LXX. STUDIES IN CALCIUM METABOLISM.

II. THE CALCIUM CONTENT OF CORPUSCLES, PLASMA, AND SERUM.

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INTRODUCTION.

ATTENTION has been directed to the presence of calcium in the serum in colloid combination and in crystalloid form. There is no direct information, however, as to whether the amount of calcium in the plasma is equal to that of the serum or whether it exists in similar forms in the two fluids. Further, there is some divergence of opinion as to the mere presence of calcium in the corpuscles, and in this connection the findings of different workers are by no means concordant. It is on account of the paucity of data and the lack of unanimity of opinion on these points that the present research has been carried out. The investigation includes quantitative analyses of the calcium in true plasma, citrated plasma, serum, and corpuscles, and a study of the physical state of the calcium in serum and in plasma.

THE CALCIUM CONTENT OF PLASMA, CITRATED PLASMA, AND SERUM.

Experimental methods. Cats, anaesthetised by paraldehyde and ether, were used as the experimental animals. Coagulation of blood was delayed and true plasma obtained in the following manner. Blood was withdrawn from the carotid artery through a paraffined needle into a paraffined glass syringe, and transferred to ice-cold centrifuge tubes under paraffin. The blood was then centrifuged at high speed for 3 minutes, the centrifuge tubes being surrounded by freezing mixture. 2 cc. samples of plasma were then immediately transferred to centrifuge tubes and oxalated for calcium estimation. To the remaining plasma sufficient sodium citrate was added to obtain a 1.5 % concentration (except in sample 3 A, where 0.6 % concentration was used), the mixture thoroughly shaken and 2 cc. samples were taken for analysis. The remainder of the citrated plasma was then centrifuged at high speed for 3 minutes and the calcium content estimated. In some cases, whole blood was citrated directly, centrifuged, and the citrated plasma analysed, and here it was found

that a concentration (0.4 %), roughly corresponding to 0.6 % in serum, was required to prevent coagulation. In all cases a sample of blood was allowed to clot in order to obtain serum therefrom. Kramer and Tisdall's [1921] method of calcium estimation was used throughout except that, following the authors' usual practice, 24 hours were allowed for precipitation of calcium oxalate. In most animals two samples of blood, withdrawn at intervals of about an hour (marked *A* and *B*, in Table I) were studied. The results are set forth in Table I, and include one set of similar observations on a human subject. Table II gives the figures for the calcium content of cat's serum after the addition of increasing amounts of sodium citrate.

Table I.

Sample	True plasma	Mg. calcium per 100 cc.		Plasma from citrated blood	Serum
		Citrated plasma	Citrated centri- fuged plasma		
1	12.10	12.00	10.80	—	10.35
2	11.80	11.8	10.66	—	10.50
3 <i>A</i>	11.70*	11.60	11.70	—	10.35
<i>B</i>	11.80	11.70	10.20	—	10.35
4 <i>A</i>	9.95	10.05	8.85	—	9.00
<i>B</i>	10.00	10.00	9.00	—	9.00
5 <i>A</i>	12.1	—	—	10.00	10.0
<i>B</i>	12.05	—	—	10.05	10.0
6	11.10	—	—	10.05	10.0
7 <i>A</i>	12.15	—	—	9.50	10.10
<i>B</i>	12.10	—	—	9.50	10.05
8 <i>A</i>	11.05	—	—	9.15	9.0
<i>B</i>	11.05	—	—	9.15	9.0
9	12.70†	—	—	11.50	11.30

* 0.6 % sodium citrate added to plasma.

† Human blood.

Table II. *Effect of sodium citrate on the calcium content of cat's serum.*

Sodium citrate in serum %	Mg. calcium per 100 cc. serum		
	I	II	III
0.00	10.0	10.35	10.05
0.5	10.0	10.35	10.00
1.0	9.9	10.1	10.00
2.0	9.1	8.0	9.05

The figures recorded in Table I clearly show that the calcium content of true plasma is from 10–20 % greater than that of the corresponding serum. The addition of sodium citrate to true plasma in a concentration of 1 % causes a certain amount of calcium to be precipitated. The evidence for this statement is that if such citrated plasma is centrifuged, the calcium content is reduced, while the calcium content of samples withdrawn for analysis before centrifuging is approximately equal to that of the true uncitrated plasma. The calcium content of citrated centrifuged plasma is roughly equal to that of the corresponding serum. Table II shows that the addition of sodium citrate to serum, in a concentration equal to that just necessary to prevent

coagulation in blood, does not alter its calcium content; on the other hand, when a 2 % concentration has been reached a definite reduction in the calcium content, due to precipitation, is brought about. These observations on serum explain the figures recorded in Table I, sample 3, *A* and *B*. In *A* the concentration of sodium citrate in the plasma is only 0.6 %, an amount which did not cause precipitation in the serum experiments, and no reduction in the calcium content of the citrated centrifuged plasma has occurred. In *B* a 1.5 % concentration of citrate has been used with the result that the calcium content of the citrated centrifuged plasma is lower than that of the true plasma and equal to that of the serum. Kramer and Tisdall [1922] state that they found the calcium content of citrated plasma to be practically the same as that of serum, and they quote Schmidt [1850] as having shown the inorganic composition of plasma to be identical with that of serum. Howland and Kramer [1921] and Halverson, Mohler and Bergeim [1917] could find no appreciable difference between the calcium content of serum and that of plasma. It may be pointed out that these workers give the normal serum value as 9–11 mg. calcium per 100 cc. serum, usually below 10.5 mg., a range which is wider than that found by the present authors [Percival and Stewart, 1926] but is of the same order. They do not state whether they examined true or citrated plasma. It has been pointed out by Kramer, Tisdall and Howland [1921] that the addition of citrate to blood may result in the precipitation of part of the calcium as phosphate, and that the amount of fluid in which the precipitation is accomplished and the length of time allowed for precipitation are important factors. Cameron and Moorhouse [1925], while stating that the calcium values in citrated plasma and in serum are usually equal, record the fact that in two of their experiments the citrated plasma values were definitely higher than those of the serum. The work described in the above and similar papers appears to have been done with plasma prevented from clotting by the addition of citrate or some other anti-coagulant and is therefore not comparable with the work described in this paper, in which true plasma was used.

It may therefore be concluded from the present work that the calcium content of plasma is greater than that of serum, and that the calcium content of citrated plasma is approximately equal to that of serum, although the closeness of this approximation depends entirely on the amount of citrate used to prevent coagulation, and their complete agreement is purely a matter of coincidence.

CALCIUM CONTENT OF CORPUSCLES.

The findings recorded in Table III are in agreement with those of Kramer and Tisdall [1922] who concluded, from estimations of the calcium content of whole blood and serum, in conjunction with haematocrit readings, that "the corpuscles of human blood do not contain appreciable amounts of calcium." In their paper they discuss the results obtained by previous workers and point out that in those instances where the presence of calcium

in the corpuscles has been reported, faulty methods of analysis have been employed. More recently Rona, Petow and Wittkower [1924] have confirmed Kramer and Tisdall's results.

On the other hand, Stanford and Wheatley [1925] claim that human corpuscles contain from 1.4-3 mg. calcium per 100 cc. These authors determined the calcium in corpuscles, whole blood and plasma (using lithium citrate as anti-coagulant) by direct analysis, and state that, though the corpuscles doubtless contained adherent plasma, a 20 % contamination would be necessary to explain the results obtained.

In our own experiments, corpuscles were obtained either by rapidly centrifuging blood drawn under paraffin into a vessel surrounded by a freezing mixture, and pouring off the uncoagulated plasma, or by centrifuging defibrinated blood.

By either method the corpuscles gave the same result when submitted to analysis. To remove adherent plasma, the corpuscles were twice washed with 0.9 % NaCl, from which they were separated by centrifuging. The washings contained very small amounts of calcium, no greater than would be expected from the adherent plasma.

Table III.

	Mg. Ca in corpuscles from 100 cc. blood
I	0.30
II	0.20
III	0.15

DISTRIBUTION OF CALCIUM IN PLASMA AND SERUM.

Having determined that the calcium content of the plasma is greater than that of the serum, it was thought advisable to ascertain, if possible, whether a similar difference in concentration exists between the diffusible and ionic fractions of the plasma-calcium and the corresponding serum fractions. Before describing the experiments made for this purpose it is desirable to survey briefly the available methods of estimating the diffusible and ionic calcium in the serum, and also the results obtained by these methods.

So far as the ionic calcium is concerned, the only methods on which any reliance can be placed are those of Neuhausen and Marshall [1922] and Brinkman and Van Dam [1920], which, as reported by those authors, have yielded roughly concordant results. The present authors have carried out a considerable number of determinations of Ca^{++} using the method of Brinkman and Van Dam, and have obtained figures (Table IV) of the same order of magnitude as those published by those authors and by Neuhausen and Marshall.

Some 80 % of the serum-calcium, then, is present in combination, part doubtless with simple inorganic radicles such as phosphate and bicarbonate, and part possibly with protein. Thus one would expect to find combined calcium partly among the crystalloids and partly among the colloids, in which

Table IV. *Concentration of calcium ions in blood-serum; determined by the method of Brinkman and Van Dam.*

		Mg. Ca ⁺⁺ per 100 cc.	
		Whole serum	Serum filtrate
Ox serum	I	2.26	2.26
	II	2.04	2.10
	III	2.54	2.40
Cat serum	I	—	1.90
	II	1.80	1.90
Human serum	I	2.26	2.18

case dialysis should effect a partial separation. The separation need not be complete, however, for disturbance of the equilibrium, it is to be expected, would be followed by a re-arrangement of the relative amounts of dialysable and non-dialysable calcium. Such a method of simple dialysis (which has been proposed by Moritz [1925], Updegraff, Greenberg and Clark [1926] and others) can give results which may approximate to the true one if the time required be short and the rate of readjustment of the equilibrium within the membrane be small; they cannot, however, give the true result. Similar objections may be raised against the method of compensatory dialysis as proposed by von Meysenberg, Pappenheimer, Zucker and Murray [1921] and others, for compensation, to be complete, must take account not only of calcium, but of all the other diffusible substances as well. However, both types of dialysis agree in showing that 50–60 % only of the total calcium is capable of passing through the semi-permeable membrane, and this figure may be accepted as indicating at any rate the order of magnitude of the amount of calcium in crystalloid form of combination.

Very similar results are obtained simply by filtering serum through a collodion membrane under pressure. Cushny [1920] found that only about 60 % of the calcium was filterable in this way, while the whole of the sodium and chlorine passed through the membrane. The pressure under which the filtration is carried out, however, exerts a considerable influence on the result obtained. Cushny used a pressure of 150 mm. of mercury; Richter-Quittner [1921] using very low pressures, found that only 5–7 % of the calcium filtered. Very high pressures, of course, are objectionable, for apart from the technical difficulty of obtaining a protein-free filtrate when they are employed, they tend to destroy any labile compounds between the protein and inorganic ions.

Cushny's figures for the collodion-filterable calcium are in good agreement with those reported by other workers using dialysis, and there can be no doubt that with proper precautions both types of method give comparable results. In measuring the diffusible calcium the present authors have always used the collodion filtration method as being reasonably rapid and sufficiently easily standardised. The collodion membrane must be capable of giving a protein-free filtrate (as determined by the biuret test) but must allow of such a rate of filtration at a pressure of 150 mm. that enough filtrate for analysis can be

obtained in 2 or 3 hours. In a previous paper Percival and Stewart [1927] have shown that the amount of filterable calcium is appreciably increased if the time of filtration is unduly prolonged, and have indicated the method of filtration which they have adopted. Briefly they wash out the envelope with the serum to be filtered, dry the outside with filter-paper, discard the filtrate of the first half hour, and again dry the outside of the envelope before collecting the sample for analysis. The constancy of the results obtained in this way, together with the lack of effect on the results of exposure to air, is shown in Table V.

Table V.

A. Membrane surface dried by filter-paper (Serum LXI).	
Time of filtration (hours)	Mg. Ca per 100 cc. filtrate
0-3	4.4
3-7	7.4
7-22	8.5
B. Membrane under pressure 1 hour before commencement of filtration and then dried (Serum LXI).	
0-1.5	7.45
1.5-7	7.60
7-24	8.70
C. Treatment as in text (Serum LV).	
$\frac{1}{2}$ -2 $\frac{1}{2}$	7.30
$\frac{1}{2}$ -2 $\frac{1}{2}$	7.40
$\frac{1}{2}$ -2 $\frac{1}{2}$	7.35
D. Treatment as described in text, but blood drawn, allowed to clot, and filtered under paraffin (Serum LV).	
$\frac{1}{2}$ -2 $\frac{1}{2}$	7.25
$\frac{1}{2}$ -2 $\frac{1}{2}$	7.35

Vines [1921] described a biological method for the determination of calcium. This method consisted in comparing the power of the fluid under investigation with that of known concentrations of calcium chloride to restore coagulability to oxalated blood. By its use he found that, whereas uncoagulated blood contains about 6 mg. of calcium per 100 cc., serum contains 10 mg. per 100 cc. and he concluded that the difference represented un-ionised calcium which was not determined by his method—*i.e.* that in blood only 60 %, but in serum 100 %, of the calcium is ionised. Now it is obvious that this conclusion is only justified if it be shown that plasma and corpuscles contain equal concentrations of calcium. Since it is now known that corpuscles contain practically no calcium, and since the plasma forms about 60 % of the total blood volume, it is evident that what Vines really did was simply to determine the total calcium in each case.

In his monograph on *The parathyroid glands in relation to disease* Vines [1924] states that about 60 % of the total *plasma*-calcium is readily precipitated by its chemical equivalent of ammonium oxalate, whereas the remaining 40 % requires a considerable excess. He terms this readily precipitable fraction the “active calcium” of the plasma, abandoning, apparently, the idea that it is ionic. In normal serum, he states, all the calcium is readily

Table VI. *Effect of varying concentrations of oxalate on precipitation of serum-calcium in 24 hours.*

Mg. calcium per 100 cc. equivalent to oxalate added	Mg. calcium per 100 cc. precipitated in 24 hours		
	I	II	III
0.00	0.00	0.00	0.00
2.4	1.30	1.20	1.50
4.8	3.20	1.50	2.60
7.5	5.50	3.55	3.95
9.9	6.40	4.28	5.10
12.5	6.80	4.45	5.85
15.0	7.60	5.25	6.40
19.8	7.60	6.70	7.00
30.0	7.80	—	7.50
∞	9.20	10.55	10.10
Diffusible calcium mg. per 100 cc.	6.20	5.30	5.00

precipitable, though in certain pathological cases this is not so. The statement that calcium is differently distributed in plasma and in serum is so important that it was necessary to subject it to further experiment. Three series of experiments were therefore carried out. In the first series ox serum was treated as for the Kramer-Tisdall determination of calcium, except that measured volumes of a $N/50$ solution of sodium oxalate were used. Precipitation was allowed to continue for 24 hours, after which the calcium oxalate was centrifuged off and determined in the usual way. The results (Table VI) showed that the amount precipitated was a function of the oxalate added, but that in no case did the oxalate precipitate its chemical equivalent of calcium. Further, the amount precipitated by oxalate equivalent to the total calcium of the serum, far from being 100 %, varied from 40–60 % of the total. The second series of experiments (Table VII) showed that the amount of calcium precipitated from serum by small amounts of oxalate depended on the time allowed for precipitation—a fact which has been shown to hold even when a huge excess of oxalate is added [Pincussen and Schimmelpfeng, 1927]. In all cases, precipitation appears to be complete after 24 hours. The third series of experiments was designed to show whether or not the calcium precipitated

Table VII. *Effect of time on the precipitation of serum-calcium by small amounts of oxalate.*

Time allowed for precipitation	Mg. calcium precipitated from 100 cc. serum	
	Oxalate equivalent to 10 mg. calcium per 100 cc.	Oxalate equivalent to 20 mg. calcium per 100 cc.
5 mins.	2.10	3.20
30 "	4.65	7.05
1 hour	4.80	7.60
1½ hours	5.12	8.02
2½ "	5.20	8.22
3 "	5.40	8.35
4 "	5.65	8.50
5 "	5.82	8.75
24 "	5.95	9.20

by small amounts of oxalate was entirely diffusible—that it might be, but was not necessarily, quantitatively equal to the diffusible calcium was shown in the experiments of the first series. To 50 cc. of serum, therefore, oxalate was added in amount approximately equivalent to the total calcium (actually to 10 mg. Ca per 100 cc.) and the mixture was allowed to stand 24 hours, until precipitation was complete. It was then centrifuged, and the total and diffusible calcium were determined in the supernatant fluid. The total and diffusible calcium in the original serum having been determined, subtraction showed (Table VIII) that the calcium precipitated by the equivalent of oxalate was derived from both the diffusible and non-diffusible calcium of the serum, though chiefly from the former. It is evident then that the amount of calcium precipitated by oxalate is a function both of the amount of oxalate and the time allowed for precipitation, and that, even when these conditions are standardised, any agreement with the amount of diffusible calcium is purely fortuitous. Further, it is evident that the whole of the serum-calcium is not “readily precipitable” in the sense that it is precipitated by its chemical equivalent of oxalate.

Table VIII. *Distribution of calcium in serum (mg. %) after partial precipitation by oxalate.*

	I	II
Total calcium in serum	9.20	9.80
Diffusible calcium in serum	6.20	5.70
Total calcium precipitated in 24 hours	6.40	6.70
Diffusible calcium precipitated in 24 hours	5.00	4.50
Non-diffusible calcium precipitated in 24 hours	1.20	2.20

From the foregoing statements it is obvious that any method of estimating the amount of ionic or diffusible calcium in serum is entirely unsuitable for similar estimations in plasma on account of the time necessary for satisfactory precipitation, filtration, or dialysis to take place, unless some anti-coagulant substance is added to the plasma. A subsequent paper shows that anti-coagulants such as citrates, fluorides, oxalates, and sodium chloride cannot be employed since they alter the ionic content and disturb the normal ratio of diffusible to non-diffusible calcium in the serum, when added in sufficient concentration to prevent the coagulation of plasma. Under these circumstances it was decided to investigate the possibility of estimating the ionic and diffusible fractions of the plasma-calcium by utilising the peritoneum of the cat as a semi-permeable membrane, carrying out the diffusion *in vivo* in the anaesthetised animal and comparing the findings with those in the ultra-filtrate of the corresponding serum.

The following is a description of the methods employed in these vividiffusion experiments. The cat having been anaesthetised with paraldehyde and ether, a long mid-line incision was made through the abdominal wall, extending down to, but not including, the peritoneum; all bleeding points were secured, and oozing was stopped by cauterisation. A small opening was made

into the peritoneal cavity, a measured quantity of either calcium-free, or calcium-containing Ringer solution was introduced, and finally the peritoneal incision was closed by means of a clip. Diffusion between this fluid in the peritoneal cavity and the circulating blood was allowed to proceed for 2-6 hours, samples of fluid being withdrawn for analysis at varying intervals. Samples of blood, for serum, were collected during the course of the experiment. The results of these experiments are recorded in Tables IX and X.

Table IX.

Exp.	Time in hours	Mg. Ca per 100 cc. serum			Fluid introduced into peritoneal cavity	Amount recovered cc.	Mg. Ca per 100 cc. fluid peritoneal cavity		
		Total	Diffusible	Ionic			Total	Diffusible	Ionic
I	0	—	—	—	100 cc. Ca-containing Ringer solution	—	8	—	—
	5	9.00	5.05	—	—	25	5.35	5.35	—
II	0	—	—	—	100 cc. Ca-containing Ringer solution	—	8	—	—
	5	9.70	6.55	—	—	20	7.00	7.00	—
III	0	11.1	—	—	100 cc. Ca-containing Ringer solution	—	5.65	—	—
	2	11.3	8.1	—	—	60	8.0	—	—
IV	0	11.6	8.7	—	100 cc. Ca-containing Ringer solution	—	5.65	—	—
	2	—	—	—	—	62	8.8	8.7	—
V	0	—	—	—	100 cc. Ca-free Ringer solution	—	0	0	0
	5	9.25	6.20	1.8	—	15	6.5	6.1	1.9
VI	0	—	—	—	100 cc. Ca-free Ringer solution	—	0	0	0
	5	9.30	6.35	1.9	—	20	5.90	5.90	1.8
VII	0	9.9	—	—	100 cc. Ca-free Ringer solution	—	0	0	0
	1½	9.9	—	—	—	—	—	—	—
	3	9.9	6.7	—	—	45	6.4	6.4	—

Table X.

Exp.	Time in hours	Mg. Ca per 100 cc. serum		Amount of fluid introduced into peritoneal cavity	Mg. Ca per 100 cc. fluid in peritoneal cavity	
		Total	Diffusible		Total	Diffusible
VIII	0	11.40	6.25	100 cc. Ca-free Ringer solution	0	0
	1	—	—	—	3.15	—
	2	—	—	—	5.50	—
	3	—	—	—	6.55	—
IX	0	9.15	5.45	120 cc. Ca-free Ringer solution	0	0
	1	—	—	—	2.60	—
	2	—	—	—	3.40	—
	3	—	—	—	4.30	—
	4	—	—	—	5.25	—
	5	—	—	—	6.2*	—
	7	—	—	—	6.3*	5.40
X	0	10.1	7.6	100 cc. Ca-free Ringer solution	0	0
	1	—	—	—	3.27	—
	3	—	—	—	5.30	—
	3½	—	—	—	7.10	—
	4	9.6	7.2	—	7.60	7.5

* Biuret test positive.

From the figures recorded in Tables IX and X, and those represented graphically in Fig. 1, it is evident that calcium diffuses from the circulating plasma through the peritoneal membrane into the fluid present in the peritoneal cavity. Exps. V-VII show that enough calcium has diffused into the

calcium-free fluid during a period of 5 hours to reach a concentration in the latter equal to the diffusible calcium of the serum. In Exps. I-IV, where Ringer solution having a calcium concentration of 8 mg. calcium per 100 cc. was used, there was a reduction in this concentration of such a degree that at the end of 5 hours the concentration was equal to that of the diffusible calcium in the serum. Conversely in Exps. III and IV, where a Ringer solution containing 5.65 mg. calcium per 100 cc. was used, this concentration was raised to a similar level. A study of Fig. 1 shows that the increase in the calcium concentration of originally calcium-free Ringer solution, allowed to remain in the peritoneal cavity, is rapid and steady for the first 3 hours, after which it diminishes and tends to remain constant when it approximately equals that of the diffusible fraction of the serum-calcium.

With the exception of one experiment the fluid recovered from the peritoneal cavity at the end of the experiment gave practically no biuret reaction, and any small amount of protein present could either be derived from the

Fig. 1.

secretion of the membrane itself, or result from contamination with traces of blood. In Exp. IX, where the biuret test was definitely positive, the last samples were obviously contaminated with blood and in this case the diffusible calcium in the last sample was slightly lower than the total calcium, while in all the other experiments in which this estimation was carried out the whole of the calcium present was found to be diffusible.

The facts observed may be summarised as follows. In all cases a calcium-containing fluid, in which the calcium concentration equals that of the diffusible calcium of the serum, has been recovered from the peritoneal cavity, irrespective of whether the fluid originally contained either no calcium, or quantities of calcium in excess of, or slightly less than, the diffusible calcium of the serum. The curve of diffusion definitely tends to reach a constant level. The whole of the calcium which has entered the peritoneal cavity can be recovered in a biuret-free collodion ultra-filtrate and is therefore not in colloidal combination. Lastly, some of the calcium which has diffused through the peritoneal membrane is in the form of calcium ions.

It seems justifiable to conclude, therefore, that a fraction of the calcium present in the plasma exists as a non-colloidal diffusible complex which is partly in the ionised state, and that this diffusible complex and also the ionised calcium is equal in amount to that which is present in the corresponding serum.

Conversely these results strongly suggest that the distribution of calcium in the serum between crystalloid and colloid as determined by collodion ultra-filtration is quantitatively comparable with that in plasma.

From the evidence put forward in this paper it would appear that some calcium is withdrawn from the plasma during the process of clotting. Since the findings suggest that the amount of diffusible and ionised calcium in the plasma is equal to that of the serum, it seems probable that it is the non-diffusible complex which is involved.

SUMMARY.

True plasma contains 10–20 % more calcium than does the corresponding serum. In citrated plasma the amount is less than in true plasma to an extent depending on the amount of citrate added.

The corpuscles contain no more than traces of calcium. In plasma the concentration of ionic and of diffusible calcium appears to be equal to that in serum. It is suggested that during the coagulation of blood some of the non-diffusible calcium is precipitated.

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REFERENCES.

- Brinkman and Van Dam (1920). *Proc. K. Akad. Wetensch. Amsterdam*, **22**, 762.
Cameron and Moorhouse (1925). *J. Biol. Chem.* **63**, 687.
Cushny (1920). *J. Physiol.* **53**, 391.
Halverson, Mohler, and Bergeim (1917). *J. Amer. Med. Assoc.* **68**, 1309.
Howland and Kramer (1921). *Amer. J. Dis. Child.* **22**, 105.
Kramer and Tisdall (1921). *J. Biol. Chem.* **47**, 479.
—— (1922). *J. Biol. Chem.* **53**, 241.
Kramer, Tisdall and Howland (1921). *Amer. J. Dis. Child.* **22**, 560.
von Meysenberg, Pappenheimer, Zucker and Murray (1921). *J. Biol. Chem.* **47**, 529.
Moritz (1925). *J. Biol. Chem.* **64**, 81.
Neuhausen and Marshall (1922). *J. Biol. Chem.* **53**, 365.
Percival and Stewart (1926). *Quart. J. Med.* **19**, 235.
—— (1927). *Quart. J. Med.* **20**, 349.
Pincussen and Schimmelpfeng (1927). *Biochem. Z.* **183**, 42.
Richter-Quittner (1921). *Biochem. Z.* **124**, 106; **126**, 97.
Rona, Petow and Wittkower (1924). *Biochem. Z.* **150**, 468.
Schmidt (1850). *Charakteristik epidem. Cholera* (Leipsic and Milan).
Stanford and Wheatley (1925). *Biochem. J.* **19**, 710.
Updegraff, Greenberg and Clark (1926). *J. Biol. Chem.* **71**, 87.
Vines (1921). *J. Physiol.* **55**, 86.
—— (1924). *Parathyroid glands in relation to disease*, 28.