calories. After stopping the extract, the metabolic rate in 4 days fell to 470 calories and 5 days later it was found to have the same value. This represents a decrease of 39% from the original level. There was then a slow, gradual rise of the rate to a new level of about 740 calories, which was attained within 6 weeks. Although the response of this rat was greatest in magnitude, it was otherwise typical of the responses in the other rats.

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Differentiation of the in Vivo and in Vitro Actions of Tissue Extracts.

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In our earlier work[1, 2] on tissue extracts, we found them active in promoting clotting of the blood *in vitro* or when intravenously injected, and also when administered to animals or man subcutaneously, intraperitoneally or orally during fasting conditions. During those studies we were dealing with fresh extracts or freshly purified fractions. More recently we have noticed that certain batches of tissue fibrinogen, after weeks of storage, lose entirely their effectiveness on intraperitoneal injections into animals, although they retain their full activity *in vitro*. Other batches made apparently in the same manner, are active with all forms of administration.

This raises the question as to whether we are dealing with 2 different actions of tissue fibrinogen, one of which is destroyed on standing, or whether 2 separate coagulants are present, one acting only when added directly to blood, and the other capable of accelerating clotting after absorption into the blood through the intestine or peritoneum. We offer evidence here that there are 2 distinct substances of this nature existing in crude tissue extracts or fresh preparations of tissue fibrinogen.

Healthy rabbits of about 4 pounds body weight, and fasted for 24 hours, were used for the injection tests. Two rabbits were used for the testing of each sample and for control in each series. In

[1] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 135, 167.

[2] Mills, C. A., Dorst, S. E., Mynchenberg, G., and Nakayama, J., *Am. J. Physiol.*, 1923, **63**, 484.

cases of any considerable disagreement in the results for a given test, a second 2 rabbits were used in similar fashion. Blood was carefully withdrawn from the heart in oiled syringes just prior to the injections and again one hour afterward. One cc. portions of these blood samples were allowed to clot in clean Pyrex-Corning test tubes (1 cm. x 10 cm.) in a water bath at 40° C. Two such tubes were prepared at each bleeding, and thus checks were obtained on the reading of the clotting time. The rabbits were first accustomed to handling so that the heart puncture could be made without struggling, as it was found that even a few seconds of vigorous struggling or effort on the part of the rabbit greatly shortened the clotting time. The ingestion of food, or infection, had a similar effect.

In vitro tests for tissue fibrinogen activity were carried out in the usual way in citrated horse plasma.[3] The tissue fibrinogen consisted of the purified globulin fraction of fresh calf lung extracts.

Effects of exposure to ultra violet radiations were first studied, with a view to a possible use of this as a means of sterilizing the coagulant. Much to our surprise we found that even a few min-

TABLE I.
Effect of Ultra Violet Light on Tissue Fibrinogen.

Citrated plasma	Tissue fibrinogen	Irradiated for	$CaCl_2$ 1%	Clotting time
0.5 cc.			0.15 cc.	2 min. 40 sec.
" "	0.1 cc. in saline	0 min.	" "	30 "
" "	" " " "	5 "	" "	31 "
" "	" " " "	15 "	" "	30 "
" "	" " " "	30 "	" "	34 "
0.5 cc.	0.1 cc. in phosphate buffer	0 min.	0.15 cc.	34 sec.
" "	" " " " "	5 "	" "	36 "
" "	" " " " "	10 "	" "	36 "
" "	" " " " "	15 "	" "	32 "
" "	" " " " "	30 "	" "	38 "

Tested by Rabbit Injection

Tissue fibrinogen	Irradiated for	Reduction Clotting time 1 hour after injection
2 cc. in saline	0 min.	50%
	5 "	58%
	10 "	28%
	30 "	0%
2 cc. in phosphate buffer	0 min.	50%
	5 "	25%
	15 "	0%
	30 "	0%

[3] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 135.

utes exposure, at 15 inches distance, completely inactivated the solution so far as its effects after absorption into an animal were concerned. Ten cc. portions of the tissue fibrinogen (1.5% strength) in 0.9% NaCl solution, and in phosphate buffer at pH 7.1, were exposed in open sterile petri dishes at a distance of 15 inches from a mercury quartz tube for 5 to 30 minutes. These various portions were then tested for their coagulative activity *in vitro* and *in vivo,* with the results shown in Table I.

It is evident from the data in Table I that the irradiation up to 30 minutes does not produce any significant change in the *in vitro* activity of the tissue fibrinogen either in saline solution or in the phosphate buffer. However, the *in vivo* effectiveness is destroyed by the light within 30 minutes in the saline medium, and within 15 minutes in the phosphate buffer. These results would indicate beyond doubt that we are dealing either with 2 different substances, or with 2 separate functions of one compound.

Treatment of the tissue fibrinogen with various adsorbents gave further interesting information. Adsorbents used were Fuller's earth, Norite, and Lloyds' reagent. They were shaken with the tissue fibrinogen in saline solution and then centrifuged. The supernatant liquid was tested by injection into rabbits with the following results:

Fuller's earth,	fluid	opalescent,	29%	reduction in	clotting	time.	
Norite,	"	"	28%	"	"	"	"
Lloyd's reagent,							
(a) small amount,	"	"	59%	"	"	"	"
(b) in excess,	"	clear,	40%	"	"	"	"
Original tissue fibrinogen,	"	opalescent,	37%	"	"	"	"

With Lloyds' reagent we succeeded in ridding the tissue fibrinogen of all its suspended material and opalescence, leaving it watery clear, but with its effectiveness *in vivo* unimpaired. Treatment with a small amount of this reagent always served to increase the *in vivo* activity of the tissue fibrinogen, but there was always present some opalescence, although not nearly so much as in the original solution. These same solutions, tested for their *in vitro* activity, gave the following results:

		$CaCl_2$ 1%	Clotting Time
0.5 cc. plasma,	0.5 cc original tissue fibrinogen	0.15 cc	0′ 15″
" " "	" "	"	4′ 45″
" " "	" " supernatant liquid (Fuller's Earth)	"	0′ 50″
" " "	" " " " (Norite)	"	0′ 28″
" " "	" " " " (Lloyd's Reagent)		
	(a) (small amount)	"	1′ 25″
	(b) (in excess)	"	4′ 10″

With Lloyds' reagent we accomplished just the reverse of the ultraviolet light effect, that is, the factor effective *in vitro* is removed, while that effective *in vivo* is left unharmed, perhaps even increased in potency.

Boiling the tissue fibrinogen solution vigorously for a few minutes, and filtering, yielded a clear filtrate which was without effect on blood clotting *in vitro;* but which gave a marked inhibition of clotting when injected intraperitoneally.

We feel justified in concluding that tissue fibrinogen, as we previously prepared it, contains 2 substances, one of which is associated with the turbidity of the solution and acts as a strong blood coagulant only when added directly to the blood. The other can be obtained in a crystal clear solution, is without coagulative activity *in vitro* but acts well in the body. This latter substance is readily inactivated by ultraviolet light, while the former is resistant to such effects.

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Delayed Reproduction in Amblystoma Punctatum.

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Nine specimens of adult *Amblystoma punctatum,* captured in pools in the Pocono Mountains, near the village of Effort, Pennsylvania, during the breeding season (the first week of April) of 1929, were kept in the laboratory of the Morris Biological Farm of the Wistar Institute until February 14, 1930, when they were placed in a mechanical refrigerator. On May 28 two females and one male were found frozen to death. The others of the lot, one female and 5 males, were placed in an aquarium. On the morning of May 29 numerous spermathecae were found deposited on sphagnum in the aquarium, and during the night of May 29-30 two clutches of eggs were deposited. On the morning of May 31 another clutch, very small, was found, but it was probably deposited on the first night, for the eggs did not appear to differ from the others in degree of development.

The eggs were examined critically on June 7. There proved to be 125 in all. Seventy-six of these were developing normally in various neural groove and early tube stages (Harrison's stages 15 to 19);