described phenomena; in fact, some of our observations as yet elude adequate explanation. Nonetheless, it has been demonstrated that mitochondria have an individual K metabolism and certain apparently specific mechanisms have been elicited. The observations on the effects of DNP at high concentrations suggest that complexes may be formed between mitochondria, phosphorylated derivatives and K; breakdown and resynthesis of such complexes could be responsible for the observed exchange of mitochondrial K. If such a mechanism occurs in the intact cell it could conceivably be utilized for the function of K accumulation; these mitochondrial mechanisms may constitute an "ion-complex carrier" for K.

Summary. Rabbit liver mitochondria contain K which cannot be removed by repeated washings with NaCl solutions at 2°C. This K fraction exchanges with ambient K under appropriate metabolic conditions, indicating a dependence of K metabolism on aerobic oxidation. Orthophosphate depresses the mitochondrial K level. A newly observed polyphasic action of DNP indicates a specific relationship between mitochondrial ester phosphate and K exchange.

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Determination of Deuterium Exchange Rates Between Maternal Circulation and Amniotic Fluid.* (20217)

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The function of the amniotic fluid is clearly defined but its origin, rate of formation and its fate represent controversial subjects among investigators in this field. Morphologic studies on cellular structures involved did not supply an adequate body of information to explain the formation of large amounts of amniotic fluid in certain pathologic states nor could it explain the rapid rate of replacement in the normal pregnant organism as demonstrated by Flexner and his associates(1). These authors reached the conclusion that the amniotic fluid is completely replaced once every 2.9 hours. Such a rapid turnover rate is contrary to the prevailing opinion that the amniotic fluid represents a stagnant pool or consists of fetal urine which is swallowed by the baby. Since it would be difficult to present an acceptable technical, mechanical or morphologic explanation for the phenomenon described by Flexner, a critical examination of these experiments is in order. As a working hypothesis, one may regard the pregnant organism as a multi-compartment system which should lend itself to a mathematical approach provided certain reservations are kept in mind. Flexner and associates considered this approach but apparently discarded it in favor of approximations which are not entirely above criticism. The theoretically correct treatment of multicompartment sys-

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tems of the type described requires the exponential approach which has been investigated theoretically(2) and practically(3). It is known that labelling of the water in maternal plasma results in a gradual rise of the tracer in amniotic fluid and, conversely, one may assume that labelling the water of amniotic fluid eventually will result in an increase of tracer in the water of maternal plasma. For the purpose of the present treatment the desired information can be obtained by simplification ("lumping") to a two compartment system consisting of (1) the amniotic fluid and (2) the total body water of mother and fetus. Such a two compartment system should lend itself to the proper treatment in order to calculate the exchange (transfer) rates, a calculation which would be based on a series of determinations throughout the whole experimental period, and not on a single initial reading, as in the experiments of Flexner.

In order to determine the exchange rates or transfer rates of a compound or element in a 2-compartment system, 2 isotopes are theoretically necessary, if the rates in both directions are to be determined simultaneously. An alternate approach would be the application of one isotope in 2 different experiments where the isotope is added first to Compartment 1 and then, in a second experiment, added to Compartment 2. Since we had only one isotope of hydrogen available, the second approach was used. The treatment of multicompartment systems as outlined by Sheppard and Householder, can be used to calculate the transfer rates. The assumptions necessary for the application of Sheppard's equations are essentially those of any application of tracer experiments to biologic problems(2,4,5) and, in addition, it is assumed that deuterium is exchanged at equal and opposite rates. For this specific case the integrated equations are:

$$\frac{a_1}{a_{1(0)}} = \frac{S_1 - S_2 e^{-\rho t(1/S_1 + 1/S_2)}}{S_1 + S_2}$$

$$\frac{a_2}{a_{1(0)}} = \frac{S_1\left(1 - e^{-\rho t(1/S_1 + 1/S_2)}\right)}{S_1 + S_2} \qquad (1)$$

where a_1 and a_2 refer to the deuterium oxide concentrations in compartment 1 and 2 at time t, $a_{1(0)}$ the initial deuterium oxide concentration in compartment 1, ρ the exchange rate and, S_1 and S_2 the quantity of exchangeable species in compartment 1 and 2. Subtracting these equations leads to the expression

$$\mathrm{Log_e}\left[\frac{a - a}{a_{1(0)}}\right] = \rho t(1/S_1 + 1/S_2) = \frac{0.69\,t}{T_{1/2}} \qquad (2)$$

A knowledge of the equilibrium D_2O concentration in maternal blood or amniotic fluid can be used to determine the total water *i.e.*, the sum of S_1 and S_2. The volume of the amniotic fluid can be determined by direct measurement after delivery or by extrapolation of the function

$$\mathrm{Log_e}\left[\frac{(D_2O)_t}{(D_2O)_{eq.}} - 1\right] = k\,t \qquad (3)$$

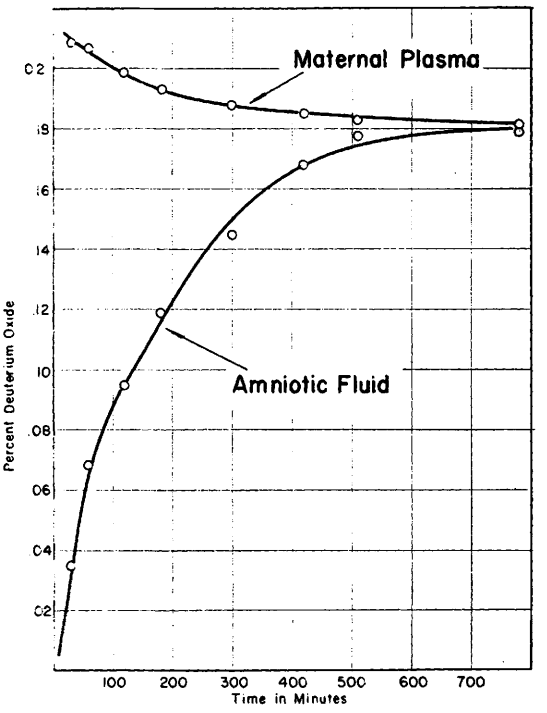

FIG. 1. Change in deuterium oxide concentration in maternal plasma and amniotic fluid following intravenous administration of 60 cc of deuterium oxide.

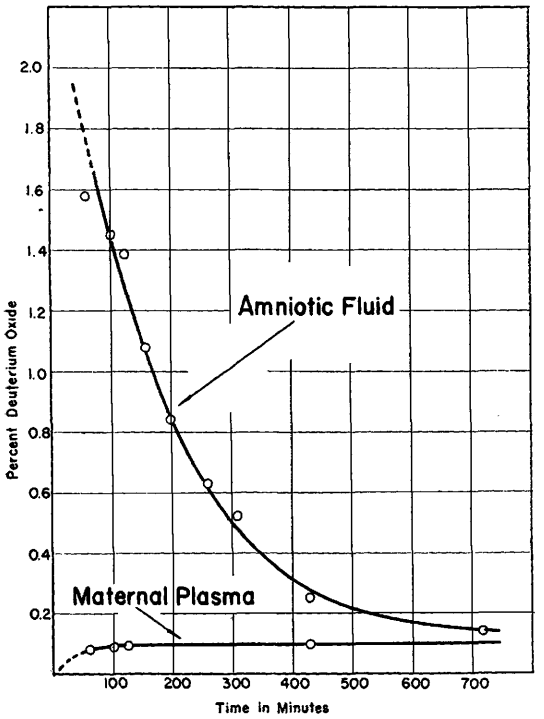

FIG. 2. Change in deuterium oxide concentration in maternal plasma and amniotic fluid following administration of 40 cc of deuterium oxide into the amniotic sac.

to zero time(5). If the assumption of equal and opposite rates is correct, substitution of the appropriate values in equation (2) should give a straight line when the values $a_1 - a_2/a_1(0)$ are plotted against time on a semi-logarithmic scale. The slope of this line then determines the half value time ($T_{½}$) and therefore the exchange rate ρ.

Experimental. (1) In the first of these experiments a normal ante-partum patient at term received 60 cc of deuterium oxide intravenously several hours before an elective Caesarean section. A sterile polyethylene catheter was introduced into the amniotic sac after transabdominal puncture by means of a Huber tip needle. Samples of amniotic fluid and maternal venous blood were then collected at pre-determined intervals and the concentration of the tracer determined by methods previously described(6,7). The results of this experiment are reproduced graphically in Fig. 1.

(2) In order to determine the transfer rate from amniotic fluid to maternal and fetal systems, the conditions of the experiment were essentially the same except that here the tracer was placed into the amniotic sac rather than the maternal system. Because of the smaller volume of the primary compartment in this experiment, slightly less deuterium oxide was used. The results of this experiment are given in graphic form in Fig. 2.

Both experiments terminated at the time of delivery by Caesarean section. A sample of cord blood, obtained just before clamping the cord, was analyzed for its deuterium oxide content. In both experiments the concentration of tracer in cord blood was found to be the same as that of the maternal plasma.

The half value time was then calculated from a plot of the function $a_1 - a_2/a_{1(0)}$ against time (Fig. 3). Substitution of the appropriate values for S_1 and S_2 in equation (2) was then used to calculate the exchange rate ρ, as 9.71 cc/min for the first experiment and 10.8 cc/min for the second. This corresponds to an exchange rate of 582 cc/hr and 648 cc/hr respectively. These values are in close agreement with, though somewhat higher

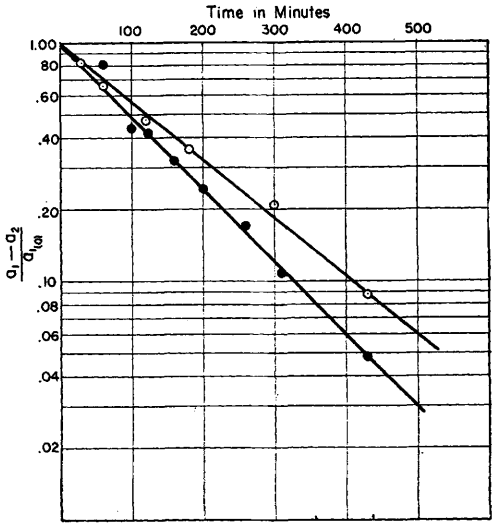

FIG. 3. Semi-log. plot of the function $a_1 - a_2/a_{1(0)}$ against time. Open circles correspond to values recorded in Fig. 1 and solid circles to those of Fig. 2. Index figure "1" refers to the primary compartment *i.e.*, the compartment which originally contained all the tracer while index figure "2" is intended to designate the secondary compartment, originally containing none of the tracer. In the 2 experiments, the order is appropriately reversed.

than, the estimations of Flexner and his associates.

Summary and conclusions. 1. Regarding the pregnant organism as a multi-compartment system the exchange rate of the water in amniotic fluid with the water of the maternal and fetal systems, was determined. By the application of trans-abdominal catheters, placed into the amniotic sac of pregnant women at term, amniotic fluid could be withdrawn at desired intervals without disturbing the continuity of the system. The mechanism of tracer (deuterium) exchange was demonstrated to be that demanded by theory, and the application of well known theoretic equations to the data so obtained permitted the calculation of absolute exchange rates. 2. In 2 experiments where the isotopic tracer was first placed in one compartment (mother) and then in the other (amniotic fluid) the exchange rates in both directions were found to be about 600 cc per hour. The observations of Flexner and his associates on the exchange rate of the water of the amniotic fluid are thus confirmed in principle.

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Effects of Sex Hormones on Serum Lipoproteins in Rabbits.* (20218)

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Ultracentrifugal analyses of blood serum in human beings have indicated differences in the lipoprotein pattern between male and female: beta-lipoprotein concentrations are approximately 25% smaller in women than men (1) and, "The incidence of measurable concentrations of the Sf 10-20 class is significantly higher in males from 20 to 40 years of age than in females of the same age group"(2). In similar investigations, Barr, *et al.*(3) using alcohol fractionation technic, found that administration of estrogens to atherosclerotic men resulted in an increase in alpha and a decrease in beta-lipoproteins. However, Glass, *et al.*(4), did not observe any change in the serum lipids or low density lipoproteins (Sf 12-20) following treatment with estradiol of 55-65-year-old male and female patients.

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There are thus indications that androgens and estrogens may have opposite effects on lipoproteins and, therefore, be important in the regulation of lipoprotein concentration. Since androgens have not been studied, the present investigation was undertaken in rabbits subjected to alternate treatment with stilbestrol and testosterone.

Methods. Nine sexually immature rabbits (5 males and 4 females) of mixed strain with an average body weight of 1.35 Kg were kept without treatment during a preliminary period of 15 days, at the end of which they were castrated. During the following 5 months they were treated with stilbestrol or testosterone; each period of treatment was followed by a control period in order to permit a complete resorption of the drug. Diethylstilbestrol in oil solution was injected subcutaneously at the daily dose of 1 mg; testosterone in aqueous crystalline suspension was injected at the