the third one evacuated most of its lipiodol in 27 minutes and disposed of the rest within the next 19 minutes. Nothing comparable to this initial rate of emptying has been found in any other species except man[4] and definitely establishes the supremacy of the primate gall bladder.

6240

Alterations of Protein Distribution Between Corpuscles and Plasma by Isotonic and Hypertonic Solutions.

C. J. BELLIS AND F. H. SCOTT.

From the Department of Physiology, University of Minnesota.

One of us (Scott[1]) observed that upon the addition of isotonic saline to whole blood *in vitro,* the total protein of the plasma was greater than in the original plasma; in other words, some protein had entered the plasma from the corpuscles. We have repeated these experiments and confirmed the observations as regards isotonic solutions, but when hypertonic solutions are used, the reverse occurs; there is a decrease in the total plasma protein, indicating that some protein had entered the corpuscles from the plasma. When hypertonic solutions were added to plasma alone, a decrease of protein did not occur, the total protein in the diluted specimen corresponding to the total in the control. Whether isotonic or hypertonic solutions were added to blood, the non-protein nitrogen of the plasma invariably increased.

The principle used was that employed by Scott. The corpuscle and plasma percentages were determined in duplicate by the hematocrit. The nitrogen determinations were made in duplicate by the modified micro-Kjeldahl method of Cavett,[2] including the use of H_2O_2[3] to complete the oxidation. 0.8 cc. of plasma was used for the total nitrogen determination and what would be equivalent to 2.5 cc. of plasma for the non-protein nitrogen, the protein-free filtrate being prepared according to the tungstic acid precipitation of Folin and Wu.[4] Duplicate blanks were run in all the determinations.

---

[4] Boyden, E. A., *Anat. Rec.*, 1928, **40**, 147.

[1] Scott, F. H., *J. Physiol.*, 1915, **50**, 128.

[2] Cavett, J. W., *J. Lab. Clin. Med.*, 1931, **17**, 79.

[3] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **56**, 2066.

[4] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **81**, 38.

Oxalated blood was used in all experiments except where the procedure involved the use of $CaCl_2$ as a diluent, in which case defibrinated blood was used. Similar results were obtained with either type of blood.

A typical experiment is outlined below:

Oxalated ox blood. 68.25% plasma.

0.8 cc. plasma = .0095598 gm. N of which .0002232 gm. is NPN.

∴ protein nitrogen = .0093366 gm.

90 cc. original blood was added to 10 cc. 50% dextrose solution and allowed to stand 21 hours (in most experiments 30 minutes).

Plasma = 74.735%.

0.8 cc. plasma = .00763 gm. N of which .0001984 gm. is NPN.

∴ protein nitrogen = .0074316 gm.

90 cc. original blood contained 61.425 cc. plasma, ∴ the total protein nitrogen = 61.425 × .0093366 × 10/8 = .7168911 gm.

Total non-protein nitrogen = .0171 gm.

In the diluted blood (plasma = 74.735%) the total protein nitrogen = 74.735 × .0074316 × 10/8 = .694213 gm.

Total non-protein nitrogen = .01853 gm.

∴ decrease protein N = .0226781 gm. = .1417 gm. protein.

∴ increase non-protein nitrogen = .0014 gm.

To make the results comparable, we have expressed them as if in each experiment we started with enough blood to have 100 cc. of plasma. The following table represents the protein and non-protein nitrogen changes as effected by the various solutions:

TABLE I.

Series	Diluent %	To Blood cc. cc.	Aver. Exp.	Protein change in Plasma gm.	NPN in mg.
1	50 dextrose	10:90	11	— .513	+ 3.9
2	” ”	25:75	1	— .143	+ 3.1
3	5.5 ”	10:90	2	+ .369	+ 1.2
4	” ”	20:80	2	+ .256	+ 1.1
5	” ”	50:50	2	+ .719	+ 3.9
6	” ”	70:30	2	+ .703	+10.4
7	8.1 NaCl	10:90	15	— .326	+ 2.5
8	0.9 ”	10:90	3	+ .243	+ 1.2
9	” ”	20:80	3	+ .576	+ 2.6
10	” ”	50:50	2	+1.603	+ 5.7
11	12.8 $CaCl_2$	10:90	1	— .029	+ 4.0
12	1.4 ”	10:90	1	+ .166	+ 1.2
13	” ”	20:80	1	+ .171	+ 2.0
14	” ”	50:50	1	+ .215	+10.2
15	” ”	70:30	1	+ .417	+18.9
16	2.65 $BaCl_2$ (isotonic)	10:90	2	+ .000	+ .1
17	” ”	20:80	2	+ .140	+ 1.2
18	” ”	50:50	2	+ .913	+ 5.1
19	” ”	70:30	2	+1.191	+11.0

+ = increase. — = decrease.

With the strength of hypertonic solutions used, it is not possible to dilute blood much more than 10% or some laking results. In the case of $BaCl_2$, 24% solution even in 10% dilution caused laking.

In the various experiments, the figures were all in the same direction. Thus, in the 11 experiments with hypertonic dextrose (Series 1), every experiment showed decreases of total protein which varied between .079 gm. and 1.913 gm. with an average decrease of .513 gm. In the 15 experiments using hypertonic NaCl (Series 7), the decreases varied between .052 gm. and 1.003 gm. with an average decrease of .326 gm. These phenomena are probably connected either with a reversal of charges on the corpuscles and plasma protein in concentrated solutions or with an alteration of the lipoid protein equilibrium between the cells and plasma. These possibilities are being investigated.

6241

Effect of Saliva on Coagulation of the Blood.

C. J. BELLIS, W. BIRNBAUM AND F. H. SCOTT.

From the Department of Physiology, University of Minnesota.

It was observed that the addition of human saliva to dog blood greatly shortened the coagulation time. Hunter[1] alone observed that the addition of his own saliva to his own blood hastened the coagulation. He did not try to determine the nature of the active substance. There is nothing specific about this material. Saliva hastened the coagulation of the following types of blood: (1) Other humans; (2) recalcified oxalated human blood of another individual; (3) dog blood; (4) recalcified oxalated ox blood; (5) recalcified oxalated ox plasma. Figures for dog blood are given below.

Human saliva was obtained from various individuals after rinsing the mouth with warm physiological saline and discarding the first samples. Salivary flow was increased by chewing paraffin. All samples of saliva were centrifuged for 30 minutes before using. Unless otherwise indicated, the saliva was unheated. When heated, the saliva was placed on a water bath for 20 to 45 minutes and then diluted to its original volume, cooled to room temperature, and filtered before using. Blood was obtained directly from the cannulated femoral artery of dogs into tubes containing the known

[1] Hunter, John B., *Brit. J. Surg.*, 1928, **16**, 203.