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State of Cytidine 3', 5' Cyclic Monophosphate (Cyclic CMP) Research

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extra expenses of multiple seining and sorting common to most polyculture situations. Any remaining trout not captured during the spring harvest would not be totally wasted in catfish production ponds.

Rainbow trout can be successfully and economically reared during the winter season in southern Arkansas. Profits of \$44.00 per 0.9 m³ cage and \$780.00 per hectare are obtainable. Producers should stock trout weighing at least 113 g to obtain marketable size fish in one growing season. Smaller operations should sell their fish on local markets to both obtain higher prices and take advantage of the maximum length of the growing season. Most trout growth is obtained during November and December and again near the latter part of the season during March and April when water temperatures range between 10-16° C.

Table 1. Net production, food conversion efficiency, percent survival, and average weight gained for rainbow trout reared in cages.

Cage	Stocked weight (kg)	Harvested weight (kg)	Weight gain (kg)	FCR	Percent survival	Individual Stocked (kg)	Average Harvested (kg)	Gain (g)
1	18.18	29.55	11.37	1.63	89	122	218	96
2	18.64	29.30	10.66	1.70	87	122	224	102
3	18.45	28.27	9.82	1.69	91	122	207	85
Average	18.41	28.05	10.64	1.75	89	122	216	95

Table 2. Net production, food conversion efficiency, percent survival, and average weight gain for rainbow trout production in Arkansas ponds.

Pond	Stocked weight (kg)	Harvested weight (kg)	Weight gain (kg)	FCR	Percent survival	Individual Stocked (kg)	Average Harvested (kg)	Gain (g)
1	60	113	44	1.93	100	136	227	91
2	88	130	42	1.37	99	136	264	128
Average	88.5	122	53	1.65	99.5	136	245	109

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THE STATE OF CYTIDINE 3', 5' CYCLIC MONOPHOSPHATE (CYCLIC CMP) RESEARCH

The status of cyclic CMP is unclear although the presence of cyclic CMP radioimmunoactive material (CRIRM) has been demonstrated in a variety of biological tissues and fluids. Furthermore, marked changes in cyclic CMP and CRIRM have occurred at certain times after partial hepatectomy during liver regeneration. In addition, CRIRM is known to increase in cell free systems thought to synthesize the nucleotide from cytidine triphosphate. Several cyclic CMP phosphodiesterases have been demonstrated. Argument about the occurrence of cyclic CMP has arisen because although CRIRM co-chromatographs with both unlabeled authentic cyclic CMP and ³H-cyclic CMP on a number of TLC systems, CRIRM has a different R_f from that of ³H-cyclic CMP or unlabeled cyclic CMP on Dowex-1-formate anion exchange chromatography of acid soluble extracts of biological tissue.

The discovery of cyclic AMP and the development of the second messenger concept constituted a revolution in biological thinking (Sutherland, 1972). In contrast, after almost ten years of research in cyclic CMP, it remains uncertain that this nucleotide is a naturally occurring compound or that it has any biological function at all. For example, in 1980 the only publications in this area were two abstracts on cyclic CMP phosphodiesterases (PDE) (Conrad and Bloch, 1980; Helfman et al., 1980), and another communication (Murphy and Stone, 1980) on apparent changes in cyclic CMP concentration during liver regeneration. In 1981, only two communications have appeared so far; (Wikberg and Wingren, 1981) one on non-identity of CRIRM with authentic cyclic CMP and (Scavennec et al., 1981) the other on the occurrence of cyclic CMP in the urine of

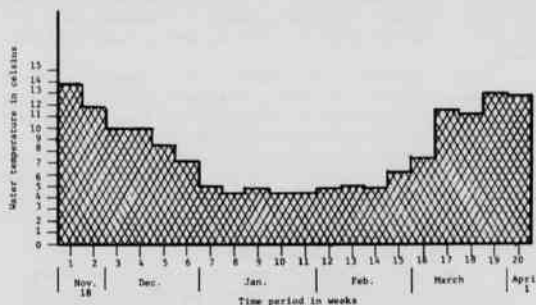


Figure 1. Average weekly water temperature of a 1.6 ha pond at 0.5 meter depth in southeast Arkansas during the winter of 1979-80.

normal and leukemic patients. Thus, it is possible to review the salient points of the literature in this area in a very short paper and, in so doing, explain the reasons for the difficulties.

Cyclic CMP was synthesized in 1961 by Smith et al., along with the cyclic analogs of the monophosphates of adenosine, guanosine and uridine. When Steiner and his group (1972) developed radioimmunoassays for most of the cyclic nucleotides, cyclic CMP was omitted. The reason was that cyclic CMP is insoluble in pyridine, the solvent used in the succinylation reaction which attaches a succinyl moiety at the 2' position of the ribose while succinic anhydride serves as the reagent. The succinyl moiety at position 2 serves several very important functions in the development and function of a radioimmunoassay: 1) It serves as a point of attachment for a large protein molecule which makes the compound antigenic when injected, along with Freund's reagent, into the toe pads of rabbits; 2) It serves as a point of attachment for a methyl tyrosine moiety, which, in turn, is iodinated with ^{125}I . The succinylation with succinic anhydride, surprisingly, could have been carried out in water. The reactions are shown in Figure 1. The highly radioactive ^{125}I methyl tyrosine ester of 2' succinyl cyclic CMP binds to the antibody in the assay and can be displaced by cyclic CMP either as known standards or cyclic CMP present in unknown samples. Figure 2 demonstrates a radioimmunoassay control curve.

Figure 3 shows the cross reactivity of the antibody with other cyclic nucleotides, most of which, with the exception of cyclic AMP, present no problems. Radioimmunoassays of this type were developed almost simultaneously during 1978-79 (Cailla et al., 1978; Murphy and Stone, 1979) and again recently (Wikberg and Wingren, 1981). A commercial assay from Collaborative Research, Waltham, Massachusetts, is also available.

However, it should be emphasized that the founder of this area of research is actually Alexander Bloch of the Roswell Park Memorial Institute, who, from 1970 to the present, has accomplished the following basic research:

- 1) He has demonstrated that cyclic CMP is present in relatively large amounts (nMoles) in mammalian liver, L 1210 cells and that it increases in regenerating liver. Bloch characterized cyclic CMP in a number of ways: Rf values in eight different thin layer chromatographic (TLC) solvent systems, electrophoretic mobility in three different buffers, UV spectra, and mass spectral analysis (Bloch, 1974a).

- 2) He has demonstrated that the addition of cyclic CMP to L 1210 cells, which had previously been synchronized by cooling to 4°C, produced a burst of mitotic activity. He concluded that the cultures had been synchronized at the G₂ phase since the cells could not likely traverse the cell cycle in 15-30 min. Thus, Bloch was the first to associate cyclic CMP with the mitotic phase. He also showed that the addition of cyclic CMP reversed the inhibition of growth produced by cyclic AMP in the L 1210 cultures (Bloch et al., 1974).

Bloch considered that, despite all these data, definitive proof of cyclic CMP as a normal physiological cellular component had to await the demonstration of its enzymatic synthesis in the cell from which it had been isolated. Bloch did not have a radioimmunoassay available but was forced to rely on TLC which is less accurate quantitatively and much more time consuming. Cyclic CMP has also been found in surprisingly high concentrations (0.05-2.0 nM) in bacterial culture media from *Corynebacterium murisepticus* or *microbacterium* species (Ishiyama, 1975). The biosynthesis of a molecule by a cell in which it has been found is important in proving its status as a normal metabolite, because otherwise, one might conclude that caffeine, nicotine or aspirin are normal human metabolites. These compounds can be isolated from many individuals, are metabolized by the body and have many pharmacological effects which might easily be mistaken for physiological functions.

A number of different phosphodiesterases (PDE's) have been described for cyclic CMP. One, which has been purified by Conrad and Bloch (1980), is of relatively high molecular weight and low K_m and is apparently specific for cyclic CMP only. Another PDE, which has been purified by Helfman et al., (1980), hydrolyzes both cyclic AMP and cyclic CMP at different sites and has a molecular weight of only 33,000. Interestingly enough, the cyclic CMP PDE's are not inhibited by the methyl xanthines as are the PDE's of the purine cyclic nucleotides.

Cech and Ignarro (1977 and 1978) using alpha- ^{32}P labeled CTP as substrate with either Mg^{++} or Fe^{++} as cofactors at neutral pH, reported the biosynthesis of cyclic CMP in both mouse and rat liver homogenates, both normal and regenerating, as well as in other rodent tissues. The product was reported as having been characterized with alumina column chromatography, on Dowex-1-formate, PEI cellulose, and recrystallization to constant specific activity in two solvents. However, Krishna (1979) showed that the presumed ^{32}P cyclic CMP had a different Rf from the ^3H cyclic CMP marking pool. We investigated a similar system and found a large number of radioimmunoactive compounds among the products of the reaction. Thus, this area is in complete disarray at the present time. Recently Wikberg et al., (1981) has demonstrated the problem quite clearly (Fig. 4 and 5).

Figure 4 shows work carried out by Wikberg, who has developed a radioimmunoassay quite similar to the others, but which uses ^3H cyclic CMP as the labelled compound to be displaced from the antibody rather than ^{125}I methyl tyrosine derivatives used in the other systems. This figure compares to different chromatographs carried out on identical 0.9×15 cm AG1-X-8-formate columns eluted with 0.2 N formic acid. One column, the solid line, was loaded with perchloric acid soluble fraction from 3-5 grams of rat liver, and an identically loaded column was further charged with 10 pMoles of ^3H cyclic CMP. The solid line represents radioimmunoactivity, and the dotted line, radioactivity. It is evident that these peaks do not coincide. Figure 5 is similar, but the tandem column was loaded with 10 pMoles of unlabeled cyclic CMP. In this figure, the dotted line represents radioimmunoactivity on the tandem column.

The treatment of CRIRM with pronase, a proteolytic enzyme, increases the radioimmunoactive cyclic CMP material by a factor of 4 but does not change its Rf. It should be evident that a radioimmunoassay which does not distinguish between the compound it was designed to quantitate and a different uncharacterized material is of doubtful value.

CRIRM has been noted by others but has been assumed to be a peptide which binds cyclic CMP and which competes with the antibody for the nucleotide. On the other hand, the material may be a peptide which is an artifact of extraction with perchloric acid and which has a sequence of amino acids identical to some portion of the carrier protein molecule, originally used to make the antibody. This is not likely since a variety of proteins have been used as carriers among the radioimmunoassays in use.

Figure 6 (Murphy and Stone, 1980) was produced by measuring the changes in cyclic CMP in regenerating liver after partial hepatectomy. The animals were accompanied by suitable control groups. The perchlorate soluble supernatants were chromatographed upon 3×1 cm Dowex-1-formate columns with 0.1 formic acid. The samples in the peak tubes were further characterized by TLC in which the CRIRM material was co-chromatographed with ^3H cyclic CMP and unlabeled cyclic CMP.

It would be most interesting to ascertain if radioimmunoassayable reactive material similar to that isolated in cyclic radioimmunoassay would be found for cyclic AMP and cyclic GMP in Wikberg type systems. The close approximation of the Rf of Wikberg's CRIRM peak with cyclic CMP itself is a technical problem which must be solved before this area can progress.

It is the opinion of Bloch, and also of the present authors, that cyclic CMP is a rather common cyclic nucleotide, but it is usually bound to some cellular constituents(s), and it may appear in free form for only relatively short periods during some physiological process in a manner analogous to norepinephrine or acetylcholine.

General Notes

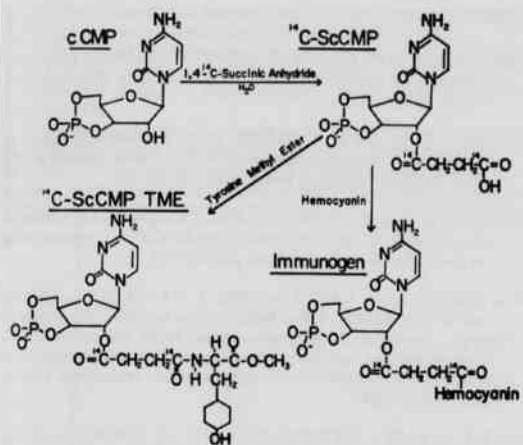


Figure 1. Structures of cyclic CMP derivatives synthesized.

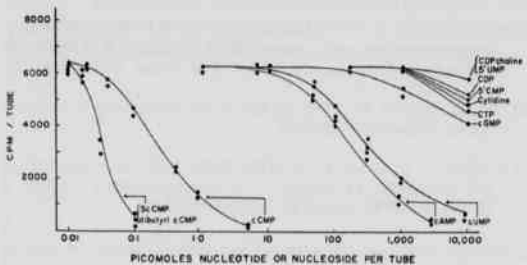


Figure 3. Cross-reactivity of the succinyl cyclic CMP antiserum.

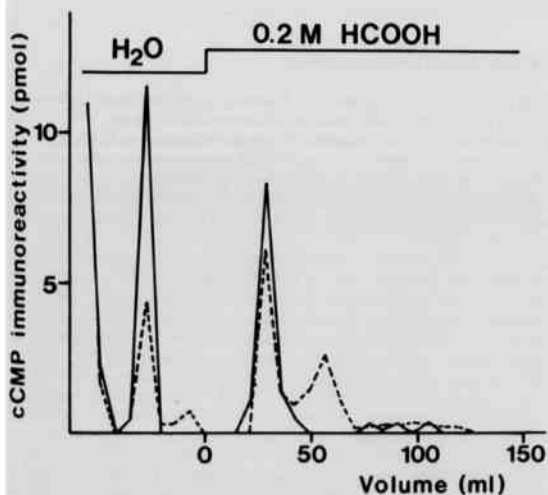


Figure 5. Chromatography of cold perchloric acid extracts from rat liver with unlabelled cyclic CMP added to tandem column. (From Wikberg et al., 1981).

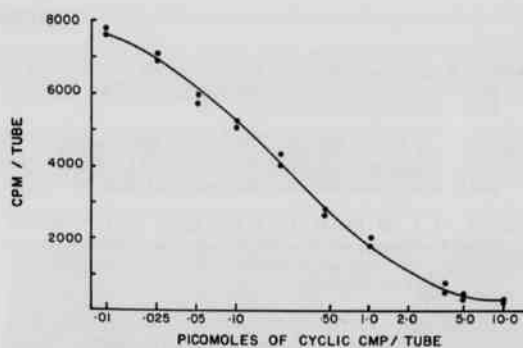


Figure 2. Standard immunoassay curve for cyclic CMP.

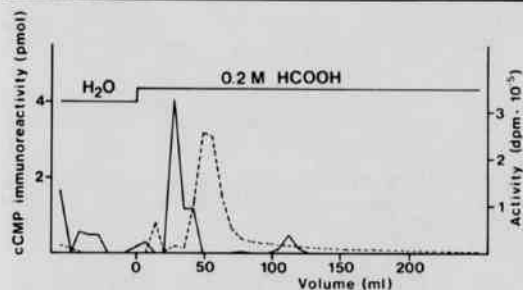


Figure 4. Chromatography of cold perchloric acid extracts of rat liver on large with ³H cyclic CMP marking pool added. AG 1-X8 columns. (From Wikberg et al., 1981).

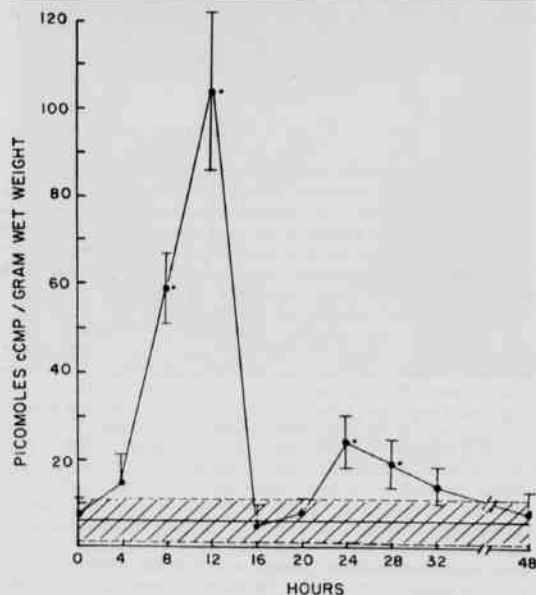


Figure 6. Changes in the cyclic CMP of rat liver following 70% hepatectomy. (From Murphy and Stone, 1980).

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ANOMALIES OF BOBCAT SKULLS (*FELIS RUFUS*) IN ARKANSAS

Examination of 275 bobcat skulls (*Felis rufus*) from Arkansas, preserved in the Collection of Recent Mammals, Arkansas State University Museum of Zoology (ASUMZ), revealed five anomalous forms which ranged from dental irregularity to supernumerary cranial bones.

Sutural anomalies were found in several skulls. The normal junction of the coronal (C) and sagittal (S) sutures is illustrated in Figure 1, # 6926. This bregmatic junction forms after fontanelle ossification of the frontal and parietal bones. In the fetal skull, cartilagenous "soft spots" or fontanelles exist at the future junctional site, and if ossification among the bones is uneven, waved or otherwise malformed sutures may result. Two of the more pronounced sutural anomalies found in Arkansas bobcats are illustrated in Figure 1, #'s 7706 and 6765. Bregmatic bone formation can also cause abnormal junctions if ankylosis obliterates one or more sutures (Pratt, 1942; Manville, 1959). We did not attempt to distinguish between anomalies caused by these factors.

Bregmatic bones (those formed at the anterior fontanelle) occur commonly in the beaver (*Castor*) and porcupine (*Erethizon*) (Schultz, 1923) and result from one or more ossification centers developing in the anterior fontanelle, thereby forming additional bones as the parietals and frontals complete ossification around them. Occurrences of bregmatic bones in bobcats are discussed in the literature (Pratt, 1942; Manville, 1959; Mahan, 1980). Hall and Kelson (1959) (probably unknowingly) depicted a bregmatic bone in their illustration of a bobcat skull. Bregmatic fontanelle bones were found in 41 of 275 (14.9%) Arkansas bobcat skulls examined, and varied in size, shape, and number. Representatives of variations seen in Arkansas skulls are illustrated in Figure 2. The nature of these bones in Arkansas bobcats is similar to reports from other areas: 14.7% in Nebraska (Mahan, 1980); 16.8% in Oregon, 14.6% in Nevada, 15.5% nationally (as represented in the U. S. National Museum) (Manville, 1959). Manville also pointed out deviations from this apparent trend: 37.5% of 32 specimens from West Virginia, 44.0% of nine from Mississippi and 7.0% of 158 from Texas. Pratt (1942) found anomalous bones in 17.5% of his museum study material, and Progulské (1952) found 15 of 72 (20.8%) skulls from Virginia and North Carolina to have anomalous bones. Usually, only a single extra bone occurs; however, in the Arkansas material examined, one skull (Fig. 2, # 7471) exhibited two additional bones (0.4% of sample). Similarly, Mahan (1980) found only one such pair of bones (0.9%) in Nebraska and Pratt (1942) reported two (0.12% of his sample). Furthermore, Pratt reported only one incidence involving three anomalous bones in a sample of 2154 skulls. ASUMZ 7734 (Fig. 2) illustrates an Arkansas specimen exhibiting this trinity of extra bones (0.4% of sample), representing the second documented record. No correlation was found between sex or geographic location and presence of anomalous bones in Arkansas bobcats.

Wormian bones are those which are sutural in origin, as contrasted with bregmatic bones which are fontanellic in origin. It is sometimes