Effect of Nicotine and Epinephrine on *in Vivo* Coagulation Time in Rabbits

By DUANE G. WENZEL and JASBIR SINGH

The intravenous administration of 0.01 or 0.02 mg./Kg. of nicotine to rabbits induced significant reductions in the time for blood to coagulate as measured by the Lee-White capillary tube method. Epinephrine induced a biphasic response with significant reductions resulting from small doses (0.01 and 0.02 mg./Kg.) and an increase from a larger dose (0.08 mg./Kg.). Intermediate doses (0.04 and 0.06 mg./Kg.) did not produce changes that were significantly different from control values. The combined treatment with nicotine, 0.01 mg./Kg., and epinephrine, 0.04 or 0.06 mg./Kg., increased the time to coagulate over that of the smaller doses of epinephrine or nicotine alone. The administration of piperoxan, 0.25 mg./Kg., 10 minutes before either nicotine or epinephrine, 0.01 mg./Kg., blocked the increased rate of coagulation.

Previous investigations have demonstrated that the chronic administration of nicotine to rabbits, in doses roughly comparable to the amount absorbed during smoking, produces a significant reduction in the blood coagulation time (1, 2). Because of the considerable epidemiological evidence relating smoking to the mortality from coronary heart disease, it is suggested that nicotine may serve as a precipitating factor through its effect upon the coagulation time.

Observations of the acute effects of nicotine upon blood coagulation have been few and generally contradictory. Takatsuki (3) reported that the subcutaneous administration of nicotine to rabbits in doses of from 0.5 to 5.0 mg./Kg. resulted in a decreased coagulation time. When he injected larger doses of from 10 to 30 mg./Kg. the coagulation times were then lengthened. The increase in rate as induced by the smaller quantities was found to be augmented by epinephrine and by cocaine, and either inhibited or reversed by yohimbine or ergotamine. From these findings Takatsuki concluded that the effect of nicotine on blood coagulation was dependent upon epinephrine release. In a later study, Kanowoka (4) administered nicotine intravenously to rabbits in doses of from 0.05 to 2 mg./Kg. and observed that the coagulation time was lengthened in a majority of the animals. The results, however, were inconsistent for a given dose and some animals responded to the treatment with a fall in the time to coagulate. The amount of nicotine employed was so large as to induce convulsions in most of the animals Doses of this magnitude, in addition to being unrelated to the amount of nicotine absorbed during smoking, are decidedly unphysiological and the extreme central stimulation may have induced secondary effects on the coagulation time.

The reported effects of smoking upon blood coagulation in humans have been at least as contradictory as those from animal studies. Grassi and Caltabiano (5) have presented evidence to indicate that smoking one cigarette results in an increased platelet count, shortened plasma coagulation and clot retraction times, and an augmentation of prothrombin activity and utilization. These findings are in accord with those of Eisen (6) who determined that smoking three to four cigarettes after a month's abstinence results in a coagulation time reduction to about one-half of the presmoking level. Blackburn, Orma, Härtel, and Punsar (7), on the other hand, were unable to demonstrate a significant effect of smoking upon blood coagulation as measured by the recalcified plasma Stypven time.

The primary purpose of this study was to reexamine the effect and the mechanism of action of acute nicotine administration upon *in vivo* blood coagulation. In order to establish the relationship of the nicotine effects to epinephrine release, actions of the latter agent were also examined. Despite the fact that epinephrine's role in blood coagulation has been studied by a number of investigators, there is still some confusion regarding its effects. That epinephrine influenced the coagulation time was first recognized by Vosburgh and Richards in 1903 (8). This work could not be confirmed by Wiggers (9), but was later verified by Van den Velden (10). It was in 1914 that Cannon and Gray (11) performed their classical studies indicating that epinephrine in small doses (0.001 mg./Kg.) shortened the coagulation time, but in larger doses (0.03 mg./Kg.) lengthened it. This experiment and many others which followed were performed with the coagulometer of Cannon and

Received December 18, 1961, from the Department of Pharmacology, The University of Kansas, School of Pharmacy, Lawrence.
Accepted for publication January 3, 1962.
Supported by a grant from the Life Insurance Medical Research Fund.

Mendenhall (12). The preceding observations regarding the clot accelerating effect of epinephrine have been found, by Forwell and Ingram (13), to be reproducible in anesthetized dogs or cats when the coagulometer principle was employed. The results could not be verified, however, under the same conditions by the standard Lee-White test tube method. For reasons that are not apparent, the administration of epinephrine to humans by these same investigators resulted in a shortened time when measured by the Lee-White tests. Furthermore, a significant fall in the time to coagulate with humans was observed only when silicon-coated tubes were employed. From this and additional evidence, Forwell and Ingram suggested that the action of epinephrine, at least in part, results from an increase in factor V activity. It was also suggested that the epinephrine effect may be duplicated by the unpleasant stimulus of blood withdrawal. Härtel (14), employing the plasma Stypven time previously found to be unaffected by smoking (7), observed that the subcutaneous injection of 0.7 mg. of adrenaline in humans caused a significant shortening. In this study all apparatus was siliconized.

As previous studies of the chronic effects of nicotine on blood coagulation had been satisfactorily performed using uncoated capillary tubes and with unanesthetized animals (1, 2), it was accordingly decided to determine the effects of nicotine and epinephrine in the same manner for this experiment.

EXPERIMENTAL

Six unanesthetized, adult, female, albino rabbits were employed for each drug treatment. To determine the mean coagulation times, three replications were made with each animal at each test period. Control mean clotting times were measured for each group immediately prior to the administration of the test agent. Blood was obtained from a puncture wound in the tail. After lightly and quickly blotting the first few drops of blood, an uncoated capillary tube with an internal diameter of approximately 1 mm. was used to collect blood to a length of about 3 cm. Capillary tubes were thoroughly washed with distilled water and dried before being employed. The end of the tube containing the blood first withdrawn was repeatedly broken off in small sections at a speed as great as was consistent with the observation of the fibrin thread. It was found that this could be accurately performed once in about every 2.5 seconds. Time was measured by a foot-actuated stopwatch.

Following the control coagulation time determinations and the administration of the appropriate drug dose, the measurements were repeated at 1 minute, 15 minutes, and every 15 minutes thereafter until the time to coagulate had returned to within 1 to 2 seconds difference from that of the control value. For some of the treatments, testing was continued beyond this point in order to demonstrate the absence of any delayed action. As three replications were performed at each period tested, the first determination was started shortly before the indicated time and the last one shortly thereafter. Each mean coagulation time therefore represents three determinations upon each of six animals for a total of 18 individual values. The *t*-tests and standard errors of the mean were calculated on the basis of six mean values for each test.

Drugs were administered in normal saline with a total volume of 0.25 ml. It has been suggested that, by what may be a stress response, repeated venepuncture alone may induce a reduction in the coagulation time. The injection of 0.25 ml. of saline followed by repeated blood collections did not produce a significant change in the time at any of the periods tested.

The following drug doses and combinations of drugs expressed as mg. drug per Kg. body weight were tested for their effect upon the coagulation time: nicotine 0.01 and 0.02; epinephrine 0.01, 0.02, 0.04, 0.06, and 0.08; nicotine 0.01 and epinephrine 0.04; nicotine 0.01 and epinephrine 0.06; piperoxan 0.25; piperoxan 0.25 and nicotine 0.01; and piperoxan 0.25 and epinephrine 0.01. Administration of the piperoxan preceded that of the

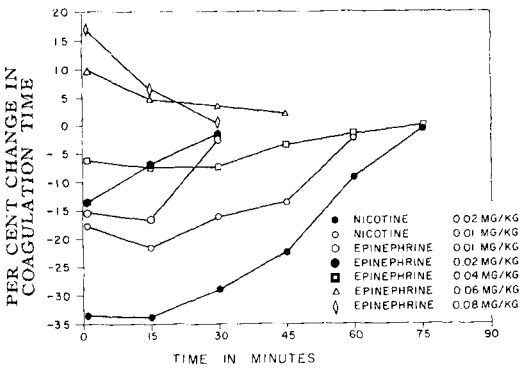

Fig. 1.—Effects of several doses of nicotine and epinephrine upon the mean blood coagulation time in rabbits. The first reading was obtained at 1 minute.

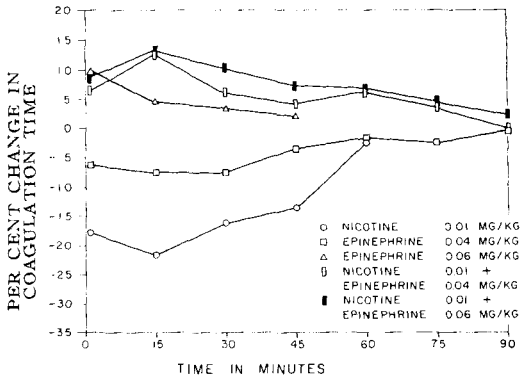

Fig. 2.—Effects of the combined administration of nicotine and epinephrine upon the mean blood coagulation time of rabbits. The first reading was obtained at 1 minute.

TABLE I.—COAGULATION TIMES IN RABBITS TREATED WITH NICOTINE AND EPINEPHRINE[a]

Treatment, mg./Kg.	Coagulation Times, sec. ± S.E. Initial Time	1 Min. After Treatment	% Change	P
Saline	28.8 ± 1.3	28.4 ± 1.6	−1.4	>0.50
Nicotine 0.01	37.4 ± 1.1	30.7 ± 1.0	−17.9	<0.01[b]
Nicotine 0.02	34.7 ± 0.34	23.1 ± 0.67	−33.4	<0.001[b]
Epinephrine 0.01	28.9 ± 1.3	24.2 ± 1.1	−16.3	<0.02[b]
Epinephrine 0.02	30.9 ± 0.86	26.7 ± 1.27	−13.6	<0.02[b]
Epinephrine 0.04	31.0 ± 1.4	28.9 ± 1.9	−7.0	>0.05
Epinephrine 0.06	33.1 ± 2.2	36.4 ± 1.3	+9.1	>0.05
Epinephrine 0.08	30.1 ± 0.75	34.5 ± 1.5	+12.8	<0.02[b]
Nicotine 0.01, epinephrine 0.04	29.4 ± 1.2	31.4 ± 1.2	+6.4	>0.05
Nicotine 0.01, epinephrine 0.06	30.9 ± 1.88	33.4 ± 1.89	+7.5	>0.05
Piperoxan 0.25	31.7 ± 0.89	29.6 ± 0.85	+6.6	>0.05
Piperoxan 0.25, epinephrine 0.01	31.2 ± 1.87	30.0 ± 2.08	−3.8	>0.05
Piperoxan 0.25, nicotine 0.01	29.4 ± 1.58	29.4 ± 1.25	0	>0.05

[a] Coagulation times were measured using the Lee-White capillary tube method. Six animals were employed for each treatment tested with three replications at each period of testing. [b] Significant values.

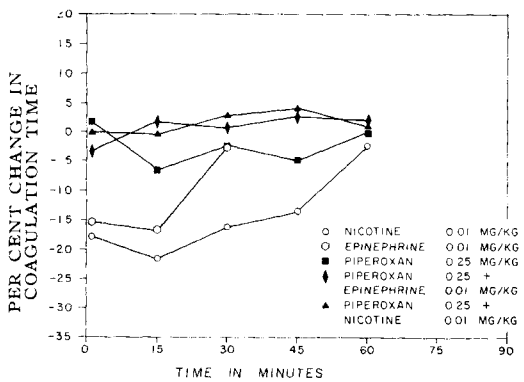

Fig. 3.—The effect of piperoxan upon the mean blood coagulation response of rabbits to nicotine and epinephrine. The first reading was obtained at 1 minute.

nicotine or epinephrine by 10 minutes in order to allow adequate time for the development of adrenergic blockade. The times at which the rate of clotting was measured for the combined treatment with piperoxan were relative to the time of nicotine or epinephrine administration.

RESULTS AND DISCUSSION

The mean initial coagulation times (controls), the times at the 1-minute period, their standard errors, the per cent change in coagulation time during this period, and the level of significance of the difference between the means are given in Table I. As the greatest drug-induced effects, with few exceptions, were apparent at the 1-minute test period, these mean experimental values were compared by the *t*-test to those of the corresponding controls. The results are treated graphically in Figs. 1, 2, and 3 to present the time-related pattern of the response.

Separate Nicotine and Epinephrine Effects.—Doses of nicotine were selected so as to induce changes in the coagulation time without the convulsive seizures reported by previous investigators (3, 4). Initial exploratory experiments indicated that the intravenous administration of 0.03 or 0.04 mg./Kg. of nicotine induced tremors in many of the animals tested. It was further determined that neither nicotine nor epinephrine in intravenous doses up to 0.02 and 0.08 mg./Kg., respectively, would cause excessive stimulation with the exception of occasional animals which responded with respiratory stimulation. These doses were then selected as the arbitrary limits to be employed.

From the values in Table I and the points in Fig. 1, it is apparent that nicotine at both of the doses tested significantly reduced the time required for blood to coagulate ($P < 0.01$ and 0.001). It seems likely that the intravenous doses of 0.01 and 0.02 mg./Kg. as employed in this study are comparable to larger subcutaneous doses reported to induce a similar effect (3). Others (4) have noted that the intravenous injection of larger doses (0.5 to 2mg./Kg.) of nicotine results in a lengthened time to coagulate, although some of the animals tested responded with reduction in the time. Such variance was not encountered with the smaller doses used in this study as all animals, without exception, responded by an increased rate of coagulation.

Epinephrine in doses of 0.01 and 0.02 mg./Kg. also significantly reduced the coagulation times ($P < 0.02$). The response to these doses was strikingly similar except for a longer significant reduction with the larger dose. Doses of 0.04 and 0.06 mg./Kg. induced a fall and an increase, respectively, in the times to coagulate. While these differences were not significant in the statistical sense, from an examination of the time-effect curves in Fig. 1 it appears unlikely that the changes were not attributable to the treatments involved. As the largest dose of the epinephrine 0.08 mg./Kg. resulted in a significant extension of the coagulation time ($P < 0.02$), it is apparent that the results of the epinephrine treatment are in agreement with those of Cannon and Gray (11) who first demonstrated the biphasic response. The data provide no explanation for the inability of Forwell and Ingram to observe an effect upon coagulation time in animals when using the Lee-White test tube method

(13). While the capillary method was employed, the tubes were uncoated and the animals were unanesthetized which, if anything, would be expected to mask the effect of epinephrine rather than to increase the sensitivity.

Combined Nicotine-Epinephrine Effects.—The results obtained by combining 0.01 mg./Kg. of nicotine with either 0.04 or 0.06 mg./Kg. of epinephrine are shown in Fig. 2 and Table I. Changes in both instances were in the direction of an increased time to coagulate, although both drugs individually had depressed the coagulation time. It is perhaps worthy of note that the combined nicotine-epinephrine, while not inducing a significant increase in the coagulation time as measured by the *t*-test, apparently prolonged the duration of the response. Further, the direction of the change is what would be anticipated if nicotine exerts its action through catecholamine release.

Modification of Nicotine and Epinephrine Responses by Piperoxan.—The injection of piperoxan alone in a dose of 0.25 mg./Kg. caused a minimal and nonsignificant increase in the rate of coagulation (Table I and Fig. 3). Administration of this dose 10 minutes prior to either 0.01 mg./Kg. of epinephrine or nicotine resulted in coagulation times which varied no more than did repeated control determinations. This blockade of the nicotine effect is consistent with the results of Takatsuki (3), demonstrating that nicotine exerts its characteristic action on the coagulation time through the release of catecholamines. As the actions of nicotine and epinephrine are both apparently mediated through the latter agent, and as the response to their combined administration indicates an additive effect, it is likely that the combination of smaller doses of each would result in a shorter coagulation time. Although the experimental evidence does not directly support the contention, it is further likely that the amounts of nicotine absorbed during smoking would increase rather than decrease the rate of coagulation, even in the presence of systemic catecholamine release. In man, Johnston has reported that 0.08 to 0.13 mg. of nicotine intravenously produces effects similar to those induced by one deep inhalation of cigarette smoke (15).

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Use of 3-Azabicyclo[3.2.2.]nonane in the Mannich Reaction I

Substituted β-Amino Ketones

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A group of Mannich bases, indicated as types I, II, and III, have been prepared utilizing 3-azabicyclo[3.2.2.]nonane as the amine component. These β-aminoketones are to be screened for possible pharmacodynamic activity.

RECORDED in the literature (1–11) are numerous ketonic Mannich bases, prepared for pharmacological testing as antispasmodics, analgesics, chemotherapeutic agents, and local anesthetics. Such compounds may, in general, be prepared easily by means of the Mannich reaction which utilizes the appropriate ketone, formaldehyde, or paraformaldehyde, and the desired amine. This may be illustrated as follows

$$RCOCH_3 + HCHO + R'_2NH \cdot HCl \rightarrow RCOCH_2CH_2NR'_2 \cdot HCl + H_2O$$

Received October 23, 1961, from the School of Pharmacy, The University of Mississippi, University.
Accepted for publication November 24, 1961.

The rather extensive literature dealing with this reaction has been reviewed by Blicke (12), Karbe (13), Reichert (14), and more recently by Hellmann and Opitz (15).

The great versatility of these ketonic Mannich bases prompted us to prepare a number of Mannich bases of types I, II, and III, in which the amine moiety is 3-azabicyclo[3.2.2.]nonane. In addition, the concept of vinylogous substances, as set forth by Fuson (16), has led us to prepare several vinylogs of types I and II. (See Tables I–III.)

To study more fully the effect of structural variants, Mannich bases have been prepared of