the Prunt descent seems to be out of trend. Nevertheless it seems permissible to combine the 2 series. The data of Table I are plotted in Fig. 1 and the slope of the combined data is also given (slope 5.5). This slope has a statistically significant difference from slope of "O".

Data for litters 1-7 were combined and compared to similar data for mice of the 8-15th litters. This comparison shows a "t" value of 2.3 which is statistically significant at the 5% level.

The new data thus permit a verification of the correlation reached in the earlier series. There appears to be a gradual loss of ability of a mouse to develop an invasive fibrosarcoma in the successive litters born to the same parents, when subjected to 1 mg of methylcholanthrene dissolved in 0.1 cc of sesame oil at 60 days of age. There is a complete loss of this ability in mice of the 8th and later litters of the Prunt descent, and in mice of the 10th and later litters of the 2Prunt stock. The present phenomenon of changes in invasiveness may also be interpreted by mice of successive litters acquiring an increased resistance to chemically induced fibrosarcomas.

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Action of Coumingine on Heart Muscle.* (19043)

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It is often stated that the erythrophleum alkaloids are the only alkaloids that have a digitalis-like action. Chemically these alkaloids have the cyclopentenophenanthrene ring system common to the digitalis glycosides, but do not contain the unsaturated lactone group said to be responsible for digitalis action. However, the usually accepted criteria of digitalis action, slowing of the heart, systolic arrest and emesis following parenteral administration, cannot be considered adequate, none being specific. Straub(1) long ago emphasized that "so-called systolic standstill" was not a specific digitalis action. More recently Pisanty(2) has studied the systolic arrest and skeletal muscle shortening caused by a variety of substances and the enzyme mechanisms involved. Observations to date(3,4) have indicated that the constant, and perhaps specific, measurable action of digitalis glycosides in experimental preparations and in therapeutic usage in man is to shorten the refractory period, or systole, of heart muscle without impairment of contractility. In their study of the erythrophleum alkaloids, Maling and Krayer(5) found the outstanding action was to decrease diastolic volume. Coumingine had a positive inotropic effect in lower concentrations, but a greater tendency to cause irregularity of rhythm than the other alkaloids of the group. They concluded that as yet there was no evidence to justify the assumption that in congestive heart failure the action of erythrophleum alkaloids was truly digitalis-like.

The present paper reports observations on the effect of coumingine on the primary properties of heart muscle of the turtle *Pseudemys*

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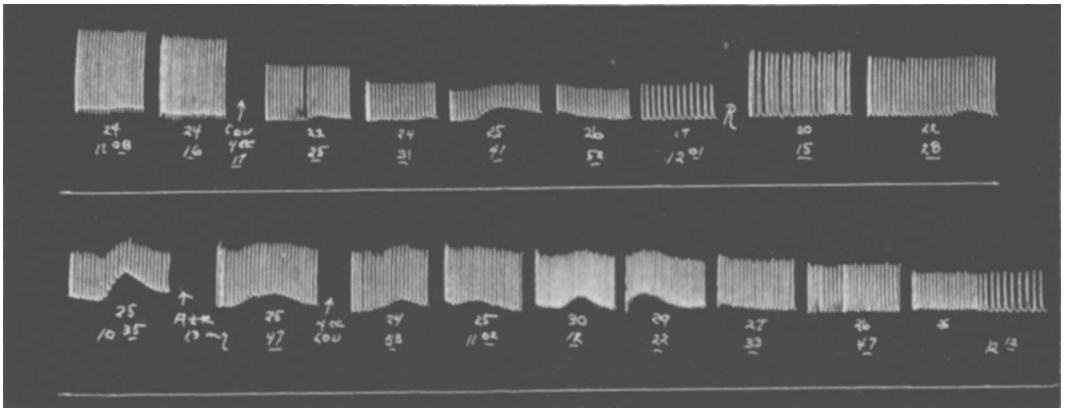

FIG. 1. A. Upper line: The response of a spontaneously beating auricle to coumingine 1:.6 million, at arrow. At R bath was changed to fresh Ringer solution. B. Lower line: The response of an atropinized auricle to the same concentration of coumingine, at second arrow. Rate increased 25 min after addition of drug. Upper figures, rate; lower, time.

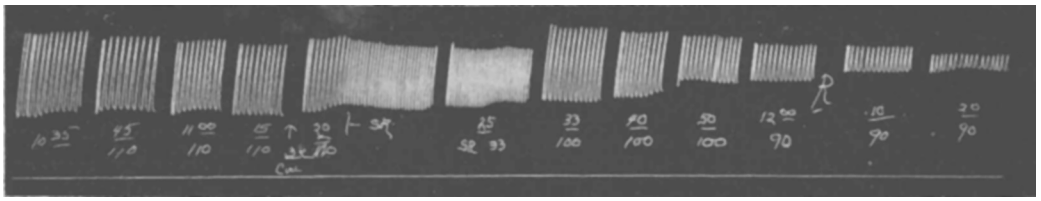

FIG. 2. The response of a rhythmically driven ventricular strip to coumingine 1:1 million, at arrow. Driven rate, 14 per min. SR, spontaneous rhythm, rate 33. Upper figures, time; lower, relative threshold readings.

elegans. With none of the drugs studied to date in this laboratory has there been the variability of response and want of relation between drug concentration and intensity of action that has been exhibited by coumingine. The drug has a pronounced vagal action on the auricle. Shortening of ventricular muscle was its outstanding action. While this effect might be expected to increase cardiac output in the dilated heart, the tendency to develop tachycardias would preclude the use of the drug in clinical medicine. The characteristic shortening of refractory period produced by digitalis bodies was not seen.

Action on auricle. The response of the isolated spontaneously beating auricle to concentrations ranging from 1:20 to 1:0.5 million was very variable. The most consistent action was a vagal negative inotropic effect. Vagal slowing or stoppage occurred with high concentrations, either early or 45 minutes to an hour after the drug had been added. Recovery followed atropine or at times merely changing to fresh Ringer solution (Fig. 1A). In one experiment stoppage was followed by tachycardia. Occasionally there was a positive inotropic action in the stimulated auricle; that action appeared late and then soon declined. Diastolic shortening was not marked, even with high concentrations; it was more apt to appear in the atropinized auricle. Tachycardias occasionally appeared with high concentrations of the drug, again more frequently after atropine (Fig. 1B).

Action on ventricle. With concentrations up to 1:10 million spontaneous tachycardias and extrasystoles often appeared in driven ventricular strips, usually soon after the addition of the drug (Fig. 2).

The effect on contractility was studied in rhythmically stimulated ventricular strips suspended in a bath and the beat recorded by a kymograph. Diastolic shortening, with concentrations as low as 1:40 million, was the constant irreversible action of the drug. Transient slight positive inotropic effects were oc-

TABLE I. Effect of Coumingine on Beat Size and Threshold for Electrical Stimulation.

Exp.	Concentration, parts/million	Min after drug	% decrease in beat size		% of basal threshold
			Systolic	Diastolic	
1	1: .8	50	52	34	354
2	1: .9	50	11	22	180
3	1: 1	45	0	58	82
4	1: 2	45	25	50	128
5	1: 2	30	29	43	160
6	1: 5	40	35	28	140
7	1: 5*	50	26	20	140
8	1:10	60	0	52	200
9	1:10*	60	0	22	150
10	1:20	60	12	54	75
11	1:40	75	12	56	58

* Glutathione added to bath before coumingine.

casionally seen, and when tachycardias appeared early, beat size was well maintained with the increased rate. Even late, systolic peaks usually were not greatly lowered, reduced beat size being largely due to diastolic shortening (Table I). When such muscle shortening is due to action on SH groups Pisanty(2) found that it could be prevented by certain sulfhydryl compounds. The shortening caused by coumingine was not prevented by the previous addition to the bath of glutathione or cysteine in relatively high concentrations, *e.g.* the presence of glutathione 1:3000, did not prevent shortening and rise in threshold by coumingine 1:10 million. In general, the tolerance of auricular muscle to this alkaloid greatly exceeded that of the ventricle. The difference did not appear to be related to rate of beating or to the effect of stimulation.

The effect on the threshold for electrical stimulation was also studied in those experiments in which contraction was recorded. With this type of preparation a basal threshold is usually reached about one-half to an hour after beginning stimulation. Control experiments indicate an expected rise to 120% of the basal threshold after an additional 2 hours of stimulation. In most experiments with higher concentrations coumingine caused a definite rise (Table I). The raised threshold is probably concomitant with depression of conductivity (Table II).

The effects on latent period, refractory period and conduction were studied in rhythmically driven strips from which two unipolar electrograms were simultaneously recorded, using small wick electrodes with an inter-electrode distance of about 25 mm. The Q-T interval of such electrograms has been shown to be a reliable measure of the refractory period of that area of muscle. Normally, there is shortening of about 8% per hour after the first hour due to driving itself. All noxious agents appear to shorten refractory period. The results of experiments given in Table II show only a single example in which there was shortening of refractory period comparable to that produced by digitalis glycosides. The effect on conduction was followed by changes in the Q-Q interval of these electrograms. An appreciable rise in conduction time was the rule (Table II).

Summary. The action of the erythrophleum alkaloid coumingine has been studied on the turtle heart. It has a pronounced vagal effect on the auricle. Diastolic shorten-

TABLE II. Effect of Coumingine on Latent Period, Refractory Period and Conduction Time.

Exp.	Concentration, parts/million	Min after drug	% change of basal intervals		
			S–Q	Q–T	Q–Q
1	1: .4	70	+58	— 6	— 14
2	1: .5	50	NC	NC	+ 41
3	1:1	35	—	+ 3	—
4	1:1	60	—40	—11	+ 66
5	1:1	40	+16	—33	+300
6	1:1.5	50	NC	—14	+112
7	1:2	60	+33	+ 7	+ 33
		90	+66	+ 3	+100
8	1:10*	40	—	— 4	+ 22
9	1:10*	60	—	+ 5	+ 12

S–Q, latent period, shock to Q interval. Q–T interval, refractory period. Q–Q interval, conduction time for inter-electrode distance.

* Spontaneous rhythms appeared during experiments.

NC = no change.

ing of ventricular muscle was the outstanding action. Conduction time in ventricular muscle was prolonged and the threshold for electrical stimulation raised. The characteristic shortening of refractory period produced by digitalis glycosides did not occur. The tendency to cause spontaneous tachycardias would preclude the use of coumingine in clinical medicine.

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Failure of Transfusion of Hemophilic Plasma to Induce Abnormalities of Hemostatic Mechanism in Normal Individuals. (19044)

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Most investigators agree that the fundamental defect in hemophilia is represented by the deficiency of a specific plasmatic factor(1). This factor has been variously defined as "antihemophilic globulin", "thrombocytolysin", "plasmakinin", "thromboplastinogen", etc. Much experimental evidence has accumulated in support of this theory, the most significant observation being perhaps that small amounts of "antihemophilic globulin" are able to normalize temporarily the clotting defect of hemophiliacs. On the other hand, some evidence has been presented to show that hemophilia might be due to the presence of a circulating anticoagulant; a significant finding in support of this theory is that hemophilic plasma may be made to clot normally by simple dilution with physiological saline(2).

The favorable effect of transfusion of fresh normal plasma on the clotting activity of hemophiliacs is well known. On the other hand, the effect of transfusion of hemophilic plasma on the clotting mechanism of normal subjects does not seem to have been investigated. Should a circulating anticoagulant be responsible for the clotting defect of hemophilia, the transfusion of sufficient amount of hemophilic plasma in normal subjects could be expected to be followed by detectable abnormalities of the coagulation mechanism.

Materials and methods. Two patients with classical hemophilia as confirmed by clinical and laboratory data were used as donors in this study. Both patients presented the usual response to transfusion of fresh human plasma and did not show precipitating antibodies(3) against human plasma or "antihemophilic globulin". Five hundred ml of blood were collected in Baxter vacuum bottles containing 100 ml of ACD solution, the donors fasting for at least 4 hours prior to donation. The blood was centrifuged at 2,000 r.p.m. for one hour in refrigerated centrifuge and the plasma separated with sterile technic. Within 2 hours from the time of collection the plasma was injected into compatible normal subjects. An amount of 5 ml of plasma per kilo weight was administered at a speed of 150-200 drops per minute. In the hemophiliacs prior to donation and in the normal subjects before, immediately after the transfusion and one hour later the following determinations were made: (a) bleeding time, according to the method of Ivy *et al.* (4); (b) tourniquet test, according to the method of Frugoni and Giugni(5), maintain-

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