CHANGES IN THE OSMOTIC ACTIVITY OF LIVER AND OF KIDNEY TISSUE CAUSED BY PASSAGE OF SODIUM CHLORIDE, UREA, AND SOME OTHER SUBSTANCES INTO CELLS

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Foregoing studies (1) have shown that slices of liver, kidney, pancreas, submaxillary gland, and muscle, including smooth, cardiac, and skeletal muscle, when immersed in solutions of sodium chloride are in water equilibrium with a concentration considerably greater than that of physiological salt solution, and take up water from Ringer's solution, or blood serum because the cells of the part maintain a higher osmotic pressure than that of the fluid surrounding them. The occurrence of this hypertonicity has been confirmed by Robinson (2), Aebi (3), Bartley, Davies, and Krebs (4), and others. Liver tissue is isotonic with solution, having more than twice the concentration of sodium chloride in blood plasma, and kidney cortex with a solution with somewhat less than twice this concentration. If in accord with widely accepted opinion, fluid of the interstitial tissue has the characters of an ultrafiltrate of plasma (5), hypertonicity of liver tissue or of kidney cortex is referable to the cells of the part. Parenchymatous cells of the organs that have been mentioned tend to maintain a constant level of osmotic activity (osmotic homeostasis) (2) which like regulation of temperature may be modified by a variety of conditions (6). This level of osmotic activity is presumably maintained by the metabolism of the cells concerned.

Most of those who have discussed the osmotic activity of cells and the movement of water between cells and extracellular fluid have emphasized the outstanding significance of electrolytes. Though potassium predominates within the cell, and sodium in the extracellular fluid, evidence has accumulated to show that the sum of the ionic concentration of sodium, potassium, calcium, and magnesium is the same in cells and in extracellular fluid. This relation has seemed to give some support to the opinion that tissue cells, like erythrocytes, are isotonic with physiological salt solution—an opinion formerly almost universally accepted. Less attention has been given to the possibility that products of metabolism aid in sustaining a level of osmotic activity above that of the surrounding fluid.

Methods

Solutions of sodium chloride varying in concentration from 0.1 to 0.5 molar have been used to measure water exchange of tissues immersed in them. The relation between the concentration of the salt and the per cent loss or gain of water, determined when possible in duplicate, pursues as plotted, a linear course which marks the concentration isotonic with the tissue. When liver or kidney cortex is immersed in isotonic solutions of sodium chloride water equilibrium is attained after 15 or 20 minutes (7), and subsequent swelling doubtless indicates that injury has ensued. Evidence that the osmotic activity of the tissue present during life is preserved during this short period of immersion has been assembled in earlier publications, in which the method employed has been fully described (1, 6).

Experiments with Sodium Chloride

In the experiments that will be described attempts have been made to introduce an electrolyte into the body in quantity sufficient to bring about its entrance into liver or kidney cortex and thus raise the level of isotonicity of immersed slices of these organs.

When sodium chloride, forming from 2.9 to 17.4 per cent of the food, was fed by Gamble and his associates (8) to white rats, the water consumed increased continuously from 17.2 cc. daily, when no salt was added to the diet, to 118 cc. with the largest salt ingestion. This quantity of water was about half of the weight of the animal. The water ingested following administration of urea was much less than that consumed when corresponding osmolar quantities of sodium chloride were given.

Hypertension has been produced by Saperstein (9) and coworkers in white rats after from 4 to 6 weeks by administration of drinking water containing 1.5 to 2.5 per cent of sodium chloride. When Meneely (10) and associates fed during periods from 9 to 12 months diets containing from 7 to 9.8 per cent of sodium chloride, hypertension occurred together with conspicuous increase of fluid intake and polyuria. In 18 per cent of the animals, edema, indicated by a sharp increase of body weight, was followed by precipitate loss of weight and death. In the kidneys of all of the animals with edema, and of some others with no edema, advanced nephrosis was associated with changes in glomeruli and in tubular epithelium. The "sodium space" of the body, determined by use of the radioisotope, Na^{24}, was found in control animals to be from 21.6 to 23.8 per cent of the gross body weight. It was raised slightly, (25.8 to 26.1 per cent), in animals which with no edema received diets with high salt content and to 57.5 per cent in animals with edema.

Isotonicity of Liver and of Kidney Cortex in Animals That Have Received Sodium Chloride in Drinking Water.—When white rats have received drinking water containing sodium chloride the quantity of water consumed and the volume of the urine have increased with increase of the ingested sodium chloride. Its rapid elimination presumably tends to prevent its accumulation in the blood and in the extracellular fluid.

When animals have had access to drinking water supplied through a tube from a water bottle and have received a routine laboratory diet of bread wet with milk the quantity of water consumed daily has been 9.4 cc. per 100 gm. of body weight but when the food has been dried at 37°C. the corresponding daily intake of water has been about 16 cc. It is noteworthy that there is some proportional decrease of water intake when body weight increases and during a period of several weeks the average quantity consumed daily by an animal weighing 175 gm. has been 19.3 cc.; of one weighing 210 gm., 17 cc.; and of a third weighing 325 gm. 12.5 cc. For the purpose of the experiments it has not been necessary to measure exactly the intake of water and output of urine.

When rats have received as drinking water 0.25 molar sodium chloride (1.5 per cent) together with dry food during 5 weeks, the water taken in per 100 gm. of body weight in 10 animals has not increased being approximately 15 cc. and during the same period the weight of these animals has remained essentially unchanged. Administration of 0.35 molar salt solution (2 per cent) has been followed by prompt increase of water intake and of urine output. The water ingested has varied widely in different animals and from week to week. Within the first 2 months the average water drunk has been about 30 cc./100 gm., reaching an average daily maximum of 40 cc. during some weeks. When these have lived 3 or 4 months the daily average has been much greater reaching 75 cc./100 gm. Body weight is maintained with slight diminution during 1 month and after 3 months is usually considerably diminished. Later in all instances weight has diminished. Fat has in large part disappeared and the thymus has atrophied. Animals that have received 0.425 molar sodium chloride have drunk about the same quantity of fluid as those that have received 0.35 molar but diminution of body weight has occurred more rapidly.

To determine whether the electrolyte content of liver or of kidney tissue is increased by the administration of sodium chloride in drinking water the isotonicity of slices of the two organs has been measured by immersing them in solutions of varying concentration. These experiments have been listed in Table I in the order of increasing duration of the period of salt ingestion and are tentatively separated into three groups in which this period has been respectively, (*a*) less than 50 days with average isotonicity of 0.306 molar sodium chloride; (*b*) from 50 to 100 days with average isotonicity of 0.325 molar; and (*c*) more than 100 days with average isotonicity of 0.367 molar. The isotonicity of kidney tissue has increased in the third period.

The significance of the figures recorded in Table I may be questioned. They suggest that the long continued ingestion of sodium chloride in drinking water may finally overcome the conditions favorable for its elimination so that in the period after 100 days the electrolyte may reach a concentration somewhat greater than that of normal liver or kidney tissue. The experiments offer no evidence concerning the location of the presumably increased electrolyte content of liver or of kidney because its relation to intra- or extracellular space is uncertain.

Isotonicity of Liver and of Kidney Tissue Following Increase of Sodium Chloride in the Food.—When white rats with free access to water are given dried food with sodium chloride forming from 7 to 11 per cent of its dry weight the quantity of water consumed varies widely in different animals and at different times in the same animal.

Over a prolonged period the average intake of water estimated in relation to 100 gm. of body weight has varied from 30 to 60 cc. per day and has differed little from the quantity of fluid consumed by animals that have received drinking water containing sodium chloride. Nevertheless the adjustment between the ingested sodium chloride and water intake seems to be made with greater difficulty when the salt is combined with the food. When sodium chloride has constituted 9.8 per cent of the food it has evidently been injurious in some instances, (Table II Experiments 1, 3, 4, 5, and 6), and body weight has diminished during the first few weeks of its administration at a time when little water has been consumed. Later when the animal drinks water in larger quantity body weight increases and sometimes rises considerably above the original weight. Restriction of water intake to 16 cc. daily has been followed immediately by diminution of body weight.

TABLE I

Sodium Chloride in Drinking Water

No. of experiment	Concentration of sodium chloride in drinking water	Duration of sodium chloride ingestion	Change in body weight	Isotonicity of liver	Isotonicity of kidney
	M	*days*	*gm.*	M	M
1	0.35	23	−1	0.268	0.260
2	0.35	29	−10	0.316	0.272
3	0.35	36	−9	0.298	0.230
4	0.25; 0.35; 0.4	37	−117	0.335	0.298
5	0.25	41	−32	0.293	0.268
6	0.25; 0.35; 0.4	42	−100	0.340	0.316
Average				0.306	0.276
7	0.35; 0.425	50	−39	0.367	0.247
8	0.35; 0.425	57	−47	0.346	0.266
9	0.35; 0.425	64	−32	0.253	0.260
10	0.25; 0.35; 0.4	64	−86	0.330	0.283
11	0.35; 0.425	71	−90	0.364	0.281
12	0.35; 0.425	75	−52	0.315	0.259
13	0.35; 0.425	82	−92	0.349	0.255
14	0.25; 0.35; 0.425	90	−6	0.314	0.265
15	0.25; 0.35; 0.425	92	−33	0.288	0.270
Average				0.325	0.265
16	0.25; 0.35; 0.425	110	−102	0.390	0.353
17	0.25; 0.35; 0.425	123	−66	0.343	0.356
18	0.35	124	−87	0.402	0.287
19	0.35	126	+22	0.317	0.234
20	0.35	131	−25	0.385	0.307
Average				0.367	0.307

The administration of sodium chloride with food (Table II) has not increased the level of isotonicity of the tissues examined, the average of the figures being for liver, 0.288 molar, which is below that of normal liver and for kidney, 0.261 molar, which is approximately normal. In several instances isotonicity has been less than 0.3 molar and in these (Table II, Experiments 3, 4, and 5) the animal has received salt in high concentration (10.5 or 11.2 per cent). The lowest level of isotonicity (Experiment 3) was found in an animal which received a high concentration of salt and restricted drinking water. Sodium chloride under these conditions may act as an injurious agent like chloroform or carbon

TABLE II

Sodium Chloride in Food

No. of experiment	Concentration of sodium chloride in dried food	Duration of sodium chloride ingestion	Change in body weight	Isotonicity	
				of liver	of kidney
	per cent	*days*	*gm.*	M	M
1	7.0	145	−5	0.289	0.286
	10.5	104			
2	9.8	308	20	0.344	0.289
	(Drinking water restricted)	(51)*			
3	9.8	48	−44	0.191	0.248
	11.2	59			
	(Drinking water restricted)	(41)			
4	9.8	119	−33	0.267	0.262
	11.2	23			
5	9.8	119	−30	0.285	0.222
	11.2	27			
6	9.8	113	+18	0.318	0.227
	(0.85 per cent sodium chloride as drinking water)	(78)			
7	11.2	119	+17	0.326	0.290
	(Drinking water restricted)	(42)			
Average				0.288	0.261

* Figures in parenthesis are included in the period of sodium chloride administration and represent its terminal part.

tetrachloride (11) and diminish the ability of the liver to maintain its normally high level of isotonicity. It is noteworthy that in one instance (Experiment 4) isotonicity of liver remained at an approximately normal level even though the concentration of salt in the food was high (11.2 per cent) and water was restricted.

Isotonicity of Liver and of Kidney Tissue Following Subcutaneous Injection of Sodium Chloride.—Elimination of sodium chloride by way of the kidneys is evidently increased in most instances when the salt is administered with drinking water or with food. Experiments have been undertaken to determine whether concentrated solutions of sodium chloride injected into the subcutaneous tissue raise the level of isotonicity of liver or of kidney tissue (Table III) as in this case there is evidently less opportunity for the renal elimination of the salt.

A solution of sodium chloride, containing 25 gm. in 100 cc., was introduced by multiple small injections into the subcutaneous tissue of the flank and back on both sides. The time recorded in Table III is that between the last of the multiple injections and death. The animals were killed by bleeding from the vessels of the neck after symptoms had appeared as indicated in the table, save in one instance (Experiment 7) in which breathing stopped before the animal was bled. Convulsions occurred in animals given quantities of sodium chloride up to 500 mg. per 100 gm. of body weight and those that received larger quantities became comatose with no convulsive movements. After injection of 250 mg. sodium chloride per 100 gm. of body weight, recovery occurred in some instances, but larger quantities caused death.

TABLE III

Sodium Chloride Subcutaneously

No. of experiment	Body weight	Quantity of sodium chloride injected per 100 gm. of body weight	Symptoms	Period of survival	Isotonicity	
					of liver	of kidney
	gm.	*mg.*		*min.*	M	M
1	176	300	Convulsions and coma	159	0.387	0.272
2	185	350	Convulsive movements	133	0.431	
3	152	500	Coma	61	0.462	
4	277	500	Convulsion	32	0.426	0.275
5	200	500	Coma	44	0.700	0.350
6	165	1000	Coma	30	0.450	>0.50
7	170	1500	Death	26	0.442	0.377
Average					0.471	>0.355

Comparison of the figures of Table III, recording the average isotonicity of liver slices, with that representing the average for normal liver, namely, 0.34 molar (1, 3) and with similar figures in Tables I and II shows that the level of isotonicity following the subcutaneous injection of sodium chloride is considerably increased, the average in 7 instances being 0.471 molar sodium chloride. The average isotonicity of kidney namely, 0.375 molar (Table III), has been much increased above the average for normal kidney tissue (0.23 molar) (2) and above the corresponding figure for animals that have ingested sodium chloride (Tables I and II).

TABLE IV

Urea Subcutaneously

No. of experiment	Body weight	Quantity of urea injected per 100 gm. of body weight	Symptoms	Period of survival	Isotonicity of liver	Isotonicity of kidney
	gm.	*mg.*		*min.*	M	M
1	145	500	None	79	0.345	0.300
2	112	500	"	77	0.348	0.270
3	110	500	"	60	0.357	0.234
4	100	500	"	60	0.316	0.219
5	225	500	"	60	0.310	0.186
6	162	600	"	60	0.421	0.226
7	151	600	"	60	0.393	0.213
Average					0.356	0.235
8	114	750	Convulsive movements	58	0.361	0.318
9	185	750	Convulsion	60	0.455	0.363
10	181	750	Convulsion	60	0.465	0.295
11	161	750	Muscular rigidity	180	0.334	0.282
12	141	750	Twitching of muscles	182	0.350	0.315
13	147	750	Twitching of muscles	60	0.410	0.268
14	141	750	Convulsive movements	180	0.383	0.260
15	230	750	Convulsive movements	60	0.502	0.342
16	225	750	Convulsive movements	60	0.444	0.293
Average					0.412	0.304
17	148	1000	Convulsion	39	0.380	0.292
18	139	1000	Tremor	82	0.412	0.273
19	130	1000	Convulsive movements	93	0.438	0.360
20	145	1500	Coma	34	0.650	0.342
21	156	1500	Convulsion	45	0.395	0.349
22	155	1500	Convulsion	54	0.388	0.357
23	167	1500	Convulsion	56	0.427	0.291
24	405	1500	Convulsion	73	0.371	0.332
Average					0.433	0.326

Experiments with Urea and Some Other Substances

In view of the readiness with which the level of isotonicity of liver tissue has been raised by the introduction of an electrolyte into the body under conditions that avoid its rapid elimination experiments have been undertaken to determine whether the same result can be obtained with a non-electrolyte that is freely disseminated throughout the tissues. For this purpose urea has been injected into the subcutaneous tissue of the back and flanks divided into small quantities distributed over a wide area in order to hasten absorption (Table IV). A 2 per cent solution of urea (20 mg. in 1 cc.) in 0.15 molar sodium chloride has been used.

Urea injected in quantities of 500 or 600 mg. per 100 gm. of body weight has produced no symptoms whereas larger quantities have caused various disturbances referable to the muscular system, as tremor, twitching or rigidity of muscles, convulsive movements or generalized convulsions. In animals that have received the smaller quantities of urea and, having no symptoms, have been examined after about 1 hour, the average levels of isotonicity of liver tissue (0.356 molar sodium chloride) and of kidney cortex (0.235 molar) have been approximately those of the corresponding normal tissues. In animals that have received 750 mg. per 100 gm. of body weight convulsions have occurred after

TABLE V

Creatinin Subcutaneously

No. of experiment	Body weight	Quantity of creatinin injected per 100 gm. body weight	Symptoms	Period of survival	Isotonicity	
					of liver	of kidney
	gm.	*mg.*		*min.*	M	M
1	198	500	None	57	0.432	0.241
2	142	1000	None	35	0.360	0.226
3	177	1000	Convulsion	41	0.406	0.236
4	205	1200	None	58	0.375	0.233
Average					0.393	0.234

from 1 to 3 hours and the average isotonicity of liver tissue in 8 animals has been 0.408 molar sodium chloride. When larger quantities of urea have been administered, that is, 1000 or 1500 mg. per 100 gm., convulsions have occurred in most instances and the average isotonicity of liver tissue has increased to 0.433 molar. Similarly the average isotonicity of kidney tissue has increased with increasing quantities of urea and has been respectively, 0.235, 0.305, and 0.326 molar.

Creatinin.—The effect of urea upon the isotonicity of liver and of kidney has suggested the desirability of similar experiments with creatinin, which is present in the normal blood serum and is increased under some pathological conditions. Table V shows that isotonicity of the liver of animals that have received creatinin subcutaneously may be raised above the normal level, the average being 0.393 molar sodium chloride in 4 instances. The isotonicity of kidney cortex has remained unchanged.

Creatinin injected in the proportion of 5 or 15 mg. per 100 gm. of body weight in combination with urea, 750 mg. per 100 gm. of body weight, (Table VI), has not changed significantly the levels of isotonicity of liver or of kidney found after injection of the same quantity of urea alone. The average isotonicity of liver of animals that received urea with creatinin has been with 0.422 molar sodium chloride, whereas that of animals receiving urea alone in the same

TABLE VI

Urea with Creatinin Subcutaneously

No. of experiment	Body weight	Quantity of urea injected per 100 gm. of body weight	Quantity of creatinin injected per 100 gm. of body weight	Symptoms	Period of survival	Isotonicity	
						of liver	of kidney
	gm.	mg.	mg.		min.	M	M
1	140	750	5	Tremor	61	0.451	0.283
2	141	750	5	Twitching	121	0.385	0.272
3	144	750	5	Tremor	60	0.388	0.236
4	222	750	15	Convulsion	74	0.462	0.334
Average						0.422	0.281

TABLE VII

Glycine Subcutaneously

No. of experiment	Body weight	Quantity of glycine injected per 100 gm. body weight	Symptoms	Period of survival	Isotonicity	
					of liver	of kidney
	gm.	mg.		min.	M	M
1	180	750	Weakness	62	0.307	0.282
2	195	750	Convulsive movements	32	0.322	0.272
3	213	1000	Twitching	90	0.289	0.244
4	156	1000	Weakness	25	0.326	0.280
Average					0.311	0.270

quantity has been 0.412 molar. The isotonicity of kidney of animals receiving urea with and with no creatinin has differed little.

Glycine and Arginine.—Glycine, when injected subcutaneously in multiple injections in quantities from 400 to 1000 mg. per 100 gm. of body weight, has caused weakness, apathy, muscular twitching, and convulsive movements. In experiments in which the isotonicity of liver and kidney has been determined no significant change from the usual figures has been found (Table VII).

Another amino acid, arginine, closely related to urea, has not increased the levels of isotonicity of liver or of kidney cortex (Table VIII).

TABLE VIII

Arginine Subcutaneously

No. of experiment	Body weight	Quantity of arginine injected per 100 gm. body weight	Symptoms	Period of survival	Isotonicity	
					of liver	of kidney
	gm.	*mg.*		*min.*	M	M
1	196	750	None	61	0.358	0.197
2	199	1000	Weakness	60	0.314	0.213
3	167	1000	Weakness	56	0.258	0.249
4	200	1000	Weakness	43	0.259	0.210
Average					0.297	0.217

RECAPITULATION AND DISCUSSION

In earlier experiments the tonicity of liver tissue tested by immersion of slices in solutions of sodium chloride has been reduced by the administration of two substances, chloroform and carbon tetrachloride (11) which cause injury of liver tissue, and similar change occurs in kidney cortex when it is injured by potassium chromate. Ligation of the common bile duct or of one ureter has temporarily reduced the level of isotonicity of liver or of kidney tissue (5). When tumors of the liver are produced by the action of dimethylaminoazobenzene (butter yellow) on liver cells, the hepatoma cells have lost the hypertonicity of liver cells and their level of isotonicity has fallen to that of blood serum (12). In the present experiments the attempt has been made to increase the level of isotonicity of parenchymatous cells when measured by immersion in solutions of sodium chloride.

The opinion that the osmotic activity of tissues is in large part determined by electrolytes has been widely accepted and in the experiments that have been described, sodium chloride has been administered in large quantity in order to determine whether the tonicity of liver or kidney cells can be increased by it. When the salt is administered with drinking water or with food its elimination is accomplished with astonishing success, chiefly at least by the kidneys. It is doubtful whether the level of isotonicity of liver slices has been significantly increased by sodium chloride given in large quantity with drinking water, and when it has been administered with the food no increase has been found; but within a short interval after subcutaneous injection of sodium chloride the isotonicity of liver slices has risen much above the average level of normal liver and that of kidney has shown similar elevation.

Among those who have attempted to determine the distribution of sodium chloride in intra- and extracellular water following its parenteral administration there has been much difference of opinion. In the present experiments sodium chloride following subcutaneous injection has evidently entered the extracellular space of the liver, but it is uncertain what part of the sodium or chloride ions have penetrated into the cells.

Abundant evidence indicates that urea enters freely all of the tissues of the body. Marshall and Davis (13) found the urea content of tissues approximately uniform almost immediately after its injection into the blood stream of dogs and noted that it was very rapidly eliminated. When Conway and Fitzgerald (14) injected inulin intravenously into rabbits it presumably occupied from 8 to 9 per cent of the water of skeletal muscle; when chloride was injected it entered 15 per cent, and when urea was similarly administered, 93 per cent. The quantity of urea taken up by muscle which P. Eggleton (15) immersed in Ringer's solution containing urea, indicated that urea had occupied all of the water of the muscle. Some investigators maintain the opinion that a large proportion of the body water is entered when sodium chloride is administered parenterally. Hetherington (16) injected intravenously into cats 8 gm. of sodium chloride in 100 cc. of water and according to his estimate the mean value of the water available for the dilution of the salt was 59 per cent of the body weight, that is, nearly all of the water present in the tissues of the body. After injection of urea and of sodium chloride into the blood stream, M. G. Eggleton (17) measured the urea directly and determined the distribution of chloride by electrometric titration. The urea apparently occupied 63 per cent of the body water, that is, approximately all of it, and the chloride, 49.5 per cent.

Non-electrolytes formed by disintegration of protein during metabolism may have a part in maintaining osmotic activity within cells. This suggestion is supported by the changes which follow autolysis of liver tissue. Slices prepared from liver that has been allowed to undergo autolysis at 37°C. take up water from solutions of sodium chloride much more concentrated than those that are isotonic with slices from liver kept at a lower temperature (1). The increased level of osmotic activity is presumably referable to increased molecular concentration accompanying protein disintegration. In the present experiments a substance, urea, with molecular weight little greater than that of sodium or potassium chloride has been introduced into the body under conditions that bring about its accumulation in excess of its elimination, and in accord with widely accepted opinion it is distributed throughout both intra- and extracellular water. The average levels of isotonicity of liver and of kidney tissue as measured by immersion in solutions of sodium chloride has risen much above the corresponding averages for the normal tissues. Under similar conditions another metabolite, creatinin, has increased the level of isotonicity of liver tissue. No increase of these levels has been found in similar experiments with two amino acids, glycine and arginine.

SUMMARY AND CONCLUSIONS

The osmotic activity of liver tissue and of kidney cortex tested within 10 minutes after immersion in solutions of sodium chloride has been increased by

procedures which introduce sodium chloride, urea or creatinin into the body in excess of its elimination.

A substance formed by cell metabolism, namely urea, can increase the osmotic activity of liver and of kidney cells.

The amino acids, glycine and arginine, under similar conditions have not increased the osmotic activity of liver or of kidney cortex.

BIBLIOGRAPHY

1. Opie, E. L., *J. Exp. Med.*, 1949, **89,** 185.
2. Robinson, J. R., *Proc. Roy. Soc. London, Series B*, 1950, **137,** 378; 1952, **140,** 135.
3. Aebi, H., *Helvet. Physiol. et Pharm. Acta*, 1953, **11,** 96.
4. Bartley, Davies, R. E., and Krebs, H. A., *Proc. Roy. Soc. London, Series B*, 1954, **142,** 187.
5. Hastings, A. B., *Harvey Lectures*, 1940–41, **36,** 91.
6. Opie, E. L., and Rothbard, M. B., *J. Exp. Med.*, 1953, **97,** 483.
7. Opie, E. L., *J. Exp. Med.*, 1948, **87,** 425.
8. Gamble, J. L., Putnam, M. C., and McKhann, C. F., *Am. J. Physiol.*, 1929, **88,** 580.
9. Saperstein, L. A., Brandt, W. L., and Dowy, D. R., *Proc. Soc. Exp. Biol. and Med.*, 1950, **73,** 82.
10. Meneely, G. R., Tucker, R. G., and Darby, W. J., *J. Nutrition*, 1951, **48,** 489; *J. Exp. Med.*, 1953, **98,** 71.
11. Opie, E. L., *J. Exp. Med.*, 1950, **91,** 285.
12. Opie, E. L., *J. Exp. Med.*, 1949, **89,** 209.
13. Marshall, E. K., and Davis, D. M., *J. Biol. Chem.*, 1914, **18,** 53.
14. Conway, E. J., and Fitzgerald, O., *J. Physiol.*, 1942, **101,** 86.
15. Eggleton, P., *J. Physiol.*, 1930, **70,** 294.
16. Hetherington, M., *J. Physiol.*, 1931, **73,** 184.
17. Eggleton, M. G., *J. Physiol.*, 1951, **115,** 482.