did not employ many strains of *Ps. aeruginosa* in their experiments. It was observed that lecithin, suspended with the aid of glycerin in nutrient broth, was most effective in inactivating polymyxin B sulfate. The possible mechanism of action is similar to that given under benzalkonium chloride. It was also noted that nutrient broth containing lecithin solubilized by either Tween 20 or Tween 80 was not as effective as the above-mentioned inactivating medium. This observation was also noted by Riegelman, *et al.* (17), and Bliss and Worth (23). A possible explanation may be that the effective charge of the lecithin molecule is reduced when it is solubilized by the Tweens. This reduction in the inhibiting action of lecithin by the Tweens was not observed with benzalkonium chloride. The Tweens, by themselves, were capable of reducing the antibacterial action of benzalkonium chloride, while they had no such effect on polymyxin B sulfate.

SUMMARY

1. Various *in vitro* studies were carried out to determine the antibacterial effectiveness of various chemical agents to be used as preservatives in ophthalmic solutions against 13 strains of *Ps. aeruginosa*. Methods were devised to (*a*) differentiate between the bacteriostatic and bactericidal properties of the chemical preservatives by formulating suitable inactivating media, (*b*) determine the sterilizing time for each chemical preservative, and (*c*) determine whether the volume of inoculum taken for sampling in the experiment was adequate.

2. An *in vivo* procedure was employed in evaluating the chemical agents to note whether findings would be in agreement with the final results obtained in the *in vitro* studies.

3. The following antibacterial agents used or recommended for use in ophthalmic solutions were thoroughly investigated: chlorobutanol, benzalkonium chloride, thimerosol, combinations of methylparaben and propylparaben, phenylmercuric nitrate, phenylethyl alcohol, and polymyxin B sulfate. Of these, only benzalkonium chloride possessed a sterilizing time against *Ps. aeruginosa* of less than 1 hour.

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Effect of Certain Drugs on Perfused Human Placentas II

Vasodilators

By HENRY P. CIUCHTA and RONALD F. GAUTIERI

Perfusion studies conducted on full term human placentas produced the following results: marked vasodilation with isosorbide dinitrate, isoproterenol hydrochloride, nitroglycerin and papaverine hydrochloride, slight vasodilation, and vasoconstriction with cyclandelate and isoxsuprine hydrochloride. Except for alcohol, which produced a slight decrease in the volume flow rate through the placenta, the solvents employed with these drugs had no effect on placental vasculature.

During the past 40 years the action of various agents on placental vasculature has been studied by numerous investigators, including Kosakae (1), Ueda (2), von Euler (3), Schmitt (4), Eliasson and Astrom (5), and Astrom and Samelius (6). The results of these experiments are rather inconsistent. This undoubtedly is partly due to the employment of different experimental procedures and the inability at times to obtain placentas within a short time after delivery. More than likely, the

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major reason for varying results is the sensitivity of the placenta itself.

Schmitt (4), besides demonstrating that the placenta is devoid of innervation, showed that histamine, posterior pituitary extract, and barium chloride constricted placental vasculature, while amyl nitrite caused a dilatation and adrenaline produced no effect. von Euler (3) conducted extensive studies on "nerve free" vessels of the placenta and found only a constrictor action with epinephrine, acetylcholine, histamine, pitressin, and barium chloride. He postulated that these drugs may act on some intermediate mechanism of the contractile element.

In an earlier work (7) von Euler observed that acetylcholine constricted pulmonary vessels in the rabbit. From these investigations (3, 7) he proposed that a correlation may exist between the responses of placental and pulmonary vessels, since arteries of both organs carry venous blood and are structurally adapted to withstand moderate pressures. Smith and Coxe (8) submitted supporting evidence in observing that pulmonary vessels in cat, dog, swine, and human lungs were constricted with epinephrine, acetylcholine, and histamine.

Experiments conducted by Dornhorst and Young (9) on placental vessels of guinea pig *in situ* indicated that adrenaline and noradrenaline caused a constriction of these vessels by a direct action, thus impairing placental circulation.

Eliasson and Astrom (5) observed vasoconstriction in placental vessels with epinephrine, norepinephrine, dihydroergotamine, acetylcholine, and histamine. Astrom and Samelius (6), in addition to testing the effects of lysergic acid, chlorpromazine, tryptamine, yohimbine, mescaline, phentolamine, phenbenzamine, reserpine, ganglionic blocking agents, and heparin, established the potent vasoconstrictor activity of serotonin on placental vasculature.

Previously in this laboratory (10) it was demonstrated that the vasculature of the placenta undergoes various degrees of vasoconstriction in the presence of narcotic analgesics, serotonin, and relaxin.

Since the actions of many vasoconstrictors on placental vessels have been intensively investigated, a study of the effects of vasodilators on this preparation is indicated. Proof exists that morphine (10) and histamine (5), which usually elicit vasodilation in man, cause vasoconstriction in placental vessels. Therefore, there is a need to determine whether certain vasodilators may also follow a similar pattern of producing an opposite effect on placental vasculature. The placenta provides a valid technique for ascertaining the action of musculotropic drugs because it is devoid of nervous tissue.

Therefore, it was the purpose of this investigation to observe the effects of clinically proved vasodilators alone on placental vasculature, and in certain cases, in the presence of drug induced spasm.

MATERIALS AND METHODS

Full term human placentas were obtained from the hospital 5–15 minutes after normal delivery. Each was transported to the laboratory in a container of 38° preheated Tyrode's solution.

The preparation was flushed free of blood by injecting 10% sodium citrate solution into one of the arteries. Cannulas were inserted into the vein and one artery of the umbilical cord 5–10 cm. from the stalk of the placenta and the remaining umbilical artery was ligated. The whole preparation was then placed in a perfusion chamber containing aerated Tyrode's solution which had a pH range of 7.2 to 7.4 and was maintained at a constant 37° by a Bronwill thermoregulator. The arterial cannula was connected with rubber tubing to a hydrostatic reservoir which was adjusted to yield pressures ranging between 40–60 mm. Hg. The reservoir contained Tyrode's solution which was modified by the addition of 0.525% polyvinylpyrrolidone (Plasdone C). The venous outflow, ranging between 10–60 ml./minute, was measured directly by means of a graduated cylinder and not reperfused.

Two placentas were discarded because the outflow per minute at the onset of the experiment was far below average; it was considered that the volume changes would not be of significant value.

TABLE I.—THE EFFECTS OF DRUGS ON VOLUME FLOW IN PERFUSED HUMAN PLACENTAS

	Composite Results					
Drug	No. of Prepns.	Dose	Vol. Flow (Range), % Increase	Decrease	Av. %	S. D.
Cyclandelate	4	1–4 mg.	11–19	...	15.5	2.6
Cyclandelate	11	1–4 mg.	...	5–15	10.2	3.5
Isoxsuprine	10	1–10 mg.	5–19	...	9.4	4.0
Isoxsuprine	5	1–10 mg.	...	5–11	7.0	2.3
Isosorbide	5	1–2 mg.	25–116	...	49.6	37.4
Isoproterenol	11	2.0 mg.	5–84	...	41.7	28.6
Nitroglycerin (spirit)	5	0.2 ml.	18–159	...	73.4	67.0
Nitroglycerin (aqueous)	6	1–4 mg.	5–36	...	20.3	11.6
Papaverine	9	4–5 mg.	5–65	...	30.4	15.0
Serotonin	9	50–100 mcg.	...	7–81	28.8	24.6

In 85 successful experiments the average duration of each preparation was between 2 and 3 hours.

The following drugs were injected into the rubber tubing adjacent to the arterial cannula in a volume of distilled water not exceeding 1 ml.: cyclandelate,[1] isoxsuprine hydrochloride,[2] isosorbide dinitrate,[1] isoproterenol hydrochloride,[3] nitroglycerin (alcoholic and aqueous vehicles), papaverine hydrochloride, and 5-hydroxytryptamine creatinine sulfate (serotonin). In addition, alcohol and propylene glycol were injected to observe if these vehicles elicited action of their own.

RESULTS

Cyclandelate.—Cyclandelate in doses of 1–4 mg. (four preparations) produced a slight vasodilation of placental vessels. The increase in volume outflow ranged from 11–19% (Table I). However, in 11 preparations there was a decrease in outflow ranging from 5–15% (Table I). After cyclandelate administration, three preparations yielded a change in volume outflow below 5% and were not counted.

The administration of serotonin after pretreatment of a preparation with cyclandelate elicited a typical vasoconstrictor effect. Isoproterenol injection caused an increase in volume outflow after a volume decrease due to cyclandelate.

Isoxsuprine.—The administration of isoxsuprine hydrochloride (10 cases) in doses of 1–10 mg. produced an increase in volume outflow of 5–19% (Table I). In five cases there was a 5–11% decrease in the outflow rate (Table I). A change in the outflow rate below 5% was recorded in 10 preparations.

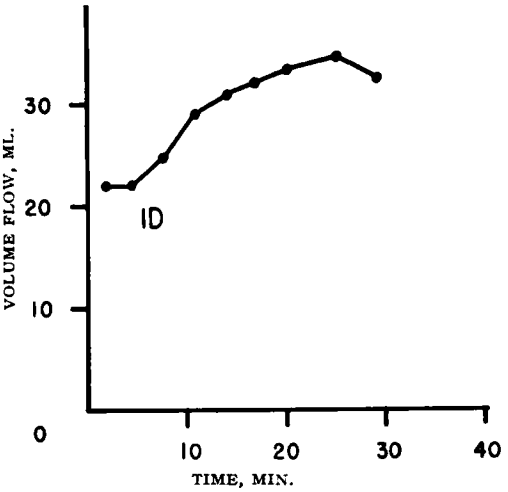

Fig.1—Effect of isosorbide dinitrate (1 mg.) on volume flow rate in vessels of the perfused human placenta.

After a decrease or increase in outflow rate due to isoxsuprine, the administration of serotonin in certain cases brought about vasoconstriction, while subsequent injections of papaverine increased the volume outflow.

Isosorbide.—In a total of five experiments isosorbide dinitrate in doses of 1–2 mg. produced an increase in volume outflow (Fig. 1). The range of outflow was increased 25–116% (Table I). Only one preparation yielded a change in outflow rate below 5%.

The subsequent administration of nitroglycerin caused an even greater increase in the volume outflow in certain preparations.

Isoproterenol.—The administration of 2 mg. of isoproterenol hydrochloride (11 preparations) brought about a powerful vasodilation (Fig. 2) which was of a long duration and not increased by the subsequent injection of cyclandelate in certain cases. The outflow rate increased from 5–84% (Table I). Results obtained from three preparations were under 5%.

Nitroglycerin.—In five cases dramatic increases in volume outflow (Fig. 3) were obtained with the administration of 0.2 ml. of nitroglycerin spirit (92% alcohol as the vehicle). The per cent increase in volume outflow ranged from 18–159 (Table I).

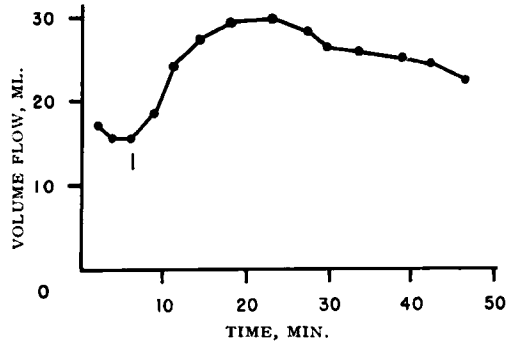

Fig. 2—Effect of isoproterenol (2 mg.) on volume flow rate in vessels of the perfused human placenta.

The administration of 1–4 mg. of nitroglycerin (aqueous vehicle) produced an increase in volume outflow in six cases, having a range of 5–36% (Table I). All preparations yielded increases of 5% or more.

The administration of serotonin in certain preparations following alcoholic nitroglycerin brought about a slight and transient vasoconstriction. Treatment of a preparation with isosorbide after aqueous nitroglycerin caused a significant increase (32%) in volume outflow.

Papaverine.—In nine preparations doses of 4–5 mg. of papaverine hydrochloride produced an increase in volume outflow ranging from 5–65% (Table I). The change in outflow rate of two preparations was under 5%.

The administration of barium chloride (Fig. 4) and serotonin (Fig. 5) following papaverine injection elicited vasoconstriction.

Serotonin.—The administration of serotonin in 50–100-mcg. doses in nine cases evoked a marked decrease in volume outflow ranging from 7–91% (Table I). Significant responses were not obtained in two preparations.

Alcohol.—Injections of 0.2 ml. of alcohol (95%) produced a predominant decrease in outflow in four cases having a range of 4–10%. No change in volume outflow was recorded in two instances.

[1] Supplied through the courtesy of Ives-Cameron Laboratories, New York, N. Y.
[2] Supplied through the courtesy of Mead Johnson Research Center, Evansville, Ind.
[3] Supplied through the courtesy of Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Propylene Glycol.—Propylene glycol caused a slight increase in outflow in two cases and a moderate decrease in two preparations.

DISCUSSION

The action of vasodilating agents is mediated through the activation of several different mechanisms, including (*a*) blood vessel musculature, (*b*) specific autonomic receptors and/or their chemical mediators, and (*c*) the central nervous system and its pathways. For example, guanethidine acts predominantly at the nerve-arteriole junction (11, 12) where it depletes norepinephrine in sympathetic nerve endings, thereby bringing about a vasodilating effect. Epinephrine, in small concentrations, produces a hypotensive effect which is mediated through stimulation of the inhibitory β-receptors (13). Papaverine (14), isosorbide (15), nitroglycerin (16), and cyclandelate (17) supposedly

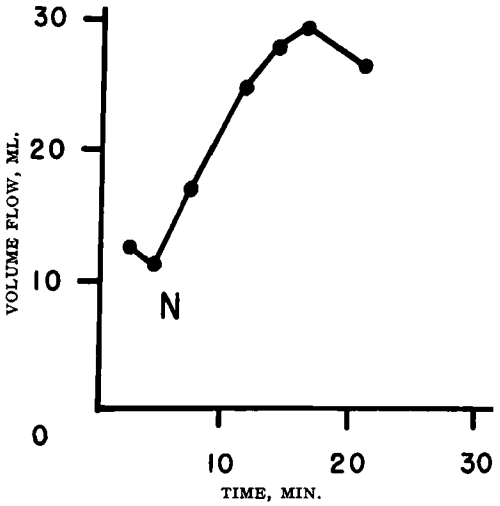

Fig. 3.—Effect of nitroglycerin spirit (2 mg.) on volume flow rate in vessels of the perfused human placenta.

inhibit or depress vessel musculature directly to elicit vasodilation. Isoxsuprine (18), in addition to its direct depressant action (papaverine-like) on vasculature, is thought to activate β-adrenergic receptors. The results of this investigation indicate that, generally, gross qualitative differences do not exist between the activity of the majority of vasodilators on placental vasculature and other circulatory beds.

The administration of cyclandelate to 15 different preparations produced either slight vasodilation or vasoconstriction. In four of these experiments the volume outflow increased above 10%, while in 11 preparations there was a decrease in volume outflow to 15%. In addition, the change in the volume outflow rate after cyclandelate administration in three other preparations was below 5%. Since responses obtained with cyclandelate were rather unpredictable and relatively slight, it is likely that placental vasculature does not possess the specific mechanism upon which this drug exerts its action. Furthermore, since consistent increases in volume outflow were obtained with papaverine and nitroglycerin, agents which affect vascular musculature directly, it follows that cyclandelate does not possess marked depressive musculotropic activity. Therefore, vasodilation that occurs in man after cyclandelate administration may be attributed mainly to some other pathway or mechanism that is not present in isolated placental preparations.

Isoxsuprine produced an increase in volume out-

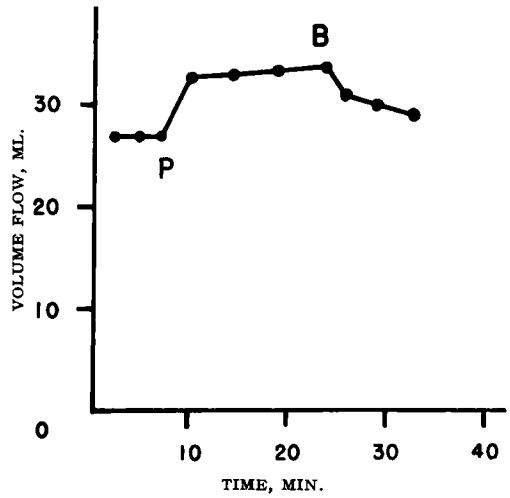

Fig. 4.—Effect of papaverine (5 mg.) and barium chloride (12.5 mg.) on volume flow rate in vessels of the perfused human placenta.

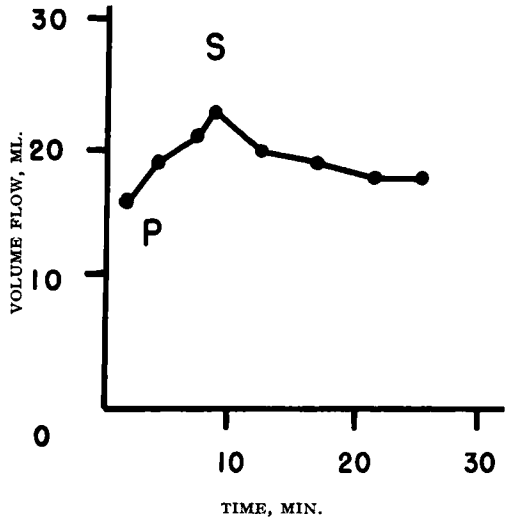

Fig. 5.—Effect of papaverine (5 mg.) and serotonin (50 mcg.) on volume flow rate in vessels of the perfused human placenta.

flow of over 5% in 10 preparations, a decrease of 5–11% in five, and either an increase or decrease of under 5% in 10. These varied responses would seem to indicate that, as with cyclandelate, placental vessels are devoid of a specific pathway upon which isoxsuprine acts. The activity of this agent on β-adrenergic receptors appears relatively slight since the degree and consistency of vasodilation are not comparable to that of isoproterenol which causes dilatation by activating these inhibitory receptors.

Vasodilation of the same magnitude as that produced by papaverine was never observed with isoxsuprine. Therefore, any papaverine-like activity that this agent may possess is relatively weak.

Treatment of placental preparations with isosorbide evoked responses that were qualitatively and quantitatively similar to those obtained from coronary artery studies in isolated hearts (19). These cardiac experiments showed that isosorbide produced an average increase in flow of 30–40% through coronary vessels, while in our investigation the average increment in volume outflow through placental vessels was 49%. Since both types of isolated preparations respond favorably to isosorbide administration, it appears that the effect of this agent depends mostly on its direct action on vascular musculature.

Isoproterenol brought about a sustained dilatation of vessels. However, this increase was not comparable to that occurring in response to alcoholic nitroglycerin or isosorbide. The mechanism of action of isoproterenol *in vivo* is due to stimulation of inhibitory β-receptors (12) and this logically could be the basis for its action on placental vessels.

Organic nitrates are known to be very active in causing dilatation of vessels; therefore, the increase in volume outflow produced in the placenta by nitroglycerin was anticipated. However, nitroglycerin spirit elicited a greater increase in flow than the aqueous preparation. It is possible that alcohol may have increased permeability in a manner to allow more effective cell contact, with the concomitant augmentation in response. This is not in accordance with the fact that the administration of 0.2 ml. of 95% alcohol did not cause dilatation of vessels but on the contrary caused appreciable vasoconstriction. The vasodilating action of alcoholic nitroglycerin was so powerful that subsequent administration of serotonin produced only very slight vasoconstriction.

Studies conducted by Eckenhoff and Hafkenschiel (20) indicate that papaverine is the most efficient coronary dilator. It should be noted that drugs which produce coronary dilation exert similar responses on placental vasculature. Likewise, in this experiment it was found that papaverine caused a prolonged dilation resulting in a marked increase in volume outflow. Furthermore, the action of papaverine was so intense that barium chloride and serotonin did not alter the dilation to any appreciable degree (Figs. 4 and 5).

The possibility exists, as indicated by von Euler (3), that the action of drugs on placental vasculature may correspond to their action on pulmonary vessels. An interesting aspect that should be explored is testing various agents on pulmonary and placental arteries and veins individually. It may be possible that these agents affect pulmonary and placental arteries and veins in a slightly different manner than other systemic arteries and veins which have a completely different oxygen content. Therefore, a correlation may exist between these two types of vessels and their reactions to drugs.

As indicated in a previous investigation (10), the possibility exists that fetal metabolism may be affected in part because of the action of drugs on placental vasculature. This is of significance if directly acting vasoconstricting agents are employed, since constriction of placental vessels may impair fetal tissue oxygenation. It should be kept in mind that in a pregnant patient with a previous history of hypotension the administration of vasoconstrictors may sensitize the placental vasculature and cause subsequent disturbance in fetal circulation. If placental vasoconstriction is suspected *in vivo*, certain of the vasodilators used in this investigation may be employed to counteract it.

Throughout this investigation, as in von Euler's study (3), a valid dose/response relationship does not appear to exist, as similar degrees of action occur with relatively small and large doses. This probably is because of the individual sensistivity of the placenta as well as to a probable lack of specific receptor sites.

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