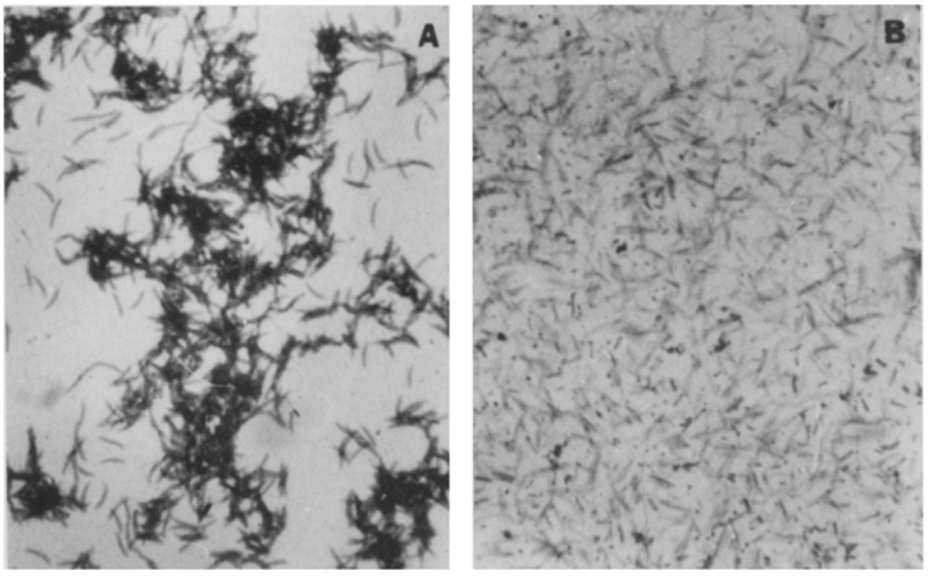

Fig. 2. Representative photographs of spores challenged with *Fusarium solani* antisera. A) *Fusarium solani;* B) *Fusarium roseum.* ×630.

animals. After 1 week, a 2nd inoculation, similar to the 1st, was administered i.p. and a 3rd inoculation, also i.p., was given 2 weeks after initial inoculation. One week following the 3rd inoculation, 15 ml of blood was obtained from the lateral ear vein.

Agglutination reactions were performed on ordinary glass slides and at room temperatures. To each slide was added a 0.05 ml of spore suspension and 0.05 ml of either normal rabbit or *F. solani* antiserum. The slides were gently swirled for 2 min to assure maximum cell contact. At the end of this period, the agglutination reactions were photographed.

Results and discussion. As seen in Figure 1, control (normal rabbit) sera did not agglutinate to any degree condida of either *F. solani* or *F. roseum*. However, as seen in Figure 2, *F. solani* antisera massively agglutinated the conidida of *F. solani*, while *F. roseum* spores showed no agglutination. It is of interest to note that the agglutination reactions of the spores did not decrease following repeated washings with PBS. This evidence indicates distinct antigenic differences between the spores of these 2 taxonomically related, though host-distinct, plant pathogens and is probably reflective of their host specificities.

It would appear that the above described method of assay of the antigenic properties of fungal spores may be an important investigative technique and should be exploited in future studies as the agglutination reaction is one of the most sensitive assays known. Thus, when this methodology can be applied to such large cells as fungal spores (4 to 5 times more volume than erythrocytes) with such apparent specificity, the intimacies of the host-parasite recognition and invasion mechanisms can be more easily investigated and elucidated.

Zusammenfassung. Immunologische Agglutinations-Reaktionen eignen sich als Methoden zur Untersuchung von spezies-spezifischen Antigenen von Pilzsporen.

S. J. Kleinschuster, D.C. Lueker and R. Baker[14]

Departments of Anatomy, Microbiology, Botany and Plant Pathology, Colorado State University, Fort Collins (Colorado 80521, USA),
29 April 1974.

[14] We wish to acknowledge C.S.U.–F.I.C. grant No. 13-2018-826 for support of this study.

A Convenient Colourimetric Method for Routine Assay of Brain Adenylate Cyclase

Brain is a tissue rich in adenylate cyclase and enzyme activity has been measured with unlabelled substrate by ion exchange and UV-spectroscopy[1]. To provide a simpler method suitable for undergraduate teaching a direct nonchromatographic method of similar sensitivity and precision was devised.

Materials and methods. Brain adenylate cyclase: Whole, freshly obtained cow brains were immediately immersed in icecold buffer consisting of glycylglycine (glygly), 2×10^{-3} *M*, pH 7.5; $MgSO_4$, 1×10^{-3} *M*; and NaCl, 2×10^{-3} *M*. The cortex was minced, and homogenized in a Teflon glass Potter-Elvehjem apparatus, according to Bitensky et al.[2] and the homogenate was processed according to Sutherland et al.[3] and stored at −20 °C.

The standard incubation medium (4.0 ml) consists of ATP, 1.0×10^{-3} *M*; $MgSO_4$, 3.6×10^{-3} *M*; caffeine, 5.0 ×

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10^{-2} *M*; and glygly, 6.0×10^{-2} *M*, pH 7.5. NaF (6.0×10^{-3} *M*) can be included or omitted. The reaction is initiated by adding 0.2 ml of particulate adenylate cyclase suspension (approximately 4.0 mg total protein[4]); this reaction mixture is shaken for 20 min at 25 °C in a Dubnoff incubator and then stopped by rapid heating to 100 °C for 3.5 min in a 15 ml centrifuge tube. After rapid cooling, protein is sedimented at 10,000 *g* for 40 min at 4.0 °C and the supernatant fluid transferred to a 25 ml flask containing 0.2 ml of $NaIO_4$ solution (100 mg/ml). The stoppered flask is kept at 40–45 °C in the dark 18–22 h, and 0.2 ml of freshly prepared $NaBH_4$ solution (60 mg/ml) is added at 25 °C; 30 min later, 1 ml of 0.2 *M* lead acetate and 20 µl distilled 2-octanol are added. The suspension is centrifuged at 1,000 *g* for 20 min at 25 °C; 4 ml of the clear supernatant fluid is drawn into a 5 ml syringe, and transferred to a 25 ml boiling tube. A modified orcinol test for ribose[5] is applied: 4 ml of 0.2% $FeCl_3$ in concentration. HCl and 0.4 ml of orcinol solution (100 mg/ml water) are stirred into each 4 ml aliquot. The samples are heated to 100 °C for 40 min, cooled, and extracted with 4.5 ml of redistilled *n*-butyl acetate. The absorbance of the clear upper phase is read spectrophotometrically at 670 nm.

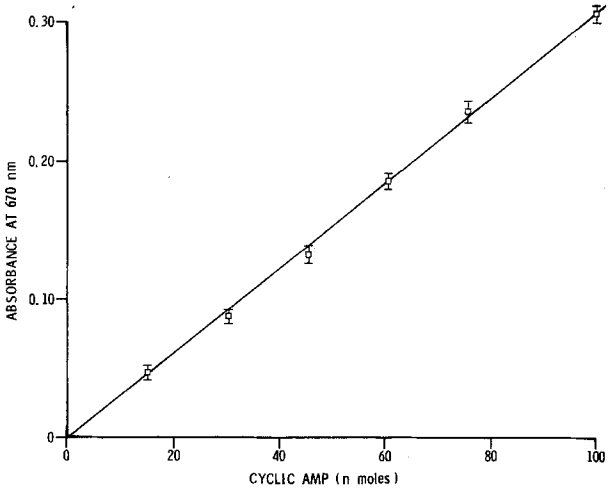

Fig. 1. Standard curve for cAMP in the presence of 3×10^{-3} *M* ATP. Each point is the mean value of triplicate experiments.

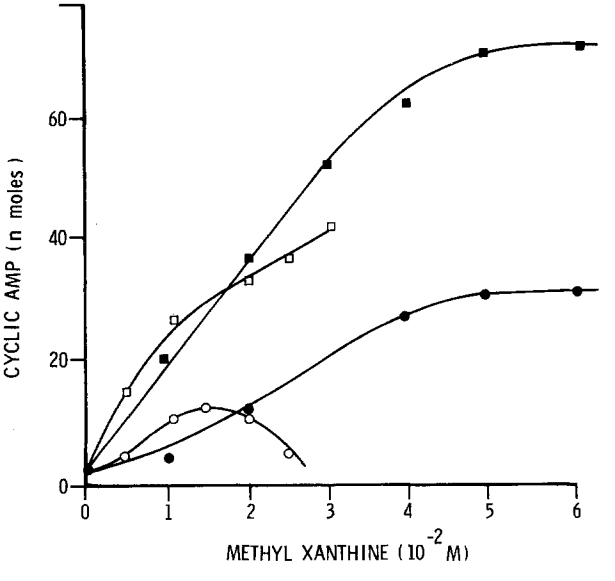

Fig. 2. Effect of methylxanthines on the cAMP levels. cAMP levels as a function of caffeine with (■) and without (●) 6.0×10^{-3} *M* NaF and theophylline with (□) and without (○) NaF. Protein concentration, 1.0 mg/ml. Each point represents a single reading.

Results. The standard curve for cyclic AMP (cAMP) (Figure 1) was unaffected by the presence (100–500-fold excess) or absence of ATP; Beer's law applied over a wide range of concentrations. The limit of sensitivity was about 10 nmoles (2.5×10^{-6} *M*). An identical curve was obtained with equivalent concentrations of ribose or cAMP but without periodate oxidation confirming the complete recovery of chromophore derived from cAMP. Blanks were unchanged by the addition of protein (1.0 mg/ml), caffeine (5×10^{-2} *M*), or epinephrine (1×10^{-4} *M*). The mean absorbance of 25 blanks (10 with ATP but without protein and 10 vice versa, and 5 with neither) was 0.023 ± 0.002 at 670 nm.

As shown in Figure 1, triplicate determinations of known concentrations of cAMP were reproducible ±6% at the lowest cAMP concentration of the standard curve, with increasing accuracy at higher concentrations (±3% at 60 nmoles). Furthermore, triplicate determinations (not shown) of cAMP in reaction mixtures containing 10^{-3} *M* ATP, with and without fluoride, were reproducible ±3%.

The relationship between cAMP synthesis and brain-cortex protein concentration was linear until about 1.2 mg/ml protein, (the useful range being 0.6 to 1.2 mg/ml). With or without NaF, recovery of cAMP was greater with 10^{-2} *M* theophylline than with 10^{-2} *M* caffeine, and with 5×10^{-2} *M* caffeine was twice that with 10^{-2} *M* theophylline (Figure 2). Under the various assay conditions, the amount of cAMP synthesized was linear, within experimental error, for 20 min. The addition of 6.0×10^{-3} *M* NaF increased cAMP synthesis approximately twofold (Figure 3), without significant *Km* effect ($Km = 3 \times 10^{-4}$ *M*).

Discussion. The method is based on the selective oxidation of glycol units to dialdehydes by periodate, first described in 1926 by MALAPRADE[6] and resistance of cAMP to periodate attack[7]. Complete oxidation of non-cyclic nucleotides can be demonstrated by blank determinations with ATP or AMP and no enzyme. Excess $NaIO_4$ and oxidation products are reduced by $NaBH_4$; resulting iodide is precipitated by excess lead acetate before quantification of intact ribose. Sensitivity (approximately 10 nmoles) is comparable to that of the assay of BRADHAM and WOOLLEY[1], is cheaper, simpler, easier to perform, faster (36 reaction min assayed routinely in 2 days) and reproducible. The low sensitivity renders the assay useful for highly active[1,3,8] or purified[9–11] cyclase

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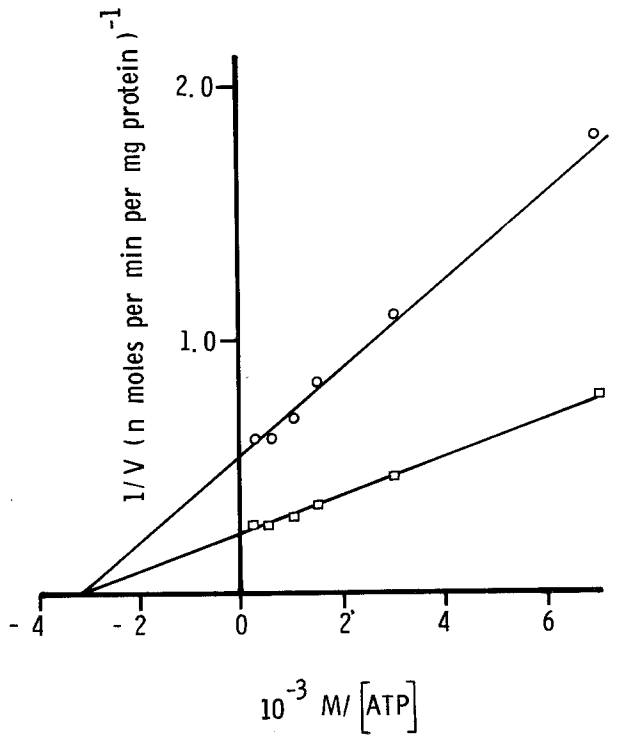

Fig. 3. Lineweaver-Burk plot for adenylate cyclase activity. cAMP synthesis as a function of ATP with (□) and wihtout (○) 6.0×10^{-3} *M* NaF. Caffeine concentration 5×10^{-2} *M*; protein concentration, 1.0 mg/ml. Each point represents a single reading.

preparations particularly where undergraduate teaching, in vitro pharmaceutical trials or kinetic studies are involved. Excess IO_4^- – consuming materials (sucrose or glycerol), excess anions forming insoluble lead salts ($> 2 \times 10^{-3}$ *M*) and cGMP interfere. Over broad concentration ranges many chemical effecters: catecholamines, F^-, Mg^{++}, Ca^{++}, Zn^{++}, Mn^{++}, Co^{++}, Fe^{++}, purines, pyrimidenes and xanthines do not affect the assay. Sulphydryl inhibitors which can interfere in other assays[12,13] were without effect.

Loss of linearity with high protein concentrations is likely due to excess diesterase and ATPase activities under these conditions. Caffeine at 5×10^{-2} *M* is optimal in minimizing interference by the former (Figure 2). Substantially lower caffeine concentrations were employed in earlier brain cyclase studies[3,8,14]. Similar to adipose adenylate cyclase[15], brain cyclase displays a V_{max} increase and no *Km* effect by the addition of NaF to the reaction medium (Figure 3)[16].

Zusammenfassung. Es wird eine direkte einfache chemische Methode zur Bestimmung von Gehirn Adenylatcyclase beschrieben, die auf der Neigung nichtzyklischer Nukleotiden zur Per-Jod-Oxydation im Gegensatz zur zyklischen AMP basiert. Empfindlichkeit: 10–100 nmol zyklischer AMP.

I. A. MACDONALD[17]

Department of Chemistry, University of Ottawa, Ottawa 2 (Ontario, Canada), 2 May 1974.

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[17] Address: Dalhousie Clinical Research Centre, 5849 University Avenue, Halifax (Nova Scotia, Canada).

Surface Area and Effect of Drying Temperature Related to Dry Weight of Jejunal Tissue in Rat

Various workers have used different techniques to estimate the dry weight of jejunal tissue. Often, however, as is the convention in short-circuit work, ion movements are expressed per unit surface area. In the course of other experiments, the effect of drying above or below the boiling point of water was investigated and also the relationship of gut dry weight to wet surface area. Since results in the literature are often expressed per tissue weight incorporating different drying techniques, it was felt that a brief investigation of this and the elucidation of the regression of surface area on estimated dry weight might facilitate comparisons and transformations and be of general interest in transport studies.

Methods. The proximal jejunum was removed from narcotized rats of about 200–250 g body weight and a section cut from sac I[1], 5 cm distal to the duodenal-

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Tissue dry weight as percentage wet weight under various drying conditions

	Dry weight (%)	Significance (p)
a) 120 °C for 4 h (not incubated)	17.45 ± 0.83 (16)	a) against b)
b) 80 °C for 4 h (not incubated)	19.35 ± 0.66 (16)	< 0.001
c) Freeze-dried (not incubated)	18.62 ± 0.22 (4)	c) against d)
d) Freeze-dried (incubated)	15.15 ± 0.40 (4)	< 0.001
e) 80 °C for 24 h (incubated)	17.25 ± 0.40 (12)	e) against f)
f) 80 °C for 48 h (incubated)	15.65 ± 0.41 (12)	< 0.02

Percentage given with standard error of mean and the number of animals in brackets.