Effect of Glyceryl Trinitrate on Extracellular Fluid Space of Aortic Strips

By O. J. LORENZETTI*, ARTHUR TYE, and JOHN W. NELSON

Extracellular fluid space (ECF) was determined in untreated rabbit aortic tissue and in tissue exposed to 0.1 per cent glyceryl trinitrate. The ECF space determinations were made in tissues bathed in a normal Krebs solution, calcium-free Krebs solution, magnesium-free Krebs solution, and calcium–magnesium-free Krebs solutions. The glyceryl trinitrate increased the ECF space in all solutions except the magnesium-free solution.

THE INCREASING importance of the concentration and distribution of ions in tissue during the state of contraction and relaxation necessitates the determination of distribution of tissue water (1, 2). Of importance in studying some effects of glyceryl trinitrate on smooth muscle relaxation was the determination of the extracellular fluid space (ECF).

The direct methods used to determine the distribution of water between extracellular and intracellular compartments are based on the measurement of the volume of distribution of an impermeable solute molecule, *e.g.*, urea, mannitol, inulin, and radio-iodinated serum albumin. Although these substances penetrate the tissue at various rates dependent on their molecular size, the use of any one substance will give an estimate of the relative distribution of the water in the extracellular fluid space.

For the authors' measurements the ECF space was determined by use of inulin, using the anthrone color reaction for spectrophotometric analysis of the inulin concentration (3). The anthrone color reaction is highly sensitive for determinations of small quantities of inulin, as low as 5 mcg./ml. of solution.

The purpose of this paper is to provide data on the measurement of the ECF space of the rabbit aorta exposed to various ionic media, before and after treatment with glyceryl trinitrate.

MATERIALS AND METHODS

Male albino rabbits weighing 2–3 Kg. were killed by a blow to the back of the neck. The thorax and abdomen were opened, and the descending aorta from the arch extending down 9 cm. was isolated from surrounding tissue. The thoracic aorta was mounted directly onto a glass rod and kept moist with Krebs–bicarbonate solution at room temperature.

The aortic strips were cut by rotating the rod against a fixed scalpel blade according to the method described by Furchgott (4). All of the aortic strips conformed to the following dimensions: 2.0 ± 0.55 mm. wide, 2.0 ± 0.3 cm. long, and 450 ±50 μ thick as noted under a microscope. Throughout this procedure the tissue was kept moist with Krebs–bicarbonate solution and gassed with 95% oxygen and 5% carbon dioxide.

The isolated aortic tissue was placed individually into 12-ml. weighing bottles containing 5 ml. of 1.0% w/v inulin in modified Krebs solution. Inulin was substituted for glucose in all Krebs solutions. Three solutions with various concentrations of electrolytes were employed in addition to the normal Krebs solution. The composition of these modified solutions is shown in Table I. The osmotic concentration of the solutions was between 314.5 and 317.5 milliosmoles. The ionic strength ranged from 0.168 to 0.174. The pH was adjusted to 7.36 ± 0.08. Some aortic muscle strips were exposed to 0.1% glyceryl trinitrate and were called treated strips.

TABLE I.—COMPOSITION OF BATH MEDIA FOR AORTIC STRIPS IN mmoles/L.[a]

	Krebs-HCO_3	Calcium-Free	Magnesium-Free	Calcium and Magnesium-Free
NaCl	118.5	120.0	120.0	128.0
KCl	4.8	4.8	4.8	4.8
$CaCl_2 \cdot 2H_2O$	1.9	...	1.9	...
KH_2PO_4	1.2	1.2	1.2	1.2
NaH_2PO_4	...	...	1.2	...
$MgSO_4 \cdot 7H_2O$	1.2	1.2	...	...
$NaHCO_3$	25.0	25.0	25.0	25.0
Dextrose	10.0	10.0	10.0	10.0

[a] Ionic strength, 0.168 to 0.174; osmolarity, 314.5 to 317.5; pH, 7.28 to 7.44.

Both treated and untreated aortic strips were allowed to equilibrate for 12 hr. The tissue was then removed, blotted, and placed in 5 ml. of distilled water contained in another weighing bottle. At the end of a 12-hr. period in the distilled water, the tissue was removed from its container, was blotted, and was weighed. The tissue was then carefully homogenized in 2 ml. of distilled water using a 10-ml. hand homogenizer. The homogenate was centrifuged at 6000 r.p.m.'s for 5 min., and the supernatant was decanted and analyzed for inulin as described below.

Inulin Assay.—Two samples of the distilled water–inulin solution were removed, placed in 22 × 175-mm. Pyrex test tubes, and cooled in a cold water bath to 8 ± 1° for 10 min. Then 2 ml. of freshly prepared anthrone reagent was added directly to the solution in the test tube and mixed by swirling. The anthrone reagent consisted of 0.02% w/v anthrone and 0.1% w/v thiourea (to prevent oxidation of the active enol tautomer of anthrone) in 96% sulfuric acid. The anthrone reagent was precooled to 8 ± 1°. Then the samples were immediately heated for 6 min. at 90 ± 3° on a water bath. The tubes were cooled for 5 min. in an ice bath to 8° and were allowed to come to room temperature. The resulting turquoise-green solutions were read on a Beckman spectrophotometer at 620 mμ within 20 min.

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of their removal from the water bath, against a distilled water–tissue blank. The blanks gave a reading of 0.97 ± 0.04% ECF per wet weight of tissue, as determined on 20 tissue blanks taken at random. The inulin concentration was read from a standard curve, constructed from determinations on inulin in distilled water. The inulin space is equivalent to the extracellular fluid space and was expressed as the mg. of inulin per 100 mg. of aortic tissue, wet weight, divided by the mg. $ml.^{-1}$ of inulin in the

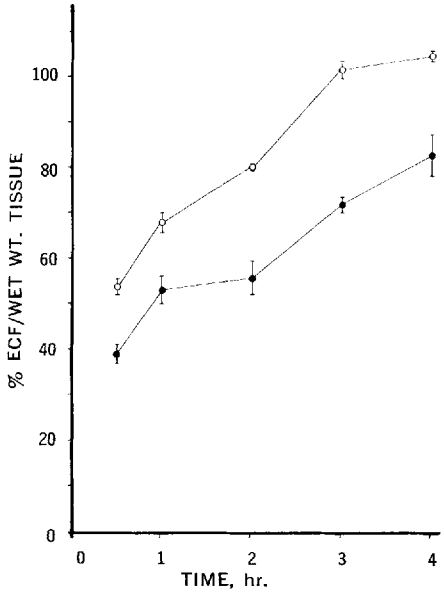

Fig. 1.—Extracellular fluid space of aortic strips in Krebs solution. Key: ●, untreated; ○, glyceryl trinitrate (1 mg.) added; I, standard deviation.

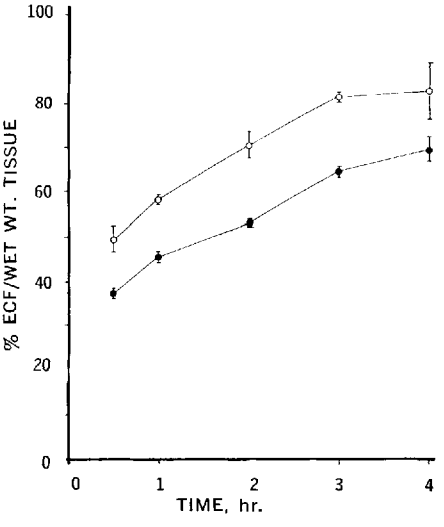

Fig. 2.—Extracellular fluid space of aortic strips in calcium-free Krebs solution. Key: ●, untreated; ○, glyceryl trinitrate (1 mg.) added; I, standard deviation.

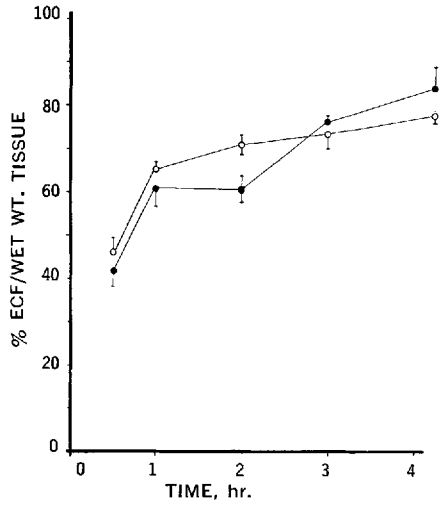

Fig. 3.—Extracellular fluid space of aortic strips in magnesium-free Krebs solution. Key: ●, untreated; ○, glyceryl trinitrate (1 mg.) added; I, standard deviation.

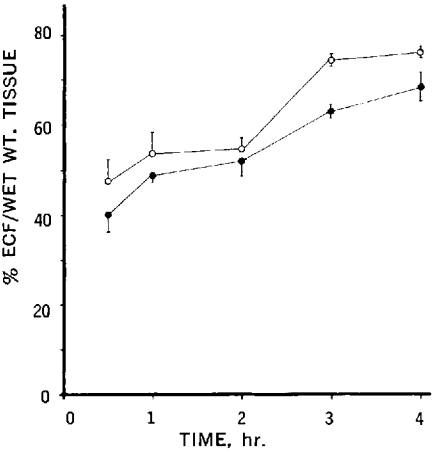

Fig. 4.—Extracellular fluid space of aortic strips in calcium and magnesium-free Krebs solution. Key: ●, untreated; ○, glyceryl trinitrate (1 mg.) added; I, standard deviation.

ECF times 100 to give per cent ECF per wet weight of tissue (5).

In the tissues exposed to 0.1% glyceryl trinitrate, it was found that the nitrates interfered with the assay. The solutions obtained from tissue treated with glyceryl trinitrate were passed through an anion exchange resin,[1] contained in a 3-cm. (i.d.) × 30-cm. column. The resulting glyceryl trinitrate-free solution was then assayed for inulin as described.

RESULTS

The rate of permeability indicated by a plot of the per cent of extracellular fluid space per 100 mg. tissue wet weight *versus* time (Figs. 1–4) shows that

[1] Marketed as Amberlite IR-45 by Mallinckrodt, St. Louis, Mo.

saturation of the inulin spaces occurs in approximately 4 hr.

The results for the normal Krebs solution in Fig. 1 show that the presence of glyceryl trinitrate increases the permeability of the cell membrane in the tissue as represented by the increase in ECF. The increase was significant from the first to the fourth hour. The values obtained were high relative to those reported in the literature by the use of other methods. It was not intended that the measurement would be an exact value of the extracellular fluid space since the incomplete penetration of inulin into the connective tissue has not been considered. Nichols and co-workers have shown that the ECF space measurements obtained with chloride are better estimations, since chloride penetrates much more rapidly than inulin (6).

The muscle exposed to glyceryl trinitrate in a calcium-free medium also shows a significant increase in the extracellular fluid (Fig. 2). The results indicate that absence of calcium does not affect the increased ECF space induced by the presence of glyceryl trinitrate.

The magnesium-free Krebs media produced an inhibition of the ability of glyceryl trinitrate to increase the ECF space of the smooth muscle over most of the 4-hr. period (Fig. 3). The Krebs media without calcium or magnesium showed no change in the ECF space for the first 2 hr., then a significant increase in ECF in the last 2 hr. as seen in the unmodified Krebs medium (Fig. 4).

The approximately $\pm 5\%$ of the calculated mean in the ECF space determination reflects some variability among the multiple samples. That may be attributed to an actual section-to-section variation within the aorta, with respect to the inulin space or endogenous carbohydrates.

DISCUSSION

Although the velocity of inulin distribution is not affected by incubation of the muscle with glyceryl trinitrate, the apparent volume of distribution of inulin is increased. This indicates an increased permeability of the tissue in the presence of nitrate and nitrite. The increased permeability was not observed in the absence of magnesium with calcium present.

In all the bathing solutions, an early rapid phase and a late slow phase was noted before equilibrium was attained. This could be attributed to different water compartments, but recent studies have indicated that there are more than the two classic water compartments (7) indicated in this study. Possibly, the phases noted could be characteristic of the tissue investigated, indicative of latent penetration of inulin into more complex extracellular structures as suggested by Page (8) for heart muscle.

Saturation of the inulin space occurred in 3 to 4 hr. in these studies. Even after this prolonged time, nitrates and nitrites can still exert an effect on the cell membrane (9).

SUMMARY

Glyceryl trinitrate increases the extracellular fluid space of rabbit aortic tissue in normal Krebs and calcium–magnesium-free Krebs solutions. Only the magnesium-free Krebs solution failed to produce an elevation of the extracellular space of aortic tissue after treatment with glyceryl trinitrate.

REFERENCES

(1) Bohr, D. F., *Pharmacol. Rev.*, **16**, 85(1964).
(2) Bohr, D. F., Filo, R. S., and Guthe, K. F., *Physiol. Rev., Suppl. 98*, **42**, 1962.
(3) Dreywood, R., *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).
(4) Furchgott, R. F., *Methods Med. Res.*, **8**, 177(1960).
(5) Normal, A., Rondell, P. A., and Bohr, D. F., *Am. J. Clin. Pathol.*, **32**, 465(1959).
(6) Nichols, G., Jr., Nichols, N., Weil, W. B., and Wallace, W. M., *J. Clin. Invest.*, **32**, 1299(1953).
(7) Bozler, E., *Am. J. Physiol.*, **200**, 651(1961).
(8) Page, E., *J. Gen. Physiol.*, **46**, 201(1962).
(9) Lorenzetti, O. J., Tye, A., and Nelson, J. W., *J. Pharm. Sci.*, **55**, 105(1966).

Interactions of Surfactants with Lipoproteins

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The interactions of benzalkonium chloride and sodium lauryl sulfate with α- and β-serum lipoproteins have been studied. Both fractions, below their isoelectric points, form insoluble complexes with the anionic surfactant. At higher concentrations of the surfactant the insoluble complexes are resolubilized completely. Above the isoelectric point, the lipoprotein fractions exhibit the same phenomenon with benzalkonium chloride. The charge and the hydrophilic nature of the macromolecules are the major factors in these interactions. The formation of insoluble complexes and the resolubilization of these complexes are modified considerably by the addition of urea to the systems.

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