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The use of the *in vivo* trachea preparation of the guinea-pig to assess drug action on lung

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A method for recording the effects of drugs on an isolated *in vivo* segment of trachea and on lung pressure of a deeply anaesthetized animal is described. Acetylcholine, angiotensin, histamine, 5-hydroxytryptamine, prostaglandin (PG) $F_{2\alpha}$ and slow-reacting substance of anaphylaxis, but not bradykinin, caused an increase in tracheal segment pressure and an increase in lung pressure. Bradykinin caused an increase in lung pressure but a fall in tracheal segment pressure. It was concluded that bradykinin acts mainly on the smaller airways and $PGF_{2\alpha}$ mainly on the larger airways. The tracheal segment responded to adrenaline, aminophylline, ephedrine, isoprenaline, papaverine, PGE_1 and PGE_2. Propranolol reduced or abolished responses to all these bronchodilators except PGE_1 and PGE_2.

For many years the preparation of Konzett-Rössler (1940) has been used to assess the effects of drugs on lung (Collier, Holgate, & others, 1960; Bhoola, Collier & others, 1962; Collier & Shorley, 1963; Berry & Collier, 1964; Collier, James & Schneider, 1966; James, 1967). Although the method has been modified (Collier & others, 1960) and made more sensitive (Rosenthale & Dervinis, 1968), since it estimates changes in lung volume, it remains more sensitive to changes in lung compliance (Widdicombe, 1963) than to changes in airway resistance. The Konzett-Rössler preparation does not respond directly to the effects of bronchodilator drugs. To assess these effects a bronchoconstrictor agent is normally used (James, 1967). A more accurate assessment of bronchodilator action can be made by measuring airway resistance (Diamond, 1967; Familiar, Wardell & Greene, 1967).

In the experiments now reported, measurements were made of lung pressure and of pressure inside a segment of trachea *in vivo* (Kahn, 1907; Green & Widdicombe, 1966), using artificially ventilated guinea-pigs. An assessment of drug effect on lung compliance and airway resistance could thus be made. The effects of bronchoconstrictor and bronchodilator drugs on this preparation have been investigated and compared.

EXPERIMENTAL

Materials

The following were used as bronchoconstrictor agents: acetylcholine bromide, angiotensin, bradykinin (synthesized by Nicolaides & De Wald, 1961), histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Dr. J. E. Pike of the Upjohn Co.)

The following were used as bronchodilator agents: adrenaline tartrate, aminophylline, ephedrine hydrochloride, isoprenaline sulphate, papaverine sulphate and prostaglandins E_1 and E_2 (PGE_1 and PGE_2) (Dr. J. E. Pike of the Upjohn Co.). As antagonists, atropine sulphate, meclofenamic acid [*N*-(2,6-dichloro-*m*-tolyl)anthranilic acid] (Winder, Wax & Welford, 1965) as the sodium salt, mepyramine maleate, methysergide bimaleate, propranolol hydrochloride and tolazoline hydrochloride were used.

Procedure

Guinea-pigs (Duncan-Hartley strain), 600–1000 g, were anaesthetized with 60 mg/kg of pentobarbitone sodium given intraperitoneally. A cannula (ventilation cannula, Fig. 1) was inserted into the lower trachea as near as was possible to the thorax. The

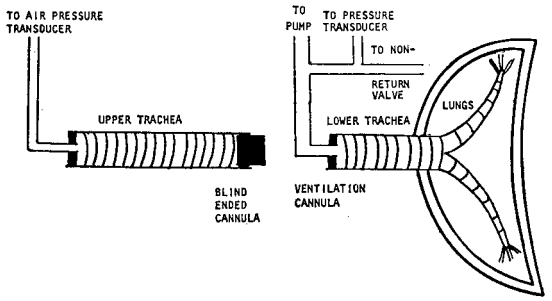

FIG. 1. Diagram to show the arrangement of the apparatus. For description see Experimental Procedure.

guinea-pig was ventilated through this cannula with a Starling miniature respiratory pump of 8 ml stroke volume and at a rate of 72 strokes/min. The side arm of the cannula was connected to a non-return water valve set for a pressure of 10 cm of water. Intrapulmonary pressure (lung pressure) was measured by a differential air pressure transducer attached to the cannula and recordings were made on a multi-channel electronic recorder. A second cannula (blind ended) was inserted into the trachea directed towards the larynx (see Fig. 1). A third cannula was inserted into the upper trachea as far as was possible from the lungs and was connected to a very sensitive air pressure transducer. Great care was taken when inserting the cannulae, to avoid complete interruption of the nerve or blood supply to the "isolated" segment of trachea. Measurements of pressure within the segment of trachea (tracheal segment pressure) were made to assess the "tone" of the segment and tracings were made on the multi-channel recorder. For intravenous administration of drugs, the jugular vein was cannulated. For intra-arterial administration, a cannula was inserted into the aortic arch via the carotid artery. All drugs were given intravenously except where specified.

RESULTS

Anaesthetic. Animals were anaesthetized with 60 mg/kg intraperitoneally of pentobarbitone sodium. At this level of anaesthesia, bronchoconstrictor agents such as histamine produced an increase followed by a decrease of pressure in the tracheal segment. Increasing the depth of anaesthesia with intravenous doses of pentobarbitone sodium did not materially alter quantitatively or qualitatively the response obtained (Fig. 2).

Bronchoconstrictor agents

Acetylcholine. 1–10 μg caused a slight increase in lung pressure and a small increase followed by a decrease of tracheal segment pressure. Both increases in pressure were abolished by 1 mg/kg of atropine. The decrease in pressure in the trachea after atropine and acetylcholine was still present but much reduced.

Angiotensin. 2–5 μg caused an increase in lung pressure and an increase followed by a decrease of tracheal segment pressure. Neither response was antagonized by meclofenamate.

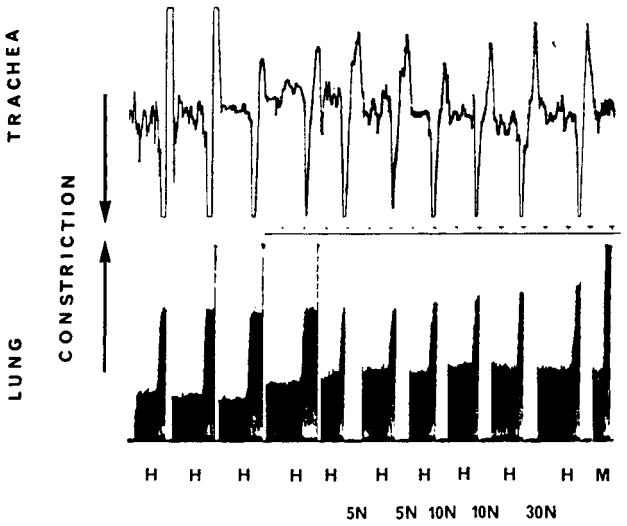

FIG. 2. Effect of anaesthetic on responses to histamine on lung pressure and tracheal segment pressure in the guinea-pig. Upper tracing—tracheal pressure, lower tracing—lung pressure. Guinea-pig of weight 800 g anaesthetized with pentobarbitone sodium 60 mg/kg i.p. and artificially ventilated. All doses were given intravenously. H, 4 μg histamine; N, dose of pentobarbitone sodium in mg/kg; M, maximum response of lung pressure obtained by clamping the trachea. All doses were given at 10 min intervals ↑ direction of increased pressure; time 1 min. The breaks in the lung pressure tracing are due to movement of the recording paper when no record was being made.

Bradykinin. Given intravenously, bradykinin (2–20 μg) increased the lung pressure and induced a small decrease in the tracheal segment pressure. Meclofenamate (2 mg/kg) antagonized the response to bradykinin on the lung pressure (Fig. 3a). Propranolol pretreatment increased the response to bradykinin on the lung pressure and abolished the decrease of the tracheal segment pressure (Fig. 3b left). Given intra-arterially, bradkyinin induced a decrease in the tracheal pressure, but did not affect the lung pressure (Fig. 3b right).

Histamine. 2–4 μg caused an increase in lung pressure and an increase followed by a decrease in tracheal segment pressure (Fig. 2). Both responses were antagonized by 2 mg/kg mepyramine, but not by 2 mg/kg meclofenamate.

5-Hydroxytryptamine, 2–4 μg gave similar responses to those obtained with histamine and could be antagonized by pretreatment with 1 mg/kg of methysergide.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). 20–50 μg in the absence of propranolol caused a small pressure increase in the trachea, with very little effect on the lung pressure. In the presence of propranolol, $PGF_{2\alpha}$ increased the lung pressure and caused a large increase in tracheal segment pressure. Neither response was antagonized by 2 mg/kg meclofenamate (Fig. 4).

Slow-reacting substance in anaphylaxis (SRS-A). 0·5–4 mg of partially purified material caused an increase in lung pressure and tracheal segment pressure. These responses were antagonized by 2 mg/kg of meclofenamate (Fig. 5). In this figure a small decrease in tracheal pressure after meclofenamate may be observed.

Bronchodilator agents

Adrenaline. 0·05–0·2 μg produced a small increase followed by a large decrease of the tracheal segment pressure. The decrease was antagonized by pretreatment with

propranolol and the increase by pretreatment with tolazoline. Adrenaline did not produce a detectable change in the lung pressure (Fig. 6a).

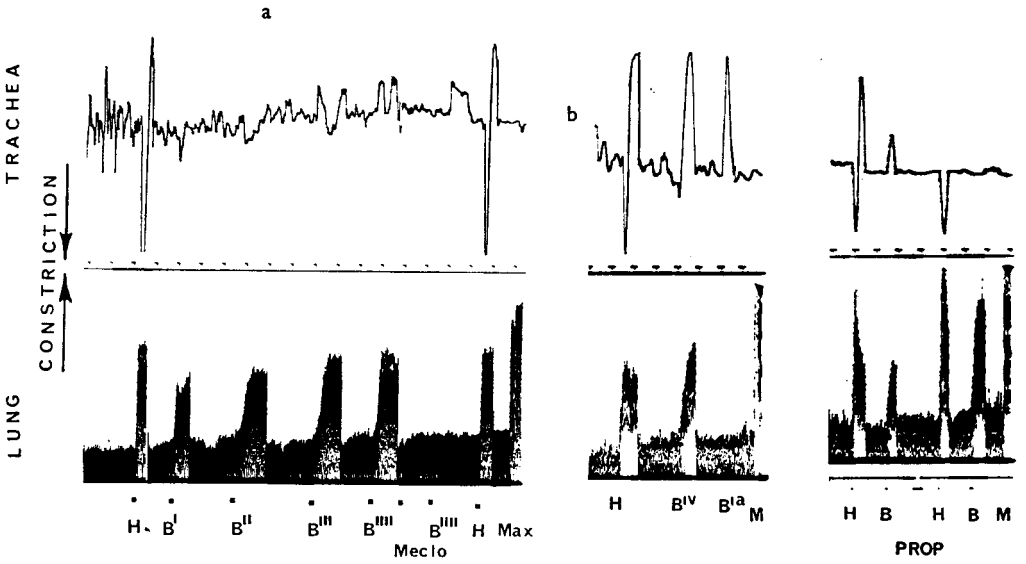

FIG. 3. a. Effect and antagonism of bradykinin on lung pressure and tracheal segment pressure in a guinea-pig, 710 g. H, 2μg histamine; B′, 2 μg bradykinin; B″, 4μg bradykinin; B‴, 8 μ bradykinin; B⁗, 20 μg bradykinin; Meclo, 2 mg/kg meclofenamate.

b. Effect of propranolol on the response to intravenous bradykinin and the effect of bradykinin given intra-arterially on lung pressure and tracheal segment pressure in the guinea-pig. Right-hand panel: Guinea-pig, 920 g. H, 3 μg histamine; B, 2 μg bradykinin; PROP, 5 mg/kg of propranolol. All substances were given intravenously. Left-hand panel: Guinea-pig 850 g. H, 3 μg histamine; B, i.v. 5 μg bradykinin given intravenously; B i.a., 5 μg bradykinin given intra-arterially.

Other details as for Fig. 2.

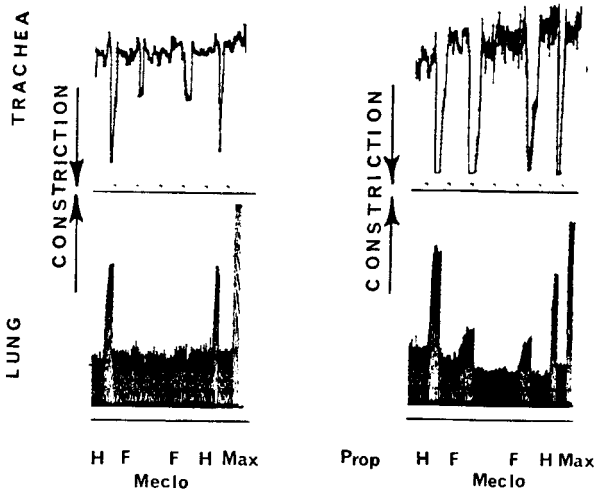

FIG. 4. Effect of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) on lung pressure and tracheal segment pressure in the guinea-pig. Left-hand tracing: Guinea-pig, 840 g. H, 2 μg histamine; F, 50 μg $PGF_2\alpha$; Meclo, 2 mg/kg meclofenamate sodium. Right-hand tracing: Guinea-pig, 750 g, pretreated with propranolol (Prop), 5 mg/kg intravenously.

Other details as for Fig. 2.

Aminophylline. Doses of 1–3 mg caused a decrease in tracheal segment pressure but did not alter the lung pressure. After pretreatment with propranolol, the change in tracheal segment pressure due to aminophylline was reduced.

Ephedrine. 0·5–1 mg caused a decrease in the tracheal segment pressure but no recordable effect on lung pressure. The tracheal response was antagonized by propranolol.

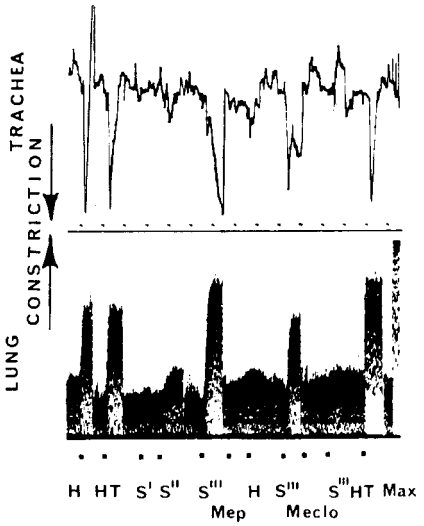

FIG. 5. Effect and antagonism of slow-reacting substance in anaphylaxis (SRS-A) on lung pressure and tracheal segment pressure in the guinea-pig. Guinea-pig, 860 g. H, 3 μg histamine; HT, 2 μg 5-hydroxytryptamine; S', 0·5 mg SRS-A; S'', 1 mg SRS-A; S''', 3 mg SRS-A; Mep, 2 mg/kg mepyramine; Meclo, 2 mg/kg meclofenamate sodium. Other details as for Fig. 2.

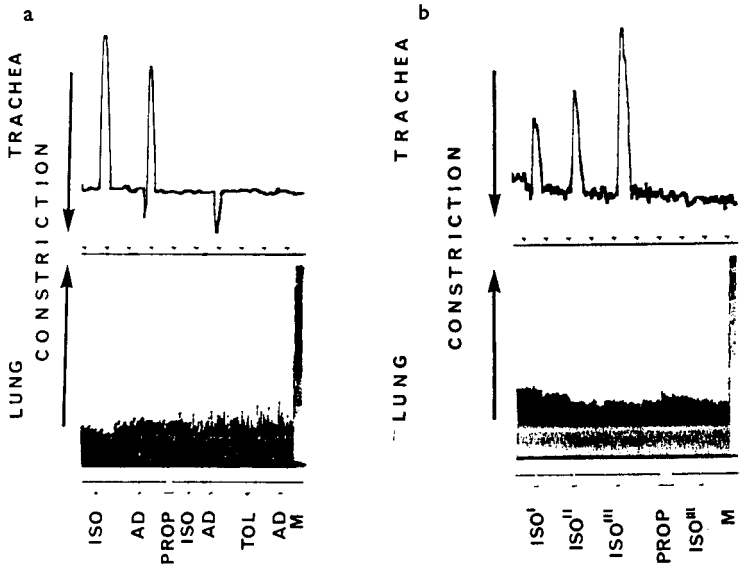

FIG. 6. a. Effect and antagonism of adrenaline on lung pressure and tracheal segment pressure in a guinea-pig, 920 g. ISO, 0·01 μg isoprenaline; AD, 0·1 μg adrenaline; PROP, 5 mg/kg propranolol; TOL, 5 mg/kg tolazoline.

b. Effect and antagonism of isoprenaline on lung pressure and tracheal segment pressure in a guinea-pig, 870 g. ISO', 0·002 μg isoprenaline; ISO'', 0·004 μg isoprenaline; ISO''', 0·01 μg isoprenaline; PROP, 5 mg/kg propranolol.

Other details as for Fig. 2.

Isoprenaline. 2–10 ng caused a dose-related pressure fall in the trachea, but no response on the lung pressure. The tracheal response was antagonized by propranolol (Fig. 6b).

Papaverine. 0·5–3 mg gave similar results to those obtained with aminophylline. The pressure fall in the trachea was only partially antagonized by propranolol.

Prostaglandin E_1 and E_2 (PGE_1 and PGE_2). Both prostaglandins caused a decrease in tracheal segment pressure with no recordable effect on lung pressure. The responses were not antagonized by 5 mg/kg of propranolol. PGE_1 was approximately twice as potent as PGE_2 in this test (Fig. 7).

The results obtained with the above drugs are summarized in Table 1.

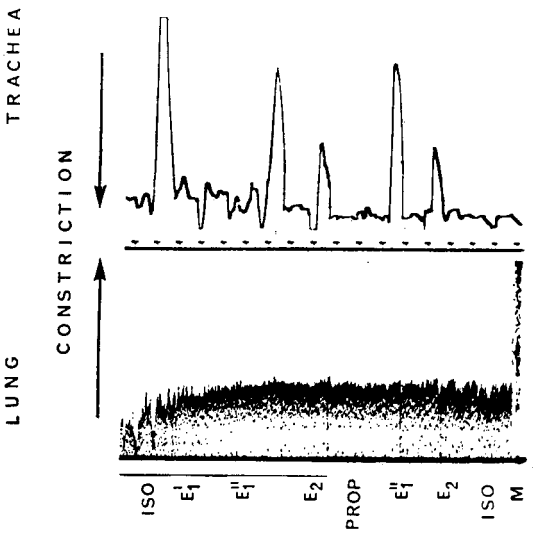

FIG. 7. Effects of prostaglandins E_1 and E_2 on lung pressure and tracheal segment pressure in a guinea-pig, 840 g. ISO, 0·05 μg isoprenaline; E'_1, 5 μg prostaglandin E_1; E''_1, 10 μg prostaglandin E_1; E_2, 10 μg prostaglandin E_2; PROP, 5 mg/kg propranolol. Other details as for Fig. 2.

DISCUSSION

With the exception of the prostaglandins, the effects of the drugs used in these experiments on the Konzett-Rössler preparation have been well documented (Collier & others, 1960; Holgate & Warner, 1960; Berry & Collier, 1964). These effects are similar to those on lung pressure described above. This discussion will therefore be mainly confined to the effects of drugs on the tracheal preparation. The pressure rise in the trachea caused by acetylcholine, angiotensin, histamine and 5-HT as well as their effects on lung pressure indicate that these drugs act on both large and small airways. These results for acetylcholine, histamine and 5-HT confirm those obtained by Colebatch, Olsen & Nadel (1966) in the cat. The fall in tracheal segment pressure following the increase due to these drugs could be abolished by propranolol. Most preparations responded biphasically.

Fig. 3 shows that bradykinin did not increase tracheal segment pressure after large doses, although changes in lung pressure were obtained and could be antagonized. Because bradykinin may have been inactivated before reaching the trachea (Ferreira & Vane, 1967) it was given intra-arterially; but still it produced no pressure increase. To check that the lack of response was not due to a physiological antagonism between

Table 1. *Effect of some drugs on lung pressure and tracheal segment pressure in the guinea-pig.* Each result was derived from at least 3 experiments All drugs were given intravenously except for bradykinin, i.v., intravenous; i.a., intra-arterial; + pressure increase; − pressure fall; +−increase followed by a decrease in pressure; 0, no effect

	Effect on			Effect of drug after antagonist on	
Drug	Trachea	Lung	Antagonist	Trachea	Lung
Acetylcholine	+−	+	Atropine	−	0
Angiotensin	+−	+	Meclofenamate	+−	+
Bradykinin i.v.	−	+	Meclofenamate	−	0
Bradykinin i.v.	−	+	Propranolol	0	+
Bradykinin i.a.	−	0	—		
Histamine	+−	+	Mepyramine	−	0
5-HT	+−	+	Methysergide	−	0
$PGF_{2\alpha}$	+	+	Meclofenamate	+	+
SRS-A	+	+	Meclofenamate	0	0
Adrenaline	+−	0	Propranolol	+	0
Adrenaline	+−	0	Propranolol + tolazoline	0	0
Aminophylline	−	0	Propranolol	− (reduced)	0
Ephedrine	−	0	Propranolol	0	0
Isoprenaline	−	0	Propranolol	0	0
Papaverine	−	0	Propranolol	− (reduced)	0
PGE_1	−	0	Propranolol	−	0
PGE_2	−	0	Propranolol	−	0

the effect of bradykinin and the effect of subsequently released catecholamines, propranolol was given before a dose of bradykinin. Even under these conditions, bradykinin did not induce a rise in pressure in the trachea. Bradykinin appears therefore to exert its effect mainly on the smaller airways. This conclusion confirms the finding of Jankala & Virtama (1963) using bronchography. The pressure fall in the trachea after the administration of bradykinin was probably due to catecholamine discharge (Collier, James & Piper, 1965; Piper, Collier & Vane, 1967) and could be abolished by propranolol pretreatment.

The large increase in pressure in the trachea due to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) confirms previous observations of the effect of this compound in guinea-pig lung (Änggård & Bergström, 1963) and for human lung (Sweatman & Collier, 1968). The response was not antagonized by meclofenamate, indicating a difference between human isolated bronchial muscle (Sweatman & Collier, 1968) and this *in vivo* preparation of guinea-pig lung. The greater effect of $PGF_{2\alpha}$ on the trachea than on the lung indicates that $PGF_{2\alpha}$ acts mainly on the larger airways. This may explain why Änggård & Bergström (1963) failed to show an effect on cat isolated bronchial chain, but obtained a response on cat isolated tracheal chain.

Slow-reacting substance of anaphylaxis (SRS-A) caused a pressure increase in both trachea and lung and in this respect differed from the responses obtained with bradykinin. These responses were antagonized by meclofenamate, thus confirming the findings of Berry & Collier (1964) with the Konzett-Rössler preparation of guinea-pig lungs *in vivo*.

The slight fall in the tracheal segment pressure following intravenous administration of meclofenamate confirms similar results obtained in this laboratory using human isolated bronchial muscle preparations.

The action of bronchodilator drugs could clearly be demonstrated on the tracheal preparation. Their action and antagonism on this preparation confirms and extends

those previously reported on the Konzett-Rössler preparation (James, 1967). A real advantage of the present technique over previous *in vivo* ones in demonstrating these effects is that no bronchoconstrictor agent need be used. The tracheal preparation responds repeatedly and in a dose-dependent way to bronchodilator substances.

The fall in tracheal segment pressure after administration of prostaglandins E_1 and E_2 (PGE_1 and PGE_2) confirms previous observations (Main, 1964; Khairallah, Page & Türker, 1967; Sheard 1968; Sweatman & Collier, 1968). In the present preparation, PGE_1 was approximately twice as potent as PGE_2. Neither response was antagonized by propranolol.

The response of the trachea to adrenaline showed the presence of a dilator component that was antagonized by propranolol and of a constrictor component that was antagonized by tolazoline. This result confirms those previously published (Nagasaka, Bouckaert & others 1964; James, 1967).

This preparation was found useful to determine roughly the site of action of bronchoconstrictor drugs on the airway system of the guinea-pig and to determine directly the mode of action and potency of bronchodilator drugs.

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