9. DeDuve, C., ibid., 1959, Suppl. 7, 169.

10. Burstone, M. S., J. Nat. Cancer Inst., 1958, v21, 523.

11. Bitenski, L., Quart. J. Microscop. Sci., 1962, v103, 205.

12. Hannoun, C., Fernandes, M. V., Maceira-Coelho, A., submitted for publication.

13. Hayflick, L., Exp. Cell Res., in press.

14. Koprowski, H., Discussion in Sabin, A. B., Spec. Publ. New York Acad. Sci., 1957, v5, 128.

15. Weber, R., Experientia, 1957, v13, 153.

16. Wachstein, M., Meisel, E., Falcon, C., Am. J. Path., 1962, v40, 219.

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Effects of Hypertonic Mannitol on Renal Vascular Resistance.* (30259)

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The renal hemodynamic effects of hypertonic mannitol infusions have recently become of interest. Infusions of this 6 carbon sugar alcohol appear to have the property of preventing postoperative oliguria(1) and acute tubular necrosis in cases of crossclamping of the abdominal aorta(2,3). Experimentally, these infusions have resulted in increased total renal blood flow in hypotensive(4,5) and normotensive(6) dogs as measured by clearance techniques, and in medullary blood flow in normotensive animals as determined by decreased dye transit time(7).

It has been suggested(7,8,9) that mannitol infusions act by decreasing renal vascular resistance. The present study was undertaken in an attempt to determine the mechanism whereby hypertonic mannitol exerts its effect on renal vascular resistance in the *in situ* perfused kidney preparation.

Methods. Ninety-three studies were carried out in 34 mongrel dogs (10.5-16.4 mg/kg) of both sexes. After intravenous pentobarbital anesthesia (25 mg/kg) and positive pressure endotracheal respiration with room air were established, the left kidney was exposed through a flank incision.

This kidney was perfused *in situ* (Fig. 1) by means of plastic tubing passing from the left carotid artery through a Sigmamotor pump into the abdominal aorta. The distal part of this system was a plastic cannula tied into the aorta 2 cm below the left renal artery. The aorta was occluded just above the left kidney by means of a Potts clamp, and all lumbar vessels in the area were ligated. The only tissue perfused by this system was the left kidney. Since this pump was capable of supplying a constant flow against variations in resistance pressure from 0 to 400 mm Hg, any changes in renal vascular resistance were directly reflected in changes in pressure in the circuit. Flows were adjusted to maintain renal perfusion pressure close to control arterial blood pressure. During measurements of changes in renal vascular resistance, flow rates were not changed from control values.

Pressures were measured in the brachial artery and in the perfusion tubing at a point between the Sigmamotor pump and the site of aortic cannulation. The latter pressure was taken as equivalent to renal artery pressure. Renal vascular resistance was calculated as the mean renal artery pressure (mm Hg) divided by pump flow (ml/min).

Isolation of the perfusion system was confirmed in each experiment by momentarily (1-3 seconds) turning the Sigmamotor pump off and observing the fall in renal artery pressure to approximately 5 mm Hg.

By means of an infusion pump, mannitol was infused directly into the renal artery as a 6.6% solution in distilled water at a rate of 1.9 ml/min. This was calculated to be

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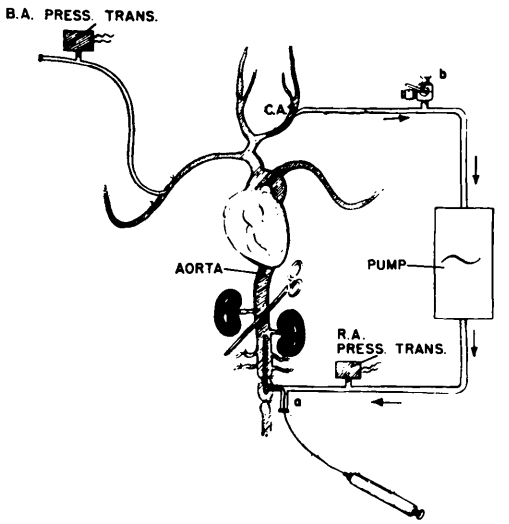

FIG. 1. Schematic illustration of *in situ* kidney perfusion. Pressures are measured in brachial artery (B.A.) and in tubing between Sigmamotor pump and site of aortic cannulation (R.A.). Infusions into renal artery are made through a thin plastic tubing inserted into perfusion tubing at "a." Infusions into renal perfusion circuit are made at "b."

approximately equivalent in concentration and amount to the mannitol presented to each kidney by an intravenous infusion of 20% mannitol at 5 ml/min.

Mannitol was also administered intravenously as a 20% solution at 5 ml/min following a 15 ml prime. Dextran (average molecular weight = 75,000) was infused intravenously as a 6% solution in 5% glucose and water at 15 ml/min following a 45 ml prime. This amount of dextran was calculated to be equivalent to the intravenous dose of 20% mannitol in plasma expanding properties.‡ Whole blood was also infused intravenously at 15 ml/min following a 45 ml prime.

The effects of mannitol and dextran infusions on renal artery pressure were observed before and after intravenous administration of hexamethonium (3 mg/kg) and the instillation of procaine around the renal pedicle.

In a subsequent series of cross transfusion experiments donor dogs were given 20% mannitol or 6% dextran intravenously at the same rates utilized previously. One minute later the blood from the donor dog was shunted into the inflow circuit of the perfusion pump (into "b" of Fig. 1) in a recipient dog, whose renal circulation was perfused by the pump. At the same time, the recipient dog was bled *via* the carotid artery at a rate designed to keep its blood volume constant. During this interval the recipient's kidney was perfused only with blood from the donor dog. This maneuver was performed before and after administration of mannitol or dextran to the donor dog, so that each recipient preparation could serve as its own control.

In another series of experiments, in addition to renal resistance measurements, changes in hematocrit of the blood perfusing the kidney were observed as whole blood (15 ml/min), 20% mannitol (5 ml/min), or 6% dextran (15 ml/min) was given intravenously or 6.6% mannitol, isotonic saline, or 6% dextran was infused at 15 ml/min into "b" of the perfusion circuit by means of an infusion pump. Blood samples for hematocrit were taken from "a" at 30-second intervals.

In the last study, a femoral artery was perfused by the pump with blood from a carotid artery in the same dog, and changes in hind limb vascular resistance were observed after an intravenous infusion of 20% mannitol at 5 ml/min following a 15 ml prime.

The duration of each infusion was from 1½ to 5 minutes. Each dog received one to four infusions at intervals of 20-40 minutes.

Results. When 6.6% mannitol was infused directly into the renal artery (Table I, Procedure 1; Fig. 2), renal vascular resistance either rose or remained constant. In no instance was the resistance lowered by this procedure, and the mean renal resistance in the 6 dogs rose 30%. When 20% mannitol was infused intravenously (Table I, Procedure 2; Fig. 3), in 31 out of 32 experiments the renal vascular resistance was significantly reduced. This occurred in most instances

‡ If all the mannitol remained within the vascular system, 5 ml of the 20% solution would be expected to draw approximately 15 ml of water into the blood. Assuming that ¼ of the mannitol escapes extravascularly, the osmotic effect of the intravascular mannitol would result in the movement of 10 ml of water into the circulation, resulting in a total expansion of approximately 15 ml.

TABLE I. Effects of Various Procedures on Renal Vascular Resistance.

Procedure	No. of exp	Control values (mean ± S.E.) Renal artery press (mm Hg)	Control values (mean ± S.E.) Renal artery flow (ml/min)	Max change (mean ± S.E.) in renal artery press (mm Hg)	Max % change (mean ± S.E.) in renal vascular resistance‡	Time (mean ± S.E.) of max change in resistance (min)
1. Renal artery infusion of 6.6% mannitol (1.9 ml/min)	6	147 ± 20	107 ± 18	+37 ± 10*	+30 ± 11*	1.4 ± .4
2. Intravenous infusion of 20% mannitol (5 ml/min)	32	159 ± 5	118 ± 10	−49 ± 4†	−32 ± 2†	1.5 ± .1
3. Intravenous infusion of 6% dextran (15 ml/min)	5	155 ± 6	72 ± 8	−83 ± 9†	−33 ± 4†	3.0 ± .4
4. Intravenous infusion of 20% mannitol after procaine and hexamethonium (5 ml/min)	12	131 ± 11	126 ± 19	−28 ± 4†	−22 ± 2†	1.8 ± .3
5. Intravenous infusion of 6% dextran after procaine and hexamethonium (15 ml/min)	4	126 ± 16	111 ± 26	−18 ± 9	−12 ± 5	1.9 ± .4
6. Renal circuit infusion of blood from donor dogs which had received 20% mannitol I.V.	6	145 ± 12	79 ± 16	−68 ± 9†	−47 ± 5†	1.2 ± .2
7. Renal circuit infusion of blood from donor dogs which had received 6% dextran I.V.	3	160 ± 18	52 ± 7	−75 ± 16*	−48 ± 12*	1.7 ± .2

* $P < .05$.
† $P < .001$.
‡ These were calculated from individual experiments rather than from mean changes.

after a lag period of 30 to 60 seconds, the maximum effect appearing at 90 seconds. Directionally similar results were also observed after intravenous administration of

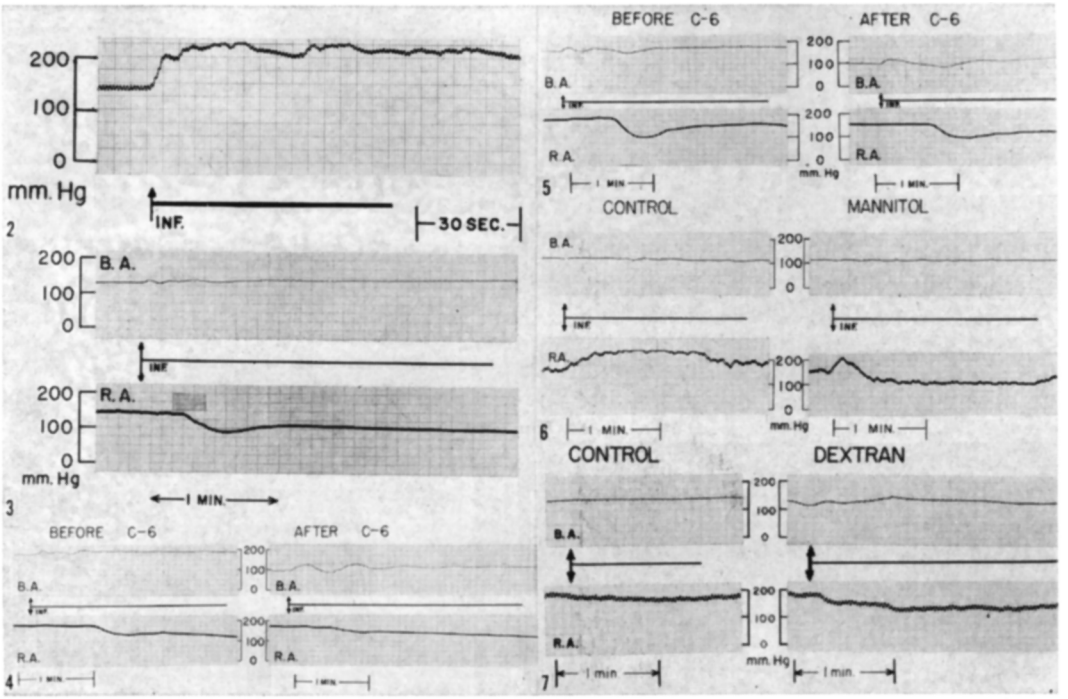

FIG. 2. Effect of renal artery infusion of 6.6% mannitol on renal artery pressure. Duration of infusion is indicated by solid black line INF. Immediately after start of infusion renal artery pressure (and renal vascular resistance) rose and stayed elevated during and after infusion.

FIG. 3. Effect of intravenous infusion of 20% mannitol on renal artery pressure (R.A.) and brachial artery pressure (B.A.). Duration of infusion is indicated by a solid black line (INF.). Twenty seconds following start of infusion, renal artery pressure (and renal vascular resistance) started to fall, declining to 65% of pre-infusion values at 40 seconds, and leveling off at 75% of pre-infusion values at 60 seconds. Brachial artery pressure was not changed significantly.

FIG. 4. Effect of intravenous infusion of 6% dextran on renal artery pressure (R.A.) and brachial artery pressure (B.A.) before and after local procaine around renal pedicle and intravenous hexamethonium (C-6). Durations of dextran infusions are indicated by solid black lines (INF.). Renal artery pressure and renal vascular resistance were decreased by the dextran infusions before and after procaine and hexamethonium.

FIG. 5. Effect of intravenous infusion of 20% mannitol on renal artery pressure (R.A.) and brachial artery pressure (B.A.) before and after local procaine around renal pedicle and intravenous hexamethonium (C-6). Durations of mannitol infusions are indicated by solid black lines (INF.). Renal artery pressure and renal vascular resistance were decreased by mannitol infusions before and after procaine and hexamethonium.

FIG. 6. Effect on renal artery pressure (R.A.) and brachial artery pressure (B.A.) of renal artery infusion (INF.) of blood from a donor dog before (CONTROL) and after an intravenous infusion of 20% mannitol had been given to donor dog (MANNITOL). Renal artery infusion of donor dog blood alone in control period resulted in an immediate and sustained elevation of renal artery pressure (and renal vascular resistance). After donor dog received an intravenous infusion of mannitol, renal artery infusion of donor dog blood resulted in an immediate increase in R.A. pressure followed by a significant decrease lasting throughout the infusion.

FIG. 7. Effect on renal artery pressure (R.A.) and brachial artery pressure (B.A.) of renal artery infusion (INF.) of blood from a donor dog before (CONTROL) and after intravenous infusion of 6% dextran had been given to donor dog (DEXTRAN). Renal artery infusion of donor dog blood alone in control period resulted in a slight decrease in renal artery pressure (and renal vascular resistance). After donor dog had received an intravenous infusion of dextran, renal artery infusion of donor dog blood resulted in a significant decrease in R.A. pressure.

6% dextran (Table I, Procedure 3; Fig. 4). These effects of mannitol and dextran were not prevented by the ganglioplegia of intravenous hexamethonium and renal nerve blockade with local procaine (Table I, Procedures 4, 5; Fig. 4 and 5).

In the cross-circulation experiments, the renal vascular resistances of the recipients were lowered significantly by donor dog blood after the donor dog had received either 20% mannitol or 6% dextran (Table I, Procedures 6, 7; Fig. 6 and 7). The changes in resistance in these experiments were immediate (after correction for catheter time delay) in contrast to the lag period observed after intravenous infusions.

The intravenous infusion of whole blood alone (Table II, Procedure 1) did not produce a decrease in either renal vascular resistance or hematocrit.

In 11 out of 12 experiments involving intravenous infusions of 20% mannitol, the hematocrit and the vascular resistance fell simultaneously (Table II, Procedure 2). In the one instance in which the hematocrit did not fall, the renal vascular resistance was not significantly reduced.

When 6% dextran was infused intravenously (Table II, Procedure 3), the renal vascular resistance was significantly reduced along with a reduction in hematocrit.

When 6.6% mannitol, isotonic saline, or 6% dextran was infused into the renal perfusion circuit for at least one minute (Table II, Procedures 4, 5, 6), decreases in renal vascular resistance were always associated with decreases in the hematocrit.

In the last procedure, changes in hind limb vascular resistance and hematocrit were observed following an intravenous infusion of 20% mannitol (Table III). The vascular resistance and the hematocrit fell significantly during the infusion.

Discussion. The results clearly demonstrate that intravenous infusions of mannitol and dextran can lower renal vascular resistance. However, the experiments involving infusion of mannitol into the renal artery showed that this substance has no direct dilating effect on renal vasculature. In contrast, when mannitol or dextran was given intravenously, either directly or in cross transfusion experiments, renal vascular resistance was lowered. This at first was interpreted as indicating the release or the production of a renal vasodilating substance, possibly as a result of plasma volume expansion, or a diminution in renal sympathetic tone. Since renal vascular resistance was decreased with or without ganglioplegia and renal nerve blockade, this latter possibility was ruled out. The release or production of a renal vasodilating substance could not be eliminated as a possible mechanism. However, since whole blood infusions did not lower renal vascular resistance, the stimulus for the production or release of such a renal vasodilating substance could not be plasma volume expansion.

Other possible explanations of the effects of mannitol and dextran must be considered. It has ben suggested by Pappenheimer and Kinter(10) that intra-renal vascular resistance is a function of the separation of red blood cells and plasma within the kidney. The infusion of mannitol or dextran may in some unknown way alter the process of red cell and plasma separation and thereby alter renal vascular resistance.

However, since hind limb vascular resistance also decreased after intravenous mannitol, and since renal vascular resistance decreased whenever the hematocrit was lowered by any method (*vide infra*), a more likely possibility is that mannitol and dextran alter physical properties of the blood, especially the viscosity.

When either mannitol or dextran was infused intravenously or into the renal perfusion circuit, or when saline was infused into the perfusion circuit, renal vascular resistance and the hematocrit fell. Maximum changes in both occurred simultaneously or within 30 seconds of each other. The data were analyzed to determine the correlation among the maximum per cent changes in renal vascular resistance (y), the maximum per cent change in hematocrit (x_1), and the initial hematocrit level (x_2), without regard to the method used to alter renal resistance. The equation of the regression line was found to $y = 0.8156\ (x_1) + 0.0162\ (x_2) + 19.32$. The correlation coefficient of y on x_1 was

TABLE II. Effects of Various Procedures on Renal Vascular Resistance and Hematocrit.

Procedure	No. of exp	Control values (mean ± S.E.) Renal artery pressure (mm Hg)	Renal artery flow (ml/min)	Hematocrit (%)	Max change (mean ± S.E.) in renal artery press (mm Hg)	Max % change (mean ± S.E.) in renal vasc resist‡	Time (mean ± S.E.) of max change in resistance (min)	Max % change (mean ± S.E.) in hematocrit	Time (mean) of max change in hematocrit (min)
1. Intravenous infusion of whole blood (15 ml/min)	5	136 ± 11	102 ± 19	46 ± 2	+ 5 ± 13	+ 4 ± 9	2.1 ± .4	— 1 ± 2	2.3
2. Intravenous infusion of 20% mannitol (5 ml /min)	12	152 ± 9	120 ± 14	36 ± 2	—49 ± 7†	—32 ± 4†	1.5 ± .2	—11 ± 2†	1.3
3. Intravenous infusion of 6% dextran (15 ml/min)	4	149 ± 10	125 ± 28	38 ± 5	—41 ± 9*	—29 ± 6*	1.5 ± .6	— 7 ± 1*	1.5
4. Infusion of 6.6% mannitol into renal perfusion circuit (15 ml/min)	3	182 ± 10	91 ± 0	34 ± 1	—85 ± 23*	—46 ± 10*	2.2 ± .5	—26 ± 9	2.2
5. Infusion of isotonic saline into renal perfusion circuit (15 ml/min)	4	141 ± 10	145 ± 24	34 ± 2	—40 ± 19	—27 ± 11	1.1 ± .3	—12 ± 7	1.1
6. Infusion of 6% dextran into renal perfusion circuit (15 ml/min)	3	123 ± 2	137 ± 33	30 ± 1	—28 ± 14	—23 ± 11	1.3 ± .6	—14 ±10	1.3

* P <.05.
† P <.001.
‡ These were calculated from the individual experiments rather than from mean changes.

TABLE III. Effects of Intravenous Infusion of 20% Mannitol on Hind Limb Vascular Resistance and Hematocrit.

No. of exp	Mean control values: Femoral artery pressure (mm Hg)	Femoral artery flow (ml/min)	Hematocrit (%)	Max change (mean) in femoral artery press (mm Hg)	Max % change (mean) in hind limb vascular resistance	Time (mean) of max change in resistance (min)	Max % change (mean) in hematocrit	Time (mean) of max change in hematocrit (min)
6	130 ± 7	54 ± 16	40 ± 2	—18 ± 2†	—16 ± 3†	1.5 ± .3	—9 ± 4*	1.3

* P <.05.
† P <.001.

0.56, $p<0.001$; that of y on x_2 was 0.03, $p>0.8$; and that of x_1 on x_2 was 0.04, $p>0.8$. Thus, there was a significant correlation of change in renal vascular resistance with change in hematocrit.

Lilien *et al*(11) in 4 dogs found a 21% decrease in hematocrit following intravenous infusion of 25% mannitol. In our study, however, infusion of 20% mannitol resulted in an 11% (± 2) reduction of hematocrit. At a hematocrit of 36 (± 2), the average in this study, blood viscosity should vary with the 1.17 (± 0.017) power of the red cell concentration(12). This would mean that the observed 11% decrease in hematocrit should result in a 16% (± 4) decrease in blood viscosity. From Poiseuille's law it would follow that the vascular resistance should also decrease 16%, if blood is considered a Newtonian fluid at physiologic rates of flow(13). However, since the resistance fell 32% (± 4) ($p<0.02$), on the basis of the above assumptions the observed change in hematocrit is not sufficient to explain the effects of the mannitol infusions.

In addition to a decrease in viscosity, Lilien *et al*(11) have proposed that hypertonic mannitol in the renal tubules decreases interstitial pressure and that this results in decreased compression of renal vessels and decreased vascular resistance. However, Winton(14) has shown that renal interstitial pressure is significantly increased by an osmotic diuretic. Ureteral obstruction also increases interstitial pressure(15), and both these factors result in an increased rather than a decreased renal blood flow(4,5,6,7,15). These phenomena may be explained by the hypothesis recently proposed by Kiil and Auckland(16). According to this theory, tonus of afferent arterioles is a direct function of the arteriolar transmural pressure. Thus, decreased transmural pressure brought about by an increased interstitial pressure should result in vasodilation and a reduction in renal vascular resistance. This may be an explanation for the greater fall in renal vascular resistance observed in this study than can be accounted for by hematocrit changes alone, although the possibility of non-Newtonian behavior of the blood cannot be ruled out.

Summary and conclusions. The renal hemodynamic effects of mannitol were studied in dogs by infusing one kidney *in situ* with a controlled sinusoidal flow pump, observing changes in pressure, and calculating changes in renal vascular resistance. Intravenous infusions of hypertonic mannitol consistently resulted in diminished renal vascular resistance, both before and after blockade of renal nerves and autonomic ganglia. Decreased renal vascular resistance was also seen after intravenous dextran, and after addition of mannitol, dextran, or saline into the renal perfusion circuit. When hind limb vascular resistance was measured, it too was decreased by intravenous mannitol. These decreases in vascular resistance correlated with diminished hematocrits of the perfusion fluids. In cross circulation experiments in which whole blood from a donor dog which had received mannitol or dextran intravenously was used as the renal perfusion fluid in the recipient dog, decreased renal vascular resistance was observed. However, when whole blood was infused intravenously to intact preparations, renal resistance was not lowered.

These results are considered consistent with the hypothesis that the decrease in renal vascular resistance produced by hypertonic mannitol is not mediated *via* the autonomic nervous system and is probably due to a combination of decreased blood viscosity and renal vasodilation. The former results from decreased hematocrit, and the latter may be due to decreased transmural vascular pressure associated with diuresis or the presence of a circulating renal vasodilating substance.

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1. Barry, K. G., Molloy, J. P., J.A.M.A., 1962, v179, 510.

2. Barry, K. G., Cohen, A., LeBlanc, P. C., Surgery, 1961, v50, 335.

3. Barry, K. G., Cohen, A., Knochel, J. P., Whelan, T. J., Beisel, W. R., Vargas, C. A., LeBlanc, P. C., New Engl. J. Med., 1961, v264, 967.

4. Braun, W. E., Lilienfield, L. S., Proc. Soc. Exp.

Biol. and Med., 1963, v114, 1.

5. Peters, G., Brunner, H., Am. J. Physiol., 1963, v204, 555.

6. Lilienfield, L. S., Braun, W. E., Clin. Res., 1962, v10, 67.

7. Thurau, K., Deetjen, P., Kramer, K., Pfluger's Arch. ges. Physiol., 1960, v270, 270.

8. Hostnik, W. J., Powers, S. R., Jr., Boba, A., Stein, A. A., Surg. Forum, 1959, v10, 872.

9. Teschan, P. E., Gagnon, J. A., Murphy, G. P., Clin. Res., 1963, v11, 241.

10. Pappenheimer, J. R., Kinter, W. B., Am. J. Physiol., 1956, v185, 377.

11. Lilien, O. M., Jones, S. G., Mueller, C. B., Surg. Gynec. Obstet., 1963, v117, 221.

12. Bayliss, L. E., in Flow Properties of Blood and Other Biological Systems, Copley, A. L., Stansby, G., ed., Pergamon Press, New York, 1960, p39.

13. Haynes, R. H., Burton, A. C., Am. J. Physiol., 1959, v197, 943.

14. Winton, F. R., in Modern Views on the Secretion of Urine, Winton, F. R., ed., Little & Brown, Boston, 1956, p84.

15. Selkurt, E. E., Am. J. Physiol., 1963, v205, 286.

16. Kiil, F., Auckland, K., Scand. J. Clin. Lab. Invest., 1961, v13, 276.

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ATEe Esterase Activity of Human Serum. Concentration from Serum, Assay, Properties and Inhibitor. (30260)

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Benzoyl arginine methyl ester (BAMe), benzoyl arginine ethyl ester (BAEe), tosyl arginine methyl ester (TAMe), lysine methyl ester (LMe) and many other synthetic substrates(1) have been used widely for determination of the proteolytic activity of human serum. The hydrolysis of acetyl tyrosine ethyl ester (ATEe) has been found to be a reliable indicator of the activity of C′ 1 esterase of human serum(2,3,4). There is considerable interest in ATEe esterase because of its potential role in immune phenomena and in vascular permeability(5). A strong inhibitor in human serum, however, handicaps the study of the activity of the enzyme. Donaldson(2) has introduced a technic for determination of C′ 1 esterase activity, based on the hydrolysis of ATEe after treatment of serum with diethyl ether. The method, while accurate, cannot be applied to routine use in the clinical laboratory.

The present paper (a) describes a simple method for concentration of ATEe esterase activity of human serum; (b) confirms previous observations on some of the properties of ATEe esterase and of its inhibitor in human serum; (c) reports observations on the activity of the enzyme in some disease states.

Materials and methods. (a) *Substrates* used in this study were TAMe, BAEe, BAMe, LMe, ATEe, acetyl tryptophane ethyl ester (ATrEe) and acetyl phenylalanine ethyl ester (APhEe). ATEe,‡ ATrEe‡ and APhEe‡ were dissolved in 10% methyl cellosolve,§ while TAMe, BAEe and LMe were dissolved in distilled water to make a 0.02 M solution. The esterase activity of human serum and of its fractions was studied with a modification of the colorimetric method described by Brown(6) for the study of the BAEe esterase activity of human serum. A 0.1 M sodium barbital buffer (pH 7.8) was prepared

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‡ Mann Research Corp., New York.

§ Eastman-Kodak Co., Rochester, N. Y. Methyl cellosolve was freshly obtained and, after solution, was kept in dark colored bottle, stoppered, at 4°C. Methyl cellosolve allowed to age contains esters which are capable of reacting with hydroxylamine.