tolic blood pressure in the rat are of a magnitude which deserves attention. A pressure of 120 mm Hg, normal for a rat weighing 300 to 500 g, is definitely hypertensive for a rat weighing less than 100 g.

The blood pressure in man increases in the corresponding age groups of infancy and early childhood in a similar fashion.[4,5] The consideration of this fact is common practice in pediatric experience.

Summary. The systolic blood pressure has been recorded in 100 unanesthetized, unheated rats of various ages. It increases with weight and age and normal values were established.

[4] Graham, Archibald W., Hines, Edgar A., Jr., and Gage, Robert P., *Am. J. Dis. Child.*, 1945, **69**, 203.

[5] Downing, Elizabeth M., *Am. J. Dis. Child.*, 1947, **73**, 293.

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Budding of Thrombocytes from Megakaryocytes.* (17375)

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It is now generally agreed that the megakaryocyte is the source of the thrombocyte.[1] Dameshek and Miller[2] discuss the changes that occur in the marrow in idiopathic thrombocytopenic purpura. The latter authors found that, in the marrows of 10 normal subjects, an average of 68.6% of the megakaryocytes showed thrombocyte budding. Cartwright and associates[3] found thrombocyte budding in 75% of megakaryocytes in normal marrows. In a recent study of bone marrow films made by the citrate technic, as described below, from 12 patients with idiopathic thrombocytopenic purpura before operation, it was found that 0.14% of the megakaryocytes showed evidence of thrombocyte budding. Eighteen postoperative films, made at varying intervals after operation on 10 of these patients showed thrombocyte budding from 9.2% of megakaryocytes. When the bone marrows of 7 normal control patients without hematological abnormality were examined, however, thrombocyte budding was seen in only 0.43% of the megakaryocytes. This discrepancy in the results obtained in the normal suggested that the differences might be due to variations in the technic used. Since the routine method for making bone marrow films in this Institute involves the use of a dry syringe, further investigations were carried out on material obtained here in this way.

Methods. Citrate method. The puncture of the sternum was performed with a Salah needle introduced at the level of the third or fourth intercostal space. The syringe and needle were first washed out with sterile 3.8% solution of sodium citrate, and aspirated material, usually 1 cc or less, was expressed on a watch glass which also had been rinsed with sodium citrate. Flecks of marrow were gently lifted with tweezers, and placed on carefully cleaned glass slides. They were then spread by means of another glass slide which was laid on top of the marrow flecks and slid rapidly across the surface of the first slide. The marrow films were dried in air. In the investigations referred to above, May-Grunwald-Giemsa stain had been used. In the investigations described below, Wright's stain was used, and the technic differed in that, following aspiration with a dry syringe, a sec-

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[1] Tocantins, L. M., *Medicine*, 1938, **17**, 155.

[2] Dameshek, W., and Miller, E. B., *Blood*, 1946, **1**, 27.

[3] Cartwright, G. E., Chung, Hui-Lan, and Chang, An, *Blood*, 1948, **3**, 249.

TABLE I.
Ten Normal Marrows Studied by the Dry Technic.

	Fields with megakaryocytes		Fields without megakaryocytes		
	Thrombocytes per 100 megakaryocytes	Clumps of thrombocytes per 100 meg.	Thrombocytes per 100 fields	Clumps of thrombocytes per 100 fields	% of meg. with thrombocytes
Areas with structure	387	16	369	8	25
Areas with no structure	1496	24	887	8	65

TABLE II.
Four Cases of Idiopathic Thrombocytopenic Purpura Studied by the Dry Technic.

	Fields with megakaryocytes		Fields without megakaryocytes		
	Thrombocytes per 100 megakaryocytes	Clumps of thrombocytes per 100 meg.	Thrombocytes per 100 fields	Clumps of thrombocytes per 100 fields	% of meg. with thrombocytes
		Presplenectomy			
Areas with structure	nil	nil	nil	nil	nil
Areas with no structure	9	nil	7	nil	nil
		Postsplenectomy			
Areas with structure	36	nil	13	nil	2
Areas with no structure	1117	12	475	nil	59

ond syringe that had been washed out with sodium citrate was used to aspirate a second sample of marrow, the further steps being those just described.

Dry method. The puncture was performed as before. A perfectly dry needle was used and a dry syringe attached to it. A sample of marrow, usually 1 cc or less, was rapidly aspirated. A small amount was then quickly expressed through another needle on to a clean cover slip. Excess fluid was withdrawn by means of the syringe, and the film was spread by means of a second cover slip. The films were stained with Wright's stain, and mounted on glass slides.

Ten normal marrow films made by the dry technic were first studied. Regardless of the technic used, it is common to find areas in which there is some preservation of marrow structure; fat spaces may be seen in many instances, although at other times there is merely a large aggregation of cells which can be observed with the naked eye. There are also other areas in which the cells lie quite free as in a blood film. The percentage of megakaryocytes that showed budding in areas with marrow structure and in areas without such structure were estimated separately. In addition, each time a megakaryocyte was encountered, another field 3 oil immersion fields distant from it was studied, and the number of thrombocytes contained therein was enumerated. Sometimes it was possible to count individual thrombocytes. At other times they had to be counted as clumps. In the normal marrows, a total of 100 megakaryocytes was examined in areas with marrow structure, and 100 in areas without such structure. In the other films it was possible to examine 25 megakaryocytes in each such area, and in some marrows, where budding was scanty, larger numbers of cells were used. Budding was recorded as being present when it appeared to be fairly definite, but this is a matter about which there may be divergence of opinion between different observers. The mere presence of megakaryocytes and thrombocytes in the same field was not considered to indicate true budding. The findings obtained by the dry technic in ten normal marrow speci-

TABLE III.
Five Patients Studied by Both the Dry and the Citrate Technics.

	Fields with megakaryocytes		Fields without megakaryocytes		
	Thromb. per 100 megak.	Thromb. clumps per 100 meg	Thromb. per 100 fields	Thromb. clumps per 100 fields	% of megakaryocytes
Pernicious anemia (thrombocytes normal)					
Dry method					
Areas with structure	248	4	556	4	20
" " no structure	748	32	476	24	52
Citrate method					
Areas with structure	120	nil	160	nil	15
" " no structure	nil	nil	8	nil	0
Pernicious anemia (thrombocytes much reduced)					
Dry method					
Areas with structure	64	nil	20	nil	8
" " no structure	332	nil	104	nil	60
Citrate method					
Areas with structure	12	nil	4	nil	2
" " no structure	nil	nil	nil	nil	0
Chronic granulocytic leukemia (thrombocytes increased)					
Dry method					
Areas with structure	168	60	312	28	52
" " no structure	1584	88	444	nil	92
Citrate method					
Areas with structure	404	44	320	20	48
" " no structure	76	12	56	4	16
Chronic granulocytic leukemia (thrombocytes much increased)					
Dry method					
Areas with structure	32	72	64	32	68
" " no structure	1696	212	1456	72	96
Citrate method					
Areas with structure	550	110	60	20	70
" " no structure	60	30	76	50	20
Idiopathic thrombocytopenic purpura (platelets very much reduced)					
Dry method					
Areas with structure	4	nil	12	nil	0
" " no structure	24	nil	4	nil	1
Citrate method					
Areas with structure	nil	nil	nil	nil	0
" " no structure	nil	nil	nil	nil	0

mens are shown on Table I.

Marrow aspirates obtained before and after splenectomy in four cases of idiopathic thrombocytopenic purpura were studied by the dry technic (Table II). In the peripheral blood, the thrombocytes were extremely few before operation. In 3 of the patients marrow films were made soon after splenectomy when the thrombocyte level was normal, and in the fourth case, the second puncture was made 3 years after operation at a time when the thrombocytes were recorded as being normal. Estimates of thrombocyte numbers rather than actual enumeration were employed because it is considered at this Institute that too much significance is apt to be attached to variations in the thrombocyte count which may, in fact, be due to technical factors or to differences in distribution of thrombocytes.

Sternal aspirations were performed on 5 patients with hematologic disorders and films were made by both dry and citrate methods. The results of the examination of the preparations are shown on Table III.

Discussion. There is obviously a very great difference between the results of thrombocyte counts on marrow aspirate obtained by the dry technic and that secured with the citrate method. It will be seen from Table III that there is less difference in areas where the marrow structure is preserved.

It would appear that when sodium citrate is used to flush out the syringe with which the sternal puncture is to be performed, many thrombocytes are removed from the marrow sample, and that this removal of thrombocytes is most marked in areas where the marrow structure is not intact. It might, therefore, be argued that the correct estimate of thrombocyte budding can be made only when a dry syringe is used. The figures given in Table III, however, indicate that even when this method is employed, the results are open to question, because here the estimates may be equally fallacious. When the thrombocyte count is high in the peripheral blood, one would expect that their number would also be increased in the marrow and in the blood which inevitably dilutes the marrow sample. The thrombocytes are sticky, and under the conditions of sternal aspiration one would expect them to adhere to one another and to the megakaryocytes, the largest cells which they encounter. Hence, it it would seem that counts performed with the dry technic are also inaccurate since much apparent thrombocyte budding is due to chance juxtaposition and adhesion of the sticky thrombocytes to the megakaryocyte. The estimate that is likely to be most valid is that made from areas with definite marrow structure. Even here, however, fields without megakaryocytes may contain an equal number of thrombocytes, and so it is not possible to say with certainty that in the normal marrow the number of megakaryocytes that show budding is as high as 25 per cent. Marrow structure is less well preserved when the citrate technic is used and it would appear that this method is unsatisfactory for investigations concerned with the budding of thrombocytes from megakaryocytes.

Summary. When marrow aspirations are performed using a syringe flushed with sodium citrate, a false impression is obtained of the amount of budding of thrombocytes from megakaryocytes because many thrombocytes are washed away from the parent cells.

When a dry technic is employed, the results are equally inaccurate because thrombocytes that by chance are in juxtaposition with megakaryocytes may stick to them and appear to be arising from them.

When aspirated marrow samples are used, the best indication of the extent of budding is obtained when a dry technic is used, and examination of the marrow is limited to areas of the film that have definite marrow structure. It is probable that less than twenty-five per cent of megakaryocytes in the normal marrow show true budding at any one time.

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