taken to remove prothrombokinase. To prepare prothrombokinase, bovine euglobulin was heated at 51°C. for 16 minutes to remove fibrinogen, most of the prothrombin was then removed by two adsorptions with barium sulfate, and finally, after dilution with distilled water, the prothrombokinase was precipitated at pH 5.2–5.5.

When calcium chloride was added to a mixture of prothrombokinase and prothrombin, there was a latent period during which little, if any, thrombin was formed. This was followed by a period of accelerated thrombin formation. It was then shown that the latent period could be eliminated by preincubation of the prothrombokinase with calcium and, contrariwise, that this could not be accomplished by preincubation of prothrombin with calcium. This made it evident that the commonly observed latent period was concerned with the activation of prothrombokinase under the influence of calcium.

When thrombokinase so prepared was added to prothrombin, production of thrombin began not only without a latent period, but with maximal velocity, and then followed a die-away pattern. The quantity of thrombokinase added to a given amount of prothrombin determined the initial rate of activation. These findings are consistent with an enzymatic interpretation. The fact that thrombokinase can be measured by the initial rate of prothrombin activation suffices for present purposes.

To follow the activation of prothrombokinase satisfactorily, it was necessary to devise a three-stage procedure based on the following principles: (I) Calcium was added to prothrombokinase, and, at intervals, (II) samples were assayed for thrombokinase by adding them to tubes of prothrombin. After a two-minute incubation, in each case, (III) a sample was transferred from the prothrombin tube to a tube of oxalated fibrinogen.

The speed of coagulation was a measure of the amount of thrombin produced during the two-minute incubation, and thus of the initial rate of prothrombin activation. In this new three-stage procedure, it will be noted that the experimental steps correspond directly to the three stages of the theoretical formulation, and that each of the active factors, thrombokinase and thrombin, is estimated in terms of its defining property.

When the activation of prothrombokinase was plotted against time, the experimental points fell close to the theoretical curve for an autocatalytic reaction. Moreover, the acceleration effected by seeding with a small quantity of activated material was striking. When a thrombokinase preparation was heated at 60° C. for 10 minutes, there were drastic losses of both the power to activate prothrombin and the capacity to accelerate the activation of prothrombokinase. Pending further study and purification of prothrombokinase, the only conclusion drawn is that the activation of prothrombokinase involves an autocatalytic or chain reaction.

Further experiments showed that the concentration of calcium was important not only for the activation of prothrombokinase but also for the activation of prothrombin. These problems involving the role of calcium in blood coagulation and optimal calcium concentrations require further investigation.

As is well known, certain plasma derivatives which have been treated to develop their fibrinolytic capacity are also able to hasten the activation of prothrombin. However, Seegers and Loomis (*7*) obtained a fibrinolytic preparation which did not activate prothrombin. It can now be reported that active thrombokinase preparations failed to cause obvious fibrinolysis in 12 hours, although a control fibrinolysin preparation produced lysis in 5 minutes. Thus, thrombokinase and fibrinolysin (plasmin) appear to be distinct; however, further inquiry is desirable.

Prothrombokinase is not a new coagulation factor. Since the early work of Dale and Walpole several investigators have recognized its existence in plasma and have called it "prothrombokinase" or by a recognizably similar name. "Factor V" well could be included, although interpreted differently by Owren (*5*). In addition to this, prothrombokinase is undoubtedly a component of several diversely-named "blood-clotting factors" which have been independently disclosed in a variety of ways. The three-stage formulation has been implicit in the contributions of many workers and, in principle, is becoming more widely accepted (*3–6*, *8*). Now that a direct three-stage method of analysis is available, it should be possible to decide whether a coagulant preparation contains prothrombokinase or thrombokinase. If this is not the fact, it should be possible to determine whether it accelerates the activation of prothrombokinase or favorably conditions the activity of thrombokinase.

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Growth *in Vitro* of Immature *Hordeum* Embryos[1]

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Experiments on the cultivation of immature plant embryos *in vitro* have met with varying success, depending on the age of the embryos used as well as on the species chosen. Most workers have found that only embryos already differentiated to some degree are able to grow on artificial media. Furthermore, in the case of older embryos, it has been observed by La Rue (*1*), working with *Zea* and *Avena*, and by others, that the plants obtained from excised embryos are smaller and weaker than those from mature seeds. This phenomenon appears to be related to the fact that an embryo removed from the seed and placed on nutrient agar tends to germinate immediately to produce a miniature seedling, rather than to continue the characteristic development leading to a fully grown embryo. Thus, the problem of growing young embryos appears to involve two interrelated questions: (1) how may the growth of young embryos be promoted, and (2) how may one

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bring about a continuation of the embryonic type of growth? It is with the latter aspect that this paper is particularly concerned.

Barley (*Hordeum vulgare* var. *Chevron*) is used as the experimental material in most of the studies. Greenhouse-grown plants provide the seeds from which embryos are aseptically removed. The basic medium contains distilled water, 2 per cent sucrose, 0.8 per cent agar, and the mixture of minerals used by Randolph and Cox (*2*). The pH of this medium is about 5.6; all of the supplemented media are adjusted with KOH and HCl to approximately the same pH. The cultures are kept in the laboratory at room temperature.

Immature embryos (about 10–15 days after pollination), when placed on the basic medium, regularly germinate within 2 or 3 days, forming seedlings of a normal appearance except for being smaller and more spindling than those from mature seeds; the younger the excised embryo, the weaker is the seedling. The transformation from embryo to seedling also finds expression in the percentage of dry matter: an embryo growing in the seed consists of about 40 per cent dry matter, whereas during 5 days on the basic medium the dry matter content of the seedling drops to about 10 per cent.

TABLE 1

GROWTH OF BARLEY EMBRYOS ON MEDIA CONTAINING VARYING AMOUNTS OF CASEIN HYDROLYSATE

Treatment	No. of embryos	Avg. wet wt. (% of control)	Avg. dry wt. (% of control)	Avg. dry matter (% of wet wt.)
Untreated				
Removed from plant at start of experiment	6	4	17	35
Removed from plant after 5 days	12	9	39	39
Grown *in vitro* for 5 days				
Casein hydrolysate (%)				
0 (control)	11	100	100	10
⅛	10	141	147	10
¼	10	169	219	11
½	11	145	264	18
1	12	69	178	25
2	12*	21	95	36

* Four of this group died without appreciable growth.

The prolongation of embryonic growth (enlargement of the scutellum and continued development of the root and leaf primordia within their embryonic sheaths) may be brought about by addition to the basic medium of an "embryo factor" found, for instance, in casein hydrolysate. On a medium containing 1 per cent of vitamin-free acid hydrolysate of casein, germination is inhibited for several days in the case of embryos about 13–15 days old; the germination of 10- to 12-day embryos is delayed for longer and sometimes indefinitely. The embryos meanwhile undergo considerable enlargement so that sometimes their size exceeds that of normal, mature embryos. Lower concentrations of casein hydrolysate give correspondingly lessened effects. Table 1 shows the results of a typical experiment.

It can be seen from Table 1 that (1) all but the highest concentrations of casein hydrolysate increase both the wet and the dry weights relative to the control; (2) with the higher concentrations the dry weight increase is relatively greater than the wet weight increase, thus giving a steady increase in the percentage of dry matter; (3) in all cases the embryos grown *in vitro* weigh more (wet *and* dry) than the original embryos at the start; and (4) the artificially cultivated embryos are heavier than they would have been had they been left to grow on the plant for the same period.

Results of the same general nature may be obtained by using water extracts of dates and bananas, wheat gluten hydrolysate, lactalbumin hydrolysate, milk, and especially tomato juice. The tomato juice may be prepared by mincing fresh, ripe tomatoes (e.g. in a Waring Blendor), centrifuging and filtering the product, autoclaving the filtrate, and then refiltering to remove the precipitate which forms the first time the juice is autoclaved. Thereafter the juice is apparently unaffected by repeated heating. Attention is called to this source of embryo factor in particular because of its clear-cut effect not only on the 10- to 15-day embryos but also on those several days younger. Embryos 7–9 days old at excision show very irregular growth on the basic medium, varying from no observable change to weak germination, with occasional callus growth; however, when placed on a medium which has been made with one-third to two-thirds of the water replaced by tomato juice, these young embryos regularly enlarge in a fashion resembling normal embryo growth. A suggestion that this growth bears a physiological as well as morphological resemblance to normal growth is found in the fact that if the embryo is removed from the supplemented medium within about 2 weeks or less, germination soon occurs and the seedling formed is much stronger than one which has grown continuously on the basic medium. The growth rate after removal is so much greater than that of a control embryo that this latter is soon overtaken by the tomato-fed plant. In some cases, young embryos showing no growth on the basic medium may be induced by this method to form small seedlings.

Growth, particularly of the younger embryos (7–9 days), was also favored by the addition of sodium nucleate from yeast. The preparation which gave the best results was one from the Schwartz Laboratories, Inc., New York City. It was effective in concentrations from 2 per cent to at least as low as .25 per cent. It had no germination inhibiting effect, but produced stronger seedling growth. As with tomato juice, it supported the growth of embryos too young to survive on the basic medium. In one typical experiment in which length of shoot was used as a measure of growth, and the height of the control seedlings was taken as 100 per cent, the average value for 19 embryos grown on a medium supplemented with 1 per cent sodium nucleate was 210 per cent.

Little can yet be said regarding the nature of the ingredients which are active in promoting embryonic growth. One question for further investigation immediately presents itself: Is the embryo factor one substance causing an all-over morphogenetic effect, or is it, e.g. in the tomato juice, a combination of two factors, one of which inhibits germination, the other promoting growth of the young organism? In either case, the temporary maintenance of the embryonic type of growth by the addition of the substances discussed appears to be of importance in rearing strong plants from immature embryos.

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