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Label Uptake in Maternal and Fetal A/jax Mouse Tissues After Maternal Injections of Tritiated Cortisol: A Liquid Scintillation Study

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LABEL UPTAKE IN MATERNAL AND FETAL A/JAX MOUSE TISSUES AFTER MATERNAL INJECTIONS OF TRITIATED CORTISOL: A LIQUID SCINTILLATION STUDY

BY

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AUTOBIOGRAPHY

Gilbert E. Dodds was born in Detroit, Michigan on August 9, 1940. His boyhood years were spent at Perth, Ontario, Canada, where he graduated from the Perth Collegiate Institute in June, 1959.

In September of the same year he enrolled at the University of Toronto in the Faculty of Engineering. A year later he transferred to the Faculty of Dentistry at the same institution where he received the degree of Doctor of Dental Surgery in 1965.

In September, 1969, he entered the Graduate School of Loyola University in Chicago, Illinois.

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CHAPTER 1

INTRODUCTION AND STATEMENT OF THE PROBLEM

When the separation between the oral and nasal cavities formed by the secondary palate is incomplete after the period of normal closure, a condition known as cleft palate results. It is one of the most common congenital malformations found in man, occurring in about one out of every eight hundred live births according to Grace (1943). In untreated cases severe handicaps develop. Speech, mastication, deglutition and respiration are affected and as a result psychologic problems develop.

In 1940, Warkany and Nelson showed that nutritional disturbances were contributing factors to cleft palate production. Fogh-Andersen (1942) demonstrated that genetic factors were also important. Strean and Peer (1956) thought that physiologic, emotional or traumatic stress played a role in human clefts by stimulating the adrenal cortex to secrete hydrocortisone. This correlates with the work of Baxter and Fraser (1950), Fraser and Fainstat (1951), and Kalter (1957) who were able to produce clefts in mice before birth by giving cortisone to pregnant A/jax mice.

Before 1967, most investigators assumed that the cleft palate producing action of cortisone was directly on the palatal shelf tissue and so almost all investigators dealt with changes in the fetal shelves. No one had attempted to determine whether cortisone injected into a

pregnant A/jax mouse actually arrived at the palatal shelves in sufficient quantities to influence their development.

In 1967, Nasjleti, Avery, Spencer and Walden injected tritiated cortisone and using autoradiography were unable to detect label in the palatal tissues of the embryos, although, they did find it in the blood channels of the fetal liver and heart. Marks (1969) and Schmitz (1970), however, were able to show conclusively that label did in fact reach the palatal shelves of the fetus after maternal injections of tritiated cortisol.

The presence of label in the fetal tissues cannot be taken as proof of the presence of cortisol. Biochemical analysis must be done on the labeled material to determine what percentage of it is cortisol, cortisone metabolites, enolization compounds or free tritium.

The purpose of this investigation is as follows:

1. a. To determine quantitatively the relative distribution of the label in various maternal and fetal tissues, such as liver, heart, kidney, spleen, brain, adrenals, maxilla and placenta, one hour post injection.

   b. To determine if there is a significant difference in the uptake of label by the various maternal tissues.

c. To determine if there is a significant difference in the uptake of label by the various fetal tissues.

d. To determine if there is a significant difference between the label in the maternal and fetal tissues, i.e., is there free exchange of label across the placenta in A/jax mice.

2. To determine the effect of a loading dose of cortisol on the degree of labeling recovered from the maternal and fetal tissues.

3. To determine if there is a significant difference in labeling between placentas from the right uterine horn and placentas from the left uterine horn.

4. To establish parameters for future chromatographic studies.

CHAPTER 2

REVIEW OF THE LITERATURE

A. Metabolism of Cortisol (Harper, 1967)

Steroid derivatives have the characteristic cyclopentanoperhydrophenanthrene nucleus. Those found in the adrenal cortex include compound E (cortisone), compound F (hydrocortisone or cortisol), 17 hydroxycorticosterone and aldosterone.

They effect the metabolism of electrolytes, protein, carbohydrates and fat. They influence basic physiologic processes such as cell permeability and the synthesis of RNA and protein.

Cortisol (compound F) is the major free circulating adrenocortical hormone in the plasma.

Renin secreted by the juxtaglomerular cells of the kidney and activated through the intermediary angiotensin stimulates the adrenal cortex and produces aldosterone.

Aldosterone is the principal mineralocorticoid and is important in the regulation of serum electrolytes. It increases the resorption of sodium and chloride by the renal tubules and decreases their excretion by sweat glands, and gastrointestinal tract.

The C-21 corticosteroids, examples of which are 17-hydroxy - 11-dehydrocortisone (compound E or cortisone) and 17-hydroxycorticosterone (compound F or hydrocortisone or cortisol), produce a number of metabolic effects. They elevate blood glucose, decrease carbohydrate oxidation, increase glycogen synthesis, decrease hepatic lipogenesis from carbohydrate, increase gluconeogenesis and increase protein catabolism. They cause involution of the thymus, eosinopenia, lymphopenia, leukocytosis and erythremia. The effects of glucocorticoids are thought to be mediated through RNA synthesis of enzymes.

About one-half of the corticosteroids in the blood are bound loosely to serum proteins. As they pass through the liver the steroids are inactivated and conjugated with glucuronic acid. Both free and conjugated corticosteroid are excreted with the bile into the intestine and may then be reabsorbed by the enterohepatic circulation. Excretion of conjugated corticosteroids takes place in the kidney.

While most steroids in the blood are loosly bound to serum proteins, their conjugates are not and have a greater aqueous solubility. This will account for the rapid excretion of conjugates by the kidney while the unconjugated material remains in the serum.

B. Cortisone Experiments on Fetal and Immature Tissue

Evans (1953) injected cortisone into the chorioallantoic membrane of chick embryos at 8 days of incubation and sacrificed at daily intervals from 10 through 18 days incubation. He found that the entire embryo showed growth inhibition. Peak inhibition was observed between 8 and 10 days.

Buno and Goyena (1955) removed the femurs from chick embryos at 7 days incubation. One they cultivated in a medium containing cortisone and the other, a control, was placed in a cortisone-free medium. They found that the anlages cultivated in the media containing cortisone were inhibited in their growth, but when they were removed and placed in a non-cortisone media they grew rapidly and made up for the retardation. Histologically there was no cellular atrophy or reduction in the intercellular substance of the cartilage. The action appeared to be a direct inhibition of cell proliferation.

Ragan et al (1949) produced wounds on the ears of rabbits. One group was used as a control group while the others were treated with cortisone I.M. The treated animals showed delayed production of granulation tissue, and there was a striking depression of new growth of all the elements of connective tissue.

Layton (1951) found that cortisone inhibited the uptake of labeled sulfate by embryonic chick heart and skeletal muscle in vitro. He also found a similar inhibition in tissue of healing wounds. Previously he had shown that wound granulation tissue had a high capacity for incorporating inorganic sulfate in the formation of chondroitin sulfate. Thus, he concluded that the cortisone inhibited the synthesis of chondroitin sulfate in connective tissue.

Mott (1968) administered cortisone to pregnant mice to induce cleft palates in the offspring. Then one hour before sacrifice he injected tritiated thymidine to label the cells as they prepared to undergo mitosis. His results indicated a decrease in mitosis in mesenchymal tissue of the palatal shelves of the cortisone-treated group and also a decrease in amount of intercellular substance, which he hypothesized was due to a concomitant decrease in the acid mucopolysaccharide content of the intercellular substance.

C. Cortisone-Induced Cleft Palate

Many cleft palate induction studies have been done with cortisol and cortisone. Marks (1969) and Schmitz (1970) reviewed this phase of the literature.

D. Use of Cortisone Isotopes in Pregnant Mice

Hanngren, Hansson, Sjostrand and Ullberg (1964) used ^{14}C-cortisone and ^{14}C-cortisol injected intravenously into mice of an unnamed strain. Each animal was given 0.14 mg. of cortisone (10 uCi) or 0.07 mg. of cortisol (5 uCi) and was sacrificed at intervals varying from 5 minutes to 24 hours after the injection.

Wholebody autoradiography (freeze-dried sections were placed on X-ray film) indicated no significant differences 20 minutes after injection between the two drugs. Label appeared in all the tissues of both ^{14}C-cortisol and ^{14}C-cortisone injected animals. Throughout the observation time, radioactivity was lower in most organs than in the blood, but in maternal liver, kidney and salivary and lacrimal glands the uptake rapidly became greater than in the blood, reaching a peak 20 minutes after injection. Thereafter the highest concentrations were in the maternal liver, kidney and intestinal contents. The central nervous system showed very little uptake. However, after five minutes there was fairly high pituitary radioactivity. The placentas showed more activity than the blood. There was fairly even distribution throughout the fetuses, but the adrenal cortex, gastric mucosa and cerebrospinal fluid showed heavier labeling than the other tissues. The period of gestation was not given.

Chromatograms from maternal liver, intestine and kidney, 20 minutes after injection, indicated that the label was associated with unidentified metabolites, except in the case of urine where cortisone was present also.

According to the author, the penetration through the placenta was relatively poor. The autoradiographic technique used in the work was not sensitive enough to yield meaningful data.

Nasjleti, Avery, Spencer and Walden (1967) used tritium labeled cortisol and A/jax mice at 12½ days gestation. They injected 10 mg. of cortisol with a specific activity of 50 uCi mg. or 500 uCi per mouse intramuscularly. Blood samples were taken from the choroid plexus in heparinized capillary tubes at intervals ranging from 30 minutes to 6½ days after injection. Fluorometric and chromatographic studies were then done on the plasma to determine whether the label was associated with cortisone or recirculating metabolites. At various time after injection the animals were sacrificed, embryos were studied autoradiographically, and the maternal livers, adrenals, spleens, kidneys and thymus glands were counted in a Packard liquid scintillation counter. Samples for scintillation counting were prepared as described by Frenkel, Whalley, Knorpp and Korst (1962). A thixotropic gelling agent was used to suspend the products of rapid hydrolysis of the tissue in the toluene-dioxane-ethanol scintillator. They reported that radioactivity in plasma reached

a peak 40 minutes after injection and chromatography indicated that 72% was associated with cortisone. They also reported that at that time all the tissues investigated showed a concentration of tritium equal to or in excess of that found in plasma at 40 minutes.

Hanngren et al (1964) found a high uptake in the maternal adrenal cortex five minutes after injection (species of mouse not identified), but twenty minutes later the adrenal medulla showed more label and very little label was left in the cortex. Nasjleti et al reported a more delayed uptake in the adrenal (A/jax mice) reaching a peak at two hours in contrast to the more rapid uptake in the other organs. However, they did not differentiate between cortex and medulla. Autoradiographic preparations of the embryos showed label in the blood channels of the liver and heart. According to the author this activity appeared $5\frac{1}{2}$ hours after radioactive injection. The author feels that it may be free tritium or a metabolite of cortisone or tritiated cortisone. He also states without proof or reference, that "the transport and transfer of hormones between mother and fetus involves complex physiologic and biochemical interactions."

In 1969, Marks, working with A/jax mice on the twelfth day of pregnancy and using autoradiographic techniques, was able to

demonstrate a significant amount of label in the fetal palatal shelves as well as the placentas. He, unlike Nasjleti et al, and Hanngren et al administered a "loading dose" of 2.5 mg. of "cold" cortisone acetate 30 minutes before the tritiated cortisol. Both injections were made intramuscularly in the left and right hamstring muscles respectively. He expected the first injection of unlabeled cortisone to overload the metabolic processes responsible for the breakdown and elimination of the drug. Then the labeled cortisol would be allowed to circulate undestroyed for a longer period of time and its chances of being found in the fetal tissues would be enhanced. Marks used the same amount of label as Nasjleti et al (500 uCi) on two mice and only 50 uCi on the remainder, but the dosage of cortisol was much lower. Animals were sacrificed five hours after the label was injected. This was the time reported by Nasjleti at which label appeared in the fetuses, although, both Hanngren and Nasjleti reported peak concentrations in the maternal tissues within 20 to 40 minutes.

Schmitz (1970), studying Marks specimens, reported a direct correlation between the amount of label in the fetal palatal processes and the amount in the placenta. Also he noted "significant differences between placentas and fetal tissues obtained from different mothers." Marks and Schmitz both reported a higher level of activity in placentas and fetuses taken from the left

side of the uterus than in those from the right side. This they attributed to possibly a better circulation on the left side but did not show that such was the case.

In 1968, Levine, Yaffe and Back compared the uptake in fetal tissues of A/jax and CBA strain mice. On the 11th day of gestation ^{14}C-A-ring labeled cortisol together with 2.5 mg. of "cold" carrier was injected subcutaneously. Animals were sacrificed at intervals of 30, 60, 120 and 240 minutes after the injection. Fetuses were individually macerated in Vortis homogenizer and counted in a liquid scintillator using dioxane, liquoflur and toluene as the suspension medium. The authors reported that in each case the A/jax strain showed a much higher fetal uptake than the CBA strain and that the biologic half-life of the labeled cortisol in the A/jax fetuses was about twice what it was in the CBA fetuses. However, the data compared was in percentage of injected dose and the two groups did not receive equal doses so this invalidates the conclusions.

By extrapolation of the graph showing the percent of the injected dose taken up by each of the two strains of fetuses at the various time intervals studied, it would appear that initially the uptakes were similar. It should be noted here though, that extrapolation in this case was not valid. Levine suggested that

this similarity in uptake is due to similarities in the placental transport mechanism of the two strains. This he feels could be "anticipated in view of the rather permissive nature of placental transport which allows the overwhelming majority of drugs with low molecular weights to pass from mother to fetus." He suggested that the difference in susceptibility of the two strains is not due to differences in the placentas but rather to differences in metabolism and/or binding by the fetuses.

Zarrow, Philpott and Denenberg (1970) working with pregnant Purdue-Wistar rats at day 21 of gestation and injecting 10 uCi of ^{14}C-4-corticosterone intravenously, reported similar concentrations of label in maternal and fetal muscle, brain and hypothalmus 30 minutes after injection. They concluded that there is "free exchange across the placenta" but did not do chromatographic tests so have no proof that the corticosterone had not been metabolized. They cited the study done by Hanngren et al as additional proof of this free exchange, but Hannegren et al reported lower levels of activity in the fetuses than in the dams. Zarrow et al prepared their tissues as described in a previous article by Zarrow, Philpott, Dennenberg and O'Connor (1968). A 3:1 mixture of hydroxide of hyamine and 30% aqueous sodium hydroxide was used to dissolve the tissue. The hydrogen peroxide was added for bleaching. 10 ml. of XDC scintillation fluid was used and the samples were counted in a

Packard Tri-Carb scintillation spectrometer. It is interesting to note that this previous experiment with two-day old rats showed a peak uptake in the tissues examined at about 30 minutes post injection. In this case ^{14}C labeled corticosterone was also used and was given intraperitoneally.

E. Preparation of Tissues for Counting

Certain problems are inherent in the use of tritium in liquid scintillation procedures. Tritium being one of the least energetic nuclides is extremely sensitive to all quenching factors in liquid scintillation counting procedures. Solubilization, suspension and emulsification procedures have been routinely utilized but a high phosphorescence and chemiluminescence may produce a high background. In addition, chemical quenching, color quenching and light absorption by particles in the counting (scintillation) solution may increase the variance of the background, thus decreasing the sensitivity of the method. Also, oxygen in the solvent of liquid scintillation preparations exerts a quenching effect and has been described by Pringle et al (1953). Mahin (1966) described procedures for removing the oxygen by freezing the scintillation fluid in liquid nitrogen and capping it when all the gas was expelled.

F. Oxidation Methods for Recovery of Label

By oxidizing the specimen the organic material is converted

into carbon dioxide and water and the amount of activity in the specimen can be counted in a solvent suitable for counting water. Since the water resulting from combustion of dry biological sample is about 60% of the sample weight, it is possible to take the water of combustion of dry tissue representing about 4-5 gm. of fresh tissue and add this to a scintillating solution capable of holding only 4% water. Thus scintillating systems of low water holding capacity can be used, these are the most efficient cocktails. With this technique color quenching is avoided and the only chemical quenching comes from the water which is easily reproducible using blank scintillation fluid so that corrections for it can be made accurately.

Many oxidation methods for preparing tritium samples have been used. Kelly et al (1961) placed dry tritium samples in a platinum basket in an oxygen filled flask and ignited the material by passing an electric current through the platinum basket by means of a transformer and platinum leads. After combustion the flask was placed on dry ice - chloroform - carbon tetrachloride bath so that the water vapor would condense and freeze. To dissolve the ice 20 ml. of toluene-phosphor solution containing 20% ethanol was added to the flask. A 15 ml. aliquote was then used for liquid scintillation counting.

As already stated, oxygen is a severe quenching agent unless removed. Dobbs (1963) reported flushing the liquid scintillation with nitrogen to remove the oxygen that dissolves in it when in the combustion flask. He found that by doing this he could get a more constant and reproducible level of counting efficiency than when the nitrogen purge was omitted. He also showed that the loss of counts due to removal of tritiated water and methanol from the liquid scintillator by the nitrogen stream was negligible.

Peets et al (1960) developed a method for the combustion of large samples. They used a continuous flow of oxygen into the flask during combustion and collected the water in a glass trap immersed in dry ice - chloroform - carbon tetrachloride. A background buildup or "memory effect" was a problem with this system after it had been operating for a time. This was due to retention of reversibly bound tritium by the glass surface of the apparatus. They found that this "memory effect" did not lower the recovery of tritium significantly, but it did result in high blank values and cross contamination of samples. They also found that this "bound" tritium could be removed by purging the tubes with steam.

CHAPTER 3

MATERIALS AND METHODS

A. Mice

A/jax mice were used in this study because they are so susceptible to cortisone-produced clefts and because most studies of cortisone-induced cleft palates have been done on this strain. A total of 30 females and 12 males eight weeks old were obtained from Jackson Laboratory in Bar Harbor, Maine.

B. Feeding and Environment

A special room in the animal quarters was used where the temperature and humidity were kept constant and the lights were automatically regulated to turn off at 6 p.m. and on at 8 a.m. Standard Purina rat chow and tap water were given ad libitum.

C. Mating

The males were isolated, each in a separate cage for five to six days and then one female was placed in each cage with a male. Checks for vaginal copulation plugs were made at 8:30 a.m. and 5 p.m. for the next five days. Females with plugs were isolated in separate cages. The day on which the plug was found was recorded as day zero. The date and weight were recorded on the

cage. After five to ten days those females which had not demonstrated copulations plugs were removed from the cages and the process of isolation of the males followed by mating was again repeated.

On the 12th day following copulation the females were again weighed and those which had gained approximately 2 gm. or more were considered pregnant and ready for injection.

D. Preparation of Stock Solution

Cortisol - 1,2-^{3}H was obtained from Nuclear Chicago in 1 ml. benzene-ethanol (9:1 v/v) suspension, vacuum sealed glass vials. The specific activity was 1 millicurie (mCi) per milliliter (ml.) (46.7 Ci/m mol.). The vials were opened and the contents placed into injection vials which were then sealed with a rubber stopper and refrigerated for storage. Each day as the solution was required, the appropriate volume was withdrawn from the storage vial with a 1 cc. tuberculin syringe and placed in another injection vial. The benzene-ethanol suspension was evaporated using an indirect air stream and sterile normal saline was added to give a concentration of 50 uCi per .05 cc.

E. Injection Procedures

Eight animals were given a loading dose of 0.1 cc. of 2.5%

cortisone acetate S.A.S. (sterile aqueous solution) (total dose 2.5 mg.) intramuscularly in the hamstring muscle of the left leg. This was followed 30 minutes later by an injection of cortisol (hydrocortisone) - 1,2 tritium. Two animals were both given 250 uCi (containing a total dose of .0021 mg.) and the remaining six were given 50 uCi (containing a total dose of .00042 mg.). Five animals were each given 50 uCi of labeled cortisone without a loading dose.

The animals were sacrificed one hour after the injection of the isotope. The carotid artery was severed and blood collected in a .1 cc. heparinized capillary tube. The uteri were removed with the embryonic sacs and the fetuses intact. Maternal livers, spleen, kidney, adrenals, heart, tongue, brain and palatal mucosa were dissected out. Then with the aid of a binocular dissecting microscope, the placentas and fetuses were separated and the fetal liver and heart, jaws, tongues and brain isolated. All the specimens were placed on pieces of preweighed Whatman number 4 filter paper and bench dried for 24 hours before the dry weights were obtained.

F. The Packard Tri-Carb Sample Oxidizer Method

The sample is placed in a platinum combustion basket in a preheated combustion chamber. A constant flow of oxygen is introduced into the chamber and the sample is ignited by heating the

platinum basket with an electric current. The water is trapped by cooling it to 0^{o} C. After combustion the oxygen is displaced from the system by a nitrogen purge and a preselected volume of scintillator is dispensed from a nitrogen purged reservoir into the counting vial. A final nitrogen purge is used to obtain a nitrogen atmosphere in the vial before the cap is secured. The instrument is also fitted with a steam purge to reduce the "memory effect."

G. Counting Technique

Counting was done in a Beckman DPM 100 ambiant temperature liquid scintillation counter. The scintillator recipe was as follows: Napthalene - 100 gm., P.P.O. - 5 gm., Dimethyl P.O.P.O.P. - 0.3 gm., Dioxane - 720 ml., Toluene - 135 ml., and Absolute Methanol - 45 ml. This cocktail will take up to 10% water. 15 ml. aliquots were used and placed in low potassium glass vials. Tritiated toluene was used as a reference standard for determining the counting efficiency. Background was determined by counting blank vials before running the tissues. Efficiency for tritium, following oxidation in the Packard Model 305 Tri-Carb sample oxidizer, was approximately 40% and the background was about 25 counts per minute.

CHAPTER 4

FINDINGS

The animals in the order in which they were sacrificed, the date of sacrifice, weight gain, amount of label and loading dose were all recorded and this data is shown in Table 1.

A. Relative Distribution of Label in Various Maternal and Fetal Tissues

The weights of the various tissues after drying at room temperature for 24 hours are shown followed by the raw c.p.m. (counts per minute) per sample; d.p.m. (decays per minute) per sample; d.p.m. per mg. and microcuries X 10^{-3} per mg. (see Table 2 in Appendix). In each case the entire organ was used for counting except in the case of the livers. The livers were too large to be oxidized in the tri-carb oxidizer and counted in total, so only a portion was used. The weights of liver segments oxidized along with the total dry weights of the livers are recorded. The d.p.m. per sample was determined by subtracting the background from the c.p.m. per sample and then multiplying by the efficiency. The efficiency was determined, as stated in the method, by adding a known number of d.p.m. in the form of tritiated toluene to a blank counting vial and observing the c.p.m.

$$\text{Efficiency} = \frac{\text{c.p.m. Tritiated Toluene X 100\%}}{\text{Known d.p.m. of Tritiated Toluene}}$$

TABLE 1

Mouse # in Order of Sacrifice	Date of Sacrifice	Weight on Day of Copulation	Weight on Day of Sacrifice in gm.	Net Gain in gm.	Loading Dose of Cortisone 1 hr. before Label in mg.	Labeling Dose $\frac{1}{2}$ hr. before Sacrifice in uCi
1	Dec. 3	21.2	23.0	1.8	2.5	250
2	Dec. 3	19.5	21.0	1.5	2.5	250
3	Dec. 6	20.5	24.6	4.1	2.5	50
4	Dec. 6	20.6	25.5	4.9	2.5	50
5	Jan. 30	19.9	22.6	2.7	-	50
6	Jan. 30	21.8	25.0	3.2	-	50
7	Jan. 30	21.6	25.0	3.4	-	50
8	Feb. 2	20.5	23.3	2.8	-	50
9	Feb. 7	20.0	23.8	3.8	-	50
10	Feb. 15	21.5	23.7	2.2	2.5	50
11	Feb. 15	22.7	24.1	1.4	2.5	50
12	Feb. 17	21.3	25.6	4.3	2.5	50
13	Feb. 17	21.7	24.4	2.7	2.5	50

The Placentas were numbered beginning at the right terminal of the uterus and proceeding around to the left terminal. Unless otherwise indicated in the table the blood volume was 0.1 ml.

The first two animals (one of which was not pregnant) were given a loading dose of 2.5 mg. of cortisone, followed one-half hour later by 250 uCi (microcuries) of label. Results indicated that this was more label than needed to obtain significant counts in both maternal and fetal tissues. Thereafter, it was decided to use only 50 uCi label per animal and these two animals were not included in the statistical analysis.

The mean uptake values in each of the tissues in uCi X 10^{-3} per mg. followed by the standard deviation for each group was figured and these results are shown in Table 3(a & b).

Analysis of variance (Table 4a) indicates that within both loading and nonloading groups, the uptake in the maternal livers was significantly higher than in all of the other tissues except the kidney. There was no significant difference between the liver and kidney uptakes within either of the groups (as determined by studentized range test). Within the two groups there was no significant difference in the uptake in the different fetal tissues. However, there was a significant difference between animals within each group both in the fetal and maternal uptakes.

TABLE 3(a)

50 uCi TRITIATED CORTISOL

NO LOADING DOSE

Tissue	Mean $(\frac{uCi \times 10^{-3}}{mg})$	Standard Deviation
	Maternal	
Tongue	1.414	1.288
Spleen	1.240	1.195
Palatal Mucosa	1.752	1.112
Liver	14.398	12.873
Brain	0.277	0.160
Kidney	8.988	9.070
Heart	2.143	2.058
Adrenal	2.019	2.405
Blood	3.915	3.775
	Placenta	
Right	4.119	2.964
Left	3.860	2.926
	Fetal	
Tongue	2.091	1.572
Jaws	1.673	1.379
Brain	1.449	1.439
Liver & Heart	2.281	1.881
Right Remainder	1.820	1.111
Left Remainder	1.751	1.530

TABLE 3(b)

50 uCi TRITIATED CORTISOL

+ A LOADING DOSE OF 2.5 mg "COLD" CORTISOL

Tissue	Mean $(\frac{uCi \times 10^{-3}}{mg})$	Standard Deviation
	Maternal	
Tongue	0.367	0.148
Spleen	0.323	0.140
Palatal Mucosa	0.351	0.143
Liver	3.568	1.452
Brain	0.059	0.024
Kidney	1.646	0.730
Heart	0.463	0.225
Adrenal	0.702	0.516
Blood	0.936	0.461
	Placenta	
Right	1.251	0.602
Left	1.242	0.646
	Fetal	
Tongue	0.486	0.371
Jaws	0.540	0.283
Brain	0.468	0.218
Liver & Heart	0.822	0.495
Right Remainder	0.678	0.369
Left Remainder	0.548	0.356

TABLE 4(a)

MATERNAL ANALYSIS OF VARIANCE

Source of Variance	DF	MS	F
Maternal Load vs. Non-Load	1	227.176	826.457*
Maternal Non-Load			
Tissues	8	109.866	5.77 *
Animals	4	124.502	6.548*
Interaction (animals X tissues)	32	19.0135	
Maternal Load			
Tissues	8	7.142	29.116*
Animals	5	1.269	5.173*
Interaction (animals X tissues)	40	0.2453	
Total	98		

* Significant at the $P < 0.01$ level.

TABLE 4(b)

FETAL ANALYSIS OF VARIANCE

Source of Variance	DF	MS	F
Fetal Load vs. Non-Load	1	26.68	371.190*
Fetal Non-Load			
Tissues	5	0.445	3.311
Animals	4	12.885	96.58 *
Interaction (animals X tissues)	20	0.1344	
Fetal Load			
Tissues	5	0.166	7.545*
Animals	5	0.663	30.136*
Interaction (animals X tissues)	25	0.022	
Total	65		

* Significant at the $P < 0.01$ level.

TABLE 4(c)

PLACENTAL ANALYSIS OF VARIANCE

Source of Variance	DF	MS	F
	Load		
Right placenta vs. left placenta	1	0.0005	0.0025
Animal rights	5	1.3591	14.23*
Animal lefts	5	1.3147	13.23*
Within rights	19	0.0999	
Within lefts	14	0.0961	
	Non-Load		
Right placenta vs. left placenta	1	0.6418	0.6418
Animal rights	4	26.1432	24.49*
Animal lefts	4	54.6165	45.80*
Within rights	9	1.0671	
Within lefts	25	1.1924	
Total	88		

* Significant at the $P < 0.01$ level.

FIGURE 1

MEAN MATERNAL UPTAKE AFTER
50 uCi TRITIATED CORTISOL INJECTION

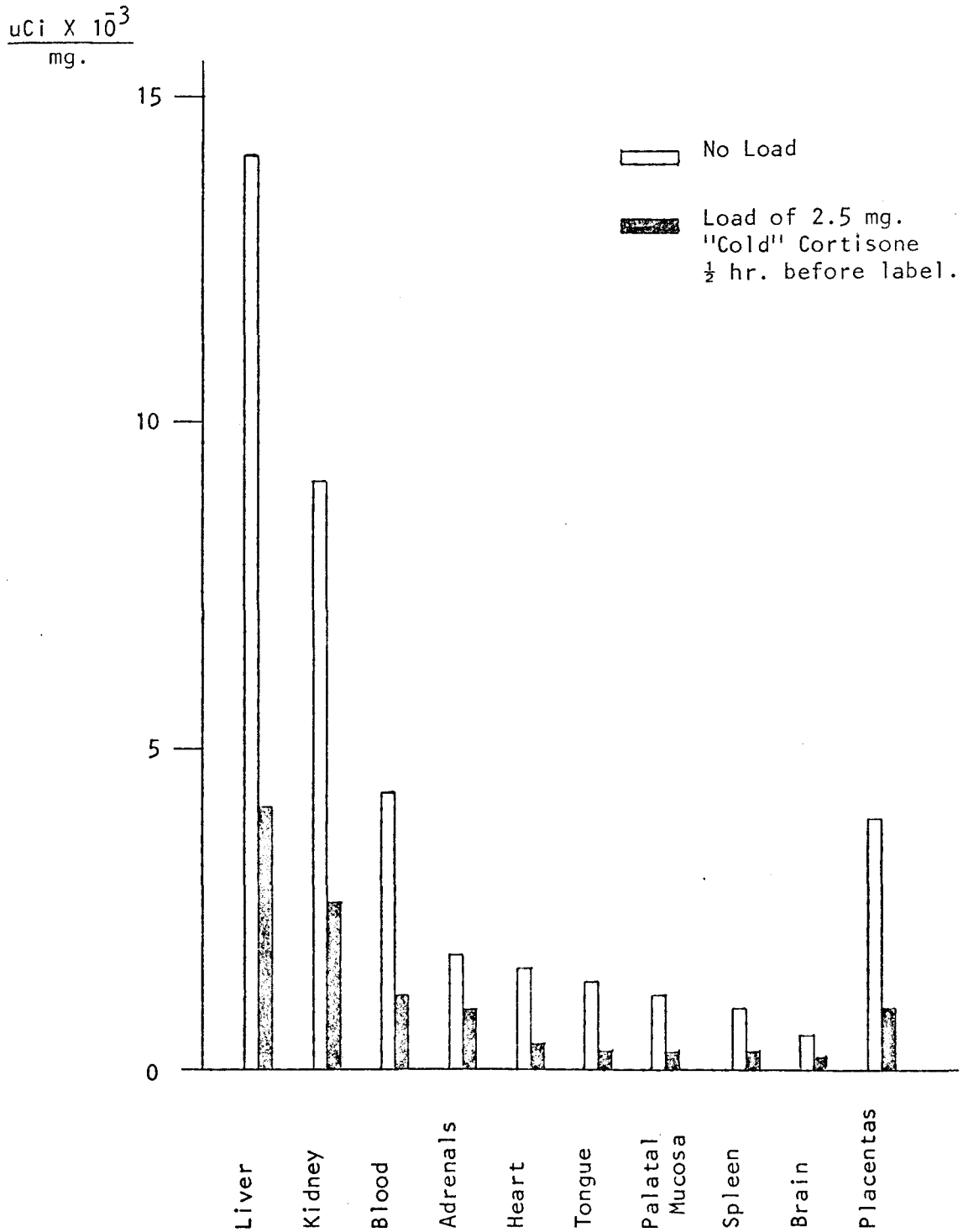
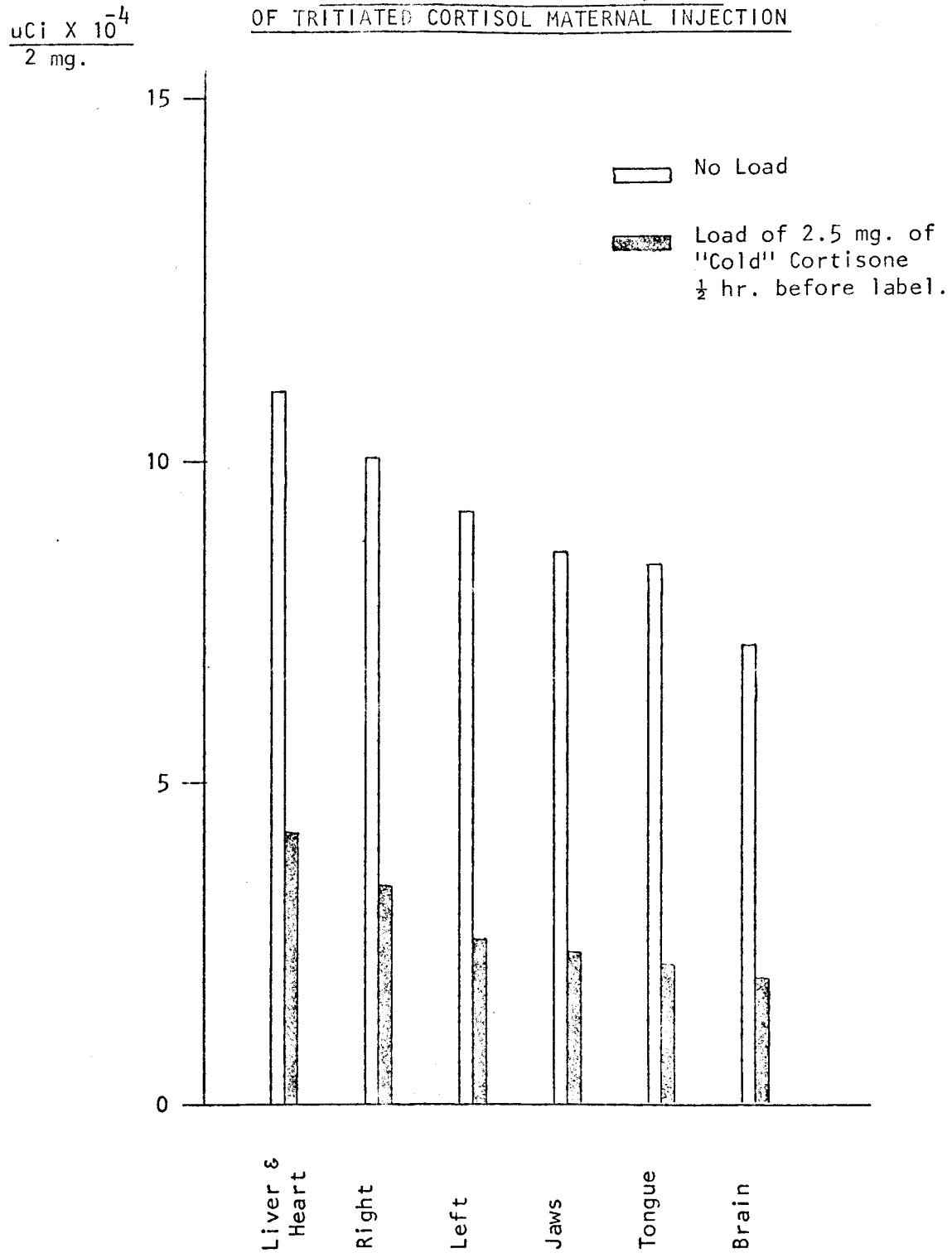


FIGURE 2

MEAN FETAL UPTAKE AFTER 50 uCi
OF TRITIATED CORTISOL MATERNAL INJECTION



In both groups there was a significantly higher uptake in the maternal tissues than in the fetal tissues (determined by student "T" test P .01). This would suggest that there is not free interchange between the mother and fetuses but that some type of placental barrier exists. Since the ratio of fetal uptake to maternal uptake in each group (loading and nonloading) was about the same, it would appear that the loading dose did not affect the placental barrier.

In both the maternal and the fetal tissues there was a statistical significant difference between the uptake in the two groups. The uptake was much higher in the nonloading group than in the loading group (Table 4a & b).

The mean uptakes in the placentas from the right and left side of the uterus as shown in Table 3(a & b) indicate that there was no significant difference between right and left sides in either of the two groups (Table 4c).

CHAPTER 5

DISCUSSION

A. The Tri-Carb Oxidizer and Liquid Scintillation Counter

The Tri-Carb oxidizer used in conjunction with the liquid scintillation counter provides an easy and accurate means of measuring the amount of tritium activity in very minute amounts of biological material. It reduces the possibility of error which is common to other techniques and in addition is much faster. Unlike autoradiography it can be used for accurate quantitative studies. Marks and Schmitz, using the same amount of activity as was used in the present study, had difficulty finding significant counts in some of their specimens, while we had no such problem. Admittedly, the failure of finding highly significant label by Marks and Schmitz may have been due to waiting too long after injection of label before sacrificing.

B. Drying and Weighing

Drying and weighing procedures were not nearly as accurate as the counting. Tissues were bench dried at room temperature and humidity. The humidity may have fluctuated considerably from one day to another. Larger specimens undoubtedly were not as dry as smaller ones at the time of weighing. The filter paper used to hold

the tissue weighed between 110 and 120 mg. while a few of the fetal tissue samples weighed less than 1 mg. Thus the moisture content of the filter paper, which was a function of the relative humidity, could be responsible for a relatively large error in the weight of the specimen. Later on in the study the size of the filter paper used with the fetal tissues and placentas was reduced to between 30 and 40 mg. Also, it was found that the balance used for weighing was only accurate to 0.5 mg., although it was calibrated in tenths of a mg.

C. Preparation of Injection Solution from Stock Solution and Measurement of Dose

As stated earlier, a tuberculin syringe was used to withdraw the ethanol-benzene suspension of tritiated cortisol from the stock solution and to place it in an injection vial as required each day. The ethanol-benzene suspension was then evaporated off and normal saline added to produce a concentration of 50 uCi per 0.05 cc. 0.05 cc. of this solution was then injected into each animal. The tuberculin syringe had a 1 cc. capacity at full scale and thus was not very accurate for measuring 0.05 cc. Early in the study plastic disposable syringes were used but these were replaced in favor of glass ones which were not as susceptible to surface absorption and thus more reliable.

Considerable error probably resulted from evaporating the ethanol-benzene suspension leaving dry cortisol powder under a stream of air. Some of the powder may have been lost at this time. A more accurate method would be to prepare the injection solution at one time. Then a small aliquot could be counted and the specific activity of the injection solution could be determined. This was not done because it was not known whether the tritiated cortisol would be stable in the saline solution. It would also be better to use the same syringe for all the injections as this would eliminate the error due to differences in syringes, i.e., surface absorption.

D. Significance of Different Uptakes in Different Animals and Different Tissues

The different uptakes between animals within the same group were probably due to differences in the physiology and differences in the dosage. The reasons for inaccuracies in the dosage have been discussed earlier. Because of the apparent inaccuracies in the dosages we were unable to show the percent of the dose taken up by the various tissues. The high concentration of label in the maternal liver and kidney probably represents the detoxification and excretion of cortisone by these organs.

E. Placental Barrier

The greater uptake in maternal tissues than in fetal tissues would tend to support the concept of a partial placental barrier to cortisone, and is in agreement with the findings of Hanngren et al, and Nasjleti et al. However, the difference may have been due to differences in the metabolism of the maternal and fetal tissues rather than to a placental barrier; but this is unlikely because there was no significant difference in label uptake among the fetal tissues. It is interesting to note that the loading dose did not affect the ratio of fetal uptake to maternal uptake. The extremely poor uptake by the maternal brain is probably due to the brain blood barrier. Apparently, though, there is still free interchange between the fetal blood and brain at this stage of fetal development.

F. Effects of Loading Dose

The loading dose as used by Marks and Schmitz did not enhance the uptake of label but rather lessened it. They hypothesized that "cold" cortisone given one-half hour before the "hot" cortisol would overload the metabolic processes responsible for its breakdown and thus the labeled material would remain unmetabolized for a longer time and would accumulate in the tissues. The results of this study indicate that the uptake in the animals which were not

given a loading dose is about three times what it is when a loading dose is used.

G. Right vs. Left

There is equal uptake in right and left sides of the placenta. This again is contrary to the finding of Marks and Schmitz, but their findings were obtained from autoradiographic studies and it is difficult to obtain reliable quantitative studies in an autoradiographic study where many specimens are used.

H. Feasibility of Chromatographic Studies

The average weight of the fetal jaws from one mouse was about 7 mg., and the mean uptake in the fetal jaws was about 1.673×10^{-3} uCi/mg. or $11.711 \text{ uCi} \times 10^{-3}$ per mouse fetal jaw tissue. The specific activity of the labeled cortisol was 46.7 Ci per m. mol., or 1 uCi per $\frac{10^{-6}}{46.7}$ m mol. This means that the fetal jaw tissue from one pregnant mouse would contain approximately 0.25×10^{-9} m. mol. of cortisone, assuming that all of the label represents cortisone. This figure can be used as a guide in future chromatographic studies. It is obvious that a very sensitive technique will have to be used. It may be necessary to use all the fetal tissue from one mouse in order to have sufficient cortisone for chromatographic analysis. It must be remembered

that in our present study we have detected label only and complete chromatographic analysis of maternal and fetal tissues will be necessary to demonstrate how much of the label is still cortisone. Our study does show that sufficient label is present and that these chromatographic studies would seem feasible.

1. Time - Uptake Study

Judging from the results of this study as well as from the findings of previous investigators it appears that one hour post injection is a better time for sacrifice than five hours as Marks and Schmitz did. However, a time - uptake study is needed to determine the time that a significant level of cortisone is present in the various tissues.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Pregnant mice were given tritiated cortisol on the 12th day of gestation, and sacrificed one hour later. The maternal tongue, spleen, palatal mucosa, liver, brain, kidney, heart, adrenals and blood; and the fetal tongue, jaws, brain and placentas were oxidized in a Packard Tri-Carb oxidizer and counted in a liquid scintillation counter.

Pertinent findings were:

a. No significant difference in the uptake in the various maternal tissues except for the liver and no significant difference in the uptake in the various fetal tissue.

b. A significantly higher uptake in maternal than in fetal tissues, suggesting the presence of a placental barrier.

c. The loading dose as used by Marks and Schmitz hinders rather than improves the uptake of label injected later.

d. There is no significant difference in uptake in placentas on the right and left sides of the uterus in contrast to that described by Marks and Schmitz.

The Packard Tri-Carb oxidizer used in conjunction with a liquid scintillation counter is an easy, accurate and efficient way of measuring uptake of tritium label in biologic specimens.

More accurate techniques for preparing the labeled material for injection and measuring the dose are needed. This would reduce the amount of variance in the study and possibly would show significant differences in the uptakes in various tissues which could not be shown at this time.

CHAPTER 7

BIBLIOGRAPHY

Baxter, H. and Fraser, P.: "The Production of Congenital Defects in the Offspring of Female Mice Treated with Cortisone," McGill Med. J. 19: 245-249, 1950.

Buno, W. and Goyena, H.: "Effect of Cortisone upon Growth in vitro of Femur of the Chick Embryo," Proc. Soc. Exp. Biol. Med., 89: 622-624, 1955.

Dobbs, H. E.: "Oxygen Flask Method for the Assay of Tritium-Carbon -14, and Sulfur -35 Labeled Compounds," Anal. Chem. 35: 783-786, 1963.

Evans, H. J.: "Action of Cortisone on Developing Chick Embryo," Proc. Soc. Exp. Biol. Med. 83: 31-34, 1953.

Fogh-Andersen, P.: "Inheritance of Harelip and Cleft Palate," Nyt Nordisk Forlag - Arnold Busck Copenhagen, 1942, (Cited from Nanda).

Fraser, F. and Fainstat, T.: "Production of Congenital Defects in Offspring of Pregnant Mice with Cortisone and Other Hormones," Pediatrics 8: 527-533, 1951.

Frenkel, E., Whalley, B., Knorpp, C., and Korst, D.: "On the Counting of Tritiated Thymidine in Tissues," J. Lab. Clin. Med. 59: 174-178, 1962.

Grace, L. G.: "Frequency of Occurrence of Cleft Palates and Harelips," Dent. Res. 22: 495-497, 1943.

Hanngren, A., Hansson, E., Sjostrand, S., and Ullberg, S.: "Autoradiographic Distribution Studies with ^{14}C-Cortisone and ^{14}C-Cortisol," Acta. Endocr. 47: 95-104, 1964.

Harper H. A.: "Review of Physiological Chemistry," Los Altos, California, 1967.

Kalter, H.: "Factors Influencing the Frequency of Cortisone Induced Cleft Palate in Mice," J. Exp. Zool. 134: 449-467, 1957.

Kelly, R. G., Peets, E. A., Gordon, S., and Buyske, D. A.: "Determination of C^{14} and H^3 in Biological Samples by Schöniger Combustion and Liquid Scintillation Techniques," Anal. Biochem. 2: 267-273, 1961.

Layton, L. L.: "Effect of Cortisone upon Chondroitin Sulfate Synthesis by Animal Tissues," Proc. Soc. Exp. Biol. Med. 76: 596-598, 1951.

Levine, A., Yaffe, S., and Back, N.: "Maternal-Fetal Distribution of Radioactive Cortisol and its Correlation with Teratogenic Effect," Proc. Soc. Exp. Biol. Med. 129: 86-88, 1968.

Mahin, D. T.: "A New Way to Reduce Oxygen Quenching in Liquid Scintillation Samples," Int. J. Appl. Radiat. 17: 185-191, 1966.

Marks, A. D.: "A Preliminary Study to Determine the Feasibility of the Use of Tritiated Cortisol for Autoradiographic Examination of A/jax Mouse Fetuses," M.S. Thesis, Loyola University, Chicago, Illinois, 1969.

Mott, W. J.: "Labeling Index and Cellular Density in Palatine Shelves of Cleft Palate Mice," M.S. Thesis, Loyola University, Chicago, Illinois, 1968.

Nanda, R.: "The Normal Palate and Induced Cleft Palate in Rat Embryos," M.D. Thesis, University of Nijmegen, The Netherlands, 1969.

Nasjleti, C. E., Avery, J. K., Spencer, H. H., and Walden, J. M.: "Tritiated Cortisone Distribution and Induced Cleft Palate in Mice," J. Oral Ther. 4: 71-82, 1967.

Peets, E. A., Florini, J. R., and Buyske, D. A.: "Tritium Radioactivity Determination of Biological Materials by a Rapid Dry Combustion Technique," Anal. Chem. 32: 1465-1468, 1960.

Pringle, R. W., Black, L. D., Funt, B. L., and Sobering: "A New Quenching Effect in Liquid Scintillators," Physical Review 92: 1582-1583, 1953.

Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., and Blunt, J. W.: "Effect of Cortisone on Production of Granulation Tissue in the Rabbit," Proc. Soc. Exp. Biol. Med. 72: 718-721, 1949.

Schmitz, D. D.: "An Autoradiographic Study to Determine Whether Label is Present in A/jax Fetal Palates, Maxillas and Placentas, after Maternal Tritiated Cortisone Injections," M.S. Thesis, Loyola University, Chicago, Illinois, 1970.

Strean, L. P. and Peer, L. A.: "Stress as an Etiologic Factor in the Development of Cleft Palate," Plast. and Reconstr. Surg. 18: 1-8, 1956.

Warkany, J. and Nelson, R. C.: "Appearance of Skeletal Abnormalities in the Offspring of Rats Reared on a Deficient Diet," Science 92: 383-384, 1940.

Zarrow, M., Philpott, J. and Denenberg, V.: "Passage of ^{14}C-4-Corticosterone from the Rat Mother to the Fetus and Neonate," Nature 226: 1058-1059, 1970.

Zarrow, M., Philpott, J., Denenberg, V., and O'Connor, W.: "Localization of ^{14}C-4-Corticosterone in the 2-Day Old Rat and a Consideration of the Mechanism Involved in Early Handling," Nature 218: 1264-1265, 1968.

CHAPTER 8

APPENDIX

TABLE 2(a)

MOUSE #1

EFFICIENCY - 38%, BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 250 uc*

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X $\bar{10}$/mg
Tongue	18.9	50,193	132,021	6,985	3.15
Spleen	26.5	57,014	149,971	5,659	2.55
Palate	0.4	5,501	14,411	36,026	16.23
Liver	140.5	2,877,000	7,570,986	53,886	24.27
Total Liver	**	-	-	-	-
Brain	86.5	34,312	90,229	1,043	.470
Kidney	76.0	750,308	1,974,429	25,979	11.70
Heart	20.9	66,232	174,229	8,336	3.76
Adrenals	3.6	21,387	56,216	15,615	7.03
Blood	18.5	114,771	301,963	16,322	7.35 X

Placenta

1 ***	2.8	45,129	118,695	42,391	19.09
2 ***	3.9	59,354	156,128	40,033	18.03
3 ***	4.1	52,520	138,145	33,694	15.18
4 ***	2.5	34,146	89,792	35,917	16.18
5 ***	4.0	53,323	140,257	35,064	15.79
6 ***	1.3	20,642	54,255	41,735	18.80
7 ***	5.0	49,378	129,876	25,975	11.70
8	2.9	31,570	83,013	28,625	12.89

Fetal

Tongue	1.0	2,464	6,418	6,418	2.89 +
Jaws	7.4	6,230	16,329	2,206	.994 +
Brain	6.9	5,737	15,032	2,178	.981 +
Liver & Heart ****	-	-	-	-	-
R. Remainder	5.3	32,546	85,581	16,147	7.27
L. Remainder	1.0	8,740	22,934	22,934	10.33

* Microcuries
** Total Weight Not Obtained
*** Intra-uterine Order Unknown
**** These tissues were not obtained as separate specimens
X Blood Volume Unknown
\+ Approximate Weight

TABLE 2(b)

MOUSE #2

EFFICIENCY - 38%, BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 250 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	28.9	21,695	57,026	1,973	.889
Spleen	10.0	7,820	20,513	2,051	.924
Palate	1.5	2,420	6,303	4,202	1.89
Liver	166.5	1,389,000	3,655,197	21,953	9.89
Total Liver	**	-	-	-	-
Brain	88.0	11,437	30,032	341	.154
Kidney	90.0	209,785	552,000	6,133	2.762
Heart	23.5	22,633	59,495	2,532	1.14
Adrenals	4.5	6,892	18,071	4,016	1.81
Blood	34.2	50,599	133,089	3,892	1.75 X

** Total Weight Not Obtained
X Blood Volume Unknown

TABLE 2(c)

MOUSE #3

EFFICIENCY - 40.09%, BACKGROUND - 30 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	22.2	4,549	11,272	508	.229
Spleen	32.2	5,204	12,905	400	.180
Palate	2.8	539	1,269	453	.204
Liver	71.5	2,632,500	6,566,400	91,838	41.3
Total Liver	382.7	-	-	-	-
Brain	58.1	1,640	4,016	691	.031
Kidney	89.7	82,195	204,951	2,284	1.03
Heart	28.1	6,132	15,220	542	.244
Adrenal	7.2	3,860	9,554	1,326	.598
Blood	21.1	11,273	28,044	1,329	.599 X

Placenta

1	9.7	9,829	24,443	2,520	1.135
2	8.1	6,429	15,962	1,971	.888
3	15.0	16,759	41,729	2,782	1.253
4	8.7	6,506	16,154	1,857	.836
5	17.2	20,837	51,901	2,966	1.336
6	5.1	2,779	6,857	1,345	.606
7	4.3	3,726	9,219	2,144	.966
8	5.7	3,144	7,768	1,363	.614
9 *	4.2	3,430	8,481	2,019	.910
10	3.0	2,911	7,186	2,395	1.079

Fetal

Tongue	1.7	857	2,063	1,213	.547
Jaws	8.9	3,235	7,995	898	.405
Brain	8.4	2,742	6,765	805	.363
Liver & Heart **	-	-	-	-	-
R. Remainder	21.1	6,098	52,891	2,507	1.129
L. Remainder	12.3	4,122	10,207	830	.374

X Blood Volume 0.1 ml.

* Nonviable fetus

** These tissues were not obtained as separate specimens

TABLE 2(d)

MOUSE #4

EFFICIENCY - 40.09, BACKGROUND - 30 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	29.1	12,673	31,536	1,084	.488
Spleen	31.3	13,036	32,442	1,036	.467
Palate	2.4	1,170	2,844	1,185	.534
Liver	33.5	146,550	365,545	10,911	4.915
Total Liver	472.0	-	-	-	-
Brain	124.4	8,695	21,613	174	.078
Kidney	96.1	203,327	507,102	5,277	2.38
Heart	29.4	17,262	42,983	1,462	.659
Adrenal	6.3	6,026	14,956	2,374	1.07
Blood	22.7	33,316	83,028	3,658	1.65 X

Placenta

1	3.8	5,632	13,974	3,677	1.656
2	5.8	9,452	23,502	4,052	1.83
3	7.4	11,764	29,269	3,955	1.78
4	11.2	18,624	46,380	4,141	1.87
5	6.0	9,631	23,949	3,991	1.80
6	6.9	8,464	21,037	3,049	1.37
7	9.3	21,953	54,684	5,880	2.64

Fetal

Tongue	1.0	1,076	2,609	2,609	1.175
Jaws	7.4	6,251	15,518	2,097	.945
Brain	6.9	4,662	11,554	1,675	.754
Liver & Heart	1.3	1,478	3,612	2,778	1.252
R. Remainder	8.2	7,525	18,695	2,280	1.027
L. Remainder	14.0	14,503	36,101	2,579	1.162

X Blood Volume 0.1 ml

TABLE 2(e)

MOUSE #5

EFFICIENCY - 39.62, BACKGROUND - 35 COUNTS PER MINUTE

NO LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	35.3	103,054	260,018	7,366	3.32
Spleen	31.5	84,957	214,341	6,804	3.07
Palate	1.8	5,494	13,778	7,655	3.45
Liver	35.8	975,860	2,462,960	68,798	30.99
Total Liver	340.0	-	-	-	-
Brain	90.8	33,336	84,051	926	.417
Kidney	84.4	1,716,600	4,332,651	51,334	23.124
Heart	29.0	127,962	322,885	11,134	5.02
Adrenal	4.0	22,060	55,591	13,898	6.26
Blood	15.5	128,877	325,194	20,980	9.45 X

Placenta

1	5.1	29,309	73,887	14,488	6.53
2	2.1	14,904	37,530	17,871	8.05
3	2.6	13,357	33,624	12,932	5.83
4	6.4	48,431	122,150	19,086	8.60
5	7.8	69,972	176,519	22,630	10.19
6	5.2	44,432	112,057	21,549	9.70
7	4.7	25,923	65,340	13,902	6.26
8	3.3	19,289	48,596	14,726	6.63
9	4.1	29,620	74,672	18,212	8.20

Fetal

Tongue	1.3	4,923	12,337	9,490	4.27
Jaws	3.7	11,902	29,952	8,095	3.64
Brain	4.0	13,146	33,091	8,273	3.73
Liver	1.8	7,854	19,735	10,964	4.94
R. Remainder	9.9	31,384	79,124	7,992	3.60
L. Remainder	8.2	28,334	71,426	8,710	3.92

X Blood Volume .07 ml

TABLE 2(f)

MOUSE #6

EFFICIENCY - 39.62, BACKGROUND - 35 COUNTS PER MINUTE

NO LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	32.5	59,540	150,189	4,621	2.08
Spleen	29.4	45,513	114,785	3,904	1.76
Palate	2.4	2,327	5,785	2,410	1.09
Liver	56.7	12,519,000	31,597,589	557,277	251.025
Total Liver	360.0	-	-	-	-
Brain	101.0	30,074	75,818	737	.332
Kidney	86.0	970,112	2,448,453	28,470	12.82
Heart	38.5	119,761	302,186	7,849	3.52
Adrenal	9.2	12,573	31,646	3,440	1.55
Blood	23.5	126,380	318,892	13,570	6.11 X

Placenta

1	5.3	27,575	69,510	13,115	5.91
2	8.1	53,769	135,623	16,743	7.54
3	4.6	25,226	63,581	13,822	6.23
4	3.6	26,643	67,158	18,655	8.40
5	2.7	12,722	32,022	11,860	5.34
6	2.8	13,882	34,952	12,482	5.62
7	2.9	15,665	39,449	13,603	6.13
8	4.3	17,987	45,310	10,537	4.75
9	8.6	43,122	108,750	12,645	5.70 *

Fetal

Tongue	0.8	2,225	5,528	6,909	3.11
Jaws	5.6	12,235	30,793	5,499	2.48
Brain	6.3	10,887	27,390	4,348	1.96
Liver & Heart	2.7	7,576	19,033	7,049	3.18
R. Remainder	4.0	7,172	18,014	4,503	2.03
L. Remainder	9.7	23,036	58,054	5,985	2.70

X Blood Volume 0.1 ml
* Fetal Resorption

TABLE 2(g)

MOUSE #7

EFFICIENCY - 39.62%, BACKGROUND - 35 COUNTS PER MINUTE

NO LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	29.5	27,167	68,481	2,321	1.05
Spleen	31.6	24,287	61,212	1,937	.873
Palate	2.0	3,497	8,738	4,370	1.97
Liver	93.8	767,359	1,936,709	20,647	9.30
Total Liver	318.0	-	-	-	-
Brain	87.4	15,300	38,528	441	.199
Kidney	96.3	425,025	1,072,665	11,139	5.02
Heart	42.9	53,551	135,073	3,149	1.42
Adrenal	2.7	2,625	6,537	2,421	1.09
Blood	23.6	49,064	123,748	5,244	2.37 X

Placenta

1	5.1	22,113	55,724	10,926	4.92
2	-	-	-	-	-
3	6.1	14,133	35,583	5,833	2.63
4	7.0	17,866	45,005	6,429	2.89
5	2.0	4,270	10,689	5,344	2.41
6	4.1	13,081	32,928	8,031	3.62
7	4.1	12,037	30,293	7,388	3.33
8	2.7	7,654	19,230	7,122	3.21
9	3.2	10,098	25,399	7,937	3.58 *

Fetal

Tongue	1.6	2,170	5,389	3,368	1.52
Jaws	2.3	2,735	6,815	2,963	1.33
Brain	5.9	4,681	11,727	1,988	.895
Liver & Heart	3.1	6,163	15,467	4,989	2.25
R. Remainder	7.4	9,631	24,220	3,273	1.47
L. Remainder	5.7	6,577	16,512	2.897	1.30

X Blood Volume 0.1 ml
* Resorption

TABLE 2(h)

MOUSE #8

EFFICIENCY - 39.62%, BACKGROUND - 35 COUNTS PER MINUTE

NO LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	31.5	8,417	21,156	671	.303
Spleen	29.9	6,047	15,174	507	.229
Palate	1.7	777	1,873	1,102	.497
Liver	42.1	100,048	252,431	5,996	2.70
Total Liver	492.0	-	-	-	-
Brain	106.8	3,304	8,251	77	.0348
Kidney	83.0	163,751	413,216	4,979	2.24
Heart	25.5	7,517	18,884	740	.334
Adrenal	1.9	1,209	2,963	1,559	.703
Blood	23.7	15,047	37,890	1,599	.720 X

Placenta

1	4.9	3,240	8,089	1,650	.744
2	5.4	3,166	7,903	1,463	.659
3	5.6	3,496	8,735	1,560	.703
4	2.0	2,000	4,960	2,480	1.12
5	4.7	3,039	7,582	1,613	.727
6	4.6	3,782	9,457	2,055	.926 *
7	4.3	2,885	7,193	1,673	.754
8	3.6	1,939	4,805	1,335	.601
9	4.1	2,103	5,220	1,273	.573

Fetal

Tongue	0.4	167	333	833	.375
Jaws	4.7	1,526	3,763	801	.361
Brain	4.3	954	2,320	539	.243
Liver & Heart	2.0	691	1,656	828	.373
R. Remainder	1.2	1,468	3,617	3,014	1.36
L. Remainder	16.5	5,457	13,685	829	.374
Fetus #6	7.3	4,586	11,487	1,574	.709

X Blood Volume 0.1 ml
* Resorption

TABLE 2(i)

MOUSE #9

EFFICIENCY - 39.62%, BACKGROUND - 35 COUNTS PER MINUTE

NO LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	28.9	8,142	20,462	708	.319
Spleen	28.5	6,840	17,176	603	.271
Palate	11.9	644	1,537	129	.0582
Liver	60.7	210,455	531,095	8,750	3.94
Total Liver	359.0	-	-	-	-
Brain	128.5	4,609	11,545	898	.040
Kidney	88.7	134,157	338,521	3,816	1.72
Heart	33.9	12,557	31,605	932	.420
Adrenal	2.9	1,284	3,152	1,087	.490
Blood	23.8	19,352	48,756	2,049	.923 X

Placenta

1	7.5	10,247	25,775	3,437	1.55
2	5.8	9,043	22,736	3,920	1.77
3	8.0	15,830	39,866	4,983	2.24 *
4	1.7	5,960	14,955	8,797	3.96
5	6.1	2,241	5,568	913	.411
6	6.5	6,571	16,497	2,538	1.14
7	5.3	5,470	13,718	2,588	1.17
8	5.5	5,386	13,506	2,456	1.11
9	7.0	7,009	17,602	2,515	1.13

Fetal

Tongue	0.9	972	2,365	2,628	1.18
Jaws	9.6	4,725	11,837	1,233	.555
Brain	8.4	3,141	7,839	933	.420
Liver & Heart	5.3	3,113	7,769	1,466	.660
R. Remainder	7.2	4,103	10,268	1,426	.642
L. Remainder	16.9	6,904	17,337	1,026	.462

X Blood Volume 0.1 ml
* Fetal Resorption

TABLE 2(j)

MOUSE #10

EFFICIENCY - 41.73%, BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	19.8	8,464	20,223	1,021	.460
Spleen	24.2	7,605	18,164	750	.338
Palate	2.2	883	2,056	935	.421
Liver	30.2	97,635	233,908	7,745	3.489
Total Liver	385.4	-	-	-	-
Brain	98.4	6,696	15,986	162	.073
Kidney	93.9	140,261	336,055	3,579	1.612
Heart	29.1	16,538	39,571	1,360	.613
Adrenals	5.5	7,919	18,915	3,440	1.549
Blood	23.8	24,443	58,514	2,458	1.107

Placenta

1	5.6	7,221	17,244	3,079	1.387
2	11.9	18,731	44,826	3,767	1.697
3	5.2	5,546	13,230	2,544	1.146
4	9.9	13,129	31,401	3,172	1.429
5	7.0	6,991	16,693	2,385	1.074
6	6.1	7,370	17,601	2,885	1.300
7	3.1	2,745	6,518	2,103	.947
8	5.8	7,469	17,838	3,075	1.385

Fetal

Tongue	1.1	459	1,040	946	.426
Jaws	5.9	2,726	6,473	1,097	.494
Brain	5.9	2,422	5,744	974	.439
Liver & Heart	3.5	2,183	5,171	1,477	.666
R. Remainder	10.2	5,463	13,031	1,278	.575
L. Remainder	4.0	2,038	4,824	1,206	.543

45 Minutes between Loading Dose and Labeled Dose

TABLE 2(k)

MOUSE #11

EFFICIENCY - 41.73%, BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	38.2	19,117	45,751	1,198	.539
Spleen	27.9	12,018	28,739	1,030	.464
Palate	2.2	951	2,219	1,008	.454
Liver	38.3	181,023	433,736	11,325	5.101
Total Liver	385.7	-	-	-	-
Brain	111.0	8,952	21,392	193	.087
Kidney	90.4	218,186	522,791	5,783	2.605
Heart	31.0	20,668	49,468	1,596	.719
Adrenals	7.4	3,549	8,445	1,141	.514
Blood	26.3	28,770	68,883	2,619	1.179

Placenta

1	3.3	4,808	11,462	3,473	1.564 *
2	6.7	18,383	43,992	6,566	2.957
3	5.2	9,010	21,531	4,141	1.865
4	6.6	12,193	29,159	4,418	1.99
5	9.7	16,616	39,758	4,099	1.846
6	5.6	10,636	25,428	4,541	2.045
7	7.5	15,572	37,256	4,967	2.238
8	3.8	5,458	13,019	3,426	1.543

Fetal

Tongue	0.8	329	728	911	.410
Jaws	5.0	3,844	9,152	1,830	.824
Brain	6.5	4,263	10,156	1,562	.704
Liver & Heart	3.2	4,601	10,966	3,427	1.544
R. Remainder	8.1	5,936	14,165	1,749	.788
L. Remainder	7.3	4,893	11,665	1,598	.720

* Fetal Resorption

TABLE 2(1)

MOUSE #12

EFFICIENCY - 41.73% - BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	46.7	8,108	19,370	415	.186
Spleen	35.2	4,443	10,587	300	.135
Palate	2.2	387	867	394	.178
Liver	17.5	23,425	56,075	3,204	1.443
Total Liver	478.2	-	-	-	-
Brain	92.6	2,585	6,135	66	.030
Kidney	115.0	79,984	191,610	1,666	.751
Heart	31.1	6,504	15,526	499	.225
Adrenal	2.4	437	987	411	.185
Blood	24.3	9,028	21,574	887	.399

Placenta

1	3.7	1,840	4,349	1,176	.530
2	6.0	2,609	6,192	1,032	.465
3	3.6	1,591	3,752	1,042	.469
4	7.3	3,404	8,097	1,109	.499
5	6.2	3,182	7,565	1,220	.549
6	7.3	2,926	6,952	952	.429

Fetal

Tongue	0.3	54	69	232	.104
Jaws	4.5	1,004	2,346	521	.235
Brain	5.5	1,012	2,365	430	.194
Liver & Heart	2.1	618	1,421	677	.305
R. Remainder	6.3	1,279	3,005	477	.215
L. Remainder	4.3	708	1,637	380	.171

TABLE 2(m)

MOUSE #13

EFFICIENCY - 41.73%, BACKGROUND - 25 COUNTS PER MINUTE

LOADING DOSE + 50 uc

Maternal

	Net Wt. (mg)	Raw Counts Per Minute	Decays Per Minute/Sample	Decays Per Minute/mg	uc X 10^{-3}/mg
Tongue	45.3	12,573	30,069	664	.299
Spleen	38.7	12,816	30,652	792	.356
Palate	2.7	807	1,874	694	.312
Liver	40.3	87,039	208,516	4,174	2.33
Total Liver	433.1	-	-	-	-
Brain	116.2	5,754	13,729	118	.053
Kidney	95.1	131,818	315,823	3,321	1.496
Heart	29.3	8,606	20,563	702	.316
Adrenal	3.1	880	2,048	661	.298
Blood	20.6	13,053	31,219	1,515	.683

Placenta

1	6.8	3,545	8,435	1,240	.558
2	3.3	1,599	3,772	1,143	.515
3	7.1	6,146	14,668	2,066	.931
4	4.6	2,877	6,834	1,486	.670
5	4.7	3,001	7,132	1,517	.683
6	5.8	4,434	10,565	1,822	.821

Fetal

Tongue	0.4	119	225	563	.254
Jaws	6.7	2,105	4,984	744	.335
Brain	5.2	1,714	4,047	778	.351
Liver & Heart	2.6	855	1,989	765	.345
R. Remainder	7.7	2,424	5,749	746	.336
L. Remainder	6.5	1,936	4,579	705	.317

APPROVAL SHEET

The thesis submitted by Dr. Gilbert E. Dodds has been read and approved by three members of the Graduate School faculty.

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 references 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 19, 1971
Date

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Signature of Advisor