previous paper.[4] This may be done because of the similar diphtheria antitoxic titre in maternal blood and placental fluid.

Conclusions. 1. The fluid obtained by squeezing the human placenta has the same diphtheria antitoxic titre as does the serum of the circulating blood of the mother drawn at the time of expulsion of the placenta. 2. This fluid is a mixture of maternal and fetal blood and tissue fluid, and possibly also a slight amount of amniotic fluid. 3. The diphtheria antitoxin titre of the placental blood, the circulating blood of the mother, and the placental tissue fluid are alike in most cases. 4. The antibody content of the placenta is probably entirely the result of transplacental transmission of the immune substance from mother to placenta, to fetus, and is probably passive in nature. 5. Since the globulin fraction carries with it both the diphtheria antitoxin and the measles antibody, it is suggested that this fluid be used in measles prophylaxis. 6. If the globulin of pooled placentas is to be used in measles prophylaxis, its dosage may be calculated by comparing its diphtheria antitoxic titre with that of the pooled placental fluid, and thus the necessity of obtaining maternal blood is obviated.

8096 C

Effects of Heavy Water on Mammalian Metabolism.

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Heavy water has hitherto not been subjected to serious pharmacological study in mammals; indeed it has been uncertain whether such activity exists to any significant extent. From its unusual physical characteristics, however, and its effects upon the behavior of lower forms of life (Urey,[1, 2] Barnes[3]) depression of function has been generally predicted in mammals. In particular, Barnes[4] has observed, for example, decrease in the rate of activity of the con-

[4] Karelitz, S., Greenwald, C. K., and Klein, A. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1359.

[1] Urey, Harold C., Cold Spring Harbor Symposia on Quantitative Biology, 1934, **2**, 47.

[2] Urey, H. C., and Teal, G. K., *Rev. Mod. Physics*, 1935, **7**, 34.

[3] Barnes, T. Cunliffe, and Jahn, Theo. L., *Quart. Rev. Biol.*, 1934, **9**, 292.

[4] Barnes, T. Cunliffe, and Gaw, H. Z., *J. Am. Chem. Soc.*, 1935, **57**, 590.

tractile vacuoles of protozoa and finds in 30% heavy water a reduced contraction rate supporting the prediction of chemists that deuterium will be found to have effects similar to those of low temperature.

The oxygen consumption of luminous bacteria in high concentrations of heavy water has been found decreased by Harvey and Taylor,[5] using the Warburg method. The same is true of yeast in 20% (or higher) D_2O. Other instances of depressed function might be cited; that reported in the highest form of life thus far is apparently the slowing of the frog heart recently described by Barnes.[6]

Our starting point in mammals has been a study of the metabolic rate of 2 white mice, each treated with several subcutaneous injections of 99% deuterium oxide. The carbon dioxide and water output, oxygen consumption and respiratory quotient have been closely followed, parallel with the variation in apparent content of the body in heavy water.

The method employed was a Haldane open metabolism chain which included some new features which will soon be published in detail by Barbour and Cochran.[7] An essential feature consists in the recovery of 97.5% of the insensibly lost water by passing it through a 100 cc. pipette, surrounded by well-insulated dry ice. Caught in this fashion, the insensibly lost water of the mouse assumes in 24 hours the appearance of a volume of snow nearly as large as the animal itself. When melted 0.02 cc. of the material suffices for a duplicate determination of the specific gravity by the falling drop method. The balance of the material may be again injected under conditions requiring low concentrations of D_2O.

The metabolic determinations were made in runs lasting from 9 to 12 hours.

The 2 mice used, No. 12 and No. 16, respectively weighed 20 and 23.8 gm. For Mouse No. 12, the routine procedure was to make 2 runs in 24 hours, separated by periods of ½ to 1 hour, in which food was allowed. Just before each run, *i. e.*, twice daily, 1.0 cc. of water was injected subcutaneously. No other water was allowed and the food uniformly employed ("Fox Chow") was practically dry. After 6 preliminary runs, Mouse No. 12 was injected with 1 cc. of 99% D_2O instead of H_2O before each of the next 4 runs (see

[5] Harvey, E. Newton, and Taylor, G. W., *J. Cell. Comp. Physiol.*, 1934, **4**, 357; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 954.

[6] Barnes, T. C., and Warren, J., *Science*, 1935, **81**, 346.

[7] Barbour, H. G., and Cochran, F., *Science*, 1935, in press.

dark block in Fig. 1). No H_2O at all was allowed during this period. The effect was so immediate that the first run showed a depression of the metabolism to about one-half of the average normal figure, a level which was maintained for the next 2 runs; the fourth and final dose of heavy water reduced the metabolism still lower. These effects are seen in the CO_2 curve and the somewhat similar water loss curve. Using for the surface area of the mouse, Benedict's[8] modification of Rubner's formula: $9.W^{2/3}$ and calculating the calories from the oxygen determinations, we find that the metabolism of this mouse fell from an average normal of 1,925 cal./sq.m./24 hr. to a minimum of 751 cal./sq.m./24 hr. Eight days after resumption of the H_2O injections, the metabolism had begun to return toward normal, but following this run the mouse was accidentally asphyxiated.

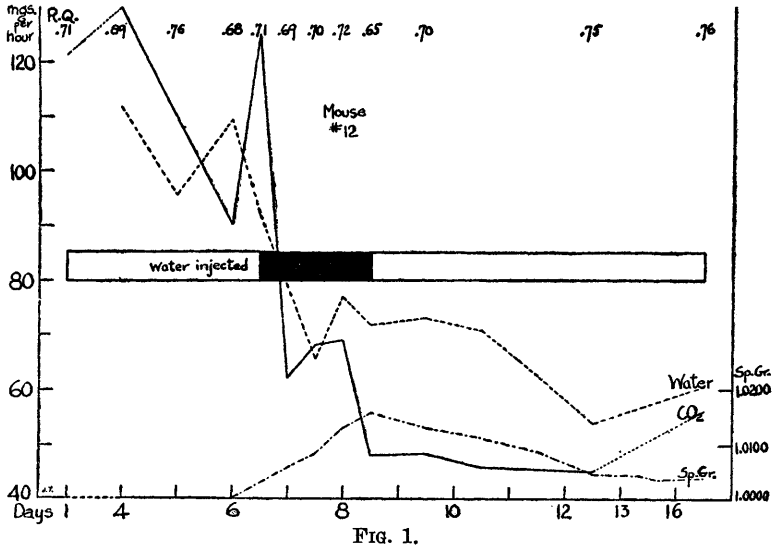

FIG. 1.

Mouse 12. Effects of heavy water on metabolism. Ordinates, mg./hr.; abscissae, days; heavy lines, CO_2 output; upper broken lines, water output; lower broken lines, specific gravity of insensibly lost water. One cc. H_2O injected twice daily during periods indicated by light blocks, D_2O dark block. Top line: R.Q. (in figures).

Finding that mouse No. 12 had received hardly enough water to meet the requirements of his combined output by urine and evaporation and desiring also that food be made accessible for a longer period each day, it was decided to provide the second mouse, No. 16,

[8] Benedict, Francis G., and Fox, Edward L., *Pflüger's Arch. f. d. ges. Physiol.*, 1932, **231**, 455.

with an over-abundance of water by injection and to make food available for at least 12 hours daily. To this end the mouse was left each day from about 7 a. m. until 7 p. m. in a beaker with abundant food and the metabolism runs were made only during the night period. After a study of the normal mouse for 6 days in this fashion, 3 successive night runs were made before each of which was given a large dose (1.5 cc.) of heavy water (see black blocks in Fig. 2). After each night's run the injection consisted chiefly of H_2O, but with the addition of sufficient D_2O to maintain approximately the same concentration of the latter in the body, as shown by the specific gravity of the insensibly lost water.

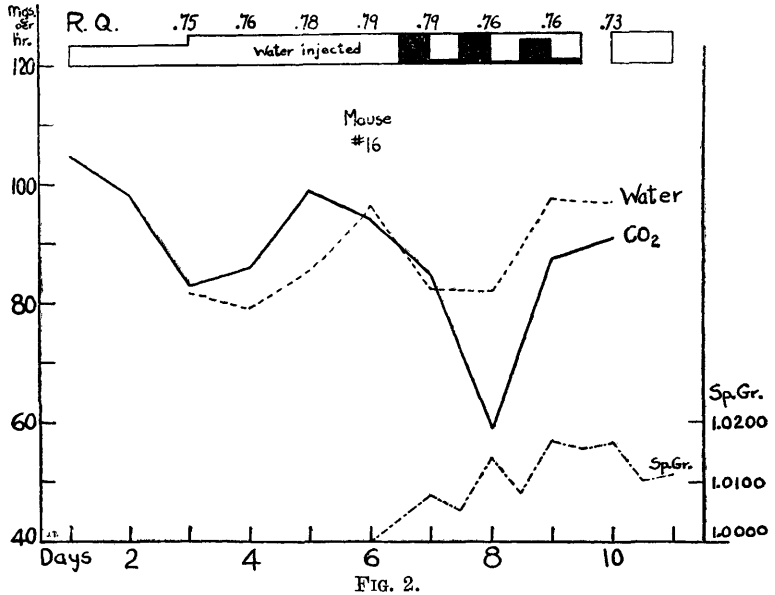

FIG. 2.

Mouse 16. Same as Fig. 1, except dose of water injected raised on third day to 1.5 cc. injected twice daily. Areas of mixed light and dark indicate proportional amounts of H_2O and D_2O injected together.

In this well-fed and well-watered mouse the depression of metabolism was much less striking. There was, however, a reduction from the normal average of 1304 cal./sq.m./24 hr. to a minimum of 868 cal./sq.m./24 hr., reached after the second night's run. Before the third night's run it was necessary (to make up the 1.5 cc. dose) to add H_2O to the D_2O injected; the metabolism of this run returned to nearly the normal level.

The specific gravity of the insensibly lost water was determined after each run and in each case a figure of over 1.0150 was reached

after the last heavy water injection. Since the specific gravity of pure deuterium oxide is 11.079, this figure is interpreted as suggesting that at this point each mouse was about 13.5% saturated with heavy water. The exact equivalence, however, of the D_2O/H_2O ratio in insensibly lost water to the ratio existing in the body remains still to be quantitatively determined.* Inasmuch as the second mouse showed a slower and less decisive fall of the metabolism, it is argued that a better condition of hydration with H_2O at the onset of a heavy water experiment tends to protect against the effect of the latter substance. This hypothesis that more subtle penetration of D_2O can be obtained in dehydrated animals remains to be tested.

So far as can be judged, both mice showed as great activity throughout the heavy water treatment as in their previous condition. The only exception to this was a slight depression in the respiratory rate observed in each mouse after the first D_2O run. They appeared otherwise to be as alert and to eat as much at all times. There is no reason to ascribe the fall in metabolism to diminution either in activity or in food intake. This statement is well borne out by the maintenance throughout the D_2O periods of essentially the same respiratory quotient as found during the immediately preceding normal periods. The question of true basal metabolism in the mouse has been dealt with by Benedict and Fox[8]; while the present work does not probably present the mice at their basal levels of metabolism, there is no reason to doubt that the chief factor in the metabolic fall seen in each case was the inhibiting effect of deuterium oxide upon the activity of the cells.

8097 C

Effects of Light and Dark Environment on Weight Changes in Normal and Hypophysectomized Frogs.

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It is a well established fact that the pituitary gland of the frog plays an important rôle in the dilatation and constriction of the melanophores in dark and light environments. It was shown by

* It may be disturbed by the loss of deuterium to carbohydrate and urea.