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Research Article

THE EFFECT OF HEXAMETHONIUM BROMIDE ON SPLANCHNIC BLOOD FLOW, OXYGEN CONSUMPTION AND GLUCOSE OUTPUT IN MAN

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The fall in blood pressure following autonomic blockade with methonium compounds could be attributed to a fall in cardiac output or to vasodilatation. In the recumbent position, however, cardiac output is usually well-maintained (1, 2, 3, 4) and blood flow through brain (5) and kidneys (1, 6) is not greatly altered. Increased blood flow has been reported in the digits and feet (1). This would hardly account for the large fall in blood pressure, and it seemed possible that the splanchnic area might be the site of the major vasodilatation. There is little information concerning splanchnic blood flow after hexamethonium. Freis and associates (1) state that it is not significantly changed, while Hoobler is said to have found a fall after tetraethylammonium chloride (7). We have therefore studied the effect of hexamethonium on splanchnic blood flow in subjects without liver disease. The opportunity has also been taken to make observations on the metabolic effects of hexamethonium on splanchnic oxygen consumption, hepatic glucose output and arterial lactic acid concentration.

METHODS AND MATERIAL

The 17 subjects had no known hepatic dysfunction or disturbance of carbohydrate metabolism, and had not previously received hexamethonium. Five had essential hypertension with diastolic blood pressures between 90 and 110 mm. Hg. Studies were made in the morning after an overnight fast, and one hour after an oral dose of 0.2 Gm. sodium amytal. A radio-opaque nylon catheter was introduced under fluoroscopic control into a radicle of the hepatic vein, and the subjects were then returned to a comfortable position in bed with the head on one small pillow. A priming dose of bromsulphalein (B.S.P.) was given and B.S.P. (4.5 to 7.0 mg. per min.) was infused at a constant rate. An in-dwelling needle was placed in a brachial or femoral artery. Thirty to forty minutes were allowed for stabilization; simultaneous hepatic vein and arterial samples were then taken for determination of B.S.P., glucose, lactic acid and oxygen. Three or four such determinations were made over twenty minutes and the results averaged to obtain the control values for splanchnic blood flow, hepatic glucose output, arterial lactic acid concentration and arterial-hepatic venous oxygen difference. The brachial blood pressure (auscultatory) and pulse rate were recorded frequently during this time. When the circulatory state was reasonably steady, hexamethonium bromide (1 mg. per Kg. body weight) was injected intramuscularly and the determinations repeated 5, 15, 30, 45 and 60 minutes later. In six subjects, observations were continued at 20 minute intervals for a further hour.

Estimated splanchnic blood flow (E.S.B.F.) was calculated with appropriate corrections for changing plasma B. S. P. levels (8). Plasma levels over 1 mg. per cent were maintained in all instances and extraction by the liver always exceeded 15 per cent. Plasma B.S.P. levels tended to rise after hexamethonium because of the marked fall in E.S.B.F. Decreasing the infusion rate by 20 to 30 per cent at the time of giving the hexamethonium usually prevented this rise. If the B.S.P. level five minutes after hexamethonium had risen in spite of this, the determination was discarded.

Hepatic glucose output (HGO) was determined by multiplying the arterial-hepatic vein glucose difference by the E.S.B.F. For the control value the average of the three or four arteriovenous glucose differences was multiplied by the average value for blood flow. Intestinal and splenic metabolism of glucose is probably negligible in the fasting state (9, 10) and the term "hepatic" rather than "splanchnic" glucose output has therefore been used throughout.

"Mean" blood pressure was expressed as half the sum of the systolic and diastolic auscultatory levels. In certain subjects this was checked by direct tracings obtained by strain gauge from the in-dwelling arterial needle.

Overall splanchnic vascular resistance was calculated by dividing the "mean" blood pressure (mm. Hg) by the splanchnic blood flow (ml. per min.) and expressed in arbitrary units.

"Portal venous pressure" was measured with a strain gauge in six subjects before and after hexamethonium by

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the technique of "occluded" hepatic vein catheterization (11, 12, 13), the catheter being advanced deep into the substance of the liver so as to occlude an hepatic vein. A close correlation has been found between the pressure recorded in this position and the portal venous pressure (13). These six subjects remained recumbent on the padded fluoroscopy table throughout the study.

Blood samples were preserved with heparin and sodium fluoride. Glucose was measured on duplicate 0.2 ml. whole blood samples (14), bromsulphalein on 1 or 2 ml. of plasma (15) and lactic acid on 2 ml. of whole blood (16). Oxygen unsaturation was determined without delay on 5 ml. blood samples collected under paraffin using the Haldane blood gas apparatus (17). Plasma volume (18) and surface area were calculated from tables.

RESULTS

Changes in Splanchnic Circulation (Table I)

In 15 subjects the reaction to hexamethonium was similar (Figure 1).

The "mean" blood pressure fell within 5 minutes, and within 30 minutes had reached levels averaging 32 per cent below the control value. A fall in blood pressure occurred in all subjects, even though recumbent. The magnitude of the fall varied considerably, being greatest in those with the highest resting values.

Pulse rate increased by an average of 11 per cent of the resting value.

E.S.B.F. initially was slightly higher than previously reported values for normal subjects under similar conditions (15). After hexamethonium the E.S.B.F. fell, reaching an average of 67 per cent of the control value at the end of 30 minutes. The fall in E.S.B.F. paralleled the fall in "mean" blood pressure and the correlation coefficient, being more than six times its standard error, was therefore highly significant (Figure 2).

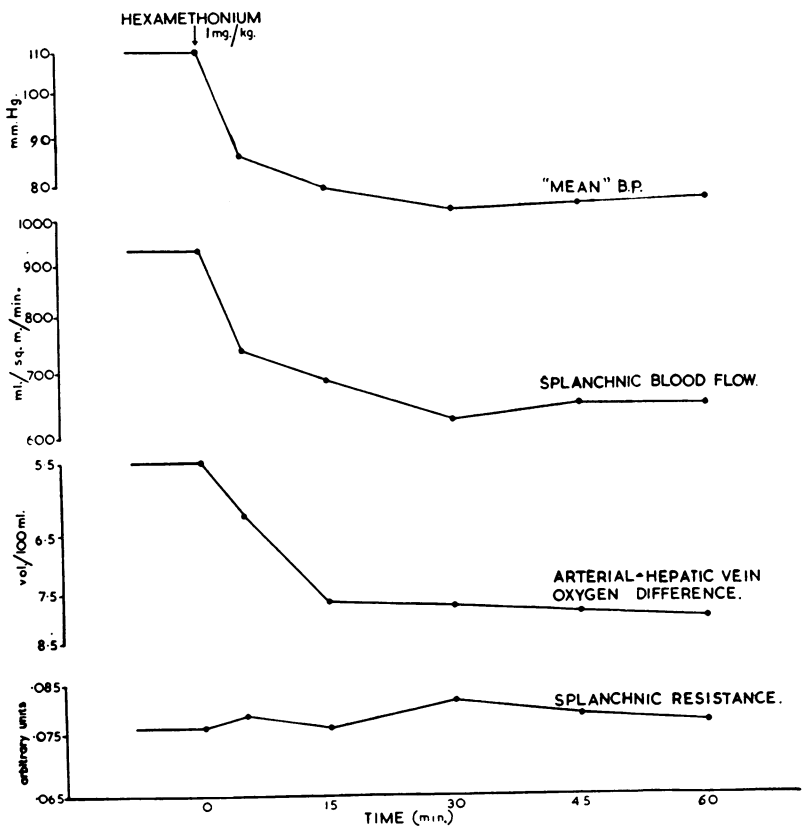

FIG. 1. MEAN RESULTS OF 15 OBSERVATIONS SHOWING EFFECT OF HEXAMETHONIUM ON "MEAN" B.P., SPLANCHNIC BLOOD FLOW (E.S.B.F.), ARTERIAL-HEPATIC VEIN OXYGEN DIFFERENCE AND SPLANCHNIC VASCULAR RESISTANCE

Semilogarithmic scale. The *increase* in arterial-hepatic vein oxygen difference is plotted downwards.

TABLE I

*Effect of hexamethonium on E.S.B.F., "mean" B.P., splanchnic vascular resistance and arterial-hepatic vein oxygen difference**

Subject	Age	Sex	E.S.B.F. (*ml./min./m²*) min. after C6						"Mean" B.P. (*mm. Hg*) min. after C6						Splanchnic Vasc. Resistance (*Arbitrary units*) min. after C6						Arterial-Hepatic Vein Oxygen Difference (*vol./100 ml.*) min. after C6					
			0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60
F. A.	35	M	940	960	880	950	1080	900	93	85	78	78	84	85	.054	.048	.048	.045	.042	.052	5.9	5.2	6.6	8.3	7.9	9.1
F. C.	42	M	1110	1070	800	810	—	820	90	83	91	87	86	88	.049	.046	.068	.065	—	.065	4.7	4.9	4.6	3.9	3.9	4.6
L. C.	46	F	720	400	430	350	490	520	125	90	68	63	61	60	.119	.155	.108	.123	.085	.079	5.3	6.4	8.4	7.8	8.3	7.1
E. C.	34	M	1070	—	1080	790	970	650	102	100	91	89	87	81	.059	—	.052	.069	.054	.075	—	—	—	—	—	—
J. D.	59	M	810	410	480	430	550	590	95	70	50	58	67	67	.068	.099	.060	.078	.070	.065	4.7	3.9	9.1	11.1	9.4	7.0
E. F.	61	M	950	850	870	820	930	950	95	83	84	75	75	65	.056	.054	.054	.051	.045	.038	5.8	5.5	6.4	5.5	6.7	8.6
J. M.	41	M	980	830	770	820	710	590	85	75	72	64	60	55	.056	.058	.060	.051	.055	.061	4.5	7.8	7.2	7.8	7.0	8.7
C. M.	33	M	960	—	840	720	710	810	105	95	94	95	92	93	.065	—	.066	.077	.077	.068	4.4	4.8	5.0	5.0	5.0	6.0
M. N.	69	F	660	490	480	440	—	—	120	105	102	87	80	75	.120	.132	.139	.130	—	—	5.9	5.1	9.9	8.0	—	6.8
A. T.	52	F	870	—	610	380	—	—	105	65	53	50	60	70	.073	—	.053	.080	—	—	4.6	—	9.5	10.2	8.7	8.2
E. W.	62	F	760	460	490	430	340	460	148	92	80	69	69	72	.119	.124	.101	.099	.125	.097	5.2	9.4	7.6	9.4	11.8	12.5
M. W.	51	F	1930	2170	1240	990	1030	790	150	127	110	100	95	90	.059	.044	.066	.076	.069	.086	4.6	4.7	5.2	5.1	4.9	5.0
A. Y.	71	M	760	540	440	460	—	570	127	68	75	75	76	85	.095	.072	.097	.093	—	.086	5.8	8.2	7.8	7.4	—	9.0
P. T.	52	M	640	—	370	450	420	470	110	78	70	70	70	72	.110	—	.119	.098	.106	.097	9.4	10.1	10.6	10.7	10.9	12.1
T. S.	41	M	810	—	510	450	360	—	99	74	68	69	70	—	.072	—	.079	.091	.113	—	5.7	—	9.4	8.9	10.4	—
Mean:			930 ±80*	820 ±170	690† ±68	620† ±59	690† ±70	680† ±55	110 ±5.2	86† ±4.2	79† ±4.4	75† ±4.0	75† ±3.7	76† ±2.1	.078 ±.007	.083 ±.013	.078 ±.007	.082 ±.006	.076 ±.008	.072 ±.005	5.5 ±.34	6.3 ±.59	7.7† ±.52	7.8† ±.60	7.9† ±.72	8.0† ±.65
T. A.‡	49	F	1510	1270	1060	1720	2110	2040	107	90	73	70	73	83	.045	.045	.043	.026	.022	.026	4.2	3.1	5.3	3.8	3.5	3.2
H. H.‡	69	M	1010	—	950	1210	1230	1220	92	75	79	73	70	70	.044	—	.041	.029	.028	.028	5.6	6.5	4.1	3.5	3.2	5.1

* In this and Table III ± values are standard errors of the mean.
† Differs significantly ($p < .05$) from control value.
‡ Subjects T. A. and H. H. reacted in an anomalous manner (see text).

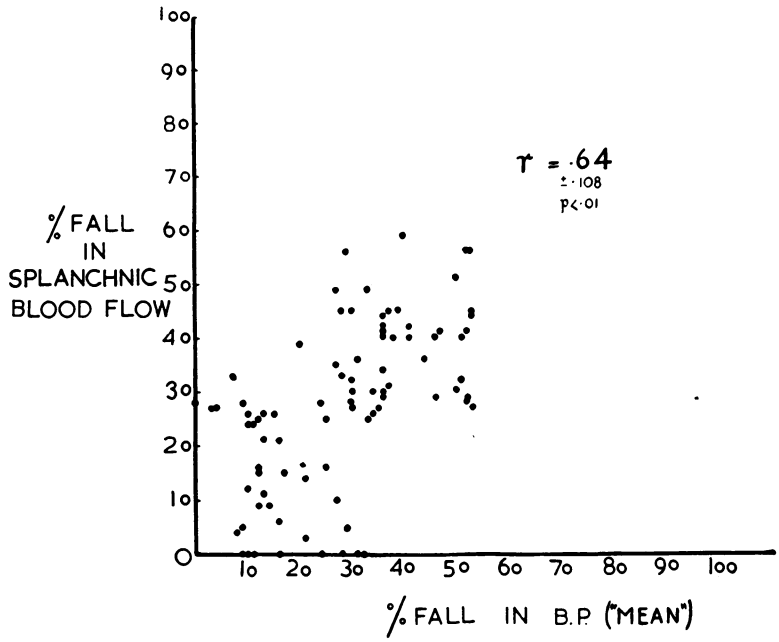

FIG. 2. RELATION BETWEEN FALL IN SPLANCHNIC BLOOD FLOW (E.S.B.F.) AND "MEAN" B.P. AFTER HEXAMETHONIUM

Splanchnic vascular resistance initially was highest in those with the highest systemic blood pressure, confirming the observations of Culbertson, Wilkins, Ingelfinger, and Bradley (19). There was no change following hexamethonium in either normotensive or hypertensive subjects.

Arterial-hepatic venous oxygen difference increased significantly (Figure 1). This increase was entirely due to a drop in hepatic venous oxygen content, for arterial oxygen unsaturation did not change during the course of the study. It coincided in time with the fall in "mean" blood pressure and in E.S.B.F.

"Portal Vein Pressure" (Table II)

In six subjects the "portal venous pressure" measured by the "occluded" hepatic vein technique fell after hexamethonium. The degree of fall in pressure varied considerably and was unrelated to the fall in E.S.B.F.

Observations during Second Hour

In six subjects in whom observations were continued for a second hour, the fall in blood pressure and E.S.B.F. and the increase in arterial-hepatic venous oxygen difference were maintained, with only a slight return towards control values.

Anomalous Results

Two subjects reacted in a different manner (T. A., H. H., Table I). After a brief initial drop, E.S.B.F. rose moderately and was accompanied by a fall in arterial-hepatic venous oxygen difference. Since the blood pressure fell, there was a drop in estimated splanchnic resistance, indicating splanchnic vasodilatation. There was no obvious explanation for this anomalous behaviour.

Changes in Splanchnic Metabolism (Table III)

Splanchnic oxygen consumption calculated by multiplying E.S.B.F. by the arterial-hepatic venous oxygen difference did not change significantly during the hour following hexamethonium.

TABLE II

"Occluded" hepatic vein pressure before and after hexamethonium

Subject	Occluded hepatic vein pressure (mm. Hg)		Fall in E.S.B.F. (% of control value)
	Before C6	After C6	
T. S.	10.2	5.0	55
A. Y.	5.3	4.1	25
E. W.	13.0	10.0	40
P. T.	9.0	5.5	27
C. M.	14.5	2.0	16
C. W.	13.3	1.6	—

Hepatic glucose output. The basal glucose output of 120 mg. per min. per sq. meter agrees well with previous findings (20). There were considerable fluctuations in output following hexamethonium, the mean values being higher than the basal output except at 30 minutes. The changes, however, were not statistically significant.

Arterial blood glucose concentration increased slightly, but again the change was not statistically significant.

Arterial lactic acid concentration rose from 8.2 mg. per cent to a maximum of 9.7 mg. per cent. This change was of doubtful significance.

DISCUSSION

The fall in E.S.B.F. after hexamethonium correlated well with the fall in "mean" blood pressure (Figure 2), and there was therefore little change in calculated splanchnic vascular resistance. Appreciable splanchnic vasodilatation apparently did not occur and splanchnic blood flow fell as a direct consequence of the drop in arterial pressure. This is rather surprising inasmuch as changes in capacity of the splanchnic bed have long been thought to play an important part in the production of the hypotensive state. The present results could have two interpretations. The dose of hexamethonium employed might have failed to block the splanchnic sympathetic ganglia. This could hardly be true of sympathetic ganglia elsewhere, for the general blood pressure fell markedly. Alternatively, splanchnic vasodilatation produced by hexamethonium might have been balanced by splanchnic vasoconstriction, in response to hypotension. For example, the hypotension initiated by changing from the recumbent to the erect posture is prevented by reflex splanchnic vasoconstriction (19). The hypotension following hexamethonium might also be balanced in this way, although this could hardly have been mediated through autonomic ganglia which are presumably already blocked.

Whatever the mechanism, the fall in E.S.B.F. was not associated with overall changes in the capacity of the splanchnic vascular bed.

Changes in splanchnic blood flow could, of course, be due to alterations in resistance in any

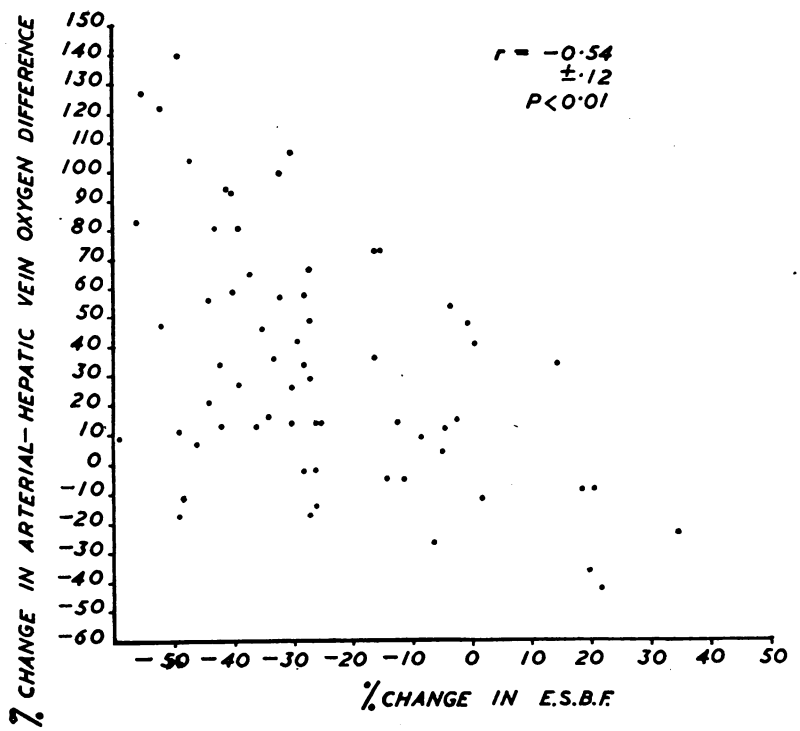

FIG. 3. RELATION BETWEEN CHANGES IN E.S.B.F. AND ARTERIAL-HEPATIC VENOUS OXYGEN DIFFERENCE AFTER HEXAMETHONIUM

TABLE III

*Effect of hexamethonium on splanchnic oxygen consumption, arterial glucose and lactic acid concentrations and hepatic glucose output**

Subject	Age	Sex	Splanchnic O_2 Consumption (*vol./min./m²*)						Arterial Glucose Concentration (*mg./100 ml.*)						Hepatic Glucose Output (*mg./min./m²*)						Arterial Lactic Acid Concentration (*mg./100 ml.*)					
			min. after C6						min. after C6						min. after C6						min. after C6					
			0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60	0	5	15	30	45	60
F. A.	35	M	56	50	58	79	85	82	78	75	90	89	88	94	113	240	211	228	281	234	9.0	6.5	7.5	9.0	6.5	4.5
F. C.	42	M	52	53	37	32	—	38	72	74	75	70	69	69	133	171	104	73	—	98	5.0	6.0	5.5	6.0	6.5	5.0
L. C.	46	F	37	25	36	27	40	37	77	84	85	103	99	100	122	52	284	126	142	78	7.0	7.5	6.0	7.5	10.0	8.5
E. C.	34	M	—	—	—	—	—	—	91	—	94	86	88	77	64	—	—	0	39	0	13.0	7.5	9.0	12.5	9.0	8.5
J. D.	59	M	38	16	44	48	52	41	93	86	81	101	93	84	78	53	86	69	154	147	12.0	11.0	11.0	21.0	16.0	16.0
E. F.	61	M	56	47	56	45	62	83	77	—	76	68	81	107	275	—	235	82	223	190	6.5	7.0	8.0	6.0	—	5.5
J. M.	41	M	44	65	55	65	49	—	82	79	93	93	81	84	235	125	185	82	177	59	8.0	6.5	5.0	8.0	9.0	6.0
C. M.	33	M	42	—	42	36	35	48	86	86	85	88	80	77	96	—	109	72	57	170	5.0	6.0	7.0	7.0	7.0	5.0
M. N.	69	F	39	—	48	36	—	—	95	92	94	82	92	95	158	118	125	123	—	—	12.0	12.0	9.0	10.0	10.0	12.5
A. T.	52	F	40	—	58	39	—	—	79	83	—	87	98	104	87	—	—	46	—	—	4.3	8.5	6.5	6.0	6.0	5.0
E. W.	62	F	40	43	37	40	40	60	86	85	89	81	86	95	46	60	64	65	126	184	10.5	13.5	17.5	19.5	25.0	25.5
M. W.	51	F	88	102	65	51	51	39	93	88	98	96	95	94	77	217	149	79	124	55	5.5	7.0	7.5	9.5	11.5	12.0
A. Y.	71	M	44	45	34	34	—	42	—	—	—	—	—	—	—	—	—	—	—	—	5.1	6.5	8.5	5.0	4.0	4.8
P. T.	52	M	60	—	40	48	46	57	81	87	86	87	81	86	96	—	44	45	64	52	5.5	4.5	5.7	6.5	5.0	8.0
T. S.	41	M	46	—	48	40	37	—	78	—	78	86	80	—	65	—	71	58	32	—	8.0	—	6.0	9.0	9.5	—
T. A.	49	F	64	40	56	65	74	65	91	92	93	93	95	93	121	102	74	206	211	163	7.5	7.5	7.5	10.0	10.0	8.0
H. H.	69	M	56	—	39	42	39	62	91	87	87	87	81	87	151	—	152	133	221	183	16.0	20.0	13.0	8.0	9.5	10.0
Mean			50 ±3.4	49 ±7.4	47 ±2.5	45 ±3.8	51 ±4.6	54 ±4.8	84 ±1.7	84 ±1.6	87 ±1.9	87 ±2.3	87 ±2.0	90 ±2.8	120 ±15.4	126 ±23.3	135 ±19.7	97 ±13.4	143 ±21.9	124 ±19.7	8.2 ±0.82	8.6 ±0.99	8.2 ±0.76	9.4 ±1.09	9.7 ±1.24	9.0 ±1.37

* None of the means differs significantly ($p < .05$) from control value.

of three systems, namely hepatic arterioles, portal vein radicles or intestinal arterioles. All parts of the splanchnic vasculature need not react similarly in producing the overall unchanged splanchnic vascular resistance after hexamethonium.

Theoretically, constriction in the portal vein radicles and dilatation in the intestinal arterioles might occur without any change in overall vascular resistance. Portal venous pressure would then be expected to rise. Hexamethonium, however, lowered "portal venous pressure," suggesting that there was no significant portal venous constriction.

On the other hand, dilatation in the portal vein radicles with intestinal arteriolar constriction might occur, and again overall vascular resistance would be unaltered. In this case there would be a fall in portal venous pressure, as in fact occurred. However, it seems unlikely that hexamethonium would cause arteriolar vasoconstriction.

Hepatic arteriolar dilatation, by raising intrasinusoidal pressure, might decrease portal venous inflow (21) and hence total splanchnic blood flow. But in this case portal venous pressure would be expected to rise.

Finally, a fall in splanchnic blood flow without any change in vessel caliber would lead to a fall in portal venous pressure, with unchanged splanchnic vascular resistance. This seems the most likely explanation of the present results.

Additional evidence for the fall in splanchnic blood flow was afforded by the arterial-hepatic venous oxygen difference. If oxygen utilization by the splanchnic area is unchanged (Table II), then the arterial-hepatic venous oxygen difference should vary inversely with the splanchnic blood flow. This in fact proved to be the case (Figure 3). The mean rise in arterial-hepatic venous oxygen difference was 31 per cent, a similar figure to that obtained for E.S.B.F. by the B.S.P. clearance technique.

The effects of hexamethonium on the splanchnic area appear to be limited to changes in circulation. Changes in hepatic glucose output and arterial glucose concentration did not occur. The failure of arterial lactic acid concentrations to rise appreciably indicates that the basal state of the subjects was maintained, since blood lactic acid increases rapidly following muscular activity or release of epinephrine (22).

SUMMARY

The effect of hexamethonium bromide (1 mg. per Kg. intramuscularly) on the splanchnic circulation, splanchnic oxygen consumption, hepatic glucose output and arterial lactic acid concentration was measured in 17 subjects.

In 15 subjects splanchnic blood flow fell in proportion to the fall in arterial blood pressure. No change occurred in splanchnic vascular resistance, indicating that there was no splanchnic vasodilatation.

In two subjects splanchnic blood flow rose, together with a fall in splanchnic vascular resistance.

Splanchnic oxygen consumption, hepatic glucose output and arterial glucose and lactic acid concentrations were not significantly altered.

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REFERENCES

1. Freis, E. D., Rose, J. C., Higgins, T. F., Kelley, R. T., Schnaper, H. W., and Johnson, R. L., The hemodynamic effects of hexamethonium in man. J. Clin. Invest., 1952, **31**, 629.
2. Gilmore, H. R., Kopelman, H., McMichael, J., and Milne, I. G., The effect of hexamethonium bromide on the cardiac output and pulmonary circulation. Lancet, 1952, **2**, 898.
3. Werkö, L., Frisk, A. R., Wade, G., and Eliasch, H., Effect of hexamethonium bromide in arterial hypertension. Lancet, 1951, **2**, 470.
4. May, L. G., Bennett, A., Gregory, R., Shih Yuan Tsai, and Lynn-Schoomer, M., The effect of tetraethylammonium bromide on the cardiac output of normotensive and hypertensive patients. J. Lab. & Clin. Med., 1949, **34**, 1622.
5. Crumpton, C. W., and Murphy, Q. R., Effects of hexamethonium bromide upon hemodynamics of cerebral and coronary circulation in hypertension. J. Clin. Invest., 1952, **31**, 622.
6. Miles, B. E., de Wardener, H. E., Churchill-Davidson, H. C., and Wylie, W. D., The effect on the renal circulation of pentamethonium bromide during anaesthesia. Clin. Sc., 1952, **11**, 73.
7. Hoobler, S. W., Unpublished observations, in Moe, G. K., and Freyburger, W. A.: Ganglion-blocking agents. Pharmacol. Rev., 1950, **2**, 61.

8. Bradley, S. E., Ingelfinger, F. J., Bradley, G. P., and Curry, J. J., The estimation of hepatic blood flow in man. J. Clin. Invest., 1945, **24**, 890.
9. Sherlock, S., and Walshe, V., The use of a portal anastomotic vein for absorption studies in man. Clin. Sc., 1946, **6**, 113.
10. Bean, W. B., Franklin, M., Embick, J. F., and Daum, K., Absorption studies using portal anastomotic veins. J. Clin. Invest., 1951, **30**, 263.
11. Myers, J. D., and Taylor, W. J., An estimation of portal venous pressure by occlusive catheterization of an hepatic venule. J. Clin. Invest., 1951, **30**, 662.
12. Friedman, E. W., and Weiner, R. S., Estimation of hepatic sinusoid pressure by means of venous catheters and estimation of portal pressure by hepatic vein catheterization. Am. J. Physiol., 1951, **165**, 527.
13. Paton, A., Reynolds, T. B., and Sherlock, S., The assessment of portal venous hypertension by hepatic vein catheterization. Lancet, 1953, **1**, 918.
14. King, E. J., Haslewood, G. A. D., Delory, G. E., and Beall, D., Micro-chemical methods of blood analysis. Lancet, 1942, **1**, 207.
15. Sherlock, S., Bearn, A. G., Billing, B. H., and Paterson, J. C. S., Splanchnic blood flow in man by the bromsulfalein method: the relation of peripheral plasma bromsulfalein level to the calculated flow. J. Lab. & Clin. Med., 1950, **35**, 923.
16. Barker, S. B., and Summerson, W. H., The colorimetric determination of lactic acid in biological material. J. Biol. Chem., 1941, **138**, 535.
17. Douglas, C. G., and Priestley, J. G., Human Physiology; A Practical Course, 3rd ed., Oxford University Press, London, 1948, p. 148.
18. Gibson, J. G., 2nd, and Evans, W. A., Jr., Clinical studies of the blood volume. I: Clinical application of a method employing the azo dye "Evans Blue" and the spectrophotometer. J. Clin. Invest., 1937, **16**, 301.
19. Culbertson, J. W., Wilkins, R. W., Ingelfinger, F. J., and Bradley, S. E., The effect of the upright posture upon hepatic blood flow in normotensive and hypertensive subjects. J. Clin. Invest., 1951, **30**, 305.
20. Bearn, A. G., Billing, B. H., and Sherlock, S., The response of the liver to insulin in normal subjects and in diabetes mellitus: hepatic vein catheterization studies. Clin. Sc., 1952, **11**, 151.
21. Bradley, S. E., Ingelfinger, F. J., and Bradley, G. P., Determinants of hepatic haemodynamics. In CIBA Foundation Symposium: Visceral Circulation, Churchill, London, 1952, p. 219.
22. Bearn, A. G., Billing, B., and Sherlock, S., The effect of adrenaline and noradrenaline on hepatic blood flow and splanchnic carbohydrate metabolism in man. J. Physiol., 1951, **115**, 430.
