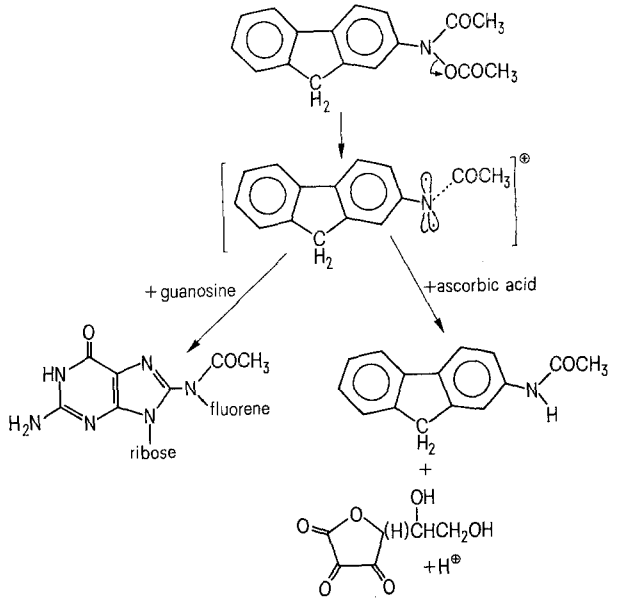
As shown in the Table, the reaction of N-acetoxy-AAF with guanosine in citric acid results in a high yield of guanosine-AAF[4,5], while the same reaction in ascorbic acid results in an 80% inhibition of guanosine-AAF formation. In contrast, the yield of product from N-acetoxy-4-acetamidostilbene and guanosine was relatively unaffected by the medium, and is comparable to that found earlier[5]. As noted earlier by LOTLIKAR[6], the reactions of esters of N-hydroxy-AAF result in small amounts of AAF, explained later as being due to hydrogen abstraction from solvent by the triplet form of the N-2-fluorenyl-N-acetylnitrenium ion (AAF^+)[7]. We observed this in the citric acid medium, but found AAF formation to be increased 5-fold in the ascorbic acid medium. When guanosine-AAF was incubated separately with ascorbic acid, no destruction of this material was seen.

Reactions of N-acetoxy-N-arylacetamides with guanosine, in the presence and absence of ascorbic acid

Compound	Medium	Yield of guanosine adduct (%)	Amide formed (%)
N-acetoxy-AAF	citric	83	6
N-acetoxy-AAF	ascorbic	17	30
N-acetoxy-AAS	citric	12	<1
N-acetoxy-AAS	ascorbic	12	<1



Reduction of N-2-fluorenyl-N-acetyl nitrenium ion by ascorbic acid.

Thus, we have noted that the reaction of N-acetoxy-AAF with guanosine is greatly inhibited by ascorbic acid, but that the reaction of N-acetoxy-AAS is not. From this, we conclude that the ascorbic acid does not act directly on unreacted N-acetoxy-N-arylacetamide. We have already shown, however, that N-acetoxy-AAF forms a triplet species, while N-acetoxy-AAS does not[7]. Thus, it appears that ascorbic acid is oxidized by triplet AAF^+ through abstraction of H from the ascorbic acid (see Figure). Such an oxidation is common for ascorbic acid, and is probably the reaction type by which ascorbic acid removes nitrous acid from a nitrosation mixture.

If this inhibition of adduct between aromatic amine and guanosine is specific for reactions involving triplet nitrenium ions, as it appears to be, the possibility of using ascorbic acid as a prophylactic against aromatic amine-induced tumors would appear to be restricted to those cases in which the nitrenium ion will fall into a triplet state. At present, it is not known how many aromatic amines would lead to such ions, nor is it known whether ascorbic acid inhibits even AAF carcinogenesis. This reaction, nonetheless, seems to be a point of control in carcinogenesis worth additional study.

Zusammenfassung. Die Reaktion des Karzinogens N-acetoxy-2-acetamidofluoren mit Guanosin wird durch Ascorbinsäure, jedoch nicht durch Zitronensäure gehemmt. Diese Hemmung bewirkt eine vermehrte Bildung von 2-Acetamidofluoren. Anscheinend reagiert Ascorbinsäure mit dem N-2-Fluorenyl-N-acetylnitrenium-triplett unter Bildung von Acetamidofluoren und oxidierter Ascorbinsäure.

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Fever Produced in Rabbits by $N^6,O^{2'}$-Dibutyryl Adenosine 3',5'-Cyclic Monophosphate

Previous experiments have shown that the fever reaction in rabbits following the i.v. injection of various exogenous or endogenous pyrogens[1,2] is associated with increased concentrations of prostaglandins of the E series (PGE) in the cerebrospinal fluid (CSF). In addition, injections of PGE into the lateral or third cerebral ventricles of different mammals were found to produce fever[3]. The hypothesis of PGE as mediators in fever genesis is supported also by the finding that inhibition of prostaglandin synthetase is the mechanism underlying the action of antipyretic, aspirin-like drugs[4]. The pharmacological effects of prostaglandins seem to be mediated in several endocrine organs via adenosine 3',5'-cyclic monophosphate (cyclic AMP) according to the 'second messen-

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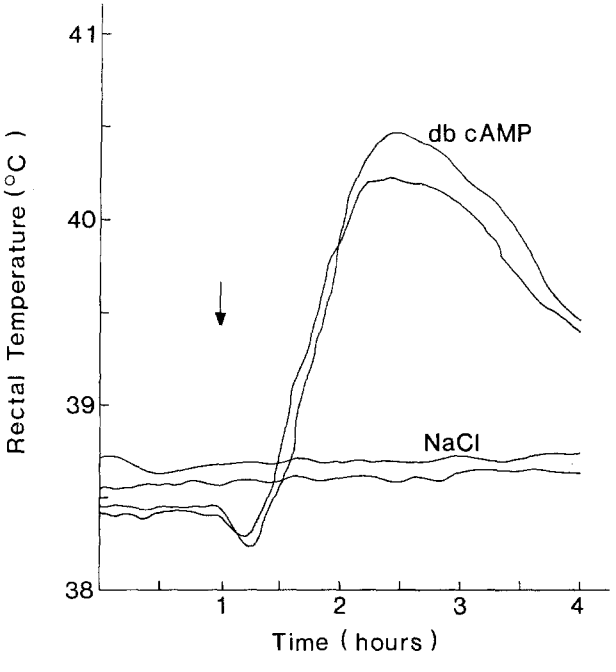
ger hypothesis' of SUTHERLAND et al.[5]. Since we found in CSF of rabbits during fever induced by *E. coli*-endotoxin[6] or Newcastle disease virus[7] 2-fold higher concentrations of cyclic AMP in comparison to normal values, it might be possible, that likewise genesis of fever depends on enhanced cerebral levels of cyclic AMP. In the present study we therefore examined, whether cyclic AMP or its derivate $N^6,O^{2'}$-dibutyryl cyclic AMP (dibutyryl cyclic AMP) have an effect on body temperature of rabbits after intraventricular and i.v. injection.

Cyclic AMP and dibutyryl cyclic AMP (Boehringer, Mannheim) were injected into conscious rabbits of uniform breeding (cross between Widder and Deutscher Riese, 2.5 to 3.0 kg) through the marginal ear veins or through modified Collison cannulas, which had been implanted permanently into the lateral cerebral ventricles as described by HASSELBLATT and SPROULL[8].

The nucleotides were dissolved in pyrogen-free sodium chloride (0.9%) and the solutions were sterilized by membrane filtering. Control animals received equal volumes of the solvent. Rectal temperature was recorded thermoelectrically and monitored continuously[2].

After the i.v. administration of cyclic AMP or dibutyryl cyclic AMP with doses up to 1.5 mg/kg, rectal temperature remained unchanged. This might imply that the nucleotides do not cross the blood-brain barrier. Likewise, injections of cyclic AMP into the lateral ventricle with doses up to 100 µg/kg had no effect upon temperature, indicating a rapid inactivation by phosphodiesterase (3′:5′-cyclic-AMP 5′-nucleotidohydrolase, EC 3.1.4.17), which is abundant in all brain regions except cerebellum[9]. Intraventricular injections of dibutyryl cyclic AMP produced no fever with doses up to 15 µg/kg. However, with 25 or 50 µg/kg, the effect shown in the Figure was observed. A slight decrease of temperature, which lasted for about 20 min was followed by an elevation of temperature of about 2 °C. This reaction was associated with increased ventilation and locomotor activity. 2 h after the injection, temperature began to decrease slowly and the animals returned to normal behavior. Injections with higher doses (75 to 200 µg/kg) of dibutyryl cyclic AMP produced a rapid increase of temperature and cyclically recurring episodes of catatonia, hyperactivity and convulsions. 1 h after the injection temperature was above 41 °C and the animals died after generalized convulsions.



Effect of dibutyryl cyclic AMP (db cAMP, 50 µg/kg, 0.1 ml) injected into the left lateral ventricle on rectal temperature of conscious rabbits ($n = 2$). Control animals received 0.1 ml of 0.9% NaCl ($n = 2$).

The present results suggest a relation of cyclic AMP levels in brain regions adjoining the cerebral ventricle system to fever production. The fact that the effects observed were only evoked by dibutyryl cyclic AMP and were of relative long duration, implies that once inside the cells this derivate is not easily destroyed by phosphodiesterase. The doses needed to obtain any effect at all are considerably above the physiological range. However, this does not necessarily mean that the results obtained have no physiological significance. For instance, peripheral tissues contain the cyclic nucleotide in concentrations ranging from 10^{-6} to 10^{-8} M[10], yet the concentrations of exogenous cyclic AMP or its derivatives used in vitro to mimic the effects of various hormones is often in the order of 10^{-3} M[11].

As prostaglandins of the E series are known to increase levels of cyclic AMP in various cultured cells of the central nervous system[12], the present results favor the concept that the pyrogenic effect of prostaglandins is mediated via increased cyclic AMP levels in brain regions responsible for temperature regulation. It should be mentioned that the intraventricular injections of prostaglandins into cats[13] produced vegetative effects and changes in behavior, some of which were similar to those observed during our study and which might also have been mediated by cyclic AMP[14].

Zusammenfassung. Die Injektion von N^6-2′-*O*-Dibutyryl-adenosin-3′5′-monophosphat in den lateralen Hirnventrikel von Kaninchen führt zu einer langanhaltenden Fieberreaktion, was vermuten lässt, dass zyklisches Adenosin-3′:5′-monophosphat als weiterer Mediator in der Genese des Fiebers fungiert.

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