STUDIES IN EDEMA.

I. Comparative Investigation into the Action of Calcium Chloride and Sodium Chloride on the Production of Urine, Intestinal Fluid and Ascites.[1]

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Continuing the work of Jacques Loeb (1) on the antagonistic action of certain salt solutions on cellular functions, John Bruce MacCallum (2) in the laboratory of J. Loeb found that an intravenous injection of pure calcium chloride solution inhibits diuresis caused by the intravenous injection of solutions of sodium chloride and certain other salts. He (3) also has found that calcium chloride has an inhibitory effect upon the secretion[2] of fluid into the intestinal canal whether inside the body or *in vitro*. Whether the mode of action of calcium chloride consists in its direct influence upon the permeability of cells, especially of certain gland cells, as MacCallum believed, or whether its influence is merely an indirect one, excited through change in the circulatory system, we shall not here discuss.

The following experiments were undertaken in order to determine whether or not calcium chloride has the same inhibitory action upon the production of edema, especially of ascites, in experimentally produced hydræmic plethora. Does calcium chloride influence the endothelial cells of the blood vessels or of the peritoneal cavity in a way that would lead to an alteration of the permeability of the endothelial surfaces or to an alteration of a real secretory function of the cells, if such secretory function should exist, in a similar

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[2] The word secretion is used here and at some other places throughout this paper not as implying necessarily a specific cellular function, but as indicating in a general way the transmission of fluid through an epithelial surface.

manner as it influences directly or indirectly the renal cells and intestinal mucosa? Or if calcium chloride acts mainly through the circulatory system do the changes in the circulation caused by its injection affect alike the secretion of urine and intestinal fluid and the production of ascites?

To determine this question we injected into one set of rabbits considerable quantities of pure sodium chloride solutions and into another set mixtures of isotonic sodium chloride and calcium chloride solutions. This plan of injecting the calcium chloride solutions seems more perfectly comparable to natural conditions than an alternating injection of the two solutions, since under natural conditions calcium chloride is always associated with sodium chloride in the living organism.

In all the experiments rabbits were used, and with but few exceptions, the weight of these ranged between 1,600 and 1,800 grams, although it was found in the course of the experiments that the weight of the animal had no influence on the amount of ascites, intestinal fluid or urine. Most of the animals had been made to fast for twenty-four hours previous to the experiment, but nevertheless a small amount of solid matter was always found in the small intestines; in a few unfasting animals, which were killed for other purposes, the intestinal contents which were mostly solid were rarely found to exceed ten cubic centimeters.

In all cases where the intestinal contents were measured, the intestines were closed, before the infusion was started, near the pylorus and at the ileo-cecal valve by vein clamps, they having first been emptied by light stripping of as much of their contents as was possible without inflicting injury to the mucosa.

In those cases where it was desired that the kidneys should not functionate, the renal vessels and ureter were ligatured, the operation being done, after anesthesia, through an anterior incision into the peritoneal cavity. Although at first the kidneys were removed, this was later given up on account of the impossibility of preventing some of the blood from the operation getting into the peritoneal cavity.

Blood counts were taken during the course of the experiments. In the earlier experiments these were made from the blood of the ear vein, but we found this unreliable on account of the congestion and stasis which resulted from the continued dependent position of the ears. We therefore decided to use the jugular vein for the blood determinations. At first both hæmoglobin and red corpuscle counts were made—the first by the Fleischl-Miescher hæmoglobinometer, the latter by the usual Thoma apparatus. We came to rely in our later experiments on the hæmoglobin estimations alone. The first counts were always taken before the animal was anesthetized in order to avoid the increase of erythrocytes which results from the etherization (4, 5, 6). The second count was taken during the course, or at the end of the experiment, and the percentage of decrease of hæmoglobin or erythrocytes was determined.

The fluid was infused through a canula in the right jugular vein. The fluid was allowed to flow in from a 100 cubic centimeter burette which was kept in an oblique position and which was filled when necessary from a larger reservoir; from this burette the fluid passed through a metal coil which was in a hot water bath; further on there was a thermometer bathed by the solution indicating the temperature which was kept between 35° and 39° C. The rate of inflow was controlled by a screw clamp and this rate was always, except in a few cases when otherwise stated, approximately four cubic centimeters per minute.

The fluids used were a solution of 0.85 per cent. sodium chloride and solutions of 1.11 per cent., 1.22 per cent.,[3] and 1.45 per cent. calcium chloride. The calcium chloride solution was used only in combination with sodium chloride solution, usually in proportion of one volume of the former to five volumes of the latter. The quantities infused varied between 500 c.c. and 1,000 c.c., but in the majority of the experiments 600 c.c. or 800 c.c. were allowed to flow in.

When it was desired to measure the quantity of urine in male rabbits a catheter was passed into the bladder; in female animals the urine was expressed from the bladder every few minutes by gentle pressure. Since we were measuring the urine for periods of twenty-five minutes and we usually obtained large quantities of urine, these methods were considered sufficiently exact.

[3] An 1.22 per cent. $CaCl_2$ solution ($CaCl_2$ free from water of crystallization being used) is approximately isotonic with an 0.85 per cent. NaCl solution.

The ascites was removed at the end of the experiment by opening widely the abdomen. The error of the measurement did not exceed five cubic centimeters. To obtain the intestinal fluid the clamped small intestines were removed from the body and the intestines lightly stripped. The contents were allowed to settle in a graduate and after approximately twenty hours the relation between solid and fluid contents was determined. The former usually represented only a small fraction of the fluid. The solid matter was subtracted from the total intestinal contents, and the remainder was called the intestinal fluid and used exclusively for computing the results.

INFUSIONS INTO NON-NEPHRECTOMIZED ANIMALS.

In the first series the intestinal fluid was not determined; in the second series the intestinal fluid was measured. In the first series of non-nephrectomized animals infused with pure NaCl solution we find per 1,000 c.c. of retained fluid, 88 c.c. of ascites and 670 c.c. of urine (we obtained the figure for the "fluid retained" by subtracting the urine from the injected fluid). If we exclude one exceptional case in which there were 280 c.c. of ascites (an amount never approached in any other case) the ascites is reduced to 35 c.c. per 1,000 c.c. of retained fluid; in the exceptional case the amount of urine excreted was very small and there is reason to assume that some pathological conditions were present in this animal.

In the second series we find per 1,000 c.c. of retained fluid 810 c.c. of urine, 46 c.c. of ascites and 53 c.c. of intestinal fluid.

Considering both series (when the averages of several series or groups are to be obtained the results of all experiments in both or all the series or groups are added and the figures per 1,000 c.c. made up from these sums) we obtain for each 1,000 c.c. of fluid retained 69 c.c. of ascites and 730 c.c. of urine (the intestinal fluid is noted only in the second series) and for every 1,000 c.c. of inflow of NaCl solution 420 c.c. of urine.

In the first series of $CaCl_2$ experiments in which $CaCl_2$ solutions of 1.11 per cent. were used in proportions of 1 volume of $CaCl_2$ solution to 7, 5 or 3 volumes of NaCl solutions, the urine per 1,000 c.c. of retained fluid is 207 c.c. and the ascites, 94 c.c.

TABLE I.

Non-Nephrectomized Animals Infused with 0.85 per cent. NaCl Solution.

First Series.

Inflow.	Ascites.	Urine.
768 c.c.	0 c.c.	520 c.c.
787 c.c.	8 c.c.	464 c.c.
480 c.c.	50 c.c.	110 c.c.
1022 c.c.	0 c.c.	723 c.c.
440 c.c.	20 c.c.	0 c.c.
982 c.c.	0 c.c.	490 c.c
1024 c.c.	15 c.c.	365 c.c.
792 c.c.	12 c.c.	128 c.c.
912 c.c.	280 c.c.	135 c.c.

Per 1,000 c.c. retained: Ascites 88 c.c.
Per 1,000 c.c. retained: Urine 670 c.c.

Second Series.

Inflow.	Ascites.	Int. Fld.	Urine.
900 c.c.	10 c.c.	12 c.c.	690 c.c.
900 c.c.	2 c.c.	8 c.c.	550 c.c.
800 c.c.	10 c.c.	5 c.c.	441 c.c.
640 c.c.	18 c.c.	56 c.c.	250 c.c.
800 c.c.	15 c.c.	8 c.c.	366 c.c.
900 c.c.	15 c.c.	35 c.c.	411 c.c
820 c.c.	40 c.c.	20 c.c.	320 c.c.
800 c.c.	54 c.c.	25 c.c.	176 c.c.
720 c.c.	23 c.c.	45 c.c.	68 c.c.

Per 1,000 c.c. retained fluid: Ascites 46 c.c.
Per 1,000 c.c. retained fluid: Int. Fld. 53 c.c.
Per 1,000 c.c. retained fluid: Urine 810 c.c.

First and Second Series.

Per 1,000 c.c. retained fluid: Ascites 68 c.c.
Per 1,000 c.c. retained fluid: Urine 730 c.c.
Per 1,000 c.c. infused fluid: Urine 420 c.c.

In the second series in one group in which $CaCl_2$ solution of 1.11 per cent. was used in mixtures of 1 volume of $CaCl_2$ solution to 4 volumes of NaCl solution, the urine was 240 c.c., the ascites 101 c.c. and the intestinal fluid 56 c.c. per 1,000 c.c. retained. In another group where a $CaCl_2$ solution of 1.45 per cent in a mixture of 1 volume of $CaCl_2$ solution and 5 volumes of NaCl solution was used we find per 1,000 c.c. of retained fluid, 180 c.c. of urine, 86 c.c. of ascites and 28 c.c. of intestinal fluid. In a third group where a $CaCl_2$

solution of 1.22 per cent. in a mixture of 4 volumes of NaCl solution was used, the urine was 430 c.c., the ascites 123 c.c. and the intestinal fluid 25 c.c. per 1,000 c.c. of retained fluid.

Summarizing the experiments with non-nephrectomized animals treated with NaCl—$CaCl_2$ mixtures we find per 1,000 c.c. of fluid retained, 240 c.c. of urine, 98 c.c. of ascites and (in the second series) 44 c.c. of intestinal fluid. The urine per 1,000 c.c. of infused fluid is 190 c.c.

We find therefore a marked decrease of the urine when $CaCl_2$ solutions were added to the NaCl solutions, also a decrease in the amount of intestinal fluid, but on the other hand an increase of ascites when the NaCl—$CaCl_2$ solutions were infused. However, the decrease in the amount of urine is considerably more marked than is the decrease in the amount of intestinal fluid.

TABLE II.

Using 7 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Urine.
158 c.c.	0 c.c.	3 c.c.
443 c.c.	8 c.c.	20 c.c.
500 c.c.	90 c.c.	65 c.c.
500 c.c.	55 c.c.	0 c.c.
811 c.c.	90 c.c.	300 c.c.
700 c.c.	50 c.c.	112 c.c.
1062 c.c.	130 c.c.	235 c.c.
864 c.c.	70 c.c.	13 c.c.

Using 5 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

100 c.c.	0 c.c.	0 c.c.
190 c.c.	0 c.c.	0 c.c.
466 c.c.	40 c.c.	77 c.c.
500 c.c.	0 c.c.	144 c.c.
600 c.c.	115 c.c.	125 c.c.
1000 c.c.	10 c.c.	500 c.c.
654 c.c.	46 c.c.	358 c.c.
800 c.c.	60 c.c.	193 c.c.
700 c.c.	225 c.c.	80 c.c.
900 c.c.	110 c.c.	37 c.c.

Using 3 Vol. 0.85 per cent. NaCl and 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Urine.
180 c.c.	0 c.c.	72 c.c.
214 c.c.	13 c.c.	30 c.c.
232 c.c.	0 c.c.	10 c.c.
277 c.c.	0 c.c.	30 c.c.
331 c.c.	25 c.c.	0 c.c.
338 c.c.	10 c.c.	54 c.c.
462 c.c.	20 c.c.	80 c.c.
500 c.c.	2 c.c.	70 c.c.
614 c.c.	35 c.c.	180 c.c.
450 c.c.	30 c.c.	0 c.c.
1039 c.c.	40 c.c.	380 c.c.
1080 c.c.	100 c.c.	55 c.c.

Per 1,000 c.c. fluid retained: Ascites 94 c.c.
Per 1,000 c.c. fluid retained: Urine 207 c.c.

Second Series.

Using 4 Vol. 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

Inflow.	Ascites.	Int. Fld.	Urine.
350 c.c.	10 c.c.	25 c.c.	13 c.c.
800 c.c.	20 c.c.	8 c.c.	393 c.c.
850 c.c.	15 c.c.	3 c.c.	417 c.c.
650 c.c.	85 c.c.	20 c.c.	24 c.c.
800 c.c.	75 c.c.	14 c.c.	140 c.c.
800 c.c.	85 c.c.	98 c.c.	106 c.c.
800 c.c.	90 c.c.	35 c.c.	42 c.c.
800 c.c.	95 c.c.	62 c.c.	26 c.c.

Per 1,000 c.c. fluid retained: Ascites 101 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 56 c.c.
Per 1,000 c.c. fluid retained: Urine 240 c.c.

Using 5 Vol. 0.85 per cent. NaCl and 1 Vol. 1.45 per cent. $CaCl_2$.

450 c.c.	35 c.c.	21 c.c.	10 c.c.
600 c.c.	55 c.c.	7 c.c.	50 c.c.
800 c.c.	45 c.c.	16 c.c.	230 c.c.

Per 1,000 c.c. fluid retained: Ascites 86 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 28 c.c.
Per 1,000 c.c. fluid retained: Urine 180 c.c.

Using 4 Vol. 0.85 per cent. NaCl and 1 Vol. 1.22 per cent. $CaCl_2$.

500 c.c.	52 c.c.	10 c.c.	150 c.c.
620 c.c.	42 c.c.	8 c.c.	245 c.c.
780 c.c.	78 c.c.	10 c.c.	255 c.c.
360 c.c.	38 c.c.	10 c.c.	130 c.c.
500 c.c.	45 c.c.	15 c.c.	105 c.c.

Per 1,000 c.c. fluid retained: Ascites 123 c.c.
Per 1,000 c.c. fluid retained: Int. Fld. 25 c.c.
Per 1,000 c.c. fluid retained: Urine 430 c.c.

First and Second Series.

Per 1,000 c.c. fluid retained:	Ascites	98 c.c.
Per 1,000 c.c. fluid retained:	Int. Fld.	44 c.c. (2nd series)
Per 1,000 c.c. fluid retained:	Urine	240 c.c.
Per 1,000 c.c. fluid infused:	Urine	190 c.c.

In order to make the experiments still more comparable we carried on a series of experiments with both NaCl and NaCl—$CaCl_2$ solutions in which the same quantities of fluid were retained in the two series and compared the amounts of ascitic and intestinal fluid in these experiments. Comparing the results of these two groups of five experiments we find per 1,000 c.c. of fluid retained 41 c.c. of ascitic and 47 c.c. of intestinal fluid when the animals were infused with pure NaCl solutions and 123 c.c. of ascitic and 25 c.c. intestinal fluid when they were infused with NaCl—$CaCl_2$ solutions; certainly a marked difference between the two solutions which, however, merely confirms our former results.

TABLE III.

Comparison of Ascites and Intestinal Fluid in Animals Infused with NaCl Solution or Mixtures of NaCl—$CaCl_2$ with like Amounts of Fluid Retained.

	NaCl.		$CaCl_2$.	
Fluid Retained.	Ascites.	Int. Fld.	Ascites.	Int. Fld.
350 c.c.	2 c.c.	8 c.c.	52 c.c.	10 c.c.
390 c.c.	10 c.c.	5 c.c.	42 c.c.	8 c.c.
390 c.c.	18 c.c.	56 c.c.	45 c.c.	15 c.c.
430 c.c.	15 c.c.	8 c.c.	38 c.c.	10 c.c.
500 c.c.	40 c.c.	20 c.c.	78 c.c.	10 c.c.
Per 1,000 c.c. retained fluid:				
	41 c.c.	47 c.c.	123 c.c.	25 c.c.

But even in these experiments we have one variable factor which differs in the experiments with NaCl and those with NaCl—$CaCl_2$, namely, the time necessary for the same amount of fluid to be retained. The time necessary for the same amount of fluid to be retained is greater in the NaCl series, for here more urine is secreted; this is equivalent to a slower rate of inflow in the NaCl series. As we shall see later on, the rate is of some importance in determining the amount of intestinal fluid but the rate of inflow influences the amount of ascitic fluid only to a relatively slight degree. This time factor would be unable to account for the great differences in ascitic fluid which we find here between the series with NaCl and $CaCl_2$ solutions. This conclusion is strengthened through the fact that $CaCl_2$, as will be shown in the following experiment, exerts the same effect in nephrectomized animals in which the time factor is eliminated.

INFUSIONS INTO NEPHRECTOMIZED ANIMALS.

The first series in which 0.85 per cent. NaCl solution was used includes experiments in which various amounts of fluid from 500 c.c. to 1,000 c.c. were infused. We note a progressive increase of the ascites with the increase in the amount of fluid infused; namely, between 500 c.c. and 600 c.c. of infused fluid, 50 c.c. of ascites; between 600 and 700 c.c., 62 c.c. of ascites; between 700 and 800 c.c., 75 c.c. of ascites; and between 800 and 900 c.c., 95 c.c. of ascites; but the ascites increases in proportion more than does the amount of infused fluid. The intestinal fluid also increases with the amount of infused fluid, but more irregularly. Between 500 c.c. and 600 c.c. of infused fluid, we find 50 c.c. of intestinal fluid; between 600 and 700 c.c., 60 c.c. of intestinal fluid; between 700 and 800 c.c., 72 c.c. of intestinal fluid; and between 800 and 900 c.c., 85 c.c. of intestinal fluid. The intestinal fluid seems to increase in proportion less than the amount of fluid injected and certainly does not increase as rapidly as does the ascites.

Considering only the experiments in this first series, with an inflow of either 600 or 800 c.c., we find at 600 c.c. of inflow 106 c.c. of ascitic fluid and 81 c.c. of intestinal fluid per 1,000 c.c. of infused fluid; and at 800 c.c., 91 c.c. of ascites and 99 c.c. of intestinal fluid per 1,000 c.c. of infused fluid.

For all the experiments in this first set of infusions of NaCl solution into nephrectomized animals we find per 1,000 c.c. of infused fluid 109 c.c. of ascitic and 102 c.c. of intestinal fluid.

Considering now the second series in which experiments with only 600 c.c. or 800 c.c. of inflow were made, we find for 600 c.c. of infused solution 109 c.c. of ascites and 76 c.c. of intestinal fluid and for 800 c.c. of infused solution 147 c.c. of ascites and 76 c.c. of intestinal fluid, per 1,000 c.c. of fluid injected.

TABLE IV.

Nephrectomized Animals Infused with NaCl Solution.

First Series.

Inflow.		Ascites.	Int. Fld.
500 c.c.		25 c.c.	30 c.c.
		50 c.c.	40 c.c.
		62 c.c.	70 c.c.
	Average,	45.6 c.c.	46.6 c.c.
600 c.c.		46 c.c.	40 c.c.
		85 c.c.	57 c.c.
		60 c.c.	50 c.c.
	Average,	63.6 c.c.	49 c.c.
700 c.c.		95 c.c.	85 c.c.
		80 c.c.	90 c.c.
730 c.c.		70 c.c.	60 c.c.
750 c.c.		63 c.c.	60 c.c.
750 c.c.		53 c.c.	110 c.c.
750 c.c.		55 c.c.	55 c.c.
780 c.c.		104 c.o	13 c.c.
800 c.c.		60 c.c.	63 c.c.
		98 c.c.	80 c.c.
		85 c.c.	53 c.c.
		49 c.c.	65 c.c.
		69 c.c.	75 c.c.
		65 c.c.	90 c.c.
		75 c.c.	43 c.c.
		55 c.c.	64 c.c.
		90 c.c.	83 c.c.
	Average,	72.6 c.c.	79 c.c.
900 c.c.		150 c.c.	75 c.c.
		125 c.c.	85 c.c.
940 c.c.		60 c.c.	60 c.c.
945 c.c.		150 c.c.	143 c.c.
950 c.c.		80 c.c.	140 c.c.
1000 c.c.		100 c.c.	100 c.c.

Per 1,000 c.c. infused fluid: Ascites 109 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 102 c.c.

Second Series.

Inflow.		Ascites.	Int. Fld.
600 c.c.		71 c.c.	68 c.c.
		64 c.c.	47 c.c.
		31 c.c.	55 c.c.
		74 c.c.	57 c.c.
		77 c.c.	35 c.c.
		60 c.c.	35 c.c.
		80 c.c.	34 c.c.
	Average,	65.3 c.c.	47.3 c.c.
800 c.c.		50 c.c.	82 c.c.
		75 c.c.	75 c.c.
		88 c.c.	74 c.c.
		155 c.c.	62 c.c.
		150 c.c.	58 c.c.
		135 c.c.	110 c.c.
		138 c.c.	30 c.c.
		136 c.c.	12 c.c.
		110 c.c.	88 c.c.
		147 c.c.	60 c.c.
		98 c.c.	38 c.c.
		135 c.c.	110 c.c.
		117 c.c.	70 c.c.
	Average,	118 c.c.	66.7 c.c.

Per 1,000 c.c. infused fluid: Ascites 131 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 82 c.c.

First and second series

Per 1,000 c.c. infused fluid: Ascites 109 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 94 c.c.

Considering those experiments in which 600 c.c. or 800 c.c. were infused into the animal in both the first and second series, we find the average per 1,000 c.c. of injected fluid at 600 c.c. of inflow 114 c.c. of ascites and 79 c.c. of intestinal fluid and at 800 c.c. of inflow, 124 c.c. of ascites and 90 c.c. of intestinal fluid.

Adding all experiments with various amounts of inflow we find per 1,000 c.c. of injected fluid 109 c.c. of ascitic fluid and 94 c.c. of intestinal fluid.

It may be noted that in the second series of experiments we find the figures for the amount of ascites considerably higher than in the earlier series, this being most marked with an inflow of 800 c.c.

The second series was done during the early summer, in June, when the average temperature was high, while the first series was done during the winter months. It is possible that differences in season are of importance. Comparable to our observation is perhaps the fact noted by Meltzer and Salant (7) that absorption from the peritoneal cavity is slower during the summer months than during the cooler seasons.

In the first set of experiments with the injection of mixtures of NaCl and $CaCl_2$ solutions into nephrectomized animals some were done with mixtures of 1 volume of 1.11 per cent. $CaCl_2$ and 4 or 5 volumes of 0.85 per cent. NaCl, some with mixtures of 1 volume of 1.45 per cent. $CaCl_2$ solution and 5 volumes of NaCl solution. In these experiments in which the less concentrated $CaCl_2$ solutions were used, we find 118 c.c. of ascites and 76 c.c. of intestinal fluid per 1,000 c.c. of infused fluid; in those in which the stronger $CaCl_2$ solutions were employed, 130 c.c. of ascitic and 60.3 c.c. of intestinal fluid per 1,000 c.c. infused. Thus it would seem that the stronger $CaCl_2$ solution increased the ascites and lessened the intestinal fluid more markedly than did the weak solution.

Considering those experiments in which 600 c.c. of the solution were allowed to flow in, we find 133 c.c. of ascites and 62 c.c. of intestinal fluid per 1,000 c.c. of inflow; with 800 c.c. of inflow, 150 c.c. of ascites and 58 c.c. of intestinal fluid, also per 1,000 inflow.

In the second series, where the infused fluid was a mixture of 1 volume of 1.22 per cent. $CaCl_2$ solution and 4 volumes of NaCl solution, we find per 1,000 c.c. of infused fluid 185 c.c. of ascites and 76 c.c. of intestinal fluid, corresponding to 600 c.c. of inflow and 208 c.c. of ascites and 27 c.c. of intestinal fluid to 800 c.c. of inflow.

Considering the experiments in both series in which there was 600 c.c. or 800 c.c. of inflow, we find per 1,000 c.c. retained 170 c.c. of ascites and 69 c.c. of intestinal fluid at 600 c.c. of inflow, and 180 c.c. of ascites and 47 c.c. of intestinal fluid at 800 c.c. of infused fluid.

In these experiments with NaCl—$CaCl_2$ mixtures, we note as in the NaCl experiments that the amounts of ascitic fluid in the second series are greater than in the first series and the same explanation perhaps holds in this case, namely, the influence of the season during which the experiments were performed.

Comparing now those experiments on nephrectomized animals into which NaCl solutions were infused with those on nephrectomized rabbits into which $CaCl_2$ mixtures were infused, we note that in those infused with NaCl solution the ascites and intestinal fluid are approximately the same or the intestinal fluid is but slightly less; in the experiments with NaCl—$CaCl_2$ mixtures, the ascites is considerably greater than the intestinal fluid, and the amount of ascitic fluid is greater and the amount of intestinal fluid smaller in the $CaCl_2$—NaCl series than in the NaCl series. The $CaCl_2$ has caused a decrease in the amount of the intestinal and an increase in the amount of the ascitic fluid.

Referring to the observations above in regard to the amount of intestinal fluid after 600 c.c. or 800 c.c. have been allowed to flow in, we note that in the animals infused with NaCl solutions we obtain 79 c.c. intestinal fluid at 600 c.c. and 90 c.c. intestinal fluid at 800 c.c., while in the animals infused with $CaCl_2$ solution we obtain 69 c.c. intestinal fluid at 600 c.c. and 47 c.c. intestinal fluid at 800 c.c. In the animals injected with NaCl solutions, the intestinal fluid has increased between 600 c.c. and 800 c.c. of inflow, and in the animals injected with NaCl—$CaCl_2$ solutions, it has decreased between 600 c.c. and 800 c.c. inflow. We must consider the amount of fluid in the intestinal cavity dependent upon two factors —the secretion into and the absorption from the intestinal cavity; the amount found at the end of the experiment represents the difference between those two factors. Our experiments do not permit any analysis of those two factors. The fact that we find less intestinal fluid after 800 c.c. inflow than after 600 c.c. of inflow of NaCl—$CaCl_2$ solution is accounted for by assuming that in using mixtures of NaCl—$CaCl_2$, the absorption from the intestinal cavity is less inhibited than is the secretion into this cavity.

Both Höber (8) and Cushny and Wallace (9) have shown that the fluid from solutions of $CaCl_2$ is absorbed more slowly by the intestines than is the fluid of NaCl solutions. It may therefore be that the secretion into the intestine becomes gradually more inhibited under the influence of $CaCl_2$ than the absorption from the

TABLE V.

Nephrectomized Animals Infused with NaCl—$CaCl_2$ Solution.

First Series.

Using 4 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

	Inflow.	Ascites.	Int. Fld.
	450 c.c.	45 c.c.	22 c.c.
	530 c.c.	67 c.c.	15 c.c.
	600 c.c.	63 c.c.	29 c.c.
	700 c.c.	65 c.c.	52 c.c.
	800 c.c.	92 c.c.	84 c.c.
	"	103 c.c.	75 c.c.

Using 5 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.11 per cent. $CaCl_2$.

	500 c.c.	95 c.c.	—
	600 c.c.	92 c.c.	37 c.c.
	770 c.c.	75 c.c.	72 c.c.
	800 c.c.	110 c.c.	—
	"	85 c.c.	—
	"	117 c.c.	68 c.c.

Per 1,000 c.c. infused fluid: Ascites 118 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 76 c.c.

Using 5 Vols. of 0.85 per cent. NaCl and 1 Vol. 1.45 per cent. $CaCl_2$.

	350 c.c.	40 c.c.	10 c.c.
	380 c.c.	45 c.c.	33 c.c.
	470 c.c.	72 c.c.	20 c.c.
	550 c.c.	50 c.c.	40 c.c.
	600 c.c.	70 c.c.	30 c.c.
	600 c.c.	85 c.c.	43 c.c.
	600 c.c.	90 c.c.	47 c.c.
Average,	600 c.c.	81.6 c.c.	40 c.c.
	700 c.c.	45 c.c.	61 c.c.
	740 c.c.	80 c.c.	70 c.c.
	750 c.c.	99 c.c.	14 c.c.
	770 c.c.	105 c.c.	70 c.c.
	800 c.c.	145 c.c.	35 c.c.
	800 c.c.	95 c.c.	33 c.c.
	800 c.c.	103 c.c.	55 c.c.
	800 c.c.	117 c.c.	16 c.c.
	800 c.c.	180 c.c.	57 c.c.
Average,	800 c.c.	128 c.c.	39.2 c.c.

Per 1,000 c.c. infused fluid: Ascites 130 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 60.3 c.c.

Experiments with both 1.11 per cent. and 1.45 per cent. $CaCl_2$ solutions.
Per 1,000 c.c. infused fluid: Ascites 127 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 65 c.c.

Second Series.

Using 4 Vols. NaCl and 1 Vol. 1.22 per cent. $CaCl_2$.

Inflow.	Ascites.	Int. Fld.
600 c.c.	130 c.c.	30 c.c.
600 c.c.	115 c.c.	65 c.c.
600 c.c.	105 c.c.	60 c.c.
600 c.c.	80 c.c.	35 c.c.
600 c.c.	110 c.c.	35 c.c.
600 c.c.	125 c.c.	43 c.c.
Average,	111 c.c.	44.6 c.c.
800 c.c.	155 c.c.	21 c.c.
800 c.c.	162 c.c.	22 c.c.
800 c.c.	210 c.c.	36 c.c.
800 c.c.	150 c.c.	20 c.c.
800 c.c.	180 c.c.	15 c.c.
800 c.c.	140 c.c.	20 c.c.
800 c.c.	170 c.c.	53 c.c.
Average,	166.7 c.c.	27 c.c.

Per 1,000 c.c. infused fluid: Ascites 193 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 49 c.c.

First and second series
Per 1,000 c.c. infused fluid: Ascites 145 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 59 c.c.

intestines. Whether $CaCl_2$ has the same inhibitory effect upon the absorption from the peritoneal cavity that it has upon absorption from the intestines has to our knowledge not been investigated. If $CaCl_2$ should decrease the absorption from the peritoneum, the increase in ascites which we find in our experiments under the influence of $CaCl_2$ might, in part at least, be accounted for by the decrease in the absorption from the peritoneal cavity and may not be entirely due to increased transudation into the peritoneum.

Returning now to the experiments with non-nephrectomized animals, we note in these experiments the same general facts, namely, that the ascites is greater with $CaCl_2$ solution, the intestinal fluid is greater with NaCl solution, and the relation of ascitic fluid to the intestinal fluid is greater under the influence of $CaCl_2$. The $CaCl_2$ acts more strongly in inhibiting the action of the kidneys than it does in the intestines for it reduced the amount of urine secreted by more than half, it reduces the amount of intestinal fluid only by a quarter to a third.

If we compare the experiments on the nephrectomized and non-nephrectomized animals, we find in the former per 1,000 c.c. of retained fluid (which is equal to the infused fluid) 109 c.c. of ascitic and 94 c.c. of intestinal fluid with NaCl solution and 145 c.c. of ascitic and 59 c.c. of intestinal fluid with NaCl—$CaCl_2$ mixtures. In the latter we find per 1,000 c.c. retained fluid 68 c.c. of ascites and 53 c.c. of intestinal fluid with NaCl solution and 98 c.c. of ascitic and 44 c.c. of intestinal fluid with NaCl—$CaCl_2$; thus there are considerably less ascites and intestinal fluid in the non-nephrectomized than in the nephrectomized animals per 1,000 c.c. retained. It is to be noted that in the experiments with non-nephrectomized animals the ascitic and intestinal fluids have been considered in relation to the amount of fluid retained, not the amount of fluid infused; thus the fluid lost by secretion of urine has been taken into consideration, and the conditions in both sets of experiments are comparable. In connection with this series the observations of Magnus (10) may be of interest. This investigator found that nephrectomy performed several hours or days previous to the infusion of fluids led to edema of the skin in a similar way as would the injection of certain toxic substances. He refers this effect in both cases to some injury to the blood vessels. In the case of nephrectomized animals the substances not excreted by the kidneys are believed by him to act in a similar way on the blood vessels, as *e. g.,* arsenic; since in our experiments the infusion took place immediately after the nephrectomy it is questionable whether there was sufficient time for injury to the vessels to be thus produced. Meltzer and Auer (11) offer as an explanation of the increased absorption from the peritoneal cavity following nephrectomy an increased osmotic pressure of the blood due to retained urinary salts; but such an increased absorption would lead to exactly the opposite result from that which we obtained in our experiments, so we cannot explain this difference between the animals with and without functionating kidneys as due to the increase of the osmotic pressure of the blood. Changes in permeability of the blood vessels on the other hand might lead to such a result.

J. B. MacCallum, in the course of his experiments, was led to the conclusion that the fluid secreted by the intestines varied roughly with the rapidity of the injection; the difference in the amounts of intestinal fluid as obtained in nephrectomized and non-nephrectomized animals he would thus explain on the basis of the rapidity of inflow. If this be true, can the relation of time to the amount of infused fluid also explain the lessened amount of ascites in these non-nephrectomized animals? Since secretions of various quantities of urine during the course of the infusion would, in certain respects, act in a similar manner as a slower injection of fluid, we can in certain respects equalize the conditions in nephrectomized and non-nephrectomized animals by using in the case of the nephrectomized animal a slower rate of inflow, so that in a given time the same amount of fluid has been retained in nephrectomized and non-nephrectomized animals. To determine what bearing the rapidity of inflow might have, we conducted a series of experiments on nephrectomized animals in which the time consumed for a certain inflow corresponded to the time consumed in non-nephrectomized animals for the same amount of fluid to be retained. In this series we obtained in using solutions of NaCl, 81 c.c. of ascites and 48 c.c. of intestinal fluid per 1,000 c.c. infused, as compared with 109 c.c. of ascitic and 94 c.c. of intestinal fluid in nephrectomized animals at a rate of inflow of 4 c.c., and 68 c.c. of ascitic and 53 c.c. of intestinal fluid per 1,000 c.c. retained in experiments with non-nephrectomized animals, and using $CaCl_2$ mixtures of 138 c.c. of ascitic and 32 c.c. of intestinal fluid per 1,000 c.c. infused, as compared with 145 c.c. of ascitic and 59 c.c. of intestinal fluid in nephrectomized rabbits at a rate of 4 c.c. per minute and 98 c.c. of ascitic and 44 c.c. of intestinal fluid in experiments with non-nephrectomized animals. Thus with slow inflow in both the NaCl and NaCl—$CaCl_2$ series we obtained a larger amount of ascites than in the non-nephrectmized series with the usual rate of inflow, if equal amounts of fluid have been retained in the same period of time in both cases; therefore, so we may set aside the suggestion that the decrease of transudation into the peritoneal cavity was due entirely to the decrease in the rapidity of the injection. The rapidity of inflow may nevertheless have some influence because the ascites is not so great with slow inflow as with the usual rate of inflow in nephrectomized animals. We must, however, remember that in almost all of the experiments with the slow rate of inflow only relatively small quantities of fluid were infused and we have already noted that the ascitic fluid increases disproportionately to the amount of fluid infused so that this fact may account for the smaller ascites in this group with slow inflow. We cannot, however, exclude the influence of the rate of inflow as one factor which determines the increase of ascitic fluid in nephrectomized animals.

TABLE V.

Infusions into Nephrectomized Animals at a Slow Rate of Inflow.

Infused with 0.85 per cent. NaCl Solution.

Inflow.	Rate.	Ascites.	Int. Fld.
320 c.c.	2–2½ c.c. per min.	8 c.c.	9 c.c.
430 c.c.	2 c.c. per min.	40 c.c.	18 c.c.
489 c.c.	2–2½ c.c. per min.	11 c.c.	8 c.c.
489 c.c.	2½ c.c. per min.	25 c.c.	30 c.c.
400 c.c.	2½ c.c. per min.	25 c.c.	27 c.c.
400 c.c.	2¼ c.c. per min.	48 c.c.	18 c.c.
500 c.c.	2½ c.c. per min.	71 c.c.	21 c.c.
624 c.c.	3 c.c. per min.	71 c.c.	48 c.c.

Per 1,000 c.c. infused fluid: Ascites 81 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 48 c.c.

TABLE VI.

Infused with 4 Vol. of 0.85 per cent. NaCl and 1 Vol. of 1.22 per cent. $CaCl_2$.

340 c.c.	2½ c.c. per min.	43 c.c.	4 c.c.
400 c.c.	2½ c.c. per min.	68 c.c.	14 c.c.
400 c.c.	2½ c.c. per min.	73 c.c.	20 c.c.
425 c.c.	2½ c.c. per min.	30 c.c.	18 c.c.
500 c.c.	2½ c.c. per min.	85 c.c.	19 c.c.
500 c.c.	2½ c.c. per min.	57 c.c.	8 c.c.

Per 1,000 c.c. infused fluid: Ascites 138 c.c.
Per 1,000 c.c. infused fluid: Int. Fld. 32 c.c.

TABLE VII.

Blood Counts. Taken after 600 c.c. of inflow.

	Per Cent. Hæmoglobin Reduction.	Per Cent. Erythrocyte Reduction.
	20 per cent.	17 per cent.
	39 per cent.	28 per cent.
	35 per cent.	26 per cent.
	25 per cent.	28 per cent.
	40 per cent.	25 per cent.
	29 per cent.	24 per cent.
	25 per cent.	20 per cent.
	26 per cent.	
	28 per cent.	
	34 per cent.	
Average	30 per cent.	23 per cent.

Taken after 700 c.c. of inflow.

	18 per cent.	18 per cent.
	39 per cent.	35 per cent.
	20 per cent.	32 per cent.
	25 per cent.	18 per cent.
	33 per cent.	
	29 per cent.	
	27 per cent.	
	30 per cent.	
	27 per cent.	
Average	29 per cent.	25 per cent.

Taken after 800 c.c. of inflow.

	27 per cent.	19 per cent.
	44 per cent.	30 per cent.
	31 per cent.	13 per cent.
	37 per cent.	32 per cent.
	38 per cent.	28 per cent.
	32 per cent.	27 per cent.
	43 per cent.	26 per cent.
	19 per cent.	
	25 per cent.	
	23 per cent.	
	25 per cent.	
	31 per cent.	
Average	32 per cent.	24 per cent.

We find, however, in this series with a slower rate of inflow than the usual 4 c.c. per minute that the quantity intestinal fluid is lower than in non-nephrectomized animals in both NaCl and $CaCl_2$ series for the same amount of fluid retained. Thus the increase in intestinal fluid in nephrectomized animals may be accounted for by the relatively larger amount of fluid retained in a given time.

The effect of the infusions in diluting the blood was found to have no relation to the chemical character of the infused fluid, nor the condition of the rabbit—whether nephrectomized or not if we compare the dilution of the blood at the beginning and at the end of the experiment. The blood was taken in the manner above described in various experiments after either 600 c.c., 700 c.c. or 800 c.c. of fluid had been infused. It was found that the amount of inflow had, within the limits indicated above, but little influence upon the reduction of either hæmoglobin or erythrocytes, for, with the above amounts of inflow, the average reduction of hæmoglobin varied between 29 and 32 per cent., and of erythrocytes between 23 and 25 per cent. However, we found quite marked individual variations. In some experiments not noted here the reduction of hæmoglobin was as marked after 300 c.c. of inflow as in the above experiments after 600 to 800 c.c. of inflow.

Magnus (12) in infusing fluid into dogs found more reduction of the hæmoglobin than do we—from 25 to 66 per cent.—but since he used NaCl solutions of varying concentration, hypotonic and hypertonic as well as isotonic, the two sets of experiments are not quite analogous. It is certain that the infusions dilute the blood, but in no manner definitely referable to the chemical character of the solutions, the amount of the infused fluid or the individual animals. Yet when we consider that in these cases the amount of fluid infused is equal to as much as 6 to 7 times the amount of blood of the animal, the dilution of the blood seems insignificant and the tissues must rapidly care for a large part of the infused fluid.

In a considerable number of cases the animals died during the experiments of edema of the lung, but this occurrence was more frequent in those cases in which $CaCl_2$ solutions had been added than when pure NaCl solutions were used. Edema of the lung appeared in 25 per cent. of the NaCl experiments and in 44 per cent. of the $CaCl_2$ experiments; it was severe in 13 per cent. of the experiments with NaCl solutions and in 25 per cent. of those with NaCl—$CaCl_2$ solutions. The number of cases of edema has diminished as the technique of the experiments has become more perfect (that is, the control of the temperature and rate of inflow) but the edema of the lung still continued to be more frequent in the experiments with $CaCl_2$ solutions.

Hydrothorax was found in a large majority of the animals. The amount of fluid, however, was usually small and its occurrence and amount had no relation to the various solutions infused. It is peculiar that Cohnheim and Lichtheim (13), although they speak of the occurrence of ascites and of edema of the organs which we also found, say they did not find hydrothorax.

Turning now to the original question whether $CaCl_2$ exerts the same influence on the endothelial cells of the peritoneal cavity as it does on the kidneys and intestines, we find this answered in the negative, for although there is an inhibition of the secretion of fluid through renal cells and into the intestinal canal we find an increase in the production of ascites. Calcium chloride may be considered to increase the ascitic fluid in two ways: (1) indirectly through diminution of the amount of urine and intestinal fluid; (2) through increasing the ascitic fluid independently of its action on the kidney, etc., inasmuch as we found a marked increase of ascites after nephrectomy. It may be noted in all the experiments with non-nephrectomized animals that there exists a certain relation between the amount of urine excreted and the amount of ascites; to a certain degree the urine and ascites increase inversely. This is true not only in the animals infused with $NaCl$—$CaCl_2$ mixtures but also in animals infused with pure $NaCl$ solutions; this decrease of urine under the influence of $CaCl_2$ is, as stated above, one of the causes of the increase of ascites under the influence of $CaCl_2$. We find in nephrectomized animals that the decrease of intestinal fluid was as marked as the increase of ascites, while in the non-nephrectomized animals it was not so marked.

Attention should be called to the considerable individual variations of the animals in regard to power to bear the infusions of the fluids as well as to the production of urine, intestinal fluid and ascites; which variations necessitated the large number of experiments. However, in averaging series of nine or ten experiments these variations were found to be practically eliminated and the average of the different experiments agree well with each other.

CONCLUSIONS.

1. The secretion of urine and the elimination of fluid through the intestinal canal which are caused by the intravenous injection of solution of 0.85 per cent. sodium chloride are decreased by the addition of calcium chloride to the sodium chloride solution. The secretion of urine is more markedly inhibited than is the elimination of fluid through the intestines.

2. In contradistinction to the decreased elimination of fluid through the kidneys and intestines, addition of calcium chloride to the sodium chloride solution increases markedly the transudation of fluid into the peritoneal cavity. To a certain degree the urine and ascites may be said to increase in an inverse proportion.

3. Although calcium chloride inhibits both absorption from and secretion into the intestines it seems to decrease the secretion more markedly than the absorption.

4. The action of calcium chloride in increasing the ascitic fluid is a double one: first, by diminishing the amount of urine secreted; secondly, by increasing the ascites independently of its action on the kidneys. The latter may be a direct action on the endothelial cells of the peritoneal cavity; this, however, must be determined by further investigations.

5. Addition of calcium chloride to the infused fluids increases the tendency to the occurrence of edema of the lungs.

6. Infusion of large quantities of fluid into animals dilutes the blood, but this dilution seems to be carried only to a certain degree—about 30 per cent.—and to be independent of the chemical character of the solution and of the function of the kidneys.

7. The presence or absence of the kidneys has a marked influence on the intestinal and ascitic fluids. When the averages of ascitic and intestinal fluids per 1,000 c.c. of retained fluid in non-nephrectomized animals and these fluids per 1,000 c.c. of infused fluid in nephrectomized animals are compared, more fluid is found in the case of the nephrectomized animals. This fact can only be explained in part by the shorter time necessary for the same amount of fluid

to be retained in the case of the nephrectomized animals. Nephrectomy causes an increase of the ascites and the intestinal fluids through a mechanism which will have to be investigated by means of further experiments.

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