Br. J. Pharmac. (1971), **41**, 113–121.

β-Adrenoceptor antagonist activity of 3-methoxyisoprenaline

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Summary

1. The β-adrenoceptor antagonist activity of 3-methoxyisoprenaline, the O-methylated metabolite of isoprenaline, was studied on isolated driven atrial strip and tracheal chain preparations of the guinea-pig and on the hind limb blood flow of the dog.

2. On both the atrial strip and tracheal chain preparations the blockade of responses to isoprenaline fulfilled the criteria for simple competitive inhibition.

3. 3-Methoxyisoprenaline decreased the vasodilator response to isoprenaline in the dog hind limb, but did not affect the response to noradrenaline.

4. 3-Methoxyisoprenaline had about 1/3,700 of the potency of propranolol as a β-adrenoceptor antagonist on the tracheal chain preparation, 1/1,000 on the atrial strip preparation and less than 1/400 on the hind limb blood flow.

5. The antagonist activity of 3-methoxyisoprenaline showed a slight specificity for cardiac β-adrenoceptors, being 4·3 times more active on guinea-pig atria than on trachea.

6. Although 3-methoxyisoprenaline antagonized the actions of isoprenaline in the three preparations, its activity was extremely weak. It is unlikely that the formation of 3-methoxyisoprenaline from isoprenaline, administered therapeutically, could lead to β-adrenoceptor blockade.

Introduction

Isoprenaline is metabolized to 3-methoxyisoprenaline in several mammalian species, including man (Sjoerdsma, 1961; Hertting, 1964; Conway, Minatoya, Lands & Shekosky, 1968). The metabolite, 3-methoxyisoprenaline, has been reported to be a weak antagonist of the depressor action of isoprenaline in the cat (Philippott, Bacq & Sulman, 1965) and to have one-tenth the potency of propranolol in antagonizing the chronotropic action of isoprenaline in the dog (Paterson, Conolly, Davies & Dollery, 1968). Paterson *et al.* (1968) suggested that asthmatics who use isoprenaline may become resistant to its cardiac stimulant effects because of the β-adrenoceptor blocking activity of 3-methoxyisoprenaline formed metabolically. Also, in asthmatics, β-adrenoceptor antagonists cause a much greater increase in airway resistance than they do in normal subjects (McNeill & Ingram, 1966). If

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the repeated use of large doses of isoprenaline resulted in the formation of sufficient amounts of 3-methoxyisoprenaline to produce appreciable β-adrenoceptor blockade, then increased airway obstruction and a progressive deterioration of the asthmatic state would be expected. Paterson *et al.* (1968) suggested that the formation of 3-methoxyisoprenaline might explain the reported increase in asthma deaths since the introduction of isoprenaline aerosol preparations.

It is of practical importance, therefore, to determine quantitatively the β-adrenoceptor blocking activity of 3-methoxyisoprenaline on smooth muscle of the respiratory tract; for this, the isolated guinea-pig tracheal chain preparation was used. In addition, the antagonist activity of 3-methoxyisoprenaline was studied using the isolated electrically driven guinea-pig atrial strip preparation and the hind limb blood flow in the anaesthetized dog. The potency of 3-methoxyisoprenaline was compared with that of the β-adrenoceptor antagonist propranolol.

Methods

Isolated tissues

Preparations of trachea and atria were obtained from guinea-pigs of either sex weighing 450–700 g after dislocating the neck. The preparations were suspended in Krebs-Henseleit solution gassed with 5% carbon dioxide in oxygen. The solution had the following composition: NaCl, 118 mM; KCl, 4·7 mM; $NaHCO_3$, 25·0 mM; $MgSO_4$, 0·45 mM; KH_2PO_4, 1·03 mM; $CaCl_2$, 2·5 mM; and D (+) glucose, 11·1 mM. Tracheal chain preparations were maintained at 37° C and atrial preparations at 32·5° C.

Tracheal chain. The trachea was removed and opened along its ventral surface. Alternate cuts were made from each side between every second cartilage so as to form a strip of tissue containing seven–ten segments of smooth muscle. After setting up the tissue it was allowed to equilibrate for 30–60 min until it attained a constant tone. Changes in length of the preparation were measured isotonically, at a tension of 300 mg, with a Brush displacement transducer and were recorded by a potentiometric pen recorder.

Electrically driven atrial strip. The technique was similar to that described by Blinks (1966). A triangular segment, with a base of 5 mm and height of 7 mm, was cut from the wall of the left atrium. The base of this segment was clamped in an electrode assembly and the apex connected to an isotonic strain gauge transducer. The atrial segment was stimulated with pulses of 1 ms duration at a frequency of 1 Hz delivered through a punctate platinum electrode. The voltage was just sufficient to elicit contractions (1·5–3 V). Contraction amplitude was recorded by a Brush MK 240 recorder.

Log dose-response curves for isoprenaline were obtained on both tracheal chain and atrial strip preparations before and in the presence of either 3-methoxyisoprenaline or propranolol. The isoprenaline was added to the bath in cumulatively increasing concentrations. The response to each concentration was allowed to equilibrate before a subsequent addition of isoprenaline. Responses were expressed as percentages of the maximal response determined for each curve. Regression lines were fitted to the linear portions of the curves by the method of least squares. Each pair of lines, before and in the presence of antagonist, was tested statistically for coincidence and parallelism and then used to calculate the dose ratio for isoprenaline.

In order to compare the potency of 3-methoxyisoprenaline and propranolol as β-adrenoceptor antagonists, pA_2 values against isoprenaline were calculated for both compounds. The experimental design used for this study was similar to that described by Arunlakshana & Schild (1959) and Blinks (1967) for the study of competitive antagonism. To minimize errors due to time dependent changes in tissue sensitivity and to make sure that the antagonist had equilibrated with the tissue, only one concentration of antagonist was tested, and a single dose ratio calculated, in each experiment. Consistent log dose response curves for the agonist were obtained before and after the addition of the antagonist, the dose ratio being calculated as the reciprocal of the potency ratio obtained from parallel, non-coincident regression lines.

Schild (1957) proposed that for competitive antagonism two criteria should be fulfilled. First, parallel log dose response curves should be obtained for the agonist in the absence and presence of the antagonist. Second, the antagonism should obey the equation,

$$\log (x-1)=\log k_2+n \log B \qquad (1)$$

where x is the dose ratio, k_2 is the affinity constant, B is the molar concentration of the antagonist and n is a constant equal to 1 for simple competitive antagonism.

Thus in the case of competitive antagonism, a plot of log $(x-1)$ against log B should give a straight line with a gradient of 1. Also, since by definition $pA_x=-\log B$ (Schild, 1949), when $n=1$, $pA_2=-\log k_2$ and $pA_2-pA_{10}=0{\cdot}95$. The values determined for the log of the affinity constant using equation (1) should be constant over the concentration range of antagonist tested.

If the antagonism of the response to isoprenaline by 3-methoxyisoprenaline proved to be competitive in nature then values for pA_2 could be determined from the values of log k_2 as well as from the plot of log $(x-1)$ against log B. For competitive antagonism the linear regression line obtained from such a plot should intersect the log B axis at a point corresponding to pA_2 (Arunlakshana & Schild, 1959 ; Blinks, 1967).

Dog hind limb blood flow

Four dogs, weighing between 10·1 and 11·5 kg, were anaesthetized by intravenous injection of sodium pentobarbitone (35 mg/kg). The trachea was cannulated and artificial ventilation given. Blood pressure was measured from the right common carotid artery in mmHg (1 mmHg≡1·333 mbar) by means of a cannula connected to a Statham P23Db pressure transducer. Blood flow was measured in the abdominal aorta, just proximal to the origins of the external iliac arteries, with a Beckmann electromagnetic flow meter and external probe. Blood pressure and mean blood flow were recorded by a Beckmann dynograph. Intra-arterial injections were given through a cannula inserted into the left femoral artery with its tip in the aorta adjacent to the origins of the external iliac arteries. The left external and internal iliac arteries were ligated to prevent blood flow through these arteries without occluding the cannula. Intra-arterial injections of drugs, dissolved in 0·9% NaCl solution, were given in volumes not exceeding 0·1 ml and were flushed in with 0·3 ml of 0·9% NaCl solution. Alternate submaximal blood flow responses were obtained to isoprenaline and noradrenaline. The effects of propranolol and 3-methoxyisoprenaline were studied in each of two animals. The antagonists were

given 5 min before a dose of isoprenaline. The response to isoprenaline was allowed to return to control levels before further administration of the antagonist.

Drugs

(±)-Isoprenaline hydrochloride (Riker); (−)-noradrenaline hydrochloride, (Sigma); propranolol hydrochloride (Inderal, I.C.I.). The 3-methoxyisoprenaline hydrochloride was prepared in the medicinal chemistry department of the Riker Research Laboratories and its structure and purity were confirmed by mass spectrometry, N.M.R. and chemical analysis.

Results

Guinea-pig isolated tracheal chain

Antagonist activity of 3-methoxyisoprenaline reached equilibrium within 30 min. In experiments with propranolol a 45 min contact time was required to obtain reproduceable effects. Foster (1966) also reported difficulties in equilibrating propranolol on the guinea-pig trachea.

3-Methoxyisoprenaline. The log dose-response curves for isoprenaline in the the absence and presence of 3-methoxyisoprenaline were parallel within the range of concentration of antagonist tested (1×10^{-4} to 1×10^{-3}M). The value of log K_2 was calculated for each experiment (Table 1) and did not vary significantly (correlation coefficient $r=0{\cdot}087$, $P>0{\cdot}80$). The regression line, fitted by the method of least squares to the plot of log (dose ratio −1) against log (antagonist concentration), was found to be linear with a slope of 1·03 (Fig. 1). The difference pA_2–pA_{10} was calculated to be 0·92 compared with the theoretical value of 0·95 expected for competitive antagonism at equilibrium (Schild, 1957). The preceding results are consistent with 3-methoxyisoprenaline being a competitive antagonist of isoprenaline on this tissue and, therefore, it was valid to proceed to calculate pA_2 values by the methods described.

The value of pA_2 calculated from the regression equation (Fig. 1) was 4·30. This value is in close agreement with that obtained from the mean of the log K_2 determinations, pA_2 (±S.E.)$=4{\cdot}34 \pm 0{\cdot}04$.

TABLE 1. *Antagonism by 3-methoxyisoprenaline and propranolol of the isoprenaline response on guinea-pig trachea*

Antagonist	Bath concentration (molar)	Dose ratio	95% Confidence limits	Log K_2*
3-Methoxyisoprenaline	1×10^{-4}	2·50	2·18–2·86	4·18
		3·90	3·54–4·29	4·46
	5×10^{-4}	11·70	10·97–12·47	4·33
		11·12	10·52–11·76	4·31
	1×10^{-3}	28·89	25·67–32·54	4·44
		19·69	18·71–20·72	4·29
Propranolol	1×10^{-7}	5·95	5·29–6·69	7·69
		12·29	11·13–13·57	8·05
	1×10^{-6}	82·83	65·51–104·7	7·93
		49·15	43·86–55·09	7·68
		55·88	53·87–57·96	7·74
		77·93	71·61–84·81	7·89

* Log $K_2 = \log \frac{(\text{dose ratio} - 1)}{(\text{antagonist concentration})}$

Propranolol. Propranolol has been shown by Foster (1966) to be a competitive antagonist of isoprenaline on the isolated tracheal chain. Values for pA_2 could, therefore, be obtained in the same manner as with 3-methoxyisoprenaline.

The calculated values of log K_2 ($=pA_2$), at the various concentrations of propranolol, are shown in Table 1. The mean pA_2 ($\pm$S.E.) was $7{\cdot}83 \pm 0{\cdot}06$. The regression equation obtained from the plot of log (dose ratio -1) versus log (antagonist concentration) yielded a pA_2 value of 7·93.

On trachea, therefore, 3-methoxyisoprenaline was approximately 3,700 times less potent than propranolol as a β-adrenoceptor antagonist, the potency ratio being the mean of the ratios calculated from pA_2 values obtained from the mean log K_2 and from the plot of log (dose ratio -1) against log (antagonist concentration).

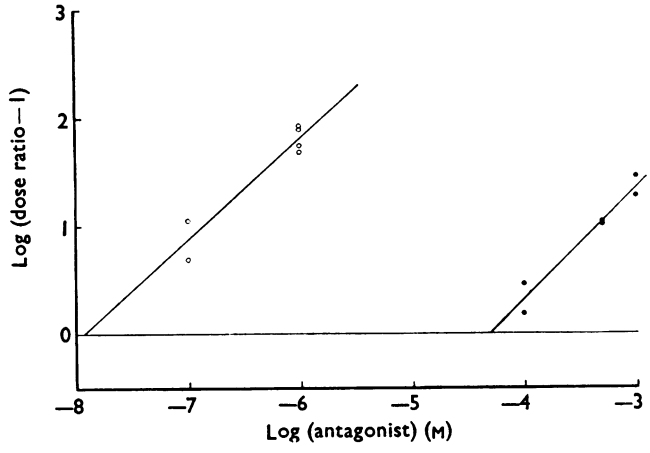

FIG. 1. Effect of 3-methoxyisoprenaline and propranolol on the isoprenaline response of the guinea-pig tracheal chain. The graphical pA_2 values are obtained from the point of intersection of the regression line and the log (antagonist concentration) axis when log (dose ratio -1)$=0$, ($pA_x = -\log B$). ●——●, 3-Methoxyisoprenaline (regression equation: $Y = 1{\cdot}032X + 4{\cdot}443$); ○——○, propranolol (regression equation: $Y = 0{\cdot}940X + 7{\cdot}450$).

TABLE 2. *Antagonism by 3-methoxyisoprenaline and propranolol of the isoprenaline response on guinea-pig atrial strip*

Antagonist	Bath concentration (molar)	Dose ratio	95% Confidence limits	Log K_2*
3-Methoxyisoprenaline	1×10^{-5}	1·85	1·61–2·12	4·93
	5×10^{-5}	4·98	4·37–5·67	4·90
		7·19	6·50–7·96	5·09
	1×10^{-4}	12·25	12·19–12·31	5·05
		8·17	7·83–8·53	4·86
	5×10^{-4}	34·46	31·67–37·49	4·83
		42·43	38·39–46·90	4·92
	1×10^{-3}	187·6	177·3–198·4	5·27
		99·1	87·3–112·4	4·99
Propranolol	1×10^{-7}	9·90	9·43–10·39	7·95
	$1{\cdot}34 \times 10^{-6}$	145·3	127·0–166·2	8·03
		118·2	109·8–126·7	7·95
	5×10^{-6}	409·6	387·3–433·2	7·91

* Log $K_2 = \log \dfrac{(\text{dose ratio} - 1)}{(\text{antagonist concentration})}$

Guinea-pig isolated atrial strip

On the atrial preparation, both 3-methoxyisoprenaline and propranolol had equilibrated within 30 minutes.

3-Methoxyisoprenaline. The log dose-response curves for isoprenaline in the absence and presence of 3-methoxyisoprenaline were parallel within the range of concentration of antagonist tested (1×10^{-5} to 1×10^{-3}M). The calculated values of log K_2 for each experiment are shown in Table 2, no significant variations being observed (correlation coefficient, $r=0{\cdot}151$, $P>0{\cdot}70$). The regression line obtained from the plot of log (dose ratio -1) against log (antagonist concentration) was linear with a slope of 1·05 (Fig. 2). The value pA_2-pA_{10} was calculated to be 0·93. The preceding results are consistent with 3-methoxyisoprenaline being a competitive antagonist of isoprenaline on the atrial strip preparation.

The value of pA_2 calculated from the linear regression equation (Fig. 2) was 4·94, while the value determined from the mean log K_2 ($\pm$S.E.) was $4{\cdot}98 \pm 0{\cdot}05$.

Propranolol. Propranolol has been shown to be a competitive antagonist of isoprenaline on the electrically driven guinea-pig atrial strip preparation (Blinks, 1967).

The calculated values of log K_2 at various concentrations of propranolol are shown in Table 2. The value of pA_2 obtained from the mean log K_2 value ($\pm$S.E.) was $7{\cdot}96 \pm 0{\cdot}03$. The regression equation obtained from the plot of log (dose ratio -1) against log (antagonist concentration) gave a pA_2 value of 7·98.

On atria, 3-methoxyisoprenaline was approximately 1,000 times less potent as a β-adrenoceptor antagonist than propranolol.

Dog hind limb blood flow

The doses of isoprenaline injected intra-arterially to produce submaximal vasodilation varied from 7 to 9×10^{-7} g. The doses of noradrenaline producing sub-

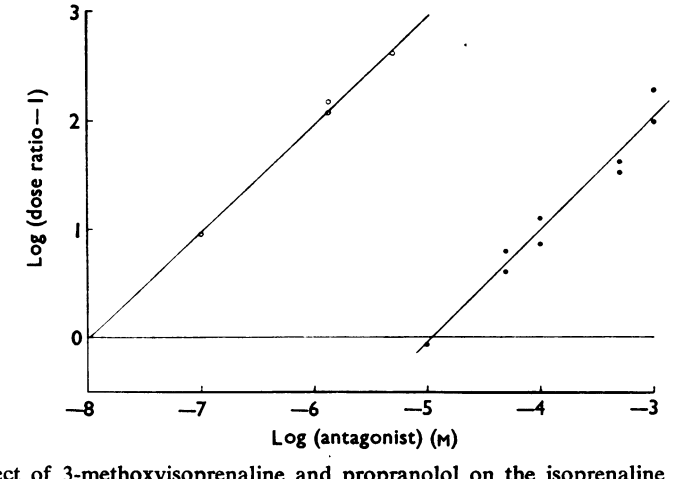

FIG. 2. Effect of 3-methoxyisoprenaline and propranolol on the isoprenaline response of the driven guinea-pig atrial strip. The graphical pA_2 values are obtained from the point of intersection of the regression line and the log (antagonist concentration) axis when log (dose ratio -1)$=0$, ($pA_x=-\log B$). ●——●, 3-Methoxyisoprenaline (regression equation: $Y=1{\cdot}046X+5{\cdot}163$); ○——○, propranolol (regression equation: $Y=0{\cdot}988X+7{\cdot}884$).

maximal vasoconstriction varied from 5×10^{-7} to 10^{-6} g. Neither isoprenaline nor noradrenaline had any appreciable effect on the systemic blood pressure.

The effects of 3-methoxyisoprenaline and propranolol on responses to isoprenaline and noradrenaline are shown in Fig. 3. In two experiments, 2×10^{-6} mol of 3-methoxyisoprenaline had no effect on the response to either isoprenaline or noradrenaline, but 2×10^{-5} mol depressed the vasodilator responses to isoprenaline by 18 and 26% without affecting the vasoconstrictor responses to noradrenaline. Propranolol, 5×10^{-8} mol, reduced the responses to isoprenaline by 29 and 42% in two experiments, but responses to noradrenaline were unaffected. A higher dose of propranolol (2×10^{-7} mol) almost abolished the response to isoprenaline without altering the response to noradrenaline and 2×10^{-6} mol completely abolished the response to isoprenaline.

The potency of 3-methoxyisoprenaline in antagonizing the vasodilator action of isoprenaline was less than 1/400th that of propranolol.

Discussion

3-Methoxyisoprenaline was found to be a weak β-adrenoceptor antagonist on all three preparations used in this study. On atria and trachea the antagonism of the response to isoprenaline by 3-methoxyisoprenaline was competitive, like propranolol, since it fulfilled the following criteria: it produced parallel shifts in the log dose response curves for isoprenaline; the regression lines calculated for the plot of log (dose ratio − 1) against log (antagonist concentration) were linear and had gradients of 1·05 and 1·03 for atria and trachea, respectively; there was no significant change

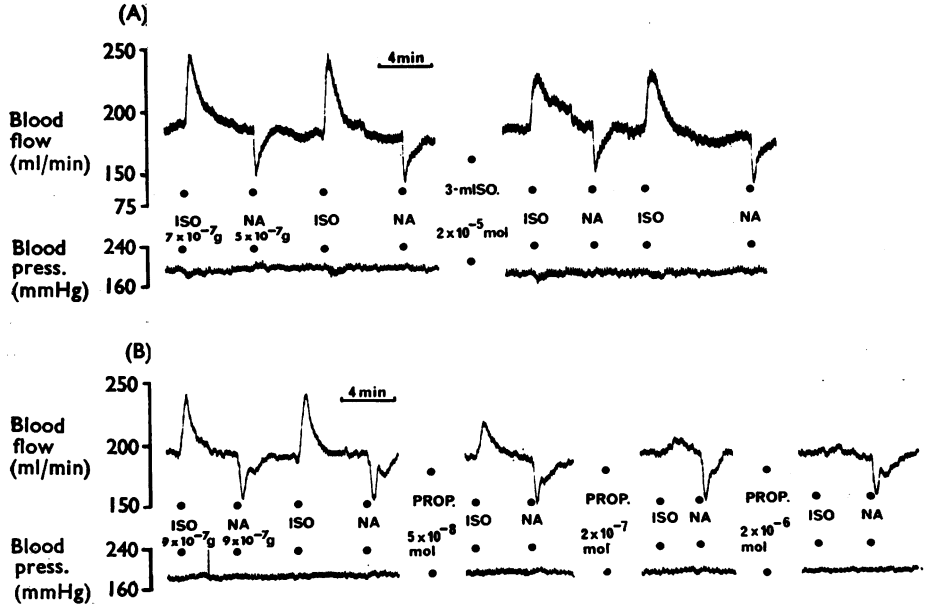

FIG. 3. Effect of (A) 3-methoxyisoprenaline and (B) propranolol on the dog hind limb blood flow response to close arterial injection of isoprenaline (ISO) and noradrenaline (NA). The time interval between injection of antagonist and the following injection of isoprenaline is 5 min in each case. Isoprenaline responses were allowed to recover to control levels between doses of propranolol.

in the values of log K_2 within the dose range of antagonist tested; the values of pA_2–pA_{10} were 0·92 and 0·93, respectively, for trachea and atria. Since the antagonism of the actions of isoprenaline by 3-methoxyisoprenaline was competitive in nature, it was valid to calculate values for pA_2 from the mean values of log K_2 and from the plot of log (dose ratio − 1) against log (antagonist concentration) and to compare its potency with that of propranolol.

On tracheal smooth muscle, 3-methoxyisoprenaline was approximately 1/3,700 as potent as propranolol as a β-adrenoceptor antagonist; on atria it was approximately 1/1,000 as potent and on blood vessels of the dog hind limb it was less than 1/400 as potent. The finding that 3-methoxyisoprenaline was such a weak β-adrenoceptor antagonist is in contrast with that of Paterson *et al.* (1968), who reported the results of two experiments in which tachycardia in response to isoprenaline was measured; they concluded that 3-methoxyisoprenaline had one-tenth the β-adrenoceptor antagonistic potency of propranolol. Paterson *et al.* (1968) suggested that the β-adrenoceptor blocking activity of 3-methoxyisoprenaline, formed from the metabolism of isoprenaline, may cause increased airway obstruction in asthmatic patients using isoprenaline aerosols. However, the antagonist potency of 3-methoxyisoprenaline is so low that it is unlikely to be of clinical importance and it is difficult to attribute either the resistance to isoprenaline sometimes seen after prolonged use of aerosols, or the reported increase in asthma deaths, to formation of 3-methoxyisoprenaline. Furthermore, the metabolic products of isoprenaline are known to be excreted fairly rapidly in both urine and bile. In man, Sjoerdsma (1961) found that between 41 and 62% of a dose of isoprenaline, given by intravenous infusion, was excreted in the urine as 3-methoxyisoprenaline within 12 hours. In rats, Hertting (1964) found that 92% of an intravenous dose of isoprenaline was excreted over an 8 h period in bile and urine, either as isoprenaline or 3-methoxyisoprenaline, unconjugated or as glucuronide conjugates. Although the metabolic routes and excretion rate of isoprenaline metabolites may be quite different if the isoprenaline is administered by inhalation, it is extremely unlikely that sufficient amounts of such a weak antagonist as 3-methoxyisoprenaline could accumulate to produce a significant β-adrenoceptor blockade.

3-Methoxyisoprenaline exhibited a slight relative specificity towards antagonism of cardiac β-adrenoceptors, being 4·3 times more active on guinea-pig atria than on trachea. No such specificity was observed with propranolol. Lands & Brown (1964) proposed that the β-adrenoceptors of the cardiovascular system differ from those of respiratory smooth muscle, since there were differences in the relative potencies of various agonists on the two types of tissue. It would appear that β-adrenoceptors show selectivity towards antagonists as well as agonists. This finding is in agreement with those of Farmer & Levy (1970).

The author gratefully acknowledges the skilled technical assistance of Miss R. Colmer and Mr. E. Alcock and wishes to thank Professor M. J. Rand and Dr. A. G. Bolt for their constructive criticism of this manuscript.

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(*Received June* 30, 1970)