absence of glucose. 2. The presence or absence of glucose produces no significant difference in the oxygen consumption of denervated muscle *in vitro*. 3. No statistically significant difference in the respiratory quotients of innervated and denervated muscle with and without glucose in the medium was found.

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Microdetermination of Carbon Dioxide Content of Serum or Plasma with a Glass Electrode. (19687)

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The estimation of carbon dioxide content of serum by titration(1) has been little used in the past, chiefly because gasometric estimation is usually more convenient. Titrimetric micromethods(2), however, have been adopted recently. It occurred to us that the titration curves of different sera would be quite similar in the region of pH 5 to 7 and would enable one to estimate the carbon dioxide content from the pH after the addition of a known amount of acid to low pH, followed by a known amount of alkali to this region of pH. In the present paper we describe a rapid method which has been developed for the determination of carbon dioxide in this way on samples of 0.1 and 0.2 ml of serum or plasma.

Experimental. Titration curves were determined in 2 ml samples of serum or plasma obtained under oil from patients in various states of acid-base balance. The samples were diluted with 0.9% saline, and the pH was determined with a Cambridge Research model glass electrode after interval additions of about 0.02 ml of standard 0.2 N hydrochloric acid until the pH was in the region of 4.0. Then standard 0.2 N sodium hydroxide was added in the same way and the pH was determined until the initial pH of the diluted serum was reached. Fig. 1 shows a representative curve drawn on cross-section paper with pH on one axis and 0.2 N acid or alkali on the other. Only the curve for the back titration with alkali, curve B, would be required to calculate the content of carbon dioxide from the difference in the amount of acid and alkali added and the initial pH. It may be seen in Fig. 1 that the carbon dioxide content is equivalent to the amount of acid or alkali between curves

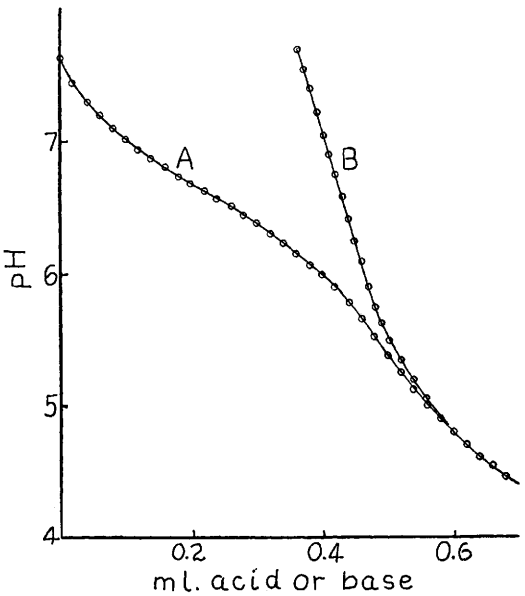

FIG. 1. Representative curve for titration of 2 ml of plasma with 0.2 N hydrochloride and 0.2 N sodium hydroxide.

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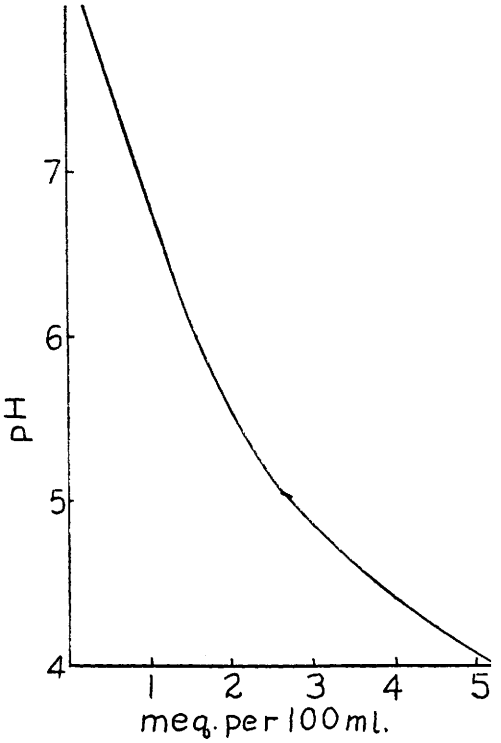

FIG. 2. Average composite curve for the back titration of plasma or serum with sodium hydroxide.

A and B at the initial pH. The concentration of acid and alkali used in the method was fixed so that the difference would always be equal to 4 milliequivalents per 100 ml of serum or plasma. If then the initial pH is assumed to be 7.60 which is the approximate average pH at room temperature, the carbon dioxide content in volumes per cent is obtained by multiplying 22.4 by 4.0 minus the difference in the amount of base in milliequivalents found on curve B between pH 7.60 and the final pH. All the B curves of samples, in which protein and carbon dioxide contents varied considerably, were corrected for the amounts of acid or alkali required to attain the same initial pH, and an average composite curve was drawn. This curve, shown in Fig. 2, in terms of milliequivalents of sodium hydroxide per 100 ml of plasma or serum, was used to calculate the subsequent values of carbon dioxide content.

We have found that a small amount of heparin sufficient to prevent clotting does not appreciably affect the initial pH or the curve, and enables a determination to be made promptly. Sodium fluoride, which inhibits glycolysis and the subsequent small change in pH, has also been found to have negligible effect. The required amount of a solution containing 100 mg of sodium heparin (Connaught) in 25 ml of 2% sodium fluoride (10 mg NaF and 0.02 mg heparin per ml of blood) was dried in tubes at 50°C, and blood drawn under oil was immediately stirred with the residues and centrifuged under oil. 0.3 ml of 0.9% sodium chloride was added to 0.2 ml samples of the plasma in small beakers. 0.1 ml of 0.1600 N hydrochloric acid was added carefully under the liquid from Kirk type micro pipettes with vigorous mixing. After thorough stirring, 0.1 ml of 0.0800 N sodium hydroxide was added in the same way. The solution was then transferred promptly to the micro MacInnis-Belcher type glass electrode, and the pH was determined. For 0.1 ml samples the procedure was the same except that 0.4 ml of sodium chloride solution and one-half the concentration of acid and alkali were used. The content of carbon dioxide was then calculated from Fig. 2 as described above.

Table I shows that the values agree quite well with those obtained by the Van Slyke manometric method and the same determinations calculated from the experimental initial pH and individual titration curves of each sample. Table II shows that the results also

TABLE I. Comparison of Values of CO_2 Content Obtained by the New Method with Those Found by the Van Slyke Method and Those Calculated from the Experimental Initial pH and Individual Titration Curves in 12 Experiments.

		CO_2 calculated from		
		Avg curve	Individual curve	
No.	pH	Vol %		Van Slyke
1	7.71	61.8	62.2	62.6
2	7.57	56.5	57.5	57.2
3	7.63	69.2	71.0	71.5
4	7.59	67.6	71.4	68.5
5	7.60	61.5	61.5	63.0
6	7.63	74.0	74.5	75.5
7	7.62	82.7	81.6	81.4
8	7.59	63.9	64.2	65.6
9	7.55	44.8	47.0	46.5
10	7.59	56.2	56.5	56.7
11	7.68	59.5	60.5	60.0
12	7.76	60.2	60.7	60.2

TABLE II. Comparison of Values of CO_2 Content Obtained by the New Method with Those Determined by the Van Slyke Method in 12 Samples of Varying Protein Content.

No.	Total protein, g/100 ml	CO_2 content, vol % — New method	CO_2 content, vol % — Van Slyke
1	6.5	65.7	65.6
2	6.4	63.5	63.3
3	7.2	77.6	76.7
4	6.2	65.4	65.8
5	5.4	55.7	55.5
6	5.5	57.8	56.1
7	6.7	58.9	58.4
8	5.7	70.9	72.0
9	5.1	61.3	62.2
10	5.5	61.1	61.6
11	5.2	55.5	56.1
12	7.0	60.2	62.0

agree quite well with the Van Slyke method in samples of varying protein contents. Of 307 determinations analyzed to date, 74% agreed within 2 volumes per cent of values found by the Van Slyke method. The agreement was always good between 40 and 80 volumes per cent, but there was an appreciable discrepancy in samples in which the carbon dioxide content was outside this range.

Summary. A method is described for the determination of carbon dioxide content of 0.1 and 0.2 ml samples of serum or plasma by adding 0.1 ml of standard acid and 0.1 ml of standard alkali and estimating the pH with a glass electrode. The values of carbon dioxide are then calculated from the amount of alkali found on an average composite curve for plasma or serum between an average initial pH at room temperature of 7.60 and the experimental final pH. Data are presented to show that the results obtained by this method agree quite well with values determined by the Van Slyke manometric method.

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Effect of Parathyroids on Radio-Calcium Uptake and Exchange in Rat Tissues.* (19688)

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These studies were undertaken to determine the uptake of radio calcium (Ca^{45}) by various tissues of the rat in normal and hypoparathyroid animals in the hope that further light might be thrown on the effect of the parathyroids on calcium metabolism. Until recently the Ca^{45} available for biological studies contained varying amounts of additional stable calcium. Since calcium in small amounts has marked effects on mammalian physiology, the carrier accompanying the radio-isotope could conceivably have a major influence on the results obtained. In this regard, L'Heureux *et al.*(1) found that the ratio of excreted urinary Ca^{45} to fecal Ca^{45} could be changed from 1:20 to 1:1 by increasing the amount of stable calcium administered with the radio-isotope. Lansing *et al.*(2) studied the uptake and exchange of Ca^{45} in mouse tissues in which the results obtained could be attributed to the large amount of stable calcium administered with the Ca^{45}. Tweedy *et al.*(3), in studying the excretion and distribution of radio-calcium in normal and thyroparathyroidectomized rats, administered Ca^{45} accompanied by total calcium in amounts ranging from 0.21 mg to 7.0 mg. Because of this apparent problem in the use of this isotope, it was thought best to first study the difference in the uptake by tissues of Ca^{45} when accompanied by widely varying amounts of carrier calcium.

Material and methods. A total of over 150 male rats, weighing between 200 and 250 g,

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