téines varie entre 18 et 55 mg/ml, celle du sérum entre 46 et 62 mg/ml.

Tableau II

Comparaison de la teneur en acide sialique et en haptoglobine du sérum et de l'exsudat chez le cobaye traité à la carrageenine. Les chiffres représentent la moyenne et l'erreur standard de la moyenne

	Indice d'hapto-globine	mg d'hapto-globine p. 100 mg protéines totales	mg acide sialique p. 100 ml	mg acide sialique p. 100 mg protéines totales
Sérum	0,99±0,13	2,05±0,32	105 ±3,5	2,0 ±0,11
Exsudat . . .	0,78±0,04	2,42±0,43	73,4±4,8	2,23±0,33

En comparant le diagramme d'électrophorèse sur papier (tampon véronal pH 8,6) de l'exsudat et du sérum du même animal, on constate le plus souvent une diminution très nette de l'intensité de la tache des β- et γ-globulines dans l'exsudat (Figure). La région des α-globulines est difficile à comparer car les exsudats sont le plus souvent hémolysées. On observe alors une bande forte correspondant au complexe Hb-Hp.

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avec la collaboration technique de
Mlle MONIQUE DE MONCUIT

Service de Biochimie Médicale, Faculté de Médecine, Paris, le 11 juin 1959.

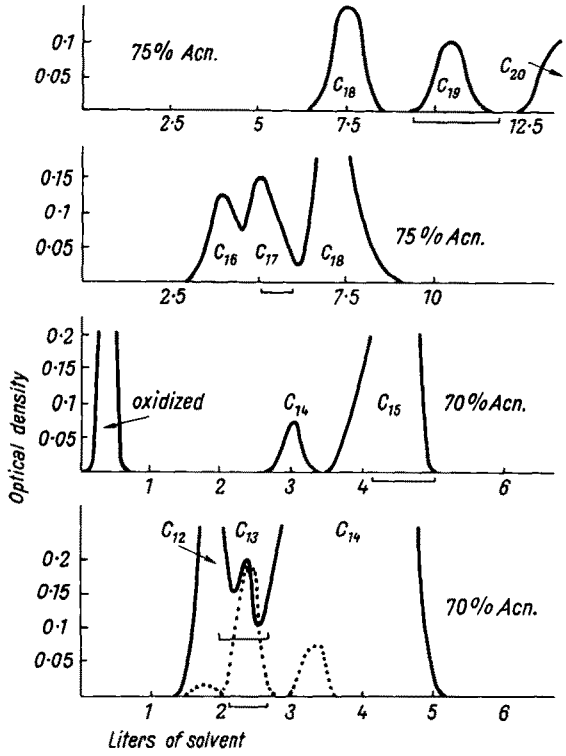
Summary

We studied serum glycoproteins in rats and guinea pigs during the development of granulomas, provoked by carrageenin injection. In rats total serum sialic acid was raised as well as sialic acid soluble in sulfosalicylic acid and haptoglobin. The rise of these 2 fractions, seromucoid and haptoglobine accounts quantitatively for the rise of the total sialic acid. In guinea pigs total serum sialic acid was unchanged, but sulfosalicylosoluble sialic acid as well as haptoglobin were much higher than basal values. Quantitatively the serum glycoprotein reaction was much higher in rats than in guinea pigs.

Column Chromatography of Lipides: Odd-Numbered Straight-Chain Fatty Acids of Menhaden Oil[1]

The occurrence of *n*-nona-, *n*-hepta-, and *n*-pentadecanoic acids in menhaden oil fatty acids had been indicated by microanalytical methods but positive identification was lacking[2,3]. The isolation of these acids and of *n*-tridecanoic acid from menhaden oil is reported here. The essential step after an initial distillation is partition in a reversed-phase column. A novel procedure was developed which enlarges to a preparative scale, the paper chromatographic methods described earlier for the separation of lipides[2,4].

Menhaden fatty acid methyl esters (637 g) were distilled and the fractions analyzed by paper chromatography (acetic and peracetic acid-silicone systems at 30°C and 2°C) or by column chromatography (similar to that described below). Aliquots of the fractions containing saturated normal C_{19}, C_{17} and C_{13} esters were hydrogenated before separation on the column. The fraction rich in C_{15} ester contained *n*-pentadecanoate as the only straight-chain ester of this chain length, together with some myristate and a considerable amount of unsaturated C_{16} esters. Therefore an aliquot of this fraction was oxidized with peracetic acid to facilitate the isolation of the saturated components[4,5].



The separation of methyl esters of saturated fatty acids in a column of silicone on Celite and aqueous acetonitrile (Acn.)

A column, 2·6 cm in diameter and 90 cm in length, was packed with 280 g of a mixture (1:1) of silicone (Dow Corning 200,10 c. s.) and Celite (Johns-Manville). The mobile phase was 70 or 75 % aqueous acetonitrile. The separations represented in the Figure were performed at 25°C with one packing; sample sizes were, in the order of decreasing chain lengths, 0·6, 0·85, 1·4, and 3·9 g of esters. A few milliliters of effluent fractions were diluted with equal volumes of water and the presence of esters was determined under standardized conditions in a spectrophotometer (extinction turbidimetry). A plot of optical density against volume of effluent gave the curves shown in the Figure. Esters located in this manner were chromatographed on paper for tentative identification. The

[1] This work has been supported by a research grant from the National Institutes of Health (USPHS RG 4226) and by the Hormel Foundation.

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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.

JOANNE L. GELLERMAN and H. SCHLENK

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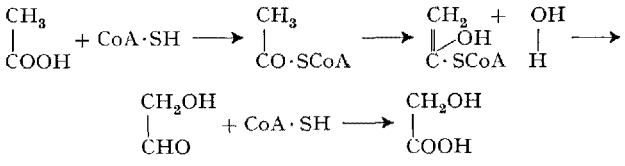
Probable Mechanism of the Oxidation of Acetate to Glycolate by the Way of the Glycolaldehyde

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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