maximum of 48 cc.) and totaled 290 cc. for the 12 hours. Sodium salicylate could not be detected. The urine totaled 1381 cc. for the same period. This contained a total of 0.584 gm. of salicylic acid, corresponding to 0.680 gm. of sodium salicylate.

Sodium salicylate did not appear in the bile in recognizable amounts following the oral administration of doses of 20 mg. per kilo of body weight to the 3 patients with disease of the biliary system. These observations are in accord with data obtained in healthy rabbits.[1] The principal route for the elimination of sodium salicylate according to the above observations is the urinary tract. It is, therefore, reasonable to assume that whatever beneficial effect may follow the administration of this drug to patients with disease of the biliary system, is not due to the presence of salicylate in the bile.

5110

Further Observations on the Oxygen Consumption of Nerve.*

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The earlier studies of Fenn[1] and Gerard[2] on the oxygen consumption of frog nerve were not in complete quantitative agreement, even when differences in the experimental temperatures were allowed for. Fenn used American frogs *(pipiens?)* and a volumetric method, I used European species and a manometric technique, so it was desirable to ascertain whether the divergent results depended on material or methods. It also seemed useful to examine in more detail than previously the influence of various conditions on the rate of oxygen usage of nerve and to compare nerves of several animals. Observations carried on during 2 years by the Warburg technique are here summarized.

The Qo_2 (cmm. O_2 consumed per gm. of moist nerve per hour) of the sciatic of the American green frog, mainly *R. pipiens,* is lowest in December and January, $Qo_2 = 37$, and highest in June or a little earlier, $Qo_2 = 60+$. The Qo_2 shows a temperature coefficient of 2.2, as far as can be judged by experiments run at 18° to

* The present investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation.

[1] Fenn, *J. Gen. Physiol.*, 1927, x, 767.

[2] Gerard, *Am. J. Physiol.*, 1927, lxxxii, 381.

24°C. The various American species do not consume oxygen at the same rate, but no accurate record of specific differences has been kept. The average Qo_2 of 227 experiments, mostly in the winter months, at an average temperature of 21.5°C. for green frog sciatics in Ringer solution is 42, and the extreme variation is 20 to 80. Fenn's[3] latest value for these same frogs at 20°C., is 45, so our results, with different methods, are in agreement.

Any influence of various substances on nerve respiration is dependent on their ability to penetrate to the respiring regions. The great importance of the perineurium as a passive barrier to diffusion, as described by Feng and Gerard[4] was not fully realized when most of these experiments were performed; and some of the negative results obtained may have depended on the presence of the sheath. Since, however, in most cases respiration remained constant for 10 to 20 hours, and since the few experiments on nerves with the sheath split longitudinally confirmed those on intact nerves, the present results may tentatively be considered valid.

The oxygen consumption is not influenced by the pH between 7 and 9. At pH 5.3 it is decreased about half. Borate buffer does not affect respiration, phosphates appear to cause an increase of 10 to 15%, independently of pH effects. Isotonic NaCl is equivalent to Ringer solution so far as oxygen consumption is concerned. 90% NaCl plus 10% of $CaCl_2$, KCl or $MgCl_2$, or plus 5% of any two of these is equivalent to 100% NaCl. Isotonic $CaCl_2$, KCl, or $MgCl_2$ may markedly decrease oxygen consumption to below 50%. Hypertonic NaCl, up to 4 times isotonic, has little effect on the Qo_2. Hypotonic solutions cause a definite decrease which is roughly progressive with the hypotonicity to 1/3 depression at 1/4th isotonic and 2/3 depression in water.

Attempts to maintain a constant CO_2 pressure of 40 mm. in the chamber, by a special absorber for CO_2 that would be effective at higher CO_2 tensions, were not satisfactory. The effect of Na HCO_3 added to NaCl was determined by comparison with NaCl solutions brought to the same pH (8.0-8.5) with borate or phosphate buffers. Certain theoretical difficulties with this procedure cannot be discussed here, but consistent results were obtained. The bicarbonate values fell between the other 2 sets, and no definite stimulating action can be ascribed to the HCO_3^- ion.

Glucose and glucose plus insulin added to a saline medium regularly had no influence on oxygen consumption; but in a few scattered experiments an increase of several hundred percent was ob-

[3] Fenn, *Medicine*, 1928, vii, 433.

[4] Feng and Gerard, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii.

tained. Sodium lactate was also without influence in most cases but gave a 25% increase in one series of 3 experiments. The bases for these exceptional results have not been discovered. Addition of methylene blue markedly increases the Qo_2, 40% in one series on summer frogs, 70% in experiments the following spring. The methylene blue action is further enhanced by addition of sodium lactate, which caused a 95% increase (compared to 70%). The relation of methylene blue to lactic acid oxidation has been emphasized by Barron.[5] Although methylene blue penetrates the nerve sheath very poorly, experiments on nerves with split or intact sheaths gave identical figures. Cyanide, even in N/100 concentration, does not abolish more than 2/3 of the oxygen consumption, and methylene blue partly restores that lost.

Morphine (0.001 to 0.1%) has no influence on nerve respiration. Urethane causes 50% inhibition at a concentration of 5%, chloralhydrate at a concentration between 0.05 and 0.1%. The relative effectiveness of these narcotics in producing nerve block apparently cannot be correlated with their action on respiration (see also Sherif[6]). Bile salts, at a concentration of 125 mgm. %, slightly decrease the Qo_2 (Ries and Still[7]).

Bull frog sciatics have an average Qo_2 of 29 at 21°C. (Green frog nerves in comparative experiments averaged 45.) Rabbit sciatics at 38°C. average 280, but during the first hour higher values (320) are obtained and the Qo_2 slowly falls with time. Dog sciatics averaged 120 at 38° and 30 at 22°. Some of these had been allowed to degenerate a week with no distinct change in resting Qo_2. The dog nerve values may be low because the nerves were obtained from animals kept under barbital for some days.

The increased oxygen consumption by green frog sciatics on stimulation is being studied by Mr. T. H. Chang. The average of 40 experiments for continuous tetanization at 22°C. is 15. Splitting the sheath has no effect. At 280 shocks per second the increase is hardly 25% greater than at 100 per second. The increase on stimulation is abolished when only a small bit of nerve on the electrodes is stimulated, the remainder being cut off. This confirms my previous experiments in showing that the extra oxygen consumption depends on the activity of the conducting nerve and is not a local stimulus effect as suggested by Winterstein.[8, 9] Methylene blue does

[5] Barron, *J. Biol. Chem.*, 1929, lxxxi, 445.

[6] Sherif, *J. Pharm. and exp. Therap.*, 1930, xxxviii, 11.

[7] Ries and Still, *Am. J. Physiol.*, 1930, xci, 609.

[8] Winterstein, *Handb. d. norm. u. pathol. Physiologie*, 1929, ix, 393, Springer, Berlin.

[9] Holmes, Gerard and Solomon, *Am. J. Physiol.*, 1930, xciii, 342.

not affect the oxygen consumption of activity. This is in harmony with the established differences between the carbohydrate metabolism of nerve during rest and activity.

5111

The Standardization of the Hemolytic Index.

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During our studies of the effect of hemolytic amboceptors on different tissues the necessity arose for the creation of a standard for the comparison of the hemolytic index of different seras. Everyone who has worked on similar problems has no doubt encountered the disadvantages of the present system of plus symbols. Not only is it subject to the personal interpretation of the investigator, but the varying degree of the hemolytic index of a given serum will depend on the age of the serum and the temperature of preservation. Furthermore the following variants of the blood cells have to be taken into consideration: Condition of the animal at the time of taking the blood, methods of preventing coagulation, differential count of the different blood-elements, volume of the red blood-cells, time of centrifugation, temperature during centrifugation, and, the most important factor of all, the increased sensitivity of the red blood cells, which rapidly increases with the time of preservation.

In order to be independent of all these variants it was thought desirable to have a standard hemolytic chemical agent, the action of which might be compared with the hemolytic effect of a given amboceptor. For this purpose sapotoxin was prepared from Quillaja bark saponin (Merck, crude), following with minor variations the methods described by Kobert and Brandl.[1] Sapotoxin has the advantage of being more effective than saponin and the methods of purification guarantee a more uniform product than the different commercial saponins.

Two systems were prepared. The first one consisted, as usual, of the hemolytic serum in varying amounts plus complement (guinea pig), plus 1 cc. of a 5% emulsion of red blood cells, which had been washed 3 times with a 0.85% NaCl solution. The second system consisted of varying amounts of sapotoxin plus 1 cc. of the same

[1] Brandl, J., *Arch. f. Exp. Path. u. Pharm.*, 1904, liv, 245.