1969

The Effects of Hydroxyurea on Albino Rat Conceptuses

Michael R. Cozza Jr.
Loyola University Chicago

THE EFFECTS OF HYDROXYUREA ON ALBINO RAT CONCEPTUSES

by

Michael R. Cozza Jr.

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

June

1969

LIFE

Michael Richard Cozza Jr. was born in Pittsburgh, Pennsylvania on May 17, 1939. In June, 1957, he graduated from Central District Catholic High School. He attended Georgetown University for two years and then Duquesne University where he graduated with a Bachelor of Science Degree in June 1961. From 1961 until 1963 he was enrolled in the University of Pittsburgh as a non-degree student.

He entered the Department of Anatomy in the Graduate School of Loyola University in September, 1963, and was a graduate teaching assistant in 1964 and 1965. In July, 1965, he married Antoinette M. Manjoine and they have one daughter, Cheray, born June 7, 1966. From June, 1965 until September 1967 he was gainfully employed in commerce. Then he rejoined the Anatomy department and until the present he has been a graduate teaching assistant.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Dr. Leslie A. Emmert for his suggestion of the problem, his patience, encouragement, and supervision throughout the course of this thesis. His expert guidance and genuine interest in my education were a great source of strength to me.

My sincere thanks are due Dr. Joseph Thomas Velardo, Professor and Chairman of the Department of Anatomy, whose personal example of dedication and thoughtfulness for others has been a source of great inspiration.

I would also like to thank Dr. Sigfrid Zitzlsperger, Professor of Anatomy, for his interest, advice, and reassurances.

Invaluable help was given to me by my wife, Antoinette, through her personal sacrifice and her faith in me.

My parents deserve my warmest appreciation for their continued expressions of confidence.

Finally my thanks go to all members of the Department of Anatomy for their help.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

This thesis is concerned with the effects of an anticancer agent, Hydroxyurea, on the developing albino rat conceptus and in particular the placenta. Hydroxyurea, besides being an active chemotheraputic agent, is also a teratogen affecting rapidly growing and developing embryonic tissues.

Hydroxyurea, dissolved in boiled, distilled water, was administered intraperitoneally on 2 occassions at 3 day intervals, during the latter third of gestation to four groups of pregnant animals which were divided on the basis of dosage and time of injection. The groups were injected respectively on the 14th and 17th (I), 15th and 18th (II), and 17th and 20th days (IV), with 2000mg/Kg of hydroxyurea. Group III was injected with 2500mg/Kg on the 16th and 19th days. Necropsy of groups I, II, III, and IV occurred on the 18th, 20th, 20th, and 21st days respectively.

Experimental animals showed a weight gain from the first day of injection until necropsy that was approximately only half that observed in control rats. Fetuses were weighed, measured, fixed in 3 formalin fixatives and subsequently examined for malformations. It was observed that the average weight and length of experimental fetuses was smaller than controls but both were within normal limits of development. Malformations in experimental fetuses included underdeveloped ear flaps and vibrissae, short, stubby limbs and snout, enlarged shoulder and upper thoracic region, edematous trunk, and unwrinkled stretched skin.

The edematous appearance was caused by extensive accumulations of fluid especially under the skin and secondarily in the body cavity.

Placentae from non-treated and hydroxyurea-treated rats were examined by means of the following techniques: Hematoxylin and Eosin for general morphology; Periodic acid-Schiff method for glycogen plus diastase-Alcian-Blue treated controls for mucopolysaccharides and acid mucopolysaccharides; Gomori's modification of Perls' method for iron; 0.06% toluidine blue for the metachromasia and basophilia; and the Glyoxal Bis (2 hydroxyanil) method for calcium.

Placentae from experimental animals lost their button-like shape and appeared flattened and shaped more like a shallow cup but the diameter of these placentae was found to be similar to those of controls. The width of the labyrinth of experimental placentae was smaller while the width of the spongy zone varied little from that of control specimens. It appeared that the decreased width of the labyrinth may have been caused by a pulling together (retraction) of the vascular tree and its accompanying stromal tissue. Stromal cells were brought closer together and gave the appearance of an increase in the number of these cells present in the labyrinth. Thickened labyrinthine plates and roughened irregular face of the maternal sinuses are believed to be a consequence of the vascular tree retraction. Since hydroxyurea acts mainly on dividing cells, and since these changes occurred after cell division is supposed to have ceased in the placenta, the mode of action of the drug is not yet fully known. However, occassional mitoses were observed in the trophoblastic and mesodermal cells of the labyrinth and

in the endodermal cells of the villous yolk sac.

Iron, in the form of ferric ferrocyanide deposits was observed in increased intensities in the endodermal cells of the villous yolk sac and in the connective tissue stroma and trophoblastic cells of the labyrinth and in the cells of the decidua basalis in experimental specimens. Placentae from control, non-treated rats, contained almost no evidence of iron deposits in any of these areas.

Glycogen deposits in hydroxyurea-treated placentae showed no variation from those of control placentae. However, the amount of neutral mucopolysaccharides present in the labyrinth was greater in experimental than in control placentae.

Increased evidence of insoluble calcium deposits in the placenta was observed in hydroxyurea-treated rats from all four groups while those of controls were almost entirely free of calcium. Calcium and iron are both cations which can be bound to polyanionic substances like mucopolysaccharides. Staining by toluidine blue for these acid substances was too variable to allow for an accurate evaluation. However, it appears likely that increased amounts of acid compounds were present in the experimentals.

In part of group III the fetuses were dead at maternal necropsy. In these placentae greater intensities of iron, calcium, and granular basophilic accumulations were observed than in other experimentals. It appears likely that fetal distress or feto-placental dissociation (Emmert, '57) played the major role in bringing about the histochemical changes that occurred.

INTRODUCTION

It is known that cancers occur during pregnancy and in some instances are treated by chemotherapy (Sokal and Lessman, '60), (Hutchison, et al., '68), (Armstrong, et al., '64). We are concerned with the effects which anticancer agents have on the developing conceptus and in particular the placenta. Hydroxyurea (HU) is an active chemotheraputic agent with inhibitory and teratogenic effects on rapidly growing and developing tissues. First synthesized in 1869, it has been shown to be a colorless, weakly acidic, crystalline solid with a melting point of 140°C. It has a molecular weight of 76 and a chemical configuration of HO-HN-CO-NH_2. It is known to inhibit experimental tumors in mice (Stearns, Losee, and Bernstein, '63) and to be active against chronic granulocytic leukemia. Recent evidence indicates that it is inconsistently active against other malignant neoplasms in man (Krakoff, Murphy, and Savel, '63), (Lerner and Beckloff, '65), (Thurman, Bloedow, Howe, Levin, Davis, Lane, Sullivan, and Griffith, '65). In the mouse, it inhibits sarcomas, carcinomas, and leukemias.

Hydroxyurea has been given orally, intravenously, intraperitoneally and subcutaneously and was determined to be toxic to the host, its tumor(s) and conceptuses by all such routes (Wilson and Warkany, '65). In addition, HU produced neurotoxic effects such as anesthesia and/or excitation (Wilson and Warkany, '65).

Hydroxyurea has been observed to inhibit DNA synthesis but not RNA

or protein synthesis in cultured mammalian cells (Young and Hodas, '64), in regenerating rat liver (Schwartz, Garofalo, Sternberg, and Philips, '65) and in bacteria (Gale, Kendall, McLain, and DuBois, '64), (Rosenkranz, Garro, Levy, and Carr, '66), (Krakoff, Brown, and Reichard, '68).

The teratogenicity of any drug is subject to a complex number of parameters. For a drug to cause disturbances in embryonic development it must be given at the proper dose, to a suitable species at a specific stage of embryonic development. This specific stage of development can induce a discriminating sensitiveness at the level of molecules, cells, tissues, and organs in both a quantitative and qualitative interpretation. The rate at which the pregnant animal and conceptus detoxifies and excretes the drug is variable depending upon the routes and frequencies of administration. Nutrition is important and yet other undiscovered constituents probably alters responses to drugs.

A typical test for determining the teratogenicity of a drug in embryonic rats is to administer an initial dose that is the acute LD_{50} for mature rats. The LD_{50} for mature non-pregnant rats is 4.7mg/Kg. Pregnant rats in the 9th to 12th days of gestation are given this dose. Fetuses are then examined at term (21 days) for signs of life, resorption, and normalcy. If the young are alive and appear normal the drug is not considered teratogenic (Wilson and Warkany, '65).

Conceptuses, like tumor cells, manifest actively growing components. Few investigators have observed the teratogenic effects of single and multiple doses of HU on the conceptuses of experimental animals. Gaik ('67) recorded the effect of single doses of the drug on the albino

fetus and placenta. At doses ranging between 300mg/Kg and 2500mg/Kg pregnant rats were injected between the 9th and 19th days of gestation and necropsied between the 15th and 20th days. The results varied with the magnitude of the dose and the day of administration. Weight loss was observed in the pregnant rat and fetuses were resorbed or retarded in growth or presented developmental abnormalities. Placentae were examined histologically and histochemically. Increased calcium and iron deposits and decreased glycogen intensities over control placentae were demonstrated. In two instances a second dose of HU was injected three days after the first injection. In the first instance the injections were given on the 14th and 17th days and in the second instance on the 16th and 19th days of gestation. Abnormally large fetuses developed. Their gross structural development appeared edematous almost like a swollen "stuffed sausage" and their snout and limbs were abbreviated and stumpy respectively.

In this thesis the object is to observe the effect of multiple, sublethal doses of HU on conceptuses when injected into pregnant albino rats.

REVIEW OF LITERATURE

The structure and function of the placenta has for centuries intrigued investigators. A lack of understanding of its fine structural anatomy and physiology, however, has contributed to the many and varied philosophic and scientific theories and interpretations that occur in the literature.

Galen in 300 AD described in detail the human chorion, its blood supply and its relationship to the uterus (Brock, '28). Realdo Columbus in 1559 (cited by Krantz, '58) was credited with naming the organ the placenta because of the shape of the human organ.

Fabricius, in the 16th century, described the human placenta as a fleshy mass that clings very closely to the uterus and is thoroughly interwoven with the minute ends of the umbilical vein and artery. He considered its position only as a "convenience" for the umbilical vessels and that the blood flow between the uterus and fetus was continuous.

Krantz ('58) reviewed the contributions of William Harvey who in 1653 believed in the separateness of maternal and fetal circulations when he observed differences in maternal and fetal heart rates. He proposed that the placenta functioned in providing nutriment for the embryo.

Today, the placenta, of mammals, is considered as a temporary organ of fetal and maternal tissue lying in close apposition for the purpose of physiological exchange of substances between maternal and fetal blood streams.

Mathias Duval (1891) was one of the first investigators to describe the morphology of the rat placenta in great and accurate detail.

Grosser ('27) summarized comparative placentation and was the first to propose that placentae be classified as to the number of layers between maternal and fetal blood streams. As a common denomenator for classifying types of placentae he used the name of the maternal tissue that was in closest association with the chorion.

Mossman ('37) with slight modifications extended Grosser's work with the addition of more species and brought it up to date. He discovered in the rabbit that near term the syncytial membranes between the two blood streams disappear creating a hemoendothelial condition and suggested that other rodents, such as the rat and guinea pig also be classified in a separate hemoendothelial group.

However, Hard ('46), Wislocki, Deane, and Dempsey ('46), and Bridgeman ('48) in the rodent, and Wislocki and Bennet ('43) in human and monkey placentae described a syncytial layer which persists to term. Wislocki, Deane, and Dempsey ('46) described in some areas of the labyrinth silver staining fibers surrounding the fetal capillaries at term.

Amoroso ('61), using the light microscope, described the hemochorial placenta as initially composed of cytotrophoblast which later becomes syncytiotrophoblast. Separating this layer from the fetal blood vessels were fine collagenous or reticular fibers. These decrease as gestation advances and by term only a very thin layer of syncytium separates fetal endothelium and maternal blood.

Ultrastructurally, the rat placenta consists of four layers of tissue separating the maternal and fetal blood streams (Jollie '64a). From the maternal to fetal side it includes trophoblast I and trophoblast II which are cellular; element III which is syncytial and rich in lipids; and finally, IV, the fetal endothelium with its basement membrane.

The placenta is a comparably vigorous organ, more resistant to delaterious influences than the fetus. The fetus can be damaged or suffer severe, critical distress in utero while the placenta and fetal membranes will often show little or no signs of gross alteration. However, there is a mounting body of literature which indicates that there are profound microscopic changes which develop in the absence of a living fetus as will be considered below.

Changes that occur in the uterus and placenta depend upon the condition of the embryo, whether alive or dead; on the method of aborting or injuring, mechanical or chemical; and lastly on the time in gestation at which the stress was inflicted.

Pritchard and Huggett ('47) surgically removed or mechanically crushed Norwegian rat fetuses on the 10th through 19th days, and then observed subsequent placental development. Placentae of fetuses removed on the 10th day showed pronounced resorption of the omphalopleure, allantoic mesoderm and its fetal vessels and the neighboring labyrinth by the 14th day. The rest of the labyrinth and spongy zone grew until the 16th day when further growth ceased. Normal trophoblastic differentiation occurred but the placentae were spherical and subnormal in size.

Degenerative changes began to predominate; giant cell and spongy zones became destroyed by hemorrhages and thromboses. By the 19th day only the trophoblastic syncytium remained healthy. Placentae from fetuses removed on the 13th and 14th days were well vascularized by allantoic vessels, the connective tissue of which persisted to term around the clumped remains of the large fetal vessels. The shape of the placentae was discoid but the placentae were smaller in size. Placentae of fetuses removed after the 16th day differed somewhat from the above. Residual omphalopleure and entodermal sinuses of Duval and the giant cell and spongy zones remained normal but in the labyrinth degenerative changes occurred which differed from the above. Endothelial nuclei of collapsed fetal vessels did not clump up or round up but remained elongated and in linear appearance. A thick layer of exudate containing maternal RBCs and fibrin strands accumulated around these collapsed vessels, and in some places obliterated maternal blood channels causing local necrosis of the labyrinth.

Henderson ('54) found that after surgically terminating further development of rabbit embryos, in situ, on days 11, 16, or 19 post coitum the placentae were slow to show any changes. At necropsy, pregnant females which were previously treated by a subcutaneous administration of 5mg of stilboestrol in olive oil on the 11, 16, or 19th days had deciduae and placentae which showed signs of degeneration before the embryo.

Emmert ('57) performed mechanical feto-placental dissociation on cotton rats on the 15th, 20th, and 25th days of a 27 day gestation

period. Subsequent examination six hours to five days after operation showed rapid degenerative changes in amnion and yolk sac and slower deterioration of the chorionic trophoblast. The placental changes observed developed more rapidly the nearer to term dissociation occurred. These changes included at first small foci followed by a general infiltration of the stroma of the labyrinth by a granular basophilic material accompanied by deposits of calcium, iron and phosphate. These stromal alterations did not develop in the absence of maternal blood flow through the placenta. The trophoblast swelled and blistered away from the supporting connective tissue and occluded maternal blood channels causing ischemic degenerative changes (necrosis) in the spongy zone and decidua basalis as well as in the labyrinth.

Payne ('58) produced changes in the rat placenta and fetus after infection with different species of bacteria by intraperitoneal injection on the 13th day of gestation. Payne observed that the periplacental uterine wall manifested signs of ulceration and inflammatory reaction. The mesometrial surface of the placental disc became ulcerated and large accumulations of organisms, eroded cells, and debris occurred at the angle of the yolk sac wall and disc. The yolk sac endothelial cells adhering to Reichert's membrane proliferated to two to three cell layers thick in response to this accumulation of organisms and formed a barrier. With very severe infections this barrier lost its integrity, organisms penetrated the sinuses of Duval deep in the labyrinth and formed abscesses. Necrosis and abscess of the visceral yolk sac wall followed by invasion of the amniotic cavity and infection of the fetal respiratory

tract were caused by group C. streptococcus and C. pyogenes.

The teratogenic effects of HU were studied by Bendich, Borenfreund, Korngold, and Krim ('63) who reported that HU, hydroxylamine, and several other drugs caused chromosomal breaks in phage DNA in cultured mammalian cells and in mouse embryo cell cultures after an exposure time of 48 hours.

Murphy and Chaube ('64) found HU to be teratogenic for the rat, chick, and sand dollar embryonic systems. The pregnant rats were administered 50-2000mg/Kg of HU on the ninth to 12th days of gestation and killed at 21 days. Growth was retarded; hairlip, cleft palate, encephaly, retarded clubbed fore and rear appendages and a retarded tail were found. Volumes up to 0.2 ml of 0.1-0.5mg/egg of HU in saline were injected into four day old chick embryos and inspected as they died or were necropsied at 18 days. Beak defects were the only abnormality observed. In the treated sand dollar embryos normal development was observed from fertilization until early morula stage at which time development was blocked. The sea urchin embryos showed extensive chromosomal aberrations which included nuclear enlargement, elongation of metaphase chromosomes, anaphase bridging, fragmentation of chromosomes, polyploidy, and some C-mitoses (mitoses arrested in metaphase).

Gale, Kendall, McLain, and DuBois ('64) observed that when HU was added in a concentration of 1.0-1.6mg/ml to the culture medium of Pseudomonas aeruginosa in the early log phase of its growth cycle and then incubated for 18 hours, marked elongations up to 30 times the normal

length of 2.5 micra occurred. Electron microscope studies revealed that the gross morphology of both experimental and control specimens differed only in their length. Inside the treated Pseudomonas aeruginosa the amount of nuclear material was diminished or absent and the cytoplasm was more granular than in controls. In 1966 Gale observed the effects of oxymal hydroxyamic, a derivative of HU, on the gram-negative bacteria Escherichea coli. Again the gross morphologic change elicited was the enhanced length of the treated cells. This time the increase was far greater than for Pseudomonas aeruginosa.

Ferm ('65) injected pregnant hamsters on the eighth day of gestation with a single i.v. dose of 50mg HU in saline solution. Fetuses were removed and examined on the ninth or 11th days. The congenital malformations observed, in addition to the above exencephaly, included abnormally coiled cardiac tubes and a failure of the neural tube to close.

Sinclair ('67) observed that when cells of the Chinese hamster, grown in vitro, were exposed to 1.0mM HU for 1.2 hours only those cells in the DNA replication or synthesis (S) phase of the cell cycle failed to divide. These cells did resynthesize DNA after drug removal, enlarged three to four times their normal volume in the next 20-30 hours, and then lysed.

There are several theories regarding the mechanism by which HU inhibits DNA synthesis. The one having the most proponents holds that HU blocks DNA synthesis at the conversion of molecules of ribotides to desoxyribotides. Included in this group are Mohler ('64), Young and

Hodas ('64), Frenkel, Skinner, and Smiley ('64), Schwartz, Garofalo, Sternberg, and Philips ('65), Frenkel and Arthur ('67), Young, Schocketman, and Karnofsky ('67), and Krakoff, Brown, and Reichard ('68). A disparity exists in the literature between those favoring this mechanism of action for HU and the interpretations of Yarbro ('68) and Pollak and Rosenkranz ('67).

Yarbro ('68) studied the effects of the four exogenously administered desoxyribonucleosides and ribonucleosides on the incorporation of ^{32}P into DNA and RNA in the mouse ascites tumor both in vitro and in vivo. The four desoxyribonucleosides stimulated incorporation of ^{32}P into DNA and depressed incorporation into RNA. The four ribonucleosides promoted incorporation into both DNA and RNA. He observed that HU prevented the promotion of ^{32}P incorporation into DNA by the ribonucleosides. No effect was observed on incorporation into RNA. Yarbro believed that if the above theory regarding the mode of action of HU were valid then addition of the desoxyribonucleosides would overcome the HU-induced inhibition. He reasoned that some other mechanism was responsible for the inhibition.

Pollak and Rosenkranz ('67) similarly believed that adding a desoxyribonucleoside should overcome the inhibition and DNA synthesis could resume. However, they found that HU interference with DNA metabolism of BHK-21 cells transformed by polyoma virus was not reversed by the addition of radioactive desoxyribonucleoside.

On the other hand Fishbein and Carbone ('63) proposed that HU split into hydroxylamine which reacts with Acetyl coenzyme A, cleaving it and

thereby causing a decrease in oxidative phosphorylation with a concomitant lowering of ATP. Gale, Kendall, McLain and DuBois ('64) agree with this mechanism of action.

Vogler ('66) presented evidence which suggested that HU may act early in DNA synthesis by blocking the de novo synthesis of pyrimidine. Vogler administered 6-azauridine to six cancer patients. This compound is known to specifically inhibit the enzyme, ortidylic decarboxylase resulting in a marked increase in the urinary excretion of orotic acid and orotidine. Orotic acid is the precursor of pyrimidine. HU in a dose of 40-45mg/Kg per day was administered for five days and reversed the orotic acidurea.

MATERIALS AND METHODS

Twenty five sperm-positive female albino rats[1] were housed two per cage and given water and Rockland rat food ad libitum. Rats numbered 5,7, and 25 were not pregnant at necropsy. Four rats numbered 1, 2, 3, and 4 were necropsied on the 13th, 15th, 16th, and 20th days of gestation, respectively, and their placentae were fixed, sectioned, stained with Hematoxylin and Eosin and Periodic acid-Schiff (PAS) and examined histologically as a preliminary procedure. Animals numbered 1, 2, and 3 were not included in this study and animal No. 4 was used as an auxiliary control. During the latter third of gestation the remaining 18 rats were anesthetized with ether on two occassions at three day intervals and given an intraperitoneal injection of HU dissolved in boiled distilled water on each occassion.

The rats were placed in four groups on the basis of dosage and time of intraperitoneal injection (Table I). Group I was injected on the 14th and 17th days of gestation with 2000mg/Kg of HU and was necropsied on the 18th day. Group II was injected with 2000mg/Kg of HU on the 15th and 18th days and necropsied on the 20th day. Group III was injected on the 16th and 19th days with 2500mg/Kg of HU and necropsied on the 20th day. Group IV was injected with 2000mg/Kg on the 17th and 20th days and necropsied on the 21st day. The rats were weighed just prior to each injection and again just before necropsy following an

---

1. Hormone Assay Inc., Chicago, Illinois

overdose of nembutal administered intraperitoneally (Table I).

The body wall of each rat was shaved, opened and the uterine horns with their intact conceptuses were removed and immediately placed in a physiological saline solution heated to 37°C. Each fetus with its placenta was excised individually from the uterus. The two were separated and the fetus was examined for signs of life (heart beat and respiration), weighed, and measured (crown-rump length) (Tables II,III).

Fetuses and placentae were then fixed in Baker's[2] calcium formalin, alcohol formalin[3], or pyrogallol formalin (Husby, '46). Fetuses were subsequently examined under the dissecting microscope for malformations. The placentae were dehydrated, embedded in paraffin, and sectioned at 6 micra. The following staining techniques were used to study the placentae: 1) Hematoxylin and Eosin[4] for general morphology; 2) PAS[5] for glycogen; Alcian Blue-PAS-Diastase (Mowry, '63) was used for the control slides for PAS; 3) DNA was demonstrated by the Feulgen[6] method; 4) Perls' method modified by Gomori[7] was used to demonstrate ferric iron in the tissues; 5) Calcium was demonstrated by the Glyoxal Bis (2 hydroxyanil), GBHA, method (Kashiwa and House, '64); 6) Metachromasia and basophilia were demonstrated by 0.06% toluidine blue buffered to pH 3.5.

The weight and length of the fetuses were compared to the stages of Christie ('64) so as to determine whether or not these animals were

---

2. Lillie, R.D. ('65), p. 293.
3. Lillie, op. cit. p. 42.
4. Lillie, op. cit. p. 176.
5. Lillie, op. cit. p. 198.
6. Lillie, op. cit. p. 149.
7. Lillie, op. cit. pp. 405-406.

within normal limits proposed by Christie.

Due to limited circumstances the control animals from group II were necropsied 48 hours after the second injection and not 24 hours as was performed on groups I, II, and IV.

RESULTS

The number of animals in each experimental group, the dosage, and days of treatment and necropsy together with the weight of the animals at each injection and at necropsy is contained in Table I.

Maternal Weights

In each group the non-treated control animals showed a steady weight increase after receiving injections of boiled distilled water, i.e., the vehicle for HU. Control animals from group I showed a weight increase of nearly 60 grams from the first injection until necropsy. Experimental animals showed a weight increase of only 27 grams after receiving two injections of HU until necropsy.

Similar to the weight gain of control animals in group I, group II controls showed an increase of more than double that of experimental rats. Although experimental animal No. 8 had a weight loss after the first injection, it recovered to a level just above its preëxisting weight when necropsy occurred (Table I).

The animals in group III, Nos. 9, 10, 11, and 12 were treated in the old Anatomy animal quarters at 706 S. Wolcott Avenue in Chicago and the fetuses were dead at necropsy. Animals Nos. 21, 22, 23, and 24 were treated at 1400 S. First Avenue, Hines, Illinois. The first set of experimental animals in group III showed a weight gain of approximately 50% that of control No. 21 while the second set showed a weight increase of over 90% that of the control.

The weight gain in control animal No. 17 of group IV was more

than double that of experimental animals Nos. 18 and 19.

Fetal Weight and Length

The number and condition of fetuses recovered at necropsy following treatment with HU is contained in Table II. The 19 pregnant females produced 200 conceptuses with an average of 10.8 conceptuses per animal.

Fetuses from all four groups were alive at necropsy except for the following: in group I, animal No. 16 contained one dead fetus which appeared normal; in group III, animals Nos. 9, 10, 11, and 12 contained fetuses which were all dead of which two from No. 10 showed evidence of resorption; while animal No. 23 contained one dead, resorbed fetus. The most extensive evidence of resorption was observed in this fetus No. 23R, injected on the 16th and 19th days and necropsied on the 20th day of gestation. Figure 3 illustrates the extent of resorption.

The weight and crown-rump length of fetuses for both experimental and control specimens in all four groups were within the normal limits of development proposed by Christie ('64) (Table III). However, in all groups the control fetuses were larger than experimental fetuses in both measurements. In group III the dead fetuses from animals Nos. 9, 10, 11, and 12 were slightly smaller in both weight and length than those from animals Nos. 22, 23, and 24 which were alive at necropsy.

Fetal Morphology

After fixation the fetuses were examined under the dissecting microscope. In group I the control fetuses showed that vibrissae had developed, digits on fore and rear paws were separated, eyelids were sealed, ear flaps were present and the umbilical hernia was reduced.

Experimental specimens contained less developed vibrissae, limbs were shorter and appeared stumpy and the digits were not as well developed as in controls. In addition the fetuses appeared very edematous with skin stretched very thin and easily crumbled to the touch. An enlarged umbilical hernia was not present.

The control fetuses of the second group of rats had sealed eyelids, well-developed ear flaps, nails, pads on the paws and vibrissae and no umbilical herniation. Under close examination, the experimentals had ear flaps that were underdeveloped; skin was stretched; the trunk was edematous; the snout appeared short and stubby and the limbs were also short. The chest cavity was enlarged over the heart and no umbilical hernia was present (Figure 2).

The controls and experimentals in group III were similar in all respects to those of group II. These fetuses were further examined by removing the head, with a scapel, just anterior to the fore limbs. A large quantity of fluid was accumulated in the body cavities around the viscera and just beneath the skin of the experimental animals. No gross changes in the viscera were observed (Figure 4).

The animals in group IV were also similar in characteristics to those in group II.

Placentae

From a total of 200 placentae from 19 rats, 151 placentae were examined histologically and histochemically.

The definitive placenta of the rat is classified as discoidal, hemochorial, labyrinthine and chorioallantoic. Formation of the

placenta begins at the mesometrial pole of the blastocyst where there is a proliferation of ectoderm (trophoblast) to form the ectoplacental cone. This proliferation is the precursor of the fetal portion of the placenta and begins its development on the sixth day. On the seventh day of gestation, the developing blastocyst implants eccentrically in an antimesometrial crypt of the uterus. A decidual reaction in the uterine tissue surrounding the blastocyst obliterates the uterine lumen.

On the seventh day the ectoplacental cone begins invading and destroying uterine tissue and the endothelial lining of the maternal blood vessels. Blood is extravasated, becomes surrounded by trophoblastic tissue which forms sinuses.

An important area of exchange for the embryo is the inverted yolk sac or vitelline placenta which is developing from the seventh to eleventh days of gestation. The yolk sac placenta is divided into two morphologic zones: (1) an outer,non-vascular, parietal wall consisting of scattered endodermal cells which incompletely line the interior surface of Reichert's membrane (N.B.: Reichert's membrane is a thick basement membrane which adheres externally to trophoblastic giant cells), and (2) an inner, vascularized, visceral wall composed of endodermal cells adjacent to a mesenchymal layer in which are embedded the vitelline blood vessels.

By approximately the 13th day of gestation a cleft appears antimesometrially to the embryo. This cleft, which is continuous with the uterine lumen divides the decidual mass into a capsularis in contact with the developing conceptus and a decidua parietalis lining the

uterine wall. By the 14th day, the remaining decidua capsularis has degenerated along with the parietal wall of the yolk sac, including Reichert's membrane together with any residual trophoblast. This results in the exposure of the endoderm of the visceral wall of the yolk sac to the uterine epithelium for the purpose of exchange. The parietal yolk sac is retained as a covering for the fetal aspect of the chorioallantoic placenta and extends inward into the placenta as double-walled sleeves around large fetal vessels entering and leaving the placenta (the endodermal sinuses of Duval).

The mesodermal allantoic process has been growing across the exocoelom toward the base of the ectoplacental cone and on the 11th day reaches the chorionic mesoderm fusing with it. This vascular chorioallantoic mesoderm now projects at intervals into the ever expanding trophoblastic villi of the cone forming trophoblastic lamellae. The definitive chorioallantoic placenta is now formed and continues to grow and expand until term.

Three general zones are recognized in the chorioallantoic placenta: (1) the outermost layer consists of phagocytic giant cells of trophoblastic origin; the area of the endometrium adjacent to this area is known as the decidua basalis; (2) under the giant cell zone is a spongiotrophoblastic or spongy zone containing only maternal sinusoids which are surrounded by sheets of oval or polyhedral cells and clusters of polyhedral cells storing glycogen; (3) this zone partially surrounds the labyrinth which is composed of an intricate branching network of plates of fetal tissue; these plates have a mesodermal core containing

the fetal capillaries and are covered by trophoblast. Maternal blood circulates in the spaces between adjacent plates. It is in this area that most of the physiologic exchange occurs.

The maternal and fetal circulations now lie very close to one another and are separated only by the four electronmicroscopically discernible layers of trophoblast and fetal endothelium.

Fetal capillaries in this zone are small, round, lined by a layer of endothelium, and most often contain immature red blood cells. Maternal sinuses are generally larger than fetal capillaries and are irregular in outline and contain only mature red blood corpuscles.

Placental Morphology

When sections from the region of the central axis of the placentae were examined the shapes of control placentae were disc- or button-like while those of experimentals were flattened and more cup-shaped giving the appearance of having a greater diameter (Figures 4,5,6,7). However, when the diameters of the placentae were measured, in millimeters by means of a plastic ruler overlying the specimen, they were found to be similar in both control and experimental animals for all four groups (Table IV). The average diameter of experimental placentae from all four groups ranged from 9.0-11.0 mm. and from 9.6-11.6 mm. for controls. In group III, experimental placentae Nos. 9, 10, 11, and 12 carrying dead fetuses at necropsy averaged 10.0 mm. and the remaining group III specimens which carried live fetuses at necropsy averaged 11.8 mm.

Micrometer measurements of the width of the labyrinth and spongy zone taken at approximately mid-placenta were also obtained in

millimeters at 40X magnification (Table V). In each group the width of the labyrinth in the experimental specimens was smaller than in controls. In group I, the control labyrinths averaged 2.26 mm. while experimental labyrinths averaged 1.63 mm. In group II, the average of the controls was 2.38 mm. and that of experimentals was 2.15 mm. The control labyrinths of group III averaged 2.30 mm. and those of experimentals 1.99 mm. The widths of the labyrinths of Nos. 9, 10, 11, and 12 of this group were only slightly smaller than the rest of the experimental placentae in the group. In both experimental and control placentae the width of the labyrinth increased from groups I to IV. The widths of the spongy zone in control placentae varied little from the experimentals in groups I, III, and IV. In group II, the spongy zone of treated placentae was more than 30% larger than non-treated specimens. In group I, the average widths of experimentals and controls for the spongy zone were 0.75 and 0.74 mm. respectively. In group II, the control average was 0.48 while the experimental average was 0.70 mm. In group III, experimental and control averages for the spongy zone was 0.54 and 0.57 mm.respectively. In group IV, the experimentals averaged 0.42 and the controls averaged 0.50 mm. In both treated and non-treated specimens,the width of the spongy zone decreased with advancing age, i.e. the width was largest in group I necropsied on the 18th day and smallest in group IV necropsied on the 21st day. This decrease is consistent with a loss of glycogen cells from this zone.

The number of trophoblast cells in the labyrinth of the placentae of experimental animals in all four groups appeared to be less than

those of the controls. Trophoblast cell nuclei were similar in size to those in control placentae but were spaced farther apart. In addition the membranes or trophoblastic plates between fetal and maternal blood channels appeared to be thicker and to present a more irregular surface facing the maternal blood spaces than in control placentae (Figures 9,10,11,12,13). The fetal capillary endothelium was often separated from its mesodermal stroma and wholly or partially collapsed in experimental placentae. This occurred less often in control placentae.

Trace amounts of local basophilic accumulations were observed in the connective tissue of all four groups.

The following was observed concerning the quantity of blood cells in maternal and fetal blood channels in both experimental and control placentae: 1) the maternal sinuses in the labyrinth tended to contain more formed elements of the blood than did the fetal capillaries; and 2) as the center of the labyrinth was approached less and less blood was observed in both channels; the periphery of the placenta was observed to contain a more uniform quantity of blood in its sinuses.

In all groups, the cell size in the giant cell zone, spongy zone, and yolk sac placenta appeared similar as did the shape and arrangement of cells in both the treated and non-treated specimens.

Specimens examined for nuclear detail with the Feulgen stain showed results similar to those found after staining with Hematoxylin and Eosin. Connective tissue nuclei appeared to be closer together and trophoblast cells in the labyrinth of experimental specimens appeared

to be spaced farther apart than in control placentae. In addition mitotic figures were observed in some trophoblastic cells of the labyrinth and endodermal cells of the villous yolk sac in each group (Figures 14,15).

Iron

In all specimens examined the bluish-green colored ferric ferrocyanide (iron) deposits (ID) were present in the spongy zone and in the giant cell zone. In control specimens of all four groups, iron deposits were not observed in the yolk sac villous cells while experimental specimens contained moderate to substantial intensities (Table VI) (Figures 16,17).

The intensity of iron present in the placentae varied with the fixative. In all groups, the calcium formalin fixed specimens contained the most iron; alcohol formalin fixed placentae displayed a lesser intensity. Unfortunately, pyrogallol formalin fixed specimens proved histotechnically inferior for assessment. Only calcium and alcohol formalin fixed specimens were utilized in this determination. In all four groups, iron deposits in the labyrinth and decidua basalis were varied in their intensities.

In group I, moderate intensities of iron were observed in the connective tissue stroma and trophoblast cells of the labyrinth of ten experimental specimens, a trace amount in three and none in two specimens. All the control specimens were devoid of iron. In the decidua basalis, barely perceptible deposits were observed in three experimental placentae and none in the remaining 12 specimens or in the controls.

The decidua basalis in two control and nine experimental placentae of group II contained faint traces of iron, while none was observed in eight control and two experimental specimens. The labyrinth of three placentae showed trace intensities of the blue ferric iron accumulations while eight experimental specimens contained trace to moderate amounts. In the remaining treated and non-treated specimens iron deposits were not observed.

Control placentae in group III did not contain iron deposits. Similarly in nine experimentals iron was absent. In 11 experimentals, trace intensities presented, and in nine specimens moderate to substantial deposits were observed. The decidua basalis in this group, in experimentals and controls, appeared essentially negative for iron.

In group IV, two control placentae contained trace intensities and one control contained substantial deposits of iron. Iron was not present in the three remaining control placentae. In seven experimental placentae, small barely perceptible deposits were observed. In the decidua basalis, trace intensities of iron were observed in three controls and in all experimentals. The remaining controls did not contain iron.

Glycogen

There was much similarity of results obtained for the presence of glycogen in both experimental and control placentae fixed in calcium formalin and alcohol formalin throughout the four experimental groups. In the decidua basalis trace intensities of glycogen were observed in those cells which had not started to undergo degeneration. Cells in

different stages of degeneration appeared to contain greater deposits of mucopolysaccharides. The connective tissue around each giant cell in the giant cell zone appeared to contain traces of glycogen, while none was observed in the giant cells themselves. Glycogen in the form of tiny granular deposits was observed in the connective tissue and trophoblast cells of the labyrinth. That present was very small in all but a few experimental and control placentae which contained somewhat more. The yolk sac villous cells uniformly showed a picture of substantial accumulations in the apical cytoplasm of each cell.

The glycogen-bearing cells in the spongy zone appeared maximally intense. Consistent with the micrometer measurements, the amount of cells with glycogen was approximately similar in controls and experimentals. The amount of such cells present became progressively less from group I to group IV (Figures 18,19,20,21). This is consistent with the disappearance of glycogen with advancing gestation.

In all four groups, placentae of experimentals and controls treated with diastase and stained with PAS, revealed that the decidua, giant cell zone, spongy zone, and yolk sac were respectively similar.

In the decidual zone there was an abundance of magenta-colored, amorphous neutral mucopolysaccharides present in the connective tissue between the junctional zone cells. In the giant cell zone, each giant cell was rimmed with this magenta-colored material in the connective tissue. In the spongy zone, each cell, in the sheets of non-glycogen-containing cells, contained a connective tissue matrix of the bright-colored magenta material like that in the giant cell zone. In many

specimens, the connective tissue around glycogen-bearing cells was also stained. Variable intensities of amorphous, bluish-purple material containing both acid and neutral mucopolysaccharides were observed in both experimentals and controls. Patterns of depositions could not be discerned. In the yolk sac, the villous cell tips contained much neutral mucopolysaccharide, and Reichert's membrane stained bright magenta throughout.

The labyrinth in the experimentals of group I and II contained more neutral substance than did that of controls. In group III, the experimentals contained more neutral mucopolysaccharide than did the controls. (Unfortunately, the fetuses of these animals died.) Group IV was similar to groups I and II. Generally, as one proceeded from group I to group IV, more neutral polysaccharides were observed in both experimentals and controls. No distinct orientation of deposits to either maternal or fetal face, central or peripheral locales in the labyrinth was observed. Deposits were mostly widely scattered.

Metachromasia and Basophilia

Toluidine blue is an aniline dye used for locating cellular and extra-cellular basophilia,due,for instance, to ribonucleoproteins and mucopolysaccharides containing acid groups. A metachromatic color change in the dye from blue to purplish-red indicates the presence of strongly acidic substances e.g. acid mucopolysaccharides.

In all four groups minimal appearances of metachromatic material were observed in both experimentals and controls. The trace substances that were observed were irregularly present and not confined to a

specific location.

Similarly in groups I, II, IV, and part of III minimal intensities of basophilic material were observed sporadically in both experimental and control specimens. In group III the placentae of Nos. 9, 10, 11, and 12, which bore dead fetuses at necropsy, showed slight increases of stromal basophilia, and placentae from No. 10 showed the extensive testimony of nuclear fragmentation in the labyrinth that accompanies cellular degeneration.

Calcium

Observations on the occurrence and distribution of insoluble calcium deposits were limited to the 12 control and 24 experimental placentae fixed in alcohol formalin in order to avoid possible false localization attendant upon the use of the other two fixatives. Of the four possible staining methods utilizing GBHA, recommended by Kashiwa and House ('64), the "flooding method with solution II" gave the best results.

Hydroxyurea-treated specimens from all four groups showed increased evidence of insoluble calcium deposits in the placenta. In group I, control specimens contained no evidence of calcium except for No. 13A which had one small reddish-brown area in the middle of the labyrinth. Experimental placentae contained heavy but not extensive calcium deposits in the labyrinth, villous and Duval cells of the yolk sac, giant cell zone, and decidua basalis. In these and placentae from subsequent groups, calcium deposits in the labyrinth were mainly oriented toward the fetal face and toward the center of the section.

In group II, control placentae were again devoid of stainable calcium whereas experimentals contained small deposits in the giant cell zone and labyrinth.

In group III, control specimens contained no evidence of calcium except for a heavy, concentrated intensity in a necrotic area near the fetal face of the labyrinth of No. 21C. Placentae from animals Nos. 9, 10, 11, and 12, which bore dead fetuses at necropsy, showed more extensive evidence of calcium than did Nos. 22, 23, and 24 which were alive at necropsy. Calcium deposits in group III were observed mainly in the labyrinth along the fetal face with the spongy zone and giant cell zone containing only a few small deposits. No. 10C contained the most extensive evidence of calcium in the labyrinth (Figure 22).

In group IV, control specimens contained some calcium in the giant cell zone and decidua basalis whereas experimental specimens showed evidence of stainable calcium in the villous and Duval cells of the yolk sac and in the giant cell zone.

DISCUSSION

Fetuses

This study of rat conceptuses following the administration of 2000mg/Kg or 2500mg/Kg of HU intraperitoneally during the latter third of gestation, was designed to reinvestigate and expand a part of Gaik's ('67) studies. Gaik administered two doses of HU to two pregnant albino rats, one on the 14th and 17th and the other on the 16th and 19th days of gestation. Enormous fetuses were produced whose crown-rump length and weight far surpassed that of fetuses from control animals. Abnormalities such as an edematous appearance, short stubby somewhat malformed limbs and snout and an enlarged thorax were also observed in these overgrown fetuses. Similar abnormalities were observed in this study but with fetuses which were within normal weight and length range. The enlarged thoracic and shoulder region that we observed was shown to be produced largely by extensive edema in the subcutaneous tissue; although this edema was not limited to the above region (Figure 4).

An hypothesis to account for the fetal edema might be developed from the work of Sinclair ('67) and of Adamson *et al.* ('65). Sinclair found that when the cells of the Chinese hamster, grown *in vitro*, were exposed to 1.0 mM of HU for 1.2 hours only those cells in the DNA replication phase (S) of the cell cycle failed to divide. While these cells did resynthesize DNA after drug removal, they enlarged three to four times their normal volume in the next 20-30 hours and then lysed.

In this thesis and in that of Gaik the amount of HU that reaches the fetuses is unknown as is the rate of its metabolism by both mother and fetuses. However, Adamson et al. ('65) found that in non-pregnant rats and mice 70% of the HU, in a dose of 200-500mg/Kg, was excreted in the urine in three hours and 90% in 24 hours. If the in vivo metabolism of HU in rats and mice was similar to the in vitro behavior of hamster cells, cellular breakdown products resulting from cell lysis would be released in the intercellular spaces there lowering water concentration extrecellularly, thereby attracting water from the cells and thus producing edema. Twenty-four hours after the second injection necropsy was performed; this time lapse might have permitted the second injection of HU to have a lysing effect on more cells. Both injections then could have had a combined effect to produce the fetal edema observed.

It was also observed that the teratogenic effects displayed by these fetuses were mild compared to the effects observed by Murphy and Chaube ('64) on younger rat embryos. They observed that pregnant rats given 50-2000mg/Kg of HU on the 9th to 12th days, and then necropsied on the 21st day of gestation had fetuses which were retarded in growth and had a cleft palate, encephaly, hairlip, retarded clubbed fore and rear appendages and a retarded tail.

Placental Morphology

The morphological and histochemical changes in placentae after treatment with HU are similar in some respects to those obtained by Gaik ('67) whose work this thesis was designed to reinvestigate and extend.

The placentae of treated animals were flattened and more cup-shaped and appeared to be of greater diameter than control placentae. However, actual measurement proved that the diameters were similar. Gaik had observed that for single and multiple-injected animals the placentae appeared flatter and smaller than those of control animals.

Jollie ('64b),in a radioautographic study of DNA synthesis in the rat placenta with tritiated thymidine, observed that no labelling of the nuclei in the labyrinthine trophoblast and yolk sac endoderm occurred after the 12th day of gestation. This suggests that no DNA synthesis and therefore no mitosis would occur after this time in these regions. Gaik's observations on the presence of mitotic figures confirms these observations. However, in the present work mitotic figures were observed in both the labyrinth and yolk sac not only on the terminal day but also on the 18th and 20th days in both treated and control specimens. This suggests that no long lasting interference of mitosis in the labyrinth and villous yolk sac by HU occurred.

Measurements of the width of the labyrinth showed a decrease in experimental placentae. Accompanying this decrease, however, there appeared to be an increase in the number of cells in the field of view in many areas of the labyrinth. HU acts mainly on rapidly dividing cells but Bridgeman ('48), Jollie ('64), and Gaik ('67) did not observe mitotic figures in the labyrinth during this latter third of gestation. The mitotic figures observed in this work were minimal in number and should not have been expected to appreciably alter the zone size if acted upon by HU. However, in the case of group I, treated on the 14th

and 17th days, if an appreciable number of dividing cells were present on the 14th day then the action of HU on these cells might account for the large decrease in width observed in the labyrinth of this group.

Similar to Sinclair's ('67) experience with dividing cells of the Chinese hamster, i.e. those cells grown in vitro and exposed to HU, one might expect a concommitant increase in the amount of cellular fragmentation, which would be observed as Feulgen-positive, basophilic accumulations in the labyrinth, if a similar swelling and lysis had occurred here. No increase was observed in groups I, II, IV, and part of III. Nos. 9, 10, and 12 of group III showed a slight increase in the intensity of basophilic deposits present and No. 10 showed extensive accumulations in the labyrinth. The fetuses from this group of animals were all dead at necropsy. Emmert ('57), after fetalectomy in the cotton rat, observed an increase in Feulgen-positive nuclear debris together with Feulgen-negative, granular basophilic accumulations in the labyrinthine connective tissue which were accompanied by increased iron and calcium deposits. These accumulations were observed as soon as six hours after fetalectomy and became quite intense and widespread with time. It appears tenable that those placentae here investigated which showed increased accumulations did so due to the fetoplacental dissociation and not to the HU whose affects appear to have resulted in a retraction of the fetal vascular tree with its accompanying, thickened, trophoblastic plates, irregular maternal face of the sinuses, apparent increased cellularity, and torn fetal capillary endothelium.

Iron

The moderate to substantial accumulations of blue ferric ferrocyanide deposits in the yolk sac villous cells of experimental placentae in all four groups confirms the results obtained by Gaik ('67) in animals given two injections.

It is unknown whether HU had any effect on the transfer or rate of transfer of iron across the placenta. Glasser et al. ('68) observed that there is a large increase in radioactive iron (Fe^{59}) transfer from days 14 to 16 and this is coincident with the start of hemopoiesis by the fetal liver. This group also observed that neither the placenta nor yolk sac possessed a large capacity to retain the iron isotope. They reported that the greatest intensity of iron found in the yolk sac occurred on the 14th day after which the iron content fell markedly. Nylander ('53), on the other hand previously suggested that the yolk sac served as a storage depot for iron, especially near term. The yolk sacs of control animals utilized for this thesis were devoid of ferric ions. Placentae from all experimental animals contained moderate to substantial deposits of iron in the yolk sac villous cells and lesser amounts in the labyrinth. Gaik ('67) obtained similar results after both single and multiple doses of HU. Also Emmert ('57) and Glasser et al. ('68) observed increased placental iron accumulations after fetalectomy. The results of the latter two investigators suggested that the ability of the placenta to remove iron from the maternal blood stream is an active process and not dependent on an intact fetus.

The fetuses in this work were alive except for those in animals

from group III Nos. 9, 10, 11, and 12. Information at hand suggests that the accumulation of iron in the placenta could be caused by polyanionic compounds in the placenta which attract and bind the cation or that there is a derrangement in the transfer of iron from the placenta to the fetal blood. The results of the metachromatic stain, toluidine blue, for acid containing compounds, was too capricious to allow a determination as to whether an increased amount of polyanionic substances, such as acid mucopolysacharides were present to bind the ferric ions. Laurell and Morgan ('64), using 20-day pregnant rats, observed that iron is transferred from maternal plasma transferrin to the placenta and then to fetal plasma transferrin. Possibly, HU may have altered the transfer mechanism from the placenta to the fetal blood, or the combination of a possible increase in anions and an altered transfer mechanism teamed-up to produce the results obtained. Present knowledge concerning the effects and mechanisms of HU action prevents any complete explanation as to why increased iron was found in the placentae of the experimental animals.

Glycogen

The distribution and rate of disappearance of glycogen in the control and experimental placentae studied is similar to that observed by other investigators (Wislocki, Deane, and Dempsey, '46), (Bridgeman, '48), (Amoroso, '52), and (Gaik, '67). A great intensity of glycogen was observed in the junctional zone of the decidua basalis, in the epithelium of the villous portion of the yolk sac and in the spongy zone on the 18th day of gestation. In the labyrinth, a few small and

and widely separated glycogen deposits were observed in some experimental and control specimens from each group. By the 21st day of gestation, most of the glycogen disappeared from the glycogen-bearing cell zone (Figures 18,19,20,21) and from the endodermal cells of the villous yolk sac.

As previously reported, HU was not expected to have any effect on the glycogen-bearing cells since by the 14th day no mitoses are observed in these cells (Bridgeman, '48). Observations and measurements of the quantity and distribution of such cells in the spongy zone corroborated this thought. Gaik ('67) observed the absence of glycogen-containing cells from the spongy zone after injection of 2000mg/Kg of HU on the 14th and 17th days of gestation and necropsy on the 18th day. She suggested that glycogen stores were depleted to feed the outsized fetuses from that animal.

Metachromasia and Basophilia

In order to determine the amount of metachromatic substance in the placenta, specimens were stained with 0.06% toluidine blue at pH 3.5. When certain aniline dyes are bound to particular substrates they exhibit characteristic color changes known as metachromasia. These substances include nuclear and cytoplasmic ribonucleoproteins and sulfate esters of high molecular weight as are found in acid mucopolysaccharides (Bergeron and Singer, '58).

The results obtained in this work were essentially the same as those observed by Gaik ('67), i.e., metachromasia never abounded in any specimens and its presence was capricious. In Nos. 9, 10, 11, and 12,

slightly increased deposits were observed, and in No. 10 extensive basophilia was observed in the stroma of the labyrinth. It should be reiterated that these fetuses were dead at necropsy.

Calcium

Emmert observed a significant relationship between fetal distress and placental calcification in the cotton rat ('57) and in the albino rat ('58). After feto-placental dissociation in these animals increases of soluble calcium were observed in the stromal tissue of the labyrinth. These deposits continued to increase in intensity of staining and in distribution as long as the maternal blood supply to the placenta was maintained.

The increased calcification observed in the labyrinth of experimental specimens suggests that this is not a normal occurrence in the aging rat placenta of this strain. Wislocki et al. ('46) working with rats (strain unspecified), observed that argyophilic deposits, soluble in aqueous acids, were present in the decidua basalis and junctional zone of the aging placenta. They concluded that these deposits represented deposits of calcium. When degenerative changes occur in cells as they do here polyanionic substances such as nucleoproteins are freed and, along with other anions, already present in the stroma, like acid mucopolysaccharides, are able to attract cations, e.g. Ca^{++} and Fe^{+++}. In the junctional zone, decidual cells eventually degenerate and one might expect to observe calcium deposition in this area. In the placentae analyzed here similar deposits were observed but not extensively along the described margin of the placenta.

Using the Dahl method for calcium, Gaik ('67) did not observe calcium in the control placentae removed for study from animals necropsied on the 19th and 20th days of gestation. She did find that in animals injected with a single dose of either 2000mg/Kg or 2500mg/Kg of HU on days 14 to 16 the placentae contained substantial calcium deposition in the spongy zone, labyrinth, and in necrotic areas in the labyrinth. Placentae from an animal injected on the 18th day with 2174mg/Kg showed no deposits. Of the two animals receiving multiple doses of HU the animal injected with 2000mg/Kg on the 14th and 17th days showed substantial accumulations in both the labyrinth and spongy zone while the animal treated on the 16th and 19th days with 2500mg/Kg showed only barely discernible amounts in these areas.

No evidence of extensive cellular degeneration in the labyrinth was observed in any animal except No. 10 of group III which contained dead fetuses at necropsy. Increased basophilia was observed in these placentae along with extensive areas of calcification in the fetal connective tissue of the labyrinth (Figure 22). Greater deposits of calcification were observed in Nos. 9, 11, and 12 of group III than in all the other placentae, but none was as extensively calcified as No. 10. Concomitently, a slight increase in the amount of discernible acid substances was observed in the placentae of the above three animals.

Since consistent and wide-spread increased calcification was observed in this study, only in those placentae whose fetuses were dead a necropsy, it is presumed that the fetoplacental dissociation, i.e., the absence of fetal blood flow, is the major factor in producing this

calcification, and further that the thickened labyrinthine plates, the irregular maternal face of the sinuses, the apparent increase in cellularity, and the separated capillary endothelium, are alterations of placental morphology brought on by the retraction of the fetal vascular tree and its stroma, and as such are not necessarily a primary affect of HU. The sporadic occurrance of calcium in the labyrinths of the HU treated specimens is probably due to localized defects in the fetal vascular tree.

Certainly, in early gestation HU acts to produce bone defects (Murphy and Chaube, '64), but whether or not HU has a metabolic effect on the utilization of calcium and iron is not known. We do know, however, that the treatment with HU occurred at a time when the placental transfer of calcium (Feaster, '56), (Wasserman et al., '57) and iron (Glasser et al., '68) is at its highest.

SUMMARY AND CONCLUSIONS

1. The placentae and fetuses of albino pregnant rats treated with Hydroxyurea during the latter part of gestation were investigated both histologically and histochemically.
2. Weight gain in treated mothers was approximately half that of non-treated control mothers.
3. The average weight and length of fetuses from treated mothers was less than for those of control specimens, but still within normal limits.
4. Fetuses examined under the dissecting microscope showed under-developed ear flaps, short stubby snout and appendages, enlarged thoracic and shoulder area, and stretched skin. The latter two changes were caused by extensive edema observed under the skin and in the body cavities.
5. Treated placentae had lost their button-like shape and appeared flattened and shaped more like a shallow cup.
6. Diameters of treated and non-treated specimens were compared and found to be similar.
7. When the widths of the spongy zones and labyrinths were measured at mid-placentæ, the spongy zones were found to be similar in size while the labyrinths were smaller in experimental than in control placentae.
8. The labyrinth appeared to be pulled together (retracted) in treated

placentae. Trophoblastic cells appeared farther apart and mesodermal cells appeared closer together; the labyrinthine plates appeared thicker and the maternal face of the sinuses presented an irregular surface.

9. Mitoses were observed in minimal numbers in the trophoblastic and mesodermal cells of the labyrinth as well as in the endodermal cells of the villous yolk sac.

10. Increased iron deposits were observed in the villous yolk sac and labyrinth in experimental conceptuses.

11. No change in the glycogen deposition or its disappearance was observed after treatment with HU. However, the neutral mucopolysaccharide content did show some increase.

12. Metachromasia, following buffered toluidine blue staining, was highly variable and histotechnically difficult to evaluate. An increase in acid mucopolysaccharides appears likely in view of the binding of calcium and iron in the connective tissue.

13. The increased calcium, iron, and granular basophilic accumulations which were observed in the labyrinths of conceptuses bearing dead fetuses in treated rats were similar to those observed by Emmert ('57) following surgical feto-placental dissociation.

14. In the treated placentae, whose fetuses were alive at necropsy, the localized alterations in the labyrinthine stroma are interpreted as resulting from limited defects in the fetal circulation and may reflect early signs of impending fetal distress.

BIBLIOGRAPHY

Adamson, R.H., S.L. Agur, S.E. Hess, and J.D. Davidson 1965 The distribution, excretion, and metabolism of hydroxyurea-^{14}C. J. Phar. Exp. Therap. 150: 322-327.

Amoroso, E.C. 1952 Placentation. In: Marshall's Physiology of Reproduction. Ed. by A.S. Parkes Longmans, Green and Co., New York. Vol. II: 127-311.

_____ 1961 Histology of the placenta. Brit. Med. Bull. 17 (2): 81-90.

Armstrong, J.G., R.W. Dyke, P.J. Pouts, and C.J. Jansen 1964 Delivery of a normal infant during the course of oral vinblastin sulfate therapy for Hodgkin's disease. Ann. Intern. Med. 61: 106-107.

Bendich, A., Ellen Borenfreund, Grace C. Korngold, and Mathilde Krim 1963 Action of hydroxylamines on DNA and chromosomes. Fed. Proc. 22: 582.

Bergeron, J.A. and M. Singer 1958 Metachromasy: An experimental and theoretical evaluation. J. Biophys. Biochem. Cytol. 4: 433-458.

Bridgeman, Jane 1948 An histological and cytological study of the development of the chorioallantoic placenta of the white rat. J. Morph. 83: 195-224.

Brock, A.J. 1928 English translation, Galen on the Natural Faculties. William Heinemann, New York. 229.

Christie, G.A. 1964 Staged development of the rat on external form. J. Morph. 114: 263-286.

Duval, M. 1892 Les Placenta des Rongeurs. Ed. by F. A.can Ancienne Librairie Germer Bailliere et C, Paris. 640.

Emmert, L.A. 1957 Observations of the fetal membranes of the cotton rat, Sigmodon hispidus hispidus, following feto-placental dissociation by surgery. Anat. Rec. 127: 289. (Abstract)

_____ 1958 Evidence for the independence of fused placentae of the albino rat. Anat. Rec. 130: 296. (Abstract)

Fabricius, H. 1942 The Embryological Treatises of Hieronymus Fabricius of Aquapendente. A facsimile edition, translation by H.B. Adelmann Cornell Univ. Press, New York. Vol. 1: 250-251.

Feaster, A. 1956 Placental transfer of Ca^{45} in the rat. J. Nutrit. 58: 399-406.

Ferm, V.H. 1965 Teratogenic activity of hydroxyurea. Lancet 1: 1338.

Fishbein, W.N. and P.P. Carbone 1963 Hydroxyurea: Mechanism of action. Science 142: 1069-1070.

Frenkel, E.P. and C. Arthur 1967 Induced ribotide reductive conversion defect by hydroxyurea and its relationship to megaloblastosis. Can. Res. 27 (1): 1016-1019.

Frenkel, E.P., W.N. Skinner, and J.D. Smiley 1964 Studies on a metabolic defect induced by hydroxyurea. Can. Chemoth. Rep. 40: 19-22.

Gaik, Geraldine C. 1967 Observations of the effect of hydroxyurea on albino rat placentae and fetuses. Unpublished Master's Thesis, The Library, Loyola University Stritch School of Medicine, Chicago, Illinois.

Gale, G.R. 1966 Hydroxyurea derivatives: Oxamyl hydroxyamic acid. Can. Res. 26 (1): 2340-2348.

Gale, G.R., Sally M. Kendall, Helen M. McLain, and Suzanne DuBois 1964 Effect of hydroxyurea on Pseudomonas aeruginosa. Can. Res. 24: 1012-1020.

Glasser, S.R., C. Wright, and R.M. Heyssel 1968 Transfer of iron across the placenta and fetal membranes in the rat. Amer. J. Physiol. 215: 205-210.

Grosser, O. 1952 In: Marshall's Physiology of Reproduction. Ed. by A.S. Parkes Longmans, Green and Co., New York. Vol. II: 148-149.

Hard, W.L. 1946 A histochemical and quantitative study of phosphatase in the placenta and fetal membranes of the guinea pig. Am. J. Anat. 78: 47-77.

Henderson, Megan 1954 Foetal regression in rabbits; experimental studies of histolysis and phagocytosis. Proc. Roy. Soc. B. 142: 88-112.

Husby, R.A. 1946 Hydroxybenzene compounds as cytoplasmic fixatives. Proc. Soc. Exp. Biol. Med. 61: 122-215.

Jollie, W.P. 1964a Fine structural changes in placental labyrinth of the rat with increasing gestational age. J. Ultrastr. Res. 10: 27-47.

_____ 1964b Radioautographic observations on variations in DNA synthesis in rat placentae with increasing gestational age. Am. J. Anat. 114: 161-172.

Kashiwa, H.K. and J.M. House 1964 The glyoxal bis (2 hydroxyanil) method modified for localizing insoluble calcium salts. Stain Tech. 39: 359-367.

Krakoff, I.H., N.C. Brown, and P. Reichard 1968 Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. Can. Res. 28: 1559-1565.

Krakoff, I.H., M.L. Murphy, and H. Savel 1963 Preliminary trials of hydroxyurea in neoplastic diseases in man. Proc. Am. Assoc. Can. Res. 4: 35. (Abstract)

Krantz, K.E. 1958 The anatomy and endocrinology of the human placenta. In: Essentials of Human Reproduction. Ed. by J.T. Velardo Oxford Univ. Press, New York. 101-130.

Laurell, C.B. and E. Morgan 1964 Iron exchange between transferrin and the placenta in the rat. Acta Physiol. Scand. 62: 271-279.

Lerner, H.J. and G.L. Beckloff 1965 Hydroxyurea administered intermittently. J. Am. Med. Assoc. 192: 1168-1170.

Lillie, R.D. 1965 Histopathologic Technic and Practical Histochemistry. 3rd ed., McGraw Hill, New York.

Mohler, W.C. 1964 Cytotoxicity of hydroxyurea (NSC-32065) reversible by pyrimidine deoxyribosides in a mammalian cell line grown in vitro. Can. Chemoth. Rep. 34: 1-6.

Mossman, H. 1937 Comparative morphogenesis of the fetal membranes and accessory uterine structures. Contrib. Embryol. Carneg. Inst. 26: 129.

Mowry, R.W. 1963 The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue G8X and their combinations with periodic acid-Schiff reaction. Ann. N.Y. Acad. Sci. 106: 402-423.

Murphy, M.L. and S. Chaube 1964 Preliminary survey of hydroxyurea (NSC-32065) as a teratogen. Can. Chemoth. Rep. 40: 1-7.

Nylander, G. 1953 On the placental transfer of iron. An experimental study in t he rat. Acta Physiol. Scand. Suppl. 107: 1-105.

Payne, J.M. 1958 Changes in rat placenta and foetus following experimental infection with various species of bacteria. J. Path. Bact. 75: 367-386.

Pollak, R.D. and H.S. Rosenkranz 1967 Metabolic effects of hydroxyurea on BHK-21 cells transformed with polyoma virus. Can. Res. 27: 1214-1224.

Pritchard, J.J. and A. St. G. Huggett 1947 Experimental foetal death in the rat: Histological changes in the membranes. J. Anat. 81: 212-214.

Rosenkranz, H.S., A.J. Garro, J.A. Levy, and H.S. Carr 1966 Studies with hydroxyurea I. The reversible inhibition of bacterial DNA synthesis and the effect of hydroxyurea on the bactericidal action of Streptomycin. Biochem. Biophys. Acta 114: 501-515.

Schwartz, H.S., M. Garofalo, S.S. Sternberg, and F.S. Philips 1965 Hydroxyurea: Inhibition of desoxyribonucleic acid synthesis in regenerating liver of rats. Can. Res. 25: 1867-1870.

Sinclair, W.K. 1967 Hydroxyurea: Effects on Chinese hamster cells grown in culture. Can. Res. 27 (1): 297-308.

Sokal, J.E. and Ellen M. Lessman 1960 Effects of cancer chemotheraputic agents on the human fetus. JAMA 172: 1765-1771.

Stearns, Barbara, Kathryn A. Losee, and J. Bernstein 1963 Hydroxyurea: A new type of potential antitumor agent. J. Med. Chem. 6: 201.

Thurman, W.G., G. Bloedow, C.D. Howe, W.C. Levin, P. Davis, M. Lane, M.P. Sullivan, and K.M. Griffith 1963 Phase I study of hydroxyurea. Can. Chemoth. Rep. 29: 103-107.

Vogler, W.R., H.A. Bain, and C.M. Huguley, Jr. 1966 In vivo effect of hydroxyurea on orotic acid synthesis. Can. Res. 26: 1827-1831.

Wasserman, R.H., C.L. Comar, M.M. Nold, and F.W. Lengemann 1957 Placental transfer of calcium and strontium in the rat and mbbit. Am. J. Physiol. 189: 91-97.

Wilson, J.G. and J. Warkany, Eds. 1965 Teratology: Principles and Tehcniques. Univ. of Chicago Press., Chicago, Illinois. 145-168.

Wislocki, G.B. and H.S. Bennet 1943 The histology and cytology of the human and monkey placenta, with special reference to the trophoblast. Am. J. Anat. 73: 335-449.

Wislocki, G.B., H.W. Deane, and E.W. Dempsey 1946 The histochemistry of the rodent's placenta. Am. J. Anat. 78: 281-345.

Yarbro, J.W. 1968 Further studies on the mechanism of action of hydroxyurea. Can. Res. 28: 1082-1087.

Young, C.W. and Sadie Hodas 1964 Hydroxyurea: Inhibitory effect on DNA metabolism. Science 146: 1172-1174.

Young, C.W., G. Schochetman, and P.A. Karnofsky 1967 Hydroxyurea-induced metabolism of deoxyribonucleotide synthesis: Studies in intact cells. Can. Res. 27 (1): 526-534.

TABLE I

MATERNAL WEIGHTS OF RATS ON DAYS OF INJECTION AND AT NECROPSY FOLLOWING MATERNAL TREATMENT DURING GESTATION WITH HYDROXYUREA

GROUP, DOSE	RAT NO.	GESTATION DAY INJ.	WT. AT EA. INJ.	WT. AT NECROPSY	GESTATION DAY NECROPSIED
I					
	13	14, 17#	282, 327	337	18
	15	14, 17#	280, 318	324	18
2000 mg/Kg	14	14, 17	261, 287	288	18
	16	14, 17	283, 314	304	18
II					
	20	15, 18#	300, 350	370	20
2000	4*	-	- -	374	20
mg/Kg	6	15, 18	310, 330	340	20
	8	15, 18	280, 260	284	20
III					
	21	16, 19#	310, 360	366	20
2500	4	-	- -	374	20
mg/Kg	9	16, 19	242, 278	290	20
	10	16, 19	304, 356	350	20
	11	16, 19	298, 324	324	20
	12	16, 19	294, 330	350	20
	22	16, 19	320, 356	344	20
	23	16, 19	300, 326	330	20
	24	16, 19	318, 348	340	20
IV					
	17	17, 20#	307, 316	362	21
2000	18	17, 20	295, 270	300	21
mg/Kg	19	17, 20	293, 323	316	21

= vehicle control (boiled distilled water)

* = non-treated control

TABLE II

THE CONDITION AND NUMBER OF FETUSES AT NECROPSY FOLLOWING MATERNAL TREATMENT DURING GESTATION WITH HYDROXYUREA

GROUP	RAT NO.	NO. FETUSES	LIVING	DEAD	RESORBED DEAD
I: Rx 14, 17th days with 2000mg/Kg N*, 18th day.	13#	11	11	0	0
	15#	11	11	0	0
	14	11	11	0	0
	16	9	8	1	0
II: Rx 15, 18th days with 2000mg/Kg N, 20th day.	20#	12	12	0	0
	4##	10	10	0	0
	6	12	12	0	0
	8	10	10	0	0
III: Rx 16, 19th days with 2500mg/Kg N, 20th day.	21#	11	11	0	0
	4##	10	10	0	0
	9	10	0	10	0
	10	14	0	12	2
	11	8	0	8	0
	12	13	0	13	0
	22	11	11	0	0
	23	10	9	0	1
	24	12	12	0	0
IV: Rx 17, 20th days with 2000mg/Kg N, 21st day.	17#	10	10	0	0
	18	3	3	0	0
	19	12	12	0	0

* = Necropsied

= Vehicle control (boiled distilled water)

= Non-treated control

TABLE III

WEIGHT AND CROWN-RUMP LENGTH OF FETUSES AT NECROPSY FOLLOWING MATERNAL TREATMENT DURING GESTATION WITH HYDROXYUREA

GROUP	RAT NO.	DOSE	AVE. WT. IN GM.	RANGE IN GM.	AVE. LENGTH IN MM.	RANGE IN MM.
I: Rx						
14, 17th	13	veh. cont.#	1.56	1.06-2.08	25.0	22-28
days, N*,	15	veh. cont.	1.64	1.45-1.80	24.0	22-26
18th day.	14	2000mg/Kg	1.34	0.70-1.77	20.0	10-22
	16	2000mg/Kg	1.49	1.35-1.77	19.0	17-22
II: Rx						
15, 18th	20	veh. cont.	4.06	1.24-4.74	36.6	25-40
days, N,	4	-##	4.15	4.00-4.68	35.5	29-39
20th day.	6	2000mg/Kg	1.74	1.44-2.13	26.5	24-29
	8	2000mg/Kg	1.93	1.65-2.56	27.0	26-29
III: Rx						
16, 19th	21	veh. cont.	4.05	3.70-4.36	36.8	36-38
days, N,	4	-	4.16	4.00-4.68	35.5	29-39
20th day.	9	2500mg/Kg	2.70	2.16-3.34	28.5	26-30
	10	2500mg/Kg	2.62	1.31-3.46	28.1	28-31
	11	2500mg/Kg	2.42	2.14-2.80	29.4	26-31
	12	2500mg/Kg	2.51	1.90-3.22	27.0	25-30
	22	2500mg/Kg	3.43	2.88-3.85	32.4	30-39
	23	2500mg/Kg	3.52	3.20-4.30	34.5	32-41
	24	2500mg/Kg	3.50	3.00-3.98	32.0	29-34
IV: Rx						
17, 20th	17	2000mg/Kg	5.71	5.03-6.05	43.5	38-44
days, N,	18	2000mg/Kg	5.28	5.24-5.36	41.3	38-44
21st day.	19	2000mg/Kg	4.99	4.76-5.34	41.7	37-44

* = Necropsied

= Vehicle control (boiled distilled water)

= Non-treated control

TABLE IV

A COMPARISON OF PLACENTAL DIAMETERS FROM CONTROL ANIMALS AND THOSE TREATED WITH HYDROXYUREA

GROUP		EXPERIMENTAL	CONTROL
I: Rx 14, 17th days with 2000mg/Kg N*, 18th day.	No. of specimens	17	17
	Range	7.5-11mm	8-11mm
	Average	9.4mm	9.6mm
II: Rx 15, 18th days with 2000mg/Kg N, 20th day.	No. of specimens	14	13
	Range	8-11mm	7-12mm
	Average	9.0mm	10.5mm
III: Rx 16, 19th days with 2500mg/Kg N, 20th day.	No. of specimens	55	12
	Range	8-14mm	9.5-12mm
	Average	11.1mm	10.9mm
IV: Rx 17, 20th days with 2000mg/Kg N, 21st day.	No. of specimens	10	7
	Range	9-11mm	8-11mm
	Average	9.8mm	10.0mm

* = Necropsied

TABLE V

A COMPARISON OF THICKNESS OF LABYRINTHS AND SPONGY ZONES

IN PLACENTAE FROM CONTROL AND HU TREATED ANIMALS

GROUP		LABYRINTH EXP'T.	LABYRINTH CONTROL	SPONGY ZONE EXP'T.	SPONGY ZONE CONTROL
I: Rx 14, 17th days with 2000mg/Kg N*, 18th day.	No. of specimens	17	17	17	17
	Range (mm)	1.00-2.25	1.13-3.50	0.38-1.25	0.50-1.38
	Ave.**(mm)	1.63	2.26	0.75	0.74
	% change#	28% decrease		No change	
II: Rx 15, 18th days with 2000mg/Kg N, 20th day.	No. of specimens	14	13	14	13
	Range (mm)	1.25-2.50	1.88-2.70	0.38-1.00	0.38-0.75
	Ave. (mm)	2.15	2.38	0.70	0.48
	% change	10% decrease		30% increase	
III: Rx 16, 19th days with 2500mg/Kg N, 20th day.	No. of specimens	55	12	55	12
	Range (mm)	1.25-2.88	1.00-3.28	0.31-0.88	0.38-0.75
	Ave. (mm)	1.99	2.30	0.54	0.57
	% change	14% decrease		5% decrease	
IV: Rx 17, 20th days with 2000mg/Kg N, 21st day.	No. of specimens	10	7	10	7
	Range (mm)	1.75-2.38	2.25-2.75	0.38-0.50	0.38-0.63
	Ave. (mm)	2.28	2.54	0.42	0.50
	% change	10% decrease		16% decrease	

* = Necropsied

** = Average

\# = (Experimental/Control) x 100

TABLE VI

HISTOCHEMICAL LOCATION OF IRON DEPOSITS

GROUP	ZONE	-	+	++	+++
I: Rx					
14, 17th	GIANT CELL ZONE	T,V			
days with	SPONGY ZONE	T,V			
2000mg/Kg	LABYRINTH		V	T	
N*, 18th	YOLK SAC	V		T	T
day.	DECIDUA	T,V			
II: Rx					
15, 18th	GIANT CELL ZONE	T,V			
days with	SPONGY ZONE	T,V			
2000mg/Kg	LABYRINTH		V	T	
N, 20th	YOLK SAC	V		T	T
day.	DECIDUA	V	T		
III: Rx					
16, 19th	GIANT CELL ZONE	T,V			
days with	SPONGY ZONE	T,V			
2500mg/Kg	LABYRINTH	V	T,V		
N, 20th	YOLK SAC	V		T	T
day.	DECIDUA		T,V		
IV: Rx					
17, 20th	GIANT CELL ZONE	T,V			
days with	SPONGY ZONE	T,V			
2000mg/Kg	LABYRINTH	V	T,V		
N, 21st	YOLK SAC	V		T	T
day.	DECIDUA		T,V		

* = Necropsied
T = Placentae treated with Hydroxyurea
V = Placentae treated with vehicle
\- = Iron deposits not present
\+ = Trace amounts of ferric iron present
++ = Moderate amounts of iron present
+++ = Substantial amounts of iron present

TABLE VII

HISTOCHEMICAL DETECTION OF GLYCOGEN BY PAS

GROUP	ZONE	-	+	++	+++	++++
I: Rx 14, 17th days with 2000mg/Kg N*, 18th day.	GIANT CELL ZONE	T,V				
	SPONGY ZONE					T,V cell
	LABYRINTH		T		V	
	YOLK SAC				T,V	
	DECIDUA				T,V	
II: Rx 15, 18th days with 2000mg/Kg N, 20th day.	GIANT CELL ZONE	T,V				
	SPONGY ZONE					T,V cell
	LABYRINTH		T		V	
	YOLK SAC				T,V	
	DECIDUA				T,V	
III: Rx 16, 19th days with 2500mg/Kg N, 20th day.	GIANT CELL ZONE	T,V				
	SPONGY ZONE					T,V cell
	LABYRINTH		T		V	
	YOLK SAC				T,V	
	DECIDUA				T,V	
IV: Rx 17, 20th days with 2000mg/Kg N, 21st day.	GIANT CELL ZONE	T,V				
	SPONGY ZONE					T,V cell
	LABYRINTH	V	T			
	YOLK SAC				T,V	
	DECIDUA				T,V	

* = Necropsied
T = Placentae treated with Hydroxyurea
V = Placentae treated with vehicle
\- = Glycogen not present
\+ = Trace amounts of glycogen present
++ = Moderate amounts of glycogen present
+++ = Substantial amounts of glycogen present
++++ = Heavy amounts of glycogen present

FIGURE 1: Histology of the normal albino rat placenta on the 20th day of gestation.

Hematoxylin and Eosin

16X

LA = Labyrinth

SZ = Spongy zone

GC = Giant cell zone

DB = Decidua basalis

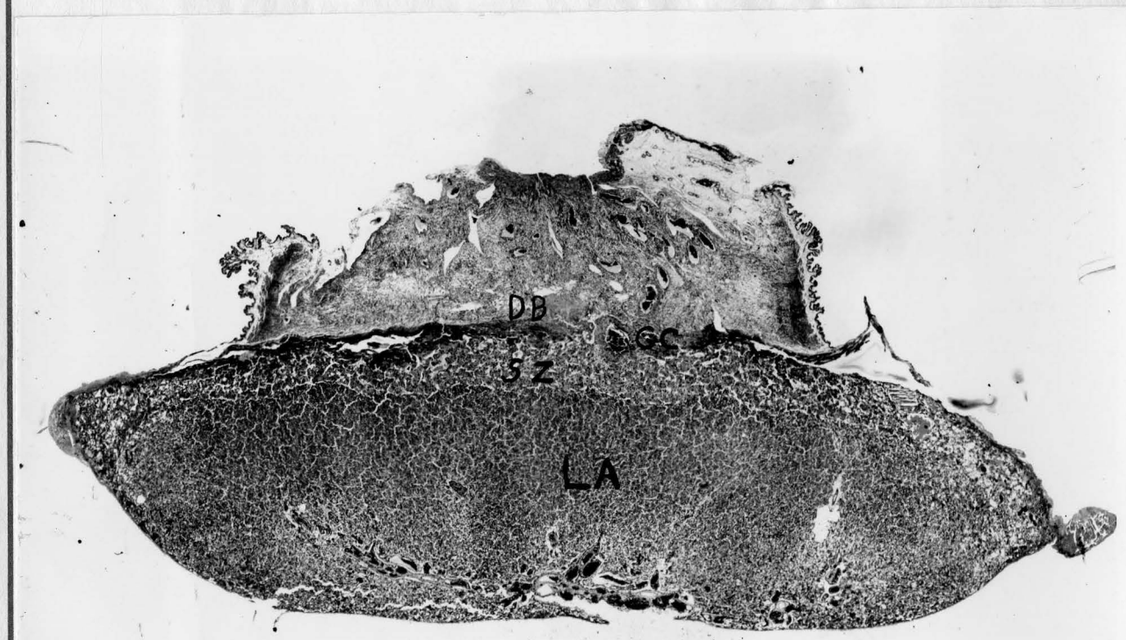

FIGURE 1

FIGURE 2: Fetuses from experimental (E) and control (C) animals which were alive on day 21 of gestation. Experimental injected on 17th, 20th days with 2000mg/Kg of HU. Observe underdeveloped fore and rear appendages, snout, and ear flaps; and the enlarged thoracic region and stretched skin of the experimental fetus.

Formalin fixed

1.3X

FIGURE 3: Fetus from an experimental animal on 20th day of gestation after treatment with 2500mg/Kg of HU on 16th and 18th days. The most marked evidence of resorption in this study.

Formalin fixed

1.3X

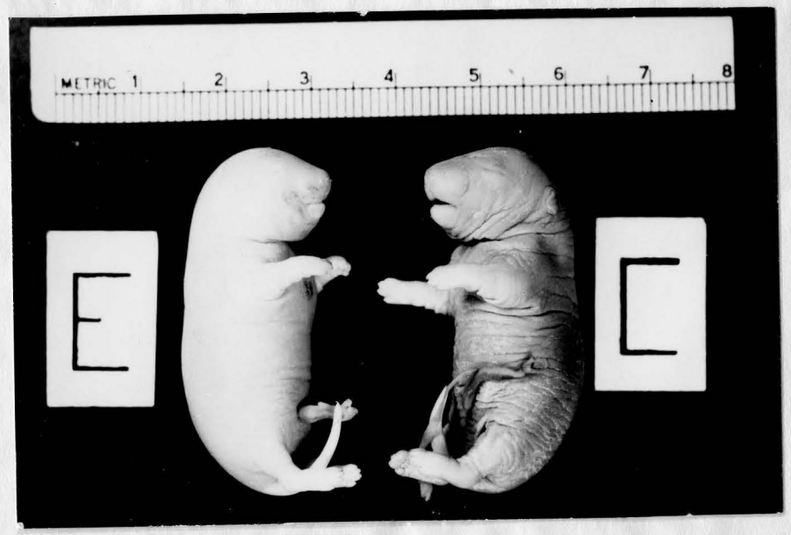

FIGURE 2

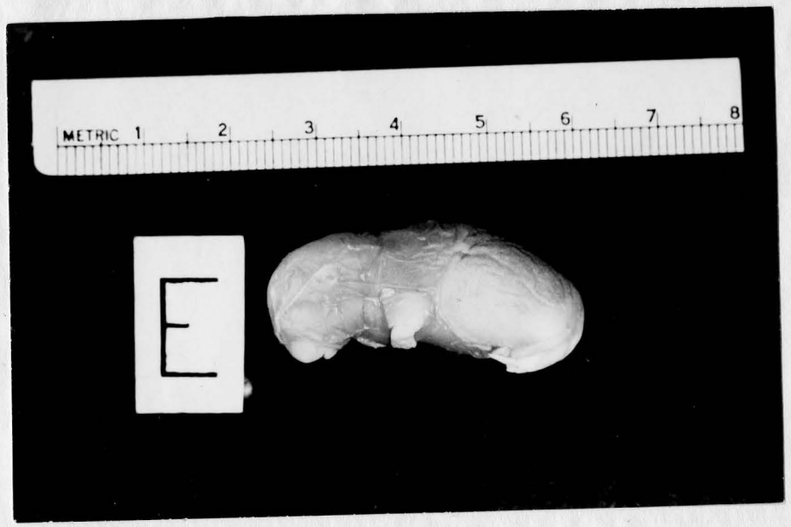

FIGURE 3

FIGURE 4: Posterior view of a cross section of 20 day control (left) and experimental (right) fetuses at the level of the forearms showing accumulation of fluid (↗ ↖) just beneath the skin on the experimental fetus. This fetus was exposed to 2500mg/Kg of HU on the 16th and 19th days of gestation. Formalin fixed

2X

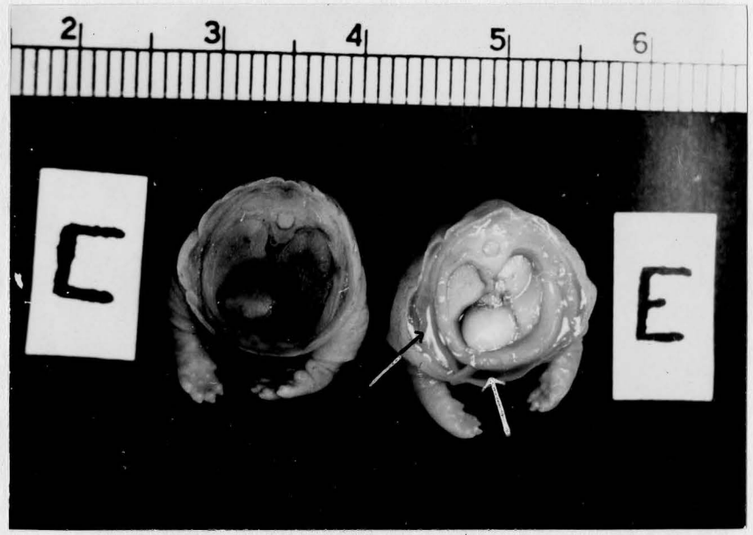

FIGURE 4

The following Figures 5 through 8 illustrate the flattening of the placental disc following treatment with HU as indicated below. In each instance the control placenta is on the left and the experimental placenta is on the right.

FIG. 5: Group I, treated 14th, 17th days with 2000mg/Kg, necropsied 18th day.

FIG. 6: Group II, treated 15th, 18th days with 2000mg/Kg, necropsied 20th day.

FIG. 7: Group III, treated 16th, 19th days with 2500mg/Kg, necropsied 20th day.

FIG. 8: Group IV, treated 17th, 20th days with 2000mg/Kg, necropsied 21st day.

Hematoxylin and Eosin

4X

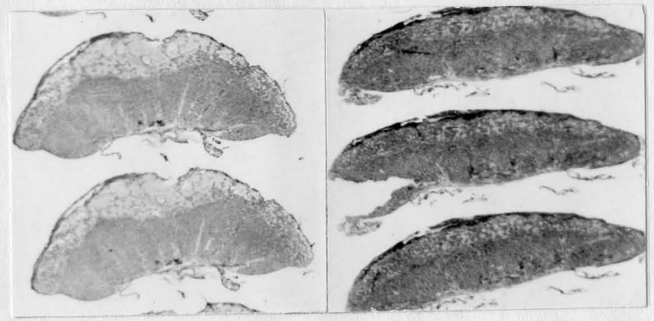

FIGURE 5

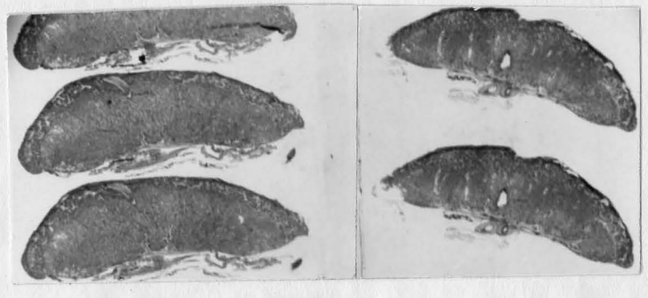

FIGURE 6

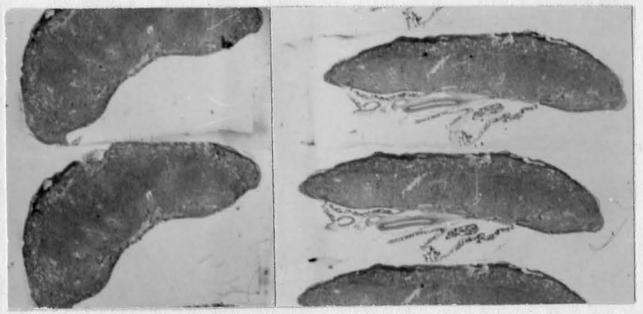

FIGURE 7

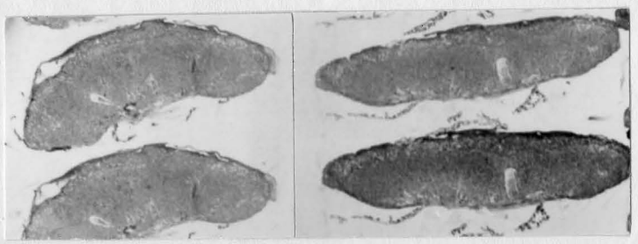

FIGURE 8

FIGURE 9: Placental labyrinth on 18th day of gestation showing thickened placental membranes (shown between arrows) after treatment with 2000mg/Kg of HU on 14th and 17th days of gestation.
Hematoxylin and Eosin
488X

FIGURE 10: Placental labyrinth on 18th day of gestation showing normal placental membranes (shown between arrows) after treatment with boiled distilled water on 14th and 17th days of gestation.
Hematoxylin and Eosin
488X

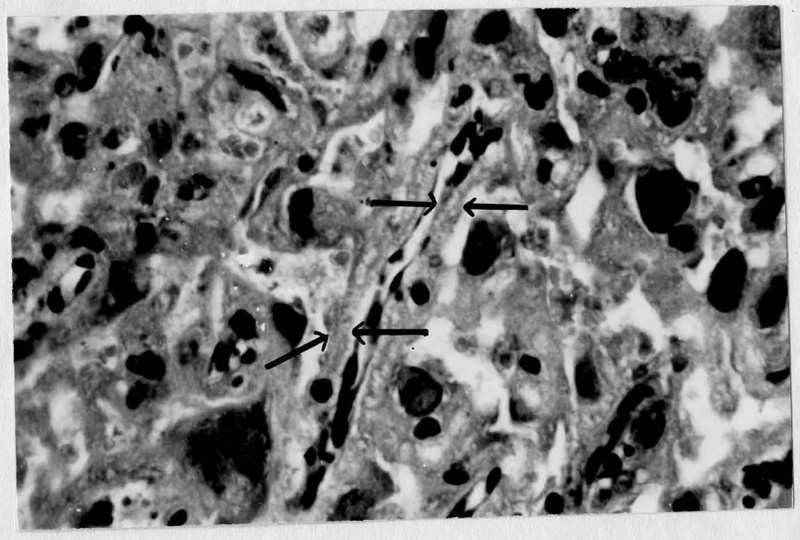

FIGURE 9

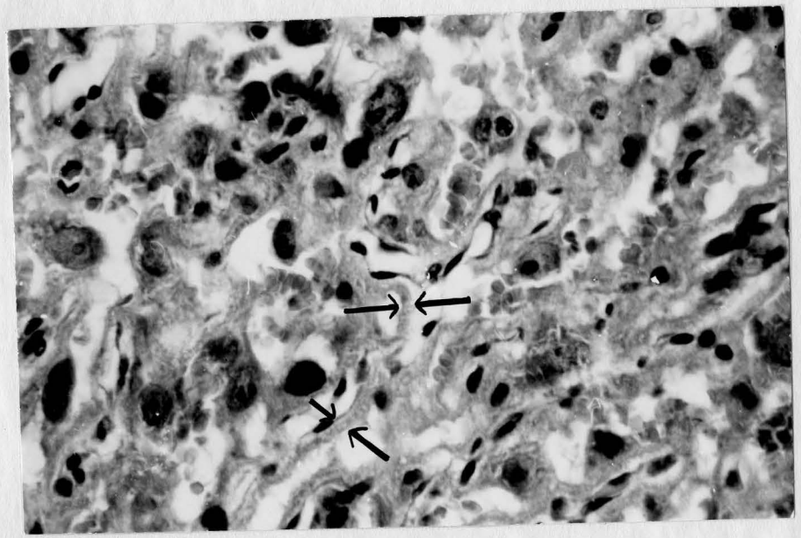

FIGURE 10

FIGURE 11: Placental labyrinth on 20th day of gestation showing thickened placental membranes (shown between arrows) after treatment with 2500mg/Kg of HU on the 16th and 19th days of gestation. Fetuses of the placentae of these rats were dead at maternal necropsy.

Hematoxylin and Eosin

488X

FIGURE 12: Placental labyrinth on 20th day of gestation showing the lack of effect of boiled distilled water (HU vehicle) on the placental membranes (shown between arrows) after treatment on 16th and 19th days of gestation.

Hematoxylin and Eosin

488X

FIGURE 13: Placental labyrinth on 20th day of gestation showing thickened placental membranes (shown between arrows) after treatment with 2500mg/Kg of HU on the 16th and 19th days of gestation. Fetuses of the placentae of these rats were alive at maternal necropsy.

Hematoxylin and Eosin

488X

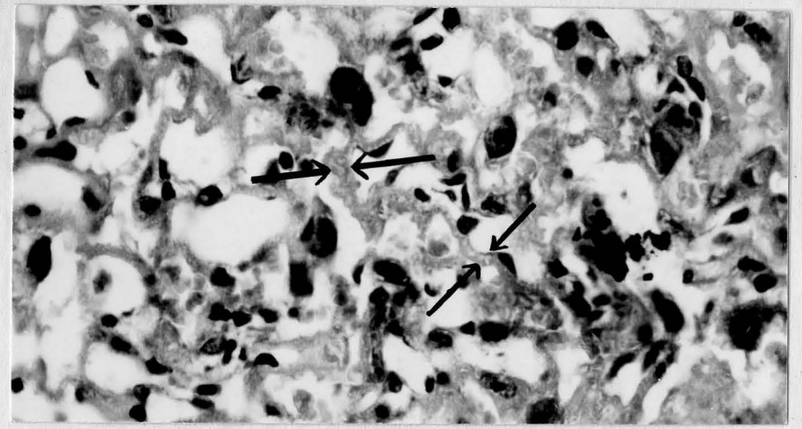

FIGURE 11

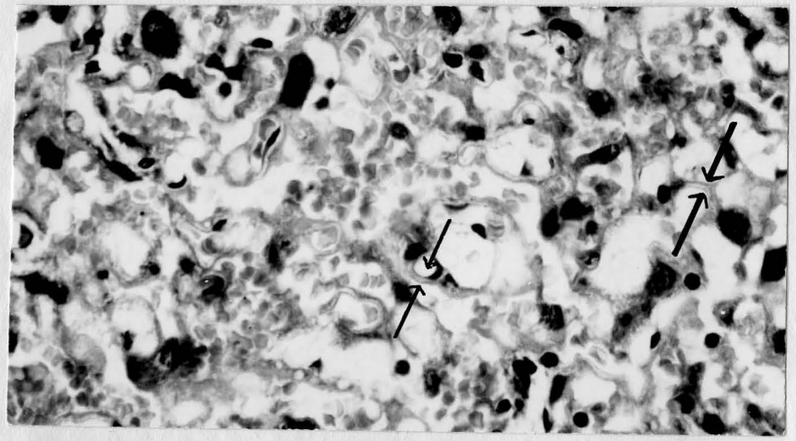

FIGURE 12

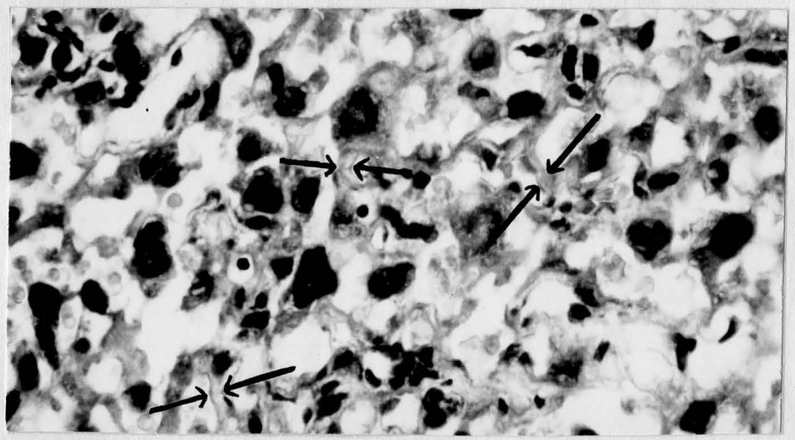

FIGURE 13

FIGURE 14: Mitotic figure in trophoblastic cell of the labyrinth in a control placenta on the 18th day of gestation.
Feulgen
1084X

FIGURE 15: Mitotic figure in an endodermal cell of villous yolk sac from a non-treated control placenta on 18th day of gestation.
Feulgen
1084X

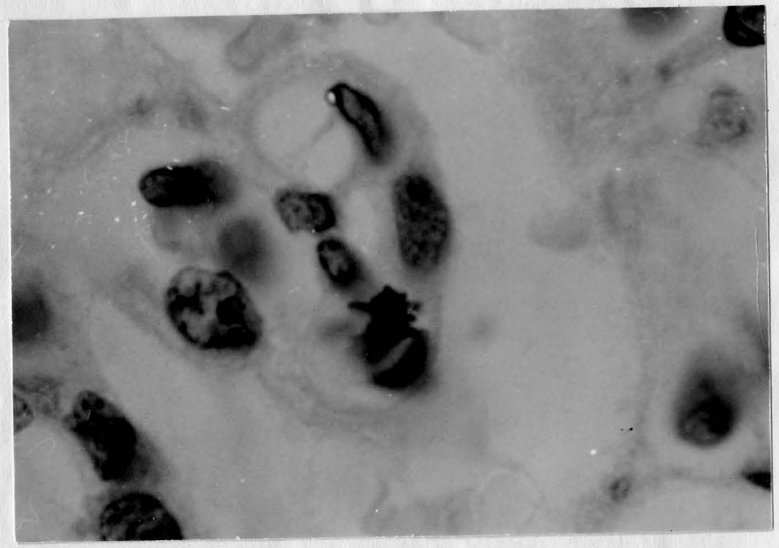

FIGURE 14

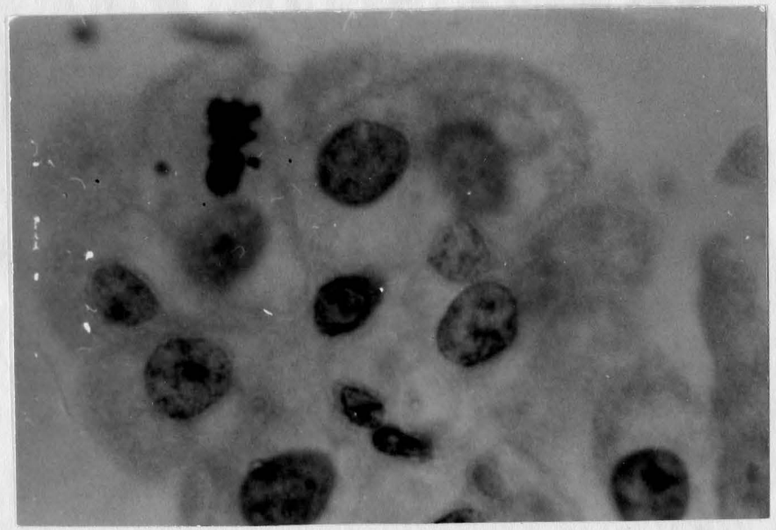

FIGURE 15

FIGURE 16: The villous yolk sac placenta on the 20th day of gestation showing absence of iron in a control animal.
Gomori modification of Perls' method.
488X

FIGURE 17: The villous yolk sac placenta on the 20th day of gestation showing deposits or iron (ID) after treatment with 2500mg/Kg of HU on the 16th and 19th days of gestation.
Gomori modification of Perls' method.
488X

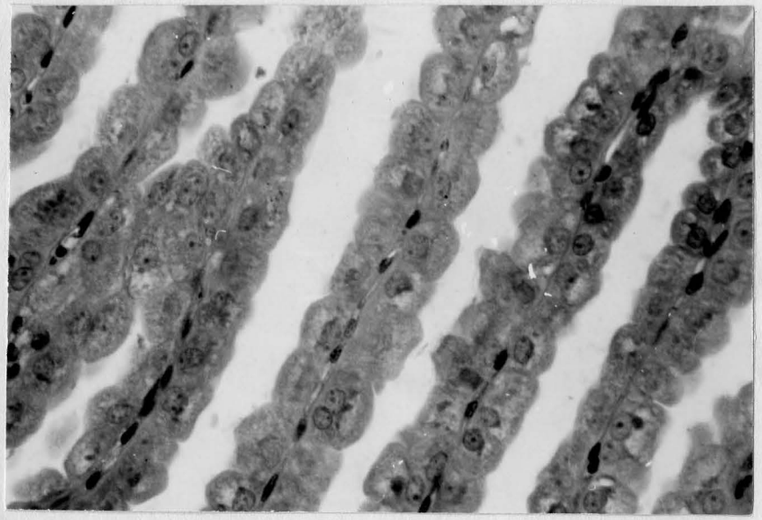

FIGURE 16

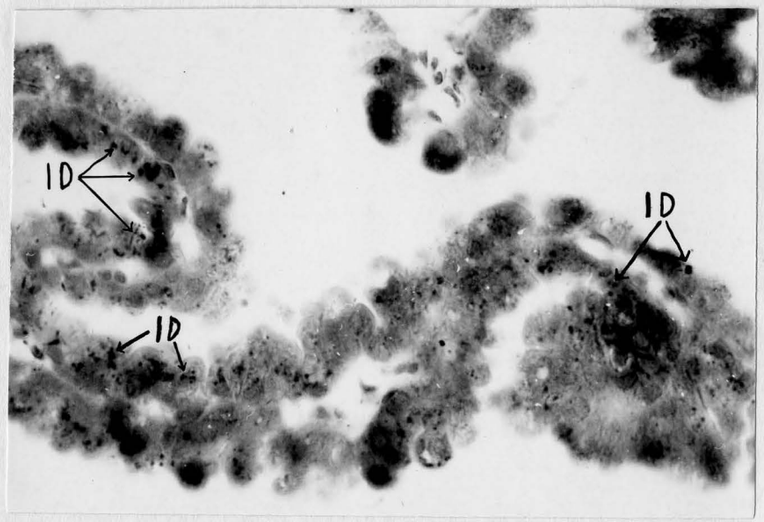

FIGURE 17

FIGURE 18: Glycogen-bearing cells (Gl C) in the spongy zone of the placenta on the 18th day after treatment with boiled distilled water (the vehicle) on the 14th and 17th days.

FIGURE 19: Glycogen-bearing cells (Gl C) in the spongy zone of the placenta on the 18th day after treatment with 2000mg/Kg of HU on the 14th and 17th days.

FIGURE 20: Glycogen-bearing cells (Gl C) in the spongy zone of the placenta on the 21st day after treatment with boiled distilled water (the vehicle) on the 17th and 20th days.

FIGURE 21: Glycogen-bearing cells (Gl C) in the spongy zone of the placenta on the 21st day after treatment with 2000mg/Kg of HU on the 17th and 20th days.

Hematoxylin and Eosin

43X

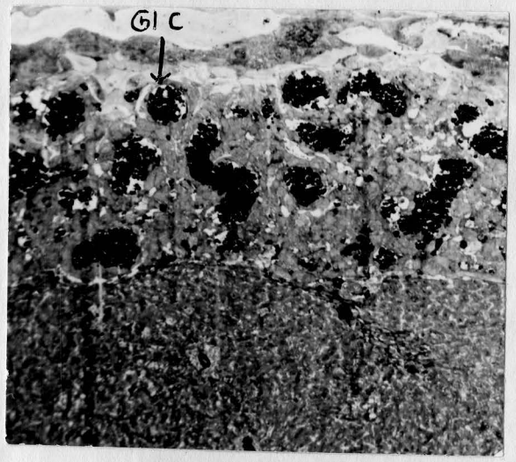

FIGURE 18

FIGURE 19

FIGURE 20

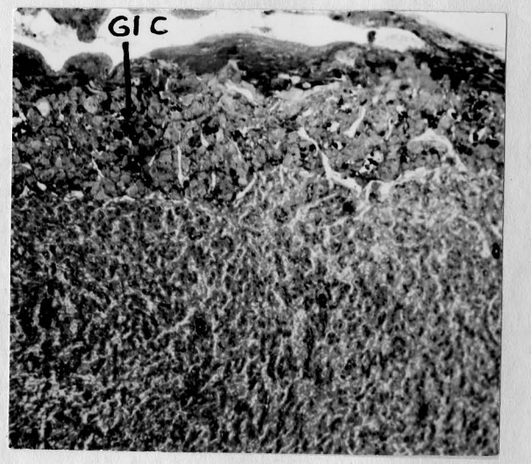

FIGURE 21

FIGURE 22: Stromal calcification (↑) of a placental labyrinth associated with a dead fetus after treatment on the 16th and 19th days of gestation with 2500mg/Kg of HU and necropsied on the 20th day.

GBHA

631X

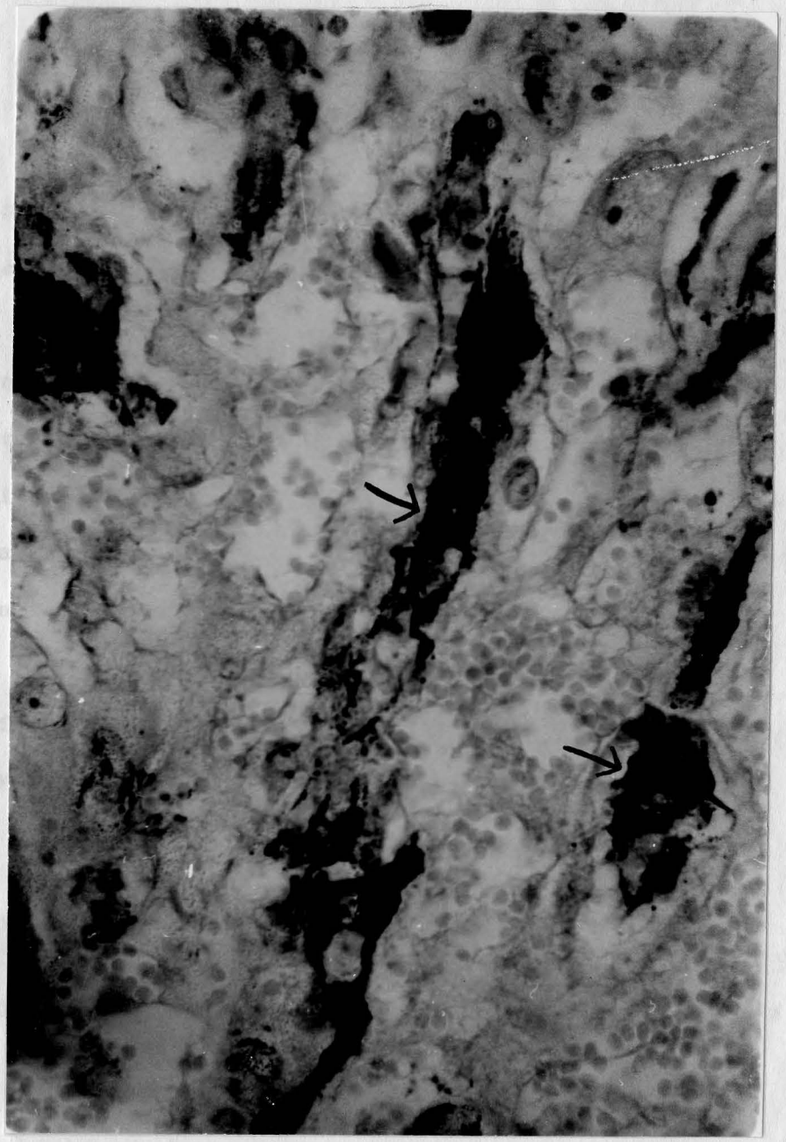

FIGURE 22

APPROVAL SHEET

This thesis submitted by Michael R. Cozza Jr. has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 15 1969
Date

Leslie A Emmert
Signature of Adviser