

capacity of the two kidneys. A modification of this method was devised by us to make the test adaptable for determining total renal functional capacity. The technic employed was elaborated in conjunction with Dr. Thomas R. Brown, who has demonstrated with it that the total daily output in urine is fairly constant.<sup>5</sup> The technic was as follows:

One-fifth of the twenty-four-hour urine collected under toluol was neutralized and diluted to 1 L. By means of a graduated 2 c.c. pipet, decreasing amounts of urine were placed in a series of twelve tubes (arranged in a rack) as follows: 1.8, 1.6, 1.4, 1.2, 1, 0.8, 0.6, 0.4 c.c. Ten c.c. of the diluted urine was further diluted to 20 c.c. and of this decreasing amounts placed in the remaining tubes of the series as follows: 0.4, 0.2, 0.1, 0.05 c.c. To each tube was added sufficient 1 per cent. NaCl solution to bring the total volume in each tube to 2 c.c. Two c.c. of a 0.1 per cent. freshly prepared soluble starch solution was added to each tube. The rack with the tubes was placed in a water-bath at 38 C. for a half-hour, then transferred to cold water for three minutes.

<sup>N</sup><sub>50</sub> — iodine solution was added drop by drop in amounts sufficient to elicit a permanent color. The occurrence of blue or violet shows incomplete digestion, while the last tube without the violet color indicates the diastatic activity of the urine and from it "d" is calculated. The diastatic activity is expressed by  $d \frac{38^\circ}{30'}$ , which represents the number of c.c. of 0.1 per cent. starch solution which 1 c.c. of the diluted urine can digest at 38 C. in thirty minutes to products not yielding a blue color with iodine.

The urea content of the twenty-four-hour specimens of urine was determined by the Marshall method.<sup>6</sup>

The principle of Marshall's method consists in the conversion of the urea into ammonium carbonate by means of an enzyme, urease, present in an extract of the soy bean, and the titration of the ammonia with standard hydrochloric acid and methyl orange, directly or after its removal with an air current.

The freezing point of serum was made in the ordinary way, utilizing the Beckman apparatus, 25 to 50 c.c. of blood usually being taken.

The blood urea was determined according to Marshall's<sup>7</sup> method.

The total non-protein nitrogen of the serum was determined as follows: Ten c.c. of serum was added to 115 c.c. of 95 per cent. alcohol, 100 c.c. of the filtrate being evaporated to dryness. The residue was subjected to Kjeldahl nitrogen determination. The result represents the nitrogen in 8 c.c. of blood.

The values which we accept as normal are as follows: Phthalein, 50 to 60 per cent. for one hour, 60 to 80 per cent. for two hours,  $d \frac{38^\circ}{30'}$  = 5 or more, freezing point of serum  $-56^\circ$ , total non-protein N of blood .22 — .26 gm. per L. as determined by Folin, and blood urea .20 — .30 gm. per L. Retention of a mild grade occurs in many conditions without apparently serious renal involvement. Only when the retention is considerable do we consider the findings of importance. Such retention we refer to as cumulative phenomena, which means that the freezing-point of blood is at least  $-0.60$  C., the total non-protein nitrogen 0.500 gm. and the urea of blood 0.550 gm. per L.

5. Brown and Smith: Bull. Johns Hopkins Hosp., July, 1914.

6. Marshall: Jour. Biol. Chem., 1913, xiv, 283; xv, 495.

7. Marshall: Jour. Biol. Chem., 1913, xv, 487.