TABLE I.
Inhibitory Effect of Procaine on Bacteriostatic Action of Sulfapyridine Against Pneumococcus III (T3-1).*

Conc. of Sulfapyridine	Concentration of procaine				
	0	.02%	.002%	.0002%	.00002%
%					
0	++++	++++	++++	++++	++++
.003	—	++++	++++	++++	—
.004	—	++++	++++	++++	+
.005	—	++++	++++	++++	—

— no growth.
+ slight growth.
++++ abundant growth.
*Growth observed at 18 hours after incubation at 37°C.

per ml of a standardized strain of Pneumococcus III (T3-1).[4] A series of concentrations of procaine were tested with varying amounts of sulfapyridine. Table I shows that 0.0002% of procaine, which is the average amount found in plural fluids of patients thus anesthetized, is sufficient to inhibit the action of 0.003, 0.004, and 0.005% of sulfapyridine.

It is possible that after procaine anesthesia sufficient procaine may be present in the remaining chest fluid to at least temporarily inhibit the action of sulfapyridine and permit bacterial growth. The effect of urethane on sulfapyridine was studied because it does not couple in the Marshall test. It was found that in concentrations as high as 0.05% urethane did not inhibit sulfapyridine.

Summary. 1. The average concentration of procaine in pleural fluids removed with this anesthetic was 0.0002%. 2. Sulfapyridine in concentrations as high as 0.005% was inhibited by procaine *in vitro*. 3. Concentrations of urethane as high as 0.05% do not inhibit the action of sulfapyridine *in vitro*.

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Influence of Prolonged Electrolyte Deprivation and Final Restoration on Fluid Intake, Balance and Distribution.

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Gilman[1] found that the intravenous injection of an hypertonic salt solution, which increased the volume of the plasma and interstitial

[1] Gilman, A., *Am. J. Physiol.*, 1937, **120**, 323.

fluid at the expense of the intracellular compartment, markedly increased the water intake of the dog. Conversely, confirming Darrow and Yannet,[2] when the extracellular compartment was dehydrated and the intracellular volume increased after the intraperitoneal injection of an isotonic glucose solution, the water intake was reduced.

In the present study, the extracellular electrolytes of 7 dogs were partially depleted by intraperitoneal glucose injections and then maintained at low levels for a period of 5 to 15 days through the use of a salt-free diet, to make observations on the following questions: (1) Whether the fluid shifted into the intracellular compartment to restore osmotic equilibrium between it and the electrolyte depleted extracellular compartment would be spontaneously returned. (2) Whether the reduced voluntary water intake would be maintained, and could be correlated with intracellular volume. (3) Whether the injection of NaCl would restore normal fluid distribution and water intake.

Methods. An intraperitoneal injection of 100 cc per kg body weight of a 5.5% glucose solution was given each animal, paracentesis being performed at the end of 5 hours. The animals were placed in metabolism cages, and water intakes, urine volumes and urine electrolyte analyses recorded daily. Serum electrolyte analyses were made at selected intervals. Food was withheld for the first few days. Then salt-free meat[3] was given in measured amounts. In constructing fluid and electrolyte balances, it was assumed that the initial total body water was 65% and the extracellular volume 27% of the body weight. Experimental extracellular volumes were derived from serum sodium and chloride concentrations[4] and from hematocrit and hemoglobin readings. It was found that for the first 7-10 days of the experiment all 4 methods of derivation gave comparable extracellular volumes, but that later hematocrit and hemoglobin readings would indicate an abnormal dilution. The discrepancy might be due to a progressive anemia of dietary origin.

Corrections for metabolic water gain and extra-renal loss were made by assumed constants based on previous experiments. Intracellular volumes were taken to be the difference between total body water and the average extracellular volume as derived from serum sodium and chloride concentrations.

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[2] Darrow, D. C., and Yannet, H., *J. Clin. Invest.*, 1935, **14**, 266.

[3] Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., *Am. J. Physiol.*, 1936, **116**, 438.

[4] Harrison, H. E., Darrow, D. C., and Yannet, H., *J. Biol. Chem.*, 1936, **113**, 515.

TABLE I.
Fluid Intake, Balance, and Distribution in Dogs Subjected to Prolonged Electrolyte Deprivation.

Date	Body wt, kg	Serum Na, m-eq/l	Serum Cl, m-eq/l	Serum K, m-eq/l	Hematocrit, %	Avg water drunk, cc	Avg urine volume, cc	Avg water balance, cc	Avg urine Cl, m-eq	Avg urine Na, m-eq	Avg urine K, m-eq	Avg extracellular volume from serum Na and Cl, l	Intracellular volume, l
						Dog 1.							
10/21[1]	11.0	146.6	113.8	4.6	53.2							2.97	4.18
10/21[2]		129.3	92.8	5.1	68.7	0	0					2.29	4.44
10/22	10.5	129.8	93.0	5.5	61.2	0	0	—422				2.28	4.44
10/25[3]	9.5	129.3	95.8	6.3	62.9	637	737	—184	9.5	0.6	28.1	2.11	3.84
11/1	9.3	133.4	92.8	3.7	57.0	1813	2005	— 16	8.3	1.4	9.5	1.90	3.83
11/2[4]						1485	1320	+465	63.2	29.2	10.7		
11/3	10.1	148.1	111.8	4.0	45.5	290	230	+360	11.4	6.5	1.5	2.75	4.18
						Dog 2.							
9/21[5]	17.2	146.7	112.8	4.7	50.6							4.64	6.54
9/21[6]		127.1	86.8	3.8	61.8	0	0					4.20	6.98
9/22	16.1	136.9	96.4	4.0	55.6	0	490	— 634	2.5	0.6	11.5	3.86	6.69
9/24[3]	15.2	138.8	99.4	6.2	53.6	460	570	— 254	3.0	0.5	12.5	3.70	6.34
9/27	15.7	137.2	97.8	5.2	53.7	970	1227	— 19	3.6	1.1	19.1	3.69	6.28
9/30		138.8	100.0	5.3	51.0	853	1197	— 106	4.3	0.8	12.5	3.58	6.30
10/7	15.4	140.7	103.8	5.3	50.0	620	850	+ 8	4.0	0.3	10.9	3.59	6.33
10/8[7]						1175	291	+1122	3.7	18.4	8.8		
10/9	16.5	149.8	117.8	4.7	41.2	40	280	— 2	20.1	13.0	10.1	4.47	6.40

[1]Injected 1100 cc glucose solution.
[2]Drained 1440 cc peritoneal fluid containing 147.3 m-eq Na, 121.0 m-eq Cl.
[3]Started salt-free diet, 500 g daily.
[4]Given 9 g NaCl, plus salted food.
[5]Injected 1720 cc glucose solution.
[6]Drained 1720 cc peritoneal fluid containing 177.3 m-eq Na, 138.8 m-eq Cl.
[7]Given 10 g NaCl plus salted food.

Results. The balance data on 2 representative dogs are given in Table I. Complete equilibrium between all parts of the extracellular fluid had often not been reached at the time of paracentesis, as evidenced by a later rise in serum electrolyte concentrations (*e. g.,* Dog 2). Constant levels had been attained by the 24th hour. Throughout the period in which NaCl was restricted these concentrations showed either no change or a slight progressive rise. In no case was there a dilution of extracellular electrolyte, as would be expected if fluid were being returned from the intracellular compartment. These results agree with those reported by Mellors, Muntwyler and Mautz,[5] based on similar experiments.

[5] Mellors, R. C., Muntwyler, E., and Mautz, F. R., *Proc. Am. Physiol. Soc.,* Chicago, 1941.

The initial response to the electrolyte deprivation was a complete absence of water intake and a persistent negative water balance. With constant extracellular volumes, this body water loss must have been contributed by the intracellular compartment. Usually by the 3rd day this compartment had lost a volume equal to that originally gained upon the intraperitoneal glucose injection, and had reached a stable minimal value. Since this initial period was one of starvation, it was to be expected that this value should be lower than that typical of the preëxperimental period.

Coincident with the attainment of this stable intracellular volume was the first voluntary taking of water, which either greatly minimized the negative water balance or restored equilibrium. Water intakes now rose steadily until they reached levels of polydipsia. Urine volumes rose accordingly, however, so that in no case was there a positive water balance established, despite the fact that the extracellular compartment was still dehydrated. Intracellular volumes were not restored to the preëxperimental level, although food was taken during this period.

When NaCl was given intravenously in amounts roughly equal to that originally removed in the peritoneal fluid, extracellular electrolyte concentrations were immediately restored to normal. The water intake on the first day of salt restoration was always high, and often twice that of previous days. A marked positive water balance was established, due in part to this increased intake and even more to a marked reduction in urine volume. The high water intake was not maintained on subsequent days, although the water balance was usually still positive. Extracellular volumes were not immediately restored to the preëxperimental level. This fall in intake might be coincident with a secondary rise in intracellular volume which occurred, with restoration to the preëxperimental levels.

Summary. Dogs partially depleted of extracellular electrolytes by intraperitoneal glucose injections and then maintained on a salt-free diet showed absence of fluid intake and a negative water balance while the intracellular volumes were above normal. When these volumes had been reduced to stable minimal levels, water was taken by mouth and the negative water balance corrected. Although the intakes now rose to polydipsic levels, a positive balance was not established despite the fact that the extracellular volumes were still reduced. Intracellular volumes were not restored to the preëxperimental level. When NaCl was given, extracellular electrolytes and volumes were restored toward normal. The initial response was an increased intake which, however, was not sustained. The intracellu-

lar compartment, originally decreased, showed a delayed rehydration to normal levels. Hence the voluntary water intake showed more positive correlation with changes in intracellular volume than with extracellular volume change. Fluid balance was affected both by intracellular hydration and electrolyte levels in the extracellular fluid.

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Widened Reactivity of Antibody Produced by Prolonged Immunization.

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A number of workers have observed that antibody becomes more cross-reactive as the course of immunization is prolonged. The first observations were apparently those of Magnus[1] on antigenic plant-extracts. Many similar results have been obtained with antigenic cells or crude mixtures of antigens; the first evidence that reasonably pure proteins can produce the same effect was that of Wells and Osborne.[2] We used 5-times recrystallized ovalbumins from hen and duck and offered additional proof[3] that the antibodies to a single "pure" antigen may develop a broadened reactivity as immunization proceeds. The antisera from later bleedings *may* show a broader equivalence-zone,[4] and the antibody, in the zone of antigen-excess, can combine with a larger quantity of antigen.[4, 5]

Three possible explanations of these effects have been considered; conceivably all of them might apply in some instances.

1. The antibody initially formed is directed toward a dominant determinant-group of the antigen, and, progressively, additional antibodies are formed for separate, minor determinants.[6-9]

2. The later antibodies differ from the earlier by the presence on

1 Magnus, *Ber. deut. Bot. Gesellsch.*, 1908, **26a**, 532 (cited in[2]).
2 Wells, H. G., and Osborne, T. B., *J. Inf. Dis.*, 1913, **12**, 341; 1916, **19**, 183.
3 Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1934, **26**, 469; 1936, **30**, 41.
4 Heidelberger, M., and Kendall, F. C., *J. Exp. Med.*, 1935, **62**, 697.
5 Malkiel, S., and Boyd, W. C., *J. Exp. Med.*, 1937, **66**, 383.
6 Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1933, **25**, 61.
7 Heidelberger, M., and Kendall, F. C., *J. Exp. Med.*, 1934, **59**, 519.
8 Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1938, **67**, 709.
9 Pauling, L., *J. Am. Chem. Soc.*, 1940, **62**, 2643.