THE DISTRIBUTION AND METABOLISM OF C^{14}-LABELED LACTIC ACID AND BICARBONATE IN PREGNANT PRIMATES *

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The oxygen consumption of the human fetus is believed to be equivalent to that of the adult organism and the amount of oxygen available to the fetus should be adequate for glycogen degradation beyond the lactic acid stage (1, 2). Although the supply of oxygen is satisfactory, an anaerobic breakdown of carbohydrate has been postulated because of the persistently elevated lactic acid concentration of fetal blood (3). This implies that lactic acid, as the end-product of carbohydrate metabolism, accumulates in the fetal organism and is transferred to the mother in a manner analogous to the removal of carbon dioxide and other waste products. The recent investigations of Hendricks (4) provide data intended to support this hypothesis.

The evidence in favor of either form of metabolic breakdown ultimately rests upon the demonstration of a concentration gradient between maternal and fetal blood. Concentration differences, however salient they may be, are not alone sufficient to prove a transfer from one system to another. Carbon^{14}-labeled compounds would be ideally suited for the examination of such problems, but their use is restricted to experimental animals. Of these, the proper subjects are pregnant primates, partly because of the anatomic and physiologic similarity to the human and more pertinently because of the accessibility of the primate fetus.

The present investigations were undertaken to demonstrate *a*) that there is an exchange rather than a unidirectional transfer of carbon dioxide and lactic acid between fetus and mother and *b*) that the fetus metabolizes lactic acid at a rate comparable to that of the adult organism.

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METHODS

Physiologic preparations. Pregnant rhesus monkeys at term were anesthetized with intravenous Nembutal and prepared in the manner previously described (5, 6). By means of polyethylene catheters placed in the amniotic sac, the interplacental artery and the maternal femoral vein, samples of amniotic fluid, fetal and maternal blood were obtained at suitable intervals. The injection of labeled materials dissolved in normal saline was accomplished in the same manner. All samples were collected under oil, and clotting of blood was prevented by the addition of crystalline heparin. *In vitro* glycolysis was inhibited with potassium fluoride. Quantitative analyses for acid volatile CO_2 and lactic acid as well as the preparation of gas samples for isotope analyses were carried out within a few hours after the collection of samples.

Analytic procedures. The incidental sodium^{22} and tritium determinations were carried out as described by Friedman, Gray, Hutchinson and Plentl (6). Conventional methods were used for the quantitative estimations of lactic acid (7) and carbon dioxide (8). The carbon dioxide necessary for isotope analyses was conveniently prepared in the manometric apparatus in the following routine manner.

A) Acid volatile carbon dioxide. The procedure was identical with the quantitative manometric method to which reference has been made, except for the omission of the last step, the reabsorption of carbon dioxide with 1 N sodium hydroxide. Instead, the crude product containing a small amount of water and noncondensable gas was collected and sealed in a previously evacuated breakseal tube attached to the side arm of the chamber. These gas samples could be stored for an indefinite length of time.

B) Lactic acid carboxyl carbon. Blood and amniotic fluid samples were deproteinized with tungstic acid and the supernatant solution treated with copper sulfate and lime, as described by Avery and Hastings (9). The filtrate, after removal of excess calcium tungstate, was introduced into the chamber of the Van Slyke manometric apparatus where the oxidation of lactic acid was accomplished by the addition of permanganate. Stirring of the oxidation mixture was continued until a constant reading was obtained. The liberated gases were transferred to breakseal tubes as described above.

C) Carbon^{14} activity. The breakseal tubes were attached to a high vacuum train and the gas allowed to expand into a series of traps cooled in dry ice and liquid nitrogen. An apparatus similar to that described by Glascock (10) was used for this purpose. The purified carbon dioxide was trapped in a suitable McLeod gauge in which 0.001 to 0.010 mmole of gas could be measured with an accuracy of at least 2 per cent. The gas samples were quantitatively transferred to Bernstein-Ballentine tubes, a constant quantity of carrier CO_2 added, and the counters filled to atmospheric pressure with methane. Counting was performed in the proportional region between 3,000 and 4,000 v. All results were expressed as specific activity, i.e., counts per minute per millimole. Standardization of the equipment was carried out at frequent intervals using carbon dioxide prepared from a standard sample of barium carbonate. On the basis of these calibrations the counters had an efficiency of 64 per cent.

Radiocarbon compounds. All radiocarbon compounds used in this study were obtained from commercial sources. Sodium-D, L-lactate-1-C^{14}, bicarbonate and urea had specific activities of 1 mc per mmole. Glucose was uniformly labeled and had a specific activity of 0.232 mc per mmole. For the biologic experiments the compounds were dissolved in normal saline, preparatory to intravenous injection.

Evaluation of degradation procedures and isotope determinations. That carbon dioxide obtained by permanganate oxidation of synthetic lactic acid is derived from the carboxyl carbon has been shown by Gibbs, Dumrose, Bennett and Bubeck (11). The oxidation of blood and amniotic filtrates presumably proceeds according to this scheme and no further experimental evidence is necessary to substantiate the mechanism:

$$CH_3-\underset{\displaystyle H}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}-C^{14}OOH \xrightarrow{KMnO_4} CH_3CHO + C^{14}O_2 + H_2O.$$

It is of importance, however, to demonstrate that the specific activity of lactic acid and acid volatile carbon dioxide can be determined in mixtures containing both and that other organic constituents normally contained within these body fluids do not interfere. In order to check the specificity of the general procedures, solutions of C^{14}-labeled organic compounds were prepared using amniotic fluid as solvent. Bicarbonate, lactic acid, glucose and urea were dissolved in amniotic fluid; each solution contained a known quantity of carrier and isotope. These solutions and mixtures of them were analyzed for the specific activity of carbon dioxide and lactate-1-C^{14}. The findings are reproduced in Table I. When bicarbonate was the only labeled compound, no activity was detectable in the carbon dioxide derived from lactic acid. When lactic acid was the labeled compound, the acid volatile carbon dioxide was devoid of any radioactivity. Radioglucose and urea did not contaminate the carbon dioxide derived by either degradation procedure. Dilutions with the same solvent led to the anticipated decline in the specific activity within the limits of error of the method.

While the liberation of carbon dioxide from blood and amniotic fluid gives a representative sample of gas originally present, the oxidation of blood filtrates with permanganate leads to a certain, known error. It can be estimated (9) that about one-tenth of the carbon dioxide obtained by permanganate oxidation of blood (but not of amniotic fluid) filtrates is derived from substances other than the carboxyl carbon of lactic acid. The specific activities for lactic acid carboxyl recorded in these experiments are therefore consistently low. The specific activities of lactate presented in the paper have not been corrected for the nonlactate carbon dioxide. The data on specific activities of lactate-1-C^{14} cannot be correlated with acid volatile carbon dioxide activities unless appropriate corrections are made.

TABLE I

*Tests for interference of lactic acid and bicarbonate in amniotic fluid**

C^{14} compounds added	No. of μc/ml $\times 10^{-5}$	Specific activity (C/min/mmole $\times 10^4$) for Bicarbonate	Specific activity (C/min/mmole $\times 10^4$) for Lactic acid
Bicarbonate	5.0	86.0	negligible
		83.5	0
Bicarbonate	2.5	46.0	0
		46.0	0
Lactic acid-1-C^{14}	5.0	0	301
		0	292
Lactic acid-1-C^{14} + bicarbonate	2.5	47.7	146
	2.5	41.5	145
Glucose (uniformly labeled)	2.32	0	0
Urea	5.0	0	0

* The solutions were prepared with amniotic fluid which contained 17.5 mEq of volatile carbon dioxide and 5.3 mEq of lactic acid per L. The radioactivity was determined by the degradation procedures described in the text.

EXPERIMENTAL RESULTS

C^{14} bicarbonate injected into the amniotic fluid. Fifty μc of isotonic bicarbonate was injected into the amniotic fluid of a 4,800 g pregnant rhesus monkey. Two hours later, a solution containing tritium and sodium^{22} was injected into the fetal circulation. Samples of maternal blood, amniotic fluid and fetal blood were then taken at predetermined intervals. After 280 minutes a cesarean section was performed and a live 285 g fetus was obtained. The specific activity of acid volatile carbon dioxide, lactic acid carboxyl, total tritium

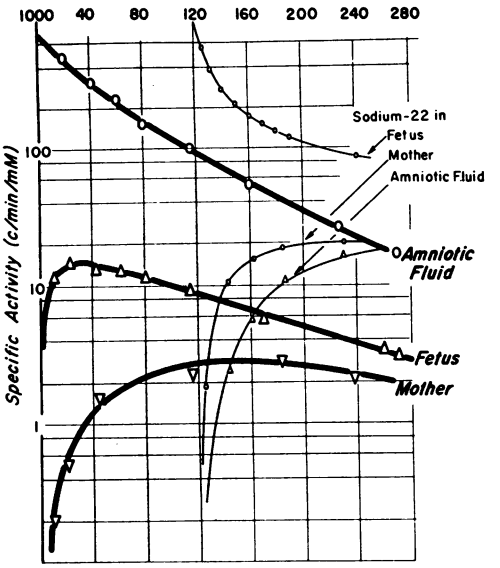


FIG. 1. CHANGES IN SPECIFIC ACTIVITY ($\times 10^4$) OF ACID VOLATILE CARBON DIOXIDE AS A FUNCTION OF TIME IN MINUTES AFTER INJECTION OF SODIUM BICARBONATE-C^{14} INTO THE AMNIOTIC FLUID.

and sodium^{22} activity for each of the samples was determined by the methods described. The results are reproduced graphically in Figure 1 in which the specific activity for acid volatile CO_2 in the three compartments is plotted against time. No activity could be demonstrated in the CO_2 obtained by permanganate oxidation of any of the samples.

The characteristic time-activity curves for sodium and tritium (6) indicate that the transfer mechanisms of the animal were normally operative; it may be assumed that this applied equally well to other transfers. Carbon dioxide disappeared from the amniotic fluid and appeared first in fetus and then in the mother. Its disappearance from the amniotic fluid points to a mechanism slightly more complex than a simple unidirectional removal since the curve is determined by at least two exponents. Fetal and maternal curves rise at unequal rates, pass through maxima and decline, and eventually all three curves seem to approach a similar slope.

C^{14} bicarbonate injected into the fetus. Twenty μc of isotonic bicarbonate in a total volume of 4.0 ml was injected into the fetal circulation of a 4,500 g rhesus monkey. As in the first experiment, a solution containing sodium^{22} and tritium was injected into the amniotic fluid to test the efficiency of the transfer mechanisms. Samples of amniotic fluid, fetal and maternal blood were taken at suitable intervals. The experiment was terminated 120 minutes after the initial injection of the tracer. A cesarean section was performed and a 365 g live fetus was obtained. No activity was demonstrated for the lactic acid carboxyl carbon in any of the samples. The specific activity for acid volatile carbon dioxide as a function of time is given in Figure 2. The specific activity of fetal carbon dioxide decreases rapidly and is associated with an equally rapid rise for the amniotic fluid carbon dioxide. At 35 minutes the two curves cross, apparently at the maximum for the amniotic fluid compartment. The maternal carbon dioxide follows a similar pattern at a considerably lower level.

Lactate-1-C^{14} injected into the fetus. Two such experiments were performed, using 3.0 and 40 μc of lactate-1-C^{14}, respectively. The lactate solutions were injected into the fetal circulation as in the preceding experiment. The fetuses also received sodium^{22} and tritium. Despite the low dosage, the data obtained in the first experiment were found to be sufficiently informative for a preliminary evaluation of the specific activity curves for fetal lactate-1-C^{14} and acid volatile CO_2 in the three compartments. The activity for fetal lactic acid declined in the form of a double exponential curve. Within a few minutes the radioactivity of fetal carbon dioxide reached a maximum and thereafter declined at a constant rate. The activity

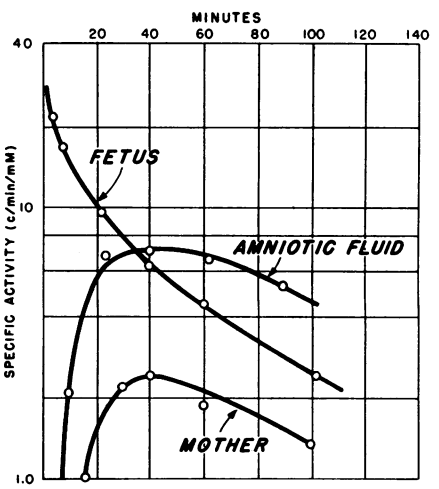


FIG. 2. CHANGES OF SPECIFIC ACTIVITY ($\times 10^4$) OF ACID VOLATILE CARBON DIOXIDE AFTER INJECTION OF SODIUM BICARBONATE-C^{14} INTO THE FETAL CIRCULATION.

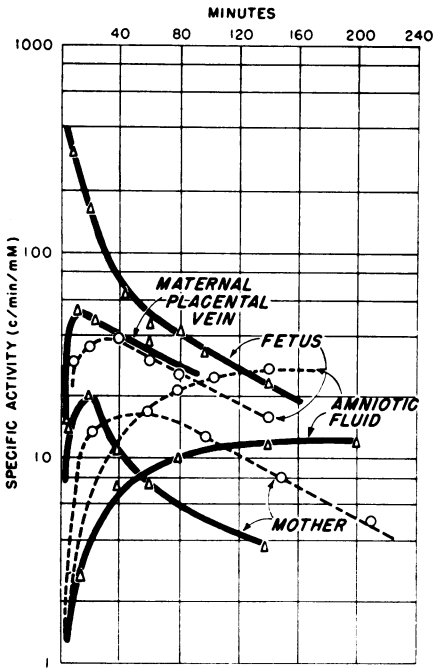


FIG. 3. CHANGES OF THE SPECIFIC ACTIVITY ($\times 10^4$) OF THE CARBOXYL CARBON OF LACTIC ACID (SOLID LINE) AND ACID VOLATILE CARBON DIOXIDE (DOTTED LINE) IN AMNIOTIC FLUID, MATERNAL AND FETAL BLOOD AFTER INJECTION OF LACTIC ACID-1-C^{14} INTO THE FETAL CIRCULATION.

curve for amniotic fluid CO_2 rose somewhat more slowly, crossed the fetal curve and then declined at the same rate as the others.

In the second experiment, reproduced in Figure 3, wherein a more suitable dosage of the radiotracer was employed, the general shape and the relative position of these curves could be confirmed. In addition, evidence for the transmission of fetal lactate-1-C^{14} to the maternal organism could be obtained. The quantity of carbon dioxide obtained and the radioactivity for all samples were within the optimal range for the analytic determinations. An appreciable radioactivity could now be demonstrated in maternal blood and amniotic fluid lactic acid carboxyl carbon.

Lactate-1-C^{14} injected into adult nonpregnant monkey. Two identical experiments were carried out in which 150 μc of lactic acid-1-C^{14} was injected into the venous system of normal adult nonpregnant female rhesus monkeys weighing 5,140 and 5,900 g, respectively. Samples of venous blood were obtained at frequent intervals up to 360 minutes. Acid volatile carbon dioxide and the carboxyl carbon of lactic acid were isolated from these samples and their specific activities determined. Since the results of the two experiments were nearly identical only one of them is reproduced in Figure 4. The specific activity curves of the carboxyl carbon and carbon dioxide closely resemble those of the preceding experiments except for the upward displacement of the latter and consequent crossing of the curves.

Lactate -1-C^{14} injected into maternal organism. Two hundred μc of lactic acid-1-C^{14} was injected into the venous system of a 4,520 g pregnant rhesus monkey. Samples of amniotic fluid, fetal and maternal blood were taken at predetermined intervals and analyzed in the manner described above. The data are presented graphically in Figure 5. The activity of maternal lactic acid declined at a rate slightly in excess of that recorded for the nonpregnant monkeys. A proportional incorporation into the acid volatile carbon dioxide is also evident. The fetal lactic acid activity increased sharply, followed by a decline parallel to the maternal curve. The time-activity curves for fetal and maternal carbon dioxide were nearly identical.

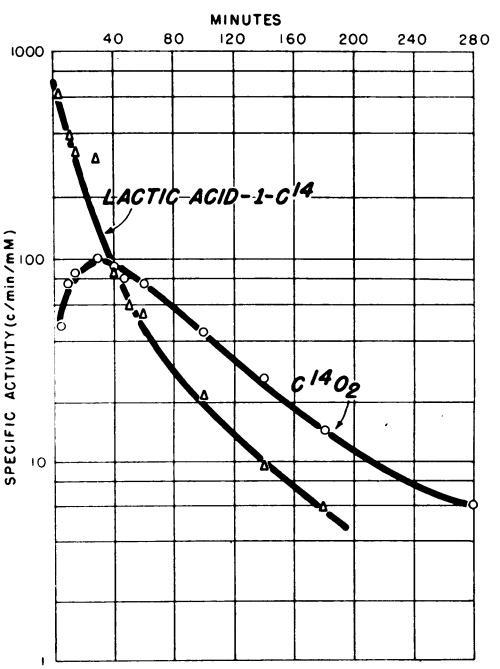


FIG. 4. CHANGES OF SPECIFIC ACTIVITY ($\times 10^4$) OF LACTIC ACID CARBOXYL AND ACID VOLATILE CARBON DIOXIDE AFTER THE INTRAVENOUS INJECTION OF LACTIC ACID-1-C^{14} INTO AN ADULT NONPREGNANT MONKEY.

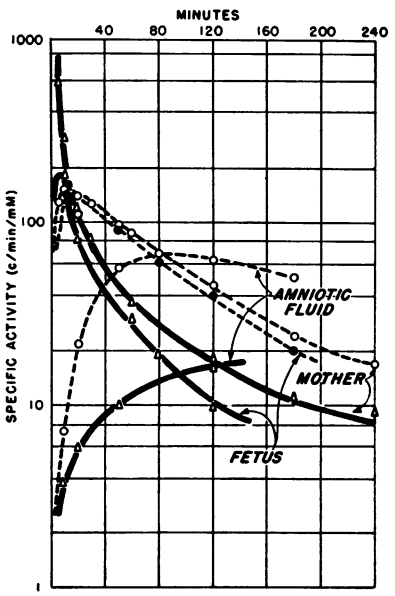


FIG. 5. CHANGES IN SPECIFIC ACTIVITY ($\times 10^4$) OF LACTIC ACID CARBOXYL CARBON (SOLID LINE) AND ACID VOLATILE CARBON DIOXIDE (DOTTED LINE) IN AMNIOTIC FLUID, MATERNAL AND FETAL BLOOD AFTER INJECTION OF LACTIC ACID-C^{14} INTO THE MATERNAL CIRCULATION.

DISCUSSION

The experiments in which labeled bicarbonate was introduced lend themselves to a relatively simple interpretation of the tracer curves. In contrast to the subsequent experiments in which labeled lactic acid was used, true metabolic processes, i.e., the incorporation of C^{14} into other compounds, play only a minor, insignificant role. The changes which take place are essentially distribution phenomena.

The injection of bicarbonate into the amniotic fluid leads to a uniform distribution within this compartment, and almost instantaneous mixing can be achieved by mechanical means. Its carbon dioxide content, which represents the size of the primary compartment, can be determined with considerable accuracy, and the theoretic CO_2 pool does not exceed anatomic boundaries. The specific activity curve for this compartment (Figure 1) can be used to estimate the amount of carbon dioxide leaving the amniotic fluid per unit of time. The curve is governed by two exponents, 0.0231 and 0.00825 per minute, which together with the integration constants define the fraction of labeled carbon dioxide at any time (t) as:

$$\text{Fraction of } C^{14}O_2 = 0.8359\, e^{-0.0231\, t} + 0.1642\, e^{-0.00825\, t}.$$

Differentiating this equation and setting $t = 0$ gives the fraction of carbon dioxide leaving the amniotic fluid per unit of time:

$$-(0.0231)(0.8358) - (0.00825)(0.1642) = -0.0207/\text{min}.$$

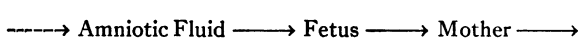
The total carbon dioxide content of the amniotic fluid in this animal was 2.6 mEq. The amount of carbon dioxide leaving the amniotic fluid compartment was therefore $(0.0207)(2.6) = 0.05382$ mEq per minute or 3.23 mEq per hour. Since there was no measurable change in the concentration of volatile carbon dioxide during the period of observation, the same quantity of carbon dioxide must have been replaced. There is either an exchange with fetus or mother or both, or the CO_2 is produced in the amniotic fluid compartment from other, nonisotopic sources.

When the tracer was injected into the fetal blood stream a time-activity curve for the fetal compartment was obtained (Figure 2) which could also be expressed as a multiple exponential equation. Calculation of the disappearance rate has less meaning than in the preceding case, because the size of the fetal pool and that of secondary compartments is not known. Furthermore, lack of instantaneous mixing renders the initial portion of the curve quite unreliable. Theoretically, an analysis of carbon dioxide exchanges similar to that reported by Steele (12) could be applied to the primate fetus but is not justified on the basis of the present data.

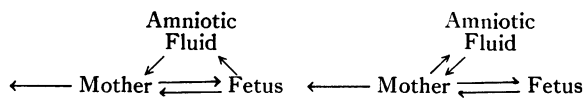
The relationship of the specific activity curves obtained in the two experiments should make it possible to derive some useful information with regard to the major transfers in this complex system. An examination of a number of theoretic models and their comparison with experimental findings would exclude some of the many possibilities and uphold others.

The shape of the retention curve for the amniotic fluid (Figure 1) or its mathematical expression as a multiple exponential equation implies that two or more transfer rates are effective in producing this phenomenon. Two unidirectional

transfers in sequence or in various combinations of exchanges between fetus and mother could be entertained as a working hypothesis. A unidirectional transfer of the type:

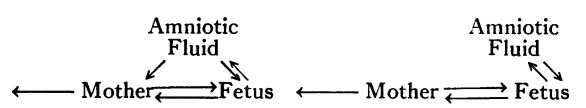


does not apply because injection of the tracer into the fetal compartment (Figure 2) gives a set of curves compatible only with a transfer from fetus to mother *and* amniotic fluid. Models involving a direct pathway from amniotic fluid to mother, e.g.,



are excluded by the relative positions of the maxima for the secondary curves of Figure 1. The maximum for the fetal curve occurs sooner (20 minutes) than that for the maternal curve (180 minutes). If either of these models was applicable, the position of the maxima should have been reversed. In Figure 2, in which the primary compartment was the fetus, the maxima for the secondary compartments occur about the same time, indicating a simultaneous transfer from fetus to mother and from fetus to amniotic fluid. Evidence for the existence of a transfer from mother to fetus is given in later experiments as will be discussed below.

Thus far, an adequate representation would be either of the following models:



of which the first model still includes a direct transfer from amniotic fluid to mother. This pathway may exist but, in view of the wide difference of t_{max} for maternal and fetal curves of Figure 1, its contribution to the removal of CO_2 from the amniotic fluid must be small or nonexistent.

Both models imply that fetal CO_2 is the only precursor of the carbon dioxide contained in the amniotic fluid, a hypothesis that can be tested with relative ease. It has been shown theoretically (13) that the specific activity of a compound and its precursor are equal when the specific activity of the former reaches its maximum. As shown in Figure 2, the specific activities of amniotic fluid CO_2 and fetal blood CO_2 are equal when the former had reached its peak. This is only possible if all, or nearly all, of the amniotic fluid carbon dioxide is derived from the fetus only. A direct transfer or exchange with maternal CO_2 and metabolic processes within this compartment probably makes minor contributions.

In the first two experiments, in which only labeled carbon dioxide was used as the primary tracer, no radioactivity could be detected in the lactic acid carboxyl carbon of any of the samples. This moiety of the lactic acid molecule must therefore be synthesized from compounds other than carbon dioxide or perhaps it requires a considerably longer period of time for synthesis. That the synthesis and degradation of lactic acid under normal conditions is a rapid process is evident from the experiments in which lactic acid-1-C^{14} was injected into the vascular tree of adult nonpregnant monkeys. As shown in Figure 4, the activity of the carboxyl carbon declined at first rather rapidly while that of the acid volatile carbon dioxide rose at a comparable rate. After the latter had reached its maximum, both curves tapered off more slowly, presumably approaching a rate representing the removal of carbon dioxide in respired air.

Only racemic lactic acid was used in these experiments and the curves obtained cannot be used for the estimation of the rate of conversion of the natural isomer to carbon dioxide. In the subsequent experiments adult and fetal organisms are compared in their ability to metabolize and distribute the racemic form.

The injection of labeled lactic acid into the fetal blood compartment results in a set of curves (Figure 3) for lactic acid carboxyl and carbon dioxide similar to those obtained in the adult nonpregnant monkey. The curves differ only in the relative position of carbon dioxide and lactic acid and the evident transfer to mother and amniotic fluid. Simultaneously with the decline in specific activity of lactic acid carboxyl there is a rapid rise in the activity of fetal carbon dioxide followed by a similar increase in the activity of the maternal and amniotic fluid compartment. Since the only tracer introduced was that originally present in fetal lactic acid carboxyl, any activity in the carbon dioxide of the three compartments must be derived from it. The decrease in activity of labeled lactic acid is, therefore, both a distribution and

a metabolic phenomenon: "metabolic" because carbon dioxide appears in fetal blood, and "distribution" because some unchanged labeled lactic acid appears in the amniotic fluid and in the maternal circulation.

Labeled lactic acid is transmitted from the fetus to the amniotic fluid and the maternal pool. The fate of the lactic acid in the amniotic fluid is not established in this experiment; it may be slowly metabolized to carbon dioxide, thus contributing to the labeled carbon dioxide in this compartment, or it may be exchanged for unlabeled lactic acid of maternal or fetal origin. Whether amniotic fluid lactic acid is metabolized to carbon dioxide within this compartment at an appreciable rate cannot be decided with certainty. The maternal carbon dioxide, on the other hand, is definitely of dual origin—part derived from labeled maternal lactic acid and part by direct transmission of fetal carbon dioxide.

The specific activity curves for carbon dioxide in the three compartments of the same animal give the interlacing pattern shown by the dotted lines in Figure 3. Since all radioactivity was derived from metabolic processes, the interpretation of these curves is quite complex. If no transmission of lactate from fetus to mother had occurred and no oxidation of maternal lactic acid to carbon dioxide had taken place, the relative positions of the maxima for fetal and maternal carbon dioxide would indicate which of them is the precursor of the other. In view of the demonstrated transfer of lactic acid and its catabolism in secondary compartments no such deductions can be made. The maxima for the carbon dioxide activity curves occur first in fetal, then in maternal, and finally in amniotic fluid carbon dioxide. The postulated exchange between amniotic fluid and fetus produced the expected crossing of the two curves, despite the fact that fetal carbon dioxide was a secondary, metabolic product.

Maternal venous blood obtained from a major vein draining the primary placenta showed an exceedingly high specific activity of its lactic acid which, after reaching its peak, declined at a rate parallel to that of fetal blood. This differs from the curve obtained with maternal peripheral blood, because the placental vein drains a constant amount of lactic acid with a negligible metabolic change, while the maternal organism as a whole transforms the lactic acid carboxyl to carbon dioxide. The high specific activity in the vein draining the placental pool indicates that most of its lactic acid is derived from the fetus. Since maternal arterial blood carries appreciable quantities of maternal lactic acid to the placental bed, this compound must have been exchanged for the same species of fetal origin. Its rate of exchange must be very rapid in order to account for this phenomenon. This notion can be put to a crucial test by injecting labeled lactic acid into the maternal rather than into the fetal circulation. The specific activity curves for lactic acid carboxyl and carbon dioxide obtained under these conditions are shown in Figure 5. The previously suspected transfer of lactic acid from maternal to fetal systems is demonstrated by the sharp rise in fetal lactic acid as the activity in the maternal circulation declined. After passing through a maximum, the fetal curve approaches a slope similar to that of the maternal species. This together with the previously demonstrated conversion to CO_2 indicates a similar metabolic pattern for adult and fetal organisms. The decline in maternal lactic acid activity and the corresponding curve for maternal carbon dioxide shows a relationship very similar to that obtained for the nonpregnant monkey (Figure 4). The activity curve for the lactic acid in amniotic fluid is not appreciably different from the pattern obtained when fetal lactic acid is the primary compartment (Figure 3). It is likely, therefore, that a direct transfer from mother to amniotic fluid does not exist, or if it does its contribution is minimal.

The fetal carbon dioxide is now derived from maternal and fetal lactic acid as shown by the relative position of fetal lactic acid to carbon dioxide curves. When fetal lactic acid is the primary source of tracer, the metabolic carbon dioxide in that compartment has a specific activity below that of the carboxyl carbon (Figure 3). In order to raise the specific activity to the levels recorded in Figure 5, most of the carbon dioxide which appears in the fetus must have been transmitted from the maternal carbon dioxide pool. The relationship of fetal and amniotic fluid curves for this metabolite (Figure 2) is not disturbed. The two curves cross in the predicted region.

The method of carbon dioxide isolation from blood and amniotic fluid does not differentiate be-

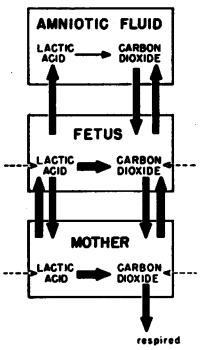


FIG. 6. SCHEMATIC PRESENTATION OF THE MAJOR DISTRIBUTION AND METABOLIC PATHWAYS OF LACTIC ACID CARBOXYL AND CARBON DIOXIDE IN THE PREGNANT PRIMATE. Dotted arrows indicate endogenous sources. The transformation of lactic acid to carbon dioxide in the amniotic fluid compartment indicated by the small arrow is probable but not established.

tween bicarbonate and dissolved carbon dioxide in these samples. Although it is likely that dissolved carbon dioxide rather than the bicarbonate ion is transmitted across the placental boundary, the present experiments do not provide information on this aspect of the transfer mechanism.

Qualitatively, several pathways have been established, a schematic presentation of which is given in Figure 6. The amniotic fluid carbon dioxide exchanges freely with that of the fetal system, whereas a direct transfer from amniotic fluid to mother, if it occurs at all, is probably insignificant. Carbon dioxide is *also* transmitted from fetus to mother and in the reverse direction at an unexpectedly rapid rate. A direct incorporation of carbon dioxide into the lactic acid does not occur in either organism. Fetus and mother exchange their lactic acid, while the lactic acid of amniotic fluid seems to be largely of fetal origin.

The transfer of lactic acid from human fetus to mother has been investigated by Hendricks (4) with the aid of conventional methods. From the difference in the lactic acid concentration of umbilical arterial and venous blood, the author found that an average of 29.63 per cent of fetal lactic acid is removed in a single passage through the placenta. Keeping in mind the species difference and method of study, a comparison of this datum with the present findings could be made by calculating the biologic half-life time for both sets of experiments. From Hendricks' data this value can be estimated in the following manner. Assuming a fetal blood volume of 300 ml and an average lactic acid concentration of 25 mg per 100 ml, the fetal blood pool is $25 \times 3 = 75$ mg of lactic acid. On the basis of the observations of Romney, Reid, Metcalfe and Burwell (2), the average blood flow is 500 ml per minute. If 30 per cent of the lactic acid contained in 500 ml (125 mg) is removed per minute, the turnover rate is 50 per cent and its half-life time $0.693/0.5 = 1.4$ minutes. From the initial slope of the activity of fetal lactate in Figure 3, a half-life time of 15 minutes can be calculated. Since this turnover represents the sum of distribution and metabolic rates, the exchange between fetus and mother is probably still slower than is indicated by a half-life of 15 minutes.

The present findings are also at variance with Hendricks' interpretation that the difference in lactic acid concentration of the products of gestation and maternal blood is related to the transfer rates or could indicate its origin. A "descending gradient" from the highest levels in the amniotic fluid to the lowest in maternal peripheral blood was thought to indicate that the lactic acid is produced by the fetus, extracted by the placenta and then transferred to the maternal circulation. The concentration of lactic acid in the amniotic fluid of human and primate is nearly five times higher than that of fetal blood, yet the present tracer experiments indicate an appreciable transfer of lactic acid from fetus to amniotic fluid, i.e., against the "gradient." Although less pronounced, the concentration gradient between maternal and fetal blood does not seem to prevent a rapid exchange of lactic acid between the two systems. Hendricks explained the persistently high concentration of lactic acid in amniotic fluid as due to fetal micturition. The present report points to a slow though consistent and continuous exchange of lactic acid and carbon dioxide between amniotic fluid and fetus. If the predominant mechanism were fetal micturition, specific activity curves as reported here would not be possible. It would seem more likely that the presence of lactic acid in the maternal organism and in products of gestation is a transitory phenomenon, and its relative concentration is not necessarily related to transfer mechanisms.

The same contradiction must arise on the subject of fetal respiration. The various calculations of carbon dioxide transport (14) from fetus to

mother are fundamentally based on concentration gradients which assume the form of pressure differentials. The present demonstration of rapid exchanges regardless of concentration levels would call for a review of many existing and generally accepted explanations of "placental transmission."

SUMMARY

Methods for the determination of the specific activity of C^{14}-labeled carbon dioxide and lactic acid in blood and in amniotic fluid were devised and tested. The transfer and exchange mechanisms of these compounds were then investigated on pregnant primates at term.

When labeled bicarbonate was injected into the amniotic fluid or the fetal circulation, time-activity curves for acid volatile carbon dioxide in amniotic fluid, fetal and maternal blood were compatible with a rapid exchange of carbon dioxide between amniotic fluid and fetus. There is a simultaneous transfer of carbon dioxide from fetus to mother which may represent a net exchange or a unidirectional transfer. Within the period of observation, no incorporation of labeled carbon dioxide into the lactic acid of conceptus or mother could be detected.

The conversion of the lactic acid carboxyl carbon to carbon dioxide was investigated on adult nonpregnant monkeys. The lactic acid carboxyl was rapidly incorporated into the acid volatile carbon dioxide. When lactic acid-1-C^{14} was injected into the maternal blood stream, unchanged labeled lactic acid and carbon dioxide of maternal origin appeared in the fetal blood stream and in the amniotic fluid. The retention curves for fetal, maternal and nonpregnant adult animals were comparable within the experimental error of the method.

Lactic acid-1-C^{14} was injected into the fetal circulation and the specific activity curves for lactic acid carboxyl and acid volatile carbon dioxide in the three compartments indicated that the carboxyl group is rapidly metabolized to carbon dioxide and that unchanged lactic acid is transferred to maternal blood and amniotic fluid.

Maternal and fetal organisms freely exchange both metabolites. The concept of a unidirectional transfer based on concentration gradients is untenable. The similarity of fetal, maternal and nonpregnant adult retention curves indicates that lactic acid is not a major end-product of fetal metabolism in primates.

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