bonyl compound[10]. Further evidence for the proximity of the non-oxidizable hydroxyl group to the lactone carbonyl was obtained when the IR-spectra of the dihydroxy compound *5*a and the hydroxyketone *6*a were recorded in very dilute carbon tetrachloride solutions. The spectrum of the ketone *6*a showed only the absorption of the intramolecularly bonded hydroxyl (ν_{max} 3573 cm^{-1}), while the spectrum of the alcohol *5*a revealed absorptions for both the free and the intramolecularly bonded hydroxyl (ν_{max} 3620, 3575 cm^{-1})[11].

Finally, it was found that the oxidation product *6*a could be converted to the corresponding acetate *6*b, which exhibited the following physical constants: m.p. 176–178°; $[\alpha]_D^{26}$ − 97° (c, 0.896); ν_{max} 1770, 1750 shoulder, 1738 and 1700 cm^{-1}. In the mass spectrum of *6*b the absorption of the molecular ion at m/e 360 as well as the prominent peaks at *M*-60 and *M*-(60 + 70) were observed[12,13].

Zusammenfassung. Die Bildung von 11β-Carboxy-3α, 11α, 17β-trihydroxy-13α-C-nor-5β-androstan 11a,17-Lakton aus 3α, 17β-Diacetoxy-11-hydroxy-12-oxo-5β-$\Delta^{9(11)}$-androsten wird beschrieben. Der Mechanismus und die Stereochemie dieser Umwandlung (Retro-Aldolkondensation und Benzilsäureumlagerung) wird diskutiert.

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The Effect of Nicotinamide Adenine Dinucleotide on Lipolysis in Adipose Tissue in vitro

Lipolysis in adipose tissue in vitro has been shown to be stimulated by a variety of sympathomimetic amines and peptide hormones[1,2]. There is evidence that these effects are mediated by the activation of adenyl cyclase, production of cyclic-3′,5′-AMP[3], activation of an adipose tissue lipase[4,5], hydrolytic cleavage of adipose tissue triglycerides and subsequent release of fatty acids and glycerol. Inhibition of lipolysis induced by the sympathomimetic amines or peptide hormones has been demonstrated with a number of compounds, including nicotinic acid[6] and various metabolites of nucleic acids[7]. Nicotinic acid has been demonstrated to be a potent lipolysis inhibitor in vitro and this effect can be observed in vivo in the form of a pronounced depression of plasma non-esterified fatty acids (NEFA)[8]. DOLE[7] has reported that ATP, 5′-AMP and adenosine inhibited lipolysis, whereas the purines, adenine and guanine, and the pyrimidines, uracil and cytosine, increased lipolysis. More recently, MATSUZAKI and RABEN[9], differing from DOLE, found that guanine inhibited the lipolytic action of epinephrine. They also reported that adenine stimulated lipolysis at high concentrations but had no significant effect at lower levels.

Nicotinic acid has been shown to be an effective precursor for nicotinamide adenine dinucleotide (NAD) synthesis[10]. The possibility exists that adenosine might also contribute to NAD synthesis. In view of the fact that both nicotinic acid and adenosine have been shown to inhibit lipolysis in vitro, it was of interest to investigate the antilipolytic effects of NAD and related compounds.

In order to define the lipolysis inhibitory effects of NAD and related compounds, epididymal adipose tissue was taken from male Sprague-Dawley rats, 180–240 g, fed ad libitum. The tissue was placed in freshly aerated Krebs-Ringer bicarbonate buffer, pH 7.4, and minced with scissors into pieces weighing approximately 10 mg. 200 ± 3 (mean ± standard deviation) mg of tissue were placed in each experimental flask containing 3 ml of freshly aerated (95% O_2-5% CO_2) Krebs-Ringer bicarbonate buffer, pH 7.4. Bovine plasma albumin, fraction IV, 1%, was used as a fatty acid acceptor in this incubation medium. Sufficient norepinephrine (20–30 ng/ml) to cause 50% of maximum fatty acid release was added to the incubation mixture. The compounds under investigation were then added at appropriate concentrations. The experimental flasks were stoppered, aerated with 95% O_2-5% CO_2 for 10 min and incubated at 37 °C for 3 h on a Dubnoff metabolic shaker. After incubation, aliquots were removed and analyzed for fatty acid content by the method of DOLE[11]. The fatty acids released into the medium by norepinephrine were compared with the release of fatty acids in flasks containing norepinephrine and the test compound. The effect of the inhibitor was expressed in terms of % inhibition.

Under the conditions of the study, NAD was shown to be a potent inhibitor of the norepinephrine-induced fatty acid release from adipose tissue (Table I). This inhibition was proportional to concentration over a range of 10^{-4} to 10^{-7} *M*. NAD is composed of nicotinamide mononucleotide (NMN) and 5′-AMP joined through a pyrophosphate bridge. NMN itself was a comparatively weak inhibitor of fatty acid release as was nicotinamide. Nicotinic acid, on the other hand, was a very potent inhibitor. The

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Table I. Inhibitory effects of various NAD-related compounds on the norepinephrine-induced release of fatty acids from rat adipose tissue in vitro

% inhibition of norepinephrine-induced release of fatty acids in vitro.

Molar concentrations	Nicotinamide adenine dinucleotide	Nicotinamide mono-nucleotide	Nicotinamide	Nicotinic acid	5'-AMP	Adenosine	Adenine
10^{-4}	96 ± 5.6[a]	40 ± 4.3	27 ± 3.9	68 ± 3.3	97 ± 6.1	96 ± 7.2	25 ± 4.4
10^{-5}	75 ± 6.1	4 ± 2.2	12 ± 3.1	68 ± 4.9	76 ± 5.7	88 ± 5.8	27 ± 4.8
10^{-6}	25 ± 3.2	0	0	66 ± 5.0	39 ± 4.6	44 ± 6.3	2 ± 1.7
10^{-7}	19 ± 2.8	0	0	28 ± 4.1	2 ± 1.4	19 ± 4.4	0

[a] Mean ± standard deviation; $n = 5$.

relative inactivity of NMN and nicotinamide suggests that neither compound is effectively converted to nicotinic acid by adipose tissue in vitro.

5'-AMP, the other half of the dinucleotide, was a very potent inhibitor of the lipolytic effects of norepinephrine. The inhibitory potency of 5'-AMP appeared to be identical with that of NAD. Adenosine, the corresponding nucleoside, also exhibited inhibitory properties similar to those of NAD. The results obtained with 5'-AMP and adenosine are in agreement with those reported by DOLE[7]. On the other hand, the results obtained with adenine, indicating that it is a weak lipolysis inhibitor, appear to differ from those reported by DOLE[7] and MATSUZAKI and RABEN[9]. They found that adenine caused an increased lipolytic effect in vitro. It should be borne in mind, however, that the concentrations of adenine used in those studies (300–500 µg/ml[9] or 1000 µg/ml[7]) were very much greater than those used in the present work (13.5 µg/ml).

The findings reported here indicate that NAD is a potent lipolysis inhibitor in vitro. In addition, a number of NAD hydrolysis products are as potent as NAD in antilipolytic activity. The similarity of the inhibitory effects of NAD, 5'-AMP, and adenosine and the relative inactivity of NMN and nicotinamide, suggest that the antagonistic action of NAD is dependent upon the presence of the adenosine rather than the nicotinic acid moiety. In addition, the results presented in Table II indicate that ADP, ATP, and NADP are also as potent as NAD, suggesting a similar dependence upon the presence of the adenosine moiety.

KAPLAN et al.[12] have demonstrated that both nicotinic acid and nicotinamide can be incorporated into mouse liver NAD. However, nicotinamide appears to be approximately five times as effective as nicotinic acid in elevating liver NAD. If the effectiveness of nicotinic acid as a lipolysis inhibitor were dependent upon its incorporation into NAD, it would be expected to be a less potent inhibitor than nicotinamide. The relative lack of antilipolytic potency of nicotinamide, in spite of its superior ability to elevate tissue NAD levels, argues against the mechanism of action requiring increased NAD synthesis. Such an argument is based upon the incorporation of these compounds into *liver* NAD but it must be borne in mind that nicotinic acid may be the more effective precursor in adipose tissue, the site of its inhibitory action.

On the basis of these data, it is not possible to distinguish between a direct action of NAD and an action dependent upon the production of an antilipolytic metabolite of NAD, e.g. adenosine. WINBURY et al.[13] demonstrated increasing coronary dilator activity in the series: adenosine, AMP, ADP, and ATP. They suggested that the adenosine moiety, but not degradation of the nucleotides to adenosine, is required for coronary dilator activity. On the other hand, ANGELAKOS and GLASSMAN[14] found that adenosine, AMP, ADP, and ATP were equipotent in decreasing heart rate, myocardial tension, and blood pressure in atropinized dogs. They concluded that adenosine, rather than the intact nucleotides, was the effective agent under in vivo conditions.

The results reported here do not establish a physiological role for adenosine (and phosphates). However, the well-known modulating effects of nucleotides on metabolism leaves open the possibility that these compounds may play a significant part in controlling the rate of intracellular lipolysis.

Table II. Inhibitory effects of ADP, ATP and NADP on the norepinephrine-induced release of fatty acids from adipose tissue in vitro

Molar concentration	% inhibition of norepinephrine-induced release of fatty acids in vitro		
	ATP	ADP	NADP
10^{-4}	93 ± 6.2[a]	96 ± 5.6	87 ± 6.4
10^{-5}	77 ± 5.7	63 ± 7.1	72 ± 4.8
10^{-6}	36 ± 4.3	29 ± 4.2	44 ± 5.3
10^{-7}	22 ± 3.7	16 ± 3.1	18 ± 4.7

[a] Mean ± standard deviation.

Zusammenfassung. Es wird gezeigt, dass Nikotinamid Adenin Dinukleotid (NAD) eine starke Hemmung auf die Lipolyse der Fettgewebe in vitro ausübt. Untersuchung der antilipolytischen Wirkung verschiedener Bestandteile des NAD ergab, dass für die Hemmungswirkung die Gegenwart des Adenosins, nicht aber die der Nikotinsäure notwendig ist.

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