more reason to suspect that the results herein reported have significance from a physiological and biochemical standpoint.

Summary. Sodium acetate or propionate, administered by venoclysis in a dose of 2.5-5.0 μM/kg/minute, had a pronounced stimulatory effect on secretion of acid from the Heidenhain pouch of the dog with total antrum resection. Higher doses of atropine were required to suppress acetate than methacholine stimulation. Acetate-stimulated secretion was lower in peptic activity than secretion produced in response to methacholine. Similarities of acetate and ethanol stimulation were recognized and discussed.

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Movement of Calcium in Both Directions Across the Primate Placenta.* (30215)

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The continuous movement of a myriad of nutrient substances and metabolic waste products across the placenta from mother to fetus and in the reverse direction is vital to the growth and development of the embryo. Radioactive tracer techniques have been used for the study of placental transport of the essential nutrient, calcium, in mice, rats, rabbits and cattle(1-4). The rapid movement of strontium in both directions across the monkey placenta has been directly demonstrated in monkeys(5). This report concerns the simultaneous transfer of isotopically labeled calcium across the placenta from mother to fetus and in the reverse direction. The primary intent was to estimate the approximate magnitudes of these transfers. Pregnant monkeys were chosen because of the close similarity of their placental structure to that of humans(6,7).

Method. Each of 3 pregnant rhesus monkeys was anesthetized with intramuscular piperidine HCl (4 mg/kg supplemented with pentobarbital 10 mg/kg body weight). Polyethylene catheters were placed in a maternal femoral vein, an interplacental vessel of the fetus and the amniotic sac in a manner previously described(6). Five μc of I^{131} labeled human serum albumin in 2 ml of isotonic saline were injected into each fetal and maternal catheter, for estimation of blood vol-

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TABLE I. Rate Constants and Compartment Factors for Blood Activity Changes.

	Disappearance of Ca^{47} from maternal blood $C = A_1e^{-b_1t} + A_2e^{-b_2t}$					Appearance of Ca^{47} in fetal blood $C = A(e^{-b_2t} - e^{-b_1t})$, or $C = A - A_1e^{-b_1t} - A_2e^{-b_2t}$				
Exp	A	A_1	b_1	A_2	b_2	A	A_1	b_1	A_2	b_2
1	1.69	1.00	.31	.69	.008	.18	.073	.77	.107	.022
2	4.24	3.50	.35	.74	.0043	.22	.057	.29	.163	.027
3	1.16	.65	.13	.51	.0043	.27	—	.12	—	.0027
	Disappearance of Ca^{45} from fetal blood					Appearance of Ca^{45} in maternal blood				
	A	A_1	b_1	A_2	b_2	A	A_1	b_1	A_2	b_2
1	19.4	16.0	.44	3.4	.031	.21	—	.077	—	.013
2	30.2	25.0	.14	5.2	.013	.35	—	.063	—	.0067
3	40.6	38.0	.17	2.6	.018	.19	—	.099	—	.0027

For *disappearance* curves, A_1 and A_2 represent the initial portions of the total blood pool (accessible to sampling) which are depleted at the experimentally observed fractional rates of b_1 and b_2. $A = A_1 + A_2$. Units of A and b are % dose/mg Ca and min^{-1}, respectively, and C is tracer concentration in % dose/mg Ca at time t.

For *appearance* curves in which a maximum tracer concentration ($A_1 + A_2 = A$) in blood was approached asymptotically during the experiment, A_1 and A_2 represent the portions of this maximum which were changing at rates expressed by b_1 and b_2. These latter constants are the rates at which the difference between the instantaneous tracer concentration and the asymptote was being reduced. For those experiments in which the incoming tracer actually reached a maximum concentration and then declined exponentially, A is the zero time intercept of this last exponential and b_2 is its rate constant. A in this case represents the theoretical maximum concentration or pool size which would have been attained if losses had not occurred simultaneously with the influx of tracer.

umes by the method of Bender(8). Static blood and heparinized saline in each catheter was removed and discarded immediately prior to sampling. Ca^{47} and Ca^{45} solutions were then administered simultaneously *via* the maternal and the fetal cannulae respectively, followed by saline to flush the tubing. Dosages ranged from 10-70 μc for Ca^{47} and from 100-500 μc for Ca^{45}, in the form of chlorides in aqueous 0.9% NaCl at a pH of approximately 6. Volumes were 1-2 ml and contained less than 2 mg of non-radioactive carrier Ca. Both isotopes were obtained from Oak Ridge National Laboratory. Beginning at 3 minutes after the radiocalcium injections and at frequent intervals thereafter, 0.5-1.0 g samples of maternal blood, fetal blood and amniotic fluid were withdrawn. The experiments were terminated by lethal injection of pentobarbital from 45 to 121 minutes after radiocalcium administration.

Ca^{47} was determined in blood and amniotic fluid by gamma scintillation counting in a NaI crystal well detector, using pulse height analysis to discriminate against the low energy gamma activity of the daughter Sc^{47} and the I^{131}. Ca^{45} was determined by Geiger-Mueller beta counting of Ca oxalate carrier precipitates prepared from the ashed samples. To avoid contributions from beta disintegration from Ca^{47} ($t_{½}$ = 4.7 days) and the daughter Sc^{47} (3.4 days), all beta counting was done at least 3 weeks after each experiment. Ca^{45} radioassays of the Ca^{47} solution used in each experiment showed that less than 2% of the observed Ca^{45} in any monkey tissue sample could be ascribed to Ca^{45} contamination accompanying the Ca^{47} which had been in that sample. All counting times were sufficiently long so that the statistical error in counting was below 3%. Non-radioactive Ca was determined by an arc emission spectrographic method having a standard error of less than ± 2%. Maternal blood plasmas averaged 10.0 mg Ca/100 ml; fetal blood plasmas 12.0 mg Ca/100 ml. Amniotic fluids (AF) were 6.4, 10.4 and 1.9 mg Ca/100 ml for experiments 1, 2 and 3 respectively.

Results. The changes in specific activity of Ca tracer in maternal blood (MB) and fetal blood (FB) with the passage of time following injection could be expressed as multi-term exponential functions. Table I lists the parameters for these equations and

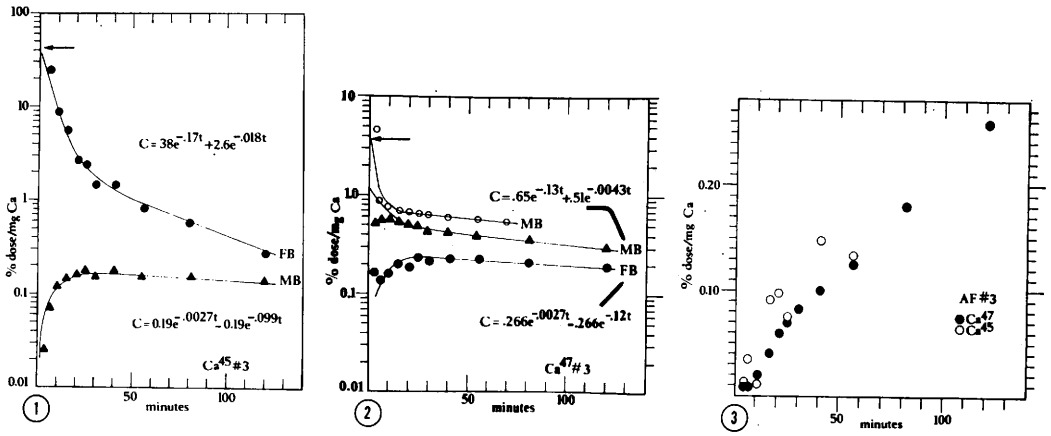

FIG. 1 and 2 present blood data for Ca^{45} and Ca^{47} respectively for Experiment #3. Arrows on ordinate scales indicate theoretical maximum values of % dose/mg of Ca calculated on the basis of instantaneous, uniform mixing of injected tracer throughout the calculated blood volume.

FIG. 3. Amniotic fluid data. MB and FB signify maternal and fetal blood, respectively. The topmost curve in Fig. 2 illustrates the initial spike in tracer concentration in Ca^{47} in MB of animal #2 (and #1) which was not observed in animal #3.

Fig. 1-3 show the curves for animal #3. Values for AF, however, were better described as direct functions of time. Ca^{47} injected into the maternal blood disappeared rapidly at first (half times 2-6 minutes) following which the rate of decline became more leisurely (half times of 87 to 160 minutes).† These changes were matched in the fetal blood by an initial rapidly rising phase (half times of 1-6 minutes for asymptotic approach to a maximum concentration) which was soon superseded by a slower rate of increase (26-31 minutes half times). In animal #3 the experiment was of sufficient duration to permit attainment of a maximum concentration of Ca^{47} in FB, after which the FB blood levels decreased at a rate (253 minutes half time) which was slower than that for the Ca^{47} tracer in maternal circulation. Similarly for Ca^{45} administered to the fetus, the initial rapid decline of activity in the primary blood system (FB in this case) was matched by a rapid appearance of tracer in the blood on the opposite side of the placental membrane (MB).‡ The half times for these initial phases were 1.6-5 minutes for the disappearance of Ca^{45} from FB and 7-11 minutes for appearance in MB, and again, these initially rapid phases soon gave way to much slower rates of change. Thus, despite the dearth of experimental points during the first few minutes it is clear that tracer concentrations in the secondary blood compartments rose from zero to at least 50% of their maximum values within 10 minutes. This obviously implies that large quantities of tracers must have left the primary blood compartments and traversed the placenta during this same short interval. Concentrations of both radiotracers in amniotic fluid rose steadily and showed little tendency to level off. Specific activities for all 3 sampled pools ap-

† The blunting of the Ca^{47} concentration *vs* time curve in MB of Exp. 3 during the first 5-6 minutes is attributed to sluggish mixing of tracer in the maternal blood circulation because of temporary mechanical entrapment in the intervillous spaces of this mature placenta. Maternal blood enters the intervillous space as jets emerging from arterial openings in the basal plate. Venous return depends upon the hydrostatic pressure differential and not on a closed, well defined net. Flow rates through the interstices are not uniform and mixing is variable(9). In the younger placentas of animals 1 and 2 an initial high concentration "spike" was observed, followed by the exponential decline as illustrated by the top curve in Fig. 2.

‡ The term "primary" signifies the particular blood pool which received the tracer by direct injection and "secondary" applies to the pool receiving the tracer by transport from the primary pool. Thus, MB was the primary pool for Ca^{47}, but the secondary pool for Ca^{45}.

peared to converge, but lengthy extrapolations were not justifiable.

Ca transfers calculated from changes in blood pool concentrations. A kinetic analysis of transfers among all the Ca compartments is not practicable even in the non-pregnant mammal and addition of a Ca permeable placenta and a Ca-absorbing fetus complicates the system still further. At least 4 compartments have been postulated to account for the 4 exponential terms observed in time-concentration curves of Ca^{45} in the blood of non-pregnant rabbits and dogs during several hours following a single intravenous injection(10-12) and physiologic identification of these compartments is difficult. However, in the present experiments with pregnant monkeys, the initial rapid decline of Ca^{47} in MB, (*i.e.*, the first exponential term with half time between 2 and 5.5 minutes) must be due at least in part to loss of Ca^{47} by transport across the placenta because the tracer appeared initially in FB with matching rapidity. Similarly, Ca^{45} crossed the placenta and appeared in MB at an initial rate which was only slightly slower than the initial rate of disappearance from FB.

Rates of departure (T_o) of Ca from the maternal and fetal blood pools *via* these rapid processes possessing half times of less than 6 minutes were estimated with equation I.§

§ I. $T_o = (Q)(A_1Q/100)(b_1)(1440)$ mg Ca/day
II. $T_B = (Q)(A/C_m)(b_1)(1440)$ mg Ca/day
III. $T_A = (Q)(S_A/S_m)(1/t)(1440)$ mg Ca/day

Where T_o = initial rate of transfer of Ca out of primary pool by all rapid processes acting in combination; T_B = rate of transfer of Ca into secondary blood pool by the most rapid process observed; T_A = rate of transfer of Ca into amniotic fluid; Q = size of total accessible Ca pool = total pool volume × mg Ca/ml in pool. In eq. I, Q refers to primary blood pool; in eq. II to secondary blood pool; and in eq. III to amniotic fluid pool; all in mg of Ca. A_1 = compartment size = constant factor for the first exponential term in the expression for disappearance of tracer from primary blood pool (Table I). A = compartment factor for appearance of tracer in secondary blood pool. Units of A_1 and A are % dose/mg Ca. The b_1 terms are the rate constants from the appropriate exponential expression for disappearance or appearance of tracer. C_m = mean conc. of tracer in primary blood pool during time interval required for conc. in secondary blood pool to reach its highest observed value, in % dose/mg Ca, and is obtained by graphical integration of primary tracer disappearance curve over this interval. S_m = mean conc. of tracer in primary blood pool for whole experiment, from integration of full tracer disappearance curve. S_A = conc. of tracer in amniotic fluid at end of experiment. The terms t and 1440 refer respectively to the duration of the experiment in minutes, and number of minutes in a day.

Computed values for T_o appear in Table II. However, only a fraction of this rapid departure of Ca and tracer from each primary blood pool can be ascribed to migration across the placenta into the secondary partner blood pool because considerable Ca (and tracer) is simultaneously being exchanged and deposited in soft tissues and bone. The initial rate of transplacental loss (T_B) can be estimated from the rate of appearance of tracer in the secondary blood pool (equation II). These T_B values for Ca placental transfer by rapid processes, calculated from the blood "appearance" curves, appear in Table II. Placental transfers to FB amounted to between 4% and 18% of the total amount of Ca leaving the MB pool per day by all rapid processes (T_B/T_o). Transfers from FB to MB accounted for 11% to 50% of the Ca leaving the FB pool.

The small transfers of Ca to amniotic fluid, from both MB and FB were approximated with equation III and are also listed in Table II: Though not measured directly, there is probably a return flow of Ca from AF back to MB and FB, as shown by Wasserman *et al* in rats and rabbits(3).

Significant fractions of the tracer doses were trapped in the placenta (3%-7% for Ca^{47} and 6%-15% for Ca^{45}). Using the mean specific activities in the primary blood pools (S_m), the quantities of Ca temporarily trapped in the placentas were estimated. The values for Ca arriving from MB were 154, 197 and 164 mg per day in the 3 animals. For Ca from FB, the values were 74, 51 and 57 mg per day. Obviously, similar amounts of Ca must leave the placenta and return to the maternal and fetal bloods or the organ would soon become calcified to an intolerable degree. However, some calcium

must deposit in the placenta because small amounts have been observed by histochemical methods(13). Therefore, in any model of the fetus-mother system, the placenta must be considered as one of the important compartments in which Ca distributes itself. It should not be regarded as a simple membrane separating the maternal from the fetal blood compartments in the way that the capillary wall separates blood from extracellular fluid.

Adequacy of placental transport to meet fetal needs. The major fetal need for Ca is to supply mineral for its growing skeleton. Daily Ca requirements can be estimated from analogous data on human fetuses because the fetal growth curve for the macaque monkey (14) is almost identical in shape to that for the human fetus(15). During the final third of the human gestation period fetal Ca increases approximately at the rate of 26 mg/day/kg body weight. For the monkey with its 165-day gestation period this corresponds to about 40 mg of Ca per day per kg body weight. On this basis, the most immature fetus (#2) was accumulating Ca at the rate of 11 mg per day, presumably laid down in newly forming bone. Fetus #1 was gaining 13 mg per day and #3, the oldest, 18 mg per day. These values are well below the amounts of Ca transported from MB to FB across the placenta, calculated from the blood disappearance curves (T_B). Thus, the amount of Ca transported daily in both directions across the placenta appears to be greatly in excess of the needs for skeletal growth. This situation is similar to the classical "safety factor" observed for placental transport of sodium by Flexner *et al*(16). For pregnant rabbits, Wasserman *et al* have estimated that the amount of Ca transferred daily to the fetuses ranges from 1.3 to 2.1 times the amount actually incorporated by daily growth(3). For the 3 monkeys of the present study the safety factor for Ca was at least 6 for the youngest fetus and more than 10 for the others. This "safety factor" has no intrinsic, absolute significance as an independent physiologic entity. The concept merely aids in visualizing the normal condition in which the flow of Ca back and forth across the placenta is apparently so copious

TABLE II. Estimation of Bi-Directional Transport of Calcium Between Mother and Fetus.

Exp		t (min)	Wt (g)	Age (days)	Blood vol* (ml)	Mean conc of tracer in primary blood pool S_m (% dose/mg Ca)	Initial tracer disappearance rate constant b_1 (min^{-1})	Total rapid Ca transport out of primary pool T_o (mg Ca/day)	Rapid Ca transport to MB and to FB T_B (mg Ca/day)	Net Ca transport to AF T_A (mg Ca/day)
1	Mother	42	6,100	—	451	.67	.305	3170	445 m→f	27 m→a
	Fetus		334	134	25	2.8	.440	330	130 f→m	5 f→a
2	Mother	75	3,875	—	180	.71	.347	1890	43 m→f	13 m→a
	Fetus		272	124	20	5.74	.139	102	25 f→m	17 f→a
3	Mother	120	11,575	—	447	.44	.126	850	118 m→f	17 m→a
	Fetus		455	153	34	2.97	.176	566	334 f→m	3 f→a

* Values for maternal blood were consistent with the 49-71 ml/kg reported for young, non-pregnant monkeys(8). However fetal blood volumes calculated from the I^{131} labeled serum albumin dilution data were obviously too low (less than 5 ml in 2 experiments) for some undetermined reason and were discarded. All fetal blood volumes therefore were assumed to be 75 ml/kg fetal body wt, similar to the top range of adult values.

that Ca removed from the fetal blood to support skeletal mineralization is rapidly replenished without seriously threatening the steady state Ca concentrations in fetal or maternal bloods.

Summary. Transfer of Ca from mother to fetus concurrent with transfer from fetus to mother was studied in 3 pregnant rhesus monkeys. Ca^{45} was injected into a fetal interplacental vessel simultaneously with intravenous injection of Ca^{47} into the anesthetized mother. Samples of maternal blood (MB), fetal blood (FB), and amniotic fluid (AF) were assayed for Ca^{45}, Ca^{47} and non-radioactive Ca. Initial loss of Ca^{47} from MB was matched by rapid initial appearance in FB ($t_{½} < 6$ min) and somewhat slower entry into AF. Similarly, Ca^{45} appeared in MB at a rate almost equal to the initial rate of loss from FB. The quantity of Ca crossing the placenta daily was at least 6-10 times that required for fetal skeletal growth.

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Effect of Wheat Germ Lipase on Human Cells Transformed *in vitro* By Simian Virus 40. (30216)

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In recent years, increased attention has been directed to surface changes as an important feature in malignant transformation. The work of Coman, Abercrombie, Ambrose and Easty has established the existence of major differences in the surface structure of normal and neoplastic cells(1-9). The striking lack of adhesiveness, of contact inhibition, *i.e.*, inhibition of movement on contact, and of organized cell arrangements which characterize most tumor cells are thought to be due to alterations in surface structure of the external membrane during transformation of a normal to a neoplastic cell(5). The finding of Ambrose *et al*(9) that wheat germ lipase (WGL) acts selectively on the growth of tumor cells in culture when compared with homologous normal cells, and other work on cell interactions with this enzyme(8,10-12), were further attempts to define the nature of this surface change. Changes in the surface membrane have also been observed in cells transformed by viruses, and are believed to be of importance in viral carcinogenesis(13),