sion of cow red cell lysate caused a decreased GFR and ERPF beyond the period of active infusion and was accompanied by tachycardia, tachypnea and a bleeding tendency. These signs and symptoms are quite similar to those seen in sensitized transfusion reactions in dogs or the reaction of dogs to massive transfusion of heterologous (human) whole blood(6).

All dogs which received the heterologous red cell lysates intraportally showed a significant fall in GFR and ERPF. In almost one half of these experiments a moderate decrease of peripheral arterial blood pressure was noted. The uniform fall in GFR and ERPF in these experiments is probably not due to the moderate vasodepressor effect since mean blood pressure remained above the critical level of 50-60 mm Hg below which the GFR and ERPF will start to fall(7). The interesting observations noted with the peripheral infusion of cows' lysed red cells did not occur during the portal infusion of this solution.

It is apparent that the 'protection' afforded the kidneys by passage of homologous lysed red cells through the liver is not present when the heterologous lysed cells are similarly infused. It would appear, therefore, that the 'protection' is not simply a mechanical slowing of the infusion's appearance in the general circulation. More likely a specific modification of the vasoconstrictor substance occurs in the liver when homologous hemoglobin is used. This mechanism is incapable of efficient modification of heterologous hemoglobin.

Summary. The renal vasoconstriction associated with peripheral intravenous infusion of homologous lysed red blood cells in dogs is primarily caused by hemoglobin although the red blood cell stroma appears to have an additive effect. Peripheral intravenous infusion of heterologous lysed red blood cells of human, cow, horse, and sheep origin cause a significant decrease in GFR and ERPF in dogs. Portal vein infusion of heterologous lysed red blood cells through the liver causes a significant decrease in GFR and ERPF in dogs and is frequently associated with moderate decrease in arterial blood pressure. These experiments suggest that the protection afforded the kidney by initial passage of homologous red cell lysates through the liver is due to specific modification of the hemoglobin, a process the liver is unable to perform with heterologous red cell lysates.

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Received July 17, 1961. P.S.E.B.M., 1961, v108.

Monoenoic Fatty Acids in Dogfish Livers: Isomers of the C_{16}, C_{18}, C_{20}, and C_{22} Series. (26868)

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Recently, interest has been expressed in the structures of unique monoenoic fatty acids from vegetable(1) and marine-animal oils(2) and from human fecal lipids(3). Dogfish (*Squalus acanthias*) liver oil is a rich source of many monoenoic fatty acids(4) whose positional isomers have not been reported. We have isolated most of these fatty acids from the liver oil and have determined their structures by oxidative degradation of the double bonds followed by analysis of the resulting fragments as methyl esters by gas-liquid chromatography (GLC). This paper reports the principal structures of the C_{16},

C_{18}, C_{20}, and C_{22} monoenoic fatty acids. Suggestions are given on the possible biogenetic origin of these isomers.

Experimental. The oil obtained from a large number of livers of dogfish caught off the coast of British Columbia was extracted by steaming and pressing(5). Methods that were applied previously to the fractionation of fatty acids from fish oils(6) were adapted to isolation of the monoenoic fatty acids. Fatty acids, free of nonsaponifiable constituents, were obtained by saponification of the oil and by extraction of the aqueous soap solution with ether prior to acidification. About 108 g of urea were placed in 270 ml of methanol, and the mixture was refluxed to effect solution. After addition of 30 g of fatty acids, the solution was mechanically stirred to promote formation of crystals and allowed to cool to 25°C. The mixture was held at this temperature for 1 hour. The filtrate was discarded, and the fatty acids from the crystals were recovered (A, 17.0 g). Overnight crystallization of A from 102 ml of acetone at −5°C yielded fatty acids from the filtrate (B, 11.4 g) that were found to be mostly monoenes (>90%) by GLC. GLC was carried out with a Research Specialties Instrument, model 600, equipped with a 10-ft. × ¼-in. column of 3% diethylene glycol succinate polymer (DEGS) on siliconized(7) Chromosorb W (80/100 mesh). The column was operated at 170°C and at a flow rate of 35 ml of argon per minute. A high-vacuum fractional distillation (750 mm spinning-band column, 0.6 mm Hg pressure at distillation head) of B after esterification with methyl alcohol and sulfuric acid yielded fractions of methyl esters that were mostly monoenes of one chain length. Since these fractions were not sufficiently pure for oxidation studies, they were fractionated further by preparative GLC in 0.5 ml amounts, and C_{18}, C_{20}, and C_{22} monoenes were isolated in high purity (98-100%). The C_{16} monoene contained a large amount of palmitate that does not interfere with oxidation studies. The preparative GLC* was carried out with a Beckman "GC-2 Gas-chromatograph" equipped with a 5-ft. × ½-in. column packed with 40% precipitated DEGS on Chromosorb (30/60 mesh)(8). The flow rate was 1300 ml of helium per minute. Infrared analysis of methyl esters from the whole oil and of each fraction obtained by GLC did not indicate the presence of *trans*isomers (10.3 μ).†

Oxidations of the monoenes were carried out by adapting the procedure described by Haverkamp-Begemann and co-workers(9). About 50 mg of methyl esters were oxidized with potassium permanganate at 25°C in 0.6 ml of glacial acetic acid. The potassium permanganate was added, a few crystals at a time, over a period of 5 hours. The reaction was conducted in a test tube that was mechanically agitated. Monocarboxylic acids and half-esters of dicarboxylic acids were isolated by the method reported recently by Fulco and Mead(10). The oxidation products were converted to methyl esters with diazomethane in ether(11) and were analyzed by GLC at column temperatures varying from 120° to 150°C, depending upon the chain lengths of the esters involved. Flow rates varied from 30 to 40 ml of argon per minute. Analyses were carried out with the Research Specialties Instrument equipped with a 6-ft. × ¼-in. column packed with 8% DEGS on Chromosorb W (80/100 mesh)(8). Pure mono- and di-carboxylic acid esters were used as standards in analysis of the fragments.

Results and discussion. The work of Haverkamp-Begemann and co-workers(9) indicated that the permanganate-acetic acid oxidation technic causes formation of small amounts of secondary oxidation products. Smith and co-workers(1), however, found this method preferable for determination of the structures of monoenoic fatty acids in seed oils. Infrared analyses indicated that double bonds in the monoenoic fatty acids are in the *cis*-form. It is recognized, however, that small amounts (<3%) of *trans*-

* The authors are indebted to Edward H. Gruger, Jr., of the Technological Laboratory, Bureau of Commercial Fisheries, Seattle, for advice on the use of preparative columns for isolation of pure fatty-acid esters.

† Infrared spectra were measured as films on sodium chloride plates using a Baird-Atomic spectrophotometer, model NKI.

TABLE I. Monoenoic Fatty Acids in *Squalus acanthias* Liver Oil.

Fatty acid	% of unsaturated fatty acids	% of total fatty acids
cis- 9-hexadecenoic acid	9.1	6.1
cis- 9-octadecenoic "	36.9	24.7
cis-11-octadecenoic "	14.6	9.8
cis- 9-eicosenoic "	6.0	4.0
cis-11-eicosenoic "	7.6	5.1
cis-11-docosenoic "	10.0	6.7
cis-13-docosenoic "	2.7	1.8

double bonds are not readily detectable by infrared analysis.

The procedure for isolation and identification of monoenoic fatty acids from dogfish-liver oil may also have application to the isolation of these fatty acids from other complex mixtures. Dogfish-liver oil is a rich source of C_{20} and C_{22} monoenoic fatty acids that are not found in significant amounts in most fish oils. Monoenoic fatty acids (Table I) were found to comprise 58% of total fatty acids and 87% of unsaturated fatty acids in dogfish-liver oil extracted from a substantial quantity of livers. The remainder of the unsaturated fatty acids are primarily C_{20} and C_{22} polyenes, and only small amounts of

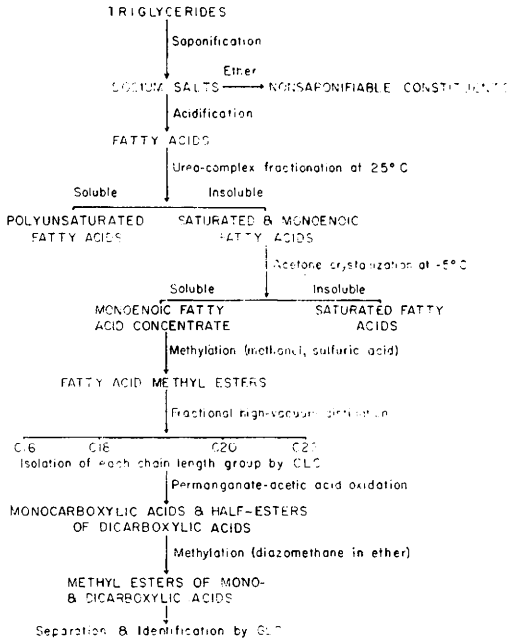

FIG. 1. Scheme for isolation and identification of monoenoic fatty acids in *Squalus acanthias* liver oil.

C_{20} and C_{22} dienoic and trienoic fatty acids were present in the oil. The monoenoic fatty acids reported here were found previously in various natural oils(12), and Hopkins and Chisholm(13) isolated 11% of 11-eicosenoic acid from liver oil of dogfish caught off the eastern coast of Canada. These authors also qualitatively identified 9-hexadecenoic acid in the same oil. The *cis*-11-octadecenoic acid has been found in animal and vegetable oils (12), but it is not known to what extent this acid is present in fish oils.

Biogenetic origins of monoenoic fatty acids. Lovern viewed the biogenesis of fatty acids in dogfish-liver oil in terms of both a hydrogenation process(14) and a chain elongation process(15). The structural analyses reported here allow a further evaluation of these concepts. The position of the double bond in the isomers reported in Table I suggests that they are formed by chain elongation by addition of acetate units. If 9-octadecenoic acid is elongated by addition of acetate units, the Δ9 double bond of the C_{18} acid becomes Δ11 in the C_{20} series and Δ13 in the C_{22} series. The 11-octadecenoic acid may be formed by elongation of 9-hexadecenoic acid with acetate units, and 11-docosenoic acid may be synthesized, similarly, by elongation of 9-eicosenoic acid. These relationships may be represented thusly:

9-hexadecenoic→ 11-octadecenoic
9-octadecenoic→ 11-eicosenoic ⟶ 13-docosenoic
9-eicosenoic ⟶ 11-docosenoic

Fatty acids having a Δ9 double bond thus appear to be the progenitors, by addition of acetate units, of most of the monoenoic fatty acids in dogfish-liver oil. Stoffel and Ahrens (6) suggested that C_{16} unsaturated fatty acids in menhaden oil may be formed from C_{18} fatty acids by loss of 2 terminal carbon atoms. The position of the double bond in the C_{16} monoenoic fatty acid from dogfish-liver oil suggests that it may have arisen similarly from oleic acid.

Summary. Structural isomers of monoenoic fatty acids in dogfish (*Squalus acanthias*) liver oil were determined. These fatty acids comprised 58% of total fatty acids and 87% of unsaturated fatty acids of the oil.

Although it is known that polyunsaturated C_{20} and C_{22} fatty acids are present in quantity in most fish oils, dogfish-liver oil was found to contain substantial amounts of C_{20} and C_{22} monoenoic fatty acids as well. The following isomers were found in the oil: *cis*-9-hexadecenoic acid (6.1%), *cis*-9-octadecenoic acid (24.7%), *cis*-11-octadecenoic acid (9.8%), *cis*-9-eicosenoic acid (4.0%), *cis*-11-eicosenoic acid (5.1%), *cis*-11-docosenoic acid (6.7%), and *cis*-13-docosenoic acid (1.8%). It is suggested that most of the monoenoic fatty acids of dogfish-liver oil may be formed by addition of acetate units to oleic acid and other fatty acids having a Δ9 double bond.

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Received July 5, 1961. P.S.E.B.M., 1961, v108.

Passive Cutaneous Anaphylaxis in Mice and Chickens. (26869)

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That the homograft reaction has many properties in common with delayed-type hypersensitivity is an established fact. Billingham *et al.*(1) demonstrated the passive transfer of the reactivity against skin homografts in guinea pigs is by cells and not by serum. The radiosensitivity of the homograft reaction(2,3) is of the same order of magnitude as that of the delayed hypersensitivity reaction(4,5), and much lower than that of soluble antibody formation(6). Nevertheless, antibodies directed against cell antigens often have been found in the serum of animals presensitized with homologous leukocytes(7) or lymphoidal cells(8,9), or bearing skin homografts(10,11). These circulating antibodies were detected by determining their cytotoxic activity *in vitro*(8,11,12) or by their ability to suppress antibody-forming activity of donor-type lymphoidal cells *in vivo* (9,13). Thus, it is apparent that the mechanism of rejection of homologous tissues is a complex one, and the relative role played by different factors is not well known; in order to understand this mechanism better, it seems necessary to use classical immunological methods along with *in vivo* assays for homograft reactions.

Recently, in other laboratories as well as in ours, transplantation antigens have been obtained in soluble form(14-16). Using these antigens, one can take a serological approach

* Portion of this work was performed during a Nat. Inst. Health Postdoctoral Fellowship.

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‡ Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.