Die Versuchsergebnisse (siehe Tabelle) ergeben: (1) MDA wird intensiv metabolisiert. (2) Beim Vergleich mit Antipyrin ist die MDA-Ausscheidung im Harn praktisch gleich, während MDA jedoch rasch aus dem Serum schwindet. (3) In den Organen werden unterschiedliche Konzentrationen gefunden, was durch verschieden schnelle Oxydation des MDA erklärt werden kann. (Auch Variationen in der Schnelligkeit des Verschwindens der Substanz aus den einzelnen Organen wurden gefunden.) (4) Eine niedrige Konzentration in den Testes fällt auf.

Kinetik des MDA in verschiedenen Organen, im Serum und Harn

	γ MDA in 1 g bzw. 1 ml in x min nach Verabreichung[a]		
	60	120	180
Leber	3,65[d]	3,29[e]	2,82[e] (20,4)
Herz	6,34[c]	6,16	3,97[d] (18,8)
Nieren	15,16[d]	11,23	7,08[d] (20,0)
Testes	1,06	0,77	0,52 (20,7)
Fett	4,42	4,51	3,63[e]
Serum	6,78[c] (26,0)	1,26[c] (26,0)	0,61[e] (26,0)

	MDA in x min ausgeschieden			
Harn	60	120	180	420
γ	66,61	132,89	169,17	243,68
%[b]	1,1	2,2	2,8 (2,0)	4,0

[a] Die Angaben in Klammern bedeuten die Konzentration bzw. Menge des Antipyrins. [b] 100% = verabreichte Menge des MDA bzw. Antipyrins. Statistische Auswertung: in Spalte 60 min sind Werte von 60 und 180 min verglichen; in Spalte 120 min sind Werte von 60 und 120 min verglichen; in Spalte 180 min sind Werte von 120 und 180 min verglichen. [c] $P \lesssim 0{,}001$. [d] $P < 0{,}01$. [e] $P \lesssim 0{,}05$.

Die Konzentration in den Organen, im Serum und Harn ist vor der Verabreichung von MDA Null. Trotzdem wir minimale Mengen von MDA bestimmen können (bis 10^{-5} mg in 1 ml) ist es uns unter anderen Versuchs-, bedingungen, z.B. bei Leberschädigung, niemals gelungen, diesen Metaboliten festzustellen. Somit zeigt sich, dass das eventuell im Organismus entstandene MDA sehr schnell ausgenützt wird. Daraus folgt, dass die Auswertung der Lipoperoxydation, mittels der Konzentration des MDA im Organismus gemessen, durch den intensiven MDA-Stoffwechsel beeinflusst sein kann.

Bei der verwendeten Menge an MDA konnten keine toxischen Reaktionen beobachtet werden.

Summary. We followed the kinetics of malonyldialdehyde in vivo and we found that it is very quickly metabolized. This may result in inaccuracy in the estimation of lipoperoxidation in the organism (reaction with 2-thiobarbituric acid).

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Polypeptides and Leuchs' Anhydrides from Carbamic Acid Derivatives of C-Activated Amino Acids

We wish to report a new reaction of carbamic acid derivatives of amino acids, namely the formation of polypeptides and Leuchs' anhydrides. This reaction may have significance in being related to biological processes[1].

It was reported previously from this laboratory that the catalytic hydrogenation product of N-carbobenzoxy-L-glutamic-1,5-anhydride rearranged to Leuchs' anhydride[2]. In this communication evidence is presented that this is a general reaction of carbamic acid derivatives of C-activated amino acids as indicated by formulae I → II → III.

C-activated N-carbobenzoxy amino acids (I) gave, on catalytic hydrogenation via the intermediate carbamic acid derivative II, the corresponding Leuchs' anhydrides III, which in certain cases polymerized immediately to polypeptides. The reaction can be followed by IR-spectro-

[1] Leuchs' anhydride was suggested to be an intermediate in the urea cycle by E. M. KOSOWER (*Molecular Biochemistry*, McGraw-Hill Book Co. Inc., New York 1962, p. 34); biotin gives a carbamic acid derivative in the 'activation' of carbon dioxide (S. H. G. ALLEN, R. STJERNHOLM, and H. G. WOOD, J. biol. Chem. *238*, PC2889 (1963)).

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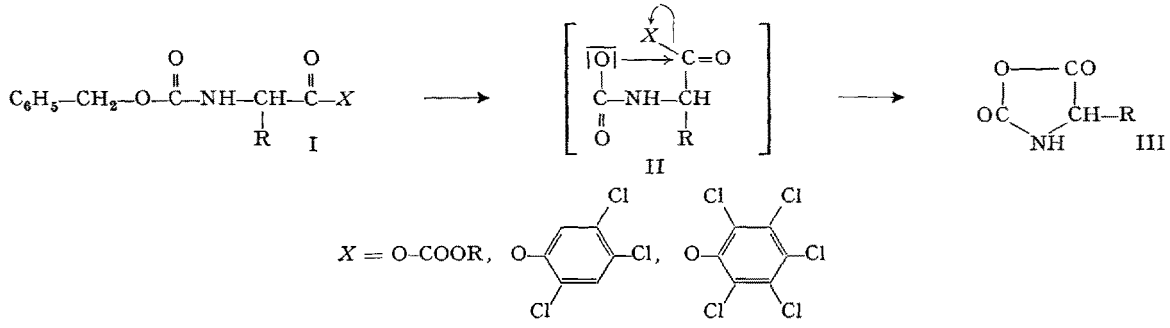

scopy; e.g. peaks of the open-chain mixed anhydrides around 5.47 and 5.67 μ disappeared during the reduction, and peaks of the Leuchs' anhydrides appeared around 5.38 and 5.58 μ[3]. In a typical example the mixed anhydride of N-carbobenzoxy-DL-phenylalanine prepared with ethylchlorocarbonate in the usual way[4] in dioxane solution was hydrogenated in the presence of 10% palladium on charcoal catalyst. After evaporation of the solvent, the Leuchs' anhydride of phenylalanine, which was identical with an authentic sample, was isolated in 60% yield. When L-phenylalanine was used in this reaction the Leuchs' anhydride polymerized immediately to poly-L-phenylalanine, however, its presence was always indicated by IR-spectroscopy. This is in agreement with earlier observations, that DL-Leuchs' anhydrides polymerize much slower than the L- or D-forms[5]. The catalytic hydrogenation of the active esters of carbobenzoxy-DL-phenylalanine, such as the pentachlorophenyl ester[6], also gave poly-DL-phenylalanine through the Leuchs' anhydride which was detected by peaks at 5.38 and 5.58 μ in the IR-spectrum[7].

Zusammenfassung. Durch katalytische Hydrierung von C-aktivierten Carbobenzoxyaminosäurederivaten, z.B. von gemischten Anhydriden und aktivierten Estern, werden via die Carbaminsäurederivate die entsprechenden Leuchsschen Anhydride und Polypeptide erhalten.

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Investigation of Smooth-Muscle Extracts by Means of Immunodiffusion

It has recently been demonstrated[1–4] that there is a difference between striated and smooth muscles as regards the interproportion and composition of proteins extracted with concentrated salt solution. Present experiments were designed to register structural discrepancies between the proteins of the different muscles by means of their specific antigenic character.

The musculature of the urinary bladders and intestines of 5 dogs, freed from connective tissue and mucosa, was homogenized for 2 min with a 0.154 M solution of KCl, and left standing in a solvent of fivefold volume for 20 h. The homogenate was then centrifuged, and its supernate used as *myogen* extract. We washed the sediment with a 0.154 M KCl solution eight times and extracted it afterwards with a Weber-Edsall solution of sixfold volume during 17 h. The supernatant fluid served as *structure-protein* extract. All the chemical procedures were performed at +4°C. The extracts had a final K-concentration of 0.5 M. They were freeze-dried and stored in ampoules. Striated muscles of the dogs (adductor femoris and rectus abdominis) and – as controls – pooled plasma and mixed fragments of renal and splenic tissue, were subjected to a similar procedure.

Groups of 4 rabbits each, were immunized with the extracts. Using Ouchterlony's double gel-diffusion method, a 1% agar gel was dissolved in a phosphate buffer of pH 7.6. The K-concentration of the agar was 0.5 M.

The well, shown in the centre of Figure 1, was filled with the serum of a rabbit immunized with smooth-muscle (SM) myogen extract. This serum had previously been absorbed by mixed-tissue myogen extract. Two bands of precipitation appeared near the SM myogen extract indicating the presence of specific antigenic components.

The metabolism of SM is known to be different from that of striated ones. It remained, however, doubtful whether a quantitative difference of the sarcoplasmic enzymes may manifest itself through such a striking discrepancy of antigenicity. JAISLE[5] succeeded in extracting with water nearly all contractile proteins from human uterine tissue. We studied this problem in another series of experiments. The sera of rabbits immunized with SM myogen extract were absorbed by a Weber-Edsall extract of SM. This done, one of the above-described precipitation bands disappeared, and the other either disappeared also or became faint; it follows that the two antigenic components, observed in the myogen extract of the SM, may partly be identical with one or more SM structure proteins (StrProt) which dissolve even at such a low ionic strength.

It can be seen in Figure 2 that the rabbit serum produced against the StrProt extract of SM induced a multiple precipitation of the SM StrProt-s and the SM myogen extract but failed to precipitate the striated muscle structure extract. This is an additional proof that one or more of the proteins contained in the SM extract of low ionic strength are identical with those found in the extract of higher ionic strength. Such proteins occur in other tissues as well (mixed-tissue control). It is, at the same time, evident that there are no such antigenic components in the StrProt extract of striated muscles.

The central well in Figure 3 contained the serum of a rabbit immunized with SM StrProt extract that had previously been absorbed by a SM myogen extract. The band of precipitation between the central well and the SM structure extract indicates the presence of a specific antigen.

NEEDHAM et al.[4] encountered in a uterine extract of 0.5 M KCl-concentration, in addition to actomyosin and myosin, other StrProt-s and also readily soluble collagen.

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