THE TOTAL BODY WATER AND THE WATER TURNOVER IN PREGNANCY STUDIED WITH DEUTERIUM OXIDE AS ISOTOPIC TRACER [1]

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The status of pregnancy profoundly affects the maternal metabolism. By means of isotopic tracers over-all changes in metabolic rates of specific substances can, with certain limitations, be studied. This investigation is concerned with the demonstration of water turnover rates during various stages of pregnancy and an effort is made to define their physiologic and pathologic variations.

Based upon clinical evidence, appreciable variations in the state of hydration are expected as pregnancy progresses and even greater variations may be anticipated under certain pathologic conditions. For this reason the water economy of the pregnant woman is of considerable interest to the clinician. Numerous investigations have been carried out to determine changes in specific fluid compartments during pregnancy. Except for one preliminary note on this subject (1) the important total body water content has not been investigated by the isotope dilution method.

Since the distribution of deuterium oxide and its disappearance rate are intimately related terms, the present study also includes an evaluation of the total body water and its changes during pregnancy.

CLINICAL MATERIAL

Six normal healthy pregnant volunteers registered in the antepartum clinic were selected for this study. They were followed throughout their ante-partum course at routine intervals and, with the exception of one patient (D. E.), their pregnancies were uneventful. Total body water and disappearance constants were determined whenever possible once during each trimester and in the immediate post-partum period. These determinations were so spaced that a reasonably even distribution of the data was obtained over the whole period of gestation. Because very few patients present themselves for registration during the first trimester, the data collected cover the period from the 13th to the 41st week of gestation.

Since it was not possible to predict, in the initial selection of patients the eventual development of toxemia of pregnancy, the "hypertensive" and "pre-eclamptic" patients were chosen from ward material after the diagnosis had been established on clinical evidence.

METHODS AND MATERIALS

Total body water: The total body water determinations were carried out according to a procedure described in the literature (2). A few minor modifications of this procedure were adapted which were intended to simplify the procedure and to increase the safety of the deuterium oxide administration.

An accurately known volume (40 to 45 cc.) of sterile pyrogen-free deuterium oxide containing 0.85 per cent sodium chloride, was administered intravenously from an analytic burette which, in turn, was connected to a slow infusion of normal saline or 5 per cent glucose. The weight of deuterium oxide administered was calculated from known specific gravity data (3).

Samples of venous blood were then withdrawn from the patient at intervals ranging from 2 hours to 20 days. The samples were allowed to clot and the sera separated in the usual manner. The samples were then stored in frozen state until ready for the determination of their deuterium oxide content.

The concentration of deuterium oxide in the sera was determined by the "Falling Drop Method" as described by Fetcher (4) and other investigators (5). Primary standards of deuterium oxide and secondary standards of sodium chloride were used for comparison. The former have the disadvantage of a certain degree of instability as evidenced by a gradual decline in the deuterium oxide concentration with time, presumably due to a slow exchange of deuterium between solution and container.

Turnover rate: Following the initial body water determinations, samples of venous blood were withdrawn at frequent intervals and the deuterium oxide concentration determined as described above. The data so obtained were then plotted on a semi-logarithmic scale. They proved to be linear within the experimental error of the method. The numerical values of the constant for the "late disappearance curve" were then calculated from the exponential equation

$$(D_2O)_t = (D_2O)_0^{-\lambda t}$$

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TABLE I

Representative experiments on the determination of the equilibrium time in normal pregnant patients during the second and third trimester

Patient	Elapsed time after D_2O infusion *min.*	D_2O concentration in plasma *wt. %*
L. W. (term)	30	0.209
	60	0.207
	120	0.198
	180	0.193
	300	0.186
	430	0.185
M. H. (term)	40	0.140
	75	0.134
	140	0.132
	195	0.128
	270	0.130
	360	0.129
M. S. (3rd trim)	25	0.140
	50	0.121
	90	0.111
	120	0.101
	180	0.100
	300	0.099
N. B. (2nd trim)	60	0.131
	120	0.124
	180	0.123
	300	0.125

where the value of λ is proportional to the "turnover rate" and represents that fraction of the total body water "turned-over" per unit of time "t." $(D_2O)_o$ is the concentration of deuterium oxide in pure water expressed in per cent obtained at the beginning of the experimental period (the equilibrium value) and $(D_2O)_t$ the concentration after an elapsed time interval of t days. The turnover rate is expressed in per cent of total body water and its dimensions are, therefore, days^{-1}. The various derivatives of this value, *e.g.,* the "turnover time" and "half life time," were calculated according to the formulae given in Table III.

RESULTS

The determination of total body water by the isotope dilution method and its clinical application has been ably discussed (5). In contrast to the normal human an additional and progressively enlarging pool of water is present during pregnancy, hence a re-examination of at least one of the basic principles of this method, the determination of the equilibrium time, seems in order.

While there is little reason to assume that the mechanism of deuterium oxide distribution would be significantly different in the pregnant and nonpregnant organism, the time necessary for the establishment of an equilibrium may be considerably prolonged. It was, therefore, necessary to determine this equilibrium time beyond which no significant decrease in deuterium oxide concentration of the serum or plasma would occur.

Deuterium oxide was administered in a series of pilot experiments to a number of normal and toxemic patients and frequent blood samples withdrawn for analytic determinations. The results on a series of these experiments are reproduced

TABLE II

A correlation of total body water (deuterium oxide space) and total body solids in a series of uncomplicated pregnancies

Period of gestation *weeks*	Body weight *Kg.*	Deuterium oxide space *L.*	Deuterium oxide space *%*	Total solids *Kg.*
Patient R. D.				
Pre-pregnancy	52.0	—	—	—
13	52.3	31.3	59.8	21.0
30	60.9	34.4	56.4	26.5
36	60.9	36.1	59.2	24.8
41 (p.p.)	55.5	29.2	52.6	26.3
Patient A. N.				
Pre-pregnancy	55.0	—	—	—
21	56.4	33.9	60.0	22.5
33	62.7	36.7	58.5	26.0
41 (p.p.)	56.8	33.0	58.1	23.8
Patient D. E.				
Pre-pregnancy	60.0	—	—	—
19	61.8	32.1	51.9	29.7
33	69.5	34.2	49.1	35.4
41 (p.p.)	62.0	31.6	50.9	30.4
Patient C. B.				
Pre-pregnancy	50.0	—	—	—
21	49.9	28.5	57.1	21.4
31	62.6	33.6	53.7	29.0
38	64.2	34.2	53.3	30.0
41 (p.p.)	57.8	31.5	54.4	26.3
Patient N. B.				
Pre-pregnancy	53.0	—	—	—
16	54.4	29.6	54.4	24.8
37	65.8	37.9	57.6	27.9
41 (p.p.)	58.6	30.6	52.2	28.0
Patient A. M.				
Pre-pregnancy	unknown	—	—	—
16	79.4	38.5	48.5	40.9
36	80.8	39.2	48.5	41.6
41 (p.p.)	76.6	33.5	43.7	43.1

in Table I which indicate that for the purpose of the present study a satisfactory distribution seems to be established within a period of two, or at the most, three hours. For routine total body water determinations the equilibrium deuterium oxide concentration was, therefore, derived from control sera and sera obtained 2 or 3 hours after the intravenous infusion of the test dose.

The large body of dependent variables makes the presentation of accumulated data in compact tabular or graphic form impractical. In Table II the data related to total body water of normal pregnant patients are arranged according to subjects rather than the corresponding period of pregnancy.

It is apparent that the relative values for the total body water show considerable variations if one individual patient is compared to another but within certain limits each patient shows a characteristic pattern of changes as pregnancy progresses. The total body water expressed as per cent of body weight exhibits no consistent changes during the second and third trimester but drops to its lowest value in the immediate post-partum period. The absolute values during the same period show a persistent rise followed by the expected drop after delivery; they have only a superficial correlation to body weights.

The total solids, representing the difference between body weight and deuterium oxide space, reflect these changes by a consistent rise during pregnancy and an appreciable net gain when the first trimester is compared with the puerperium.

The late disappearance constants and their related values are reproduced in Table III. Except during the immediate post-partum period the turnover of water remained constant within the limits designated by previous investigators (2). In one patient (D. E.), who eventually developed signs of toxemia during the end of her pregnancy, the turn-over rate was considerably and consistently higher than the upper limits of normal as defined by these authors.

In the immediate post-partum period the disappearance of injected deuterium oxide no longer follows the expected dieaway curve, the mathematical representation of which is given in the exponental relation outlined above. The linear relation of a semilog plot is lost. After the intravenous administration of deuterium oxide an equilibrium is rapidly established, but the concentration of the tracer remained almost constant over the first 24 hours. It then declined at rates which exceeded the values for the ante-partum period. An approximately linear relationship was obtained between the second or sixth day and from

TABLE III

The disappearance constants, half life of deuterium oxide, turnover time and total turnover in a series of uncomplicated pregnancies

Period of gestation	Disappearance constant in days^{-1}	Half life *days**	Turnover time in days†	Total turnover in liters/day‡
		Patient R. D.		
13	0.0836	8.28	12.0	2.62
30	0.0777	8.91	12.9	2.67
36	0.0740	9.36	13.5	2.67
41 (p.p.)	0.0810	8.55	12.3	2.36
		Patient A. N.		
21	0.0794	8.72	12.6	2.69
33	0.0880	7.86	11.4	3.23
41 (p.p.)(a)§	0.236	2.93	4.24	7.78
(b)	0.128	5.41	7.81	4.22
		Patient D. E.		
19	0.128	5.41	7.81	4.11
33	0.144	4.81	6.94	4.91
41 (p.p.)	0.096	7.21	10.4	3.03
		Patient C. B.		
21	0.079	8.77	12.6	2.25
31	0.086	8.06	11.6	2.89
38	0.084	8.25	11.9	2.87
41 (p.p.)(a)	0.173	4.00	5.78	5.45
(b)	0.089	7.78	11.24	2.80
		Patient N. B.		
16	0.075	9.24	13.33	2.22
37	0.084	8.25	11.90	3.18
41 (p.p.)	0.081	8.56	12.35	2.48
		Patient A. M.		
16	0.089	7.79	11.23	3.43
36	0.074	9.36	13.51	2.90
41 (p.p.)	0.093	7.45	10.75	3.12

* Half life time = 0.693/disappearance constant.
† Turnover time = 1.00/disappearance constant.
‡ Total turnover = (Total Body Water) (Disappearance Constant) (100).
§ (a) and (b) refer to two consecutive compensatory phases during the postpartum period. (a) is the period of diuresis.

TABLE IV

The disappearance constants, half life of deuterium oxide and turnover time in a series of toxemic patients

Patient	Disappearance constant in $days^{-1}$	Half life *days*	Turnover time *days*	Clinical diagnosis
M. W.	0.123	5.63	8.1	Hypertensive
S. M.	0.071	9.76	14.1	Hypertensive
McQ.	0.092	7.53	10.9	Hypertensive
I. R.	0.095	7.29	10.5	Hypertensive
M. B.	0.042	16.5	23.8	Pre-eclamptic
C. McG.	0.070	9.90	14.3	Pre-eclamptic
M. R.	0.040	17.3	25.0	Pre-eclamptic
E. D.	0.060	11.5	16.7	Pre-eclamptic
E. G.	0.064	10.8	15.6	Pre-eclamptic
E. D. (II)	0.065	10.6	15.4	Pre-eclamptic

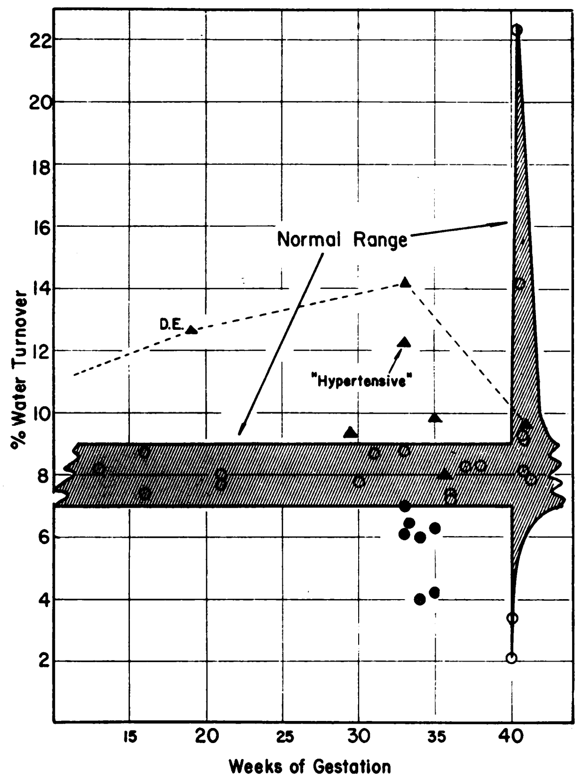

FIG. 1. PER CENT OF TOTAL BODY WATER TURNOVER AT VARIOUS GESTATIONAL PERIODS DURING NORMAL AND PATHOLOGIC PREGNANCIES

The open circles represent values obtained for normal patients. The closed circles correspond to values obtained on patients with a clinical diagnosis of pre-eclampsia and the solid triangles to hypertensive patients.

then on the rate of turnover seemed to approach the ante-partum values.

Patients with clinical diagnoses of toxemia of pregnancy, *e.g.,* hypertensive disease or pre-eclampsia, were subjected to similar tests. The turnover rate in patients who, on the basis of clinical evidence, were classified as pre-eclamptic, was appreciably lower than the turnover rate of normal pregnant women of the same gestational period. The patients with documented essential hypertension had a turnover rate which exceeded the normal range. The numerical values are given in Table IV and, in order to emphasize the difference between normal and toxemic patients, the data on both are represented graphically in Figure 1.

DISCUSSION

The relative changes in the fluid compartments during pregnancy have been the subject of numerous investigations (6–8). These changes are relative and occur within the aqueous phase; their sum should be equal to the changes in total body water which is presumably identical with changes in the "deuterium oxide space." The over-all changes of the aqueous phase have recently been investigated by the antipyrene dilution method (8) but the application of this method to an abnormal state might be subject to criticism. The determination of total body water by the isotope dilution method should be more reliable because the exchangeable species is not an exogenous or foreign substance. It may be assumed that the isotope will mix in the available pool of water in all abnormal states, but that the time necessary to reach an equilibrium may be considerably prolonged. Before applying the deuterium oxide dilution method to the pregnant organism it would be necessary to demonstrate the minimum length of time necessary for the establishment of an equilibrium. Plentl and Hutchinson (9) have shown that the water exchange between mother and amniotic fluid at term amounts to about 600 cc. per hour and that the analogous exchange between mother and fetus is an even more rapid process. Although the products of gestation constitute the largest contribution to the enlarging pool of water during pregnancy the exchange of deuterium oxide appears to be as fast as in other compartments of the pregnant or non-pregnant organism.

Table I demonstrates that for all practical purposes the equilibrium is established within a period of 2 hours which confirms the preliminary report of Haley and Woodbury (1) and the incidental data reported by Plentl and Hutchinson (9). Unless an unprecedented isotope effect or grossly abnormal conditions prevail (hydramnios) one may assume that the deuterium oxide dilution method can be used for the determination of the total body water in pregnant patients.

Although it is not surprising that there exists a rough correlation between body weight and total body water, this correlation is not as close as one might expect. The lack of parallel changes in body weight and total body water can only be explained by changes in the remaining total solids, *i.e.*, fat and lean body mass. These values cannot be determined directly and depend, therefore, upon the accuracy of the measurable indices of body weight and total body water. Since the precision of both methods is somewhat better than 2 per cent the changes for total body solids assumed significance. The assumption of a constant ratio of fat and lean body mass as advocated by Messinger and Steele (10) may hold under normal circumstances, but whether it can be applied to the abnormal circumstance of pregnancy remains to be determined. If it were applied to our data it would lead to the very plausible conclusion that a normal pregnancy is associated with a relative and absolute net gain of fat.

A similar approach cannot be taken in the study of the limited number of toxemic patients because here the continuity is lacking and a comparison to earlier stages on the same patients is not possible. An interpretation of the relative proportion of water and total body solids would have to be made by cross comparison and, therefore, require statistical interpretation for which the number of our subjects is too small.

The turnover rate of water in the animal and the human, originally investigated by Hevesy and Hofer (11), has been studied in great detail (2) in an effort to establish the limits within which variations of the normal adult human could be expected. These authors found the disappearance constant to be 0.077 per day or a turnover rate of 7.7 per cent of total body water per day with a standard deviation of $\pm$ 1.2 per cent. A difference between male and female subjects was not apparent. They suggested that changes in turnover rates might be of interest in various diseases or abnormal states even though the significance of these changes need not be clearly understood.

Haley and Woodbury (1) determined the deuterium oxide turnover rates on three normal pregnant patients. They observed that the water turnover of these patients did not materially differ from the non-pregnant. Our experiments which had been carried out concurrently with those of Haley and Woodbury, confirm these findings in principle.

The period of gestation represents a continuous change and the selection of patients at an arbitrary stage of pregnancy would not permit valid conclusions to be drawn for the whole period. We have, therefore, selected our patients in the early stages of pregnancy and determined the turnover rates at convenient intervals at least once during each trimester and the immediate post-partum period. An even distribution of these data could also be used for cross-comparison and it would seem unlikely that periods of unusual changes in water turnover have been missed. Although we have not obtained any experimental data during the earliest phases of pregnancy it may safely be assumed that at least the turnover rates (but not the total body water) would not differ appreciably from those of the later stages. Thus, during the second and third trimester and up to the onset of labor the water turnover rates of normal pregnant patients are of the same magnitude as those of non-pregnant humans, although significant changes in the fluid compartments can be demonstrated. The event of parturition is associated with an appreciable, immediate and sudden loss of water attributed to amniotic fluid, fetus and blood to which the maternal organism has to adjust itself. This adjustment apparently takes about 2 to 5 days during which time the disappearance constants exhibit a peculiar but nevertheless consistent pattern. The normal patients of this series showed only minor changes in weight during the first few post-partum days and, of course, remained constant thereafter. If deuterium oxide is administered immediately after delivery or as soon as acute water loss in whatever form had ceased, the water turnover rate is extremely slow over the first 24 hours, rises rapidly to almost twice its antepartum levels and then returns to normal sometime between the second and fifth day. These changes in the disappearance rate of deuterium oxide are explained by the well known post-partum-diuresis which is followed by a compensatory water intake. During the immediate post-partum period when no change in the deuterium oxide concentration of the patients plasma could be demonstrated, the diuresis alone was the significant event. The subsequent increase in water intake without an immediate decline in water excretion was reflected in a rise in turnover rate. When the period of diuresis was over, the water intake also resumed normal proportions and the turnover rates returned to normal levels. Thus, except for the initial period of decreased water consumption and the early phases of diuresis these patients were in a "steady state" and apparently did not change the size of the available water pool.

In the light of the above it should not be surprising to find that patients diagnosed as pre-eclamptics with frank or occult edema should have a slower turnover of water than normal patients. The turnover time for these patients, *i.e.*, the average length of time of a molecule of water or deuterium oxide to remain in the body, is considerably increased, presumably because (*1*) the available pool is larger, or (*2*) there is an inherent defect in water metabolism. Whether only one or both of these possibilities should be considered cannot be decided at this time. Our observations rest upon fairly convincing and consistent experimental evidence but its detailed explanation would have to await further study.

Whatever its explanation may be, it is hoped that a decreased water turnover may eventually be used as an objective diagnostic tool. The extreme sensitivity of this index of water metabolism could, under the proper circumstances, be used as a guide in the therapy of such patients.

Because the differential diagnosis of hypertensive and pre-eclamptic toxemia occasionally presents serious difficulties, the pattern of an objective test can only be applied with caution. Although the turnover rates of hypertensive patients were considerably higher than normal (Figure 1), this observation needs confirmation on a larger number of documented hypertensive patients. Since the well known renal pathologic changes of the hypertensive are likely to pre-dispose these patients to a more or less pronounced diuresis, an increased water turnover would not be incompatible with this picture. Conversely, hypertensive patients with edema due to excessive sodium intake, or cardiac decompensation might simulate a normal water turnover or show a depression to within the pre-eclamptic range. A knowledge of water turnover rates should, therefore, be interpreted in the light of other significant data in order to serve as a diagnostic aid.

CONCLUSIONS

1. The total body water content was determined on six normal pregnant women at least once during each trimester. The changes during normal pregnancies were found to be an absolute rise in total body water and total solids. A comparison of the early phases of pregnancy with the post-partum period indicates a relative loss of total body water and a relative gain in total body solids.

2. The time necessary to establish an equilibrium between injected deuterium oxide and body water did not differ appreciably from the equilibrium time of the non-pregnant normal human. The disappearance constants and turnover rates for deuterium oxide were found to be within the limits reported for the normal non-pregnant female.

3. The water turnover rates for pre-eclamptic patients were found to be lower than those of normal patients at comparable stages of pregnancy. Hypertensive patients showed inconsistent turnover rates which were either faster than normal or within the normal range.

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