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Alteration of Hepatic Tissue Spaces by Platelet-Activating Factor and Phenylephrine

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Mean transit times for the movement of extracellular and intracellular reference compounds through isolated perfused rat livers were determined during exposure of livers to platelet-activating factor (AGEPC; 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) and the α-adrenergic agonist phenylephrine, using the multiple indicator dilution technique. From the outflow profiles of rapid bolus injections of ^{3}H-sucrose and ^{14}C-urea given to the liver, the estimated intracellular volume of distribution of small freely permeant substances, V_i, and θ', the ratio of intracellular to extracellular space, were computed. Exposure of the liver to AGEPC decreased V_i and θ' by 32 and 34%, respectively, from control values, whereas infusion of phenylephrine increased V_i by 16% and θ' by 33%. The results indicate that the hemodynamic effects of AGEPC in perfused rat liver cause the apparent loss of tissue space accessible to small permeant compounds. Phenylephrine, although increasing hepatic vascular resistance, measured at the portal vein, by the same magnitude as AGEPC, led to an increase in the apparent tissue space accessible to this same species.

In the experimental results reported here, we have characterized the microvascular changes which occur in the perfused rat liver following administration of platelet-activating factor (AGEPC), an autacoid-type mediator of allergic and inflammatory reactions, and phenylephrine, a vasoactive hormone. Administration of these agonists led to substantial changes in the microcirculatory parameters of the liver. A multiple indicator dilution technique (1) was used to facilitate these experimental studies.

AGEPC (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) has been shown to have potent vasoactive effects (2) as well as potent glycogenolytic effects (3, 4) in isolated perfused rat liver. In other systems, such as guinea pig heart (5), guinea pig ileum (6), basophils (7–9), mast cells (10, 11), neutrophils (12) and platelets (13, 14), AGEPC has been demonstrated to be involved in a variety of biological responses. In the perfused liver, AGEPC elicits production of glucose, transient changes in oxygen consumption and increased portal vein pressure at concentrations as low as 10^{-11} *M*. In isolated hepatocytes, whereas exposure to AGEPC elicits a rapid and transient change in phosphoinositide turnover, there is no concomitant activation of glycogenolysis, suggesting the involvement of hepatic cellular components other than the hepatocyte in the response of the intact liver to AGEPC (15). We have described recently a relationship between portal pressure increases and concomitant glucose release in the perfused liver as a result of exposure to AGEPC (2).

In order to understand further the relationship between increases in hepatic vascular resistance, measured as portal vein pressure, and the metabolic sequelae of AGEPC exposure, such as the transient reduction in oxygen consumption and an increased release of glucose by the liver (2, 4), we have measured the perturbations of the microvasculature and accessible tissue spaces of the liver during stimulation by AGEPC and phenylephrine.

Using a multiple indicator dilution technique, which has proved useful in studies of liver (16, 17) and heart (18) hemodynamics, we have asked whether the liver remains well perfused during treatment with AGEPC. We have determined that at moderate to high perfusate concentrations of AGEPC (0.2 to 20 n*M*), the cellular space accessible to a small permeant species, specifically urea, is reduced by 36% from the prestimulation levels. Phenylephrine, which results also in increases of hepatic vascular resistance, does not lead to a decreased cellular space. These results support the hypothesis that exposure of livers to moderate and larger doses of AGEPC leads to localized loss of effective liver space through perturbations of the microvasculature and accessible tissue spaces of the liver.

MATERIALS AND METHODS

Rat Liver Perfusion. Male Sprague-Dawley rats (180 to 220 gm), fed *ad libitum*, were used for all of the experiments. Prior to surgery, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (7.5 mg per 100 gm). Livers were perfused *in situ* using a nonrecirculating perfusion system (19) with Krebs-Henseleit-bicarbonate buffer containing 1.25 m*M* Ca^{++}, pH 7.4, equilibrated with a mixture of oxygen:carbon dioxide (95:5%) and maintained at 37°C. Flow rate was maintained constant throughout the perfusion, using a constant flow pump adjusted to 35 ml per min at the start of each liver perfusion. Livers were perfused for at least 30 min prior to measurements to ensure stabilization of liver metabolism and

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to wash endogenous hormones from the liver. Hepatic portal vein pressure was measured continuously using a Statham P231D pressure transducer, monitored via a Grass 7P1A/70AC physiograph connected to a chart recorder, connected to the inlet perfusate line. Basal portal pressures during the experiments were in the range 4 to 7 mmHg (0.5 to 0.9 kilopascals).

Multiple Indicator Dilution Method. Tissue spaces and microvascular parameters were measured using a multiple indicator dilution technique (1) employing ^{3}H-sucrose and ^{14}C-urea as the markers for extracellular space, including vascular, and total liver space, respectively. A bolus (10 μl) of a mixture of ^{3}H-sucrose and ^{14}C-urea was injected rapidly (<0.5 sec) through the inlet cannula to the portal vein. For measurements following the infusion of AGEPC or phenylephrine, bolus injections were made at the peak of developed hepatic vascular resistance. To minimize between-liver variations, a control measurement was made in the same liver but prior to the treatment measurement. Samples of the effluent perfusate were collected at 1-sec intervals for 60 sec following the injection, and radioactivity in each fraction was determined by dual channel liquid scintillation counting using a double label correction program. For each tracer, the data were normalized for the total amount of each isotope injected, typically 2 μCi for the urea tracer and 1 μCi for the sucrose tracer. Recovery of each tracer for the different conditions, control and treatment, was determined by adding an extrapolation of the outflow profile (described below) to the cumulative tracer outflow. Calculated recoveries were greater than 95% for both tracers, during the control and the treatment measurements with either AGEPC or phenylephrine.

Two approaches, a model independent and a model dependent, were used to assess the changes in microvascular and tissue space parameters as a result of AGEPC or phenylephrine administration. Using a model-independent approach, the mean transit times for each of the tracers were calculated from the outflow fractions following the injection using the following equation:

$$\tau_i = \frac{\sum_{j=1} t_j q_i(t_j)}{\sum_{j=1} q_i(t_j)} + \frac{A(kt_f + 1)e^{-kt_f}}{k^2} \quad (1)$$

where $q_i(t_j)$ is the fraction of the injected marker i appearing at time t_j, the midpoint of the j^{th} collection interval. The term on the far right of Equation 1 represents the extrapolation of the latter portion of the outflow profile to infinite time. The constants A and k are determined by fitting the data for each of the tracers in the last 15 fractions of the outflow to a monoexponential decay function, $y(t) = Ae^{-kt}$, and t_f is the time of the last fraction. Mean transit times were corrected further for the time spent in the outflow cannula (3.4 sec).

Intracellular space, V_i, is calculated as the difference between the volumes of distribution for sucrose and urea. When all of the outflow of tracer can be collected, *i.e.* there is only one exit point for tracer, the mean transit time for a tracer can be used to calculate the volume of distribution for that tracer. For substances which enter the cellular space passively, the volume of distribution is independent of the permeability of the diffusible substance (20). The volume of distribution for sucrose includes the sinusoidal space and the space outside the cells, which includes the space of Disse. For urea, the volume of distribution includes that of sucrose as well as the cellular space. V_i was calculated as the product of the overall flow rate and the difference between the mean transit times for the two marker substances, normalized for the liver weight W:

$$V_i = F(\tau_{urea} - \tau_{sucrose})/W \quad (2)$$

where F is the perfusate flow rate (0.588 ml per sec) and τ_{urea}, $\tau_{sucrose}$ are the mean transit times for the injected markers calculated from Equation 1.

Using a model-dependent approach, that of flow-limited distribution of tracers, Θ', the ratio of intracellular space to total extracellular space, including sinusoidal space, was calculated similar to Goresky (1) from the normalized peak heights of the labeled sucrose and urea curves, q_i^{max}, as:

$$\Theta' = \frac{q_{sucr}^{max}}{q_{urea}^{max}} - 1 \quad (3)$$

Θ' can be defined using a second model-dependent parameter, t_0, the transit time for all of the marker substances through the large vessels of the liver (1), as:

$$\Theta' = \frac{t_{urea} - t_{sucrose}}{t_{sucrose} - t_0}$$

using the transit times of the tracer peaks, t_{urea} and $t_{sucrose}$. Rearranging, t_0 is defined as:

$$t_0 = \frac{(\Theta' + 1)t_{sucrose} - t_{urea}}{\Theta'} \quad (4)$$

Values for Θ' obtained during the control injections in these experiments were in the same range as values for Θ' reported previously by Schwab et al. (21) for isolated perfused rat livers.

Washed rabbit erythrocytes were used in additional experiments to assess the changes in the vascular space of the liver. Rabbit erythrocytes were prepared by several cycles of sedimentation at 1 × *g*, first in acid-citrate-dextrose buffer, then in Krebs-Henseleit buffer, removal of the supernatant and slowly sedimenting cells (buffy coat) and resuspension to 20% (v/v) until the buffer/cell interface was sharply defined. Erythrocytes were suspended to 20% (v/v) in Krebs-Henseleit buffer prior to injection. A bolus of 50 μl erythrocyte suspension was injected for each determination. Tubes containing the effluent perfusate, collected as described above, were centrifuged to pellet the erythrocytes. The amount of hemoglobin in each fraction was determined from the optical density at 410 nm after resuspending and lysing the erythrocyte pellet in 2 ml of distilled water.

AGEPC (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) was obtained from Bachem (Budendorf, Switzerland) and was determined to be of high purity using methods previously reported (22). For infusion into the liver, AGEPC was complexed with bovine serum albumin (2.5 mg per ml) in isotonic saline. AGEPC was infused at final perfusate concentrations of 0.2 or 20 n*M* as indicated in the figures. Radiolabeled sucrose and urea were purchased from ICN Corp. (Irvine, CA) and Amersham Corp. (Arlington Heights, IL), respectively. Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

In the isolated perfused rat liver, infusion of AGEPC leads to increased vascular resistance which is dose dependent and saturable. A similar dose-response relationship occurs for the production of glucose following AGEPC exposure (2). Figure 1 shows changes in oxygen consumption and vascular resistance (portal vein pressure) following infusion of AGEPC or phenylephrine. The molecular mechanism by which AGEPC effects increased portal vein pressure is unknown, however. Since, in the isolated perfused rat liver preparation used in the

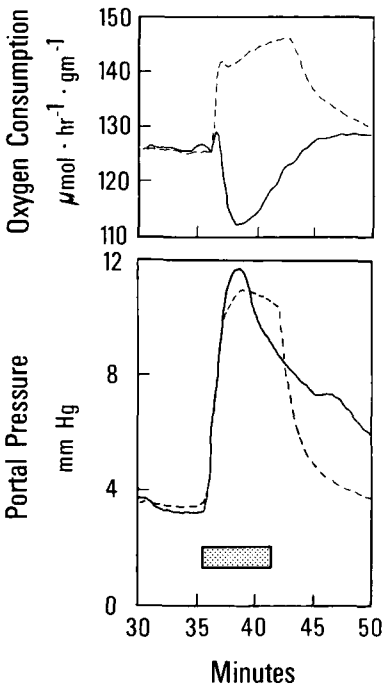

FIG. 1. Effect of AGEPC and phenylephrine on hepatic vascular resistance and oxygen consumption in the perfused rat liver. Agents were infused during the period indicated by the *bar* in the *lower panel.* —— = AGEPC (0.2 n*M*); - - - = phenylephrine (10 μ*M*).

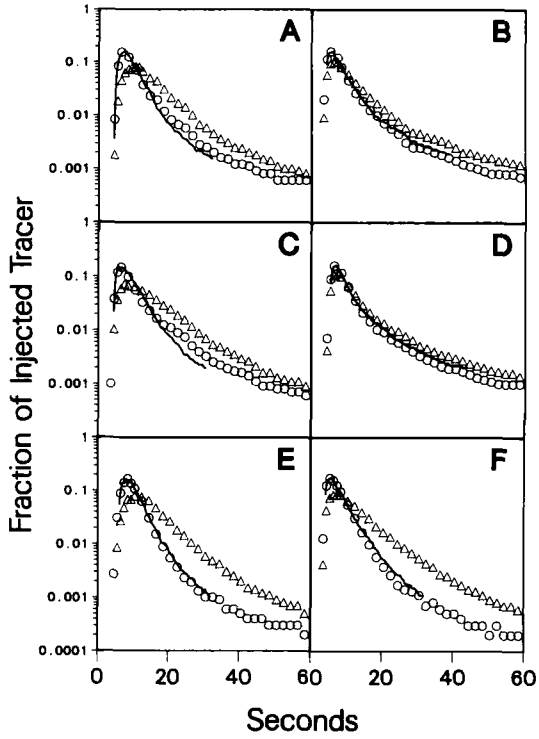

FIG. 2. Effect of AGEPC and phenylephrine on the outflow profiles of ^{3}H-sucrose and ^{14}C-urea reference tracers from perfused rat liver. Each set of *panels* presents a representative liver with control (A, C, E) and treatment (B, D. F) outflow profiles. The *solid lines* represent adjustment of the urea data using Θ' and t_0. (B) AGEPC = 0.2 n*M*; (D) AGEPC = 20 n*M*; (F) phenylephrine = 10 μ*M*. ○ = ^{3}H-sucrose; △ = ^{14}C-urea.

present study, there is no high-pressure component associated with the hepatic arterial system, there are two basic models by which hepatic vascular resistance may increase as a result of exposure to vasoactive substances. If we consider the vascular bed of the liver as a parallel collection of cylindrical elements, *i.e.* sinusoids, then decreases in the diameter of sinusoids would lead to increased resistance to flow through the sinusoids. At a constant flow rate, as was the case in the experiments illustrated here, any reduction in the radius of the sinusoid would lead to an increase in vascular resistance and a decrease in the transit time for substances passing through the sinusoidal elements due to an increased local velocity of fluid through the sinusoid. Alternatively, if some fraction of the sinusoids were to be shut down, so that no flow occurred through them, then the remaining sinusoids, at a constant flow rate, would experience increased local volume flow, hence an increased vascular resistance and a decreased transit time for traversal of the sinusoidal bed by injected marker substances. The difference between the two models is that in the first case all of the sinusoids experience flow, hence the liver is well perfused, whereas in the second case, certain portions of the liver are not perfused.

We have used the multiple indicator dilution technique to attempt to distinguish between these two cases, that is to determine whether the liver remains well perfused during treatment with AGEPC. This methodology is well suited for measurements of tissue spaces. From the outflow profiles of two injected markers, one for total tissue space (urea) and the other for extracellular space including vascular (sucrose), the intracellular volume as well as the ratio of intracellular space to extracellular space can be estimated. These parameters were calculated from the normalized peak heights of the two curves and the mean transit times for the two substances through the liver. Figure 2 shows the outflow profiles from perfused rat livers given rapid (<0.5 sec) bolus injections of ^{3}H-sucrose/^{14}C-urea in the presence and absence of stimulation by AGEPC (0.2 n*M*, 20 n*M*) and phenylephrine (10 μ*M*). The control injections were made in the same liver as the treatments but prior to infusion of either AGEPC or phenylephrine. Infusion of either AGEPC or phenylephrine resulted in hepatic vascular resistance increases which led to decreased transit times for the injected reference substances. These effects can be noted in Figure 2 as a leftward shift in the peaks of the outflow profiles for sucrose and urea. In the case of phenylephrine, there was little shift in the urea outflow profile compared to control values. In the case of AGEPC infusion, at maximum developed vascular resistance, the outflow profile for urea began to parallel the outflow profile for sucrose. Adjustment of the data for the urea outflows using the calculated values of Θ' and t_0 is shown as *solid lines* in Figure 2 for the controls and treatments. The urea curves were adjusted by multiplying each value by $(\Theta' + 1)$ and changing the time of appearance of tracer from t to $(t + \Theta' t_0)/(\Theta' + 1)$ (1). The mean calculated coefficient of variation was less than 0.02 for each of the treatments and controls, indicating a good fit of the model-dependent parameters to the data.

The recovery of tracer from the livers during the

treatments was better than 95% for AGEPC (0.2 nM, 20 nM) and phenylephrine (10 μM) for both tracers and there was no disproportionate loss of one tracer over the other during the treatments compared to controls. The calculated values for the mean transit times for sucrose and urea are shown in Table 1 for AGEPC- and phenylephrine-stimulated livers. Whereas the mean transit time of the sucrose reference decreased by 13, 34 and 21% for AGEPC concentrations of 0.2 and 20 nM and phenylephrine (10 μM) treatment, the mean transit time of the urea reference decreased 21 and 39% for AGEPC concentrations of 0.2 and 20 nM, but decreased only 5% with phenylephrine treatment. The difference between the two agents was emphasized further by the calculated values for Θ', the ratio of intracellular volumes of distribution, shown in Table 2. AGEPC treatment of the liver lowered Θ' by 34 and 42% ([AGEPC] = 0.2, 20 nM) compared to the untreated controls, whereas phenylephrine (10 μM) led to an increase of 33% over control values. Mean portal pressure increases above control values were 7.73, 8.5 and 7.51 mmHg for AGEPC (0.2, 20 nM) and phenylephrine (10 μM), respectively. These increases in vascular resistance with AGEPC are consistent with those reported previously (2).

In the experimental protocols presented here, there was only one inlet and one outlet for the liver, so that from the mean transit times for the outflow profiles of sucrose and urea, the volumes of distribution of sucrose and urea were calculated as the product of the overall flow rate, F, and the respective mean transit time. Since the volume of distribution for urea includes the volume of distribution of sucrose, the volume accessible only to urea, V_i, was calculated from the difference between the distribution volumes for urea and sucrose, normalized for liver weight (Table 2). The changes in V_i as a result of infusion of AGEPC or phenylephrine demonstrate clearly the opposite effects of AGEPC and phenylephrine on this parameter. AGEPC consistently decreased V_i, whereas the opposite was true for phenylephrine treatment, *i.e.* a consistent increase in accessible tissue space. The same behavior occurs for Θ'. Again, AGEPC decreased Θ', whereas phenylephrine increased this parameter. Figure 3 presents the differences in V_i and Θ' plotted against one another and demonstrates the correlation between the two measurements. Figure 3 indicates also that AGEPC acted to reduce the accessible tissue space, whereas phenylephrine tended to increase the accessible tissue space. Figure 3 represents treatment data paired with control measurements. Using paired comparisons, AGEPC decreased V_i and Θ' by 33 and 37% at [AGEPC] = 0.2 nM and by 48 and 42% at [AGEPC] = 20 nM, whereas phenylephrine (10 μM) treatment increased V_i and Θ' by 17 and 30%. Following removal of AGEPC or phenylephrine treatment and the subsequent return of vascular resistance to prestimulation levels, V_i and Θ' returned to their prestimulation values for AGEPC and phenylephrine. Vascular resistance returned to baseline values within 15 min of treatment removal; thus the effects of AGEPC and phenylephrine on the measured parameters were fully reversible.

Rabbit erythrocytes were used to assess changes in the vascular space of perfused rat livers treated with AGEPC or phenylephrine. Bolus injections of rabbit erythrocytes (20% v/v) were performed as described in "Materials and

TABLE 1. Changes in mean transit times following exposure to AGEPC and phenylephrine

Treatment	$\tau_{sucrose}$			τ_{urea}		
	(−)	(+)	Δ	(−)	(+)	Δ
AGEPC, 0.2 nM (n = 8)	7.03 (1.22)	6.12 (1.12)	−0.91[a] (0.98)	12.58 (1.19)	9.92 (1.83)	−2.66[b] (1.66)
AGEPC, 20 nM (n = 3)	9.18 (1.56)	6.02 (0.29)	−3.17 (1.79)	14.67 (1.97)	8.91 (1.26)	−5.76 (2.41)
Phenylephrine, 10 μM (n = 6)	7.47 (1.29)	5.93 (0.93)	−1.54[a] (1.09)	13.39 (1.25)	12.7 (1.37)	−0.67 (1.65)

Mean transit times were calculated from the outflow profiles of the injected tracers as described in "Materials and Methods." Mean transit times are given in seconds and are corrected for the cannula delay. The perfusate flow rate was 35 ml per min for each perfusion. Means are given for each treatment group [(−) controls; (+) treatments]. Significance of the differences, Δ = (+) − (−), are determined using a pairwise t test. S.D. are given in parentheses.

[a] $p < 0.05$.

[b] $p < 0.005$.

TABLE 2. Changes in Θ and V_i following exposure to AGEPC and phenylephrine

Treatment	Θ			V_i (ml/gm)			Weight (gm)
	(−)	(+)	Δ	(−)	(+)	Δ	
AGEPC, 0.2 nM (n = 8)	1.28 (0.39)	0.84 (0.45)	−0.44[a] (0.25)	0.34 (0.05)	0.23 (0.08)	−0.11[a] (0.06)	9.41 (0.73)
AGEPC, 20 nM (n = 3)	1.01 (0.04)	0.58 (0.11)	−0.42[b] (0.14)	0.35 (0.03)	0.18 (0.04)	−0.17 (0.07)	9.23 (1.47)
Phenylephrine, 10 μM (n = 6)	1.52 (0.026)	2.02 (0.69)	0.50 (0.45)	0.38 (0.06)	0.44 (0.03)	0.06 (0.05)	9.10 (1.22)

Values for Θ and V_i were calculated from the outflow profiles of the injected tracers as described in "Materials and Methods." V_i is corrected for liver weights, which are given for each treatment group. Means are given for each treatment group [(−) controls; (+) treatments]. Significance of the differences, Δ = (+) − (−), are determined using a pairwise t test. S.D. are given in parentheses.

[a] $p < 0.005$.

[b] $p < 0.05$.

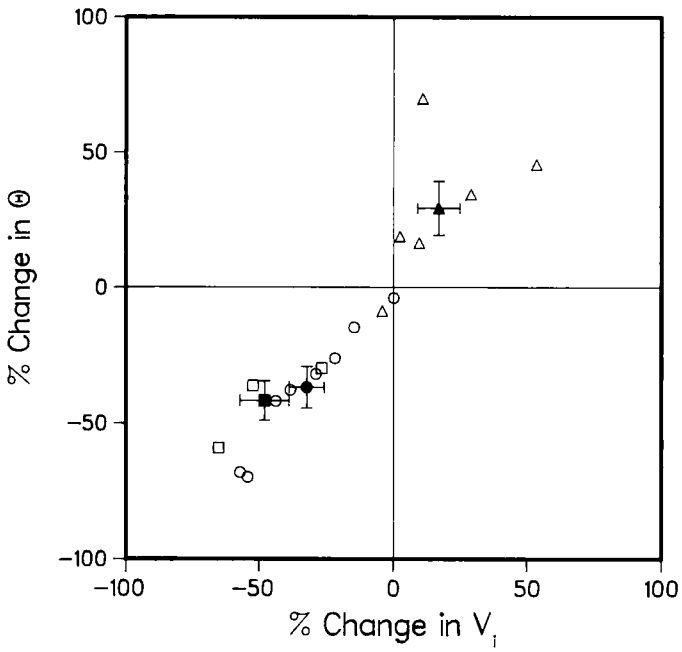

FIG. 3. Correlation between changes (% of control) in V_i and Θ' as a result of AGEPC or phenylephrine infusion in perfused rat livers. ○ = AGEPC (0.2 n*M*); □ = AGEPC (20 n*M*); △ = phenylephrine (10 μM). *Filled symbols* represent means ± S.E.

TABLE 3. Changes in erythrocyte transit times and volumes of distribution

Treatment	Control		Treatment		% change
	t_{RBC}	V_{RBC}	t_{RBC}	V_{RBC}	
AGEPC (n = 7)	3.67 (0.51)	2.14 (0.30)	2.51 (0.42)	1.47 (0.24)	−31.0 (11.8)
Phenylephrine (n = 7)	3.43 (1.07)	2.00 (0.63)	2.56 (0.95)	1.49 (0.55)	−25.9 (14.3)

Rabbit erythrocytes were prepared as described in "Materials and Methods." Bolus injections of erythrocytes (50 μl) were made using a 20% (v/v) suspension in Krebs-Henseleit buffer. The outflow of erythrocytes was measured by centifuging the tubes containing the outflow perfusate and measuring the optical density at 410 nm after resuspending and lysing the erythrocyte pellet in distilled water. S.D. are given in parentheses.

Methods." Control measurements were made on each liver prior to treatment with AGEPC or phenylephrine. Measurements during treatment with AGEPC or phenylephrine were made at the time of maximum developed vascular resistance, as before. Treatment with either AGEPC or phenylephrine decreased the transit time of the erythrocytes over control values as expected; however, there was no significant difference between the values obtained with the two agents (Table 3). Both agents lowered the transit time for erythrocytes by 25 to 30%. In these experiments AGEPC was used at a concentration of 2 n*M*.

DISCUSSION

The results presented here demonstrate clearly that one of the effects of AGEPC infusion into perfused rat livers is to reduce the space accessible to small, highly permeant molecules, such as urea. AGEPC is known to have potent vasoactive effects, particularly on vascular permeability (23, 24) and in several situations, AGEPC promotes increased vascular permeability and edema.

In the experiments reported here, AGEPC reduced both V_i, the mean-transit-time volume of tissue space, and Θ', the ratio of intracellular to extracellular volume. The close correlation between reduction of both V_i and Θ' would indicate a primary effect on cellular associated volume. Under both conditions, AGEPC and phenylephrine treatment, the transit time of labeled sucrose through the liver was decreased significantly relative to controls. The transit time of urea decreased sharply (21 and 39% for AGEPC concentrations of 0.2 and 20 n*M*, respectively) during AGEPC treatment compared to control values, more than proportionately for the decrease in transit time for the sucrose reference (13 and 34% for AGEPC concentrations of 0.2 and 20 n*M*, respectively). Phenylephrine (10 μM) treatment led to only a 5% decrease in the transit time of urea. These disproportionate changes in transit times for sucrose and urea are reflected in the calculated values for V_i and Θ'. For erythrocytes, which are confined to the vascular space, both agents decreased the transit time by the same amount. The reduction in both V_i and Θ' suggests that, during exposure to AGEPC, intracellular volume was reduced preferentially relative to the extracellular volume since V_i is related to the numerator of Θ'. However, the changes in V_i and Θ', for phenylephrine treatment, suggest the opposite case. There are several alternative hypotheses to explain these apparent changes in intracellular volume.

Exposure to AGEPC could affect the permeability of hepatocytes to urea, although this is not a likely possibility. Urea diffuses passively across the hepatocyte plasma membrane so a direct alteration of urea transport is unlikely. For substances which passively enter the cells, the measured volume of distribution should not be affected by permeability changes (20). For phenylephrine, it would seem unlikely that changes in the permeability of the hepatocytes could account for the calculated increase in Θ' or V_i. The transit time for urea does not change dramatically, so it is likely that decreases in the distribution of the sucrose tracer account for the changes observed with phenylephrine. Studies in cirrhotic human livers using the multiple indicator dilution technique have shown a reduced permeation of sucrose into extravascular space with progression of the disease (16).

Exposure of perfused livers to AGEPC could lead directly to a decrease in the volume of the hepatocyte. The most likely possibility for such an effect would be a perturbation of the ionic balances, leading to a volume decrease of the hepatocyte. AGEPC is known to affect the activity of the Ca^{++}, Mg^{++}-ATPase in certain cells (25) and could lead to conditions which result in loss of cell volume. Whether a reduction in cell volume, which has been shown to be dependent upon internal Ca^{++} under certain circumstances (26), would lead to a set of internal conditions in the parenchymal cells sufficient to trigger glycogenolysis is not known presently, but it presents an intriguing possibility. Edema, which has been reported to occur following AGEPC exposure, could result from the movement of cell water during a cellular associated volume decrease. The values obtained in the experiments reported here represent substantial de-

creases in V_i, however, so whether V_i accurately represents the volume of liver cells is not clear at this time. In a similar way, phenylephrine-induced swelling of hepatocytes could account for the observed increases in Θ' and V_i. Swelling of hepatocytes under hypoosmotic conditions in the intact liver has been documented to lead to increased portal pressure (27). Such a swelling should be expected to occlude interstitial spaces as well as the sinusoidal lumen. This would have two effects: an increase in V_i due to swelling of the hepatocytes and an increase in Θ' due to the preferential loss of sucrose-accessible space. Overall, the total urea accessible space should remain roughly constant, only partitioned differently, which can be seen in the lack of significant change in the transit time for urea under phenylephrine treatment. Direct measurement of the sinusoidal bore within livers of anesthetized rats using transillumination techniques has shown a 13% decrease in sinusoidal diameter during adrenergic stimulation (28). Predicted reductions in vascular space (32%) from that study are similar to reductions (34%) in vascular space seen in the livers of dogs due to reflex sympathetic effects using the multiple indicator dilution technique to measure the space changes (17). The results obtained here with red blood cells indicate a reduction in vascular space of similar magnitude during phenylephrine treatment, 25%.

A third possibility is that during AGEPC treatment the liver is not well perfused; that is, some portions of the liver experience little or no flow. Under these conditions, a reduced mass of liver would be exposed to the injected tracers, so that the volumes of distribution for urea and sucrose would underestimate the actual volumes of distribution for the tracers in entire liver. Reducing the availability of tissue to perfusion would also lower the effective surface area and thus the permeability of transported substances. Indeed, the close correlation between reductions in V_i and Θ', which indicate loss of intracellular space relative to extracellular space, and the reduction in the space accessible to red blood cells, *i.e.* a decreased transit time, indicate that portions of the liver are not perfused during treatment with AGEPC and that the loss of liver space is on the order of 31%. Other changes which occur during AGEPC treatment, transient reductions in oxygen utilization and irregular production of glucose (2), could be explained by this mechanism. Whether localized hypoxia would result during AGEPC treatment under conditions of altered flow and what the metabolic consequences of altered flow would be remain to be seen.

It is clear that sizable perturbations in the urea-accessible tissue space occur during AGEPC exposure. Whether the effects of perturbations of the microvasculature and tissue spaces in the liver by AGEPC lead to the increased rates of glycogenolysis observed is not clear. The glycogenolytic response begins early following exposure of livers to AGEPC, as does the increase in vascular resistance. The measurements from which V_i and Θ' were calculated were made at a point after the increases in glycogenolysis and vascular resistance had begun. Since AGEPC does not have direct effects on glycogenolysis in hepatocytes (15), the interaction of AGEPC with other cell types must be considered. In perfused livers, AGEPC induces vasoconstriction in a dose-dependent manner, which is sensitive to perfusate calcium levels, further suggesting sinusoidal cells as the site of action of AGEPC (2). Experiments with heat-aggregated IgG administered to perfused livers result in similar responses from the liver as those following AGEPC infusion (4). Moreover, although the infusion of heat-aggregated IgG leads to the production of AGEPC in the liver, the effects of heat-aggregated IgG do not seem to be mediated completely through AGEPC (4). It remains to be seen precisely which mediators are involved in the response of the liver to AGEPC which produce the dramatic metabolic and hemodynamic effects observed.

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