivors, of which 11 seemed normal at the time of fixation. They were at stages between 17 and 19. Of the eleven abnormal embryos, five were judged to have neural defects at lumbo-sacral levels, either spina bifida or multiple lumen. Some degree of abnormality of the tail region was also observed. Five embryos combined exencephaly with spinal defects. In one, the head was normal. Study of the section series showed that four of the embryos were ourenteric. Of these, two had been thought normal at the time of fixation, while in two spinal and tail defects had already been recognized.

After exposure of Romanoff cultures to $[^{14}\text{C}]$sucrose, some of the label was retained within tissues of the embryo, although special histological methods are usually necessary after treatment of tissues with a soluble tracer in order to avoid the total loss of the material during fixation and dehydration in aqueous mixtures. (Miller, Stone & Prescott, 1964; Brown, Stumpf & Roth, 1969). The fact that in the present experiments the label persisted with no special precautions to avoid loss by solution shows that sucrose in teratogenic concentrations enters the tissues of the embryo in a bound form.

The clearest instance of the penetration of the label into cells of the embryo is into the large cells of the area opaca endoderm. There, silver grains overlie areas of cytoplasm, and also the large empty spaces once occupied by intracellular
Defects in chick embryos caused by sugars

Figures 1-6

Figs. 1-6 are from transverse sections of chick embryos treated with approx. 0.5 mg of sucrose, and incubated further by Romanoff's method. The magnification of Figs. 1-3 is indicated in Fig. 1A, and of Figs. 4-6 in Fig. 4.

Fig. 1. Fixed 16 h after treatment, with flat neural plate (np), bifid at rostral tip (A) and extending caudally over a few irregular somites (s) (C). Foregut (fg) (A) and anterior intestinal portal (aip) (B) are normal in appearance. Heart tubes not yet fused within pericardial space (p) (A).

Fig. 2. Fixed 21 h after treatment. Here the rostral tip of the neural plate (np) (A) is a collapsed forebrain, which is succeeded by a midbrain (mb) with a necrotic roof (B). The hindbrain (hb) (C) is open, but the cord (sc) at thoracic levels (D) is closed. The heart (h) and foregut (fg) are normal in appearance (B-D).

Fig. 3. Fixed 28 h after treatment. Here the embryo is partially anencephalic, with the heart (h) (A) projecting forwards beyond a free neural plate (np) (B) which is much folded at hindbrain levels (np) (D) but flat at the level of the anterior intestinal portal (aip) (E). At anterior trunk levels, the neural tube is open (ont) (F) but is closed further caudally (G). Somitic mesoderm (s) is seen in (F) and (G). At the hind end of the embryo is an oureteric diverticulum (od) (H and I), and still further caudally, an upstanding tail papilla (tp) (J).

Fig. 4. Higher-power view of embryo of Fig. 1, showing mitoses on upper surface of neural plate (np).

Fig. 5. Higher power view of part of Fig. 2B, showing necrosis of midbrain roof (mbr).

Fig. 6. High-power view of part of Fig. 3I, showing oureteric diverticulum (od). Pycnotic nuclei are conspicuous in this region.
Fig. 7. Standard deviations (vertical bars) for grain counts in test area of 218 \( \mu\text{m}^2 \) over neural epithelium in chick embryos labeled with \([^{14}\text{C}]\text{sucrose}\). The mean values on scale 0–110 represent means of five counts, minus the mean background count shown with standard deviation on scale 0–10 below the \( x \) axis. The left side of each set of data refers to the head (\( fb \), forebrain; \( mb \), midbrain; \( hb \), hindbrain; in A and C), and the right to the tail region (\( sc \), normal spinal cord). (A) Two normal embryos with a general trend of decrease in grain count along the neural axis. This is not apparent in two exencephalic embryos (B), with open neural plate in head region (\( ex \)) and unclosed neural groove (\( ont \)) in thorax. A high point in one embryo (thicker bars: \( n \)) is over a path of cell degeneration in open neural plate. In (C) both embryos are normal at the anterior end, but are ourenteric (\( our \)) caudally. In one (thicker bars) there is a multiple lumen defect in the cord at lumbo-sacral levels. In (C) each of these spinal defects is accompanied by cell degeneration, with an overlying increase in grain count.

yolk. Elsewhere in the embryos, though the label is clearly associated with cells, one cannot be certain whether the isotope was mainly attached to the cell surface or to internal cytoplasmic components.

Countable levels of grain density were found over most of the 21 sectioned embryos, both among normal and abnormal specimens. In general, grain density was highest within compact epithelial tissues with no visible intercellular spaces, an observation which applies both to neuroepithelium and to that of the gut. The range of grain counts over neuroepithelium in all embryos varied from 20 to 170 within the test area. The highest levels were found among normal embryos, particularly in the two most advanced in development. Here the grain density
Defects in chick embryos caused by sugars

was too high for accurate counting and there was some evidence of image broadening due to oblique tracks of electrons (‘cross-fire’, Przybylski, 1970), with grains spreading from the walls of the spinal cord over the central canal. Otherwise, grain counts were at background level in all normal tissue spaces of the embryos, both in coelomic cavities, vascular spaces, and in the ventricles of the brain. In mesenchymal areas, counts in regions of varying cell density showed a correlation between numbers of cells and of silver grains per unit area.

Six embryos, two normal and four abnormal, were selected for serial counting. In each, counts extended over the whole length of the central nervous system. The results are shown in Fig. 7. In the normal embryos (Fig. 7A) the grain density was higher in the brain than in the spinal cord. However, in two exencephalic embryos (Fig. 7B), where the open condition of the neural tube extended to thoracic levels, there was no indication of denser labeling at the anterior end.

Fig. 7C refers to two embryos normal at the head end, but with spinal defects further caudally. Both were oureneretic with malformations at the tail region, while one showed the multiple lumen defect at lumbosacral levels. At the site of these defects are seen local areas of cell degeneration.

In such areas grain counts rise toward the foci of these defects and attain a maximum among the debris resulting from cell degeneration. In one area of an exencephalic neural plate (Fig. 7B, thicker bars) a path of necrosis is also associated with a local high in grain density.

We would suggest that these accumulations arise from repeated cycles whereby absorption into a cell is followed by liberation of materials on its degeneration, to be again succeeded by uptake and release by a new generation of cells which in their turn undergo pycnosis. Thus, in such areas the level of label continuously rises much in the way that an ecological pollutant is concentrated along a food chain.

DISCUSSION

The malformations of the neural tube and embryonic axis with which we are here concerned are among those which originate during a period of peak sensitivity relatively early in development, during the stages of gastrulation and neurulation. Such periods are most clearly demarcated where the teratogen is a physical agent the operation of which can be terminated at will. Periods of maximum sensitivity to chemical teratogens cannot be defined with the same precision. Thus, Deuchar (1952) found that a temperature shock of 4-5 °C for 5 h was most effective in causing abnormalities at 12 h of incubation, and thus at an early phase of gastrulation. The same stage was found by Stiles & Watterson (1927) as the limit of the influence of mechanical vibration in suppressing either wholly or in part the development of the neural tube. For more specific effects on the nervous system, Hadjinsky (1962) found that 600 rads of X-rays had most effect when applied at 22 h in mid-primitive streak phases.
Table 2 shows that when eggs are opened at 20 h, a high proportion of exencephalies is found both in control and in treated embryos. A few preliminary trials showed that when these manipulations are postponed until 27 h, exclusively cephalic malformations become less numerous, though exencephalies combined with those at spinal levels are still frequent. These results point to two conclusions. In the first place, they show that these neural and axial malformations are not caused by interference with gastrulation, for at 25 h this period of development is then largely over. Furthermore, at 27 h (stage 8) the neural folds have already met over the future midbrain. If exencephaly can then still result from treatment, this condition must be due to a reopening of the neural tube, a conclusion which supports the interpretation of that shown in Fig. 2B and 5 as a secondary phenomenon.

A fact which may prove of major significance is that the abnormalities which we report are similar or indeed identical with those which may be evoked by a wide variety of other means, both physical and chemical, when applied at the same period of development. Among the latter, Romanoff (1972) cites some 75 substances which have been administered to chick embryos between days 0 and 2. Of these, 68% have been shown to cause abnormalities of the neural tube and of the caudal region. They range from general agents such as ammonia (Noto, 1967) and ethanol (Sandor & Elias, 1968), to aminopterin (Blattner, Williamson, Simonsen & Robertson, 1960) and 5-fluorouracil (Puchov, 1966) among specific antimetabolites. Comparable data could be obtained from the literature of mammalian teratology. While the effects of these substances may be interpreted according to their several physiological properties where these are understood, the fact that sugars can cause the same abnormalities points to some general cellular influence common to all such teratogens.

As to the nature of such influences, the one relevant circumstance disclosed by the present experiments is that some of the labeled sucrose becomes associated with the tissues of the embryo in a bound and insoluble form. Distortion of development by osmotic imbalance thus becomes a less likely explanation. The insoluble material is probably of the nature of a glycoprotein. It is at the cell surface that such bodies are known to be assembled, and it may thus be that the teratogenic action of sugars is exerted at cell membranes, by interference with the normal processes of coupling protein and carbohydrates.

The authors wish to express their thanks to Mrs Judith Freeman for her great skill and patience in preparing serial sections, to Dr R. J. Przybylski for his valuable advice, and to the National Foundation (March of Dimes) for the support of this research.
Defects in chick embryos caused by sugars

REFERENCES


A. F. HUGHES AND OTHERS


(Received 19 March 1974. Revised 29 June 1974)