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The teratogenic effects of 5-fluoro-2-desoxyuridine (F.U.D.R.) on the Wistar rat fetus, with particular reference to cleft palate

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INTRODUCTION

In a previous paper (Ferguson, 1978) a description was given of palatal shelf elevation in Wistar rat fetuses, and a mechanism, consistent with the observations, was put forward to account for this event. Since it is widely accepted that the vast majority of cases of isolated cleft palate (i.e. without associated cleft lip or cleft primary palate) result from a delay in (or failure of) palatal shelf elevation rather than a failure of fusion of elevated shelves, experimental induction of such an abnormality is an obvious way of testing hypotheses purporting to explain normal elevation. Furthermore, since it has been established (Fogh Andersen, 1968, 1971; Ross & Johnston, 1972) that in man some 80 % of cases of isolated cleft palate are caused by environmental factors, knowledge of the process of normal shelf elevation and of ways in which cleft palate can be induced experimentally is clearly a necessary prerequisite for understanding the aetiology of, and providing rational preventive or restorative treatment for, this distressing abnormality. Therefore it was decided to induce cleft palate in the Wistar rat fetus and to study such abnormal fetuses statistically, macroscopically, microscopically, ultrastructurally and experimentally. In the course of the work it was found that other abnormalities induced at the same time exhibited some surprising and very interesting features.

MATERIALS AND METHODS

Twenty pregnant female Wistar rats (average weight 300 g) were used in this study. The onset of pregnancy was determined by vaginal smearing, the day of finding sperm in the smear being called day 0. All the female and male Wistar rats used were highly inbred, being the twelfth generation of a brother/sister mating programme.

In order to determine the primary lesion responsible for any failure of elevation induced experimentally, it was necessary to study fetuses at risk from the earliest stages of palatogenesis and, since the shelves are normally vertical from day 14 to day 16·3, this necessitated finding a teratogen which would consistently induce cleft palate in 100 % of the (> 16·3 days old) offspring. In preliminary trials it was found that a single intraperitoneal injection of 100 mg of F.U.D.R. (5 fluoro-2-desoxy-uridine dissolved in injectible water) per kilogram maternal body weight into an unanaesthetized rat at day 12·5, induced cleft palate later on in 100 % of the (> 16·3 days old) fetuses. A total of 70 living fetuses was recovered (under ether anaesthesia)

between days 14 and 19 from nine rats injected with F.U.D.R. There were 29 dead and resorbing fetuses.

As controls, a series of unanaesthetized rats were each given a single intraperitoneal injection of a similar volume of injectible water at a similar stage of gestation. The experimental and control rats were housed in separate but similar cages, under similar environmental conditions, and were fed similar pellet food and water *ad libitum*. A total of 109 fetuses and two resorption sites were recovered (under ether anaesthesia) from 11 control rats between days 14 and 19. The resorption rate in the F.U.D.R.-treated animals was 30 % as compared with 2 % in the control rats.

The techniques used in the study of both the F.U.D.R. and the control fetuses were identical with those used in the previous study of normal fetuses (Ferguson, 1978) and reference should be made to this paper for details.

OBSERVATIONS

Control rats injected with water alone

The live fetuses recovered from these rats were all normal, their appearances at all ages being similar to those described in the previous paper dealing with normal palatal development in the Wistar rat.

Statistical analysis of data from F.U.D.R.-treated fetuses

A method was described in the previous paper (Ferguson, 1978) whereby the developmental age of a normal fetus could be assessed from the smear age and certain morphological criteria. In an attempt to assess the developmental age of F.U.D.R.-treated fetuses in a similar manner, the smear age was correlated with each of the eight morphological measurements made on the 70 fresh fetuses. The correlation coefficients of each of these measurements with smear age were: fresh weight, 0·7963; weight after fixation, 0·7845; CR length, 0·8110; maximum head height, 0·7176; maximum head width, 0·8010; average length of upper limb bud, 0·6321; average length of lower limb bud, 0·5772; and distance between upper and lower limb buds, 0·7593. All these correlation coefficients are lower than those derived from normal fetuses, reflecting the greater heterogeneity within the population of F.U.D.R. fetuses recovered at a particular age. Although the strongest correlation is still obtained with CR length, the Coefficient of Determination (square of the correlation coefficient) of smear age/CR length was only 0·6577 for the F.U.D.R.-treated fetuses as compared with 0·9333 for the normal fetuses. Thus only 65·77 % of any age increase in the F.U.D.R. fetuses is associable with a corresponding increase in CR length. Therefore, although both single and multiple regression analyses were performed, the calculated ages offered little advantage over smear ages, and so the former were not used in the present paper. In any case, since palatal shelf elevation never occurs in F.U.D.R. fetuses, there is not such a need for a highly accurate dating procedure as was required for the determination of the time of shelf elevation in normal fetuses.

In order to see whether there was a statistically significant difference between the eight measurements made on each of the F.U.D.R. fetuses and the eight comparable measurements made on each of the normal fetuses, a computer program was written which performed a *t* test comparison between the two groups. The original data, computer programs, and print-outs of the *t* test, correlation and regression analysis for both the F.U.D.R. fetuses and the normal fetuses, are available for

reference in The Anatomy Department, Queen's University, Belfast. In every case the probability of the differences observed between the two groups having arisen by chance was less than 0·0001. Therefore it was concluded that there was a highly significant difference between the normal fetuses and the F.U.D.R. fetuses with regard to (1) their fresh weight, (2) their weight after fixation, (3) their CR length, (4) their head width, (5) their head height, (6) the average length of their upper limb buds, (7) the average length of their lower limb buds and (8) the distance between their limb buds. In all cases the parameters were smaller in the F.U.D.R.-treated fetuses.

Macroscopic, microscopic and ultrastructural observations on F.U.D.R. fetuses aged between 14 *and* 19 *days*

In general the F.U.D.R. fetuses were smaller, and about one day developmentally retarded as compared with their normal counterparts. Indeed, not only had the teratogen slowed down development, but it had also induced certain specific abnormalities, so that at each stage the F.U.D.R. fetuses were less differentiated as well as being abnormal in form. The fetuses showed many signs of depressed mucopolysaccharide synthesis, e.g. chondrogenesis was retarded and atypical. However, about day 17 the effects of the teratogen appeared to wear off and the fetuses showed considerable 'catch up' in growth, development and repair. The pathogenesis of each of the induced malformations will now be considered.

The secondary palate

The palatal shelves of the F.U.D.R. fetuses *never* elevated *in vivo* and eventually at day 18 they became ossified while still in the vertical position (Fig. 6).

The shelves first appeared at day 15·8 (normally at day 14) and from then on maintained essentially similar macroscopic relationships. Anteriorly they were small stubby structures directed verticomedially, but posteriorly they were longer and more strictly vertical (Fig. 4); indeed the shelves here were so long that were they to flip up they would overlap (indicating that shelf growth was not specifically impaired). At their extreme posterior limit the vertical shelves ended abruptly (merging into the lateral oral walls) and there were no small horizontal shelves which would normally form the soft palate. Macroscopically the unelevated palatal shelves were parallel (except for slight bulges anteriorly) and separated throughout their length by a wide straight cleft (Fig. 3). The primary palate and nasal septum showed a slight backward projection (Fig. 3). Developing rugae were apparent on the vertical shelves by day 17.

At all ages the palatal shelves showed a high density of mesenchymal cells and an absence of large intercellular spaces (Fig. 4). The shelf mesenchyme did *not* stain for mucopolysaccharide (alcian blue at different pHs, Hale's colloidal iron, toluidine blue, acridine orange fluorescence and hyaluronidase digestion techniques) – except extremely faintly at day 17·5 – and did *not* appear oedematous. This is in contrast to normal shelf mesenchyme which from day 14 to day 16·3 shows a progressive accumulation of mucopolysaccharide and a decrease in cell density associated with a highly oedematous matrix occupying wide intercellular spaces. Furthermore, the F.U.D.R. shelves exhibited absent or defective epithelial undercutting of the shelf base, and the maxillary and palatine osteogenic blastemata (which are normally present exterior to the shelves before flip up at day 16·3) did not appear till day 17·8 (Fig. 4). At day 18 the palatal processes of these blastemata invaded and ossified the vertical shelves (Fig. 6), in contrast to the normal at this age when the blastemata of

each side have made sutural contact with each other across the elevated and fused palate. Developing molar tooth germs appeared in the unelevated shelves at day 17 (Fig. 6).

Ultrastructurally, the abnormal mesenchymal cells showed similar cell processes to the normal but differed from the latter in several ways (Fig. 8): (1) they were more densely packed, (2) they exhibited numerous lipid-like deposits (Fig. 8) (such deposits being extremely rare in normal cells), (3) lysosomes, autophagic vacuoles, myelin figures, vacuoles and pyknotic nuclei were fairly frequent, indicating cell distintegration, (4) they exhibited few 'secretory vesicles' and (5) they contained more glycogen. As in normal fetuses the matrix was devoid of fibrous elements apart from primitive nerve fibres (Fig. 8). Both histologically and ultrastructurally the vasculature of the shelf mesenchyme appeared normal and there was no sign of haemorrhage into either the shelves or the facial region as a whole.

The epithelium of the palatal shelves, and indeed epithelium in general, was poorly attached to the underlying mesenchyme (Figs. 4, 6) and epithelial basement membranes appeared to be either disrupted or absent (presumably associated with defective mucopolysaccharide synthesis). In addition, amorphous material was occasionally seen between the mesenchyme and epithelia, particularly in the mandibular region where the epithelium was often widely separated from the mesenchyme. The shelf epithelium was 2–3 layers thick and the epithelial cells contained numerous glycogen deposits and areas of cell degeneration (Fig. 7).

At day 18, some fetuses showed fusion between the tips of the long, vertically orientated, posterior parts of the palatal shelves and the lateral walls of the oral cavity (palato-oral fusion), this involving epithelial fusion, breakdown of the resulting epithelial seam and mesenchymal continuity, simulating normal shelf–shelf fusion.

Nasal septum

The nasal septum first appeared about day 15 and by day 16·4 Jacobson's organs and the cartilages of the nasal capsule were evident. Prior to day 16·4, the nasal septum hung free for a short distance behind the primary palate before disappearing into the cranial floor. At day 16.5 the septum developed lateral flanges which fused with corresponding bulges on the lateral nasal walls (as normally occurs at this age). However, since the palatal shelves were unelevated, the septal flanges intervened between the sphenoethmoidal recesses and an undivided oronasal cavity, there being no common nasal passage as there is normally after palatal elevation (Fig. 6). In this regard it is of interest that tongue protrusion, which accompanies palatal elevation in the normal fetus, never occurred in the F.U.D.R. fetuses. The septal flanges were consolidated by cartilage at day 18 (Fig. 6), and the blastemata for the vomer and nasal bones appeared at day 17.

Lower jaw and tongue

At all ages Meckel's cartilages were completely absent except for two islands about 100 μm long at the extreme anterior and posterior ends of the mandible (Figs. 4, 6). Nevertheless the mandible exhibited reasonable forward and lateral growth, although it was somewhat retrognathic when compared with the normal at each age (Fig. 5). The mandibular bone blastemata appeared at day 16.3 and from then on the mandible showed rapidly advancing intramembranous ossification despite the virtual absence of Meckel's cartilages (Fig. 6).

Compared to the normal at each age the tongue showed poorer differentiation of its musculature, was smaller in length and was more posteriorly placed, seemingly as a consequence of its being attached to a retrognathic mandible (Fig. 5).

Ears and eyes

At all ages the inner ears were malformed. The malleus, incus and stapes were either completely absent or grossly abnormal – presumably associated with the absence of Meckel's cartilages. The external auricular appendages did not appear until day 16·5 and then they were abnormal in form and position, being situated in the neck region (Fig. 2).

Macroscopically the eyes were abnormal in size, shape and position (Fig. 2). The upper and lower eyelids were malformed and did not close over on schedule (Fig. 2). Histologically, from day 17 onwards, the lenses of the eyes showed numerous cystic lesions (Fig. 6).

Limbs and tail

At all ages the limbs were severely stunted in their growth and less differentiated than normal (Fig. 2). At day 14 the tips of the phocomelic limb buds showed areas of focal haemorrhage; and from day 14 to day 17 there were no signs of any digital differentiation, and the limb cartilages remained stunted, so that there was practically no growth of the limbs (Fig. 2). While the limbs remained consistently stunted, the haemorrhage underwent progressive resorption and organization until at day 19 some attempt at digit formation was made, although the number of digits per phocomelic limb was extremely variable and their form bizarre. The tail was also severely stunted in its growth (at all ages); indeed the genital tubercle exceeded it in length (Fig. 2).

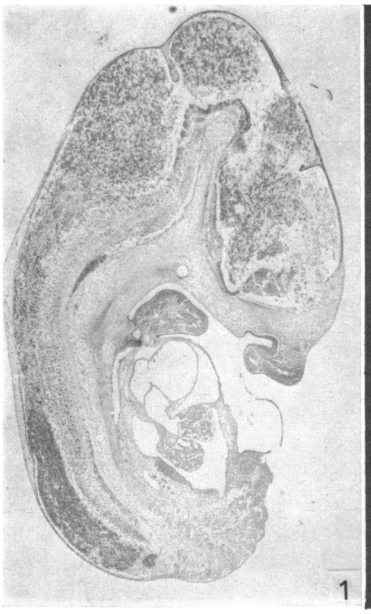
Vertebral column and cranial base

In the back, chondrogenesis was initiated at day 16·3 and initially occurred only anterior to the notochord. From this age on the vertebral bodies were represented by a solid cartilaginous rod with no signs of segmentation or disc formation.

The cranial base cartilages were first evident about day 16 and from then on they were hyperextended when compared with the normal. Indeed by day 17 the angulation between the anterior and posterior cranial base cartilages had been reduced so that the two formed a straight line, while the posterior cranial base cartilage was at approximately 140° to the vertebral column. This hyperextension was associated firstly with a small, posteriorly positioned, abnormal brain and, secondly, with a generalized straightening out of the fetus as a result of a massive subcutaneous swelling, particularly marked in the thoracic and back regions (Fig. 5). Indeed the entire heads of the F.U.D.R. fetuses were smaller than normal, and hyperextended (Figs. 2, 5). As a result of this, and the changed angulation of the cranial base, and the altered position of the brain, the normal anatomical relationships between different head structures were disturbed (e.g. coronal sections of day 18 fetuses cut the cranial base cartilages throughout most of their length and are comparable with normal horizontal sections of the cranial base).

Subcutaneous swelling

At day 16·3 the fetuses showed a mild degree of subcutaneous swelling; by day 17 the fetuses appeared grossly bloated; and by day 18 some fetuses had become so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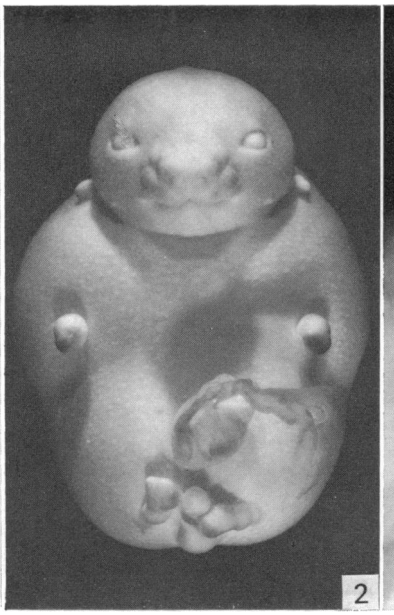
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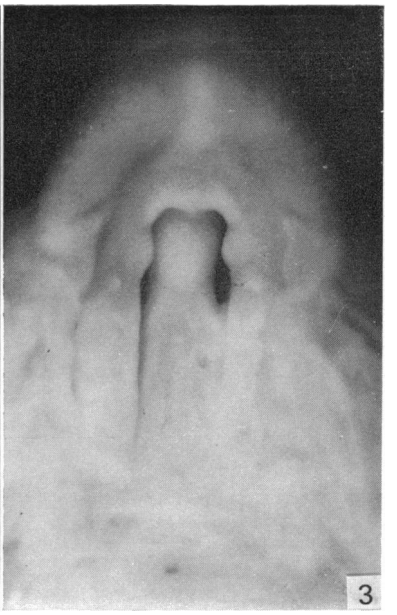
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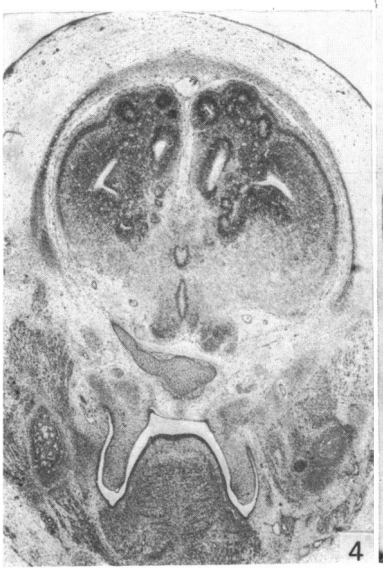
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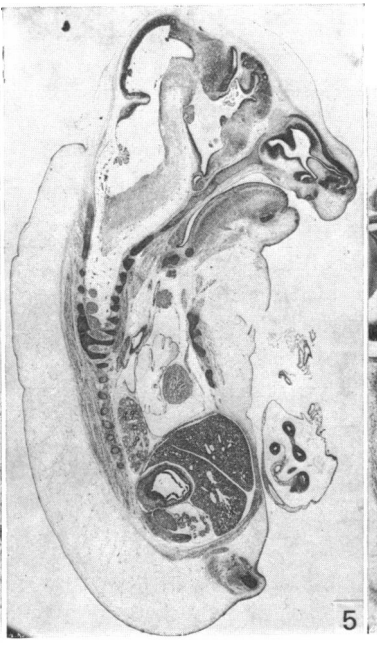
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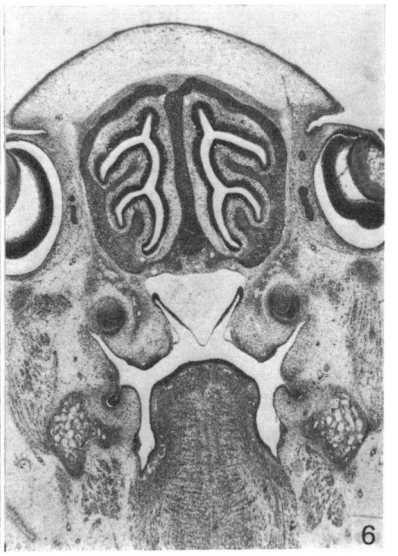
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swollen that they were crushed and folded by the restricting fetal membranes (Fig. 2). This swelling was most marked in the back and thoracic regions and resulted in a generalized uncurling of the fetuses (Figs. 2, 5). The subcutaneous material responsible for the swelling was gelatinous in consistency and did not leak fluid when incised. Histologically, the subcutaneous tissue was very loose and oedematous, containing numerous primitive mesenchymal cells, leaky blood vessels, a few extravascular red blood cells and fibrin-like material, but it did not stain for mucopolysaccharides (Fig. 5). The subcutaneous infiltrate may be an abnormal lipid, but to date it has defied chemical characterization.

The central nervous system

Perhaps the most striking of all the F.U.D.R.-induced malformations was to be found in the central nervous system. At day 14 there was a massive haemorrhage into the ventricles of the brain and the central canal of the spinal cord, the result of rupture of the internal carotid arteries in the region of the pituitary gland (Fig. 1). From the absence of alcian blue staining in vascular basement membranes it was inferred that blood vessel walls were unduly weak and easily ruptured (though, rather surprisingly, blood extravasation was restricted to the limb buds and C.N.S.). As a result of this haemorrhage the architecture of the brain and spinal cord was severely disrupted (Fig. 1). Up to day 15·5 the C.N.S. was represented in the main by masses of organizing blood clot interspersed with patches of detached neuroepithelium; the occasional presence of fresh red blood cells, however, suggested that the internal carotid arteries were still leaking. From day 14 onwards the extravasated blood underwent progressive resorption and organization, and at all ages the ventricles were filled with debris from the earlier disruption (Fig. 5). However, residual neuroepithelium proliferated (numerous mitotic figures were evident) and became organized into a brain-like structure (Figs. 1, 4 and 5). This 'brain' was grossly abnormal, with extra ventricles, small and large ependyma-lined vesicles, cystic spaces and masses of tortuous blood vessels (Figs. 4, 5); and its cortex was not laminated. The 'brain' was smaller than normal and posteriorly displaced, with the midbrain occupying a bulge at the back of the head (Fig. 5). There was practically no cervical flexure. Anteriorly the cranial cavity contained a mass of endomeningeal tissue, with very little neuroepithelium. The olfactory bulbs were smaller than normal (Fig. 5).

Fig. 1. Parasagittal section of a day 14 abnormal fetus. Note the massive haemorrhage into the brain and spinal cord. H & E and alcian blue. ×13.

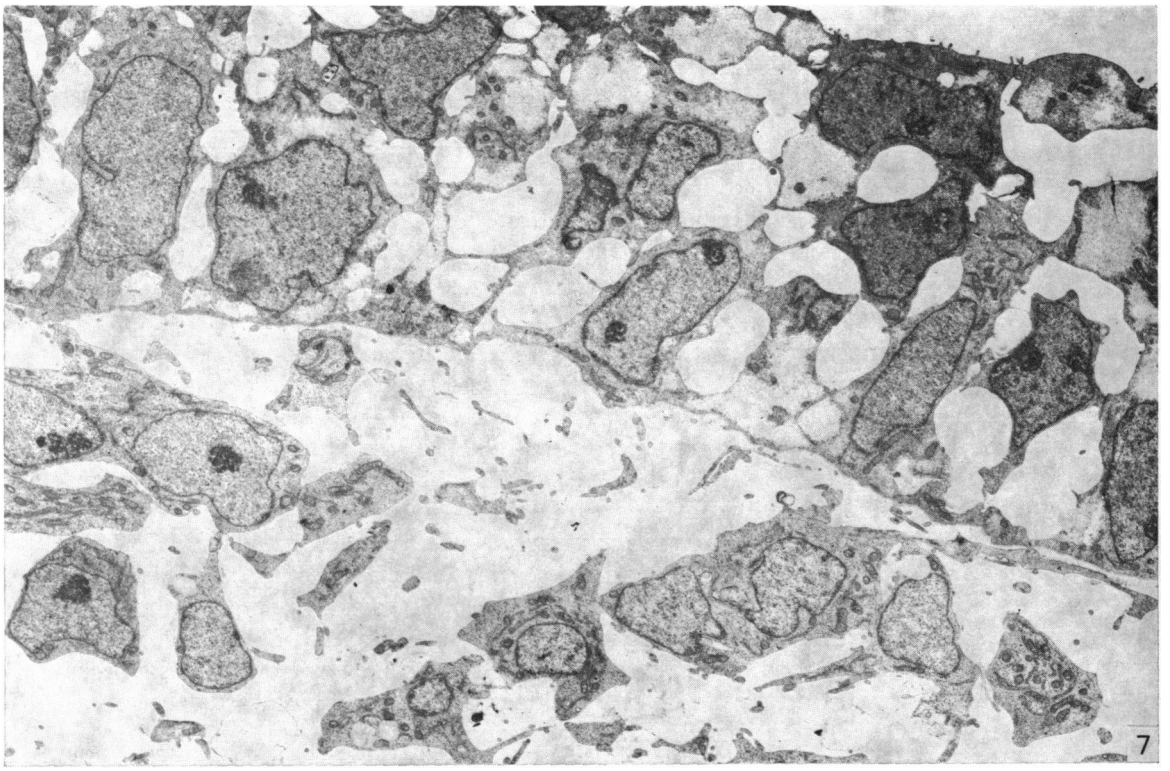
Fig. 2. Frontal view of a day 17·5 abnormal fetus, fixed in Bouin's fluid. Note the bloated appearance, phocomelia, small tail, abnormal position of the auricles and extended head. ×7.

Fig. 3. Macroscopic view of cleft palate in a day 17·5 abnormal fetus. ×10.

Fig. 4. Coronal section of a day 17·5 abnormal fetus showing the long vertical palatal shelves and the malformed brain. H & E and alcian blue. ×25.

Fig. 5. Parasagittal section of a day 17·5 abnormal fetus. Note the malformed brain and remnants of blood clot in the ventricles. The head and cranial base are hyperextended and the mandible is retrognathic. A thick layer of oedematous subcutaneous tissue (particularly marked in the back and thoracic regions) surrounds the fetus. H & E and alcian blue. ×10.

Fig. 6. Coronal section of a day 18·5 abnormal fetus. Note the unelevated palatal shelves and the vertically directed maxillary bone blastemata. Ossification of the mandible is obvious but Meckel's cartilages are not present. H & E and alcian blue. ×25.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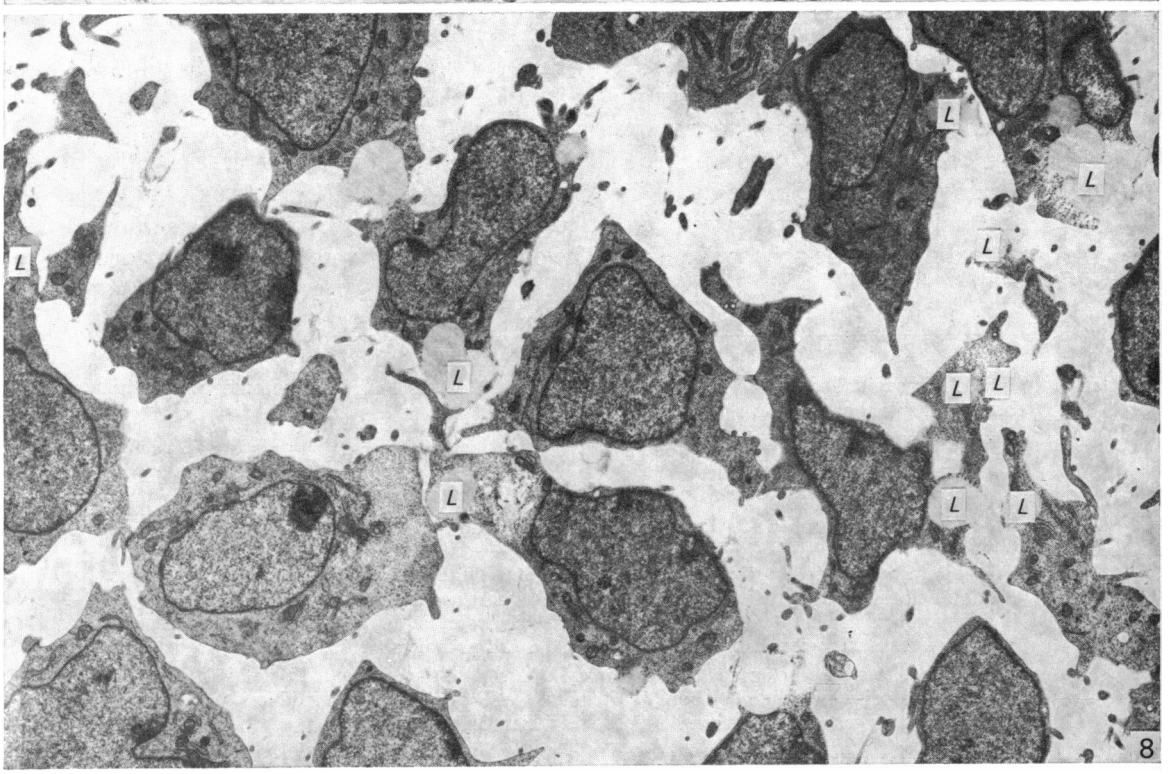
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Other observations

At day 17 mesenchymal condensations for the membrane bones of the skull vault appeared, and from then on ossification advanced rapidly (Fig. 4).

Reflex activity was sought for (using all the techniques described previously for normal fetuses – see Ferguson, 1978) in living (hearts still beating) F.U.D.R. fetuses, but none could be elicited in any of the age groups studied. In normal fetuses, however, from day 16·5 onwards an increasing number of reflexes of increasing complexity can be demonstrated.

Experimental observations on fresh F.U.D.R.-treated fetuses

Experiments similar to those carried out on normal fetuses (Ferguson, 1978) were performed on fresh 16–19 days F.U.D.R.-treated fetuses. When the tongue was depressed, or removed from between the palatal shelves, the latter did *not* elevate to the horizontal position. Indeed 'flip up' could *not* be induced experimentally by any means in any of these fetuses, and it was clear that no intrinsic shelf elevating force was present.

DISCUSSION

The pharmacological and biochemical effects of 5-fluoro-2-desoxyuridine (F.U.D.R.) are reasonably well known (Hartmann & Heidelberger, 1961; Reyes & Heidelberger, 1965; Goodman & Gilman, 1970; Santi & McHenry, 1972). F.U.D.R. is rapidly converted by an intracellular deoxyuridine kinase into fluoro-deoxyuridylic acid (F-dUMP). This fraudulent nucleotide (F-dUMP) has a very high affinity for the enzyme thymidylate synthetase which normally catalyses the transfer of a methyl group from N^5,N^{10}-methylenetetrahydrofolic acid to deoxyuridylic acid (dUMP). The F-dUMP behaves as a competitive inhibitor with dUMP, being incapable of serving as a substrate and binding 250–4000 times more tightly to the enzyme than dUMP. This unusually powerful inhibition of thymidylate synthetase (a key enzyme in thymidine synthesis) leads to a deficiency of the nucleotide thymidine and thus to an upset in DNA synthesis. Levitt, Ho & Dorfman (1974) and Dorfman *et al.* (1975) have shown that an upset in the thymidine content of DNA (produced by bromodeoxyuridine, BrdU) leads to a drastic reduction in the synthesis of mucopolysaccharide by cultured chondrocytes. These authors found that if BrdU was administered to chondrocytes *in vitro* it temporarily inhibited the synthesis of mucopolysaccharide; when all the BrdU had been replaced in the genome by thymidine, mucopolysaccharide synthesis recommenced. However, if BrdU was administered before the differentiation of mesenchyme to cartilage it *irreversibly* inhibited the acquisition of the capacity to synthesize mucopolysaccharide.

This *in vivo* study of the teratogenic effects of F.U.D.R. suggests that it may act in a comparable manner to BrdU *in vitro*. The fetuses show many signs of depressed mucopolysaccharide synthesis and poor differentiation. For example, Meckel's cartilages were absent, suggesting that F.U.D.R., like BrdU, had irreversibly

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Fig. 7. Epithelium and underlying mesenchyme at the tip of a day 16·5 abnormal palatal shelf. Note that the epithelium is several layers thick and the cells contain both glycogen deposits and areas of cell degeneration (more clearly seen at higher magnification). Lead & uranyl. ×3200.

Fig. 8. Mesenchymal core of a day 16·5 abnormal palatal shelf. Note numerous lipid-like deposits (*L*) in the mesenchymal cells. Lead & uranyl. ×5000.

blocked the differentiation of mesenchymal cells into chondrocytes. However, cartilages which had already begun to differentiate (e.g. those of the nose and cranial base) showed reversible interferences with growth and mucopolysaccharide synthesis; indeed, when the effects of F.U.D.R. had presumably worn off, these structures showed *increased* growth and mucopolysaccharide synthesis. It is interesting that, ultrastructurally, almost all the mesenchymal cells in the palatal shelves of F.U.D.R.-treated fetuses showed a number of lipid deposits, whereas these were very rare in normal cells. In this connexion it is significant that in a biochemical study of the palatal shelves of 16–17 day rat fetuses with cleft palates induced by floxuridine (also a thymidine base analogue), Stefanovich & Gianelly (1971) found a higher concentration of cholesteryl esters and diglycerides, and a lower concentration of free fatty acids, monoglycerides and total phospholipids, than normal.

There are surprisingly few detailed reports in the literature of the teratogenic effects of F.U.D.R. Chaube & Murphy (1968) carried out an extensive study in which they screened hundreds of new drugs (including F.U.D.R.) used in cancer chemotherapy, while Bro-Rasmussen *et al.* (1971) studied macroscopically the teratogenic effects of F.U.D.R. in mice. With respect to F.U.D.R., these authors stated that cleft palate, digit anomalies, skull haematomas and spina bifida were present, but they did not mention the subcutaneous oedema, or state the percentage of fetuses affected, or report the histological aspects of the malformations (e.g. brain disruption, depressed mucopolysaccharide synthesis, absence of Meckel's cartilages, palato-oral fusion). The conclusion of Bro-Rasmussen *et al.* (1971) that vascular damage is the *primary* and fundamental teratogenic effect of F.U.D.R. is not in line with current knowledge; the primary action of F.U.D.R. is now believed to be on DNA and mucopolysaccharide synthesis. The abnormalities found, namely: (1) poorly formed or absent cartilages, (2) poor basement membrane synthesis, and hence detached epithelia and rupture of blood vessels in the brain, spinal cord, limbs and tail, and (3) absence of palatal shelf turgor leading to cleft palate, are all readily explained by defective mucopolysaccharide synthesis.

In teratogenic studies like the present one it is essential to adjust dosage and timing of administration of the teratogen so as to achieve a 100 % abnormality rate, and also to study the development of the abnormality from the beginning. If abnormal fetuses are only studied from day 17 onward (as in many previous studies of cleft palate), then one can have little idea of the early pathological changes, because wearing off of the action of the drug, and reparative and compensatory growth processes (which undoubtedly occur) will almost certainly have masked the primary events.

It is interesting that the mandible of the F.U.D.R. fetuses can grow and ossify in the absence of Meckel's cartilages in view of the widespread belief that many membrane bones are induced by the cartilage in their immediate vicinity. Indeed it is difficult to reconcile the F.U.D.R. observations with the conclusions of Frommer & Margolis (1971) that Meckel's cartilage is "an essential element in normal intramembranous osteogenesis by its inductive capacity for initiating and regulating the growth of the primary intramembranous ossification center (*sic*) of the mandible". In view of the extremely bizarre brain abnormalities, and the attempts at neural reconstitution, in the F.U.D.R. fetuses, the cerebral changes warrant further investigation. Although the mechanism of production of limb deformities is uncertain, many authors believe that focal haemorrhage into the limb buds can give rise to such deformities as phocomelia, ectromelia, and syndactyly (Bagg, 1927;

Poswillo, 1976; Wolpert, 1976). Certainly the phocomelia (and greatly reduced tail growth) in the F.U.D.R.-treated fetuses can be associated with focal haematomas.

Cleft palate in man is frequently associated with abnormalities of the limbs, brain and vertebral bodies (De Myer, Zeman & Palmer, 1964; De Myer, 1975; Ross & Lindsay, 1965; Gorlin, Cervenka & Pruzansky, 1971; Gorlin & Cervenka, 1974) as in the F.U.D.R.-treated rat fetuses. One is tempted to speculate that such cleft palate-associated defects in man are likewise initiated by defective mucopolysaccharide synthesis.

The palatal shelves of F.U.D.R.-treated fetuses showed adequate growth in length, but they exhibited a high density of mesenchymal cells, absence of large intercellular spaces, little mucopolysaccharide, delayed appearance of the maxillary and palatine osteogenic bone blastemata, and defective epithelial undercutting at the shelf base. These palatal shelves never spontaneously elevated to the horizontal position, nor could elevation be obtained experimentally, indicating a very weak or absent intrinsic shelf elevating force. All these observations are consistent with the mucopolysaccharide-turgor hypothesis previously advanced to account for *normal* shelf elevation (Ferguson, 1978). It is also of interest that although both head and cranial base were extended – indeed hyperextended – the palatal shelves remained vertical, which is inconsistent with the 'cranial base theory' of shelf elevation which involves the notion of a shelf force generated by extension and straightening of the cranial base (Verrusio, 1970).

The fusion of the unelevated vertical palatal shelves to the lateral oral walls in these F.U.D.R.-treated fetuses resembled very closely the palatopharyngeal fusion in some human fetuses with cleft palate, an example of which was described in detail by Humphrey (1970). In the rat, administration of a variety of antihistamines (all metabolized to norchlorcyclizine) produces cleft palate in which the vertical palatal shelves are fused to the tongue (King, Weaver & Narrod, 1965; Steffek, King & Derr, 1966). This glosso-palatal fusion is alleged to be a consequence of destructive changes induced in the shelf and tongue epithelium (Mato & Uchiyama, 1975). Certainly the epithelium in the F.U.D.R.-treated fetuses was abnormal, and this could explain the abnormal palato-oral fusion in some of these fetuses. In man, glosso-palatal ankylosis is rare, but in some such cases not only are the vertical palatal shelves fused to the tongue, but they are ossified in the vertical position (Hayward & Avery, 1957; Spivack & Bennett, 1968). It is interesting to note that in the F.U.D.R. fetuses, though the appearance of the maxillary and palatine bone blastemata was delayed, bone eventually invaded the vertical palatal shelves. Since, in man, the majority of cases of cleft palate are associated with a delay rather than an absence of shelf 'flip-up', there clearly must be a corresponding delay in ossification of the palatal shelves, otherwise more cases of ossified vertical palatal shelves would be seen.

It is significant that numerous teratogens causing cleft palate in laboratory animals also depress mucopolysaccharide synthesis: these teratogens include cortisone and other anti-inflammatory drugs (Greene & Kochhar, 1975), Vitamin A (Woollam & Millen, 1957; Solursh & Meier, 1973), salicylates (Larsson & Bostrom, 1965), and βAPN (Kauffman & Delbalso, 1975). Folic acid deficiency (Asling *et al.* 1960; Asling, 1961) may be presumed to act in a comparable manner to F.U.D.R. since the former inhibits the formation of tetrahydrofolic acid and hence of thymidine. However, F.U.D.R. represents a more specific, more convenient, more accurate and more reliable method of inhibiting thymidine synthesis and inducing clefts than does

experimental folic acid deficiency. Furthermore, a number of the drugs which induce cleft palate in the rat and depress mucopolysaccharide synthesis have been incriminated (on epidemiological evidence) in the aetiology of human cleft palate (Saxen, 1975). There is evidence that the incidence of cleft palate in man is increasing alarmingly – it has almost doubled in the past 25 years (Fogh, Andersen, 1968, 1971; Flynt & Oakley, 1973); and since some 80 % of these clefts are believed to be caused by environmental factors, it is not difficult to conclude that the worldwide increase in drug consumption may be responsible. From the evidence presented in this paper the avoidance, during at least the earlier stages of pregnancy, of drugs known to depress mucopolysaccharide synthesis, is indicated.

SUMMARY

The teratogenic effects of 5-fluoro-2-desoxyuridine on Wistar rat fetuses were studied macroscopically, microscopically and ultrastructurally. In no case did palatal shelf elevation occur, and a palatal shelf elevating force could not be demonstrated in freshly-removed fetuses. The shelves, like the connective tissues generally, showed clear evidence of depressed mucopolysaccharide synthesis. The shelves eventually ossified in the vertical position, and in some cases their free edges fused with the lateral wall of the oral cavity (palato-oral fusion). The results were consistent with the mucopolysaccharide-turgor hypothesis advanced by the author in a previous paper to account for normal shelf elevation.

Phocomelia, brain and limb bud haemorrhages, gross subcutaneous oedema, hyperextension of the cranial base, fused vertebrae, detached epithelia, bizarre brain abnormalities (including some remarkable attempts at neural reconstitution) and growth and ossification of the mandible in the virtual absence of Meckel's cartilages were also noted in these fetuses.

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