

be a feedback mechanism which involves a porphyrin or porphyrin-like compound. The effects could be direct or through stimulation of increased production of the plasma erythropoietic factor. Further studies are required to establish the mechanism by which hemin and related compounds affect erythropoiesis.

**Summary.** 1. Regardless of age, sex, or species of the donor animals, lysed mammalian red blood cells show erythropoietic stimulating activity. 2. Comparison of the stimulating effects of red blood cell components on erythropoiesis shows that hemoglobin and hemin are active; stroma and globin are not. 3. The following compounds related to heme appear to increase erythropoiesis: compounds containing 4 pyrrole rings connected by methene bridges; an open 4-pyrrole ring configuration, as in biliverdin and bilirubin. The nature of the side-chain groups is not critical for activity; a metal moiety is not essential to activity. 4. Several precursors involved in

synthesis of the porphyrin ring, a single pyrrole ring, and cyanocobalamin which has a corrinoid ring structure, did not increase erythropoietic activity.

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### Effect of Cigarette Smoke on Epinephrine Secretion in the Dog.\* (28185)

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Various investigators have shown that large doses of nicotine, the active ingredient in tobacco smoke, when administered intravenously can cause an increase in secretion of epinephrine from the adrenal gland(1-4). It is controversial as to whether or not a similar effect can be produced as the result of inhalation of cigarette smoke(5). In an attempt to clear up this controversy the present experiments were carried out to determine whether or not inhalation of cigarette smoke could cause an increased secretion of epinephrine from the adrenal glands of dogs.

**Methods.** Twenty-two healthy mongrel dogs of either sex with weights ranging from 7.7 to 17.7 kg were used. The dogs were

anesthetized with intravenous pentobarbital sodium (30 mg/kg) or chloralose (80 mg/kg). The dogs then inhaled cigarette smoke from a holder inserted into a tracheal cannula. The rate of smoking was regulated by means of a screw clamp on a 14 mm ID rubber tube which served as a bypass for the cigarette. The same standard brand of cigarette was used in all experiments. Blood samples were collected before, during and after inhalation periods. Blood pressure was recorded from the carotid artery by means of a polyethylene catheter connected to a Statham transducer and a Sanborn 150 M recorder. Heparin (3 mg/kg) was used as an anticoagulant in all experiments.

Blood samples were taken from the adrenal vein, inferior vena cava, the femoral artery and femoral vein. The adrenal vein was cannulated using the method of Satake(7), while

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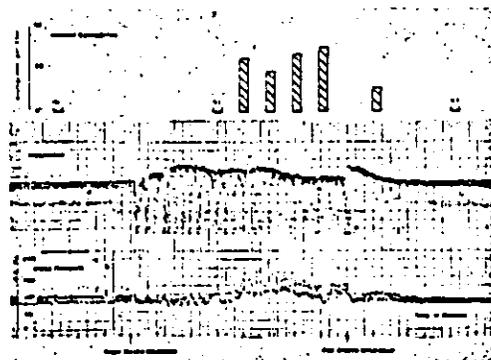


FIG. 1. Effect of slow inhalation of cigarette smoke (smoking time, 8 min) on blood pressure, respiration and arterial epinephrine levels in a representative experiment. Rate of cigarette smoke inhalation was one inhalation per 20-25 sec.

the vena cava blood was obtained by means of a catheter placed *via* the femoral vein into the vena cava to the level of the sternal notch and well above where the adrenals empty into the vena cava. The average smoking time for most experiments was 3.5 minutes; however, in certain experiments this time was prolonged to approximately 8 minutes, the slowest rate at which the cigarette could be conveniently burned.

Blood samples were taken when the blood pressure response reached maximum levels during the inhalation period. When the smoking time was 3.5 minutes 2 or 3 samples were obtained, while in the longer smoking experiments of 8 minutes duration, samples were obtained at about one-minute intervals once the blood pressure response was seen (Fig. 1). The actual inhalation rate for the faster smoking experiments was approximately one inhalation per 10 seconds, while for the longer smoking experiments, there was one inhalation per 20-25 seconds.

To eliminate other factors which might affect blood epinephrine levels, control experiments were conducted on the effect of inhaling cornsilk cigarette smoke, the effect of asphyxia and the effect of hypoxia on blood epinephrine levels.

Blood samples were assayed for epinephrine content by the rat uterus method of Gaddum and Lembeck(8) as modified by Franko, Bragg and Watts(9).

This bioassay method was well adapted for

this study since epinephrine determinations could be made on 0.5 ml serial blood samples at about 6-minute intervals. The method also has a good specificity for epinephrine and a number of studies have shown that the increase in circulating catecholamines following nicotine administration is primarily due to epinephrine(10-14). It was thus possible to assay as many as 20 samples from a single dog for epinephrine without stimulation of the sympatho-adrenal system by hemorrhage. Unfortunately, Gaddum and Lembeck's similar bioassay method employing the rat colon is not sufficiently sensitive to detect norepinephrine in peripheral blood. To obtain information on norepinephrine levels and to substantiate the epinephrine data obtained by bioassay, 2 experiments were carried out in which 40 ml peripheral arterial blood samples were assayed for their norepinephrine and epinephrine content by a fluorometric procedure. These determinations were made by the method of von Euler and Lishajko(15). Statistical analyses for significance of data were made using Fisher's t test.

*Results.* The data in Table I summarize the effect of inhalation of cigarette smoke on epinephrine secretion from the adrenals, on peripheral arterial and venous blood epinephrine levels and on blood pressure. These results show that cigarette smoke can cause a statistically significant increase in the amount of epinephrine in blood samples obtained from the adrenal vein, vena cava, femoral artery and femoral vein of the dog.

A representative slow smoking experiment is shown in Fig. 1. Even when the cigarette is inhaled over a rather long period comparable to the average cigarette smoking time in man, there is a significant increase in blood pressure and arterial epinephrine levels.

The effects of inhaling cornsilk cigarettes, of asphyxia and of hypoxia on blood epinephrine levels are shown in Table II. Inhalation of the smoke from cornsilk cigarettes had no effect on epinephrine levels of vena cava blood, the blood in which any increase could have been most readily detected. Although there was some increase in epinephrine levels during severe asphyxia and hypoxia, the amounts observed were small compared to the

TABLE I. Effect of Inhalation of Cigarette Smoke on Epinephrine Secretion from the Adrenals, on Peripheral Arterial and Venous Blood Epinephrine Levels and on Blood Pressure.

Site	Anesthetic	Smoking time (min)	No. of dogs	No. of observations	Epinephrine secretion rate of peripheral blood level $\pm$ S.E.		Blood pressure in mm Hg $\pm$ S.E.		P compared to control
					Control	Smoking	Control	Smoking	
Adrenal vein	Pentobarbital	3.5	5	15	.004 $\pm$ .0018 $\mu$ E/kg/min	.0241 $\pm$ .0053 $\mu$ E/kg/min	108 $\pm$ 3	155 $\pm$ 8	<.001
"	Chloralose	3.5	4	10	.0032 $\pm$ .0012 $\mu$ E/kg/min	.201 $\pm$ .051 $\mu$ E/kg/min	120 $\pm$ 12	133 $\pm$ 6	<.025
Vena cava*	"	3.5	8	20	<2 $\mu$ E/l	458 $\pm$ 132 $\mu$ E/l	113 $\pm$ 5	233 $\pm$ 8	<.001
Femoral artery*	"	3.5	8	27	<1 "	126 $\pm$ 28 "	113 $\pm$ 5	233 $\pm$ 8	<.001
" vein*	"	3.5	8	20	<1 "	45 $\pm$ 13 "	85 $\pm$ 3	166 $\pm$ 7	<.001
" artery	"	8.0	5	23	<1 "	25 $\pm$ 4 "	85 $\pm$ 3	160 $\pm$ 7	<.05

\* Simultaneous samples.

TABLE II. Effect of Standard Brand Cigarettes, Cornsilk Cigarettes, Asphyxia, and Hypoxia on Epinephrine Levels of Vena Cava Blood in the Dog.

Item	No. of observations	Max epinephrine levels ( $\mu$ E/l) in vena cava blood
Cigarettes	20	458
Cornsilk cigarettes	10	<1
Asphyxia—3 min	10	30
Atmospheric air	18	<1
O <sub>2</sub> —15 %	3	1.1
10 %	3	1.3
7.5 %	2	3.8
5 %	6	5.0
2.5 %	4	12.0

amounts obtained following inhalation of cigarette smoke.

In the 2 experiments in which peripheral arterial blood samples were assayed for both norepinephrine and epinephrine, the results indicate that inhalation of cigarette smoke caused an average increase in norepinephrine level from a control value of 1  $\mu$ E/l to a value of 20.2  $\mu$ E/l after smoking. The simultaneous fluorometric analysis of epinephrine showed an average increase from a control value of 1.2  $\mu$ E/l to a value of 96.8  $\mu$ E/l. At the same time the samples were taken for fluorometric analysis, another sample was also biologically assayed for its epinephrine content. The epinephrine value of 120  $\mu$ E/l obtained by bioassay during smoking compares reasonably well with the simultaneous value of 96.8  $\mu$ E/l obtained by fluorometry.

*Discussion.* These results show that inhalation of cigarette smoke can cause a significant increase in secretion of epinephrine from the adrenal gland of the dog. It is well established that nicotine is the chief pharmacologically active substance in cigarette smoke (16,17). Nicotine in large doses can cause the release of catecholamines from the adrenal gland (2,3,4). Control experiments involving no smoking and the smoking of cornsilk cigarettes had no effect on epinephrine secretion. The effect of asphyxia and hypoxia was very small when compared to cigarette smoking. The data indicate it is the nicotine in the smoke which produced the observed responses.

These experiments have shown that the adrenal medulla is more responsive to cigar-

ette smoke in dogs anesthetized with chloralose than in dogs anesthetized with pentobarbital. Exley(18) has shown that amobarbital significantly reduced transmission through sympathetic ganglia, whereas equivalent anesthetic doses of chloralose were almost devoid of such effects. Armstrong, Porter and Langston(19) found that chloralose anesthesia was associated with an average increase of 8% in reflex activity over the unanesthetized level, while pentobarbital was associated with a 9% decrease in reflex activity when compared to unanesthetized dogs. Koppermann *et al.*(20) observed that in deep barbiturate anesthesia the carotid reflex was completely eliminated, but when dogs recovered from anesthesia, the reflex returned. Chloralose, on the other hand, was found not to depress the reflex and there was a greater than normal reflex response during the recovery period. A combination of these factors apparently made the dogs under chloralose anesthesia much more sensitive to cigarette smoke than were the dogs under pentobarbital anesthesia.

The observed increase in epinephrine secretion in adrenal vein blood would lead one to expect, and these results show, a significant increase in epinephrine level in the vena cava, peripheral arterial and peripheral venous blood as the result of cigarette smoke. Since hemorrhage and surgical trauma are known to stimulate secretion of catecholamines(21-24) it was thought that the vena cava technique would eliminate errors in increased epinephrine levels due to trauma as well as substantiate the results obtained with the adrenal vein technique.

It is apparent that the increase in epinephrine content during smoking is greatest in the vena cava blood, followed by the arterial and peripheral venous blood, respectively. The vena cava blood contains the immediate secretions from the adrenal glands diluted by blood flow from the posterior part of the body only. By the time the secretions from the adrenal glands reach peripheral arterial blood, the epinephrine has been further diluted by the total venous return and possibly by inactivation in the lungs. Finally, epinephrine content is further decreased by passage through the tissues of the hind leg so the

lowest concentration appears in the peripheral venous blood. Further significance is placed on the fact that inhalation of cigarette smoke produced an increase in epinephrine levels of peripheral arterial blood since this is the blood which reaches all tissues. To simulate more closely cigarette smoking in humans, experiments were carried out in which inhalation of cigarette smoke was controlled at a slow rate. Smoking time in these experiments was 8 minutes which is comparable to the average human cigarette smoking time. The results show that from control levels of less than 1  $\mu\text{g/l}$  the slow inhalation of cigarette smoke increased epinephrine levels of peripheral arterial blood to 25.1  $\mu\text{g/l}$  ( $p < 0.001$ ). This elevation in epinephrine levels was found to increase progressively to a maximum value during the smoking period. In the previous experiments, where average smoking time was 3.5 minutes, inhalation of cigarette smoke caused an increase in arterial epinephrine level from an undetectable amount before the cigarette to a value of 126  $\mu\text{g/l}$  during the cigarette. Although consideration must be given to species and weight differences, these results indicate that a similar effect can occur in man during cigarette smoking.

Results obtained in this study on the differential release of epinephrine and norepinephrine are in agreement with results reported in the literature on release of epinephrine and norepinephrine by nicotine(10-14) in that the increase of catecholamines in the peripheral circulation is due primarily to a release of epinephrine from the adrenal medulla.

Fowler *et al.*(25) studied the effect of hypoxia upon adrenal secretion in anesthetized dogs using a nonspecific bioassay method employing the rabbit aortic strip. They observed that there was an increase in medullary secretion during hypoxia. They further observed that in 4 animals studied during asphyxia there was an increase in medullary secretion comparable to that found during hypoxia. Contrary to the above results, Toyooka and Blake(26) using a fluorometric technique found no significant differences in mean urinary excretion of plasma catecholamines in dogs subjected to hypoxia as compared to the control or recovery periods. The

results (Table II) indicate that asphyxia or hypoxia did not contribute to the overall effects of epinephrine secretion due to cigarette smoke in this present study. Only moderate increases in peripheral epinephrine levels were observed and these were seen only after severe asphyxia and hypoxia. In all smoking experiments there was a free exchange of respiratory gases; smoking always stimulated respiration and there were no indications of either asphyxia or hypoxia in the smoking experiments.

**Summary.** Inhalation of cigarette smoke causes a significant increase in epinephrine levels of the adrenal vein, vena cava, peripheral arterial and peripheral venous blood in the dog, the degree of increase being proportionally less in each area respectively.

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### Alterations of Plasma Volume, Electrolytes and Volume of Distribution of Sodium Thiocyanate Induced by Electroshock.\* (28186)

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Electroconvulsive shock induced by passage of an electric current through the brain serves as a powerful stimulus to the central

nervous system producing many and varied reactions. Thus the dog is rendered unconscious; violent muscle spasms occur necessitating restraint; respiration may be held in abeyance; profuse salivation is present; involuntary urination and defecation occur. Aside from these gross manifestations, numer-

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