The African Journal of Medicine and Medical Sciences

Volume 14
1985

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Palo Alto Melbourne

Afr. J. Med. med. Sci. (1985) **14**, 11–19.

Effect of adrenergic receptor blockers on the cardio-respiratory response to cow's urine concoction in rats

D. D. O. OYEBOLA* AND J. O. ARIWODOLA

Department of Physiology, College of Medicine, University of Ibadan, and Department of Physiological Sciences, Faculty of Health Sciences, University of Ife, Ile-Ife, Nigeria

Summary

The influence of alpha and beta adrenoceptor blockers on the cardio-respiratory effects of cow's urine concoction (CUC) in rats was studied. The study showed that phenoxybenzamine abolished the pressor response observed in the untreated animal while propranolol had no effect on blood pressure response.The alpha-blocked animals also developed significant post-injection hypotension. Electrocardiographic abnormalities seen in the control group were ameliorated by beta-blockers but alpha-blockers had no such effect. CUC caused an initial depression of respiration with short apnoeic phases and an increase in pulmonary ventilation. The latter was significantly decreased by alpha and beta adrenoceptor blockers.

It was concluded that adrenoceptors play an important role in the cardio-respiratory effects of CUC.

Résumé

On a étudié l'influence des agents alpha ou bêta-bloquants des adréno-récepteurs sur les effets cardio-respiratoires de la mixtion d'urine de vache (MUV) chez les rats.

L'étude a montré que la phénoxybenzamine annulait la réaction vaso-constrictrice observée chez l'animal non traité, alors que le propranolol n'avait aucun effet sur la tension artérielle. Les animaux alpha-bloqués ont également présenté une hypotension significative après injection. Les anomalies électrocardiographiques observées dans le groupe de contrôle ont été atténuées par les agent béta mais non par les agents alpha. La MUV a causé une inhibition initiale de la respiration marquée par de brèves apnées, ainsi qu'une augmentation de la ventilation pulmonaire. Cette dernière a été sensiblement diminuée par les agents alpha et béta.

On conclut que les adréno-récepteurs jouent un rôle important dans les effets cardio-respiratoires de MUV.

*Author to whom correspondence should be addressed.

Introduction

In Nigeria, like in many developing countries, Western-type health care services are in short supply. If they fall ill, a large percentage of the population still rely on herbal preparations obtained from traditional healers. Sometimes, some of these remedies, in spite of popular and long usage, have been found to cause poisoning. One of such remedies is cow's urine concoction (CUC) used in treating convulsive seizures in children in certain parts of Nigeria. It is not certain whether, if used in controlled doses, CUC can be beneficial in cases of convulsion. However administration of CUC has resulted in great morbidity and mortality in Nigerian children (Atalabi, 1964; Familusi & Sinnette, 1971). In order to elucidate the mechanism by which CUC produces its toxic effects, experimental studies on this herbal remedy were initiated by Oyebola and Elegbe (1975). Since then, more reports of studies on CUC poisoning have appeared in the literature. The chemical constituents of CUC were recently published (Ayorinde *et al.*, 1982).

Previous experiments in laboratory animals showed that CUC has a biphasic effect on blood

pressure (Elegbe, Bamgbose, & Oyebola, 1976; Elegbe & Oyebola, 1977) and marked depressive effects on respiration (Oyebola & Elegbe, 1975; Elegbe & Oyebol, 1977). The cardiorespiratory effects demonstrated by Elegbe and Oyebola (1977) were dose-related. Experiments in cats suggested that the pressor effect of CUC in mediated via the alpha receptors (Elegbe *et al.*, 1976), while the mechanism of the depressor effect is yet to be elucidated. The mechanism of the respiratory effects of CUC is also unknown. Since the alpha and beta adrenoceptors are important in mediating cardiorespiratory changes, the aim of the present study is to investigate the possible role of these receptors in the cardiorespiratory responses to CUC. Effects of CUC are observed for a longer period post-injection than in the earlier experiments.

Materials and methods

Experiments were carried out on male albino Wistar rats, weighing 230–285 g. Animals were fed *ad libitum* before the experiments. Anaesthesia was induced with pentobarbital sodium 36 mg/kg given intraperitonially. The trachea was cannulated to allow free breathing from room air. A carotid artery was cannulated and connected to a transducer (Nihon-kohden type MPU, 0.5 unbonded wire strain gauge, Japan) for blood pressure recording. A femoral vein was cannulated for intravenous (i.v.) injections. Electrocardiogram (ECG, lead II) was recorded using a Biophysical input panel (type RBL — 45, Nihon-kohden, Japan). The animal was heparinized (100 units heparin per 100 g body weight) to prevent blood clotting in the cannulae. Amplitude of respiratory excursions (RA) was monitored via a silk loop attached along the middle at two points 2 cm apart on the wall of the upper abdomen and lower thorax. This was the area of maximal visible respiratory movement on the body wall. This silk loop was attached via a string to a force/displacement transducer mode coupled to a carrier amplifier model R.P. — 5. Blood pressure (BP), ECG and RA were continuously recorded on a multipurpose four-channel ink-writing polygraph (model RM — 46, Nihon-kohden).

Source of CUC

Cow's urine concoction was prepared in the laboratory using the method of Oyebola and Adetuyibi (1977) 8 weeks before commencement of experiments. Previous studies showed that CUC has a shelf life of well over 2 years (Elegbe & Oyebola, 1977).

Experimental procedure

Thirty min post-surgical recovery was allowed. Basal recordings of BP, RA, and ECG were made for 10 min at a paper speed of 25 mm/min. At the end of each 5 min slow run, a fast run at 50 mm/sec was carried out for 3 sec to allow easy reading of the respiratory rate (RR), ECG pattern and heart rate (HR) from the ECG records.

Untreated group (Group 1)

Twelve rats were given 0.2 ml/100 g body weight of undiluted CUC as a slow i.v. injection. Recordings of BP, ECG and RA were continued for 70 min post-injection, with fast runs at 5 min intervals as indicated above to allow for determination of HR and RR.

Pre-treatment with propranolol (Group 2)

Eight rats were pre-treated with propranolol, 0.5 mg/kg given i.v. Fifteen min after propranolol injection, CUC (0.2ml/100g. body weight) was administered. Blood pressure, ECG pattern, HR, RA and RR were monitored for 70 min as in the untreated group.

Pre-treatment with Phenoxybenzamine (Group 3)

Seven rats were pre-treated with phenoxybenzamine 1 mg/kg body weight given i.v. The effects of alpha blockade on CUC-induced changes in BP, ECG, HR, RA and RR were also monitored for 70 min post-injection.

Saline control injections. 0.15M saline in volumes equal to those of CUC administered to the animals was used for control injections in the three groups.

Calculations

The BP, HR and RA were read at 5 min intervals and their mean (± s.e.) were calculated. Mean BP was computed from the systolic and diastolic values.

The index of pulmonary ventilation rate (IPVR) per minute was calculated as described in an earlier study (Oyebola & Ariwodola, 1984). Briefly, IPVR was obtained by calculating the difference in the product of RR and RA before and after CUC injection and expressing the value obtained as a percentage of the value before CUC injection.

The mean values of the parameters measured (± s.e.) at corresponding 5 min intervals in each of the three groups studied were compared using *t*-test of difference between two independent sample means (Bahn, 1972). The mean values of parameters measured in successive 5 min intervals were each compared with the initial value before injection using paired *t*-test. *P* values of 0.05 or less were taken as statistically significant.

Results

The results are presented in Tables 1–4 and Fig. 1–3.

Effect of CUC on blood pressure

Table 1 shows the blood pressure effects of CUC in groups 1, 2 and 3. Post-injection values that are significantly different when compared with resting values for each group are indicated by asterisks in Table 1.

The early phase of the BP response to CUC injection in the untreated and beta-blocked animals were similar (Fig. 1 and Table 1).

This is characterized by an initial depressor effect followed by a marked pressor effect lasting about 2 min. Phenoxybenzamine abolished the pressor response to CUC but not the depressor effect. Phenoxybenzamine also abolished the pressor response to adrenaline (1 μg/kg) and noradrenaline (2 μg base/kg). The absolute BP value in the transient depressor phase in group 3 is significantly lower than those of groups 1 and 2 ($P = 0.05$ and 0.01 respectively). A significant secondary hypotension was observed in all the groups in the fifth minute post-injection as well as in the tenth minute post-injection in group 3. All BP values in group 3 are significantly lower than corresponding values in groups 1 and 2 ($P < 0.01$).

Effect of CUC on Heart Rate

Table 2 shows the HR changes. Alpha blockade caused an insignificant decrease in resting HR while beta blockade caused a highly significant decrease in resting HR ($P < 0.01$). In the untreated group, injection of CUC resulted in a significant decrease in HR from the fifth to twenty-fifth minute post-injection.

The heart rate decreases recorded after CUC injection in the alpha-blocked animals were not significant.

In the beta-blocked animals, CUC injection caused a further reduction in HR which persisted till the twentieth minute post-injection. When compared with resting values for the group, this decrease was significant only on the fifth minute post-injection. All HR values in group 2 are significantly lower than corresponding values in groups 1 and 3 (*P* values are between 0.01 and 0.05). Corresponding HR values in groups 1 and 3 are not significantly different.

Effect of CUC on Respiration

Tables 3 and 4 show the respiratory changes. Alpha and beta blockade had no significant effect on resting RR. The bottomtrace in Fig. 1 shows a typical early effect of CUC on respiration. The early response to CUC is characterized by periods of apnoea lasting a few seconds. This is followed by a period of reduced RR which was however associated with a marked increase in RA. The reduction in RR was significant in the fiftieth minute post-injection in the beta-blocked group only. The late effects of CUC on respiration in the three groups consists of a moderate increase in RR. The increased RR was not significant in groups 2 and 3 but was significant in group 1 in the fiftieth, sixtieth, sixty-fifth and seventieth minutes post-injection.

Table 4 shows the IPVR values. The IPVR in group 2 was significantly different from

Table 1. Effect of CUC on Blood pressure (mm Hg) in untreated, beta-blocked and alpha-blocked rats

	Post-injection period (min)										
	Control	0.2	0.4–2	5	10	15	20	25	30	45	60
Group 1 untreated	126.8 ± 2.1	74.7* ± 11.3	153.5* ± 6.7	88.1* ± 6.8	111.2* ± 7.6	116.7 ± 9.4	120.0 ± 10.1	121.8 ± 9.7	122.8 ± 9.0	124.5 ± 7.6	122.9 ± 6.4
Group 2 (beta-block)	113.6 ± 6.4	74.3* ± 5.4	146.6* ± 7.0	81.6* ± 8.0	107.1 ± 10.1	120.1 ± 6.9	121.0 ± 6.1	114.5 ± 11.6	113.6 ± 11.7	121.2 ± 9.9	119.2 ± 10.4
Group 3 (alpha-block)	74.6 ± 3.8	44.8* ± 3.6	80.3 ± 3.3	49.4* ± 1.6	53.4* ± 2.0	66.8 ± 3.7	71.4 ± 3.0	72.3 ± 4.0	75.3 ± 3.6	75.1 ± 3.2	72.0 ± 2.4

In Tables 1–4, symbols * and † indicate significant differences of values compared with control for the group. * = $P \leq 0.02$.

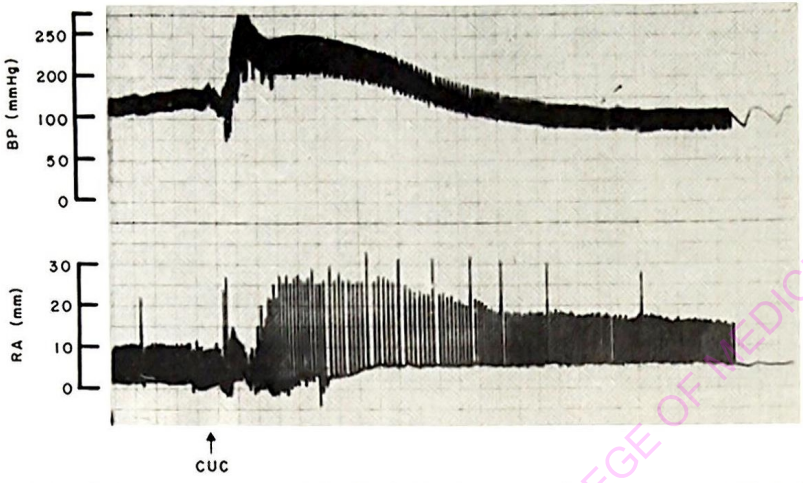

Fig. 1. A typical tracing in an untreated animal showing the blood pressure and respiratory responses in the first 5 min. after CUC injection.

corresponding values in group 1 in the fifth and tenth minutes post-injection. Although great differences existed between the other corresponding IPVR values in the three groups, the variabilities of the values were very wide. The differences were not statistically significant. If apnoea is sustained for 45–60 sec, BP fell rapidly. Failure to re-establish respiration resulted in demise of the animals within 6 min of CUC injection (Fig. 2). However, if respiration is re-established after an apnoea of a minute or less, the low BP returned to normal and the animal survived.

Effect of CUC on ECG

In less than 2 sec post-injection, the ECG changed to a bizarre pattern (Fig. 3). About 5 min post-injection, blunting of the P waves, tall QRS complexes and elevated T waves were

Table 2. Effect of CUC on Heart Rate (/min) in groups 1, 2 and 3

	Post-injection period (min)								
	Control	5	10	15	20	25	30	45	60
Group 1 (not treated)	360.4 ± 8.2	316.0† ± 10.7	318.7† ± 13.5	316.9* ± 16.0	321.1† ± 13.2	322.2* ± 14.6	327.3 ± 15.6	329.1 ± 15.8	341.6 ± 14.4
Group 2 (beta-block)	270.6 ± 8.2	243.7* ± 6.8	247.1 ± 7.5	247.5 ± 7.5	251.2 ± 7.9	261.2 ± 4.8	268.1 ± 7.4	286.2 ± 9.6	290.7 ± 12.3
Group 3 (alpha-block)	348.6 ± 15.5	321.4 ± 14.2	317.1 ± 15.5	321.4 ± 16.6	321.4 ± 17.8	321.4 ± 17.8	323.6 ± 16.9	332.8 ± 17.1	334.3 ± 17.8

*$= P<0.02$; †$= P<0.05$.

Table 3. Effect of CUC on respiratory rate (/min) in groups 1, 2 and 3

	Post-injection period (min)									
	Control	5	10	15	20	30	40	50	60	65	70
Group 1 (untreated)	58.8 ± 4.4	59.1 ± 8.1	64.3 ± 6.2	59.1 ± 5.0	64.3 ± 6.7	68.5 ± 4.8	71.1 ± 4.4	72.8* ± 4.6	74.6 ± 5.3	73.3 ± 4.2	72.8 ± 4.4
Group 2 (beta-block)	51.8 ± 2.2	41.1* ± 4.4	48.0 ± 5.2	48.0 ± 3.7	49.7 ± 3.1	56.5 ± 4.3	58.2 ± 4.1	53.1 ± 6.8	56.6 ± 5.6	63.4 ± 6.2	63.4 ± 7.7
Group 3 (alpha-block)	56.6 ± 5.0	66.0 ± 3.4	64.3 ± 5.3	62.6 ± 5.2	63.4 ± 5.7	61.7 ± 6.1	62.6 ± 5.5	59.1 ± 4.6	58.3 ± 3.8	60.0 ± 5.2	64.3 ± 7.0

* = $P<0.02$.

Table 4. Percentage changes in Index of Pulmonary Ventilation Rate (IPVR) after CUC Injection in groups 1, 2 and 3

	Post-injection period (min)									
	5	10	15	20	25	30	40	50	60	70
Group 1 (untreated)	60.2 ± 24.4	58.8 ± 12.6	29.7 ± 14.1	29.2 ± 14.5	23.7 ± 12.9	21.9 ± 12.9	23.2 ± 17.8	22.0 ± 16.9	22.2 ± 16.2	23.0 ± 19.1
Group 2 (beta-block)	0.6* ± 16.2	13.3* ± 12.9	5.4 ± 8.0	2.8 ± 7.3	8.2 ± 8.5	10.8 ± 8.5	7.9 ± 7.4	−2.9 ± 8.4	6.8 ± 9.7	15.4 ± 8.7
Group 3 (alpha-block)	35.2 ± 24.3	17.5 ± 17.9	10.1 ± 16.1	8.2 ± 13.4	8.1 ± 12.1	8.3 ± 12.4	10.5 ± 13.8	4.4 ± 13.1	3.5 ± 12.9	8.2 ± 13.4

* = $P<0.02$.

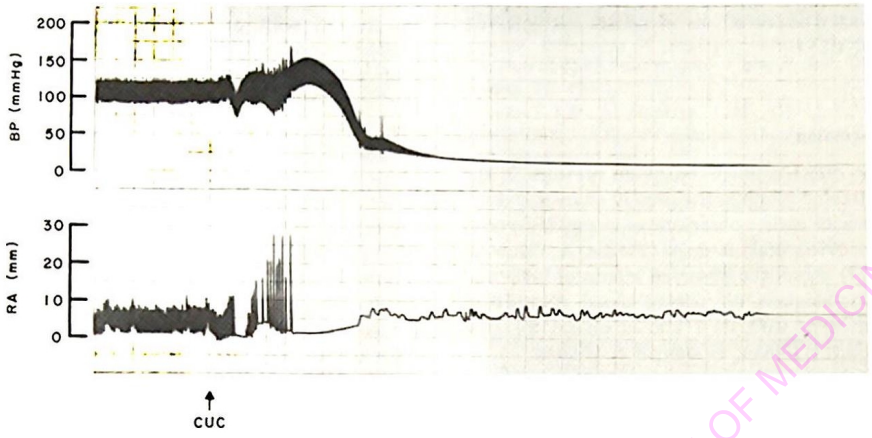

Fig. 2. Blood pressure and respiratory movement (RA) tracing in an untreated animal that died 6 min after CUC injection. Note that there was cessation of breathing before the rapid fall in BP.

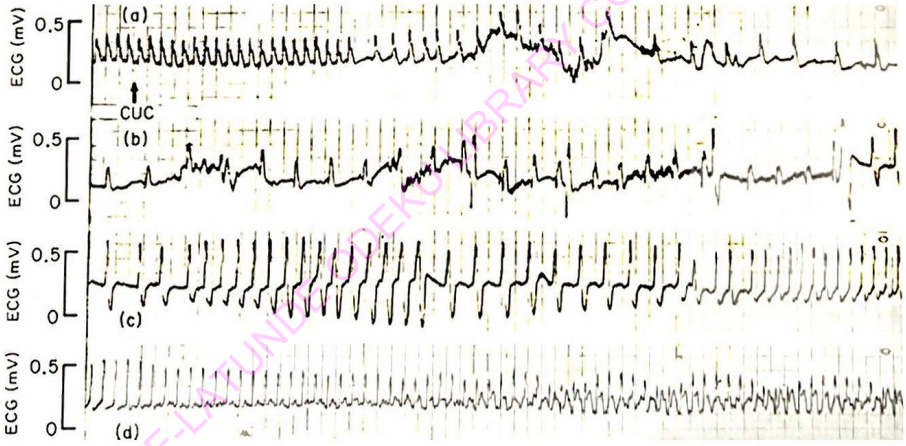

Fig. 3. A long strip of ECG recording in an untreated animal. Recording was for 25 sec at a paper speed of 50mm/sec. To improve the size and the clarity of the Fig., the strip was cut into four consecutive equal parts, a, b, c, & d. Each part represents 6.25 sec of ECG recording. Note the bizarre ECG pattern as from 1.6 sec after CUC injection.

observed. The ECG abnormalities persisted throughout the 1 h post-injection observation period in the control animals. Electrocardiographic abnormalities caused by CUC were attenuated and lasted just 7 sec in the propranolol-treated group while phenoxybenzamine had no effect. Indeed, elevation of the T waves was accentuated in the alpha-blocked group.

Effect of 0.15M Saline

0.15M saline in volumes equal to those of CUC

injected had no effects on the BP, HR, RR, RA and ECG.

Discussion

The effect of CUC on blood pressure in the untreated group in the present study is consistent with earlier observations in cats (Elegbe *et al.*, 1976) and in dogs (Elegbe & Oyebola, 1977). Also, the observed effects of beta- and alpha-blockers on resting blood pressure in consistent with their known pharmacological actions (Gilman, Goodman & Gilman, 1980). Previous studies have shown that the pressor response observed in control rats following CUC injection was absent in adrenalectomized rats (Oyebola & Ariwodola, 1984). This pointed to catecholamines released from the the adrenal glands as the mediator of CUC-induced pressor response. The abolition of the pressor response to injections of CUC, adrenaline and noradrenaline by phenoxybenzamine in the present study support this conclusion. This result also shows that the present response to CUC is mediated via the alpha adrenoceptors. Our results suggests that the depressor effect of CUC is not mediated through beta receptors since propranolol did not abolish it. The depressor effect is probably due to a direct effect of CUC in vascular and/or cardiac muscles. This needs further investigation.

The increase in HR reported earlier in dogs (Elegbe & Oyebola, 1977) was not observed in the present day. Rather, HR decreased after administration of CUC in all the animals studied. Fast runs immediately after injection of CUC in some experiments also failed to show tachycardia. This may be due to species variation between the dogs and rats. Moreover, the resting HR in the rats in the present study is 360.4 ± 8.2 beats/min (mean ± s.e.) while that of dogs was 123.4 ± 5.4 beats/min (Elegbe & Oyebola, 1977). The high resting HR in rats suggests a high level of resting sympathetic activity and/or a low level of vagal tone. It is probable that the initial reduction of HR observed in all the three groups after CUC injections is due to a direct cardio-toxic effect of CUC. Such an affect had been demonstrated in previous experiments (Elegbe *et al.*, 1976; Ojewole, 1979). Since the significant reduction in RR at 5 min post-injection seen in rats pre-treated with propranolol was absent in alpha-blocked rats (the latter in fact showed an increase) it is difficult to escape the conclusion that increase in RR causeed by CUC is mediated through the beta receptors. The early periods of apnoea observed in some animals and the total stoppage of breathing seen in others could be due to direct effect of CUC on the central neurons responsible for the control of respiration. Nicotine causes CNS stimulation followed by depression and death from nicotine results from failure of respiration due to both central paralysis and peripheral blockade of muscles of respiration (Gilman *et al.*, 1980). Although nicotine poisoning is not synonymous with CUC poisoning (Oyebola & Adetuyibi, 1977), previous studies have shown that CUC contains enough nicotine to make it act in a manner similar to nicotine (Oyebola & Elegbe, 1975).

It would appear from the results shown in Table 4 that the increase in pulmonary ventilation following CUC administration is mediated via a reflex loop which is composed largely of autonomic nerves. The fact that the increase in pulmonary ventilation is decreased by both phenoxybenzamine and propanolol lend some support to the latter conclusion. Before final conclusions can be drawn however, there is need for further studies using selective and specific adrenoceptor blockers. The location of the peripheral receptor(s) for this reflex respiratory response is unknown. The lungs and the carotid chemoreceptors are probably involved in the response. Further investigation is necessary to clarify this proposal.

The occurrence of respiratory failure before circulatory collapse and death in the present study suggests that active assistance of a victim's respiration may have a place in the management of cases of CUC poisoning.

The ECG findings in this study confirm the earlier findings of severe cardiotoxicity of CUC (Elegbe *et al.*, 1976). Although propranolol decreased the arrhythmia induced by CUC, the tendency of propranolol to produce hypotension may limit its possible value in the management of patients with CUC poisoning. Moreover, CUC itself causes hypotension (Elegbe & Oyebola, 1977).

Acknowledgements

I am grateful to the Medical Illustration Unit, University College Hospital, Ibadan, for assistance with the figures and Miss Joyce Ikekhua for secretarial help. This study was supported in part by University for Ibadan Senate Research Grant No. 2/SRG3/90 tp Dr D. D. O. Oyebola.

References

Atalabi, G. (1964) 'Cow's urine' poisoning. Dokita, No. 6, 1–4.

Ayorinde, F.O., Avery, J.W., Adekile, A.D., Ojewole, J.A. & Odebiyi, O.O. (1982) Chemistry of a Nigerian herbal preparation (cow's urine concoction) *J. Trop. Paed.* **28,** 13–17.

Bahn, Anita, K. (1972) In: *Basic Medical statistics*. p. 136. Grune & Stratton, Inc., New York.

Elegbe, R.A., Bamgbose, S.O.A. & Oyebola, D.D.O. (1976) Cow's urine poisoning in Nigeria: The cardiotoxic effects of cow's urine concoction on anaesthetized cats. *J. Trop. Paed. Environ. Child Hlth.*, **22,** 232–235.

Elegbe, R.A. & Oyebola, D.D.O. (1977) Cow's urine poisoning in Nigeria: cardiorespiratory effects of cow's urine in dogs. *Trans. R. Soc. Trop. Med.* **71,** 127–132.

Familusi, J.B. & Sinnette, C.H. (1971) Febrile convulsions in Ibadan children. *Afr. J. Med. Sci.*, **2,** 135–149.

Gilman, A.G., Goodman, L.S. & Gilman, A. (1980) The pharmacological basis of therapeutics, 6th ed. p. 180–192. Macmillan Publishers, New York.

Ojewole, J.A.O. (1979) Effect of cow's urine concoction (CUC) on isolated cardiac muscles of the guinea-pig. *J. Pharmaceut. Med. Sci.*, **3,** 43–45.

Oyebola, D.D.O. & Adetuyibi, A. (1977) Toxicity of modified preparation of cow's urine concoction in mice. *Trans. R. Soc. Trop. Med. Hyg.* **71,** 349–350.

Oyebola D.D.O. & Ariwodola, J.O. (1984) The effects of ganglionic blockade and adrenalectomy on the cardio-respiratory response to cow's urine concoction in rats. *Afr. J. Med. med. Sci.* **13,** 161–70.

Oyebola, D.D.O. & Elegbe, R.A. (1975) Cow's urine poisoning in Nigeria: experimental observations in mice. *Trop. Geogr. Med.* **27,** 194–202.

(*Received* 13 *September* 1983; *revision received* 26 *April* 1984; *accepted* 11 *May* 1984)