STUDIES OF FLUID EXCHANGES BETWEEN RAT LIVER SLICES AND SIMPLE MEDIA

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When slices of mammalian tissue are incubated under conditions which depress metabolism they take up fluid from Ringer's and hypertonic solutions which contain up to twice the solute concentration of extracellular fluid (1–4). When the metabolic activity of the slices is restored towards normal the swollen tissues lose fluid and return to their original volume (4–13). These findings have been cited as indicating the inadequacy of the classical theory of osmotic equilibrium between cells and their immediate environment and have been offered as evidence that the intracellular osmotic activity is higher than, and may be twice, that of the extracellular fluid (1–5, 15–18). Presumably this osmotic gradient is established across normal living cell membranes by a process of water expulsion which steadily counteracts inward diffusion and is maintained at the expense of the resting oxidative metabolism of the cell through the mediation of phosphate bond energy (5, 6).

However, the inverse correlation of tissue swelling and metabolic activity may also be the result of a reversible shift of both solute and solvent. The present paper reports on such a transfer of both solute and water between liver slices and simple media.

Methods

The animals used in these experiments were adult male and female rats weighing 150 to 250 gm. They had been maintained on a standard laboratory diet with free access to water. The rats were killed by bleeding and their livers removed immediately. Slices of liver were cut by hand to a thickness of approximately 0.5 mm., gently blotted with cotton gauze, weighed, and then immersed in 25 to 50 ml. of various solutions at temperatures of 4, 20, and 37°C. for periods of 1 to 60 minutes. Upon removal from the solutions the slices were blotted again, reweighed, and their sodium and potassium contents determined by flame photometry. The sodium and potassium contents of unimmersed slices were determined in parallel procedures.

In separate experiments the water content of the tissues was measured as the weight loss after drying to constant weight at 105 ± 5°C. In some of these experi-

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ments the tissue slices were dried in siliconed pyrex weighing bottles, digested in nitric acid and prepared for sodium and potassium analysis. In the remaining experiments, the chloride content of the tissues was determined in slices dried in platinum crucibles, alkalinized with 1 ml. of 0.1 M $NaHCO_3$, dried again, and then ashed at 500°C. The residue was dissolved in 1 ml. of 0.4 N H_2SO_4, transferred quantitatively to small beakers with redistilled water, and analyzed for chloride content by differential potentiometric titration (21).

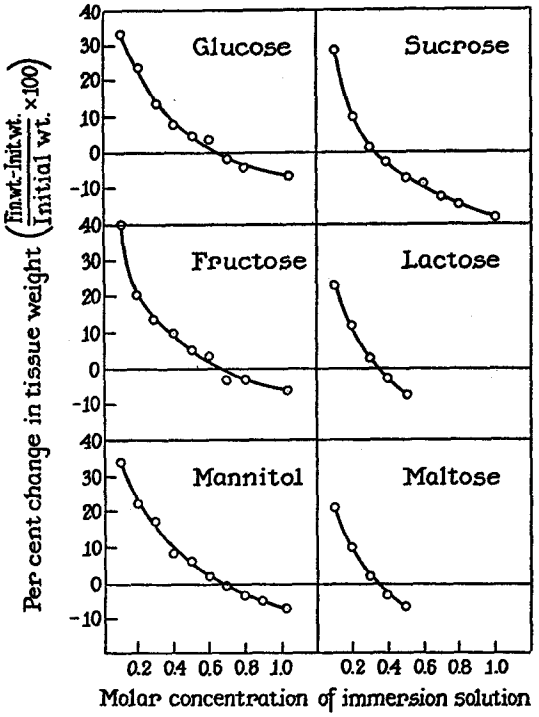

FIG. 1. Changes in the weight of liver slices immersed for 10 minutes at 20°C. in solutions of monosaccharides, and disaccharides varying in concentration from 0.1 to 1.0 molar.

RESULTS

Immersion of Fresh Liver Slices in Simple Non-Electrolyte Media.—Slices of liver immersed for 10 minutes at 20°C. in media containing glucose, fructose, or mannitol appeared to be isotonic with 0.62 to 0.68 molar solutions, since the slices gained weight in solutions which were less concentrated and lost weight in those which were more concentrated (Fig. 1, Table I). When the temperature of the immersion fluid was 37°C., the point of apparent isotonicity (P.A.I.) of the tissue did not differ significantly; but at 4°C., the P.A.I. dropped to 0.45 molar (Fig. 2). When the period of immersion was extended from 10 to 60 minutes all slices swelled significantly more at 37°

than at 20°C. although the curves were qualitatively similar at the two temperatures (Fig. 3).

When liver slices were immersed for 10 minutes in disaccharide solutions (sucrose, factose, or maltose) the P.A.I. ranged from 0.35 to 0.37 M at 20°C. and remained unchanged at temperatures of 4° and 37°C. (Figs. 1 and 4; Table I).

TABLE I

Apparent Isotonicity of Liver Slices Immersed for 10 Minutes at 4, 20, and 37°C. in Solutions of Monosaccharides, Disaccharides, and Sodium Chloride

Immersion fluid solute	Temperature	No. of experiments	Molar concentration of the apparently isoosmotic solution	
			Mean	Range
	°C.			
Glucose	4	5	0.45	0.42–0.48
	20	5	0.68	0.61–0.80
	37	5	0.64	0.60–0.68
Fructose	20	2	0.62	0.61–0.63
Mannitol	20	5	0.64	0.60–0.75
Sucrose	4	3	0.35	0.34–0.37
	20	5	0.35	0.33–0.40
	37	3	0.37	0.34–0.38
Lactose	20	4	0.36	0.31–0.36
Maltose	20	4	0.37	0.32–0.40
Sodium chloride	4	5	0.34	0.29–0.38
	20	5	0.37	0.34–0.40
	37	7	0.27	0.21–0.34

Immersion of Fresh Liver Slices in Solutions of Sodium Chloride.—When slices of liver were immersed for 10 minutes at 20°C. in 0.1 to 1.0 molar solutions of sodium chloride, fluid entered the tissues from the solutions that were less concentrated and left the tissues when the solutions were more concentrated than 0.37 (±0.02) molar. At 4°C., this point of apparent isotonicity was 0.34 (±0.04) molar; and not significantly different from the P.A.I. at 20°C. At 37°C.,[1] the P.A.I. averaged 0.27 (±0.04) molar and proved to be significantly ($p < 0.01$) lower than at 4° and 20°C. (Fig. 5).

[1] It is noteworthy that the slope of the curve relating change in tissue weight to the sodium chloride concentration of the medium was greater at 37 and 20°C. than at 4°C. rendering the measurement of the P.A.I. more sensitive at the higher temperatures.

Because of its apparent isotonicity with liver tissue, the 0.3 molar sodium chloride solution was used to study the effects of temperature and of a longer period of immersion on the shifts of water, sodium, potassium, and

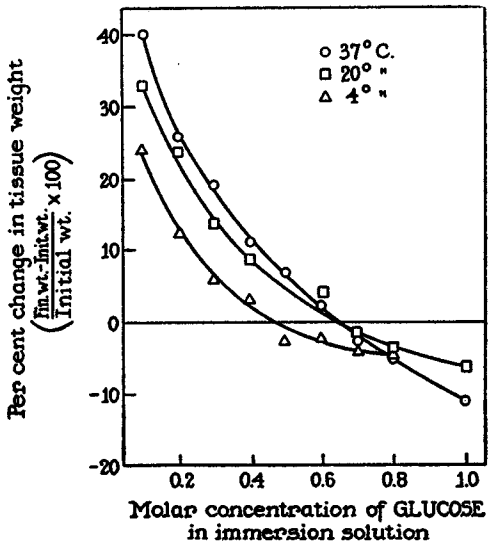

FIG. 2. Changes in the weight of liver slices immersed for 10 minutes at 4, 20, and 37°C. in solutions of glucose varying in concentration from 0.1 to 1.0 molar.

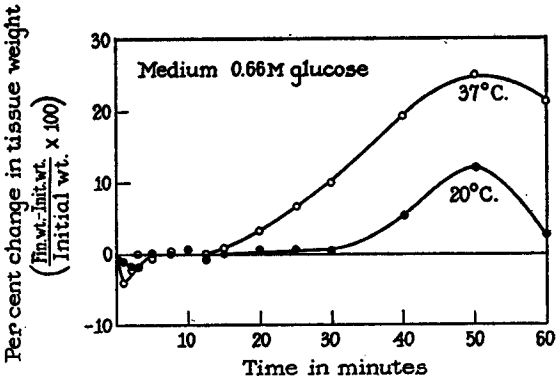

FIG. 3. Changes with time in the weight of liver slices immersed at 20 and 37°C. in 0.66 molar glucose solutions.

chloride between the slice and the bath. Typical results of this study are given in Fig. 6 and Table II. At 20°C. the slices first lost fluid for a period of 2 to 4 minutes, but after 10 to 15 minutes, began to swell. In contrast, at

37°C., the initial loss of fluid was maintained throughout the entire 60 minutes of the experiment. At all times after immersion the content of sodium and chloride was less at 37° than at 20°C., while the retention of potassium

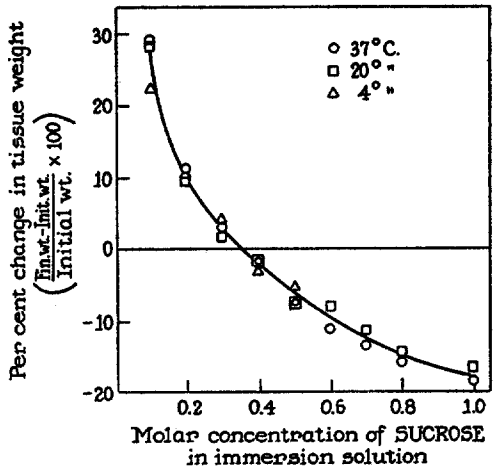

FIG. 4. Changes in the weight of liver slices immersed for 10 minutes at 4, 20, and 37°C. in solutions of sucrose varying in concentration from 0.1 to 1.0 molar.

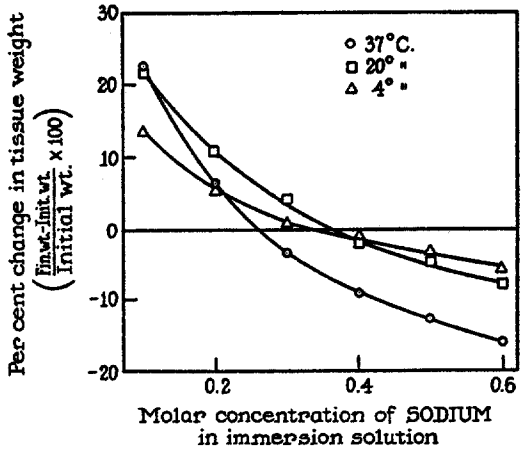

FIG. 5. Changes in the weight of liver slices immersed for 10 minutes at 4, 20, and 37°C. in solutions of sodium chloride varying in concentration from 0.05 to 0.6 molar.

was always greater at 37° than at 20°C. Thus the accumulation by the tissue of both salt and water was greater at 20° than at 37°C.

After 30 minutes of incubation at both 20 and 37°C., the sodium, potas-

sium, and water content of the slices remained constant. The chloride content of the slices was lower than the Na+K content but continued to increase for as long as 60 minutes suggesting that chloride from the medium was exchanging with other intracellular anions.

In addition to the transfer with chloride ions, sodium entered the tissue

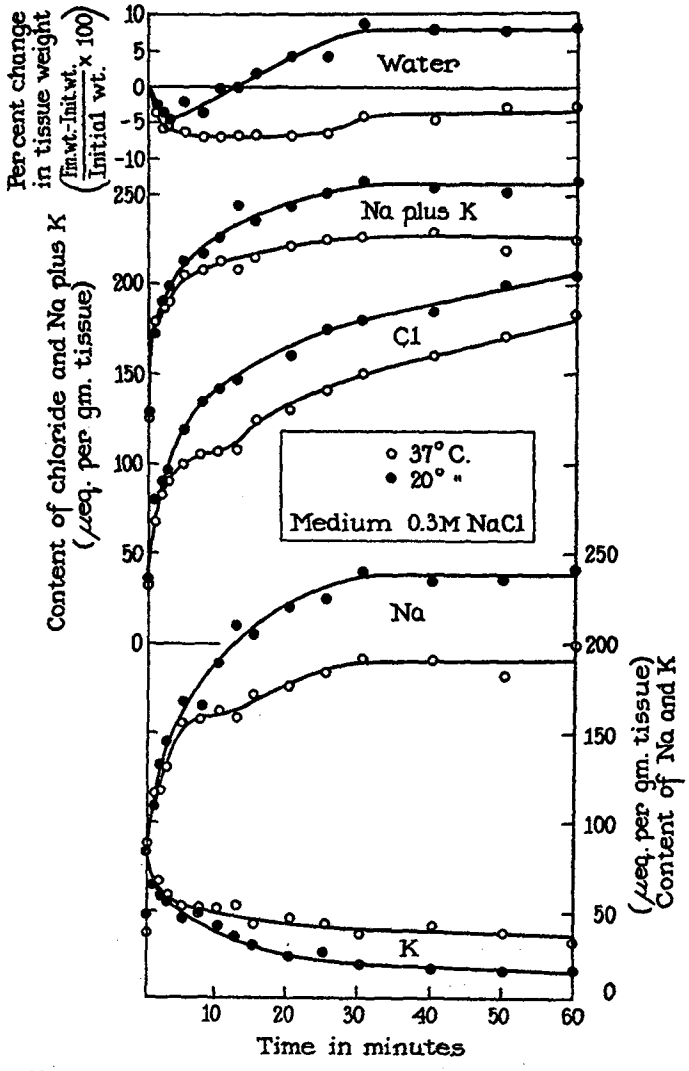

FIG. 6. Changes with time in the weight and the content of sodium, potassium and chloride (microequivalents per gram wet weight) of liver slices immersed at 20 and 37°C. in 0.3 molar sodium chloride solutions.

TABLE II

Difference in the Hydration and Na + K Content of Liver Slices Immersed at 20 and 37°C. in 0.3 Molar Sodium Chloride

	Incubation temperature	Control slices (unimmersed)	Duration of immersion period, *min.*			
			15	30	45	60
	°C.					
A. Per cent of total tissue mass remaining after immersion (observed)		100				
	20		95.6	94.7	95.6	94.9
	37		87.4	84.3	87.4	90.1
B. Per cent of dry substance before and after immersion (observed)		30.2				
	20		27.5	26.8	25.9	25.0
	37		28.6	28.3	27.4	25.7
C. Gm. of dry substance remaining after immersion of 1 gm. of original tissue ($10^{-4} \cdot A \cdot B$)	20		0.263	0.257	0.248	0.237
	37		0.250	0.239	0.239	0.232
D. Gm. of dry substance lost on immersion of 1 gm. of original tissue (0.302 − C)	20		0.039	0.045	0.054	0.065
	37		0.052	0.063	0.063	0.070
E. Per cent loss of dry substance $\left(\frac{D}{0.302} \cdot 100\right)$	20		12.9	14.9	17.8	21.5
	37		17.2	20.8	20.8	23.2
F. μeq. of Na per gm. wet weight of tissue before and after immersion (observed)		55				
	20		190	217	231	226
	37		166	191	201	218
G. μeq. of K per gm. wet weight of tissue before and after immersion (observed)		87				
	20		41	30	26	25
	37		54	46	41	29
H. μeq. of Na + K per gm. wet weight of tissue before and after immersion (F + G)		143				
	20		231	247	256	251
	37		221	235	243	247
I. μeq. of Na + K remaining after immersing 1 gm. of original tissue ($10^{-3} \cdot A \cdot H$)	20		221	234	245	238
	37		193	198	212	223
J. Increase of Na + K content (μeq.) on immersion of 1 gm. of original tissue (I − 143)	20		78	91	102	95
	37		50	55	69	80

TABLE II—*Concluded*

	Incubation temperature	Control slices (unimmersed)	Duration of immersion period, *min.*			
			15	30	45	60
	°C.					
K. Per cent tissue water before and after immersion (100 − B)		69.8				
	20		72.5	73.2	74.1	75.0
	37		71.4	71.7	72.6	74.3
L. Tissue water content after immersion expressed as gm. H_2O per gm. of original tissue. ($10^{-4} \cdot A \cdot K$)	20		0.693	0.692	0.709	0.712
	37		0.624	0.604	0.634	0.670
M. Gm. of water gained or lost from 1 gm. of original tissue after immersion. (0.698 − L)	20		−0.005	−0.006	+0.011	+0.014
	37		−0.074	−0.094	−0.064	−0.028
N. μeq. of Na + K gained by 1 gm. original tissue as a result of temperature change from 37 to 20°C. ($J_{20°C.} - J_{37°C.}$)	20–37		28	36	33	15
O. Gm. of water gained by 1 gm. of original tissue as a result of temperature change from 37 to 20°C. ($M_{20°C.} - M_{37°C.}$)	20–37		0.069	0.088	0.075	0.042
P. μeq. of Na + K per gm. of water gained by the tissue from the medium as a result of temperature change from 37 to 20°C. (N/O)	20–37		405	409	440	358

Each value represents the average of a group of five experiments.

slice by exchanging with potassium. A comparison of the analyses obtained on slices immersed at 20 and at 37°C. showed that the change in the concentration of sodium plus potassium corresponded approximately to the change in the concentration of chloride (Fig. 6); this change averaged 344 (±101) μeq. per gm. change in tissue water. Thus, with change in temperature from 37 to 20°C. or from 20 to 37°C., the slice may be considered to gain or lose 688 (±202) m.osM per kilogram of total shifting fluid. If the immersion fluid, which contains 600 m.osM per liter (activity coefficient taken as 1), be regarded as entering and leaving the slices in bulk, other mechanisms are necessary to provide additional osmotically active intracellular solute when the observed concentration of the shifting fluid is less than 600

m.osm per liter, and to inactivate such intracellular solute when the shifting fluid has a higher osmolar concentration.

DISCUSSION

Deyrup has shown that slices of rat kidney swell in physiological fluids or in equally concentrated solutions of monosaccharides, but not in 0.3 molar solutions of disaccharides (14). The results of the present study with liver slices confirm Deyrup's observations on renal tissue, and the differential effects of temperature are in accord with her suggestion that the swelling is due to ingress of water accompanying the primary entry of solute into the tissue cells. Presumably the greater swelling of liver slices in glucose solutions at 37 and 20°C. as compared with slices immersed at 4°C. is due to the more efficient inward transport, accumulation and/or metabolism of the immersion fluid solute (glucose) at the higher temperatures.

One of us has reported previously (22) on the change of the sodium content of liver slices immersed for varying periods in solutions of varying sodium concentration. When the sodium concentration of the medium, M_{Na} was held constant and the time of immersion varied, it was found that the slice gained sodium till the 8th minute, and thereafter its sodium content remained constant for 10 to 15 minutes; when M_{Na} was varied and the slice immersed for 10 minutes, the entry of sodium into the tissue was found to be proportional to M_{Na} and independent of the degree and direction of the fluid movement as judged by the change in the weight of the slice. At the time we interpreted these findings to mean that the medium had come into equilibrium with the rapidly exchanging (extracellular) phases of the slice within the first 10 minutes after immersion, but that the solute of the medium had not yet penetrated the less accessible (intracellular) phases.

This interpretation implied that the solute concentration of the intracellular phases of the slice was 0.6 to 0.8 osmoles per liter, that is, the osmolar concentration of the sodium chloride solution in which the slice neither gained nor lost weight. However, this interpretation of the earlier experiments did not account for the subsequent finding that the weight of the tissue slices immersed in hypertonic solutions first decreased before reversing to regain or exceed the original value (Figs. 3 and 6). Also the comparison of electrolyte content and hydration of liver slices at 20 and 37°C. suggested that the greater swelling at the lower temperature was due to the depressed metabolic activity of the cells with consequent failure of solute (sodium and chloride) extrusion. These latter findings do not support the assumption that liver cells respond to changes in external osmotic pressure by movement of water alone but rather support the conclusion of Mudge (12) and Leaf (23) that cells adjust to osmotic changes with electrolyte shifts and do not react as simple osmometers.

In general, it was found that there was sufficient Na+K entering or leaving the slices to account for the observed changes in tissue volume in terms of movement of medium in bulk. In some experiments the amount of Na+K accumulated in the tissue when the temperature was lowered exceeded that required for simple bulk transfer of medium. For example, in the group of experiments presented in Table II an average of 400 μeq. of Na+K accompanied each gram of water entering the slice when the temperature of incubation was lowered from 37 to 20°C. It is possible that under the conditions of these experiments there occurred a net aggregation of intracellular constituents, decreasing the intracellular osmotic activity concomitantly with or shortly after the failure of sodium chloride extrusion.

In other experiments it was found that only 250 μeq of Na+K accompanied each gram of water entering or leaving the slices. In these experiments the movement of medium in bulk would explain only five-sixths of the tissue swelling observed when the incubation temperature is lowered from 37 to 20°C.; the residual swelling may be accounted for by movement of water alone in response to a gradient of 100 m.osM per liter developed across cell and/or subcellular membranes. This magnitude of intracellular hypertonicity is in accord with the cryoscopic measurements of Appelboom, Brodsky, Dennis, Rehm, Miley, and Diamond (24) and the rapid (19) and presumably reversible (20) breakdown of ATP, hexose esters, creatine phosphate, and glycogen *in vitro*.

Inasmuch as relocation of solute depends on metabolic activity, it is difficult to consider disaggregation or breakdown of intracellular constituents and solute transport processes as independent factors influencing the water exchange between cells and extracellular fluid or between subcellular structures and cytoplasm; an adequate theoretical treatment of this problem would require detailed knowledge of the mechanisms of the major relevant solute fluxes and the precise nature, sequence, and relationship of the coupled chemical reactions. As such knowledge accumulates it may become possible to distinguish between the movement of preexisting particles and the appearance of new particles and to assess quantitatively the role of each as factors in cell hydration. For the present, however, it seems more important to develop direct methods of study, such as improved techniques for measuring the colligative properties of tissue preparations (24), which will permit a valid interpretation of the findings now offered as evidence that the free energy of water is lower within cells than in extracellular fluid.

SUMMARY

1. The exchange of fluid between slices of rat liver and solutions of monosaccharides, disaccharides, and sodium chloride has been studied in relation to the temperature of incubation. The point of apparent isotonicity (P.A.I.)

of the tissue was defined as the concentration of the solution in which the slices neither gained nor lost weight after immersion for a period of 10 minutes.

2. In solutions of glucose, the P.A.I. of the slices was significantly lower at 4°C. than at 20°C. but similar at 20°C. and 37°C. Upon immersion for 15 to 60 minutes in 0.66 molar glucose the slices always swelled more at 37°C. than at 20°C. In solutions of sucrose change in the temperature of incubation was without effect on the hydration of the tissues.

3. In solutions of sodium chloride, the P.A.I. and the content of water, chloride, and sodium plus potassium were lower at 37°C. than at 20°C.

4. These findings emphasize the role of translocation of solute in providing an osmotic gradient for the movement of water between the tissue slices and the media.

REFERENCES

1. Opie, E. L., *J. Exp. Med.*, 1949, **89,** 185.
2. Opie, E. L., and Rothbard, M. B., *J. Exp. Med.*, 1953, **97,** 483.
3. Opie, E. L., *Harvey Lectures*, 1954–55, **50,** 292.
4. Robinson, J. R., *Proc. Roy. Soc., London, Series B*, 1950, **137,** 378.
5. Robinson, J. R., *Nature*, 1950, **166,** 989.
6. Robinson, J. R., Proc. Roy. Soc., *London, Series B*, 1952, **140,** 135.
7. Stern, J. R., Eggleston, G. V., Hems, R., and Krebs, H. A., *Biochem. J.*, 1949, **44,** 410.
8. Aebi, H., *Helvet. Physiol. et Pharmacol. Acta*, 1950, **8,** 525.
9. Aebi, H., *Helvet. Physiol. et Pharmacol. Acta*, 1952, **10,** 184.
10. Aebi, H., *Helvet. Physiol. et Pharmacol. Acta*, 1953, **11,** 96.
11. Mudge, G., *Am. J. Physiol.*, 1951, **165,** 113.
12. Mudge, G., *Am. J. Physiol*, 1951, **167,** 206.
13. Deyrup, I., *Am. J. Physiol.*, 1953, **175,** 349.
14. Deyrup, I., *J. Gen. Physiol.*, 1953, **36,** 739.
15. Whittam, R., and Davies, R. E., *Biochem. J.*, 1953, **55,** 880.
16. Bartley, W., Davies, R. E., and Krebs, H. A., *Proc. Roy. Soc., London, Series B*, 1954, **142,** 187.
17. Robinson, J. R., and McCance, R. A., *Ann. Rev. Physiol.*, 1952, **14,** 115.
18. Robinson, J. R., *Biol. Rev.*, 1953, **28,** 158.
19. Conway, E. J., Geohegan, H., and McCormack, J. I., *J. Physiol.*, 1955, **130,** 427.
20. Conway, E. J., and Geohegan, H., *J. Physiol.*, 1955, **130,** 438.
21. Dole, V. P., and Thorn, N., *Anal. Chem.*, 1955, **27,** 1184.
22. Schwartz, I. L., and Opie, E. L., *Fed. Proc.*, 1954, **13,** 132.
23. Leaf, A., *Biochem. J.*, 1956, **62,** 241.
24. Brodsky, W. A., Appelboom, J. W., Dennis, W. H., Rehm, W. S., Miley, J. F., and Diamond, I., *J. Gen. Physiol.*, 1956, **40,** 183.