500 mg. of autoclaved yeast* daily. The experimental period lasted 3 to 5 weeks, the purified P.A. factor being administered orally and parenterally.

Typical data are presented in the charts. It may be seen that while 50 mg. daily of the crude liver extract gave adequate growth, 46 mg. of the purified P.A. material orally failed to support growth, nor did parenteral administration give better results. The addition of autoclaved yeast or crude liver extract was followed by immediate weight gain. Another liver extract (Lederle) presumably adequate in the antianemic factor, contained less vitamin G.

In addition to the data depicted in the graphs, assays for the vitamin G factor were performed with various fractions obtained during the preparation of the purified P.A. factor. The results were negative, which indicated that the vitamin G potency was lost during the initial stages of the purification.

It is hoped that the possible relationship between vitamin G and antianemic material from liver may be clarified by the reported preparation of nitrogen-containing fluorescent, pigment-like substances with a high degree of vitamin G activity.[4]

Summary. Purified liver extracts (P.A. factor) prepared from the Lilly extract No. 343 and of known potency in pernicious anemia were tested on vitamin G deficient rats. The extracts failed to induce growth in such animals. These experiments indicate that a purified preparation of the P.A. factor is lacking in vitamin G as tested by the growth method.

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Properties of Heated Chick Embryo Extracts When Activated.

JOSEPH T. KING. (Introduced by F. H. Scott.)

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The writer has found that addition of one volume rabbit plasma to 60-100 volumes chick embryo extract greatly increases the coagulative power of the extract[1]; that the increase in power is due to

* The yeast was obtained through the courtesy of Mr. Leo Wallerstein of the Wallerstein Laboratories, New York, N. Y.

[4] Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber. d. chem. Gesell.*, 1933, **66**, 317.

[1] King, Joseph T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

a body which reacts like thrombin in regard to the calcium ion[2]; that the plasma factor involved is prothrombin[3]; that the extract factor has the heat stability of cephalin and that the heated extract is as susceptible to activation as the unheated.[4] (Degree of activation calculated according to the method of Mills.[5])

Further study has brought out the fact that the time at which activation occurs is closely correlated with the clotting power of the extract being activated (as measured by its power to shorten the coagulation time of a recalcified citrate rabbit plasma). There appears, however, to be no such correlation between the coagulative power of such an extract and the degree of activation.

Determinations of coagulative power and activation were done as described above.[4] In that paper it was reported that there was a marked decrease in the coagulative power of chick extract as heating was done at intervals of 10°C. and that the decrease was especially marked below 60°. Above this point the power is relatively stable at a much lower level.

With the decrease in coagulative power of the extract there is a lengthening of the latent period of activation. In a typical experiment 0.05 cc. fresh unheated extract shortened the coagulation time of a recalcified citrate plasma from 15 min. to 1 min. 30 sec.; activation required 4 min. 35 sec. After heating until the coagulation time increased from 1 min. 30 sec. to 7 min. the activation required 37 min.

The degree of activation is somewhat variable but there is not sufficient decrease to permit the conclusion that it is the thermolabile constituent of the extract that is responsible for the extent of activation. The data point to this constituent as determining the time at which activation occurs while the relatively heat stable constituent determines the extent.

The preliminary studies suggest that the thermolabile factor involved is the "tissue fibrinogen" of Mills. It probably does not directly activate prothrombin but brings about clotting of the activating plasma freeing the prothrombin.

When tested on a recalcified citrate plasma the increase in clotting power is found to take place during the time the fibrin is forming. Testing on a citrate plasma without recalcifying one finds that

[2] King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1112.

[3] King, Joseph T., in press.

[4] King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 868.

[5] Mills, C. A., and Matthews, Stewart, *Am. J. Physiol.*, 1922, **60**, 193.

the ability to cause clotting appears at this same time. The control 0.05 cc. of either heated or fresh unactivated extract never causes clotting.

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Influence of Oxygen on Survival of Tissue Suspensions.

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Living cells are necessary for the growth of filterable virus. In certain instances cultures have been incubated with free access to oxygen. Li and Rivers[1] point out that a gradual simplification of the medium has taken place recently.[2, 3, 4] They also emphasize the advantages of chick embryo tissue in saline over the more complex media employed earlier. Flexner and Noguchi[5] and Long, Olitsky and Rhodes[6] on the virus of poliomyelitis are typical of work done on cultures under oil. Dochez, Mills and Kneeland[7] and Powell and Clowes[8] used chick embryo tissue under vaseline seal.

In this type of culture apparently intact cells are seen after incubation. Muckenfuss and Rivers[9] and Muckenfuss[10] found that the addition of dead cells to the virus improved survival. Eagles and McClean[11] emphasized the unsatisfactory state of our knowledge regarding the rôle of tissue which had access to oxygen.

We attempted to define more accurately the condition of the cells supposed to be living in such preparations. Saline suspensions of chick embryo were studied since they are coming into general use.

Ten-day chick embryos were shaken with freshly broken pyrex

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1 Li, C. P., and Rivers, *J. Exp. Med.*, 1930, **52**, 465.

2 Carrel, A., and Rivers, T. M., *Compt. rend. Soc. Biol.*, 1927, **96**, 848.

3 Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

4 Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

5 Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, **18**, 461.

6 Long, P. H., Olitsky, P. K., and Rhodes, C. P., *J. Exp. Med.*, 1930, **52**, 361.

7 Dochez, A. R., Mills, K. C., and Kneeland, Y., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

8 Powell, H. M., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 332.

9 Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

10 Muckenfuss, R. S., *J. Exp. Med.*, 1931, **53**, 377.

11 Eagles, G. H., and McClean, *Brit. J. Exp. Path.*, 1929, **10**, 35.