which has been shown by Neubauer[4] and confirmed by Fellows *et al.*,[5] to stimulate an increase in the excretion of glucuronic acid (conjugation?). However, glycerol itself and ethylene glycol did not produce such an increase when administered gastrically or hypodermically in rabbits.[5]

Preliminary observations would seem to indicate that myanesin is conjugated with glucuronic acid, at least in part. Neuberg and Schewket[6] have devised a method for the extraction and identification of conjugated glucuronic acid which they claim to be specific. By employing this method of extraction unequivocal reactions were obtained with naphthoresorcinol,[6,7] although inconsistent reactions were obtained with orcinol,[6,7] on urine excreted after the administration of myanesin, whereas similarly treated control urines gave only the weakly positive reactions expected of normal urine.

Summary. A colorimetric method has been presented for the determination of myanesin in plasma and in urine. The rapid decay curve of myanesin in dog plasma explains the brevity of its pharmacological action. In the dog, from 0.1 to 2.0% of the administered dose is excreted as free myanesin; from 32 to 42% of the administered dose is excreted as conjugated myanesin in 24 hours, possibly as the glucuronide.

4 Neubauer, O., *Arch. exp. Path. u. Pharmakol.*, 1901, **46**, 133.

5 Fellows, J. K., Luduena, F. P., and Hanslik, P. J., *J. Pharmacol. and Exp. Therap.*, 1947, **89**, 210.

6 Neuberg, C., and Schewket, O., *Biochem. Z.*, 1912, **44**, 502.

7 Hawk, P. B., and Bergheim, O., *Practical Physiological Chemistry*, Philadelphia, P. Blakiston's Son & Co., Inc., 11th edition, 1937, pp. 68, 658.

16057

The Null Effect of Hemorrhage on Intestinal Absorption of Chloride in the Presence of Sulfate.*

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Goldschmidt and Dayton[1] showed that sodium sulfate increases the absorption of sodium chloride in the large intestine of dogs. Burns and Visscher[2] later demonstrated that the presence of sodium sulfate and certain other anions of the lyotropic series caused the chloride ion, at a concentration below that in the blood plasma, to leave the small intestine and enter the blood against its diffusion gradient.

Van Liere, Northup, and Sleeth[3] noted that isotonic sodium chloride solution was absorbed significantly faster from the intestine of dogs which had sustained a severe hemorrhage (3.2% of their body weight). Since hemorrhage thus simulated the effect of the sulfate ion, it was of interest to study their combined effect.

Methods. Dogs, selected in pairs as nearly alike in weight and age as possible, were fasted 24 hours previous to the experiment. One served as a control and the other was subjected to hemorrhage. Fifteen experiments were performed on a total of 29 animals (one control died).

* Aided by a graant of the Ella Sachs Plotz Foundation.

1 Goldschmidt, S., and Dayton, A. B., *Am. J. Physiol.*, 1919, **48**, 459.

2 Burns, H. S., and Visscher, M. B., *Am. J. Physiol.*, 1934, **110**, 490.

3 Van Liere, E. J., Northup, D. W., and Sleeth, C. K., *Am. J. Physiol.*, 1938, **124**, 102.

TABLE I.
Effect of Hemorrhage on Intestinal Absorption of Chloride in the Presence of Sulfate.

	Control* %	Experimental† %	Difference %	"p"‡
Absorption of chloride	69	81	12	.16
" " sulfate	24	30	6	.15
" " fluid	34	44	10	.11
Final conc. of chloride	0.187	0.138	0.049	.15
" " " sulfate	1.17	1.22	0.05	.20

* 14 animals.
† 15 "
‡ Significant when "p" (according to Fisher) is 0.05 or less.

The experimental animals were etherized and blood equal to 3% of the body weight was withdrawn from the cannulated femoral artery. The artery was ligated and the incision closed. Four hours were allowed for recovery from the ether and adjustment of the circulatory mechanism. During this interim, water was allowed *ad libitum*. The control animals were subjected to the same procedure without, of course, being bled.

Under sodium barbital anesthesia (300 mg/kg intravenously) the small intestine was exposed and practically the entire ileum used for a loop. The same length of loop was used in the control and the bled animal. It was washed out with isotonic glucose solution and filled with a fluid composed of equal parts of isotonic sodium chloride and isotonic sodium sulfate solutions. The resulting mixture contained Na 0.647%; Cl 0.055%, and SO_4 1.278%.

Forty minutes later the fluid remaining in the intestine was removed and the amount carefully measured. An aliquot portion was digested with concentrated HCl and the amount of sulfate determined gravimetrically by precipitation with barium chloride. A second aliquot was used to determine the chloride as sodium chloride by the Van Slyke modification of the Volhard method.

Results and Discussion. The absorption of the chloride ion in the presence of the sulfate was not significantly increased by hemorrhage, although there was a trend in that direction. Since a hemi-isotonic sodium chloride solution was used, it was necessary for absorption to take place against a distinct gradient. Perhaps this explains why hemorrhage did not significantly accelerate the absorption of chloride as it had when isotonic solutions of sodium chloride were used.[3] As previously noted, Goldschmidt and Dayton[1] observed that chlorides were absorbed more rapidly from the colon when a sulfate was present. It has not been shown, to our knowledge, however, that this effect obtains in the small intestine.

Less absorption of chloride and fluid was noted in the control animals. As in our previous absorption studies a fair degree of parallelism was observed between the absorption of the chloride ion and fluid.

At the end of the 40-minute period both the control and experimental animals showed definite chloride impoverishment. This was more pronounced, on the average, in the latter group but, presumably because of the wide individual variation in the several animals, the difference was not statistically significant. In both groups the sulfates showed a definite increase in concentration, because more water than sulfate was absorbed. This increase was not unexpected in the control dogs. In our laboratory it has recently[4] also been shown that a pronounced hemorrhage does not significantly affect the absorption of magnesium sulfate from the small intestine.

Summary. The absorption of chloride, sulfate and fluid from a mixture of equal parts of isotonic sodium chloride and isotonic sodium sulfate solution was studied in dogs which had suffered a hemorrhage of 3% of their body weight.

The percentages of absorption in control

[4] Van Liere, E. J., Northup, D. W., Stickney, J. C., and Richard, R. E., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 62.

and experimental dogs were, respectively, 69 and 81 of the chloride; 24 and 30 of the sulfate; and 34 and 44 of the fluid.

In both control and experimental animals the sulfates showed a definite increase in concentration at the end of the experimental period, the chlorides a definite impoverishment. However, the differences between the control and the experimntal animals were not statistically significant.

16058

Influence of Iodinated Trypsin on Blood Coagulation.*

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It has been known for some time that the addition of trypsin accelerates the coagulation of normal blood[1-4] and the blood of hemophilic individuals.[5,6] Intravenously administered trypsin is, however, quite toxic as pointed out by Eagle and Harris.[4] The similarity between the symptomatology resulting from the injection of the enzyme and anaphylactic reactions was observed by Rocha e Silva[7] and these observations have been extended by Dragstedt and his associates.[8-12] Shock resulting from the injection of a relatively small amount of trypsin is indicated by a marked fall in blood pressure.

Tagnon[13] slowly injected trypsin intravenously in hemophilic patients and observed a significant shortening of the coagulation time; however, he has advised extreme caution since the dose most effective on clotting time is very near to that which is quite toxic.

In an earlier report[14] the author pointed out that the iodination of trypsin serves to decrease greatly its hypotensive effect while its proteolytic activity is decreased only about one tenth as much. Relatively large amounts of the iodinated enzyme do not cause the profound fall in blood pressure observed with the same amount of untreated trypsin even with rapid injection.

The present report deals with the influence of iodinated trypsin on the rate of blood coagulation *in vitro*.

Methods. Freshly drawn dog blood was mixed with 3.3 mg of sodium citrate per ml of blood. The plasma was separated after centrifuging for 2 hours at 3200 r.p.m. With short periods of centrifugation the clotting

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1 Douglas, S. R., and Colebrook, L., *Lancet*, 1916, **2**, 180.

2 Heard, W. N., *J. Physiol.*, 1916, **51**, 294.

3 Waldschmidt-Leitz, E., Stadler, P., and Steigerwaldt, F., *Naturwissenschaften*, 1928, **16**, 1027.

4 Eagle, H., and Harris, T. N., *J. Gen. Physiol.*, 1936-37, **20**, 543.

5 Tyson, T. L., and West, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 494.

6 Ferguson, J. H., and Erickson, B. N., *Am. J. Physiol.*, 1939, **126**, 661.

7 Rocha e Silva, M., *Arquiv. Inst. Biol.*, Sao Paulo, 1939, **10**, 93.

8 Ramirez de Arellano, M., Lawton, A. H., and Dragstedt, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 360.

9 Rocha e Silva, M., and Dragstedt, C. A., *J. Pharm. and Exp. Therap.*, 1941, **72**, 36.

10 Dragstedt, C. A., and Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 420.

11 Rocha e Silva, M., and Dragstedt, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 152.

12 Wells, J. A., Morris, H. C., and Dragstedt, C. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 209.

13 Tagnon, H. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 45.

14 Bowman, D. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 408.