lated from each tube of broth. Special anaerobic culture methods were not attempted.

Results

The results are summarized in Table 1. Death of the embryos occurred in nearly all of the eggs which received stool suspension mixed with saline or with penicillin. These eggs usually were foul smelling, and the embryos often were in various stages of decomposition. Cultures from them were positive in every instance. In the control group inoculated with saline or penicillin, about two-thirds of the embryos survived and only 3 out of 40 had positive cultures, a contamination rate of about 7 per cent.

In contrast, half of the embryos survived in the eggs inoculated with stool suspension, streptomycin, and penicillin. Practically the same rate was obtained in the corresponding group of controls. About one-third survived when inoculated with stool suspension and streptomycin, a frequency below that of the control group. The number of positive cultures from all the eggs in these groups was about the same as the contamination rate in the controls.

Conclusions

By the described method, using streptomycin and penicillin in simultaneous inoculations of stool suspensions into chick embryos for the purpose of combating bacterial growth, this study suggests that the use of streptomycin and penicillin combined is of greatest value; streptomycin alone is probably inferior; and penicillin alone has no value.

References

1. Burnet, F. M. *Brit. J. exp. Path.*, 1940, **21**, 147.
2. Florman, A. L., Weiss, A. B., and Council, F. E. *Proc. Soc. exp. Biol. Med.*, 1946, **61**, 16.
3. Hirst, G. K. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 155.
4. Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A. *Science*, 1944, **100**, 103.
5. Parker, R. F., and Diefendorf, H. W. *Proc. Soc. exp. Biol. Med.*, 1944, **57**, 351.
6. Reimann, H. A., Hodges, J. H., and Price, A. H. *J. Amer. med. Ass.*, 1945, **127**, 1–5.
7. Reimann, H. A., Price, A. H., and Elias, W. F. *Arch. int. Med.*, 1945, **76**, 269–277.

Prothrombin and Fibrinolysin

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It has been stated a number of times that fibrinolysin, derived from plasma, can activate prothrombin to thrombin in the absence of calcium ion (*2, 3*). That conclusion has an important bearing on our understanding of the blood-clotting mechanism. Consequently, we were prompted to perform a series of experiments concerning the action of fibrinolysin on prothrombin. As a result of our work we believe that the fundamental conclusion is erroneous. When proper precautions are taken to *eliminate prothrombin and thrombin* from fibrinolysin preparations, the latter do not activate prothrombin, clot oxalated plasma, or clot purified fibrinogen solutions.

Quite unexpectedly, however, a new reaction has been discovered. Fibrinolysin *destroys* prothrombin. While the destruction proceeds it is possible to demonstrate, by commonly used analytical methods, that the purified prothrombin first becomes less easily activated with thromboplastin and later becomes completely refractive. We call this less reactive prothrombin *paraprothrombin,* to convey the idea that it is a modification of native plasma prothrombin. The reaction can be illustrated as follows:

$$\text{Prothrombin} \xrightarrow{\text{Fibrinolysin}} \text{Paraprothrombin} \longrightarrow \text{Inert protein}$$

Although the water-insoluble, saline-soluble, fibrinolysin preparation contains more than one protein, there is no reason to suspect that a factor other than the plasma-derived fibrinolytic principle is involved.

Fibrinolysin does *not* inactivate thrombin.

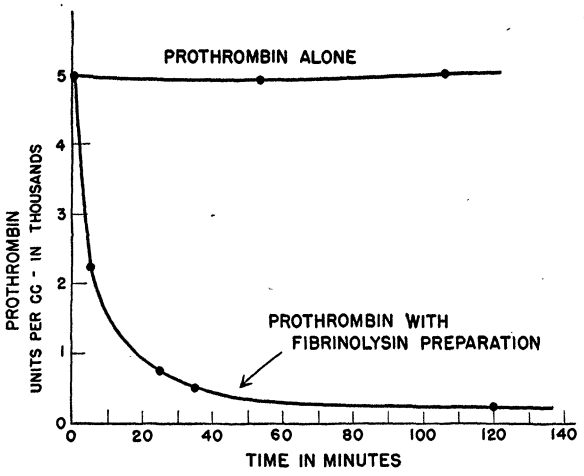

Fig. 1. The inactivation of purified prothrombin, at room temperature, with fibrinolysin.

Fig. 1 illustrates the rate of destruction of prothrombin by one of our potent lysin preparations. A 10,000-unit/ml. solution of purified prothrombin product No. 5 (*1*), dissolved in 0.9 per cent NaCl + 0.075 per cent $K_2C_2O_4 \cdot H_2O$, was mixed with an equal volume of fibrinolysin solution. The potency of the fibrinolysin solution was such that it would dissolve an equal volume of 0.3 per cent fibrin clot in less than 2 minutes. The disappearance of prothrombin was followed with the two-stage prothrombin titration technique (*4*).

The preparation of fibrinolysin and additional details will be described in another paper.

References

1. SEEGERS, W. H., LOOMIS, E. C., and VANDENBELT, J. M. *Arch. Biochem.*, 1945, **6**, 85.
2. TAGNON, H. J. *J. lab. clin. Med.*, 1942, **27**, 1119.
3. TAGNON, H. J. *Science*, 1942, **95**, 333.
4. WARNER, E. D., BRINKHOUS, K. M., and SMITH, H. P. *Amer. J. Physiol.*, 1936, **114**, 667.

Minimal Electroencephalographic Response to Metrazol as a Method for Measuring the Convulsive Threshold for Use in Human Beings

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Measurement of the convulsant threshold by direct means (induction of seizures) has been performed, chiefly on animals. The harshness of the procedure precludes or limits its use in human beings. If a method were available to measure this response indirectly in man, it might have many practical applications. It therefore occurred to one of us (E. Z.) that perhaps the first, or at least an early, minimal response to the injection of a convulsant drug, such as metrazol, indicated by the electroencephalograph might be an indirect measure of the convulsive threshold. Dr. Sjaardema assisted in the electroencephalographic recordings and interpretations at first, but later this was continued by Dr. Bercel.

The method consists of the usual electroencephalographic recording. The subject is then given an intravenous injection of metrazol of given concentration at a uniform rate, and the time at which the first recognizable change occurs in the EEG is recorded. In the rabbit we have used the first appearance of high-voltage slow waves, usually a 1- to 2-second run of waves of 4–5 cycles per second of increased amplitude. This repeats itself shortly and tends to become "established" as a recurring paroxysmal episode of increasing frequency. The injection may or may not be continued on to the point of a convulsion.

The purpose of this communication is chiefly to record the method. However, certain observations have already been noted.

In our experiments with rabbits the minimal EEG response was always obtained, and always preceded the convulsion.

When the convulsive threshold was altered in rabbits as a result of drugs, the minimal EEG response was altered in the same direction. In rabbits, phenobarbital retarded the appearance of the minimal EEG response and of the convulsion. The following is an example of such a test run:

Injection of 0.5% Solution of Metrazol (1 cc./min.)

	Minimal EEG Response	Convulsion
Before phenobarbital	0.6 cc.	1.4 cc.
2½ hr. after phenobarbital	1.2 cc.	5.4 cc.
Phenobarbital: 50 mg./kg. by stomach tube		

Dilantin did not have the anticipated effect of raising the convulsive threshold. At times a convulsion appeared more rapidly after the dilantin than before. However, the minimal EEG response also showed a lowered threshold. The following experiment is an example:

Injection of 1% Solution of Metrazol (1 cc./min.)

	Minimal EEG Response	Convulsion
Before dilantin	0.9 cc.	6.2 cc.
2 hr. after dilantin	0.5 cc.	2.3 cc.
Dilantin: 60 mg./kg. by stomach tube		

Diluting the metrazol gave a rise in the convulsive threshold and also a rise in the threshold for the minimal EEG response.

Rabbit #68	Minimal EEG Response	Convulsion
1.0% metrazol solution	3.1 cc.	5.5 cc.
0.5% " "	6.1 cc.	15.8 cc.
0.25% " "	12.7 cc.	31.3 cc.

In a few patients who were tested the threshold for the minimal EEG response was raised by the administration of phenobarbital and dilantin. The following is an example:

Injection of 10% Solution of Metrazol (1 cc./min.)

Patient With Epilepsy	Minimal EEG Response
Without medication 11 hr.	0.4 cc.
Dilantin gr. iss. 5 and 2 hr. before	1.1 cc.

It may be concluded, therefore, that metrazol and possibly other convulsant drugs and other convulsive agents produce a minimal response on the EEG before the occurrence of the seizure. The data reported tend to support the thesis that this minimal EEG response fluctuates in the same direction as the convulsive threshold under different conditions of excitation.

[1] The authors are indebted to Bilhuber-Knoll Company for contributing the metrazol used in this study.