16201

Attempts to Prevent Ergot Gangrene with Heparin and Dicumarol. Vascular Effects of Ergot by Fluorescein Technic.

L. H. ANDERSON AND J. A. WELLS.
From the Department of Pharmacology, Northwestern University Medical School, Chicago, Ill.

The suggestion that thrombosis, associated with ergot gangrene,[1-10] contributes to the vascular occlusion responsible for the resultant tissue necrosis,[3,7] prompts the hypothesis that prevention of thrombosis should modify the gangrene. This hypothesis is supported by the observation that heparin prevents necrosis resulting from experimental "frostbite," a condition with which thrombosis is also associated.[11-13]

The present study is concerned with the influence of heparin and dicumarol on the incidence of ergotamine induced gangrene in the rat.

Gangrene of the tail was produced in male and female albino rats (140-200 g) by the subcutaneous or intraperitoneal injection of a 1:1000 solution of ergotamine tartrate. Whenever a particular treatment was employed, litter mates served as controls. Preliminary studies revealed no influence of sex or route of administration of ergotamine on the incidence of gangrene.

Gangrene can be produced by a single injection of a suitable dose of ergotamine[6,14-17] and such a procedure was employed. When doses of 3.3, 12.5, 25.0, 37.5 and 50.0 mg/kg were injected it was observed that the incidence of gangrene with the smallest dose was very low, while with the largest dose a high mortality occurred. The incidences of actual necrosis in 31, 17 and 13 normal rats given 12.5, 25.0 and 37.5 mg/kg of ergotamine tartrate were 35.5, 43.3, and 15.4% respectively.

In the process of developing gangrene the appearance of the rats' tails passed progressively through several poorly defined stages. The earliest certain indication of impending necrosis was the appearance of a demarcating region of redness and tenderness, distal to which was an area of extreme pallor, with dark discoloration of the tip of the tail. This change was designated arbitrarily as the onset of gangrene, and on the basis of such criteria the time of onset of gangrene was determined in 115 rats (Fig. 1). A few animals began to develop gangrene within 24 hours of the injection of ergotamine. Approximately 50% of the animals which developed gangrene began to do so within 3 days and 100% within 7 days of the injection of ergotamine.

Rats given a single subcutaneous injection of 30 mg/kg of heparin in Pitkin's menstruum[18,19] showed prolongation of the blood

1 Kobert, R., *Arch. f. exp. Path. Pharmakol.*, 1883, **18**, 316.

2 Dale, H. H., *J. Physiol.*, 1906, **34**, 11.

3 Lewis, T., *Clin. Sci.*, 1935, **2**, 43.

4 Kaunitz, J., *Arch. Surg.*, 1932, **25**, 1135.

5 Telford, E. D., and Stopford, J. S. B., *Brit. J. Surg.*, 1931, **18**, 557.

6 McGrath, E. J., *Arch. Int. Med.*, 1935, **55**, 942.

7 Yater, W. M., and Cahill, J. A., *J. A. M. A.*, 1936, **106**, 1625.

8 Custer, R. P., *Am. J. Med. Sci.*, 1938, **195**, 452.

9 Rubin, M. J., and Rapoport, M., *Arch. Int. Med.*, 1937, **59**, 714.

10 Thomas, R. M., *Yale J. Biol. and Med.*, 1940, **12**, 415.

11 Lange, K., and Boyd, L. J., *Surg. Gyn. and Obst.*, 1945, **80**, 346.

12 Lange, K., and Loewe, L., *Surg. Gyn. and Obst.*, 1946, **82**, 256.

13 Friedman, N. B., Lange, K., and Weiner, D., *Am. J. Med. Sci.*, 1947, **213**, 61.

14 Rothlin, E., *Arch. Internat. de Pharmacodyn. et de Therap.*, 1923, **27**, 459.

15 Suzman, M. M., Freed, C. C., and Prag, J. J., *South African J. Med. Sci.*, 1938, **3**, 29.

16 Cobet, R., Ratschow, M., and Steckner, M. L., *Klin. Wchnsch.*, 1939, **18**, 278.

17 Ratschow, M., and Klosterman, H. C., *Z. f. klinische Med.*, 1939, **135**, 198.

18 Loewe, L., and Rosenblatt, P., *Am. J. Med. Sci.*, 1944, **54**, 208.

19 Evans, J. A., and Boller, R. J., *J. A. M. A.*, 1946, **131**, 879.

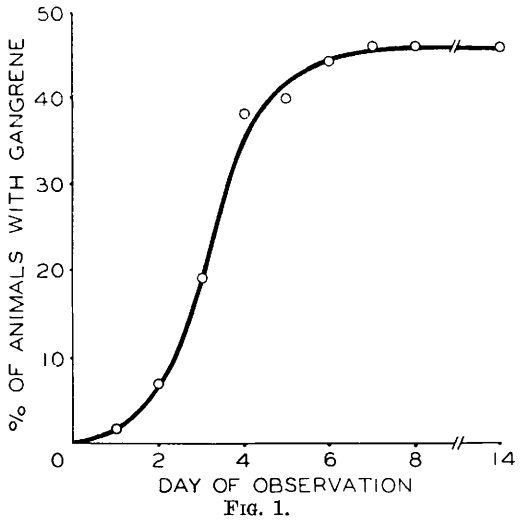

FIG. 1.

clotting time. This effect was maximum at 24 hours, and persisted for 36 hours. Clotting times were estimated by the capillary tube method on blood obtained from the tail. Following 3 daily doses of 30 mg of heparin in Pitkin's menstruum, the average clotting time exceeded 20 min. (normal = 1.04 ± 0.38 min.).

In view of the evidence that 50% of the control animals began to develop gangrene within 3 days of the injection of ergotamine, it was reasoned that therapy with anticoagulants need not be continued beyond this time. Thus, in the present series of animals, 5 daily doses of 30 mg/kg of heparin in Pitkin's menstruum were administered, beginning 2 days prior to the injection of ergotamine.

The incidences of actual necrosis in the series of 10, 8 and 5 heparin-treated rats given 12.5, 25.0 and 37.5 mg/kg of ergotamine tartrate were 30.0, 50.0 and 40.0% respectively, and all animals which developed gangrene did so within 6 days of the injection of ergotamine.

Rats given 2.5 mg of dicumarol by mouth show changes in prothrombin times which are maximum at 24 hours and last 36 hours. These animals will tolerate 10 mg of dicumarol daily for 3 days.[20]

[20] Overman, R. S., Stohman, M. A., Sullivan, W. R., Huebner, C. F., Campbell, H. A., and Link, K. P., *J. Biol. Chem.*, 1942, **142**, 941.

Preliminary to the present study a series of rats were given 5 mg of dicumarol daily for 10 doses. Clotting times determined during this course of therapy were never above 8 min. However, bleeding from the tip of the tail after collection of blood for clotting determinations was greatly prolonged and 2 of 7 animals bled to death from this minor wound. Some animals developed spontaneous hemorrhages which stopped when dicumarol was discontinued and some showed severe anemia associated with tarry stools. The daily administration of 5 mg of dicumarol thus appeared to represent an effective and near maximum tolerated dose in the rat. In the present series 5 such daily doses were administered, beginning 2 days prior to the injection of ergotamine.

The incidences of actual necrosis in the series of 13, 16 and 6 dicumarol-treated rats given 12.5, 25.0 and 37.5 mg/kg of ergotamine tartrate were 15.4, 31.3 and 100% respectively, and all animals which developed gangrene did so within 6 days of the injection of ergotamine.

Statistical analysis of the pooled data from the control, heparin and dicumarol experiments for the incidence of gangrene, as a function of the dose of ergotamine, revealed that no significant difference in the incidence of gangrene was attributable to the dose of ergotamine administered. Therefore, in evaluating the incidence of gangrene as a function of the treatment, the animals in the 3 dosage groups under any particular regime were pooled to give a figure representing the incidence for the group (Table I).

The incidences of gangrene in the control, heparin treated and dicumarol treated groups were 35.1, 39.1 and 37.1% respectively. Statistical analysis revealed no significant difference between these groups.

There would appear to be two possible explanations for the failure of heparin or dicumarol to reduce the incidence of ergotamine induced gangrene in the rat. They are (1) thrombosis does not contribute to the development of gangrene or (2) the anticoagulants employed were ineffective in preventing thrombosis.

Microscopic examination of the tails of

TABLE I.
Influence of Anticoagulants on Incidence of Ergotamine-Induced Gangrene in Rats.

Procedure	Total	Mortality No.	Mortality %	Survivors	Gangrene No.	Gangrene %
Control	82	8	9.8	74	26	35.1
Heparin	28	5	17.9	23	9	39.1
Dicumarol	38	3	7.9	35	13	37.1
X^2*		1.19			0.012	
p†		>0.50			>0.99	
Significance		None			None	

* Corrected Chi Square.
† Probability.

TABLE II.
Usefulness of Fluorescin Test in Predicting the Development of Ergot Gangrene in Rats.

	Ultimate incidence of gangrene					
	Group I (Tested 6 hr after ergotamine injection)			Group II (Tested 24 hr after ergotamine injection)		
		Gangrene			Gangrene	
Results of test*	Total	No.	%	Total	No.	%
Fluorescence	11	4	34.9	18	5	27.8
No fluorescence	66	29	44.0	19	15	79.0
X^2†		0.019			7.79	
p‡		0.90			<0.01	
Significance		None			High	
Ass'n Coef.§		—			0.417	

* Presence or absence of fluorescence of tail 1 hr after injection of 1 cc 10% fluorescein.
† Corrected Chi Square.
‡ Probability.

$$\S = \sqrt{\frac{X^2}{n + X^2}}$$

2 rats, in which gangrene was developing in spite of therapy with dicumarol, revealed that the vessels were largely free of blood and there was no evidence of thrombosis. Similar studies on 2 control animals, developing gangrene, revealed accumulated masses of red cells containing small amounts of fibrin in the large arteries, but no actual thrombosis was observed. It is thus concluded that thrombosis is incidental, rather than contributory, to the vascular occlusion resulting in ergot gangrene and the severity of the vascular impairment is to be explained solely on the basis of the vasoconstriction produced by the ergot alkaloids.

In order to gain further information on the severity of the vascular impairment which can be produced by such vasoconstriction, one cc of 10% sodium fluorescein was injected intraperitoneally into 12 normal and 23 ergotamine-treated rats. Fluorescence appeared in the ears of normal rats 2.7 ± 0.9 min. and in the tails 9.5 ± 1.91 min. after the injection of the dye. In the animals receiving ergotamine (12.5 or 25.0 mg/kg) the time required for the appearance of fluorescence in the ear was 9.32 ± 4.06 min. while in the tail 244 ± 182 min. was required. It is thus concluded that the circulation to the tail of rats receiving ergotamine is not only greatly, but preferentially impaired.

This preferential impairment of the circulation to the tail might be due either to the degree or the extent of the vasoconstriction occurring in this structure. In a series of ergotamine treated animals (37.5 mg/kg) no

bleeding occurred when the tail was amputated within 1-2 cm of the base. Normal animals, so amputated, showed vigorous hemorrhage and even spurting of blood. The failure of the major arteries of the tail to bleed when transected implies practically complete closure of these vessels by ergotamine.

The time required for fluorescence to appear in the tail of rats receiving ergotamine is not to be construed as a measure of the duration of the action of ergotamine on this vascular bed. A highly diffusable substance, such as fluorescein, could slowly accumulate to a sufficient concentration to give fluorescence of the tissues, even in the presence of severe vasoconstriction. A different type of test was devised to give information as to the duration of the vascular impairment produced by ergotamine.

Seventy-seven rats were given fluorescein within 6 hours after having received ergotamine and another group of 37 rats were given the dye 24 hours after ergotamine. It was arbitrarily decided that all animals in which the time required for fluorescence to appear in the tail was longer than one hour should be classed as having a seriously impaired circulation. In the group tested within 6 hours after the administration of ergotamine 66 or 85.7% of the animals had seriously impaired circulation to the tail. In the group tested 24 hours after the administration of ergotamine 19 or 51.1% had seriously impaired circulation to the tail. Thus, in approximately half of the animals a single dose of 12.5-25.0 mg/kg of ergotamine can cause nearly complete vascular occlusion of the tail vessels, lasting for at least 24 hours.

It can be seen in Table II that a significantly greater incidence of gangrene developed in those animals which, by the fluorescein test, still showed seriously impaired circulation to the tail 24 hours after the administration of ergotamine, than in those which did not. The coefficient of association between the performance on the fluorescein test carried out at 24 hours and the incidence of gangrene is of such magnitude (0.417) as to imply that the test is of considerable value in estimating the prognosis.

It may be assumed that an agent, to be effective against such gangrene, should be able to bring about fluorescence in a tail in which it did not appear as a consequence of the action of ergotamine. Priscol, papaverine, magnesium sulfate, aminophylline, nicotinic acid, alcohol, ether, demerol, histamine, methacholine and histidine-ascorbic acid were shown to be incapable of producing such an effect, and the tails of animals so treated remained nonfluorescent.

Summary. Twenty-four hours after the administration of a single dose of ergotamine tartrate, approximately 50% of rats showed, by means of the fluorescein test, a seriously impaired circulation to the tail, due to marked constriction of the major arteries of the tail. Forty to 50% of rats receiving a single dose of 12.5-37.5 mg/kg of ergotamine tartrate may be expected to develop gangrene of the tail and to begin to do so within one week of the injection of ergotamine. Anticoagulants, such as heparin and dicumarol, do not alter the incidence of ergotamine induced gangrene. It is concluded that the thrombosis, observed to be associated with this process by others, is incidental rather than contributory to the vascular occlusion.