10828

Absorption of Water by Liver Slices from "Physiological" Saline Solutions.

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In a recent publication Field, Belding, and Martin[1] concluded that "the variability of the Qo_2 (as determined on tissue slices), based upon wet weight determinations, is no greater than when based upon determination of dry weight." They concluded further that "organs and tissues differ significantly in respect to the mean value of wet/dry weight ratio" and suggested that "expression of Qo_2 as a function of wet weight will often be the procedure of choice." The conditions under which the wet weight was determined were not stated.* It is the purpose of this note to call attention to a source of error which may accompany the calculation of quantitative metabolic data, obtained on tissue slices, on the basis of wet weight.

In a study of cholesterol distribution in the brain, slices were left in "physiological" saline solutions (0.9% NaCl or Tyrode solution) for varying intervals before weighing for analysis. Certain discrepancies in the results led us to suspect that considerable amounts of water had been taken up. To investigate the question it was necessary to devise a method by which tissues could be sliced without wetting. This was accomplished in the following way: A wooden box was constructed with a sloping glass top, glass front and back, and holes, covered with rubber flaps and large enough to admit the hands, at each side. The dimensions were 19½" wide, 15" deep, 12½" high in front, and 8¾" high in back. The atmosphere within the box was kept saturated, or nearly so, with moisture by heating water in a metal cup or beaker by means of a steam coil. An auto-

1 Field, J., 2d, Belding, H. S., and Martin, A. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 565.

*In a personal communication Dr. Field states that excised organs were placed in Ringer's solution at once. The tissue was then sliced with a blade kept moist with Ringer's. The time between the placing of the organ in Ringer's and the removal of tissue slices from Ringer's ranged from 3 to 5 minutes; extraneous moisture was then removed by turning the slices over and over on quantitative filter paper, slightly moistened with Ringer's (to avoid the possibility of removal of water from cells by dry paper), and the slices were weighed. Dry weights were determined at the end of the respiration measurements.

mobile windshield wiper, working on the front window, made it possible to see into the box. Satisfactory conditions were indicated by heavy steaming of the glass windows, such that a visible film of moisture formed on the window within 5 seconds after stopping the wiper. This situation was obtained if the temperature of the water was kept at 60° to 80°, with the temperature of the air within the box at about 40°. No attempt was made at an exact definition of the conditions, which of course varied from day to day with fluctuations in room temperature and atmospheric humidity. The criterion of steaming was found to be adequate for satisfactory results.

Livers were removed from rats immediately after killing and transferred to a porcelain plate covered with a paper towel within the box. They could be sliced in the moist atmosphere with a dry razor blade almost as readily as with a wet blade according to the customary procedure. The slices were either immersed in saline solution (0.9% NaCl, Tyrode, or Krebs) at room temperature in small beakers, or, in control determinations, transferred directly to small tared aluminum pans, made by bending pieces of thin sheet aluminum about 1 cm square, and weighed immediately. The slices were removed from the solutions after varying intervals and turned over with a spatula on hardened filter paper until the paper showed no evidence of wetting. They were then transferred to aluminum pans and weighed. The samples were dried over night in an oven at about 110° and weighed after cooling in a desiccator.

Under the conditions described no significant variation in the water content of the tissue occurs over long periods of time. The results of 3 experiments, typical of the 9 carried out, are shown in Table I. In one of the experiments the liver remained in the box for as long as 150 minutes without change. Some slices were transferred to aluminum pans and left within the box for from 13 to 43 minutes

TABLE I.
Constancy of Water Content of Liver Kept in a Moist Atmosphere.

Exp. 3		5		6	
Time* min	Water %	Time* min	Water %	Time* min	Water %
6	69.0	0	68.6	0	69.4
7	69.8	2	69.0	1	69.1
25	69.2	22	70.7	2	69.5
33	68.9	27	70.4	15	69.0
44	69.5	42	69.7	27	68.7
51	69.6	54	69.7	49	69.7
61	69.9	69	69.6	71	68.4
73	69.6				
85	69.6				

* From placing liver in box.

before weighing. The moisture content did not vary significantly from control determinations in which the tissue was weighed immediately after slicing.

The rapid uptake of water by liver slices immersed in saline solutions is demonstrated in Fig. 1 by curves obtained from a single liver. In order to bring out the magnitude of the effect the data are expressed as amount of water taken up per unit weight of dry tissue. The calculation is illustrated by the following example: Water content of liver (control) 69.5%, or 228 mg water per 100 mg dry tissue. Water content of liver after 10.5 minutes in 0.9% NaCl solution 77.5%, or 344 mg water per 100 mg dry tissue. The difference, 116 mg, represents the water taken up per 100 mg dry tissue.

It will be seen that in the experiment represented in Fig. 1 the slices absorbed an amount of water equal to their dry weight within less than 10 minutes and equal to twice their dry weight in about 1 hour. The rate of uptake was about the same for each of the solutions studied. Similar findings were obtained in a total of 7 experiments with sodium chloride and 3 each with Tyrode and Krebs solutions. There was considerable spreading of the curves, particularly above 10 minutes, but in most experiments the tissues took up an amount of water equal to their dry weight within 10 minutes.

It is well known[2] that whole isolated muscle swells in isotonic solution. The phenomenon has been studied in detail by Parry.[3]

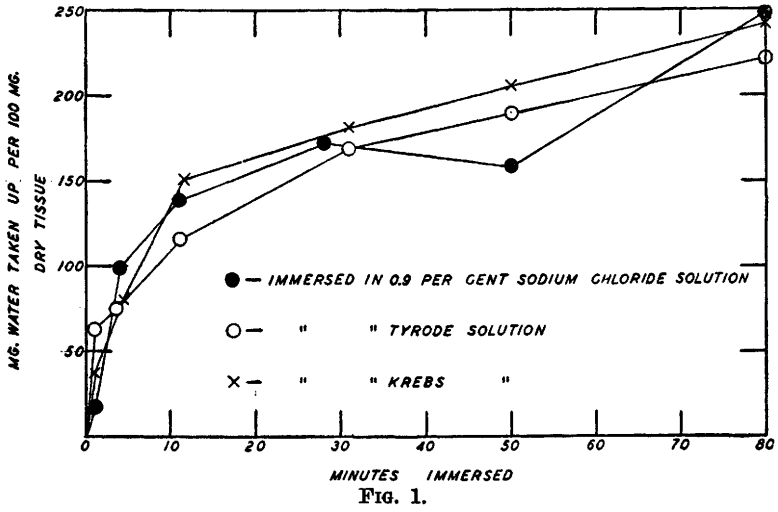

FIG. 1.

[2] Adolph, E. F., *Am. J. Physiol.*, 1931, **96**, 598.

[3] Parry, A. A., *J. Cell. Comp. Physiol.*, 1936, **8**, 277.

The possibility that tissue slices would also take up water from isotonic solutions does not appear to have been generally recognized.

Adolph[2] showed that exchange of water by muscle occurred in proportion to the surface area and to the square root of the duration of immersion. Unfortunately it is not possible to calculate the surface area of the slices employed in our experiments. The average moist weight of the control samples was about 100 mg, but there was a wide variation from about 50 to 175 mg. Furthermore, although the great majority of the slices were within the range of thickness ordinarily employed in respiration experiments, no attempt was made to keep within close limits of tolerance. In a few instances exceptionally thick slices were noted to have taken up water at a low rate. Our primary purpose was to find conditions under which the uptake of water might be avoided; we were interested in establishing the magnitude of the effect more from the qualitative than the quantitative standpoint.

It is difficult to correlate the findings of Field, *et al.*,[1] with our results. In their experiments the tissues were immersed for about 3 to 5 minutes.* It is during this period that water is taken up most rapidly and, therefore, small differences in time should have introduced a considerable variation into their results. Furthermore, amounts of water such as they report for liver (ratio of wet to dry weight 5.07) were encountered in our experience only after 30 minutes to 1 hour in saline solutions. It is possible, though unlikely, that the rate of uptake from Ringer's solution is much faster than from the solutions employed in this investigation and that the maximum uptake had been approached by the time their slices were weighed.

Summary. Rat liver slices absorb water at a rapid rate from "physiological" saline solutions. An appreciable uptake may be observed within one minute; within 10 minutes an amount of water equal to the dry weight of the tissue is usually absorbed. Conditions under which tissues may be maintained for long periods of time without significant change in water content, and may be sliced without contact with solutions are described.