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Osmotic Activity Changes of Serum and Salt Solutions Placed in the Gall Bladder.

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Dreser,[1] the first to use cryoscopy on animal fluids, made the first determination of the osmotic pressure of bile. Numerous early investigators, among them Brand,[2] Strauss,[3] Bernstein,[4] Bosquet,[5] Koziezkowsky,[6] Messadaglia and Colletti[7] determined cryoscopically the osmotic pressure of animal and human bile obtained by various methods from living and dead specimens. They came to the conclusion that the osmotic pressure of bile, both bladder and hepatic, was approximately the same as the osmotic pressure of the blood of the same animal species; *i.e.* the depression of freezing point of the bile and blood both lay in the same range (about −.54°C to −.58°C for most species). Of the many objections to the earlier work, the varied methods of collection and the inaccuracy of the cryoscopic technic are perhaps the most significant. A difference in freezing point of .01°C corresponds to a difference in osmotic pressure of nearly 100 mm of Hg. Ravdin, *et al.*,[8] state that despite the wide variance of constituents, the osmotic pressures of hepatic and bladder bile, as determined by the depression of freezing point, are approximately the same; and that the total depression of freezing point may be accounted for on the basis of the osmolar concentration of base, chloride, and bicarbonate present. Yet the difference in osmotic pressure of their hepatic and bladder bile amounts to 357.2 mm of Hg. They also conclude that the osmotic pressure of hepatic and bladder bile is approximately the same as that of serum. On the basis of experiments in which they placed various bile constituents individually into a bile-free dog's gall bladder, Ravdin *et al.*[9] came to the conclusion that regardless of the concentration of the original solution, the total osmolar concentration of the fluid in the gall

1 Dreser, *Arch. f. exp. Path. u. Pharm.*, 1892, **29**, 303.

2 Brand, *Arch. f. d. gesammte Physiol.*, 1902, **90**, 491.

3 Strauss, *Berl. Klin. Wehnsch.*, 1903, **40**, 261.

4 Bernstein, *Arch. f. d. gesammte Physiol.*, 1905, **109**, 207.

5 Bosquet, cited by Strauss.

6 Koziezkowsky, cited by Strauss.

7 Messadaglia and Colletti, cited by Strauss.

8 Ravdin, Johnston, Riegel and Wright, *Am. J. Phys.*, 1932, **100**, 317.

9 Ravdin, Johnston, Austin and Riegel, *Am. J. Phys.*, 1932, **99**, 638.

bladder after a period of time approaches that of serum. Reinhold and Wilson[10] state that: "Although the sum of the molar concentrations of anions and cations in bile exceeds that in serum, the osmotic pressure of the two fluids, as shown by the work of others, is practically the same. Actually the molar concentrations of inorganic ions are approximately the same in both. It would appear, therefore, that the principal organic ion of dog bile, taurocholic acid, either exhibits little osmotic activity or diminishes the osmotic activity of other ions." Gilman and Cowgill[11] using Hill's method found approximate isotonicity of blood and hepatic bile, and that artificially produced changes in the osmotic pressure of the blood produced parallel changes in the osmotic pressure of hepatic bile. Their osmotic pressure values are given in terms of milliequivalents of an osmotically equal NaCl solution. They state they are "confident the values for osmotic pressure—are accurate to within 1 milliequivalent;" yet their average values for the osmotic pressure of blood and hepatic bile under all conditions are 155 and 151 milliequivalents, respectively. Therefore the difference in the average osmotic pressure of blood and bile falls 3 milliequivalents outside the range of experimental error. Furthermore, the values for hepatic bile were 2-9 milliequivalents lower than for blood in 13 of 15

OSMOTIC ACTIVITY AND CHLORIDE CONCENTRATION CHANGES IN THE GALL BLADDER

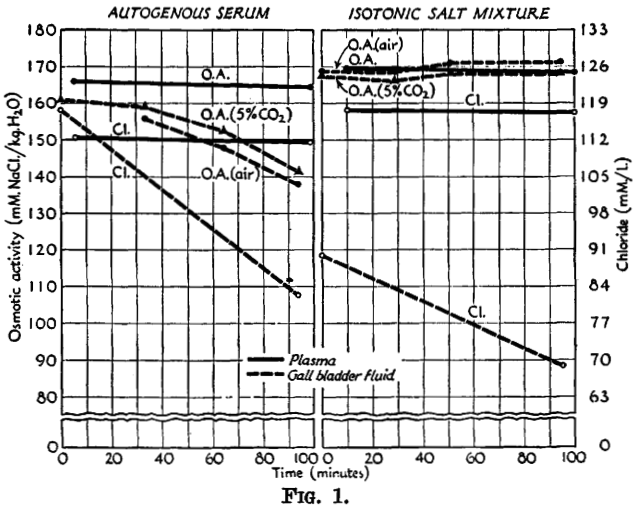

FIG. 1.

10 Reinhold and Wilson, *Am. J. Phys.*, 1934, **107**, 378.

11 Gilman and Cowgill, *Am. J. Phys.*, 1933, **104**, 476.

cases. In the other 2 cases the value for bile was 1 milliequivalent higher than for blood. In order to investigate the role of the gall bladder in osmotic processes, it was decided to study the changes in osmotic activity of serum and nearly isotonic NaCl + $NaSO_4$ solutions placed in the gall bladder by the vapor tension method.

Methods. Cats anesthetized with nembutal were used as experimental animals. The gall bladder was entered via a whistle-tip fiber ureteral catheter so inserted and tied into the cystic duct as not to injure the cystic vessels. Injections into and withdrawals from the gall bladder were then accomplished by a syringe whose needle fit closely into the open end of the catheter. When not in use, the open end of the catheter was closed by a piece of wire of similar diameter to the bore of the catheter. After the bladder bile was removed, the gall bladder was washed out several times with normal saline at body temperature then with the fluid to be injected at body temperature. Finally such an amount of fluid was injected as to moderately distend the gall bladder (usually 1.5 to 0.7 cc). The fluids injected were: (1) the cat's own serum obtained just before injection from femoral vein blood, and (2) NaCl + Na_2SO_4 (in about equiosmotic proportions) solution approximately isotonic with the cat's plasma. Small samples of gall bladder fluid were withdrawn about every ½ hour for 1½ to 2 hours. Blood plasma samples were taken as described by Roepke and Visscher[12] at the beginning and end of the experiment. All samples were protected from evaporation and CO_2 loss. Osmotic activity (see Roepke and Visscher[12]) determinations were made with Hill's thermoelectric method as modified by Baldes.[13,14] Vapor tension measurements were made with air and CO_2 mixtures in the thermocouple chamber in order to control the influence of the CO_2 tension. Chloride was determined according to the method of Van Slyke.[15]

Results. Fig. 1 shows the typical results obtained on placing 1 cc of autogenous serum in a cat's gall bladder. Within 90 minutes the volume decreases (50% to nearly 100%); the chloride falls (30% to 40%); and the osmotic activity decreases (7 to 23 mM). The osmotic activity is higher in 5% CO_2 than in air. The osmotic activity and chloride concentration of the blood remain relatively constant. If the serum is poisoned with .004 M $HgCl_2$; the volume remains practically constant, the chloride decreases (but only about

12 Roepke and Visscher, PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 500.

13 Baldes, *J. Sc. Instruments*, 1934, **11**, 223.

14 Baldes and Johnson, *Biodynamics*, 1939, No. 47, 1.

15 Van Slyke, *J. Biol. Chem.*, 1923, **58**, 523.

15%), and the osmotic activity does not change. The osmotic activity and chloride concentration of blood again remain nearly constant.

Fig. also shows the results of an experiment in which 1 cc of an isotonic mixture of osmotically equal parts of NaCl + Na_2SO_4 was placed in a cat's gall bladder. The volume decreased (50% to 75%). The chloride (25% to 50%). The osmotic activity usually rose somewhat (3 to 5 mM), and was higher in air than in 5% CO_2. The osmotic activity and chloride concentration of the blood remained relatively constant. If the salt solutions are markedly hyper- or hypotonic to the blood (8 to 10 mM), the osmotic activity of the gall bladder fluid decreases or increases, respectively, to approach that of blood. In these cases there is also a decrease in volume and chloride. If the salt solution is poisoned with .001 M $HgCl_2$; the volume increases (50%), the chloride increases (30%), and the osmotic activity increases more rapidly and markedly (8 mM). The osmotic activity of the plasma remains unchanged.

The osmotic activity of the removed gall bladder bile was usually 1 to 3 mM lower than that of blood removed 15 to 30 minutes later.

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Cultivation of the St. Louis Encephalitis Virus.*

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The virus of St. Louis encephalitis is known to grow readily on the chorioallantoic membrane of the developing hen's egg and in tissue cultures of the Li and Rivers type.[1-4] In both media, however, virus titrations have been uniformly low, usually attaining levels of 10^{-2}, with an occasional maximum of 10^{-3}.

Successful propagation of the lymphogranuloma venereum virus

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1 Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

2 Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 359.

3 Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 799.

4 Smith, M. G., and Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 323.