STUDIES ON VASOMOTOR TONE. I. THE EFFECT OF THE TETRAETHYLAMMONIUM ION ON THE PERIPHERAL BLOOD FLOW OF NORMAL SUBJECTS [1, 2]

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The animal experiments of Acheson, Moe, and Pereira have shown that the tetraethylammonium ion is capable of relatively complete blockade of sympathetic and parasympathetic impulses at the autonomic ganglia (1–2). In the dog, the infusion of 20 mg. per kg. per hour will completely prevent the cardiac response to vagal stimulation, and the response of the nictitating membrane to preganglionic stimulation of the cervical sympathetic nerve (3). That the drug would also inhibit autonomic tone in man has been demonstrated in previous reports (4–7).

The purpose of the present investigation was *1.* to study the effect of tetraethylammonium on blood flow in the extremities of normal and sympathectomized persons by the more quantitative plethysmographic technique; *2.* to establish the extent of sympathetic vasomotor blockade produced by the drug; and *3.* to compare its effect on peripheral blood flow with that of certain other vasodilating agents in common use.

METHODS

Peripheral blood flow was measured by means of a venous occlusion plethysmograph [4] according to the technique described by Abramson (8). Collecting cuff pressures about 20–30 mm. of mercury below diastolic levels were selected and all subsequent determinations in any experiment were made at the same pressure in the collecting cuff.

All blood flows were determined with the subject in the recumbent position, the room temperature at approximately 70° F and bath temperature at 32° C. Foot blood flow was determined with the subject horizontal and the plethysmograph at body level. Forearm and calf blood flows were determined after excluding return circulation from the hand and foot by means of tourniquets inflated to exceed arterial pressure for five minute periods during which consecutive blood flow measurements were made.

After the subject had rested 45 to 60 minutes, three to four series of blood flow determinations were performed. Each series consisted of five observations made at 30 second intervals in the resting state. During the experimental procedure blood flow was determined every 30 seconds for 10 to 15 minutes or until a maximum had apparently been reached. The average of at least five consecutive determinations just prior to the test procedure was designated as the *average resting blood flow* and of five consecutive determinations at the time of maximum increase as the *average maximal blood flow.*

In the sympathetic block experiments, resting blood flows were first obtained with the foot in the plethysmograph. It was then removed from the apparatus and 0.5 per cent metycaine infiltrated in the region of L_1, L_2, and L_3 on one side according to the technique described by White and Smithwick (9), until paravertebral block had begun to take effect as judged by an increase in toe temperature with abolition of the temperature gradient between toe and thigh. The foot was then replaced as rapidly as possible in the plethysmograph and observations were repeated at frequent intervals until blood flow had fallen toward resting levels. That we were able to record the maximum flow after paravertebral block seemed apparent from the fact that the blood flow continued to increase for a while after the foot had been replaced in the plethysmograph and no decline appeared until after 15 to 30 minutes of observations.

Skin temperatures in the extremity opposite to the one enclosed in the plethysmograph were recorded by means of a Leeds-Northrup potentiometer, the thermocouples being held lightly in contact with the skin on the outside margin of the tip of the big toe or index finger, and on the anterior surface of the upper arm or thigh.

The normal subjects referred to in this report consisted of seven healthy male college students in whom the effects of varying dosage of tetraethylammonium,

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[4] We are indebted to Dr. I. Arthur Mirsky for the loan of the plethysmographs used in this investigation.

other vasodilating procedures, and paravertebral block were compared. The remaining normal subjects consisted of 16 male and seven female patients ranging in age from 18 to 43, hospitalized for a variety of disorders but presenting no evidence suggesting the presence of any type of vascular disease.

OBSERVATIONS

1. *Effect of tetraethylammonium on peripheral blood flow in normal subjects*

In Table I are presented the effects of the intravenous injection of 500 mg. of tetraethylam-

TABLE I

Effect of tetraethylammonium chloride on peripheral blood flow and skin temperature in normal subjects

	Subject	Sex	Age	Blood flow* *cc./100 cc./limb/min.*		Ratio TEA to resting flow	Mean B.P. per cent change after TEA	Skin temperature gradient† ° C			
								Toe-thigh		Finger-arm	
				Before TEA	After TEA			Before TEA	After TEA	Before TEA	After TEA
Foot	H. T.	M	21	4.2	12.0	2.9	11	1.8	−2.0		
	W. P.	M	18	1.1	9.2	8.3	−12	5.6	−2.8		
	O. O.	M	31	2.9	8.1	2.8	−10	4.0	0.3		
	G. M.	M	25	1.4	6.5	4.7	− 8	4.7	−0.3		
	E. M.	M	21	2.0	6.2	3.1	0	9.0	0.6		
	D. M.	M	24	1.2	12.5	10.3	4	6.0	−2.0		
	T. G.	M	36	2.1	9.8	4.7	− 8	3.3	−2.0		
	B. F.	M	43	0.8	5.3	6.6	−10	7.0	2.0		
	R. E.	M	18	2.0	9.6	4.8	5	7.2	−0.4		
	J. B.	M	37	3.8	8.5	2.2	− 3	9.0	0.9		
	J. D.	M	21	4.4	15.0	3.4	2	2.8	−2.2		
	D. S.	F	27	1.1	6.4	5.8	−13	8.0	−0.5		
	B. M.	F	21	1.1	16.0	14.5	+ 8	6.8	−3.6		
	M. R.	F	19	0.5	14.9	33.0	+20	7.0	1.6		
	Average			2.04	10.0	7.65					
Hand	J. S.	M	37	3.1	3.8	1.2	−10			7.0	7.0
	E. W.	M	33	4.9	14.6	3.0	4			2.0	1.2
	W. R.	M	36	2.2	8.1	3.7	− 7				
	J. M.	M	30	5.0	26.3	5.2	17				
	R. F.	M	19	5.2	19.2	3.7	−17				
	H. E.	M	18	5.3	23.9	4.5	− 2				
	J. M.	F	21	1.2	10.7	9.0	9			2.7	−0.3
	M. A.	F	27	1.6	12.5	7.8	3			9.0	6.0
	Average			3.5	14.8	4.8					
Forearm	J. D.	M	21	2.6	5.0	1.9	2			1.5	1.7
	H. E.	M	18	1.9	4.9	2.6	1			5.4	−1.0
	D. M.	M	24	3.0	4.8	1.6	15			8.1	0
	E. M.	M	21	1.0	1.4	1.4	−20			1.0	1.0
	G. M.	M	26	2.0	1.8	0.9	− 3			3.0	3.0
	H. T.	M	21	1.2	2.0	1.7	0			4.5	0
	R. S.	F	22	2.1	5.2	2.5	2			4.2	0
	M. B.	F	21	2.8	3.2	1.1	0			7.9	0
	Average			2.07	3.53	1.7					
Calf	L. S.	M	18	0.7	1.1	1.6	− 7				
	A. H.	M	31	1.3	1.4	1.1	3				
	D. H.	M	16	0.5	1.2	2.4	0				
	D. W.	F	24	0.5	0.8	1.6	10				
	C. M.	F	15	0.8	1.2	1.5	5				
	C. B.	F	25	0.3	0.5	1.7	4				
	Average			0.7	1.0	1.6					

500 mg. of tetraethylammonium chloride were given intravenously in all experiments.

* Blood flow values were calculated as described in the text. The value before TEA represents the *average resting flow* and that after TEA was the *average maximal flow.*

† Skin temperatures were determined on the extremity opposite to the one enclosed in the plethysmograph. Arm or thigh temperatures were not significantly altered by injection of TEA; consequently changes in gradient represent increases in toe or finger temperatures. Negative values indicate that digital temperatures exceeded skin temperatures of arm or thigh.

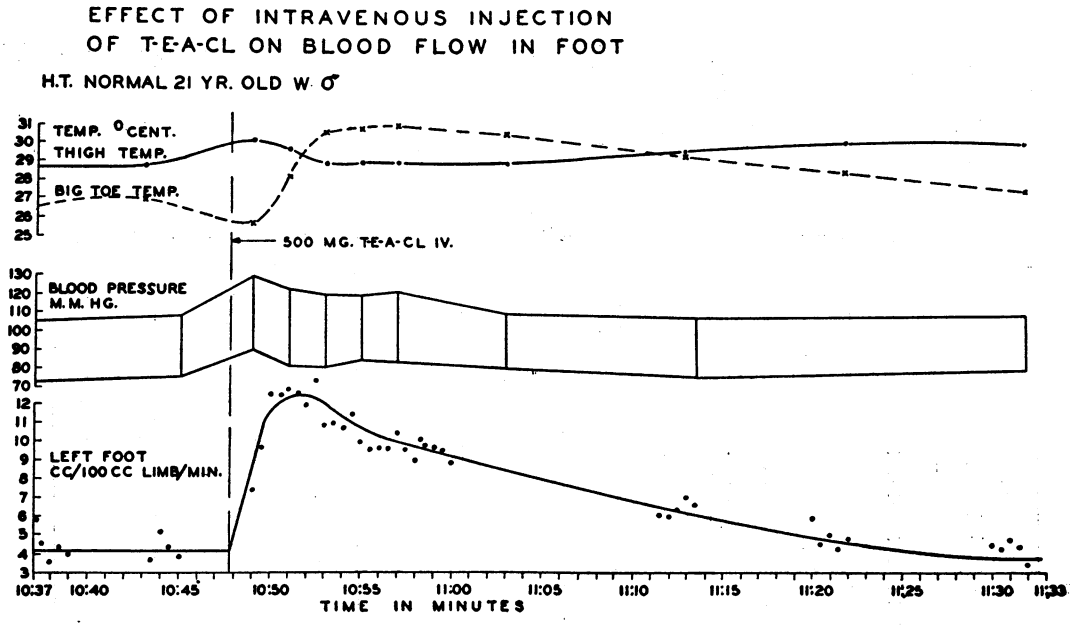

FIG. 1. TYPICAL RESPONSE OF NORMAL SUBJECT TO INTRAVENOUS INJECTION OF 500 MG. OF TETRAETHYLAMMONIUM CHLORIDE

monium chloride[5] on peripheral blood flow and skin temperature. After the rapid intravenous injection of the drug, the blood flow in the foot always increased; that in the hands usually rose; while calf and forearm blood flows were unchanged or increased only slightly. In the few female subjects studied there was a tendency for the drug to increase the blood flow in the foot and hand, more than in normal male subjects. The skin temperature gradient between toe and thigh was always reduced and often abolished while the temperature gradient between finger and forearm was less regularly affected. A typical response is shown in Figure 1. Foot volume usually increased after the administration of the drug and reached its

[5] We are indebted to the Department of Clinical Investigation, Parke, Davis & Co., for the supply of "Etamon" used in these studies.

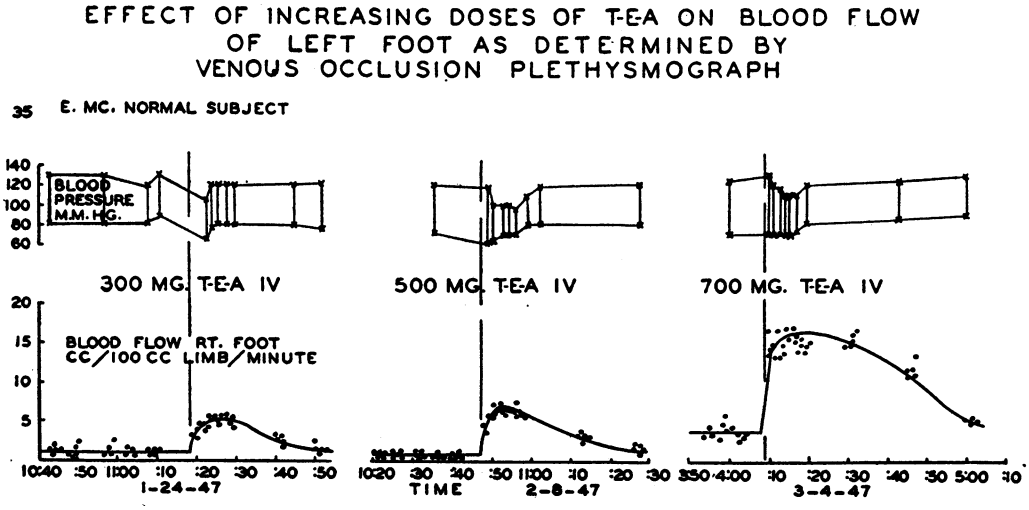

FIG. 2. INCREASE IN BLOOD FLOW IN THE FOOT AFTER TETRAETHYLAMMONIUM IS APPROXIMATELY PROPORTIONAL TO THE DOSE ADMINISTERED

peak at the time of maximum flow. The systemic blood pressure did not fall markedly in any of the normotensive subjects studied, but pulse rate increased moderately after the drug. It was shown that tachycardia per se apparently had no effect on the plethysmographic measurement since comparable increases in pulse rate induced by the administration of atropine, 1 mg. intravenously to three normal subjects, had no significant effect on the foot blood flow.

2. Effects of dosage and route of administration

In three normal subjects it was demonstrated that greater and more prolonged increases in foot blood flow were obtained when 500 mg. rather than 300 mg. of the drug were given intravenously. A 500 mg. dose was not exceeded except in one case in which 700 mg. produced a greater rise in blood flow than 500 mg. (Figure 2). In one subject the intramuscular injection of 20 mg. per kg. resulted in a more prolonged but no greater rise in blood flow than a 500 mg. dose given intravenously.

3. Effect of tetraethylammonium on reflex vasomotor changes

Following the injection of tetraethylammonium, the reflex vasoconstrictor response to pinprick and mental arithmetic was reduced or absent. Respiratory and other rhythmic changes in limb volume were reduced (Figure 3). Reflex vasoconstriction induced by suddenly immersing an opposite extremity in ice water was likewise obliterated by tetraethylammonium (Figure 4).

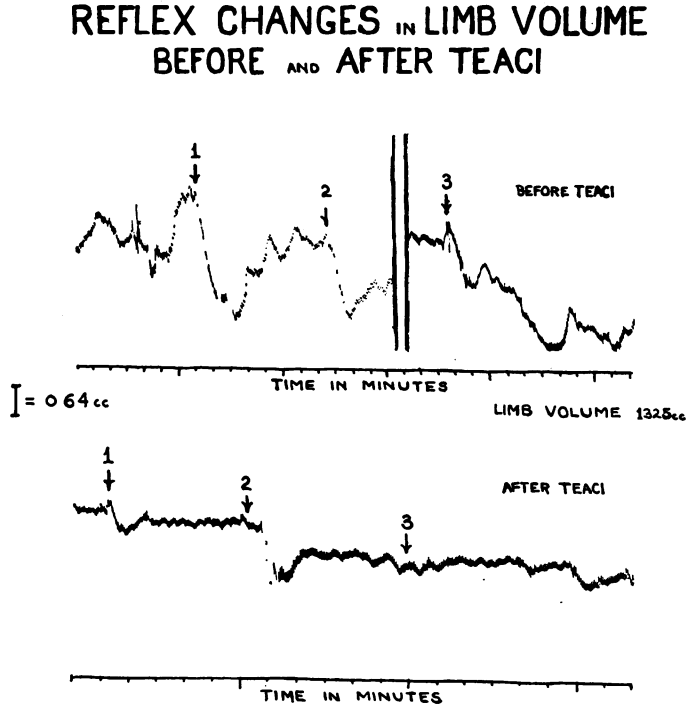

FIG. 3. REDUCTION IN REFLEX CHANGES IN LIMB VOLUME AFTER AUTONOMIC BLOCKADE WITH TETRAETHYLAMMONIUM

The upper tracing is a control plethysmographic observation. The lower record was obtained eight minutes after the administration of 500 mg. of tetraethylammonium chloride intravenously. At 1 and 2 a painful stimulus (pin prick) was applied. At 3 the patient was given a problem in mental arithmetic to perform.

Note the reduction in rhythmic volume changes and in the response to various stimuli after autonomic block.

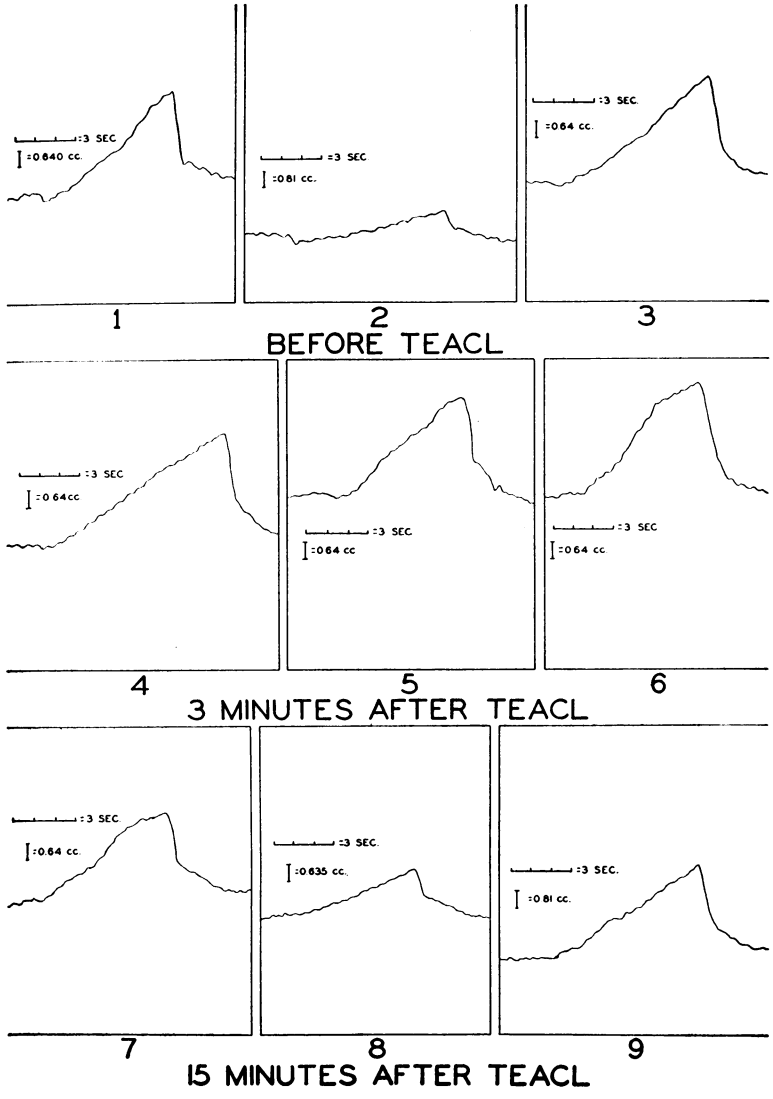

FIG. 4. BLOCKADE OF REFLEX VASOCONSTRICTION TO COLD STIMULUS AFTER AMMONIUM

Representative inflow curves in the hand have been selected from the plethysmographic tracings of a normal subject before and after 500 mg. of tetraethylammonium chloride intravenously. Numbers 2, 5, and 8 were recorded 60 seconds after immersion of the opposite hand in ice water. The tracings on the left and right represent inflow curves one minute before and 1½ minutes after cessation of the ice stimulus.

It will be noted that the reflex vasoconstriction apparent in the first series of tracings was abolished three minutes after tetraethylammonium chloride and had begun to return 15 minutes after the injection.

4. Effect of tetraethylammonium compared to that of other vasodilating procedures

(a) Heat

In seven normal subjects vasomotor tone was reduced by heating the body and three extremities under an electric blanket for 30 to 45 minutes until marked diaphoresis occurred and no further increases in blood flow in the foot could be produced. The increases obtained by this procedure showed considerably greater variation and were somewhat less than after a single injection of 500 mg. of tetraethylammonium (Figure 5). When the drug

was given at the point of maximum reflex vasodilatation, only minimal increases in blood flow were observed.

(*b*) *Vasodilating drugs*

Observations on the effect of nicotinic acid, papaverine, aminophyllin, and nitroglycerin on foot blood flow were made in several normal male subjects in whom the effects of tetraethylammonium had also been studied. These drugs had far less effect than did tetraethylammonium given to the same subjects (Figure 5).

(*c*) *Lumbar paravertebral block*

In four instances lumbar paravertebral block was induced with metycaine before or after the response of the vascular bed in the extremity to tetraethylammonium had been tested. The block was usually slightly more than twice as effective as tetraethylammonium in increasing blood flow to the foot, and the hyperemia lasted from three to four times as long (Figures 5, 6). In cases where block appeared incomplete, as judged by a delayed or incomplete rise in toe temperature before placing the foot in the plethysmograph, and by a less than usual increase in blood flow, injection of tetraethylammonium resulted in further vasodilatation. Also when sympathetic block had begun to wear off, as manifested by a decline in blood flow in the foot, an increase in blood flow could be obtained by an injection of tetraethylammonium.

5. Effect of tetraethylammonium on the sympathectomized extremity

In one patient with early Buerger's Disease, tetraethylammonium increased blood flow in the foot from 3 cc. to 10 cc. per 100 cc. of limb volume per minute. After lumbar sympathectomy blood

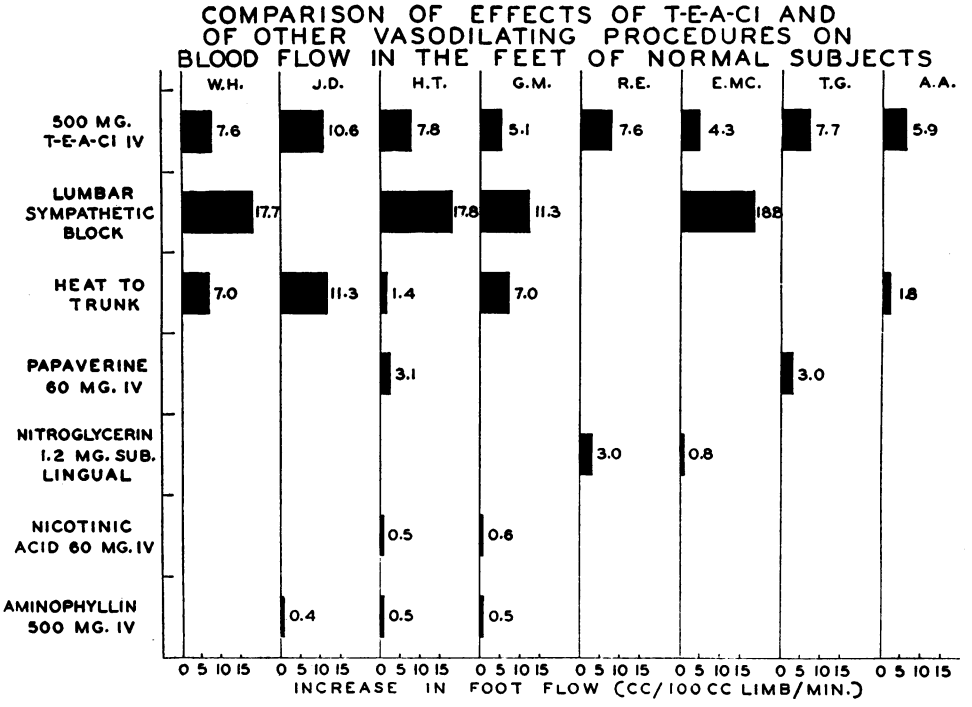

FIG. 5

Length of the solid bars indicates the increase in blood flow after the various procedures described in the left-hand margin of the figure. The increase in foot blood flow in centimeters per 100 cc. foot volume per minute is expressed numerically beside each bar. Experiments performed in the same individual on different days are arranged in vertical columns beneath the subject's initials.

It will be observed that paravertebral block was the most effective vasodilating procedure, that tetraethylammonium chloride and reflex body heating produced about one-half as great an increase in foot blood flow and that certain common vasodilator drugs were considerably less effective.

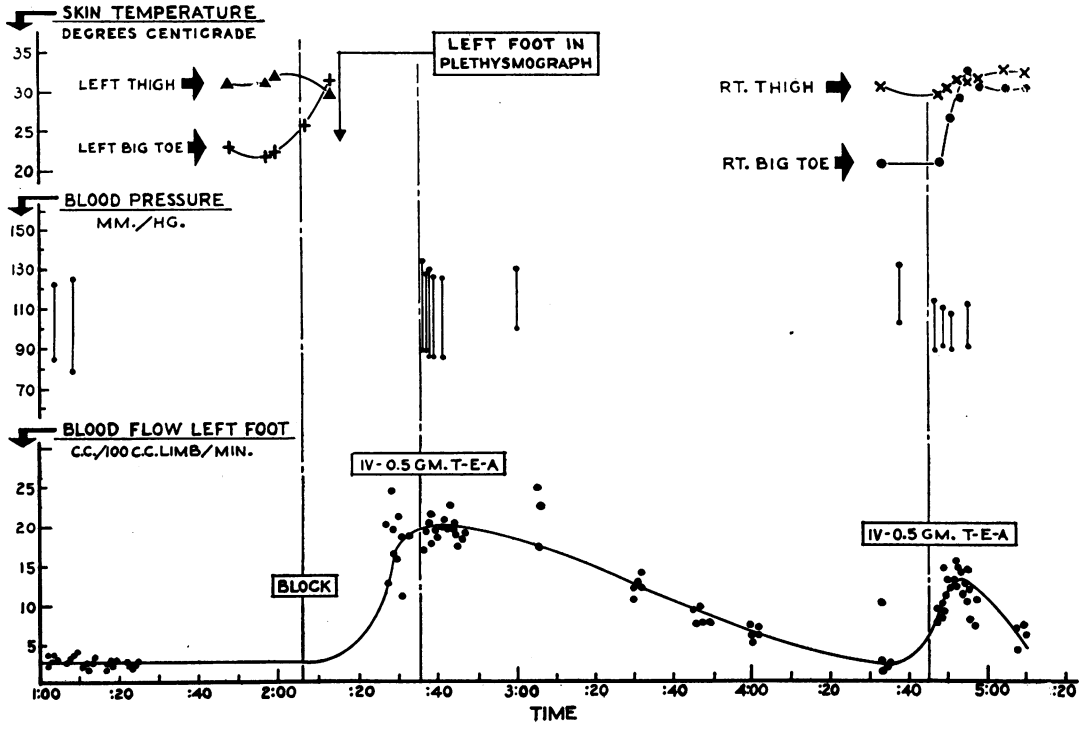

FIG. 6. COMPARATIVE EFFECT OF TETRAETHYLAMMONIUM AND OF LUMBAR PARAVERTEBRAL BLOCK WITH METYCAIN ON FOOT BLOOD FLOW IN NORMAL SUBJECT

Note that at height of sympathetic block administration of tetraethylammonium produced no significant change in blood flow but that after the effects of the metycain had worn off, tetraethylammonium produced vasodilatation of somewhat lesser magnitude and duration.

flow averaged 5 cc. per minute but injection of the drug was now without vasodilator effect (Figure 7). On 12 other occasions we have observed that sympathectomy has abolished the vasodilator response to tetraethylammonium. These observations have been made four times with respect to blood flow in the hand and eight times with respect to blood flow in the foot, and as early as the second day after operation and as late as the ninth month following sympathectomy.

DISCUSSION

Tetraethylammonium significantly increased blood flow to the foot in every normal subject studied; it produced vasodilatation in the hand in seven of eight instances; while circulation in the forearm and calf increased only slightly in most of the subjects. These plethysmographic observations correspond to those of Slaughter, Brown, and Wakim (10) who used an air displacement plethysmograph and measured circulatory changes in the hand and forearm or foot and calf.

The increases in temperature following tetraethylammonium provide confirmatory evidence of the peripheral vasodilator action of the drug. This occurred regularly in the toes and frequently in the fingers. Elevation in skin temperature in the extremities of subjects with vasospastic peripheral vascular disease has been reported previously (4–7). On the other hand, Boyd *et al.* (11), and DeBakey *et al.* (12) have found elevations of the digital temperature to occur less consistently both in the normal and in the subject with peripheral vascular disease. We are unable to explain these discrepancies but we believe that the plethysmographic observations herewith reported provide convincing evidence of the vasodilator action of the drug, at least so far as the blood flow in the entire hand or foot are concerned.

Since tetraethylammonium no longer produces vasodilatation after an extremity has been sympathectomized, it follows that the drug acts by altering sympathetic tone and not by any direct vasodilator action on the blood vessels. This conclusion

is further supported by the observations that the vasodilator response is reduced or abolished when vasoconstrictor tone is reduced by body heating and by the observation that vasoconstrictor reflexes following pain, cold, and mental activity are reduced or abolished during the period of action of the drug. Finally, the less marked increases in blood flow in the forearm or calf following tetraethylammonium can be readily explained by the relative lack of vasomotor tone to these vascular areas (8). Our clinical findings, therefore, are not at variance with the results of investigations performed in animals (1, 2). While it is impossible to prove the ganglionic site of action in the human subject as in the animal, it seems most likely that blockade of sympathetic impulses occurs in this location.

If it may be assummed from our studies that lumbar paravertebral block produced complete inhibition of vasomotor tone in the foot, the maximum blood flow under these conditions averaged 17.6 cc. per 100 cc. of foot volume in four normal subjects. This figure may be compared to an average maximum flow of 9.9 cc. after administration of 500 mg. of tetraethylammonium. It would appear that the increase in blood flow after this drug represented about one-half the possible increase had a complete chemical block been established. In larger doses it is probable that a more complete blockade could have been achieved since in one subject the response to the injection of 700 mg. of the drug was greater than after the administration of 500 mg. and approximated the degree of vasodilatation to be expected from complete sympathetic block. However, we do not believe that in the average human subject more than 500 mg. can be administered with safety by the intravenous route. The failure to achieve greater inhibition of sympathetic tone was somewhat unexpected since the autonomic nervous system of the dog can apparently be blocked almost completely to preganglionic faradic stimulation for a brief interval by doses of 4 to 8 mg. per kg. (3). If this were applied to a human subject weighing 60 kg., it would

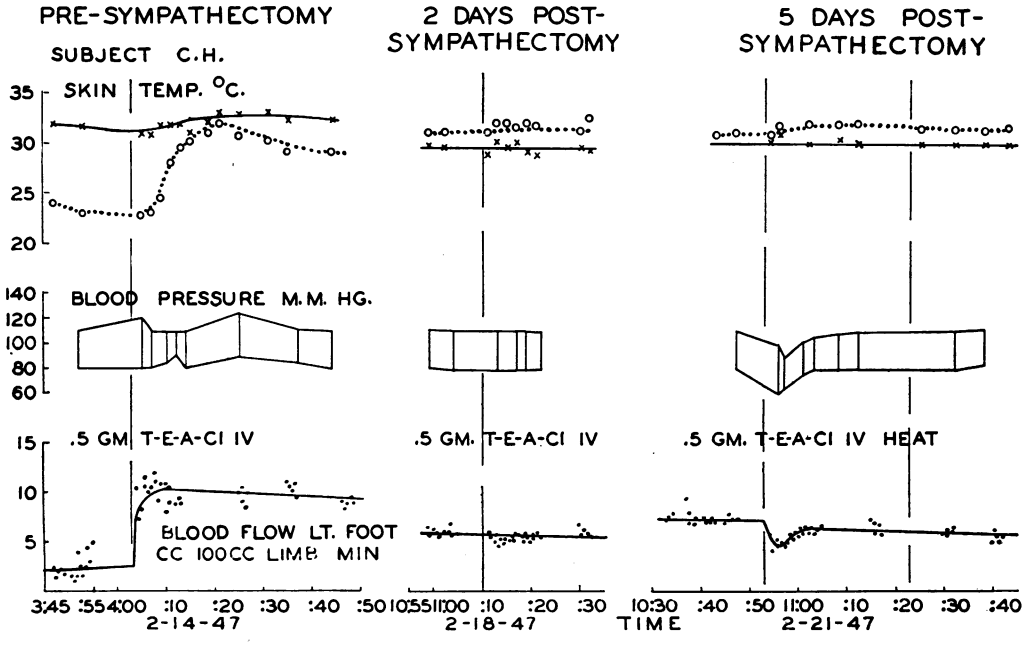

FIG. 7. EFFECT OF TETRAETHYLAMMONIUM ON BLOOD FLOW BEFORE AND AFTER SYMPATHECTOMY

Vasodilator response to tetraethylammonium occurring prior to sympathectomy in a patient with early Buerger's Disease was abolished by the operation. Failure to increase blood flow in sympathectomized extremity indicates that the drug has no direct vasodilator action in this dosage.

The slight decline in blood flow after tetraethylammonium on fifth post-operative day was probably the result of parallel decline in blood pressure. It will be noted that body heating likewise failed to produce vasodilatation on the fifth day following sympathectomy.

mean that 480 mg. given intravenously should have produced a temporary but relatively complete blockade.

Gibbon and Landis (13) and Coller and Maddock (14) have utilized reflex heat vasodilatation as a means of inhibiting sympathetic vasomotor tone. The degree of vasodilatation achieved was measured by alterations in the digital temperature. The extent of the peripheral vasodilatation, as measured in the plethysmograph, which could be induced by prolonged body heating in five normal subjects was more variable and of a lesser magnitude than the response to 500 mg. of tetraethylammonium. Despite the frequent obliteration of the temperature gradient between toe and thigh following both these procedures, the increases in blood flow were far less than after paravertebral sympathetic block. It would appear from these observations that reflex heat vasodilatation likewise does not produce a complete release of vasomotor tone and the abolition of a skin temperature gradient does not necessarily mean that a complete sympathetic blockade has been achieved.

While we made no extensive comparative studies of the effects of various vasodilator drugs on blood flow in the foot, a few observations utilizing the plethysmographic technique have convinced us that, at least in the normal subject, measures involving an inhibition in vasoconstrictor tone result in significantly greater increases in blood flow than do those vasodilating agents which act on the peripheral vessels directly. The increase in blood flow following papaverine, nitrites, nicotinic acid, and aminophyllin given intravenously was far less than that seen after lumbar sympathetic block, tetraethylammonium, or reflex heat vasodilatation. The extensive studies of Abramson and his associates (8) on the effect of various vasodilator drugs and of Gubner *et al.* (15) on the effects of glycine would suggest that increases in the peripheral blood flow of the magnitude which followed tetraethylammonium are most uncommon.

CONCLUSIONS

1. Tetraethylammonium in doses of 500 mg. intravenously produces an average seven-fold increase in foot blood flow, a four-fold increase in hand blood flow, and slight increases in forearm and calf blood flows in the normal subject as measured by the venous occlusion plethysmograph. Digital skin temperatures are increased and the temperature gradient between thigh and toe and forearm and finger are usually abolished or greatly reduced.

2. The drug does not produce vasodilatation in the sympathectomized extremity. Its vasodilator action is therefore the result of the inhibition of sympathetic vasoconstrictor tone.

3. Sympathetic blockade is probably incomplete with the usual doses of tetraethylammonium tolerated by the human subject. The vasodilatation in the foot, which follows 500 mg. of the drug, is about 50 per cent of that observed after lumbar paravertebral block.

4. In the foot of the normal subject, the increase in blood flow is of much greater magnitude after tetraethylammonium than after aminophyllin, papaverine, nicotinic acid, and nitroglycerin and is slightly greater than the vasodilatation following prolonged body heating.

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