relatively small amount of tridecanoate necessitated rechromatographing on the column.

Suitable fractions were pooled and the esters recovered. The crude materials were subjected to alembic distillations and saponified. Straight-chain odd-numbered acids were still contaminated with branched-chain acids. The straight and branched components superimpose in paper chromatography at room temperature but can be separated by developing the chromatograms at low temperature[2]. By this procedure, we found that *n*-C_{19}, C_{17}, C_{15}, and C_{13} acids occur in menhaden oil to the extent of at least 0·09, 0·65, 0·6, and 0·05% respectively.

The acids were recrystallized two or three times from acetone, and then identified by melting points, mixed melting points, and the long spacings of their crystals. Gas-liquid chromatography showed their purity to be better than 99%. All data confirmed the identity as the normal nona-, hepta-, penta-, and tridecanoic acids.

It has been found previously that C_{16} chain length represents the greatest share of the even-numbered acids in menhaden oil[2]. It is noteworthy that the maximum amount of odd-numbered acids is found with C_{15} and C_{17}. This is in accord with the concept that the latter arise from propionic acid entering into the early phase of chain formation, which then proceeds as with the even-numbered acids[6,7]. The relative amounts of other odd acids, however, are lower than those of the adjacent even acids.

Acknowledgment. The authors wish to thank Dr. L. P. WITNAUER for X-ray investigation and Dr. R. T. HOLMAN for gas-liquid chromatography of the samples.

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The Hormel Institute, University of Minnesota, April 23, 1959.

Zusammenfassung

Das Vorkommen von *n*-Nona-, *n*-Hepta-, *n*-Penta- und *n*-Tridekansäure in Menhaden-Öl wird papierchromatographisch bewiesen. Die Säuren werden nach Hydrierung oder Oxydation geeigneter Fraktionen mit Hilfe der Säulenchromatographie isoliert, eine Trennung, die auf der Verteilung der Methylester zwischen Silikon-Öl und wässerigem Acetonitril beruht. Es werden die Anteile der gerad- und ungeradzahligen Säuren des Menhaden-Öles verglichen.

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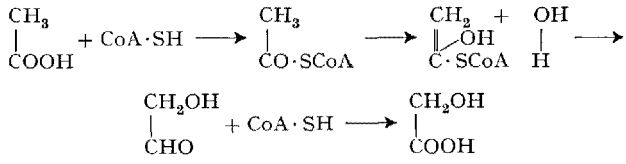
Probable Mechanism of the Oxidation of Acetate to Glycolate by the Way of the Glycolaldehyde

The possible oxidation of acetate to glycolate has been shown by CHALLENGER *et al.*[1] and by BERNHAUER and SCHEUER[2], who have obtained glycolate as one of the products of the action of *Asp. niger* on acetate. Moreover, WEINHOUSE[3] using the same mold, allowed it to metabolize labeled acetate in the presence of unlabeled glycolate has found that appreciable radioactivity was incorporated in the isolated glycolate. The same result has been obtained by us in similar experiments with yeast cells[4]. For the interest in the reaction that leads from acetate to glycolate for our monocarboxylic acid system (MAS) of respiration of acetate[4], we have studied the possible mechanism by which the reaction may occur. As a working hypothesis, we have considered that acetate is activated by the acetate-activating enzyme and that the resulting acetyl-coenzyme A may exist in solution as an equilibrium of the two tautomeric forms[5–8]. By hydration of the enolic form, glycolaldehyde is formed and it is subsequently oxidized to glycolic acid:



If our formulation were true, glycolaldehyde, in the form of its osazone, should have been trapped in experiments of the oxidation of acetate, in the presence of excess phenylhydrazine as a trapping agent. In effect, from these experiments, which were resumed in the present note, glyoxal was isolated in appreciable amounts. The result seems to demonstrate that glycolaldehyde is an intermediate of the oxidation of acetate to glycolate and is therefore also an intermediate of the MAS[4].

Another proof that supports this conclusion, is the fact that from the oxidation of glycolaldehyde by the yeast cells, in presence of phenylhydrazine, the intermediates of the MAS have been obtained, i.e. glyoxylate, formaldehyde, and the formyl group. The doubt that glycolaldehyde might arise from other intermediates of the MAS (glycolate or glyoxylate) is to be excluded, since in submitting these substrates to the oxidation by yeast cells, no glycolaldehyde (glyoxal osazone) could be isolated.

Details for the procedures employed for the experiments with acetate and glycolate[9] or glyoxylate[10] have been described in previous papers. In some experiments the yeast cells were starved by aeration in the presence of 2,4-dinitrophenol before use. The oxidation of glycolaldehyde (Fluka and California Corporation) in presence of phenylhydrazine occurred with the same procedure as was used for glyoxylate[10]. Upon incubation for 8–10 h the yeast cell suspension was centrifuged and in the clear liquid glycolaldehyde was isolated according to the following method. The liquid was concentrated to 3:1 under vacuum, made alkaline to pH 9 and continuously extracted with ether for 12 h. After evaporation of the ether, the extract was dissolved in 25 ml of ethyl alcohol and the alcoholic solution was poured with stirring into 150–200 ml of hot 1% 2,4-dinitrophenylhydrazine (2,4-DP) in 5 *N* H_2SO_4. After boiling under reflux for 30 min all the trap-

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ped phenylhydrazones or osazones were transformed in the corresponding 2,4-DP derivatives. The mixture was allowed to stand for some hours, then filtered, washed, and dried. The material was treated with boiling benzene, filtered, dried, and dissolved in 4 ml of nitrobenzene. To the solution 20 ml of a mixture of benzene, containing 25% of benzine (90–100°C), were added. After filtration, the solution was chromatographed in a Brockmann's alumina column. Development of the chromatogram was carried out with a mixture containing 67·5% of benzene, 22·5% of benzine, and 10% of nitrobenzene. Some red products remained on top of the column, the glyoxal 2,4-DP-osazone in the central portion, and methylglyoxal 2,4-DP osazone in the lower portion. The methylglyoxal arose from the triose phosphates of the endogenous respiration[11]. It was always present in all experiments in which the yeast cells used were not treated with 2,4-dinitrophenol. The glyoxal osazone was eluted with acetone and after partial evaporation of the solvent, it was precipitated with ethyl alcohol. The product melted at 325°C and no depression was observed on admixture with synthetic glyoxal-2,4-DP osazone. Amounts found, mg 20–40/l. Failure to trap larger amounts of glycolaldehyde depended on the fact that it is oxidizable also in the presence of phenylhydrazine, as mentioned above (see also[10]).

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Riassunto

Dall'ossidazione dell'acetato con cellule intatte di lievito, in presenza di un eccesso di fenilidrazina, è stato isolato l'osazone del gliossale, che rappresenta probabilmente l'aldeide glicolica. Si ritiene perciò che l'aldeide sia un intermedio dell'ossidazione dell'acetato a glicolato secondo la formulazione riportata nel testo.

Catecholamines and Histamine in Vascular Tissue of Normal and Depancreatised Dogs

Since the observations of von Euler[1], the presence of noradrenaline was demonstrated in different tissues, thus also in the walls of the blood vessels[2]. Special significance was attributed to this substance in the arteries by Burn and Rand[3], who have found that an artery deprived of its noradrenaline content by pretreating the animal with reserpine does not react in the usual way to vasoconstricting stimuli. Raab[4] supposes that the adrenaline content of the arteries is in some way connected with atherogenesis.

It seemed of interest to study the adrenaline and noradrenaline concentration in the arteries of normal and diabetic dogs, since the occurrence of late and even early vascular changes in human diabetes mellitus is well known[5].

The dogs were bled in morphine-chloralose narcosis through the carotid artery and segments of different arteries were excised. After washing with saline, drying on filter paper and weighing, the tissues were ground with quartz-sand and trichloracetic acid containing ascorbic acid. The suspension was centrifuged and the catecholamines were chromatographed on aluminium oxid. After washing, the catecholamines were eluted with acetic acid and the Euler-Floding reaction was performed. The reaction is specific for adrenaline at pH 3·45, while both catecholamines give the reaction at pH 5·45 as described previously[6]. The results are expressed in μg/g wet weight.

The results in normal dogs are summarised in Table I. With the exception of the femoral artery, the sum of the two catecholamines is 1·0–1·5 μg/g, adrenaline representing only 5–6% of the total.

Totally depancreatised dogs were maintained for 27–55 days on insulin. The treatment was abandoned three days prior to the experiment. The results are given in Table II. It is evident that, with the exception of the femoral artery, the adrenaline concentration increased significantly compared with the vessels of normal dogs ($p < 0{\cdot}05$). The noradrenaline concentration showed a decrease, but according to statistical analysis this decrease did not prove significant ($p > 0{\cdot}05$). The sum of the concentration of the two catecholamines showed a small decrease too. The most significant finding is the change in the percentage of the adrenaline fraction which proved to be 10–35% in

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Table I

	Number of dogs	Adrenaline (A)	Noradrenaline (NA)	Sum of A+NA	Percentage of A
Femoral artery	10	0·039 ± 0·005	0·470 ± 0·072	0·509 ± 0·077	7·6
Carotid artery	12	0·056 ± 0·006	1·112 ± 0·129	1·168 ± 0·135	4·8
Renal artery	11	0·073 ± 0·007	1·445 ± 0·221	1·518 ± 0·228	4·8
Coeliac artery	7	0·075 ± 0·011	1·440 ± 0·284	1·515 ± 0·295	4·9
Abdominal aorta	9	0·093 ± 0·015	1·368 ± 0·305	1·461 ± 0·320	6·3

Mean adrenaline and noradrenaline concentrations expressed in μg/g tissue with standard deviations, in normal dogs.