tents of 13 eggs which were found to be infertile when candled on the 7th day. Twenty live embryos which were sacrificed and 17 chicks which were allowed to hatch were examined without evidence being found that the virus was present. The chicks remained normal during the 21 days they were held for observation, and their sera were negative when tested for hemagglutination-inhibition antibodies.

It is of interest that clinical evidence of pneumoencephalitis infection was not observed in the flock of four-day-old chicks until several days after the 6 birds from which the virus was isolated had been selected. Two additional lots of chicks from the same hatch delivered to other farms were reported to have remained free from infection.

The isolation of pneumoencephalitis virus from the yolk sac of four-day-old chicks, chick embryos, and infertile eggs further emphasizes the possibility that hatching eggs may be a medium of transmission of the disease from breeding hens to their offspring.

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Antibacterial Action of Inactivated Ergosterol in the Guinea Pig and Man

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The antibacterial action, *in vitro*, of ergosterol and related substances has been reported (*3*). Of these substances, vitamin D (activated ergosterol) has found clinical use in tuberculosis and has often caused toxic symptoms (*1*, *2*, *4*).

In guinea pigs we have been able to show that inactivated ergosterol, like vitamin D (activated ergosterol), suppressed tuberculosis; unlike vitamin D, it never caused any toxic symptoms.

Twenty-two guinea pigs infected with virulent tubercle bacilli were injected with 20 mg. of inactivated ergosterol in oil, or 76 mg. vitamin D (viosterol) in oil, and 11 untreated pigs were used as controls. Following the injection of vitamin D up to several days, a change in the appearance of the guinea pigs receiving this substance was noted: their hair stood up, and the animals shivered, had diarrhea, refused food, and lost weight. No such change occurred after inactivated ergosterol injection. The tubercles present on autopsy were fewer in pigs treated with vitamin D than in the others.

In a second trial, infected guinea pigs received a larger dose of inactivated ergosterol (100 mg.) and less vitamin D (15 mg. D_2). These doses were repeated for three consecutive weeks, with untreated pigs as controls. In those receiving vitamin D_2 the changes outlined above occurred after each injection. No such changes were noted after the much larger dose of inactivated ergosterol. On autopsy there was more suppression of the tuberculosis in the guinea pigs treated with the larger doses of inactivated ergosterol than in those receiving smaller doses of vitamin D.

In man, 300–500 mg. of inactivated ergosterol were injected intramuscularly weekly in cases of pulmonary tuberculosis. Four cases treated thus for 6 months showed more improvement than could be expected by bed rest alone; there was retrogression and absorption of the predominantly exudative and productive disease, with conversion of the sputum to negative for tubercle bacilli (except one case with a cavity over 4 cm. in diameter).

The intravenous injection of inactivated ergosterol did not result in an exacerbation or local reaction, as seen after intravenous vitamin D application.

There were never any ill effects after injection of inactivated ergosterol, regardless of the route of injection or the type of lesion treated.

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Prothrombokinase and the Three Stages of Blood Coagulation[1]

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For four decades a two-stage theory of blood coagulation has held sway. Reviews and textbooks have clung to it for lack of agreement among investigators of the problem concerning further detail. But now sufficient unanimity is emerging to justify reformulation of the basic theory.

When plasma is diluted with distilled water and the pH brought between pH 5.0 and 5.5, the resulting euglobulin precipitate contains the essential core of the blood-clotting system. A solution of this euglobulin preparation clots promptly following the addition of ionic calcium. Even in this somewhat simplified form, the coagulation process can be shown to involve three distinct reactions:

(1) Prothrombokinase	→ Thrombokinase	(in the presence of calcium)
(2) Prothrombin	→ Thrombin	(in the presence of thrombokinase and calcium)
(3) Fibrinogen	→ Fibrin	(in the presence of thrombin)

Evidence that blood plasma contains an inactive form of thrombokinase was available as early as 1916 (*2*); and Bordet long ago detected a preliminary reaction occurring before the development of thrombin (*1*). That these important concepts have gained ground so slowly has been due to difficulties both of interpretation and technique. Among the chief of these has been the lack of a satisfactory procedure for studying the activation of prothrombokinase. Progress toward this objective is included in the following preliminary report.

Bovine prothrombin was prepared by a method involving adsorption on magnesium hydroxide, particular care being

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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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