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A Method for Distinguishing Between "Activation" and Addition Effects when Mixing Coagulants.

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Mills[1] reported results obtained on mixing serum and lung extract; in general there was noted an increase followed by a decrease in the coagulative power of the mixture. He particularly emphasized the tremendous increase in coagulative power that occurs immediately after mixing. He used the decrease in the clotting time of a recalcified citrate plasma as an index of coagulative power.

Burns, Scharles and Aitken[2] reported a study on a series of kidney extracts from different animals incubated with various sera. Using the decrease in clotting time of heparinized plasma as an index of coagulative power, they secured results somewhat similar to those obtained by Mills with lung extract.

Considerable interest attaches to the interpretation of this phenomenon in terms of coagulation theory. In order accurately to determine whether activation occurs and the extent of the activation where it does occur, it is necessary to use a method which will measure activation at its height (according to available data this is immediately after mixing). And since serum as well as tissue extract has coagulative power, the method used should permit distinguishing between addition and "activation" effects.

The following method is found to meet the above requirements. Decrease in the clotting time of recalcified citrate rabbit plasma is used as the index of coagulative power. Coagulation time is determined in 10 mm. tubes kept in a water bath at 30°C. The materials are added in the following order. Plasma, 2 drops; 1% $CaCl_2$, optimum amount; coagulant; 0.9% NaCl to make 10 drops. The strength of the coagulants is now adjusted, by dilution of the stronger with saline, until 2 drops of either decrease the clotting time of the recalcified citrate plasma to the same extent. The mixed coagulants are now tested by adding to the tube, plasma, 2 drops; $CaCl_2$, optimum amount; 1 drop of each coagulant, NaCl to 10 drops. Activation will be indicated by shortening of the coagulation time beyond that caused by 2 drops of either coagulant alone.

[1] Mills, C. A., *Am. J. Physiol.*, 1922, **60**, 193.

[2] Burns, E. L., Scharles, F. H., and Aitken, L. F., *Am. J. Physiol.*, 1931, **97**, 233.

In a typical experiment, serum mixed with tissue fibrinogen (Merrel's Fibrogen) shows no (or occasionally just a trace of) activation. The following values were obtained: 2 drops serum, coagulation time 3¼ min.; 2 drops tissue fibrinogen, 3¼ min.; 1 drop of each, 3¼ min. The same serum, used in greater concentration to render it isocoagulant with fresh chick extract, shows marked activation; 2 drops serum, 2¼ min.; 2 drops chick extract, 2¼ min.; 1 drop of each, 45 sec.

Controls are run before and after the experiment to determine whether the coagulants remained isocoagulant during the test. Plasma is citrated to 0.5%; serum is from citrate plasma freshly clotted by recalcification; chick extract is made by extracting a 10 day chick embryo in 10 cc. Ringer fluid according to a method described earlier.[3]

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Serum Calcium in Relation to Menstruation in Cases with Dysmenorrhea.*

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A study of the serum calcium in relation to the menstrual cycle in a group of 10 women who had dysmenorrhea was made. The cases selected were those having severe dysmenorrhea necessitating bed rest every month. Our purpose was to determine whether women having dysmenorrhea showed any significant variations in calcium level from women with normal menstrual periods.

Serum calcium determinations were made in duplicate on 10 dysmenorrhea cases twice a week for 4 consecutive weeks. The blood was drawn between 8 and 10 a. m. The determinations of serum calcium were made by one of us (E. M. G.) using the Clark-Collip[1] modification of the Kramer-Tisdall[2] method. A total of 80 readings were made.

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[3] King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

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[1] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[2] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.