mannitol diuresis seems therefore to exclude the second hypothesis.

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[1] Zerahn, K., *Acta Physiol. Scand.*, **36**, 300 (1956).
[2] Leaf, A., *J. Gen. Physiol.*, **43**, 1st Suppl., 175 (1960).
[3] Hess Thaysen, J., *et al.*, Kramer, K., *et al.*, *Excerpta Medica*, Internat. Congress Series, No. 29, 44, 50 (1960).
[4] Hawk, P. B., Oser, B. L., and Summerson, W. H., *Pract. Physiol. Chem.* (Blakiston and Co., 1949).
[5] Patent No. 402785, "Verfahren zur Reinigung von Milchsäure", in *Fortschritte der TeerFarbenFabrikation*, ed. by Friedländer, **14**, 282 (1921–25).
[6] Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Murray E., *Amer. J. Med.*, **24**, 730 (1958).
[7] Malvin, R. L., and Wilde, W. S., *Fed. Proc.*, **18**, 97 (1959). Ullrich, K. Y., Drenckhahn, F. O., and Yarausch, K. H., *Arch. ges. Physiol.*, **261**, 62 (1955).

Pharmacological Actions of Some Methoxyindolealkylamines

The O-methylation of catecholamines has been shown to be an inactivation process[1], but the recent characterization of melatonin (N-acetyl-5-methoxytryptamine—a potent agent in lightening frog skin) as a methoxy indole[2] indicates that this may not be true with hydroxyindolealkylamines. To investigate this possibility, we have compared the actions of a number of methoxyindolealkylamines with their corresponding analogues on smooth muscle, blood pressure and behavioural patterns.

Oxytocic activity was measured on isolated œstrous rat uterus. Gramine and its methoxy derivative were found to possess no oxytocic activity at a dose-level as great as 0·1 mgm. per ml. (Values are given as final concentration in the muscle bath.) At the same dose-level, 5-hydroxyindole acetic acid and 5-methoxyindole acetic acid caused small but demonstrable contractions. This action could not be blocked with bromolysergic acid diethylamide (*BOL*). N-Acetyl-5-methoxytryptamine also exhibited slight oxytocic effect, but only at the high dose-level of 0·1 mgm. per ml.

The four other compounds tested were serotonin, bufotenine and their respective methoxy analogues. These exhibited marked oxytocic activity at dose-levels below 10 μgm. per ml. The relative activity of these compounds is given in Table 1, and it can be seen that the methoxy derivatives were only slightly less active than their hydroxy analogues. The results obtained for bufotenine and 5-methoxytryptamine are in general agreement with those of Erspamer[3] and of Barlow and Khan[4].

Whereas N : N-dimethylation of 5-methoxytryptamine only reduces the oxytocic activity slightly, N-acetylation reduces the potency a thousand-fold.

The effect on arterial blood pressure was measured on a smoked drum from a mercury manometer. Two cats and three dogs anæsthetized with pentobarbital were used. The action of compounds was noted before and after the nervous system had been destroyed in cats by pithing, or blocked in dogs by large doses of the ganglioplegic agents : tetraethyl ammonium chloride, 30–40 mgm./kgm., or chlorisondamine ('Ecolid' : 2 mgm./kgm.).

In doses of 1–2 mgm. N-acetyl-5-methoxytryptamine, 5-hydroxyindole acetic acid, 5-methoxyindole acetic acid and 5-methoxygramine had no effect on arterial blood pressure of dogs or cats.

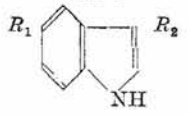
Serotonin, 5-methoxytryptamine, 5-methoxy-N : N-dimethyltryptamine in doses of 0·1 mgm. and bufotenine, 0·5 mgm., all caused depression of arterial pressure or an amphibaric response in both species while the nervous system was intact. After pithing or blocking, these compounds caused blood pressure to rise by 26–92 mm. mercury.

It has been reported that ganglioplegic agents cause powerful augmentation of a variety of pressor agents[5]. Serotonin and 5-methoxy-N : N-dimethyltryptamine were augmented to an almost identical degree, both being slightly exceeded by 5-methoxytryptamine. 5-Methoxytryptamine also exhibited a depressor component, after pithing in cats, usually following a strong pressor phase.

The relative vasopressor potency of these four compounds is : Serotonin > 5-methoxy-N : N-dimethyltryptamine > 5-methoxytryptamine > bufotenine. Thus, O-methylation reduces the vasopressor activity of serotonin but greatly increases that of bufotenine, and N-acetylation of 5-methoxytryptamine inactivates it.

To investigate behavioural disturbance, rats were conditioned to escape, in a shuttlebox, from a 535-V.

Table 1



Compound	R_1	R_2	Oxytocic*	*BOL*†	Effect on blood pressure‡ (unblocked)	(blocked)	Behavioural§ disturbance
Gramine	H	CH_2NH_2	0·0001	—	None	None	0
5-Methoxygramine	OCH_3	CH_2NH_2	0·0001	—	,,	,,	+
Serotonin	OH	$CH_2CH_2NH_2$	1	Inhib.	⇅	↑	(++)
5-Methoxytryptamine	OCH_3	$CH_2CH_2NH_2$	0·7	,,	⇅	↑	++
N-Acetyl-5-methoxytryptamine	OCH_3	$CH_2CH_2NHCOOH_3$	0·0001	—	None	None	0
5-Hydroxyindole acetic acid	OH	CH_2COOH	0·0001	No inhib.	,,	,,	—
5-Methoxyindole ,, ,,	OCH_3	CH_2COOH	0·0001	,,	,,	,,	—
Bufotenine	OH	$CH_2CH_2N(CH_3)_2$	0·49	Inhib.	↑	↑	++
5-Methoxy-N : N-dimethyltryptamine	OCH_3	$CH_2CH_2N(CH_3)_2$	0·08	,,	⇅	↑	+++++

* Potency of equimolar concentrations of the base. † Inhibitory effect of *BOL*. ‡ Blood pressure raised ↑, biphasic ⇅. § At 0·1 m*M*/kgm. dose-level each + represents 2 mistakes during 10 tests.

2-m.amp. shock in response to a warning auditory stimulus, individual trials being separated by 6-min. intervals. The criterion of completed conditioning was a correct response to the conditioning stimulus of not less than 9 out of 10 times on two consecutive days. The animals were then used for assay and the number of mistakes plotted against the dose-level. N-Acetyl-5-methoxytryptamine caused no behavioural disturbance at dose-levels of 0·2 m*M*/kgm. At dose-levels between 0·1 and 0·3 m*M*/kgm. 5-methoxygramine caused a somewhat greater degree of disturbance than did gramine. At 0·1 m*M*/kgm., 5-methoxytryptamine, bufotenine and serotonin all gave similar results, though in the case of the latter compound the paresis of the hind legs which developed introduced a physical handicap which modified the interpretation of this result. The most active compound of the series proved to be 5-methoxy-N : N-dimethyltryptamine, which caused 10 mistakes out of 10 trials at a dose-level of 0·05 m*M*/kgm. This was three times more effective than bufotenine, which has been reported to be psychotomimetic in man[5] and to affect the performance of trained rats[7]. At dose-levels exceeding 0·1 m*M*/kgm. the animals given 5-methoxy-N : N-dimethyltryptamine developed rhythmic beating of the forepaws, which tallied closely with the description given by Fabing and Hawkins[6] for the effects of bufotenine in rats which we have, however, not observed. 5-Methoxytryptamine also proved quite potent and in the same range as bufotenine. Finally, 5-methoxygramine appeared to cause more mistakes than did gramine itself.

Thus the ability of gramine, serotonin and bufotenine to cause behavioural mistakes was increased by O-methylation; and whereas N : N-dimethylation of 5-methoxytryptamine increases its potency, N-acetylation inactivated it.

It can therefore be concluded that O-methylation of hydroxyindoles is not an inactivation mechanism as is the O-methylation of catecholamines. The O-methylation of hydroxyindoles, however, changes their activity in the three assay procedures used in the following ways: oxytocic activity is still exhibited though slightly decreased; the vasopressor activity of serotonin is decreased but that of bufotenine is increased; ability to cause behavioural mistakes is increased. In addition, although O-methylation and N-acetylation are both necessary for the frog skin lightening activity of melatonin[2], it is the N-acetyl group which renders melatonin inactive towards smooth muscle, blood pressure and behaviour.

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[1] Axelrod, J., *Physiol. Rev.*, **39**, 751 (1959).
[2] Lerner, A. B., Case, J. D., and Takahashi, Y., *J. Biol. Chem.*, **235**, 1992 (1960).
[3] Erspamer, V., *Nature*, **170**, 281 (1952).
[4] Barlow, R. B., and Khan, I., *Brit. J. Pharm. Chemotherap.*, **14**, 265 (1959).
[5] Page, I. H., and Taylor, R. D., *Science*, **105**, 622 (1947).
[6] Fabing, H. B., and Hawkins, J. R., *Science*, **123**, 886 (1956).
[7] Mahler, D. S., and Humoller, F. L., *Proc. Soc. Exp. Biol. and Med.*, **102** 697 (1959).

HÆMATOLOGY

A Possible Specificity of Albumin Auto-antibodies

Anti-*I* was the name given by Wiener *et al.*[1] in 1956 to a cold antibody found in the serum of a patient suffering from cold antibody hæmolytic anæmia. Further investigations into the *I* group system were made by Jenkins *et al.*[2], Tippett *et al.*[3], Weiner *et al.*[4] and Marsh[5]. These workers showed that cold auto-antibodies (previously called non-specific cold agglutinins) had *I* specificity and that occasional powerful examples of these antibodies may be found as auto-agglutinins in cases of cold antibody hæmolytic anæmia. The *I* antigen has an almost universal distribution, Wiener *et al.* finding only five *i* people from 22,000 blood donors, while Jenkins *et al.* failed to find an example of the *i* phenotype after testing 17,000 donors. Marsh afterwards described examples of cold auto-antibodies having *i* specificity and showed that normal infants at birth possessed the *i* phenotype, which changed within 18 months of birth to *I*. He concluded that the rare *i* phenotype in adults arose from the absence of a genetically determined factor essential for the proper development of *I*. The majority of cold auto-antibodies therefore appear to have *I* specificity, a minority may be anti-*i*.

In 1956, Weiner *et al.*[6] described some examples of a rare auto-agglutinin active against all cells, but only when they were suspended in bovine albumin. These auto-agglutinins were inactive by anti-globulin or enzyme techniques and could not be absorbed by saline-suspended red cells. We have encountered two examples of the albumin auto-agglutinin described by these workers and were able to test them for *I* or *i* specificity.

Both sera were active against the patient's own cells and also all cells of a large genotyped panel, but only when albumin suspended red cells were used. One serum contained an additional trace of anti-*D* which was successfully removed by absorption with saline-washed rhesus-positive red cells.

The sera were tested against the red cells of two examples of the i_1 phenotype, one i_2 and four cord blood samples, with appropriate adult controls. The tests were all positive with about the same strength of reaction. Repeated tests with one of the sera in titration (using *AB* serum as a diluent) showed that the i_1 and i_2 cells scored the same as the adult controls; the cord samples were slightly weaker.

It is apparent from these results that while cold auto-antibodies have *I* or *i* specificity, albumin auto-agglutinins are not aimed at antigens within the *Ii* system.

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[1] Wiener, A. S., Unger, L. J., Cohen, L., and Feldman, J., *Ann. Intern. Med.*, **44**, 221 (1956).
[2] Jenkins, W. J., Marsh, W. L., Noades, J., Tippett, P., Sanger, R., and Race, R. R., *Vox Sang.*, **5**, 97 (1960).
[3] Tippett, P., Noades, J., Sanger, R., Race, R. R., Sausais, L., Holman, C. A., and Buttimer, J., *Vox Sang.*, **5**, 107 (1960).
[4] Weiner, W., Shinton, N. K., and Gray, I. R., *J. Clin. Path.*, **13**, 232 (1960).
[5] Marsh, W. L. (submitted to *Brit. J. Haemat.*).
[6] Weiner, W., Tovey, G. H., Gillespie, E. M., Lewis, H. B. M., and Holliday, T. D. S., *Vox Sang.*, **1**, 297 (1956).