

castrated for a period of time which was twice as great (30 days). Study of the frequency distribution of these cells in the mature and immature groups will show the individual variations in the different groups and demonstrate in a clear-cut fashion that the factor of age must be considered seriously when one is studying the reaction of the anterior pituitary of the female rat to castration. It is impossible at the present time to give an explanation of this interesting finding. It is pointed out that the initial levels of the basophiles are much higher in normal immature female rats than they are in mature females killed during the various phases of the oestral cycle. Also in the anterior pituitaries of immature female rats a great majority of the basophiles are completely filled with granules, while in the mature female these cells undergo cyclic changes in their granular content.

Reference to Table I will show that the mean level of eosinophiles in 39 female rats castrated for 30 days was 41.2% while the mean level of these cells in 69 mature normal female rats was 33.6%. This would indicate that in this group of castrates there was some increase in the eosinophiles. However, it is important to point out that occasionally the level of these cells in non-castrated females was slightly higher than 40%. Since the mean level of these cells in the 30-days castrates was only slightly above the upper limits of normal for normal females it seems questionable to conclude without additional data that castration in mature female rats results in an increase in the eosinophiles.

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### Suspension Stability of Erythrocytes in Solutions of Gum Acacia.\*

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It is stated that the suspension stability of erythrocytes is dependent upon variations in the albumin, globulin, and fibrinogen content of plasma.<sup>1, 2</sup> An increase in the fibrinogen, or globulin, tends to diminish the stability.<sup>3</sup> A careful study of this phenome-

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<sup>1</sup> Fahraeus, R., *Acta Med. Scand.*, 1921, **40**, Suppl.

<sup>2</sup> Westergren, A., Juhlin-Dannfelt, C., and Schnell, R., *Acta Med. Scand.*, 1932, **77**, 469.

non<sup>4</sup> reveals that although there appears to be an association between an increase in blood serum globulins and a diminished suspension stability, the real causative factor is an agglutinant which may or may not be associated with the globulins. The sedimentation of red blood corpuscles in the presence of this agglutinant, found in physiologically altered blood, may be paralleled experimentally in solutions of gum acacia.

When solutions of gum acacia are administered intravenously to human subjects it is noted that the blood becomes difficult to smear, and that the suspension stability of the erythrocytes is diminished. Similar phenomena may be observed when blood is mixed with gum acacia solutions *in vitro*.

*Method.* Human subjects with normal and shortened sedimentation rates, and subjects receiving gum acacia solutions intravenously were chosen for this study. All sedimentation experiments were done using the Friedlander tube and recording the time necessary for the column of erythrocytes to settle 18 mm. A 20 cc. sample of venous blood was withdrawn and oxalated. A sedimentation test was done directly on this sample, and another was done using the Lenzenmeier<sup>5</sup> technic. The remainder of the sample was separated by centrifugation and the corpuscular moiety washed with 3 changes of Locke's solution. Then 0.2 cc. of washed corpuscles were resuspended in 0.8 cc. of the following menstrua: plasma,

TABLE I.  
Sedimentation Rate Expressed in Minutes for a Column of Cells to Settle 18 mm.

	Slowly Sedimenting Blood 4 samples	Rapidly Sedimenting Blood 9 samples
S.R. Lenzenmeier technic	192	24
S.R. oxalated undiluted specimen	186	14
S.R. cells resuspended in plasma E.V.	44	7
S.R. " " " Locke E.V.	719	726
S.R. " " " 1% acacia E.V.	66	55
S.R. " " " 2% " E.V.	11	11
S.R. " " " 3% " E.V.	4.7	3.5
S.R. " " " 4% " E.V.	5	3.8
S.R. " " " 6% " E.V.	6.5	4.6
S.R. " " " 8% " E.V.	9.2	7
S.R. " " " 10% " E.V.	36	30

S.R. = Sedimentation rate.

E.V. = 20% cell volume.

<sup>3</sup> Westergren, C., Theorell, H., and Widstrom, G., *Z. f. d. g. Exp. Med.*, 1931, **75**, 668.

<sup>4</sup> Lucia, S. P., Gospe, S., and Brown, J. W., to be published.

<sup>5</sup> Lenzenmeier, G., *Arch. f. Gynaekologie*, 1920, **113**, 608.

Locke's solution, 1, 2, 3, 4, 6, 8 and 10% acacia in buffered Locke's solution, the resulting cell volumes being 20% or  $2.15 \pm 0.1$  million corpuscles per c.mm. The experiments are therefore corrected for a standard volume.<sup>6</sup> In all resuspension experiments the syringes were rinsed in 10% potassium oxalate solution.

*Results.* Table I records the results, expressed as averages, obtained on 13 different samples of blood.

It will be noted that although the sedimentation rates of the 2 series differ considerably, when suspended in their respective plasmas, there are no marked differences when these same cells are resuspended in Locke and acacia solutions under standard conditions of volume.

The following experiment was done in order to determine, macroscopically, the time of rouleaux formation, and the rapidity of sedimentation. For this purpose a stop-watch was used.

TABLE II.

The Sedimentation Rate Expressed in Minutes for a Selected Sample.

S.R. Lenzenmeier technic	235'
S.R. oxalated undiluted specimen	176'
S.R. cells resuspended in plasma E.V.	50' RBC's = 2,220,000/cmm.
S.R. " " " " Locke E.V.	450' RBC's = 2,110,000/cmm.

S.R. acacia in Locke	Fall in mm.	1%	2%	3%	4%	6%	8%	10%
Rouleaux formation begins at		2' 0"	1'10"	1'15"	1'02"	1'03"	2'25"	2' 0"
	mm.							
	1	5'50"	1'25"		1'15"	1'58"	3'45"	
	2	9'35"	2'19"	1'30"	2'14"	2'44"	4'15"	
	3	12'20"	3'08"			2'55"	4'30"	4'50"
	4	13'50"	4'25"			3'10"	4'45"	5'15"
	5	14'45"	4'39"	2'30"	2'39"	3'17"	4'57"	5'40"
	6	17'45"	4'52"	2'45"		3'24"	5'10"	
	7	20'30"	5'29"			3'31"	5'19"	6'15"
	8	23'15"	5'58"			3'46"	5'26"	
	9	26'25"	6'27"				5'33"	
	10		6'42"		3'04"		5'40"	
	11		6'50"				5'47"	6'45"
	12	34'10"	7'20"	3'30"	3'19"	4'26"	5'54"	6'55"
	13		7'50"				6'02"	
	14		8'23"					
	15		8'43"					7'25"
	16		9'05"					
	17		9'25"					
	18	56'0"	10'00"	4'15"	3'54"	4'56"	6'52"	8'10"

An examination of Table II reveals that at least one minute elapses before rouleaux formation is initiated. Sedimentation begins slowly at first and then progresses with an accelerated velocity followed by retardation. In rapidly sedimenting bloods the rate of sedimentation is not a rectilinear function.

<sup>6</sup> Walton, A. C. R., *J. Lab. and Clin. Med.*, 1933, **18**, 711.

When samples of corpuscles in acacia solutions are examined microscopically, it is noted that rouleaux formation is slow and the clumps small in the 1% solution; rapid with large clumps in the 10% solution.

*Summary.* The factors responsible for alterations in the suspension stability of erythrocytes is independent of the corpuscles. Solutions of gum acacia reduce the suspension stability of the blood. A solution of 3 or 4% of acacia is the minimal amount that will produce the maximal instability of erythrocyte sedimentation. The rapidity of formation and the size of agglutinated erythrocyte masses progresses parallel to the increase in concentration of acacia in solution. The curve of sedimentation for rapidly sedimenting bloods is not a rectilinear function.

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### No Anti-Hormones Against Estrin.\*

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In 1934, Collip indicated that there may exist anti-hormones, or substances produced as a result of continued hormone injection which tend to counteract the effect of the hormone. In the preparations which he used, however, the possibility of protein being present could not, with absolute certainty, be excluded. The so-called anti-hormone might then possibly have been an antibody, although evidence opposing this belief was presented.

It seemed to us desirable to investigate the possibility of anti-hormone production against estrin since we would here be dealing with a substance certainly not a protein, and also because we found in earlier work<sup>1</sup> that continued estrin administration did not cause a continued increase in size of the uterus, but rather the reverse, which might conceivably be explained on the basis of anti-hormone production.

Two series of experiments were performed. In the first, 3 groups of 10 rats each were used, one group normal, mature females, the

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<sup>1</sup> Spencer, Jack, D'Amour, Fred E., Gustavson, R. G., *Am. J. Anat.*, 1932, **50**, 129.