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[1] Pickering, B. T., and Li, C. H., *Biochim. Biophys. Acta*, **62**, 475 (1962).
[2] Lee, T. H., and Buettner-Janusch, V., *J. Biol. Chem.*, **238**, 2012 (1963).
[3] Kastin, A. J., and Ross, G. T., *Experientia*, **20**, 461 (1964).
[4] Shizume, K., Lerner, A., and Fitzpatrick, T. B., *Endocrinology*, **54**, 553 (1954).
[5] Long, J. M., and Guillemin, R., *Experientia*, **17**, 132 (1961).
[6] Hano, K., Koida, M., Kubo, K., and Yajima, H., *Biochim. Biophys. Acta*, **90**, 201 (1964).

N-Acetylmannosamine Digestion by Human Oral Bacteria

LEACH[1] showed that *N*-acetylneuraminic acid was rapidly metabolized by human saliva and ascribed this to the presence of an inducible aldolase produced by contaminating micro-organisms since such destruction was prevented by the addition of 'Ledermycin' or by heating the saliva in boiling water. This appears to be the second step in a process whereby salivary mucoproteins having *N*-acetylneuraminic acid residues as their non-reducing end groups act as inducer molecules to oral bacteria, producing first a neuraminidase and later an aldolase which cleaves the *N*-acetylneuraminic acid liberated by the neuraminidase into *N*-acetylmannosamine and pyruvic acid. Such a sequence has been found by us[2] to occur when α_1-glycoprotein, which is present in sputum, is used as an inducer molecule in cultures of *Klebsiella aerogenes*. We wish to report on the third step whereby *N*-acetylmannosamine itself is digested.

Fresh saliva samples (0·5 ml.) were incubated with sterile aliquots of *N*-acetylmannosamine (100γ in 0·1 ml.) at 37° for 2 h, dialysed against water (100 volumes) at 4° and the dialysate freeze-dried before assay with the Morgan-Elson reagents[3]. From a random group of healthy individuals including smokers and non-smokers, the amounts of *N*-acetylmannosamine remaining were with *A*, 77 μg; *B*, 34 μg; *H*, 95 μg; *M*, 90 μg; *P*, 84 μg and *W*, 51 μg. In a further incubation of saliva from *B*, only 40, 30 and 10 per cent of the original *N*-acetylmannosamine was left after 1, 2 and 4 h respectively. When saliva from *B* was assayed with incubation for 1 h before and after a mouth-wash, first with 'Dettolin' (2·5 ml./70 ml. water) and then with hydrogen peroxide (5 ml. 20 vol./70 ml. water), it was found that the first mouth-wash produced arrest of *N*-acetylmannosamine digestion (73 μg remaining) compared with saliva before washing (45 μg remaining), but this was not increased by the second mouth-wash (73 μg remaining).

When glucose (1 mg) was included in the incubation mixture in addition to *N*-acetylmannosamine (100 μg), digestion of the latter was again arrested: *B* with glucose, 63 μg; *B* without glucose, 41 μg (2 h incubation); *W* with glucose, 90 μg (2 h), 60 μg (4 h); *W* without glucose, 11 μg (4 h). Glucose is widely effective in inhibiting enzyme induction in bacteria.

The micro-organisms responsible for *N*-acetylmannosamine digestion in the saliva of *B* and *W* differed in their sensitivity to a range of antibiotics. Each antibiotic (5 mg) was incubated with the saliva for 1 h at 37° before further incubation with *N*-acetylmannosamine (100 μg) for 4 h. The amount (μg) remaining with *B* saliva was with erythromycin, 99; tetracycline, 76; streptomycin, 68; kanamycin, 51; methymycin, 50; without any antibiotic, 37. On one occasion with *W* saliva the amount (μg) remaining was, with tetracycline, 93; without any antibiotic, 65; while later with another sample no completely effective arrest was shown with streptomycin, 53; erythromycin, 45; methymycin, 26; paromomycin, 25; kanamycin, 22, compared with a control without antibiotic, 14.

Further evidence that the agent responsible for *N*-acetylmannosamine digestion was an enzyme induced in a micro-organism was obtained by comparing the amounts remaining after autoclaving salivas *B*, 99 *W*, 100, after Seitz filtration of salivas *B*, 97 *W*, 99, compared with no previous treatment of *B*, 49 *W*, 64 μg.

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[1] Leach, S. A., *Nature*, **199**, 486 (1963).
[2] Barker, S. A., Pardoe, Grace, I., and Stacey, M., *Protides of the Biological Fluids*, **11**, 284 (1963).
[3] Aminoff, D., Morgan, W. T. J., and Watkins, W. M., *Biochem. J.*, **51**, 379 (1952).

Kinetics of Cyclic Adenosine Monophosphate Changes in Rat Heart following Epinephrine Administration

ALTHOUGH it has become generally accepted[1] that epinephrine causes glycogenolysis in cardiac muscle by a series of enzymatic reactions involving the formation of cyclic AMP, activation of phosphorylase *b* kinase, and finally conversion of phosphorylase *b* to the active *a* form, direct evidence in favour of this mechanism in the intact heart is so far not conclusive[2]. Furthermore, a possible relation between cyclic AMP and the inotropic effect of catecholamines is also tentative.

In view of the recent finding[3,4] that the inotropic response of the rat heart to epinephrine precedes the glycogenolytic effect, it is clearly of great importance to determine the kinetics of the cyclic AMP changes in relation to the elevation of the contractile force and phosphorylase *a* levels in order to gain a deeper understanding of the mechanisms involved.

Hearts were taken from male, fed rats of Wistar strain (220–260 g) and perfused as described by Williamson[5]. Cyclic AMP in 500-mg samples of frozen heart powder was extracted and assayed by the method of Posner *et al.*[6]. Cyclic AMP phosphodiesterase was prepared according to Butcher and Sutherland[7]. Other analytical procedures were similar to those used by Williamson[5,8].

Fig. 1 compares the change in the level of cyclic AMP with that of the total phosphorylase in the *a* form, and that of the contractile force, following the addition of a single dose of 1 μg epinephrine. The contractile force increased to a maximum after 10–12 sec with a half-time of 3·5 sec, and afterwards decreased to a value 30 per cent above the initial at the end of 30 sec. More extensive recordings of the contractile force reported elsewhere[3,4] have shown that the pattern of the contractile force depicted in Fig. 1 is characteristic of a single large dose of epinephrine, and that the force increases again after 30 sec, reaching a plateau 60–70 per cent above the control after about 90 sec. This level is maintained for as long as 10 min. Cyclic AMP levels increased in a manner similar to the force curve ($t_{\frac{1}{2}} = 3{\cdot}5$ sec), and also paralleled it during the declining phase from 15 to 30 sec. At the end