CXCI. STUDIES ON THE CHOLESTEROL CONTENT OF NORMAL HUMAN PLASMA

VIII. A NOTE ON THE EFFECT OF ANTICOAGULANTS

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(Received 21 July 1938)

SPERRY & SCHOENHEIMER [1935] concluded that for the determination of blood cholesterol, oxalated plasma was unsuitable. In one case they estimated the cholesterol contents of serum and oxalated plasma and found 409.5 and 300 mg. per 100 ml. respectively. They then instituted further comparisons and though they did not report finding any other differences of this degree, they stated that "the average difference between the amounts of total cholesterol in heparinized and oxalated plasmas was 15.3 % of the former". They also quoted the work of Schmidt [1935] who found that the corresponding differences in the amount of phospholipid as estimated in heparinized and oxalated plasma averaged 10 % of the former.

There can be little doubt that the chief explanation of these differences is the effect of the oxalate addition in producing some shrinking of the red corpuscles; the fluid so lost diluted the plasma. This effect was noted by Gram & Norgaard [1923], and Eisenman *et al.* [1925] estimated this shrinkage by determining the serum protein after the defibrination of the blood with and without oxalate addition. They calculated the relative change in serum volume from the oxygen capacity and cell volume on the one hand, and from the protein content on the other. Both these methods demonstrated that the addition of oxalate to the blood caused a relative increase of plasma volume averaging 6 %. Eisenman [1927] showed that the addition of neutral potassium oxalate to the extent of 0.17–0.2 % of the whole blood caused a reduction of cell volume averaging 2 vol. % of the whole blood, which corresponds to a dilution of the plasma averaging 3.5 %. She also showed that with higher concentrations of oxalate, up to 0.45 %, there might be a shrinkage of the corpuscles up to 7.4 vol. % of the whole blood, equivalent to a plasma dilution of about 12 %.

Wintrobe [1931–2], in a series of haematological studies, stated that heparin is the ideal anticoagulant as it does not affect the size of the red corpuscles, but that it cannot always be depended upon unless the extremely expensive purified material is employed. He noted that there was a shrinkage of 8.2 % of the corpuscular volume in oxalated blood as compared with heparinized blood, and consequently he used the factor 1.09 to obtain the real corpuscular volume from the value obtained by using oxalated blood. Now the average corpuscular volume is 42 % of the whole blood, so that a shrinkage of 8.2 % of the volume would represent the addition of 3.44 vol. of fluid to 58 vol. of plasma, i.e. there would be a dilution of the plasma by approximately 6 %. It is interesting to note that this figure is the same as that determined by Eisenman *et al.* [1925].

Wintrobe's estimations of the change of corpuscular and plasma volumes by relatively simple physical methods are likely to be more accurate than the estimation of either cholesterol or phospholipid in the plasma by any method. As Sperry & Schoenheimer's results showed differences more than twice as large as the probable degree of plasma dilution, we thought that it would be of interest to make a further series of determinations.

Table I. *Comparison of the cholesterol content of heparinized and oxalated plasmas*

Series	Cholesterol, mg. per 100 g. plasma			% of total as ester	Mean values			
	Free	Ester	Total		Free	Ester	Total	
D 1:								
Heparin	47	135	182	74.2	—	—	—	% diminution produced by oxalate
	45	134	179	74.9	46	134.5	180.5	
% difference	4.3	0.7	1.7	—	10.9	1.1	3.6	
Oxalate	42	132	174	75.8	—	—	—	
	40	134	174	77.0	41	133	174	
% difference	4.9	1.5	0	—	—	—	—	
D 2:								
Heparin	34	118	152	77.6	—	—	—	% diminution produced by oxalate
	34	118	152	77.6	34	118	152	
% difference	0	0	0	—	4.4	3.0	3.3	
Oxalate	32	115	147	78.2	—	—	—	
	33	114	147	77.6	32.5	114.5	147	
% difference	3.1	0.9	0	—	—	—	—	
D 3:								
Heparin	32	120	152	78.8	—	—	—	% diminution produced by oxalate
	33	116	149	78.1	32.5	118	151.5	
% difference	3.0	3.3	2.0	—	4.6	1.7	3.0	
Oxalate	31	119	150	79.6	—	—	—	
	31	113	144	78.6	31	116	147	
% difference	0	5.0	4.0	—	—	—	—	
H 1:								
Heparin	71	183	254	72.1	—	—	—	% diminution produced by oxalate
	72	180	252	71.3	71.5	181.5	253	
% difference	1.4	1.6	0.8	—	3.5	5.5	5.3	
Oxalate	68	170	238	71.4	—	—	—	
	70	173	243	71.3	69	171.5	239.5	
% difference	2.9	1.8	2.0	—	—	—	—	
*H 2:								
Heparin	70	187	257	72.9	—	—	—	% diminution produced by oxalate
	69	186	255	73.0	69.5	186.5	256	
% difference	1.4	0.5	0.8	—	2.9	5.4	4.7	
Oxalate	68	176	244	72.6	—	—	—	
	67	177	244	72.2	67.5	176.5	244	
% difference	1.5	0.6	0	—	—	—	—	

* These results are expressed as mg. cholesterol per 100 ml. plasma.

Cholesterol was determined in heparinized and oxalated plasmas by the method described by us [Gardner *et al.* 1938]. Blood was taken from normal individuals or from patients who were unlikely to have any disturbance of their

cholesterol metabolism. Each specimen was divided into two portions of which one was treated with neutral potassium oxalate to the extent of 0.2 g. per 100 ml., and the other with heparin (Hyson, Westcott and Dunning's preparation) to the extent of 1 mg. per 5 ml. of blood. In all experiments, duplicate determinations were made. Cholesterol is expressed in mg. per 100 g. of plasma except in one series (H 2) where determinations were made on 10 ml. portions of plasma, and hence the results are expressed as mg. cholesterol per 100 ml. plasma.

The average diminution of total cholesterol content produced by oxalating plasma was 3.8%, whereas the average difference between duplicate determinations of total cholesterol was only 1.1%. As we do not yet understand the significance of much greater variations from the average normal, this reasonably constant error of 4% is not a very grave matter.

SUMMARY

1. Addition of neutral potassium oxalate to blood leads to a small shrinkage of the erythrocytes, with a consequent slight dilution of the plasma.

2. In cholesterol estimations on heparinized and oxalated plasma there was found to be a reduction of concentration produced by neutral potassium oxalate of about 4%. This figure is of the same order as the degree of shrinkage of the red cells.

We are indebted to the Government Grants Committee of the Royal Society for a grant in aid of this investigation.

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