Some Biochemical Aspects Of Fats

Part 2

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IN PRESENTING some biochemical aspects of fats we have considered the topic under two general headings—saponifiable and unsaponifiable matter. A discussion of the unsaponifiable portion has been presented in Part I appearing in a previous issue of this Journal (1). The present discussion is concerned with the saponifiable portion of fats.

The fundamental features of the chemical structure of the fats have been studied for more than a century, thus antedating the beginning of the scientific understanding of the diverse carbohydrates and the ubiquitous proteins. Chevreul, the pioneer in the chemistry of the fats, is credited with being the first individual to isolate butyric, caproic, caprylic, capric, palmitic, stearic, and oleic acids as well as cetyl alcohol from a variety of animal and fish oils. In the words of Tripp (2):

> "It was (also) Chevreul's ever-memorable service to show that fat and alkali reacted to produce the alkali salt of the fatty acid together with glycerin (or cetyl alcohol)."

Chevreul analyzed fats, Berthelot, another famous French chemist, was the first to synthesize glycerides. The thesis (3) of Berthelot dealt with the combinations of glycerine with acids, and the synthesis of the immediate principles of animal fats. His studies resulted in the discovery that glycerol is a polyhydric alcohol. Inasmuch as the tissue of living organisms, whether animal or plant, are composed in large measure of the proximate principles—protein, carbohydrate and fat—in addition to variable substances, it is not surprising that the chemistry of these fundamentally important components of living matter should early receive this critical consideration of scientific investigators.

Regarding the physiological significance of fats, Leathes (4) remarked:

> "The role assigned to fats in physiology is and has been for a long time threefold. In order of precedence determined by seniority these are: that they add beauty to the outline of the human form, that they serve as a protection for the body as a whole against cold; and finally, that they constitute the principal fund of reserve fuel on which the system can draw in times of shortage."

A hitherto unsuspected possible role of certain specific fatty acids in the animal organism, has been presented for serious consideration, through various investigations including those of Burr and Burr (5). As a result of these studies, certain fatty acids assume the dignity of indispensability for living animal organisms. Burr and Burr maintain that:

> "When rats were reared on a fat-free diet, a deficiency disease developed which had not been previously described. This disease is rather specific since the scaly condition of the skin develops, while growth is continuing at an approximately normal rate. Later the tail often becomes necrotic and the kidneys degenerate, allowing the passage of blood into the urine." p. 587.)

Saturated fatty acids are ineffective, but either linoleic acid or linolenic acid, is the outstanding curative or prophylactic agent. They conclude that warm-blooded animals are unable to synthesize linoleic acid and possibly some of the other more highly unsaturated acids.

The general thesis that these acids are indispensable has been challenged by a number of students of the problem. Gregory and Drummond (6) who have made a study of fat metabolism with special reference to nutrition on diets devoid of fat, attribute the abnormal condition to a deficiency of one or more factors of the vitamin B complex rather than to an inability to synthesize linoleic acid. In commenting on various studies related to this problem, Burr and Brown (7) later remark that differences in technique may readily account for lack of agreement of results. Evans and coworkers (8) who have been active in this field of investigation continue to present evidence to show the vital need of the body for certain unsaturated fatty acids.

The observations of Burr and Burr and others present a somewhat novel aspect of the biochemistry of fats; the fundamental relationship between food fat and body fat in the animal has continued to be a theme of significant study. Inasmuch as large amounts of hydrogenated oils are currently consumed, it is of interest to obtain information regarding the deposition and utilization in the animal body of isooleic acid, a constituent of hardened oil and fats. Barbour (9) has reported such a study in which he found that isooleic acid was deposited and utilized by fasting rats. Apparently this isomer or mixture of isomers of oleic acid may serve as a source of energy as satisfactorily as other fatty acid components of dietary fats.

The same investigator (10) has also studied the relation of saturated fatty acid content of the diet to the composition of body fat as well as of fecal fat. When, for example, lard and cottonseed oil containing 36 per cent and 22 per cent of saturated fatty acids respectively were fed to rats at a 20 per cent level of the diet, the respective body fats contained nearly 26 and 25 per cent saturated fatty acids. Under the experimental conditions employed, Barbour concludes that the saturated fatty acid content of the body fat cannot be raised be-

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yond a level of 25 to 27 per cent by feedng fats containing a larger amount of saturated fatty acids but can be decreased by feeding fats, e.g., linseed and peanut oil, of low saturated acid content. Analyses of feces showed that animals fed lard excreted the most fat (13.5 per cent) and that this fecal fat contained 60 per cent saturated fatty acids which was 16 per cent greater than the content of the fecal fat of animals which consumed cottonseed oil. The existence of a threshold for saturated fatty acids in the body fat is thus indicated. It was also observed that the arachidic acid of peanut oil was excreted almost quantitatively.

Regarding the absorption of fats in relation to their character, Holt and coworkers (11) have presented recent clinical evidence to show that the chemical constitution of the fat rather than the melting point is the important factor in fat absorption. These investigators fed partially hydrogenated corn oil (melting point 52° C. and iodine number 50.2), and a tripalmitintristearin mixture (melting point 52° C. and iodine number 2.4) and found the retention of the hydrogenated product was 83 per cent as compared with 62 per cent for the saturated fat mixture.

The influence of diet upon the character of fat produced in the rat has been studied also by Banks and coworkers (12) who fed the following diets: (a) "fat-free"; (b) containing hydrogenated fat in which unsaturated fats were absent; and (c) containing 2 to 15 per cent of cod liver oil. The fat produced on all three dietary regimens contained 23-30 per cent of palmitic acid and only 2-3 per cent of stearic acid. In the fat of pigs, sheep and oxen stearic is definitely a major component of the fatty acids, occurring in widely varying amounts, whereas in rat body fats it is equally clearly a minor component. Lack of sufficient material made it impossible to obtain decisive evidence regarding the palmitoleic acid content. Body fat produced on cod liver oil rations (in most cases the diet included 5 per cent of this fish liver oil), contained 8.5 per cent of unsaturated C_{20-22} acids and only 4 per cent of the less unsaturated constituent, namely, linoleic acid.

In a study conducted by Almquist et al. (13), on the relation of depot fat to egg yolk fat in hens fed crude cottonseed oil, the evidence, based on the Halphen test, showed that depot fat is not utilized to any important extent in the formation of yolk fat. Cruickshank (14) reports the results of feeding a number of dietary fats including palm oil and mutton fat which contain 46 and 51 per cent of solid acids, respectively. Eggs produced by hens fed these fats at a 28 per cent level, contained about 28 per cent and 29 per cent of "solid" acids, respectively. When linseed oil with a "solid" acid content of about 9 per cent was consumed the yolk contained 24 per cent of "solid" acids, a figure which is not markedly less than that obtained when fats high in "solid" acids are fed. Egg oil of so-called normal composition, obtained from yolks produced on a control ration including cereal products and fish meal contained 31 per cent of "solid" acids. Grossfield (15) had previously reported a similar value for egg oil obtained from market eggs of Danish origin. Cruickshank concludes that "the ingestion of saturated acids had relatively little effect in altering the normal composition of the mixed fatty acids of the egg fat."

Body fats (abdominal, gizzard, and neck) of hens have been studied by Hilditch and coworkers (16) who report the component palmitoleic acid $CH_3(CH_2)_5CH:CH(CH_2)_7COOH$. Apparently this acid which is a feature of marine animal oils is not dependent on the ration of the birds. "The presence of 65-68 per cent of unsaturated acids in the component acids of the depot fats of an animal with so high a body temperature as the hen affords another interesting example of the fact that body temperature is by no means the sole determining factor of the relative saturation of depot fats." The composition of all three body fats was almost identical. According to these English investigators, the specific type of depot fat seems to depend primarily on the biological species; the influence of body temperature is to be regarded as a secondary factor which may modify the composition of body fat.

Brown and Sheldon (17) who remind us of the general belief that the body fats and oils of land animals are composed principally of glycerides of oleic, stearic and palmitic acids, have recently reported the presence of highly unsaturated fatty acids in fowl (goose, turkey, duck and chicken) oils as well as in animal (lamb, calf, beef and reindeer) fats. Linolenic and arachidonic acids were found in some of the specimens of avian oils and traces of acids more highly unsaturated than arachidonic acid were discovered in all of the animal fats. As a result of these findings and those of other investigators including Eckstein and Ellis and Isbell, Brown and Sheldon believe that small amounts of highly unsaturated fatty acids occur quite generally in animal fats.

Klenk (18) claims to describe accurately for the first time the fatty acids of amphibian fat. The fat of the fat body (a lobulated mass of fatty tissue attached to the genital gland of amphibians) of male and female frogs (Rana temporaria) was found to contain the following fatty acids:

	Saturated Per cent	Unsaturated Per cent
C_{14}	4	trace
C_{16}	11	15
C_{18}	3	52
C_{20}	0	15 (C_{20} + C_{22})
C_{22}	0	

Apparently this fat is characterized by a considerable quantity of palmitoleic acid as is the oil of fresh water fish examined by Lovern. Regarding the origin of this acid, Klenk believes that it possibly may be explained by the β oxidation of the higher unsaturated fatty acids.

Inasmuch as existing data of horse fat (depot and whole carcass) were confined to that of the domestic work animal, a recent report by Schuette and coworkers (19) on the abdominal fat of the untamed Western range horse, an animal left to its own devices in the matter of foraging for food, assumes considerable importance. The glycerides of the fat obtained from the abdominal cavities of young wild horses (not over 4 years of age) "were found to contain five acids in the amounts indicated, viz., palmitic 26.28 per cent, stearic 4.50 per cent, oleic 46.86 per cent, linoleic 11.88 per cent, and linolenic 4.48 per cent."

Fat metabolism in a number of fishes has been studied by Lovern (20) who finds considerable variations in fatty acid composition in different fats from one animal; this observation indicates a highly selective formation of fat deposits. Evidence is presented to show that zoologically related species of sea organisms exhibit definite similarities in fat composition. In this connection it is interesting to recall the work of Hilditch (21) who showed similarities in fat composition of related plants. Porpoise and dolphin fats are unique in containing large amounts of isovaleric acid; dolphin jaw-oil, a valuable lubricant, was previously reported by Gill and Tucker (22) to contain approximately 90 per cent of this acid in various combinations. In the porpoise, the heart fat contains a more unsaturated group than the liver fat, a finding not supporting the theory of desaturation in the liver.

An investigation of the fat metabolism in both fresh water and marine species of fish (and marine mammals) has been made by Lovern (23) who remarks that "palmitoleic acid is a more important component of all fresh-water fats than of the marine ones with the exception of halibut-liver oil which is unique." The latter fish liver oil has an apparent lack of linoleic acid, according to this investigator; whether or not this fatty acid is present in the depot fat of the halibut has not been determined. Recently halibut liver oil, which has a high though fluctuating vitamin A potency, and is also a rich source of the vitamin D factor, has been accepted as a valuable accessory to human nutrition (24, 25).

In the analysis of the peritoneal fat (main storage fat), pancreatic and liver fats of a sturgeon, a fish which inhabits both fresh and salt water and which, moreover, does most of its feeding in fresh water, Lovern (26) found that "in respect of the high content of palmitoleic acid, and the constant proportion of total C_{16} acids, these oils resemble those of the fresh-water fish rather than those of marine species." Furthermore, "the high content of oleic and linoleic acids, together with the reduced amounts of C_{20} and C_{22} acids, is also suggestive of a fresh-water fish." Inasmuch as the liver fat was no more unsaturated than the main depot fat of the peritoneal cavity the theory of desaturation in the liver was not supported (27). The proposed inter-relationship between the main saturated acid component which has an unsaturated derivative, for example, stearic and oleic acids in hogs, etc., and palmitic and palmitoleic in the case of fish, is substantiated.

Interesting studies of the physiology of the spawning migration of salmon by numerous investigators have shown marked changes in fat and other body constituents. Depot fat is used as a source of energy required to make long journeys against swift currents to spawning grounds; in these migrations no food whatever is eaten. Greene (28), a student and reviewer of the literature of this subject, presents data regarding the changes in muscle tissue fat of the king salmon during spawning migration up the Columbia River. The phospholipin and neutral fat figures (on fresh tissue basis) at the beginning of the journey were 0.93 per cent and 15.5 per cent, respectively. At the spawning grounds the corresponding figures were 0.43 per cent and 2.2 per cent, respectively. The phospholipin and neutral fat content of the ovary of the spawning king salmon was reported to be about 2.5 per cent and 10.5 per cent, respectively; depot fat is transferred to the rapidly developing gonads. A comparison of ovarian and depot fats of salmon shows a higher degree of unsaturation in the former than in either liver or muscle fat. Regarding the Manx herring, Channon and El Saby (29) remark "that the degree of unsaturation of the fatty acids of the ovaries and testes steadily rise to a maximum before the fish spawns, when the iodine value has risen from 137 to 200". Iodine values for fatty acids of other tissues remained constant. It is further noted that cholesterol is synthesized in at least the last stages of maturation; and that the milt contains considerably more cholesterol than the roe.

Lovern (30, 31) who has studied fat metabolism in young and adult salmon, finds the content of unsaponifiable matter of the fat of parr is only 5 per cent as compared with a figure of 18 per cent for that of smolts. (Parr are young salmon which hatch out and live 1 to 4 years in fresh water, whereas smolts are young salmon which at an average age of 2 or 3 years, swim down stream and out to sea, becoming a marine species.) Contrasting the two groups further, it was observed that the fat content of parr and smolts are 3.9 per cent and 1.2 per cent, respectively; smolt fat has a low proportion of palmitoleic acid (about 8 per cent) as compared with nearly 22 per cent of parr fat; smolt fat has a high component C_{22} unsaturated acid (about 18 per cent) whereas parr fat has only about 10 per cent. Both fats are very highly unsaturated. Lovern remarks that different food and growth rates might well affect and explain the variations in fat content of parr and smolts. "It is suggested that when salmon changes from the parr stage to the smolt stage, its metabolic requirements become those of adult fish, and thus the composition of its depot fat takes on the peculiarities characterizing the salmon fats as a whole."

The foregoing discussion which deals exclusively with a few of the recent studies in animal fat metabolism, may serve to emphasize the vigorous investigation currently taking place in this field of fat chemistry. For a recent comprehensive review of animal and vegetable fats, we are indebted to Hilditch for his jubilee memorial lecture, "The Fats: New Lines in an Old Chapter of Organic Chemistry" (32)

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