liver glycogen in overdigitalized animals, the per cent of glycogen decreasing somewhat as the animals approached death. These results seem to indicate that increased glycogenesis occurred in the liver of rats given non-toxic doses of digitoxin, while toxic doses of the drug resulted in a decrease in the amount of liver glycogen in rats in which symptoms of over-digitalization are manifested.

Summary. There was no marked difference in the distribution of the acid-soluble phosphorylated intermediates of glycolysis in the heart muscle of normal and digitoxin-poisoned rats. Small differences in the glycogen values of heart values were noted, but there was an increased amount of liver glycogen in the digitalized animals as compared with the controls as indicated by an average of 2.39% of glycogen in the former and 1.85% in the liver of the latter. Neither was there any difference in the rate of breakdown of glycogen in the excised liver tissue in normal and digitoxin treated rats. Increased glycogenesis occurred in the liver of rats given non-toxic doses of digitoxin while toxic doses of the drug resulted in a decrease in the amount of liver glycogen in rats in which symptoms of over-digitalization were manifested.

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Effect of Fibrinolysin upon Oxytocin, Vasopressin and Hypertensinogen.

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Plasma fibrinolysin has been considered a tryptase. Its proteolytic effect may be observed not only in the dissolution of fibrin but also in the digestion of fibrinogen, gelatin and casein, which is shown by the production of non-protein nitrogen when it acts upon these substrates (Tagnon *et al.*;[1] Kaplan *et al.*;[2] Christiensen,[3] Ferguson,[4] Shinowara,[5]). Nevertheless fibrinolysin does not inactivate hypertensin (Croxatto and Badia,[6]) a substrate which is attacked by all proteolytic enzymes tested up to now, including crystallized pancreatic trypsin (Croxatto[7]).

As fibrinolysin may appear in the bloodstream in its active form, an experimental study was carried out by observing its effect upon the hormones of the neurohypophysis-oxytocin and vasopressin- and upon hypertensinogen, in a similar way to that used before for trypsin. This also has the advantage of permitting a more detailed comparison between the effects of fibrinolysin and pancreatic trypsin. It has already been established that the latter is capable of inactivating hypertensin and vasopressin but not oxytocin (Croxatto[7]).

Hypertensinogen is attacked by trypsin but without the formation of hypertensin. Moreover after this enzyme has been used the substrate is incapable of producing hypertensin even when renin is added.

The results published in this paper show that fibrinolysin, just as trypsin, destroys the pressor effect of vasopressin but does not, under the same conditions, alter the effect of

1 Tagnon, H. J., Davidson, C. S., and Taylor, H. L., *J. Clin. Invest.*, 1942, **21**, 525.

2 Kaplan, M. H., Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., *J. Clin. Invest.*, 1942, **21**, 533.

3 Christiensen, R. L., *J. Gen. Physiol.*, 1945, **28**, 363.

4 Ferguson, J. H., *Am. J. Med.*, 1947, **3**, 67.

5 Shinowara, J. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 456.

6 Croxatto, H., Badía, W., and Croxatto, R., *Bol. Soc. Biol.*, Santiago, 1948, **5**, 82.

7 Croxatto, H., *Revista de Medicina Aliment.*, 1943, **5**, 259.

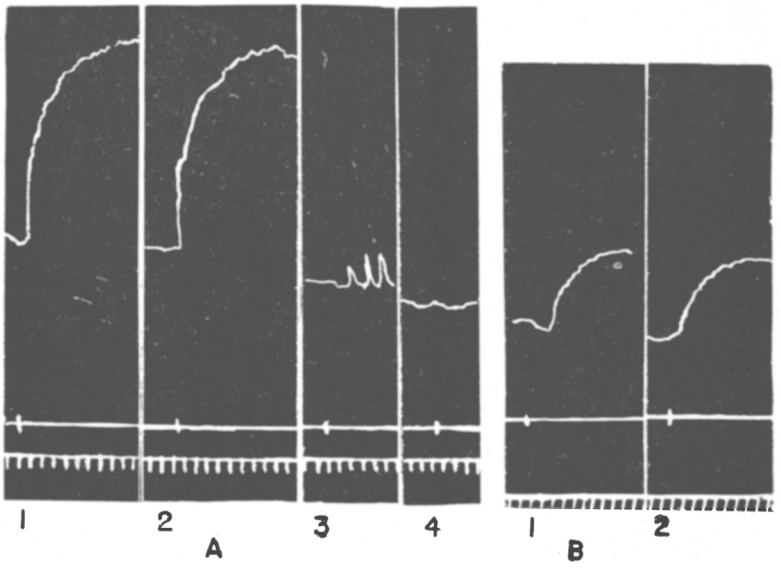

FIG. 1.

Contraction of the isolated guinea pig uterus. A, 1, 0.005 of 1.5 U Oxytocin; 2, 0.005 of 1.5 U Oxytocin + 5 mg fibrinolysin; 3, 0.005 of 1.5 U Oxytocin + 0.5 cc serum from patient in 9th month of pregnancy + 5 mg fibrinolysin; 4, 0.005 of 1.5 U Oxytocin + 0.5 cc pregnant woman serum. B, 1, 0.005 of 1.5 U Oxytocin + 20 mg fibrinolysin + 5 mg glutathione; 2, 0.005 of 1.5 U Oxytocin + 20 mg fibrinolysin. In A the incubation period lasted 24 hours, and 30 hours in B.

oxytocin. However hypertensinogen is not attacked by this enzyme, and after the addition of fibrinolysin it is still capable of producing hypertensin when renin is added.

Material and method. Three different samples of fibrinolysin were used, one prepared by us from ox plasma, activated with chloroform by the Loomis technic,[8] and two others obtained by fractionation of human plasma prepared by the Physical Chemical Department of Harvard Medical School.

The sample with which we did most of the experiments, (Squibb III-3-1) contained approximately 0.5 U per mg, according to Loomis. The substrates were: a) purified extract of neurohypophysis which contained 10 U.v. of oxytocin and 10 U of vasopressin per cc and b) human and horse hypertensinogen. In order to study the oxytocin fibrinolysin reaction a guinea pig uterus suspended in oxygenated Tyrode's solution was used. The blood pressure of the cat was employed in order to determine the inactivation of vasopressin by the enzyme. This same test was used to titrate the amount of hypertensin formed by the interaction of hypertensinogen with the enzymes:

Effect of fibrinolysin upon oxytocin. Different amounts of fibrinolysin: 2, 5 and 10 mg in 0.9% NaCl were made to act *in vitro* upon 2 U.v. of oxytocin; the solution was adjusted to pH 7.3 by means of a solution of sodium phosphate (0.2 normal). The total volume was brought up to 2.5 cc. The control contained 2 U.v. of oxytocin without the enzyme. The tubes were incubated at 37°C from 4 to 72 hours. When the incubation time exceeded 4 hours a drop of sodium merthiolate (Lilly) was added to each tube, as a bactericidal agent. As seen in Fig. 1 the oxytocic activity remains unchanged even after an incubation of 72 hours in the presence of 10 U of fibrinolysin, therefore it is quite clear that oxytocin is not hydrolyzed by fibrinolysin.

In experiments with pregnant woman serum it was proved fibrinolysin does not increase the destruction of oxytocin by the

[8] Loomis, C. E., George, C. H., and Ryder, A., *Arch. Bioch.*, **1947, 1, 12.**

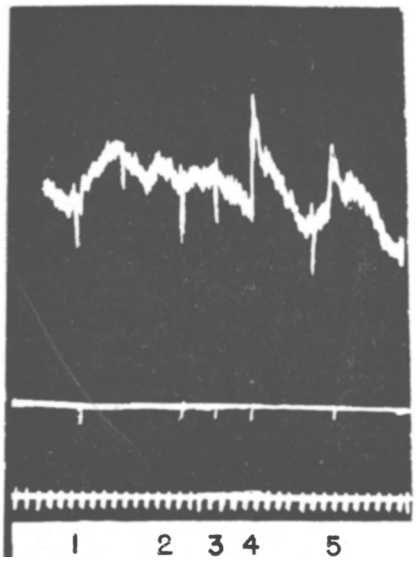

FIG. 2.
Arterial blood pressure of the cat. Injections into the femoral vein. 1, 0.3 of 1.5 U vasopressin + 0.1 mg trypsin; 2, 0.3 of 1.5 U vasopressin + 20 mg fibrinolyhin + 0.1 mg trypsin; 3, 0.3 of 1.5 U vasopressin + 20 mg fibrinolysin; 4, the same as in 3, but the different components were incubated separately. In all cases the incubation period lasted 24 hours at pH 7.3.

serum oxytocinase (Fig. 1).

Effect of fibrinolysin upon vasopressin. The incubation of vasopressin under similar conditions to those described above shows that fibrinolysin produces an appreciable inactivation of the pressor hormone. The titration tests were done by means of the blood pressure of the cat, anesthetized by dial. The solution was injected intravenously; in a few cases it was boiled previously. In order to discover whether fibrinolysin itself could have any influence upon the sensitivity of the cat to the pressor hormone, a mixture of fibrinolysin and vasopressin, incubated separately, was injected. Great care was taken to inject the same doses of substrate and enzyme as those which had been incubated.

As a general rule, each animal received only a few injections due to the tachyphylaxis produced by this hormone. In any case, by using a sufficiently large number of animals (3 or 4) it was clearly established that after 8 to 10 hours of incubation 10 to 20% of the vasopressin is inactivated when 10 U of fibrinolysin are used with 2 U of the pressor hormone. After 24 hours 40% is destroyed, and after 72 hours 80 to 100%. If crystallized trypsin is used in a dose equivalent to the fibrinolytic effect, it will be seen that 0.08 mg of this enzyme (containing 37% $MgSO_4$) which equals one fibrinolytic unit, destroys 0.8 U of vasopressin after 24 hours of incubation, *i. e.* the effect of 10 U of fibrinolysin, (Fig. 2).

The addition of 4 mg cysteine to 10 mg fibrinolysin, does not alter the effect of this enzyme upon vasopressin or oxytocin. This distinguishes it from blood plasma which shows a notable increase of its vasopressinase and oxytocinase effect when cysteine or glutathione are added (Croxatto, Reyes,[9]).

Effect upon hypertensinogen. Human and ox hypertensinogen were prepared as described, by precipitation with $(NH_4)_2SO_4$ at 0.5 saturation of the plasma, dialyzed with cold tap water and then filtered. The pH was brought to 7.4 by means of NaOH using a potentiometer. One hundred cc of this preparation incubated 15 minutes with 2 cc of the standard solution of pig renin gave 18 to 25 U of hypertensin. When hypertensinogen was treated with fibrinolysin for periods varying from 10 minutes to 12 hours, no pressor substance was produced. In order to investigate the possible formation of hypertensin, the mixtures of 50 cc of hypertensinogen with 1, 5, 10, and 20 mg of fibrinolysin were treated by the usual procedure for extracting hypertensin (Braun-Menéndez *et al.*[10]). The final extract was injected intravenously; the dose was calculated to be the equivalent of 10 to 20 cc of hypertensinogen. The results showed that the extract of hypertensinogen and fibrinolysin obtained produced exactly the same effect as the extract prepared from hypertensinogen alone, *i.e.* without hypertensin. In view of a previous paper (Croxatto and Croxatto,[11]) which demon-

[9] Croxatto, H., and Reyes, M., *Bol. Soc. Biol.*, Santiago, 1948, **5**, 80.

[10] Braun-Menéndez, E., Fasciolo, J. C., Leloir, L. F., and Muñoz, J. M., *J. Physiol.*, 1940, **133**, 731.

[11] Croxatto, H., and Croxatto, R., *Rev. argent. de Biol.*, 1948, in press.

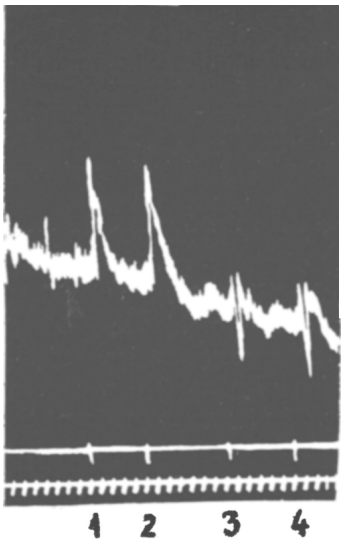

FIG. 3.
Arterial blood pressure of the cat. 1, 1/5 of 40 cc hypertensinogen at pH 6.8 + 3 cc renin, incubated during 10 minutes; 2, 1/5 of 40 cc hypertensinogen at pH 6.8 + 10 mg fibrinolysin + 3 cc renin, incubated for 10 minutes; 3, 1/5 of 40 cc hypertensinogen pH 6.8; 4, 1/5 of 40 cc hypertensinogen + 10 mg fibrinolysin incubated for 10 minutes.

strated that the addition of ether or acetone increased the production of hypertensin by favouring the renin-hypertensinogen reaction, it appeared necessary to add these solvents to the mixture of hypertensinogen and renin. However the results were no different from those obtained without the solvents.

It was also shown that if hypertensinogen is first treated with fibrinolysin and afterwards incubated with 2 cc renin for 15 minutes, the production of hypertensin is the same as when fibrinolysin is not used. Therefore this enzyme, unlike trypsin does not modify the capacity of hypertensinogen to serve as a substrate for renin (Fig. 3).

Discussion. The effect of fibrinolysin upon the hormones of the neurohypophysis is qualitatively, comparable to that of crystallized pancreatic trypsin, however it differs from the latter by its inability to alter the hypertensinogen substrate. This last result discards the possibility that fibrinolysin could have an effect comparable to renin. The proteolytic activity of fibrinolysin is limited to only certain substrates, and it does not appear to strengthen the activity of other plasma enzymes also thought to be proteolytic, such as renin, oxytocinase, hypertensinase, etc.

Even though fibrinolysin destroys vasopressin it does not have such a high vasopressinase activity as plasma during normal pregnancy. However it is possible that the increase of fibrinolytic activity observed in the plasma of pregnant women complicated by hypertensive toxemia (Willson and Munnell,[12]) may be related to a defense mechanism brought about by the capacity of this enzyme to inactive vasopressin.

It could be presumed that this effect upon the pressor hormone of the hypophysis is not due to fibrinolysin itself but to some other accompanying enzyme as yet unidentified. It cannot be hypertensinase because the addition of cysteine or glutathion to fibrinolysin does not modify its activity upon vasopressin or oxytocin (Fig. 1).

Conclusions. 10 U of fibrinolysin obtained from human and ox plasma, added to 1 U of oxytocin, does not alter the uterotonic effect of this hormone even after 72 hours of incubation at 37°C at pH 7.3 On the other hand the same preparation of fibrinolysin, under similar conditions produces a progressive destruction of vasopressin. Fibrinolysin added to human or horse hypertensinogen does not produce hypertensin, neither does it incapacitate the hypertensinogen for producing hypertensin when renin is added immediately after.

We wish to thank the Rockefeller Foundation and Dr. Edwin Cohn for the aid they have given us in the work here reported.

12 Willson, J. H., and Munnell, E. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 277.