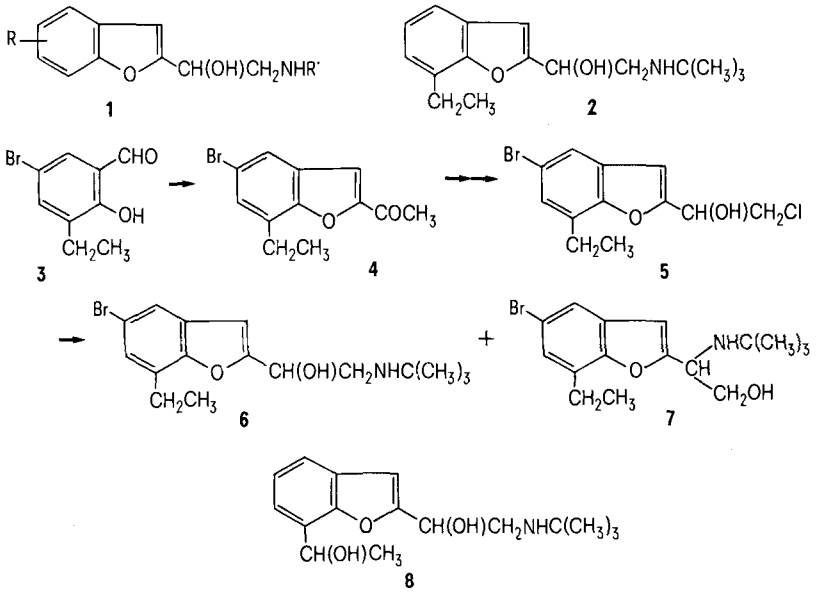
afforded methyl 5-bromo-7-ethylbenzofuranyl ketone **4**. Chlorination with sulphuryl chloride followed by reduction with sodium borohydride gave 1-(5-bromo-7-ethylbenzofuran-2-yl)-2-chloro-1-hydroxyethane **5**. Reaction with an excess of *tert*-butylamine led to the formation of the amino-alcohol **6**, separated from a small quantity of the isomeric primary ethanolamine **7** by fractional crystallization. Debromination was effected by catalytic hydrogenation over palladium/charcoal and the racemic alcohol **2** was isolated as the crystalline hydrochloride, antiarrhythmic activity, but is devoid of α-adrenoceptor blocking activity. Bufuralol antagonizes the catecholamine-induced release of free fatty acids from isolated fat cells and in the intact animal. It also blocks the activation of glycogen phosphorylase by catecholamines in rat diaphragm in vitro. and single doses of the drug cause raised glycogen levels in a number of tissues as a consequence of this action.

In man the biological half-life of bufuralol is 3 to 5 h. The principal metabolite after oral or i.v. administration



m.p. 149–150°, for which satisfactory microanalytical and spectroscopic data were obtained.

Bufuralol has been resolved into its optical isomers by fractional crystallization from ethanol of the diastereoisomeric salts formed with (+)- and (−)-di-*p*-toluoyl tartaric acid. β-Adrenoceptor blocking activity resides only in the laevorotatory form, [hydrochloride, m.p. 122–123°; $[\alpha]_{365}^{20} - 136.0°$ (c = 1, ethanol)], but local anaesthetic and antiarrhythmic properties are observed in both enantiomers.

Pharmacological studies in several species of experimental animals show that bufuralol exhibits non-selective β-adrenoceptor blocking properties similar to those of propranolol[2] and of comparable potency. These effects are exerted on the β-adrenoceptor stimulant actions of injected amines and of sympathetic nerve stimulation. However bufuralol, unlike propranolol, possesses some instrinsic sympathomimetic activity as indicated by tachycardia in reserpinized rats. Bufuralol shows membrane-stabilizing properties, such as local anaesthetic and is a monohydroxylated compound **8** which also possesses potent β-adrenoceptor blocking activity; this metabolite is present in both urine and plasma. Bufuralol is an active β-adrenoceptor blocking agent and antihypertensive in man.

Summary. The synthesis and biological properties of bufuralol, 1-(7-ethylbenzofuran-2-yl)-2-*tert*-butylamino-1-hydroxy-ethane, a new, non-selective β-adrenoceptor blocking agent, are described.

G. A. Fothergill, R. J. Francis, T. C. Hamilton, J. M. Osbond and M. W. Parkes

Roche Products Limited, Welwyn Garden City, Hertfordshire (England), 19 June 1975.

[2] J. W. Black, W. A. M. Duncan and R. G. Shanks, Br. J. Pharmac. *25*, 577 (1965).

The Influence of Pigmentation of Rats and Guinea-Pigs on the Ototoxicity of Kanamycin and Neomycin

Numerous studies have shown a variety of substances, particularly the aminoglycoside antibiotics, to be toxic to the inner ear. The rate and extent of accumulation of these compounds in inner ear fluids (endolymph and perilymph) have been studied in guinea-pigs[1,2] and evidence has been provided that this is due to their affinity for melanin pigment which is present, in the cochlea, mainly in the stria vascularis – one of the sites where endolymph is thought to be produced[3]. In vitro studies showed that kanamycin had a very high affinity for melanin and by

[1] Y. Watanabe, R. Nakajina, R. Oda, M. Uno and T. Naito, Med. J. Osaka Univ. *21*, 257 (1971).

[2] H. Stupp, K. Küpper, F. Lagler, H. Sous and M. Quante, Audiology *12*, 350 (1973).

[3] L. Dencker, N. G. Lindquist and S. Ullberg, Experientia *29*, 1362 (1973).

Table I. Mean startle reactions (± SEM) to a 1 kHz tone (106 dB, re 0.0002 dyn/cm²) of groups of 6 guinea-pigs

Group	Day of test				
	1	14	22	28	34
Pigmented (neomycin, 150 mg/kg)	11.40 ± 2.03	12.47 ± 2.45	5.20 ± 1.56[a]	2.27 ± 0.83[c]	0.67 ± 0.19[c]
Albino (neomycin, 150 mg/kg)	8.60 ± 1.87	10.93 ± 3.40	5.00 ± 2.20	2.72 ± 1.19[b]	0.87 ± 0.27[c]
Pigmented, control (saline, 1 ml/kg)	11.50 ± 2.16	10.94 ± 2.14	10.94 ± 2.68	9.67 ± 2.00	11.78 ± 2.98

Daily s.c. injections were begun on day 3. [a]$P < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. P-values relate to the significance of reductions between the responses and the initial (control) response of the same group.

Table II. Mean startle reactions (± SEM) to an 8 kHz tone (90 dB, re 0.0002 dyn/cm²) of groups of 5 Wistar albino or Lister hooded (pigmented) rats

Group	Day of test			
	1	20	36	101
Pigmented (kanamycin, 250 mg/kg)	20.48 ± 3.01	15.32 ± 2.28	14.40 ± 1.75[a]	10.24 ± 2.01[b]
Pigmented, control (saline, 1 ml/kg)	13.80 ± 1.54	14.12 ± 2.12	12.96 ± 2.03	11.35 ± 2.35
Albino (kanamycin, 250 mg/kg)	7.45 ± 1.43	11.50 ± 2.36	1.10 ± 0.36[c]	0.05 ± 0.05[c]
Albino, control (saline, 1 ml/kg)	6.56 ± 0.59	9.08 ± 1.13	8.04 ± 1.46	7.64 ± 1.62

Daily s.c. injections were commenced after the test on day 1 and terminated on day 35. [a]$P < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. P-values relate to the significance of reductions between the responses and the initial (control) response of the same group.

histopathological investigations of guinea-pigs injected with kanamycin it was seen that serious damage resulted in the stria of pigmented animals but not in albinos[3].

Other workers have noted lesions in the stria vascularis of guinea-pigs injected with ototoxic drugs[4–6], but they were unaware of the involvement of melanin pigment. It is now thought that damage to the hair cells in the organ of Corti (resulting in deafness) may occur secondary to the pathological changes in the stria vascularis.

In our laboratories, over the past 3 years, studies have been conducted into the effects of ototoxic drugs on the hearing of rats and guinea-pigs, both albino and pigmented. A progressive diminution of acoustic startle reaction was taken to be indicative of the development of hearing impairment. The results of these studies were subsequently examined to determine whether or not there might be any difference between the incidence or degree of deafness (as detected by our methods) occurring in albino and pigmented animals.

A description of the apparatus and methods for the measurement of startle reaction appears elsewhere[7]. In experiments involving Lister hooded (pigmented) rats there was conclusive evidence of deafness after kanamycin administration, whereas studies of the effect of kanamycin on Wistar albino rats produced no significant evidence of deafness[7]. However, direct comparisons could not be made since neither the doses of the drugs nor the conditions of the experiments were identical for the albino and pigmented animals.

Direct comparisons of the results were possible in an experiment involving the use of a group of 6 random bred, pigmented guinea-pigs and a group of 6 Hartley albino guinea-pigs. During and after daily s.c. administration of neomycin (150 mg/kg) the startle reactions of these animals were assessed to a short series of 240 msec tones presented randomly in the frequency range, 1 to 8 kHz (maximum sound pressure level – 106 dB re 0.0002 dyn/cm²). Both groups showed marked startle reaction decrements at some frequencies, for example, 1 kHz (Table I). By the use of paired t-tests, it was shown that there was a significant ($p < 0.05$) reduction in the mean startle reaction of the group of pigmented animals at a time when there was a non-significant reduction in the albino group (day 22). However, when the drug administration was continued the mean startle reactions of both groups eventually showed very highly significant ($p < 0.001$) reductions from the respective initial control values. The stable responses of a control group (Table I) demonstrated that habituation was not a factor in these reductions which were therefore assumed to have resulted from drug-induced hearing impairment.

Thus the available evidence, both circumstantial and direct, suggested that albino animals might be less susceptible to the effects of ototoxic drugs, which was consistent with earlier findings[3]. In order to confirm this, a controlled comparison between albino and pigmented rats was carried out. The magnitude of the animals' startle reactions to 8 kHz tones of 240 msec duration and presented at a sound pressure level of 90 dB (re 0.0002 dyn/cm²) were taken as a measure of their hearing acuity. 4 groups of animals, 1 drug and 1 control group for each of the Wistar and Lister hooded rats, were included. The animals in the drug groups were given daily s.c. injections of kanamycin (250 mg/kg) and the control animals, injections of an equivalent volume of saline. Before the commencement of injections, young immature animals were screened to exclude those with intrinsically low startle

[4] N. RISKAER, E. CHRISTENSEN, P. V. PETERSEN and H. WEIDMAN, Acta oto-lar. *46*, 137 (1956).
[5] L.-G. JOHNSSON and J. E. HAWKINS, jr. Laryngoscope, St Louis *82*, 1105 (1972).
[6] J. E. HAWKINS, jr. Audiology *12*, 383 (1973).
[7] E. S. HARPUR. Br. J. Pharmac. *52*, 137P (1974).

reactions. The mean weights of the 4 groups did not differ by more than 24 g (overall mean was 181.8 g). However, the initial mean startle reactions of the albino animals were much smaller than those of the pigmented group (Table II). This difference occurred despite the fact that 43 albino rats were screened to obtain the 10 experimental animals, whereas the 10 pigmented animals were selected from just 14 rats tested. Since the stimulus and the test procedure were the same in both cases, this result could imply some inherent difference between the strains of rat in their acoustic startle reaction.

In this experiment, there were no significant reductions in response in either control group (Table II). By the use of paired t-tests, it was shown that there was a significant ($p < 0.05$) reduction in the mean startle reaction of the pigmented drug group at day 36 and a highly significant ($p < 0.01$) reduction at day 101. However, at these times, there were very highly significant ($p < 0.001$) reductions in the mean startle reaction of the albino drug group compared with the initial control value; these significant reductions in startle reaction were taken to have resulted from drug-induced hearing impairment.

These latter results certainly question the hypothesis that, during chronic intoxication with kanamycin, albino animals are less likely than pigmented animals to suffer cochlear lesions resulting in hearing impairment. However, this interesting possibility is being further explored in our laboratories using a more refined method for testing hearing – namely, operant conditioning of tone discrimination. The resolution of this problem could lead to a fuller understanding of the mechanism of toxic action of such drugs on the inner ear.

Summary. Following the finding that melanin pigment played a role in the accumulation of ototoxic drugs in the inner ear, an investigation was made of the possible influence of the pigmentation of animals on their susceptibility to the ototoxic effects of drugs. Hearing acuity was assessed by measurement of acoustic startle reaction. Preliminary experiments suggested that pigmented animals might be more likely to suffer hearing impairment following ototoxic drug administration. However, in a controlled study using rats treated with kanamycin, it was not possible to confirm this and albino animals appeared no less vulnerable than pigmented animals to kanamycin-induced deafness.

E. S. HARPUR[8] and P. F. D'ARCY

Department of Pharmacy
The Queen's University of Belfast,
Medical Biology Centre, 97, Lisburn Road,
Belfast BT9 7BL (Northern Ireland), 11 July 1975.

[8] This work is supported by a grant from the Department of Health and Social Services (Northern Ireland).

The Effects of Dopamine, Piribedil (ET-495) and its Metabolite S-584 on Retinal Adenylate Cyclase

It has been suggested that in rats adenylate cyclase might be the possible dopamine-receptor within the caudate nucleus of the central nervous system[1]. As a result of this, striatal homogenates of various other species, including man, have been used by numerous workers to investigate the mechanism of action of neuroleptic agents, which are supposed to exert their effects by blockade of dopamine-receptors[2–4]. In a broad sense, a correlation between potency of these agents in vitro, as inhibitors of dopamine-sensitive adenylate cyclase, and their effects in vivo as neuroleptics, seems to exist, although some discrepancies have been encountered[2,4]. We have recently found that intact or homogenized retinae of the rabbit may provide another useful system for such investigations in vitro[5,6], since the neuronal catecholamine in the retina of this species seems to be mostly dopamine[7]. The increased selectivity (and sensitivity) of our model, compared with other striatal or retinal preparations[8,9], also makes it suitable for elucidating the mechanism of action of other drugs, such as experimental or clinical antiparkinsonian agents. Moreover, this can be done at the cellular or molecular level. For example, we have recently shown that apomorphine, a typical "dopamine-like" drug, is a very potent dopamine-receptor agonist, in intact cells as well as in homogenates of rabbit retina[5,6]. In contrast, we have been able to demonstrate that the mechanism of action of amantadine, a clinical antiparkinsonian drug, is not related to direct stimulation of dopamine-receptors[5], although other authors have suggested this possibility[10].

Among the antiparkinsonian drugs acting directly on dopamine-receptors, L-dopa is the most specific. Several attempts have been made to synthesize other dopaminomimetics, such as apomorphine, or piribedil (ET-495) [1-(3,4-methylene dioxybenzyl)-4-(2-pyrimidinyl)piperazine], a non-catechol analogue of dopamine. The agonistic activity of the latter seems to be questionable[11], although it has been proposed that its metabolite formed in vivo, namely S-584 [1-(3,4-dihydroxybenzyl)-4-(2-pyrimidinyl)piperazine] can directly activate striatal dopamine-receptors[12]. To test this hypothesis, we have investigated the effects of both piribedil as well as S-584 on either intact cells or homogenates of rabbit retina, under conditions where the activity of the dopamine is maximal.

Methods. The procedure for the dissection of the retina has already been described[5]. When intact retinae were used, the following modifications of the previous experimental procedure[5] were introduced. Oxygenation with 95% O_2–5% CO_2 during the final 10 min incubation (in the presence of drugs) was not applied, in order to maintain the very easily breakable retina in good shape. This

[1] J. W. KEBABIAN, G. L. PETZOLD and P. GREENGARD, Proc. natn. Acad. Sci., USA *69*, 2145 (1972).
[2] Y. C. CLEMENT-CORMIER, J. W. KEBABIAN, G. L. PETZOLD and P. GREENGARD, Proc. natn. Acad. Sci., USA *71*, 1113 (1974).
[3] R. J. MILLER, A. S. HORN and L. L. IVERSEN, Molec. Pharmac. *10*, 759 (1974).
[4] M. KAROBATH and H. LEITICH, Proc. natn. Acad. Sci., USA *71*, 2915 (1974).
[5] M.-B. BUCHER and M. SCHORDERET, Biochem. Pharmac. *23*, 3079 (1974).
[6] M.-B. BUCHER and M. SCHORDERET, Naunyn. Schmiedebergs Arch. Pharmak. *288*, 103 (1975).
[7] J. HÄGGENDAL and T. MALMFORS, Acta physiol. scand. *64*, 58 (1965).
[8] J. H. BROWN and M. H. MAKMAN, Proc. natn. Acad. Sci., USA *69*, 539 (1972).
[9] J. H. BROWN and M. H. MAKMAN, J. Neurochem. *21*, 477 (1973).
[10] R. PAPESCHI, Neuropharmacology *13*, 77 (1974).
[11] O. HORNYKIEWICZ, Biochem. Pharmac. *24*, 1061 (1975).
[12] R. J. MILLER and L. L. IVERSEN, Naunyn Schmiedebergs Arch. Pharmak. *282*, 213 (1974).