markedly lowered the tone level and, with the stronger ones, completely abolished rhythmic activity. A high tone previously induced by pituitrin was immediately brought back to control level or lower by these concentrations. Following a primary dose of sodium amytal in this range the tissue responded to pituitrin either in a curtailed fashion or not at all, and usually was unable to respond even to barium.

While the quantitative reaction varied considerably, and those of the uterus were the most spectacular, the directional responses were consistent. Specimens used included guinea pig uterus (virgin and pregnant), rabbit duodenum, rabbit uterus (non-pregnant), kangaroo* uterus and intestine, and portions of the digestive tract of the barn owl.

5470

Delayed Differential Counting of the White Blood Cells by a Modified Supravital Technique.

ALBERT E. CASEY AND PAUL D. ROSAHN. (Introduced by L. Pearce.)
From the Laboratories of The Rockefeller Institute for Medical Research.

It is a common experience in doing differential white blood cell counts by the supravital method[1, 2] that the cells cannot be identified after about 2 hours in the hot-box, since by this time nearly all the cells have taken up large amounts of the dye.

It has been found that by a relatively slight modification of the usual technique, differential counts may be made with great facility as long as 12 to 24 hours after taking the blood. This has been accomplished (1) by reducing the concentration of the dye used, (2) by placing the blood smears in the refrigerator until ready for counting, and (3) by eliminating the hot-box. Through the use of this procedure, all cells have been found to be actively motile and to retain their morphological viable characteristics; a 24 hour preparation which has been kept in the icebox appears to be entirely similar to a fresh smear and cannot be distinguished from it.

Thirty drops of a saturated solution of neutral red iodide No. 2

* This was an old female which had been in paralytic shock for 3 days. She and some of the other animals were available by courtesy of the Milwaukee Zoo.

[1] Simpson, M. E., *Univ. of Calif. Pub. in Anat.*, 1921, **1**, 1.

[2] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, **34**, 277.

in 10 cc. of absolute alcohol is a satisfactory concentration for rabbit's blood instead of the usual 50 to 100 drops; as much as 100 drops per 10 cc. of absolute alcohol has been used with success in the case of normal human blood. Smears left in the open laboratory were found to last about four hours or twice as long as those kept at 37° in the hot-box. Further reduction of the temperature to 5°-10° (refrigerator) effected the preservation of the cells for as long as 24 hours. When the preserved smears are examined with an ordinary electric bulb as the source of illumination, active motility of the white cells is evident. Consequently, it has been found possible to dispense with the hot-box.

Parallel observations on 6 male rabbits were made. A series of 16 smears were counted immediately after taking the blood, with the usual technique, and a duplicate set of smears was counted after 24 hours in the icebox with the modifications here reported. In making this comparison, more than 5,000 white cells were counted upon the total of 32 smears. The means and standard errors of the means of the two series of counts are given in the following tabulation:

TABLE I.

	Neutrophiles		Basophiles		Eosinophiles		Lymphocytes		Monocytes	
Age of Smear in hours	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24
Means	59.0	57.6	3.6	4.7	0.9	0.8	23.4	24.7	12.5	12.0
Standard error of the mean	±2.1	±1.4	±0.4	±0.6	±0.2	±0.2	±2.2	±1.9	±1.1	±1.4

That comparable results were obtained is seen from the fact that no significant difference between the respective means was obtained, and in no case was this difference equal to twice its standard error. A number of counts upon normal human blood has also been made with similar results.

The time limits to which blood smears may be successfully preserved have not yet been worked out, and dyes other than neutral red have not been used; but it seems that the present studies justify the statement that accurate differential white cell counts may be made with the supravital technique as long as 24 hours after making the preparations.