

## THE PLACENTA AND PROTEIN METABOLISM

### TRANSFER STUDIES USING CARBON<sup>14</sup>-LABELED PROTEINS IN DOGS\*, †

BY G. H. WHIPPLE, M.D., R. B. HILL, JR., R. TERRY, M.D., F. V. LUCAS, M.D.  
AND C. L. YUILE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

(Received for publication, February 7, 1955)

The main theme of this paper is that the *placenta is an active organ* and that its parenchyma (the trophoblastic chorionic epithelium) has important functions related to protein production and fetal protein nutrition. This paper follows logically other contributions from this laboratory dealing with protein metabolism and protein transfer across cell and other membranes (5, 7, 10). Lysine labeled with C<sup>14</sup> is used to label dog plasma proteins which then are given by vein or mouth to the pregnant dog. Iodine-131 and Evans blue dye (T-1824) are used as protein labels in some experiments.

It is well known that certain antibody proteins may pass the placental barrier between maternal and fetal blood in some species, and interest in the broad problem of plasma protein transfer across the placenta has centered almost exclusively on its immunologic aspects (2-4). It has been shown that the placentas of human beings, rabbits, and guinea pigs readily permit the passage of antibodies from mother to fetus, whereas the placentas of dogs, horses, sheep, goats, and pigs are impermeable to the passage of these proteins.

The present study indicates that the placenta of the dog utilizes both amino acids and plasma proteins present in the maternal circulation for incorporation into its own cells, possible modification, and transmission to the fetus. In these respects amino acids are used more effectively, from a quantitative standpoint.

#### *Methods*

Data concerning the experiments involving the use of C<sup>14</sup> and I<sup>131</sup> were obtained from 5 healthy pregnant bitches not previously used for experimental purposes. General details of the experimental procedures are shown in Table I. In 2 cases (PA and PC) the actual date of mating was known, while in the other 3, it could be closely approximated. For 2 days prior to the start of each experiment, all animals were fed a very low protein diet (containing 0.12 per cent N). Three of the animals received C<sup>14</sup>-labeled dog plasma intravenously as indicated in Table I, to which in 2 instances (dogs PA and PC) small amounts of I<sup>131</sup>-labeled

\* This work has been supported in part by the Office of Naval Research.

† We are also indebted to Eli Lilly and Company and the Ernest L. Woodward Fund for aid in conducting this work.

dog plasma, containing approximately 6,000,000 C.P.M., were added. The 2 other animals were given  $C^{14}$ -labeled lysine by stomach tube. In the case of dog 52-65 the isotope was incorporated in homologous plasma protein while dog PD was fed an aqueous solution of L-lysine- $\epsilon$ - $C^{14}$  mixed with amigen. At periods varying from 2 to 4 days thereafter, the pregnancies were terminated either by Caesarian section or by viviperfusion and autopsy, or by intravenous chloroform and autopsy. The labeled plasma proteins were obtained from donor dogs fed DL-lysine- $\epsilon$ - $C^{14}$ , as previously described (10). Small quantities of dog and rabbit plasma were labeled with  $I^{131}$  by adding specially treated  $I^{131}$  to the protein in borate or phosphate buffer, followed by dialysis (6). In the final product, approximately 92 per cent of the  $I^{131}$  was bound to protein.<sup>1</sup>

TABLE I  
*Labeled Plasma Protein by Vein and by Mouth*

Dog	Weight kg.	Dose	Time of gestation	Dura- tion of exper- iment days	Termination of experiment	No. of fetuses
<i>Labeled Material Administered Intravenously</i>						
PA	15.3	120 ml. plasma ( $C^{14}$ -2.34 $\mu$ c. in 7.0 gm. protein); 2 ml. plasma ( $I^{131}$ -ca. 6,000,000 C.P.M.)	5 wks.	2	Viviperfusion and autopsy Light ether anesthesia	11
PC	18.2	175 ml. plasma ( $C^{14}$ -1.57 $\mu$ c. in 6.0 gm. protein); 5 ml. plasma ( $I^{131}$ -ca. 6,000,000 C.P.M.)	Term	3	Viviperfusion and autopsy Light ether anesthesia	7
49-158	18.8	137 ml. plasma ( $C^{14}$ -2.60 $\mu$ c. in 7.4 gm. protein)	Near term	2	Caesarian section Nembutal anesthesia	11
<i>Labeled Material Administered Orally</i>						
52-65	7.4	92 ml. plasma ( $C^{14}$ -0.73 $\mu$ c. in 4.3 gm. protein)	About 6 wks.	4	Intravenous $CHCl_3$ and autopsy	4
PD	10.5	3 mg. L-lysine- $\epsilon$ - $C^{14}$ (2.42 $\mu$ c.) plus 5 gm. amigen	Near term	4	Intravenous $CHCl_3$ and autopsy	3

Samples of maternal venous blood were collected in 1.4 per cent sodium oxalate at suitable intervals for nitrogen and  $C^{14}$  analyses of whole plasma, total plasma protein, albumin, and globulin. The  $I^{131}$  activity of maternal whole plasma and plasma albumin was determined when this isotope was used.

Pooled fetal blood from each litter was obtained by severing the jugular veins immediately following delivery. This was collected in saturated sodium citrate. Analyses of nitrogen concentration and  $C^{14}$  activity in fetal plasma, total plasma protein, albumin, and globulin were made.  $I^{131}$  activity was also measured in whole fetal plasma and plasma protein in appropriate instances.

Amniotic fluid was collected as quantitatively as possible. Total protein concentration,  $C^{14}$  activity and  $I^{131}$  activity, when necessary, were determined.

Measurements of total and specific protein  $C^{14}$  activity were made on lyophilized finely

<sup>1</sup> We are indebted to Dr. W. F. Bale for the preparation of this material.

ground maternal tissues and on selected fetal tissues similarly prepared (10). The total weight of all fetuses in each litter was determined, and the lyophilized material from at least one intact fetus was ground in a mill of mesh size 40/inch.  $C^{14}$  activity and total nitrogen were then measured in triplicate on suitable aliquots.

Two animals (PA and PC) were viviperfused with modified Ringer's solution plus glucose under light ether anesthesia. Estimated recovery of plasma protein  $C^{14}$  was 83 per cent and 90 per cent of the amount circulating at the time of perfusion for PA and PC respectively.

All methods pertaining to chemical determination of plasma protein, albumin, and globulin as well as preparation of samples for  $C^{14}$  analysis have been described in detail elsewhere (10).

$C^{14}$  analyses were made by the method of Bale, which utilizes the quantitative conversion of organic material to  $CO_2$  by the reagent of Van Slyke and Folch (8), and then transfers the carbon dioxide to an ionization chamber for radioactivity measurement.  $I^{131}$  was counted on a well-type scintillation counter as described by Anger (1).

Two similar experiments involving pregnant rabbits were also carried out. Mature healthy does not previously used for experimental purposes were mated, and within a week of the expected date of delivery were given small doses of  $I^{131}$ -labeled rabbit plasma proteins by vein. Two days later they were sacrificed with intravenous chloroform and autopsied im-

TABLE II  
*Evans Blue Dye (T-1824) by Vein*

Dog	Weight	Vol. of dye injected	Time before delivery	Size of litter
50-8	16.7	20 ml.	10 min.	7*
50-39	11.4	20 ml./day	6 days	7‡
49-140	12.0	20 ml./day	3 days	4

\* Also received 260 cc. homologous plasma each day for 3 days prior to delivery.

‡ Last injection 24 hours before delivery.

mediately. A third experiment attempted at mid-term was unsatisfactory owing to the small size of the fetuses.

Samples of maternal plasma, amniotic fluid, and fetal plasma were collected from these rabbits and measurements of total nitrogen concentration and  $I^{131}$  activity were made as in the dog experiments.

Three additional pregnant dogs (50-8, 50-37, and 49-140) received from 1 to 6 intravenous injections of 20 ml. of Evans' blue dye (T-1824), (0.3 per cent in 0.9 per cent NaCl), at intervals prior to spontaneous full term delivery indicated in Table II. The injections were sufficient to produce a deep blue color in the maternal plasma. Blood was collected from the jugular veins of newborn pups immediately after delivery and the plasma examined for visible evidence of dye.

Dog 50-8 also received 260 ml. of homologous plasma intravenously each day for 3 days prior to delivery, a total of 52.5 gm. of plasma protein being injected during this 3 day period. Techniques used have been previously described (7). Maternal plasma samples were analyzed for total protein before the injection period and on the day of delivery while serum protein levels in the fetuses were determined on blood collected by jugular venesection after birth.

#### EXPERIMENTAL OBSERVATIONS

Data concerning the disappearance of  $C^{14}$ -labeled proteins from maternal plasma following intravenous and oral administration are not included. The

short duration of the present experiments makes a detailed analysis of this material impossible. However, the findings appear to differ in certain important particulars from those *observed in normal dogs* similarly prepared (10, 11). Based on greatly reduced half-life values, *it is probable that the pregnant dog metabolizes considerably more plasma protein per unit of time than does the normal dog*. This increased protein metabolic rate is apparently a reflection of an internal shift, being accompanied by an increased transfer from plasma proteins to tissue proteins, but not by increased loss of metabolites as shown by activities in urine and expired air comparable to those in non-pregnant animals. These unexpected effects of pregnancy on maternal physiology warrant further investigation.

Fig. A illustrates the relative  $C^{14}$  activities of maternal and fetal plasma protein, selected organ proteins, and red cell hemoglobin in 2 dogs given  $C^{14}$ -labeled plasma by vein after 5 (dog PA) and 9 (dog PC) weeks of gestation. The difference in maternal plasma protein activity noted between these 2 dogs is accounted for in part by the greater body weight of dog PC (Table I) and in part by the longer interval between injection of labeled plasma and termination of the experiment on this animal. Both these factors would tend to reduce plasma protein specific  $C^{14}$  activity.

As seen in Fig. A., the  $C^{14}$  activity of maternal plasma protein is approximately 10 times greater than that in fetal plasma protein. Both maternal and fetal tissue proteins show approximately the same activity as fetal plasma protein. Values shown in Fig. A for maternal and fetal tissues are approximately, though not absolutely, comparable since those from the mothers, after viviperfusion, contain all "lymph" protein and some residual plasma protein of relatively high activity, and some whole blood of low activity remains in the fetal tissues. It is felt, however, that the discrepancy is not great. The tissues selected are among those found previously to have relatively high  $C^{14}$  activity (10).

Blood hemoglobin activities are also shown in Fig. A. The higher  $C^{14}$  levels in fetal red cells probably reflect more rapid synthesis and the very low activity of maternal hemoglobin in dog PA, 2 days after injection of labeled plasma as compared to dog PC, after a 3 day interval, is doubtless related to the time required for maturation and discharge of labeled red cells into the circulation.

The observed placental concentrations of labeled proteins are about the same as noted in the liver, (Figs. A and B) and it is reasonable to suppose that most of this activity resides within epithelial cells. The trophoblastic chorionic epithelium makes up a considerably smaller proportion of the total placental mass than do the polygonal cells of the liver so that the *placental parenchyma is relatively richer in labeled protein than the hepatic epithelium*. Thus the chorionic epithelium, gram for gram, is probably 2 or 3 times more active than the liver in the manufacture and storage of protein from either amino acid or plasma protein sources.

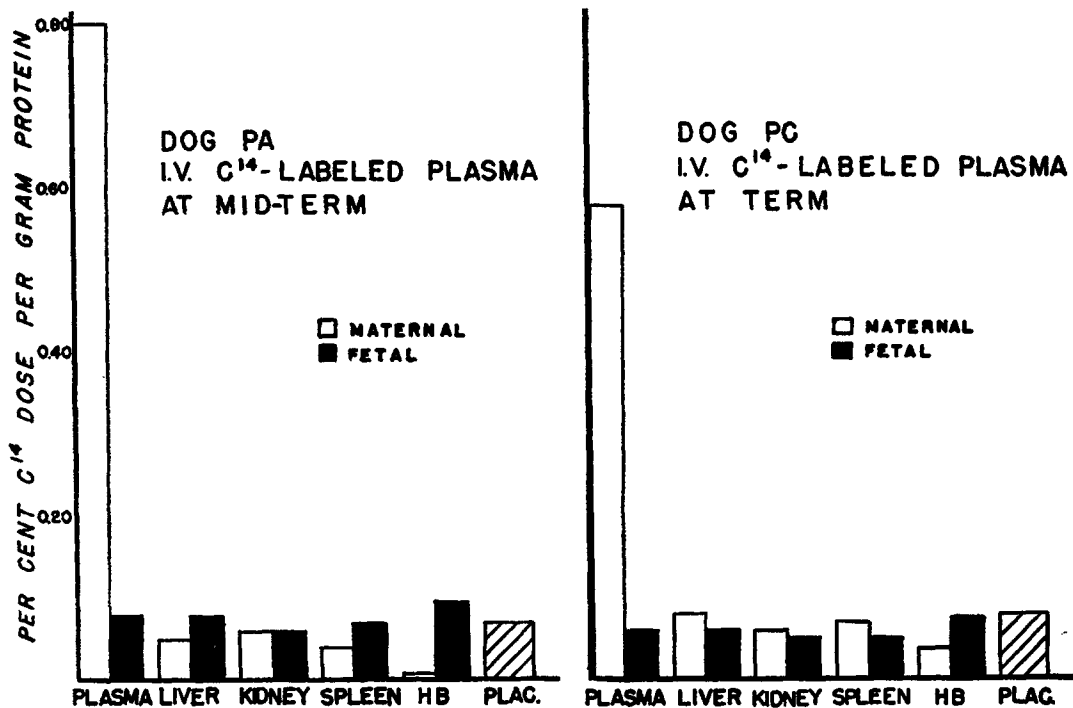


FIG. A. Labeled plasma proteins given intravenously to 2 pregnant dogs at mid-term and full term. Comparison of specific C<sup>14</sup> activities of maternal and fetal plasma and selected tissue proteins at time of delivery. Placental C<sup>14</sup> activities are also included.

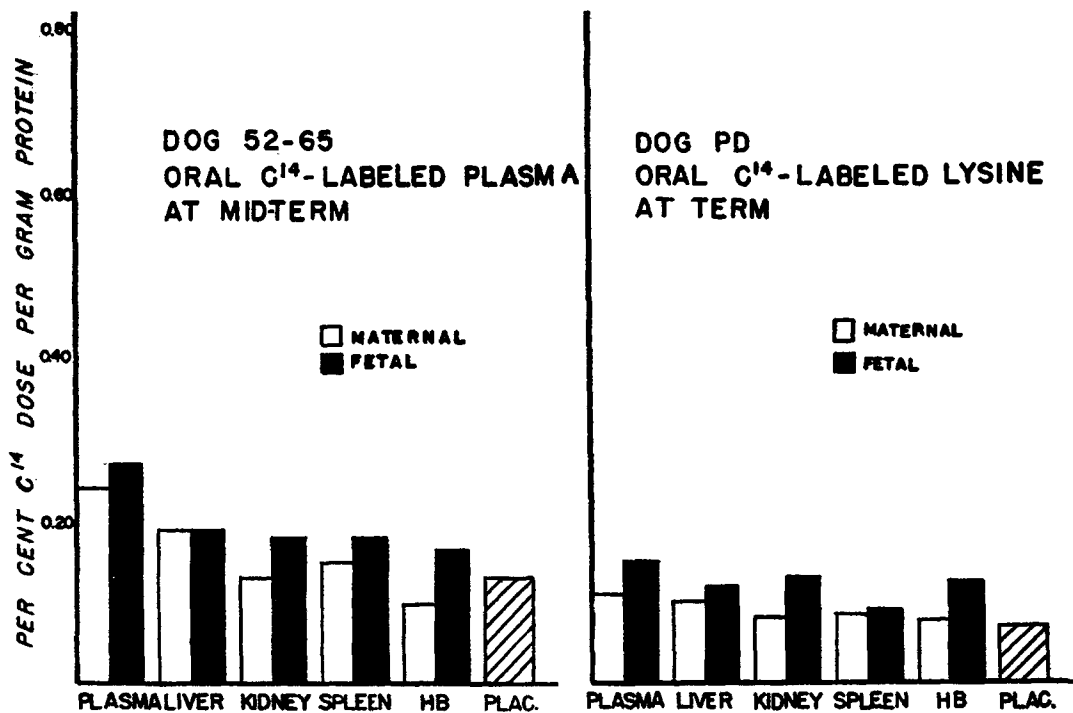


FIG. B. Maternal and fetal plasma and selected tissue protein C<sup>14</sup> activities 4 days after oral administration of labeled material. Dog 52-65 received C<sup>14</sup> labeled plasma protein at mid-term, Dog PD received C<sup>14</sup> labeled lysine mixed with amigen at full term. Placental C<sup>14</sup> activities are also included.

Fig. B illustrates data, similar to that shown in Fig. A, for 2 dogs which received the  $C^{14}$  by mouth in the form indicated (labeled plasma and labeled lysine, Table I). Since both maternal and fetal tissues from these animals contained approximately the same percentage of residual, equally radioactive whole blood, the relative values here are directly comparable. The lower level of all values for dog PD is a reflection of greater isotope dilution due to greater body weight (Table I).

Under these experimental conditions, the isotope initially reaches the maternal circulation in the form of  $C^{14}$ -labeled lysine, absorbed from the gastrointestinal tract. As found in normal dogs receiving  $C^{14}$ -labeled plasma or lysine by mouth (11), the maternal plasma protein activities seen in Fig. B are approximately the same as the maternal tissues selected. Fetal plasma and tissue

TABLE III  
Total  $C^{14}$  Transfer across Placenta Expressed as per Cent of Dose

Dog	Duration of experiment	No. of fetuses	Total $C^{14}$ in all fetuses	Total $C^{14}$ in amniotic fluid	Total $C^{14}$ in fetuses plus amniotic fluid	$C^{14}$ transfer per fetus	$C^{14}$ transfer per fetus per day
	days						
<i>Intravenous</i>							
PA	2	11	3.32	0.45	3.77	0.34	0.17
PC	3	7	5.32	0.44	5.76	0.82	0.27
49-158	2	11	5.60	0.20*	5.80	0.53	0.27
<i>Oral</i>							
52-65	4	3	6.50	0.14	6.64	2.21	0.55
PD	4	4	15.68	0.24	15.92	3.98	1.00

\* Estimated. Total volume of amniotic fluid not determined.

activities in these animals are also essentially similar to those in maternal plasma proteins. As in the intravenous group, hemoglobin  $C^{14}$  is higher in the fetus than in the mother.

Data relating to the quantitative transfer of  $C^{14}$  across the placenta, following both intravenous and oral administration to dogs, are shown in Table III. The figures for total  $C^{14}$  in all fetuses were derived by multiplying the  $C^{14}$  activity per gram of wet weight by the wet weight of the entire litter. The radioactivity per gram of 2 or more fetuses, separate or combined depending upon size, was determined in all but one instance (PD) when only 1 large fetus was used. Specific activities of separate fetuses from the same litter were very similar in all cases. Assuming that most, if not all, of the activity found in amniotic fluid had first crossed the placenta, the combined fetal and amniotic fluid activities were taken to represent total placental transfer. From these figures, (column 6, Table III), divided by the number of fetuses and days in

each experiment, the amount of  $C^{14}$  transferred to each fetus per day was derived.

It will be noted (Table III, final column) that the average daily amount of  $C^{14}$  which crosses each full term placenta (dogs PC, 49-158, PD) is approximately twice as much as that crossing the smaller, less mature placentas present after 5 to 6 weeks of gestation (dogs PA and 52-65). A striking relationship is also noted between the magnitude of  $C^{14}$  transfer and the route of administration of labeled plasma protein. After feeding, the activity in each fetus is 5 to 7 times greater than that found after intravenous injection of a similar amount of labeled plasma protein. This does not give a true indication of the relative rates of transfer, however, since, after oral administration, most of the activity

TABLE IV  
*I<sup>131</sup> Transfer across Placenta in Dogs and Rabbits*

	Maternal plasma	Fetal plasma					Amniotic fluid				
	I <sup>131</sup> per cent dose per 100 ml. at delivery	I <sup>131</sup> per cent dose per 100 ml.	Protein I <sup>131</sup> per cent dose per 100 ml.	Estimated total fetal plasma volume	I <sup>131</sup> per cent dose circulating in plasma		I <sup>131</sup> per cent dose per 100 ml.	Protein I <sup>131</sup> per cent dose per 100 ml.	Total volume	I <sup>131</sup> per cent dose in fluid	
					Total	Protein bound				Total	Protein bound
Dog PA	4.26	0.28	0.01	38.7	0.108	0.004	—	—	1450	—	—
Dog PC	2.70	0.10	0	66.2	0.066	0	0.5	0.03	560	2.80	0.2
Rabbit 1	14.2	10.6	2.4	17.2	1.82	0.40	8.4	2.9	50	4.2	1.5
Rabbit 2	12.1	6.3	2.9	10.5	0.63	0.31	8.6	5.0	38*	3.3	1.9

Rabbit 1, 12 fetuses; Rabbit 2, 8 fetuses.

\* Estimate.

crossing the placenta must reach it during the first few hours, while appreciable plasma levels of  $C^{14}$ -labeled lysine are present; and since subsequent maternal plasma protein  $C^{14}$  activities never exceed 10 per cent of the values obtained at similar time intervals after intravenous injection. The  $C^{14}$  activities transferred to each fetus per day in the intravenous group (final column, Table III) are equivalent to from 0.15 to 0.30 gm. of maternal plasma protein.

Table IV contains data concerning transfer of  $I^{131}$  after intravenous injection of plasma labeled with this isotope in 2 dogs and 2 rabbits. Significant differences are readily observed between the two species. Very little  $I^{131}$  is found in the dog fetal plasma and amniotic fluid, and virtually all of this is not bound to protein. On the other hand, relatively high concentrations of  $I^{131}$  are found in the fetal plasma and amniotic fluid of the rabbits and appreciable proportions of this  $I^{131}$  are attached to protein. Variable amounts of unbound  $I^{131}$ , ranging

from 6 to 10 per cent of the total, were present in the samples of labeled plasma injected.

No Evans blue dye was seen in the gross in the plasma of any of the puppies in the litters from 3 dogs given large amounts of this dye intravenously (Table II) despite the fact that the maternal plasma was dark blue in color.

The circulating maternal plasma protein concentration in dog (50-8), also given 3 large daily doses of homologous plasma intravenously, rose from a level of 5.8 gm. per cent to 9.1 gm. per cent on the day of delivery without raising the concentration of fetal plasma protein above the levels found in a comparable control litter.

#### DISCUSSION

The placenta is the most interesting and probably the least understood of the various maternal and fetal tissues included in this study. The placenta in mid- and late term development consists essentially of maternal venous sinuses into which dip the chorionic villi carrying the fetal vessels. The maternal and fetal bloods are separated by the *trophoblastic chorionic epithelium*,—the essential *parenchyma of the placenta*,—and a variable amount of connective tissue stroma. Actual bulk of the epithelium, when compared with the rest of the placenta, probably does not exceed 40 per cent.

Until quite recently the placenta was considered to function as a semi-permeable membrane and when anything was observed which could not be explained by the function of such a membrane, it was concluded that breaks or epithelial injury in the villi was responsible. Recent evidence indicates that the placenta does permit passage of proteins, for example, antibodies, in certain species (4) and it probably elaborates some specific hormones.

The assumption that the placenta is *an organ* with other tasks besides the obvious is supported by much evidence (9) and the observations on dogs described in this paper give very specific evidence that it is concerned with *protein metabolism of the mother and fetus*.

After introduction of labeled proteins into the maternal circulation, C<sup>14</sup>-labeled proteins appear in fetal plasma and organs (Fig. A). This, it is believed, must be related to the great activity of the placental parenchymal epithelium which accepts and possibly modifies the labeled plasma protein before passing it to the fetus.

Despite this important finding, it is nevertheless noteworthy that from a quantitative standpoint the placenta of the dog acts as a relatively effective barrier between the circulations of mother and fetus as far as plasma proteins are concerned. This is clearly indicated by the fact that not more than 200 to 300 mg. of maternal plasma protein or its equivalent C<sup>14</sup> reaches each fetus daily while the maternal surface of the placental villi must come in contact with many hundred grams of plasma protein in a 24 hour period.



Dietary  $C^{14}$ , on the other hand, which first reaches the maternal circulation after protein digestion, is passed in considerably greater quantity to the fetus, presumably in a relatively short space of time. These data suggest that under normal circumstances the major fetal nitrogen requirements are supplied more or less directly by the mother's diet. The high  $C^{14}$  content of the placenta in the feeding experiments also points to an active role played by this organ in the utilization of amino acids in fetal protein metabolism.

The experiments with  $I^{131}$ -labeled plasma protein indicate merely that a significant transfer of unaltered protein from maternal to fetal plasma occurs in the rabbit but not in the dog. This is in accord with the observed difference between the 2 species with respect to antibodies. The findings, however, are not directly comparable with those from the  $C^{14}$  experiments. That differences should occur is to be expected since in one instance (lysine) the *proteins are naturally labeled by the body* and in the other  $I^{131}$  is artificially attached to the proteins *in vitro*.

#### SUMMARY

Plasma proteins tagged *in vivo* by feeding D-L-lysine- $\epsilon$ - $C^{14}$  to donor dogs have been administered to pregnant dogs by both oral and intravenous routes.

A relatively small percentage of the  $C^{14}$  activity originally incorporated in these proteins is found to pass from mother to fetus after intravenous injection. The amount transferred tends to increase with the length of gestation period and total number of fetuses.

Plasma protein labeled with  $I^{131}$  does not cross the placenta in the dog, but does in the rabbit.

Evans blue dye does not cross the placenta of the dog.

After oral administration of labeled plasma protein or lysine,  $C^{14}$  is transferred promptly and in considerable quantity to the fetus.

Labeled plasma proteins disappear more rapidly from the circulation of pregnant than of normal dogs. This increased metabolic turnover occurs without excretion of any excess waste metabolites.

The *chorionic epithelium*, gram for gram, is probably 2 to 3 times as active as the hepatic epithelium in protein metabolism.

These findings indicate an important placental function related to maternal and fetal protein metabolism. While the placenta utilizes maternal plasma proteins and amino acids, in a quantitative sense the latter appear to supply the major nitrogen needs of the growing fetus.

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