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An Instrument Suitable for Measurement of Osmotic Pressure of Biological Fluids. (19473)

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In previous papers(1) I have reported on measurements of osmotic concentration of the extraocular and intraocular fluids. The instrument constructed for this special need has not been described and might be a valuable tool for similar investigations.

Niederl and Levy(2) published a simplified method for the determination of molar concentrations, a modification of the well known original method of Barger. The method is based upon the fact that in a closed system, harboring two solutions of different molarity,

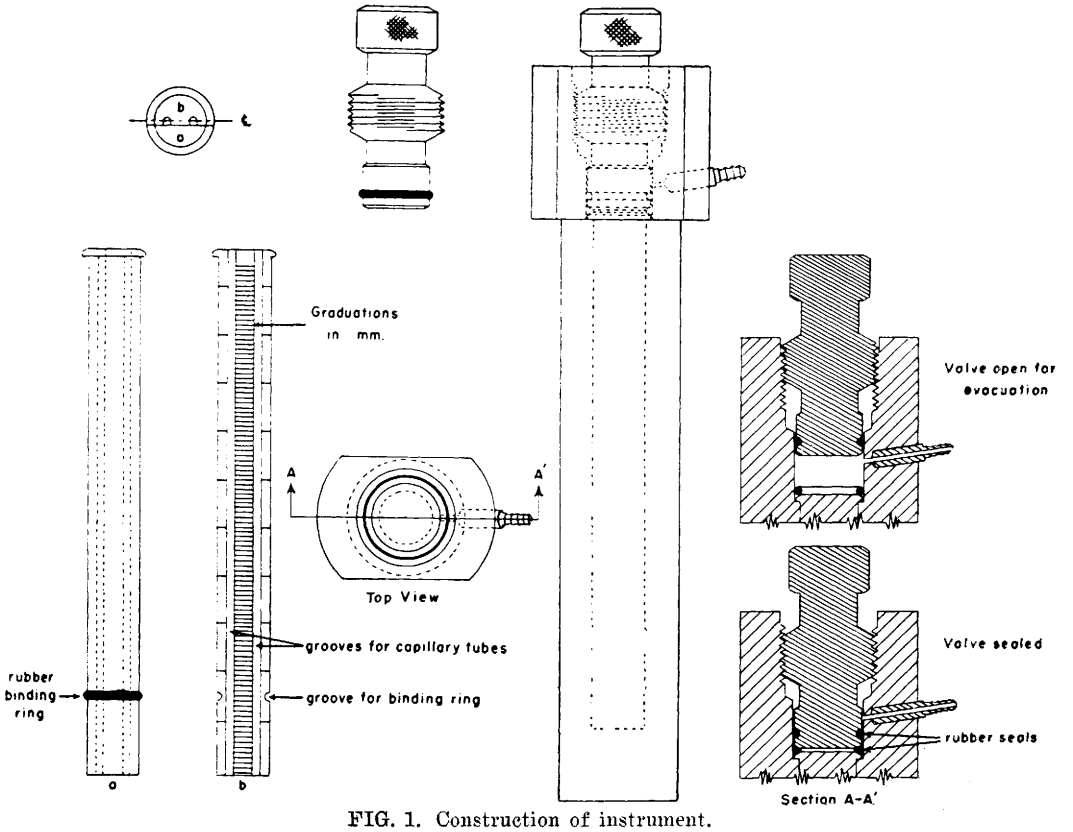

FIG. 1. Construction of instrument.

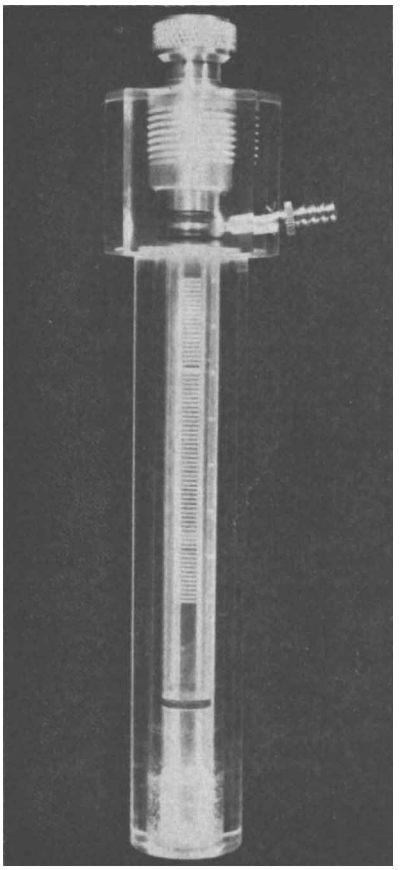

FIG. 2. Assembled instrument.

a change in the proportional volumes will occur due to a differential distribution of water vapor in the two solutions. The system tends towards an osmotic equilibrium, and the solution with higher molarity will gain, while the solution with lower molarity will lose in relative volume until equilibrium is reached. In this method 2 capillary tubes are filled with the relative solutions. On one end the capillaries are sealed and placed so that the menisci of the columns are exactly at the same level. The capillary tubes are now placed in a suitable external tubing which is sealed on one end, and the other is sealed only after evacuation of air to about 15 mm pressure. The system stays at room temperature for 4 days, when the distance between the 2 menisci, originally at the same level, is measured under low-powered microscopic magnification, using a micrometer scale in the eyepiece; the measurement is subsequently repeated every day for a week.

Applying the method for determination of osmotic pressure of biological fluids, we met with certain technical difficulties, one of which was the setting up of the capillary tubes in such a way that by handling or especially by centrifugation the menisci should not change their relative position. Exact arrangement of the two tubes was somewhat cumbersome. Another difficulty arose from the fact that biological material within such long periods of time as 4 to 10 days at room temperature, undergoes chemical changes in which larger molecules are broken down to smaller particles, disturbing the final result considerably. For this reason an improvement of the method of Niederl and Levy was attempted that would facilitate its application, and the time period necessary to obtain equilibrium in the system was considerably shortened. This last requirement for our purposes was fulfilled by reducing the volume of the evacuated space in which the evaporation and subsequent establishment of osmotic equilibrium take place. It can be assumed that the rate of exchange with time, t, of the amount of fluid evaporated, m, is given by a constant, α, minus a quantity which is proportional to the vapor pressure. This pressure is inversely proportional to the volume v. This gives a differential equation

$$\frac{dm}{dt} = \alpha - \frac{\gamma}{v} m \qquad (1)$$

the integral solution of which is:

$$m = \frac{\alpha v}{\gamma} (1 - e^{-\frac{\gamma}{v} t}) \qquad (2)$$

Therefore, if all other conditions are unchanged, reduction of the volume reduces the time in which equilibrium is reached. On the other hand, because the total amount of fluid evaporated is also reduced, the gain in time is somewhat biased by loss of sensitivity. If the reduction of volume is not excessive, this handicap is not considerable. In applying the technic of Niederl and Levy, in our hands the volume consisted of 5 or more cc, which in our modification could be reduced to 0.25 to 0.5

cc or any size desirable. This meant a reduction of the reading time by at least 10 times the original method. Leaving the system for 12 hours, *e.g.* overnight, yielded a satisfactory reading. No complete equilibrium was aimed at. In applying low microscopic magnification, estimation of differences less than 0.01 molarities can be obtained.

The instrument is constructed of lucite plastic. It can be used repeatedly for successive determinations. The transparency of the tubes allows the determination of the length of the fluid columns in the capillaries with the aid of low power magnification. The inner tube harboring the 2 capillaries possesses a recording grid. (The instrument may be obtained from Mr. V. L. Frykman at the Precision Instrument Shop of the University of Southern California in Los Angeles.)

Summary. The present paper describes an instrument constructed for determination of osmotic concentration in biological fluids. It estimates differences less than 0.1 molarities.*

* I take the opportunity to acknowledge the contribution of Dr. J. P. Meehn (Physiological Institute of the University of Southern California) in the design of this instrument.

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Potentiating Effect of Ascorbic Acid on Cortisone-Induced Gluconeogenesis. (19474)

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Previous work from this laboratory suggested that ascorbic acid treatment of rats, mice, and guinea pigs can prevent activation of the pituitary-adrenal axis in response to stressors(1-4). It was observed that the vitamin fails to alter the leukocyte response to cortisone and to adrenocorticotrophin. Furthermore, it was demonstrated that the vitamin does not possess any specific anti-epinephrin action to explain failure of the activation of the pituitary-adrenal axis(4). Those data suggested that the ascorbic acid prevents the activation of the axis by either directly or indirectly rendering the pituitary inert to certain stimuli. Preliminary data indicated that ascorbic acid prolongs the leukocytic effects, and intensifies the gluconeogenic actions, of cortisone. The present communication reports data which indicate that the gluconeogenic action of cortisone is potentiated by ascorbic acid.

Materials and methods. These experiments were conducted on young adult male Swiss mice. The animals were bilaterally adrenalectomized under ether or nembutal anesthesia at least 3 days, and not more than 3 weeks prior to experimentation and maintained on an *ad libitum* fare of Purina Laboratory Chow and 0.9% NaCl solution. At the end of the periods of experimentation the animals were killed by a blow on the head, and the liver extirpated and plunged into a 30% solution of potassium hydroxide; the glycogen was then hydrolyzed by the method of Somogyi *et al.*(6), and the glucose determined by the method of Folin(5).

Experimental designs and results. The first experiment was conducted on male Swiss mice which had been adrenalectomized 3 weeks previously, and were maintained on Chow and saline. Body weight range at time of experiment was 25-30 g. Group I mice received single intraperitoneal injections of ascorbic acid (sodium ascorbate, vit. C, injectable, Roche), 100 mg per 100 g body weight, on the previous evening. Early the next morning these animals received similar amounts of the vitamin. The first injection