## The influence of cellular metabolism on the α- and β-adrenoceptor responses of the rabbit small intestine

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The relaxation of the rabbit small intestine produced by sympathomimetics involves (depending on the drug used) interaction with α- and/or β-adrenoceptors (Bowman & Hall, 1970). These two receptor systems are not necessarily coupled to the same intracellular mechanisms.

Manifestation of the β-adrenoceptor effect on the rabbit small intestine was found to be dependent on the integrity of cellular metabolism. Partial glycogen depletion using the methods described by Bueding, Bülbring & others (1967) or treatment with the glycolysis inhibitor iodoacetate (5 mM) abolished the β-adrenoceptor effects of isoprenaline but not the α-adrenoceptor effects of phenylephrine or the α-adrenoceptor-like effects of ATP. Andersson & Mohme-Lundholm (1969, 1970) have recently obtained similar results after glycogen depletion of the rabbit colon and taeniae coli. In the continued presence of iodoacetate the isoprenaline effect was restored when the block was by-passed by adding pyruvate (8 mM) to the organ bath. Although iodoacetate or glycogen depletion did not reduce the extent of the inhibitory response produced by phenylephrine or ATP, they did hasten the onset of recovery from the inhibitory response to these two drugs and did augment the overshoot on washout which is found with these two drugs (Bowman & Hall, 1970). Thus it appears that these two conditions can block the β-adrenoceptor mediated inhibition of the rabbit small intestine and also potentiate the stimulatory phase of the α-adrenoceptor mediated response or of the response to ATP.

The conditions used in these experiments never abolished the spontaneous activity of the rabbit gut, nor did they diminish the inhibitory response of drugs acting on α-adrenoceptors. However the inhibitory effects of β-adrenoceptor agonists were reduced by glycogen depletion and by iodoacetate. Thus it would seem that one of the intermediary products of glycogenolysis is necessary for the expression of β-adrenoceptor mediated effects in the rabbit small intestine, whereas α-adrenoceptor mediated effects utilize an entirely different mechanism. This provides an explanation for the observation that towards the end of a prolonged experiment on an isolated tissue, or with tissues that have been lying on the bench for several hours in a beaker of oxygenated Krebs solution, β-adrenoceptor mediated responses were found to gradually diminish in size. It was shown that glycogen stores are significantly decreased under the latter conditions.

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## The extraction and estimation of histamine from *Gossypium* species

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This investigation was a development of work by Lloyd & Nicholls (1964) and others on the constituents of cotton mill dusts likely to cause byssinosis in cotton workers. In this case, authenticated material from *Gossypium hirsutum* L., the Upland cotton, and *G. arboreum* L., tree cotton, was used and the experimental work concerned with the histamine content of the cotton plant.

Histamine was estimated fluorimetrically using a method by Shore, Burkhalter & Cohn (1959) based on a fluorogenic reaction between histamine and *ortho*-phthalaldehyde, (oPT). oPT was known to form a fluorescent complex with substances other than histamine which frequently occurred in biological materials (Turner & Wightman, 1968), and it was found that most of the extraction procedures which had been used to recover histamine from animal tissues also recovered interfering substances from the cotton plant material.

An extraction procedure was devised which excluded the interfering substances but gave a low recovery of histamine. This involved a combination of ion exchange (Huff, Davis & Brown, 1966) and solvent extraction (Shore, Burkhalter & Cohn, 1959): plant material was extracted with N trichloroacetic acid, the extract adjusted to pH 7·5 and passed through Amberlite CG 50 anionic exchange resin, the eluate was subjected to solvent extraction before the fluorogenic reaction with oPT.

The histamine content of fresh tissue of *Gossypium* species was estimated by this procedure: *G. hirsutum*, old leaf, 87 μg histamine/g fresh leaf; *G. hirsutum*, young mature leaf, 101 μg/g; *G. arboreum*, young mature leaf, 113 μg/g. Fresh mature bracts of *G. hirsutum* were estimated to contain 26 μg histamine/g of *G. arboreum*, 6 μg histamine/g.

By comparison, dried leaf of *G. hirsutum* was estimated to contain 1760 μg histamine/g which corresponded to 330 μg histamine/g fresh leaf.

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## The estimation of rauwolfia alkaloids by quantitative thin-layer chromatography

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Previous work on the estimation of rauwolfia alkaloids (Harris, Stewart & Court, 1968; Los & Court, 1969) involved thin-layer chromatographic separation followed by ultraviolet spectrophotometric estimation of the eluted alkaloids. Substances extracted from the adsorbent are known to interfere with the spectrophotometric measurements (Harris, 1966) and coloured complexes of the alkaloids can be formed and measured at wavelengths such that interference is minimal (Court, 1968).

Table 1. *Alkaloid content of* Rauwolfia *root bark.*

| | *R. caffra* | | *R. vomitoria* | |
|---|---|---|---|---|
| Alkaloid | Mean percentage | Coefficient of variation | Mean percentage | Coefficient of variation |
| Ajmalicine | 0·016 | 2·20 | — | — |
| Ajmaline | 0·239 | 1·17 | 0·090 | 3·33 |
| Rescinnamine | 0·013 | 1·43 | 0·105 | 1·33 |
| Reserpiline | — | — | 1·090 | 1·01 |
| Reserpine | 0·016 | 1·97 | 0·218 | 0·46 |
| Serpentine | 0·180 | 1·24 | — | — |
| | (based on 5 determinations) | | (based on 10 determinations) | |

In this work 10 rauwolfia alkaloids were separated using various chromatographic systems employing silicagel G layers 250 μm thick. The individual alkaloids were recovered by elution in alkaline chloroform and complexed with iodine in citrate-phosphate buffer (pH 4·1). The absorption measurements of the alkaloid-complex solutions were recorded at appropriate wavelengths in the range 365–396 nm. Results were calculated from compensated standard curves.

Recovery of the alkaloids by elution from the plates was investigated and the method was applied to samples of *Rauwolfia caffra* and *R. vomitoria* root barks (Table 1).

The method is more rapid and accurate than the earlier method and yields lower results due to reduced interference.

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