5. Mosoro, E. J., Chaikoff, I. L., Chernick, S. S., Felts, J. M., *J. Biol. Chem.,* 1950, v185, 845.

6. Kerby, M., Ottoway, J. H., *J. Physiol.,* 1954, v123, 534.

7. Whitney, J. E., Roberts, S., Beaver, E. L., *Am. J. Physiol.,* 1955, v182, 51.

8. Astwood, E. B., Harvey Lectures, 1944-45, v45, 195.

9. Umbreit, W. W., Burris, R. H., Stauffer, J. F., Manometric techniques and selected methods for study of tissue metabolism. Minneapolis, Burgess, 1945.

10. Edson, N. L., *Biochem. J.,* 1935, v29, 2052.

11. Good, C. A., Kramer, H., Somogyi, M., *J. Biol. Chem.,* 1933, v100, 485.

12. Hagedorn, H., Jenson, B. N., *Biochem. Z.,* 1923, v35, 46.

13. West, E. S., *J. Biol. Chem.,* 1927, v74, 1927.

14. Nath, M. C., Chitale, R. P., Belavady, B., *Nature,* 1952, v170, 545.

15. Tepperman, J., Tepperman, H. M., Schulman, M. P., *J. Physiol.,* 1956, v184, 80.

16. Frends, C. E., Ingram, R. H., Uran, J. A., Borron, G. P., Swift, R. W., *J. Nutr.,* 1953, v51, 329.

17. Barker, S. B., *Physiol. Rev.,* 1951, v31, 205.

18. Nath, M. C., Khanade, J. M., *J. Sci. Ind. Res.,* 1959, v18c, 169.

19. Kennelly, B., Maynard, L. A., *J. Nutr.,* 1953, v49, 599.

Received July 15, 1960. P.S.E.B.M., 1960, v105.

Effect of Mephentermine on Venomotor Tone, Blood Flow and Arterial Pressure in Forearm of Man.* (26179)

A. W. HORSLEY† AND JOHN W. ECKSTEIN‡ (Introduced by Henry E. Hamilton)
Dept. of Internal Medicine, State University of Iowa College of Medicine, Iowa City

Mephentermine sulfate§ is used effectively in treatment of hypotension(1-3), but there is confusion regarding the means by which the drug restores arterial pressure. A pressor amine may elevate arterial pressure by increasing arteriolar tone, increasing cardiac output or a combination of both(4). Welch *et al.* reported that mephentermine had little effect on peripheral arteriolar resistance in the dog but exerted its effect primarily on myocardial contractility(4). Several authors reported an increase in myocardial contractility after administration of mephentermine without reference to its action on the peripheral arterioles(5,6). Brofman *et al.*(3) found little change in cardiac output in man and dogs and attributed the elevation in arterial pressure to increased peripheral resistance. Aviado(7) reported vasodilation in the dog after intraarterial injection of mephentermine but Borden found vasoconstriction(8).

The effect of a pressor amine on cardiac output will depend not only on response of the myocardium, but also upon the changes induced in venous tone. Venous constriction with a shift of blood from the periphery would increase amount of blood available to the heart and help increase output. Data regarding the effects of mephentermine administration on venous tone in man are not available.

Because of these discrepancies, and since there may be a species difference, it seemed important to study the effect of mephentermine on forearm venous tone and blood flow in man.

Method. Normal male students were studied while lying supine. Room temperature was maintained at 79-81°F and every effort was made to keep the subjects comfortable. They remained in the recumbent position for at least 60 minutes before experiments were begun. Arterial pressure was measured intermittently with a cuff on the right arm. Venous pressure was measured in the antecubital vein of the dependent right arm with a strain gauge. The data were ana-

* Supported by research grant from Nat. Heart Inst., P.H.S., and from Iowa Heart Assn.

† Research Fellow of Am. Coll. of Physicians.

‡ Established Investigator of Am. Heart Assn.

§ Wyamine®, Wyeth Labs., Philadelphia, Pa.

lyzed by the methods described by Fisher (11).

Blood flow was measured in the left forearm of 5 subjects by venous occlusion plethysmography (9,10). Temperature of water in the plethysmograph was maintained at 32°C. A cuff was placed distal to the plethysmograph and inflated above systolic pressure during measurements to exclude the circulation in the hand. The congesting cuff was placed close to proximal end of the plethysmograph and optimal collecting pressure, usually about 40 mm Hg, was selected by making preliminary flow measurements. Rates of change of arm volume were recorded with a sensitive pressure transducer, which measured changes in water level in a cylinder attached to the top of the instrument. Blood flow was calculated in ml/min/100 ml of forearm tissue. Flow measurements were made until a steady state was achieved. Control values were then obtained over a 20 minute period by making 4 flow measurements for each 5 minute period. The control value for each experiment is the average of all control measurements. Blood flow measurements after mephentermine were made at the rate of 3 per minute and values reported are averages of flows during the second, third and fourth minute following drug administration. This period includes the peak response but avoids giving undue weight to single observations. Mean blood pressure was calculated by adding one-third of pulse pressure to diastolic pressure. Observations after mephentermine are average values obtained during the second, third and fourth minute after single intravenous injection of 25 mg of the drug. Peripheral resistance was calculated by dividing mean pressure in mm Hg by flow in ml/100 ml for forearm tissue and expressed in arbitrary units.

Venous tone was measured in 5 subjects by the plethysmographic method previously described (12). This method yields information which permits construction of venous pressure-volume curves. These curves express in ml/100 ml of forearm tissue the volume of blood in the forearm veins at any level of transmural pressure between 0 and 30 mm Hg. The final point on the curve, venous volume at 30 mm Hg, is termed arbitrarily *venous distensibility*. A high value represents dilatation or reduced venous tone, a low value represents constriction or increased tone. Since venous pressure-volume characteristics are the same in both arms, the naturally occurring venous volume of the forearm is the volume co-ordinate at the point on the curve which corresponds to venous pressure measured in the arm not in the plethysmograph. This volume co-ordinate is determined by drawing a perpendicular from the point on the pressure axis which corresponds to the level of measured venous pressure. Four control observations were made over a 30 minute period and average values are reported. The recorded forearm venous responses after drug administration represent maximal changes observed after injection of 25 mg.

Results. (Tables I and II) Arterial pressure increased in each experiment. Mean blood pressure increased by an average of 30 mm Hg ($p<0.01$) or 31.8%. Venous pressure increased in each experiment. The average change was 1.7 mm Hg ($p<0.02$) or 13.2%.

TABLE I. Forearm Venous Responses to Mephentermine Administration.

Exp.	Venous pressure, mm Hg		Venous distensibility, mm/100 ml		Venous vol, mm/100 ml	
	Before	After	Before	After	Before	After
1	11.0	12.5	4.5	4.1	2.5	2.0
2	19.0	20.0	6.0	4.8	4.8	3.7
3	12.8	16.0	3.8	3.2	2.6	2.3
4	11.5	13.5	4.7	3.7	2.7	2.3
5	15.0	16.0	4.5	3.5	3.0	2.3
Mean	13.9	15.6	4.7	3.9	3.1	2.5
Stand. dev.	3.26	2.90	.81	.62	.96	.67
Mean diff.	+1.7		−.8		−.6	
Stand. error	.41		.14		.14	
Probability	<.02		<.01		<.02	

TABLE II. Effect of Mephentermine on Forearm Blood Flow and Arterial Pressure.

Exp.	Forearm blood flow, ml/100 ml		Blood pressure, mm Hg		Resistance, arbitrary units*	
	Before	After†	Before	After	Before	After
1	4.0	3.2	98	115	24.5	35.9
2	2.0	1.5	86	104	43.0	69.3
3	2.3	1.8	95	130	41.3	72.2
4	4.3	1.7	95	131	22.0	77.0
5	4.6	2.2	98	143	21.3	65.0
Mean	3.4	2.1	94	125	30.4	63.9
Stand. dev.	1.2	.7	4.9	15.2	10.8	16.2
Mean diff.	−1.4		+30.0		+33.5	
Stand. error	.47		5.47		7.46	
Probability	<.05		<.01		<.02	

* Ratio of mean arterial pressure to blood flow.
† Avg values for 2nd, 3rd, and 4th min. after drug admin.

Venous distensibility decreased in each of the 8 experiments. Average decrease in distensibility was 0.8 ml/100 ml of forearm tissue ($p<0.01$), a change of 22.1%. The naturally occurring venous volume decreased from an average of 3.1 ml/100 ml to 2.5 ml ($p<0.02$), a decrease of 18.5%. Even though the pressure distending the veins increased, venous constriction was sufficient to shift blood from the forearm. Blood flow for the second, third and fourth minutes after drug administration decreased in each of the 5 experiments. Decrease in flow averaged 26.6% or 1.4 ml/100 ml of forearm tissue ($p<0.05$). Peak reduction in flow occurred within 1 to 2 minutes and returned to near control levels within about 10 minutes.

Calculated peripheral resistance increased from an average of 30 units during control periods to 64 units after mephentermine administration ($p<0.02$).

Discussion. These results show that forearm blood flow decreases in normal men after intravenous administration of mephentermine. Rapid single injections of the drug were used for this study but similar results were obtained in one subject when the experiment was repeated with a 5 minute intravenous infusion of mephentermine. These observations are in agreement with Borden (8) and Brofman(3). They do not indicate, however, that the same response should or would be expected in the subject with hypotension. In many forms of acute hypotension, there may be loss of venomotor tone and pooling of blood in the periphery(13). This reduces venous return and leads to a fall in cardiac output. Mephentermine produces peripheral venous constriction with a shift of blood from the periphery as these experiments show. Since it also increases contractility(4, 5,6), it would be expected to restore cardiac output. This increase in total blood flow could restore arterial pressure without an associated increase in arterial resistance.

We measured cardiac output in 14 normotensive and hypotensive dogs and found that mephentermine caused it to increase in each instance. In 5 dogs, however, arterial pressure increased appreciably immediately after the drug before cardiac output changed. This indicates that mephentermine produced an increase in arteriolar tone initially. The later effect was increased resistance as well as increased arterial pressure and total blood flow.

The changes in forearm venous tone and blood flow after mephentermine could be due in part to an effect on the central nervous system, a direct action on the vessel or reflex neural adjustment secondary to an alteration in cardiac output. Swaine(15) reported evidence that mephentermine increases cardiac output primarily by releasing catechol amines. He suggested that this effect was limited to the myocardium and did not influence response of the peripheral vessels. If mephentermine exerts its effect on the myocardium by releasing catechol amines, it might act in the same way on the peripheral vessels. Such a hypothesis would be plausible since norepinephrine has been reported to produce venous constriction(12) and to reduce blood flow in the extremity(16).

Summary. Forearm blood flow and venomotor responses to intravenous injection of mephentermine were studied in normal men. In each experiment blood flow decreased

while arterial pressure increased. Venous pressure increased while forearm venous distensibility and venous volume decreased in each subject. In each case blood shifted from the forearm. These experiments indicate that mephentermine administration increases both peripheral resistance and venous tone in man.

1. Hellerstein, H. K., Brofman, B. L., Caskey, W. H., *Am. Heart J.*, 1952, v44, 407.
2. Blundell, A. E., Pillon, J. W., Ansbro, F. P., *Anesth. and Analg.*, 1956, v35, 483.
3. Brofman, B. L., Hellerstein, H. K., Caskey, W. H., *Am. Heart J.*, 1952, v44, 396.
4. Welch, G. H., Braunwald, E., Case, R. B., Sarnoff, S. J., *Am. J. Med.*, 1958, v24, 871.
5. Gazes, P. C., Goldberg, L. I., Darby, T. D., *Circ.*, 1953, v8, 883.
6. Goldberg, L. I., Cotten, M., Darby, T. D., *J. Pharmacol. and Exp. Therap.*, 1953, v108, 177.
7. Aviado, D. M., *Anesth.*, 1959, v20, 71.
8. Borden, C., Haddy, F. J., *Clin. Res.*, 1959, v7, 237.
9. Wilkins, R. W., Eichna, L. W., *Bull. Johns Hopkins Hosp.* 1941, v68, 425.
10. Eckstein, J. W., Wood, J. E., Wilkins, R. W., *Am. Heart J.*, 1957, v53, 455.
11. Fisher, R. A., *Statistical Methods for Research Workers*, 10th Ed., Oliver and Boyd Ltd., Edinburgh, 1946.
12. Eckstein, J. W., Hamilton, W. K., *J. Clin. Invest.*, 1957, v36, 1663.
13. Weil, M. H., *Circ.*, 1957, v16, 1097.
14. Horsley, A. W., Eckstein, J. W., *Clin. Res.*, 1959, v7, 238.
15. Swaine, C. R., Perlmutter, J., Ellis, S., *Fed. Proc.*, 1960, v19, 122.
16. Doyle, A. E., Fraser, J. R., Marshall, R. J., *Clin. Sci.*, 1959, v18, 441.

Received July 15, 1960. P.S.E.B.M., 1960, v105.

A Species Comparison of Serum Proteins and Enzymes by Starch Gel Electrophoresis.* (26180)

S. H. LAWRENCE, P. J. MELNICK AND H. E. WEIMER
(Introduced by Charles M. Carpenter)
Biochemistry Research Laboratory and Laboratory Service, San Fernando Veterans Hospital, and Depts. of Infectious Diseases and Pathology, University of California, Los Angeles

Employment of starch gel electrophoresis as a means of protein separation has found wide application. Serum proteins(1), hormones(2), serum(3,4) and tissue(5) enzymes have been characterized by this technic. Although several recent investigations have been concerned with the enzymes of human serum(3,4), little information is available on the enzymes of laboratory animals. Protein migration in a number of animals(6-11) and esterase migration in starch block in several species(12) have been published. In the present study the occurrence and distribution of esterase, cholinesterase, aminopeptidase, cytochrome oxidase, beta-glucuronidase, acid and alkaline phosphatase and ceruloplasmin oxidase in the sera of man and 6 species of experimental animals are compared. Succinic, lactic, beta-hydroxybutyric and glutamic dehydrogenases were also studied in human serum. To provide a broader basis of comparison and for orientation, serum protein patterns are also included.

Materials and methods. Species studied and number of individuals in each follow: Man (Caucasian) 30, monkey (Cynomologus from Philippines) 3, dog (mongrel) 3, rabbit (New Zealand White) 8, guinea pig (hybrid) 8, rat (Sprague-Dawley) 8, mouse (Webster strain of Swiss) 10. All subjects were adult males.

Starch gel electrophoresis was carried out under conditions described by Smithies(1). Specimens were absorbed on filter paper and inserted in a slot 10 cm from the cathode in parallel with a specimen of human serum. Following electrophoresis, the gels were sliced in half longitudinally. One half was stained for protein with Amido-black 10 B and enzymatic activity was determined in the other (4). Gels were photographed and schematic representations prepared. Differences in mi-

* Supported in part by a grant from California Tuberculosis and Health Assn.