tually devoid of anticonvulsant activity as measured by a number of diverse laboratory screening procedures.

1. Tower, D. B., *Neurology,* 1955, v5, 113.
2. Swinyard, E. A., *J. Am. Pharm. A.* (Sc. Ed.), 1949, v38, 201.
3. Swinyard, E. A., Brown, W. C., and Goodman, L. S., *J. Pharmacol. and Exp. Therap.,* 1952, v106, 319.
4. Brown, W. C., Schiffman, D. O., Swinyard, E. A., and Goodman, L. S., *ibid.,* 1952, v107, 273.
5. Goodman, L. S., Grewal, M. S., Brown, W. C., and Swinyard, E. A., *ibid.,* 1953, v108, 168.
6. Woodbury, L. A., and Davenport, V. D., *Arch. internat. de pharmacodyn. et de thérap.,* 1952, v92, 97.
7. Swinyard, E. A., Schiffman, D. O., and Goodman, L. S., *J. Pharmacol. and Exp. Therap.,* 1955, v114, 190.
8. Davenport, V. D., and Davenport, H. W., *J. Nutrition,* 1948, v36, 139.
9. Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharmacol. and Exp. Therap.,* 1949, v96, 99.
10. Ginsberg, B. E., *Res. Publ. Assn. nerv. ment. Dis.,* 1954, v33, 39.

Received November 2, 1956. P.S.E.B.M., 1957, v94.

D_2O Equilibration Rates in Thermally Injured Animals.* (22843)

CHARLES S. HARRISON (Introduced by Helene W. Toolan)
Surgical Research Unit, Brooke Army Medical Center, Ft. Sam Houston, Texas.

There have been numerous attempts to make *in vivo* dissections of the human body to understand better the phenomena which occur in both normal and pathologic states. Isotopes have facilitated this work; but no isotope is ideal since each has its own peculiar advantages and disadvantages. Most investigators consider deuterium oxide the isotope of choice in measuring total body water. The principle of isotope dilution is well known and accepted; and it is dependent upon knowing the amount of substance injected, amount of substance excreted prior to equilibrium, and concentration of this substance in the body at equilibrium. In the majority of cases, equilibrium of D_2O in the normal state will be attained in 1 to 2 hours. Some investigators have allowed 3 hours for D_2O to equilibrate in certain pathologic states; thus they imply some delay in equilibration(6). Others insist that time to equilibrium is even longer(5). This is particularly true with ascites.

It is most important to determine total body water in any study of profound alterations in fluids and electrolytes following thermal injury. Determinations of total body water are also used with determinations of extracellular fluid space, to measure intracellular water compartment. It has been questioned as to whether or not D_2O would equilibrate within a 2- or 3-hour period in subjects when there were large edematous areas as a result of thermal injury. For this reason, equilibration studies were made on thermally-traumatized animals to determine whether or not the time to equilibrium was prolonged. Each animal acted as its own control.

Materials and methods. Sixteen adult mongrel dogs were used, average weight 16.8 kg, ranging from 12.7 to 23.0 kg. Each animal was weighed 3 times a week until its weight had stabilized, usually in 4 to 6 weeks. Food was withheld from animals for 12 hours prior to experiment; however, all animals had free access to water until 4 hours prior to experiment. Dogs were anesthetized with intravenous phenobarbital anesthesia (30 mg/kg). Femoral vessels were exposed through small incision in the groin and both femoral artery and femoral vein were cannulated with polyethylene tubing. Sterile D_2O (purity greater than 99.5%) as isotonic saline was obtained in 50 g ampules (Abbott). Twenty cc of D_2O-saline solution were injected into femoral vein through a calibrated syringe. The polyethylene tubing was rinsed repeatedly with

* The author appreciates assistance of Hubert Lindsay.

TABLE I. Comparison of D_2O Equilibration Rates in 16 Thermally Injured Animals.

Pre-injury				Post-injury				Diff. in time to equilibrium
Wt (kg)	Vol of distribution	% body wt	Min. to equilibrium	Wt (kg)	Vol of distribution	% body wt	Min. to equilibrium	
15.0	8.92	59.47	240	15.6	10.60	67.95	150	− 90
14.3	8.20	57.34	90	**16.3**	**7.53**	**46.20**	**90**	**0**
16.7	10.85	64.97	180	16.7	11.03	66.25	180	0
14.8	9.17	61.96	150	13.3	9.81	73.76	150	0
14.1	8.77	62.20	90	14.7	9.36	63.67	240	+150
18.2	9.71	53.35	90	18.7	10.91	58.34	90	0
16.9	10.94	64.73	150	17.5	10.64	60.80	90	− 60
12.7	6.94	54.65	150	13.9	6.85	49.28	150	0
17.2	11.51	66.92	150	17.8	12.03	67.58	150	0
19.7	9.62	48.83	90	19.1	10.38	54.35	150	+ 60
16.0	9.60	60.00	90	19.5	9.66	49.54	180	+ 90
22.5	11.83	52.58	150	22.1	13.41	60.68	150	0
23.0	15.77	68.57	90	24.1	17.39	72.16	180	+ 90
19.9	13.87	69.70	90	21.1	14.00	66.35	90	0
14.8	9.87	66.69	90	14.5	10.74	74.07	150	+ 60
13.3	7.88	59.25	180	13.1	7.91	60.38	180	0
Avg 16.8	10.22	60.70	129.4	17.37	10.77	61.96	148.1	18.75
		S.D. ± 46.11				S.D. ± 41.67		

small amounts of normal saline. Blood samples were taken from femoral artery at 90, 150, 180, 240, 270, and 300 minutes following injection. Blood samples were collected in small glass ampules, flame-sealed immediately after collection. At least 3 weeks elapsed between studies. In some cases the interval was as long as 8 weeks. The animals were anesthetized again in identical manner as previously. A standard, body-surface injury was produced by immersing the lower half of the animal to a point midway between groin and umbilicus in water at 90°C for 20 seconds. Following thermal injury, animals were observed for 8 hours during which time no fluid therapy was administered. Minimal amounts of pentobarbital were given to keep animals comfortable. *Femoral vessels* in unoperated leg were exposed and cannulated at the end of 8-hour waiting period, the cannulae being threaded well up into the common iliac vessels. Twenty cc of D_2O-saline solution were injected into the femoral vein from a calibrated syringe. Blood samples were collected from femoral artery catheter by the same method and time intervals as those in the initial study. *Plasma samples* were prepared by vacuum-distillation technic and the D_2O levels of these samples were determined by the falling drop method(2).

Results of the experiment are summarized in Table I. The average weight of animals in the preburn series was 16.8 kg; in postburn series 17.4 kg. The smallest animal weighed 12.7 kg. Smaller animals were not used because the mortality rate during postthermal injury 8-hour waiting period was much greater than in larger animals. Weight variation of each animal was directly related to length of time between the two procedures, *i.e.*, dog No. 11 gained 3.5 kg in 2-month interval.

The average D_2O volume of distribution was 10.32 l in the preburn series and 10.77 in the postburn series. Using the paired "t" test, a statistical analysis was made on differences of columns of distribution between preburn and postburn series using animals as their own controls. There was no statistically significant difference. The standard deviation of differences of volumes of distribution was 0.707 liter. The standard deviation of the mean of the differences was 0.177 liter. Replicate determinations of volumes of distribution of D_2O in normal animals have been made in this laboratory and the standard deviation was 0.510 liter. The time interval between these replicate determinations ranged from 3 to 8 weeks.

In this experiment, volume of distribution as per cent of body weight was 60.7 in the preburn series and 61.96 in the postburn se-

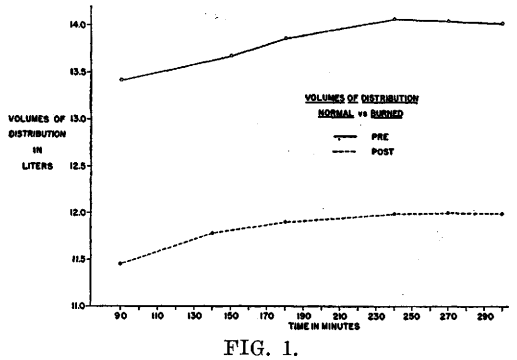

FIG. 1.

ries. Using the paired "t" test, a statistical analysis was made. There were no significant differences between volumes of distribution recorded (as per cent of body weight) between preburn and postburn series. The standard deviation was 6.67% of body weight.

D_2O concentration in the plasma specimen was determined; and volume of distribution was calculated at each time interval. The volumes of distribution were plotted on arithmetic paper. The time to equilibrium was selected as that interval when volume of distribution was within 5% of final volume of distribution at 300 minutes (Fig. 1).

The average time for D_2O to equilibrate in the preburn animal was 129.4 minutes. Standard deviation was 46.11 minutes. The time necessary for equilibration was somewhat longer in the postburn series. The average time was 148.1 minutes, with standard deviation of 41.67 minutes. Using the paired "t" test, a statistical analysis disclosed no statistically significant differences between the time necessary for equilibration of D_2O in preburn *vs.* the postburn series. The standard deviation of differences of time to equilibrium was 58.9 minutes.

Discussion. Schloerb *et al.*(4) found that equilibration was complete in 2 hours in the normal human. Edelman *et al.*(8) studied the time necessary for D_2O to penetrate a human bone and to come into equilibrium with the serum and found that it took approximately 4 hours. Thus, there is apparently no difficulty for D_2O to penetrate remote and inaccessible areas of the normal body and to equilibrate with these areas with considerable rapidity.

Since 1950, most investigators have assumed that equilibration was complete in 2 or 3 hours regardless of the pathologic state (6). The possibility that sampling at 3 hours did not allow adequate time for equilibration was suggested by Last *et al.*(9) who found greatly increased time to equilibration using inulin and mannitol in edematous states. Faller *et al.*(5) showed that the time to equilibrium for D_2O was prolonged in several pathologic states particularly in ascites. Edema fluid following a burn is entirely different insofar as the D_2O has a greater opportunity to reach equilibrium with the edema fluid because the area for diffusion in comparison to the pericapillary area is much greater than in the case of ascites with peritoneal surface. In this experiment the time required for equilibration in both unburned and burned animals showed considerable variation, range being 90-240 minutes. The average time for equilibration in the unburned animal was 129.4 minutes while in the postburned animal it was 148.1 minutes. There was no statistical significance between the time required for equilibration between animals in preburn and postburn series. The variation in time for equilibration should be stressed in order to show that more than 2 hours is necessary for equilibration in any study involving the D_2O analysis.

The postburn animals had virtually the same volume of distribution as in the normal state. The 1.5 l variation as shown in Fig. 1 was somewhat greater than in the majority of cases since the standard deviation of the differences was 0.707 liters. Because of the severity of the burn there were marked changes in the blood picture. All blood samples showed marked hemolysis of red cells, and the hematocrit postburn varied between 60 to 75%. The average volume of distribution of D_2O as per cent of body in the normal animal was 60.7(2,5).

From the above findings it appears that 2 hours for equilibration is inadequate. While 3 hours is adequate, in most instances, blood samples should be taken for 5 hours because of considerable variation in circulatory efficiency in normal and pathologic states.

Equilibrium cannot be assumed to be attained in either normal or pathologic states unless consecutive blood samples show little or no variation either in volumes of distribution or concentration of D_2O. It is the normal variation, rather than edema formation following burns, which necessitates a longer time interval for equilibration.

Summary. 1. The average time to equilibrium following intravenous injection of D_2O in 16 normal adult mongrel dogs was 129.4 minutes. Equilibrium was established in thermally injured dog in 148.1 minutes. There were no statistically significant differences between animals before and after thermal injury. 2. Equilibrium should not be assumed to be complete at 2 hours in either normal or pathologic states. Consecutive blood samples should be taken over a longer period of time, preferably up to 5 hours. A point of equilibrium should be selected when variation is within 5% of final volume of distribution at 5 hours. 3. Because of wide variation in time to equilibrium in both normal and pathologic states, any conclusions regarding changes in "efficiency of total circulation" should be studied carefully and interpreted cautiously.

1. Hutchinson, D. L., Plentl, A. A., and Taylor, H. C., Jr., *J. Clin. Invest.*, 1954, v33, 235.

2. Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Sheldon, D. B., and Moore, F. D., *J. Lab. and Clin. Med.*, 1951, v37, 653.

3. Levitt, M. F., and Gaudino, M., *Am. J. Med*, 1950, v9, 208.

4. Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Solomon, A. K., and Moore, F. D., *J. Clin. Invest.*, 1950, v29, 1296.

5. Faller, I. L., Petty, D., Last, J. H., Pascale, L. R., and Bond, E. E., *J. Lab. and Clin. Med.*, 1955, v45, 748.

6. Gilder, H., Redo, S. F., Barr, D., and Child, C. G. III, *J. Clin. Invest.*, 1954, v33, 555.

7. Edelman, I. S., Haley, H. B., Schloerb, P. R., Sheldon, D. B., Friis-Hansen, B. J., Stoll, G., and Moore, F. D., *Surg., Gynec. and Obst.*, 1952, v95, 1.

8. Edelman, I. S., James, A. H., Baden, H., and Moore, F. D., *J. Clin. Invest.*, 1954, v33, 122.

9. Last, J. H., McDonald, G. O., Jones, R. A., and Bond, E. E., *J. Lab. and Clin. Med.*, 1952, v39, 62.

Received April 25, 1956. P.S.E.B.M., 1957, v94.

Effects of Rhythm and Rate Changes on Inotropic Action of Ouabain.* (22844)

SOLOMON GARB AND MARIO PENNA† (With the technical assistance of Ann Kirk)
Department of Pharmacology, Cornell University Medical College, New York City.

These studies were performed to determine the relationship of changes in rhythm and rate to the inotropic action of digitalis glycosides. In the experiments on rhythm, the relationship of the inotropic action of ouabain to postextrasystolic potentiation was studied. This latter phenomenon was first described by Woodworth(1) in the dog heart. He found that when an extra contraction was interpolated in a series of regular contractions, the regular contraction immediately following the extra contraction was augmented. Cattell and Gold(2,3) subsequently redirected attention to this phenomenon and clarified many of its aspects. Recently several other studies of this response have been reported(4-8) in both atrial and ventricular musculature of different species. The term postextrasystolic potentiation was first used by Hoffman *et al.*(8). The occurrence of postextrasystolic potentiation indicates that there are sources of muscular power which can be demonstrated by alterations in the pattern of stimulation, and it was of interest to determine whether the inotropic action of a glycoside involved these sources of energy. In the second part of the experiment, the re-

* This investigation was supported in part by a research grant [H1016(C)] from N.H.I., Bethesda, Md., and New York Heart Assn.

† Kellogg Foundation Fellow from University of Chile.