SOFT PORK STUDIES.

III. THE EFFECT OF FOOD FAT UPON BODY FAT, AS SHOWN BY THE SEPARATION OF THE INDIVIDUAL FATTY ACIDS OF THE BODY FAT.

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In a previous paper (1) the writers reported data on the effects of widely different rations on the composition of the body fat of the hog. The characteristic differences in the composition of the lard suggested the need of a more complete analysis on a few typical samples. This study has been made on six samples including certain ones already briefly reported, as a further contribution to the information on fat formation in the hog. The types subjected for complete analysis were obtained from well matured and fattened hogs fed on rations (1) very low in fat, (2) moderately low in fat, and (3) high in fat.

The feeds used to obtain the desired results were from different sources and were, respectively, (*a*) brewers' rice and tankage, (*b*) corn and skim milk, (*c*) soy beans alone, and peanuts alone, both represented by two samples each. Where necessary, the rations were supplemented with mineral mixtures. In the case of the first two rations, the hogs were also allowed access to green feed. Table I gives the weights, gains, carcass grades, and other data on the six lots. The length of the feeding periods and the gains were all of sufficient duration and size to make the fat samples typical for the feed used. This is particularly true of Samples 1, 2, 4, and 6. Sample 3 is representative of the 2 months feeding period used in practice for grazing the peanut crop to be later followed by a hardening period on corn. Likewise Sample 5 is representative of the system of grazing soy beans.

TABLE I.

Weights, Gains, and Carcass Grades of Hogs.

	Sample No. and basic feed.					
	1 Brewers' rice.	2 Corn.	3 Peanuts.	4 Peanuts.	5 Soy beans.	6 Soy beans.
No. of hogs in lot	11	6	3	3	9	1
Days on experiment	84	188	80	277	52	53
Average weight at start of experiment, *lbs.*	59	30	99	52	100	83
Total gain on experiment, *lbs.*	178	201	58	149	65	98
Slaughter weight, *lbs.*	242	231	151	201	152	175
Carcass grade	Hard.	Hard.	Oily.	Oily.	Oily.	Oily.

TABLE II.

Chemical and Physical Characteristics of the Fat.

	Sample No. and basic feed.					
	1 Brewers' rice.	2 Corn.	3 Peanuts.	4 Peanuts.	5 Soy beans.	6 Soy beans.
Saponification value	195.3	195.9	194.2	194.4	195.3	194.3
Iodine No.	52.6	58.8	84.1	91.8	90.7	100.6
Refractive index, 40°C.	1.4582	1.4587	1.4619	1.4633	1.4628	1.4636
Melting point, °C.	39.7	37.5	22.5	Liquid at 5°	22.0	28.1
Acetyl value	3.8	4.1	12.1	16.3	7.7	7.6
Acid value	1.0	0.9		0.7	0.8	5.2
Polenske No.	3.7	5.2		0.3	0.5	0.6
Reichert-Meissl No.	3.3	1.0		0.1	1.5	0.2
Specific gravity, 100°C.	0.8942	0.8957	0.8979	0.9007	0.9000	0.9003
Unsaponifiable matter, *per cent*		0.02		0.03		0.33
Insoluble acids, *per cent*	96.0	95.6	95.8	94.5	95.1	95.4

Analyses were made on the meat fat of the corn-fed lot and the two peanut-fed lots and on the back fat of the remaining three lots. In addition to the fat constants determined in the previous study, the acetyl value, acid value, Polenske number, Reichert-Meissl

number, and per cent of unsaponifiable matter were determined. The chemical and physical characteristics of the fat are given in Table II.[1]

From 200 to 300 gm. of fat from each sample were separated by the lead salt-ether method into saturated and unsaturated fractions for use in determining the individual fatty acids.[2] The percentages of total saturated and total unsaturated acids were always checked by duplicate determinations using smaller quantities of fat, and, in certain cases, duplicate separations of large quantities were made for duplicate distillations of the saturated acid esters. In calculating the percentages of the fractions, corrections of the unsaturated acids contained in the saturated acids were always made.

Procedure for Separation of the Unsaturated Acids.

2 to 3 gm. of the liquid acids were weighed into a previously carefully dried and weighed test-tube and 20 cc. of a 10 per cent solution of glacial acetic acid in absolute ether added. The solution was cooled in an ice bath, and, while stirring the solution, bromine was slowly added to a decided red tint. After standing overnight in the ice box, the precipitated bromides were separated by centrifugation, washed three times with 10 cc. portions of chilled ether, after which they were extracted three times with 10, then 5 and 5 cc. portions of hot benzene. This benzene was then evaporated off and the residue reextracted with 3, then 2 and 2 cc. portions of hot benzene in order to recover any octabromide which may have dissolved in the first treatment with the larger volumes of benzene. After evaporating off the benzene, the bromide fractions were dried in a vacuum oven to constant weight, and melting points and per cents of bromine determined.

The ether-soluble bromides were washed with a dilute solution of sodium thiosulfate followed by water to remove the excess

[1] The analytical procedures followed in the determination of the chemical and physical characteristics of the fat were the same as those used in previous work (1): namely, the Official and provisional methods of analysis, *Assn. Off. Agric. Chem., Washington, 1920.*

[2] In the separation of the fatty acids, the technique described by Jamieson and Baughman (2) has been generally followed. The authors wish to express their thanks to Dr. G. S. Jamieson of the Bureau of Chemistry for helpful suggestions in technique.

bromine. After evaporation of the ether and acetic acid, the mixture of bromides was dried and weighed. They were then dissolved in 25 cc. of petroleum ether and the solution allowed to stand in the ice box for a week. In case the tetrabromide failed to start crystallizing, the sides of the flask were scratched or part of the petroleum ether was evaporated and the solution seeded with a trace of tetrabromide crystals.

The crystallized bromides were filtered in the cold, washed with chilled petroleum ether, dried, and weighed. The melting points and per cent of bromine were then determined. The method used throughout this work for the determination of bromine was that of Drogin and Rosanoff (3).

The bromide contents of the various fractions indicated a fairly clean cut separation in most cases. The bromine in the octabromide averaged 67.1 per cent and was interpreted as corresponding to a compound of the formula $C_{20}H_{32}O_2Br_8$ which has a bromine content of 67.76 per cent. The theoretical per cents of acid in the octabromide (32.24), the hexabromide (37.67), and the tetrabromide (46.67) were used in calculating the respective acids. The soluble residue usually contained appreciable quantities of tetrabromide along with the dibromide so the proportionate amounts of the linolic and oleic acids were calculated, using 63.82 per cent as the per cent of oleic acid in a pure dibromide.

The percentages of each acid were further checked by calculations from the iodine numbers of the fat and the total unsaturated acids. In previous work, only the octa- and hexabromides were isolated leaving the oleic and linolic acids to be calculated from iodine numbers.

Procedure for the Separation of the Saturated Acids.

The saturated acids were separated by the vacuum distillation of their methyl esters according to the following procedure.

The acids were dissolved in twice their weight of purified methyl alcohol. The solution was kept warm on the steam bath and a current of washed and dried hydrochloric acid gas was bubbled through the mixture for 12 hours. After washing the esters with water followed by a dilute solution of sodium bicarbonate, about 75 gm. were filtered (to remove traces of water) into a distilling flask. The esters were then subjected to fractional distillation at

a pressure of 1 to 2 mm. The fractions were cut at points where abrupt rises in temperature occurred or where convenient volumes of distillate were obtained. After weighing the fractions, iodine and saponification values were determined. Wherever the saponification value indicated a possibility of three saturated acids occurring in a single fraction a further separation was made to insure having not more than two saturated acids to a fraction. The mean molecular weights were calculated and from these values and the iodine numbers the per cent of each saturated acid was determined.

The acids were also further identified by fractional crystallizations from alcohol and the determination of the melting point. Although the errors in the determinations may sometimes be large, the duplicate runs checked very satisfactorily. One great chance of error lies in the possibility of having three saturated acids in one fraction. This possibility was tested by making duplicate distillations on one of the samples in which the distillate in one case was cut into ten fractions and in the other into three fractions. It was thought that the former procedure would eliminate the chances of three acids occurring together and the latter procedure would favor such a possibility. However, the results of the two distillations checked surprisingly well. All other samples were separated into six to eight fractions.

The general differences in the characteristics of the various fats shown in Table II are further illustrated by the titer, refractive index of insoluble acids, and per cents of saturated and unsaturated acids given in Table III. While not all of these characteristics show variations for the different types of lard, they do indicate the ranges likely to be encountered in lards produced on the rations outlined.

It will be noted in Table III that oleic, linolic, linolenic, arachidonic, myristic, palmitic, stearic, and arachidic acids were isolated. The occurrence of oleic, linolic, palmitic, and stearic acids has been commonly accepted but the presence of the other acids has either not been noted or has been questioned.

The linolenic acid was found in lards from hogs fed soy beans. In the case of Sample 5, there was a loss when isolating the bromide so that the amount actually recovered was little more than a trace; the 0.5 per cent found in Sample 6 was easily identified by melting point and bromine content. The acid yielding an octa-

bromide and thought to be arachidonic acid occurred in all samples in small amounts.

Arachidic acid was identified in the two samples from peanut

TABLE III.

Characteristics and Percentage Composition of the Fatty Acids.

	Sample No. and basic feed.					
	1 Brewers' rice.	2 Corn.	3 Peanuts.	4 Peanuts.	5 Soy beans.	6 Soy beans.
Titer	41.4	39.1	30.7	24.5	34.2	32.2
Refractive index of insoluble acids, 40°C.	1.4420	1.4424	1.4452	1.4465	1.4472	
Unsaturated acids, per cent of fat (corrected).	57.0	58.8	73.0	79.6	69.2	72.3
Saturated acids, per cent of fat (corrected)	38.6	36.9	22.6	14.9	26.3	20.8
Iodine No. of unsaturated acids	92.0	100.8	113.7	111.5	130.6	136.2
Iodine No. of saturated acids as separated	4.8	5.3	13.4	12.4	4.8	11.2
Acids in fat:	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Oleic	55.9	52.0	54.3	61.7	38.7	36.1
Linolic	1.2	6.7	18.6	18.8	30.5	35.6
Linolenic	0.0	0.0	0.0	0.0	0.2	0.5
Arachidonic	0.02	0.06	0.12	0.05	0.08	0.05
Myristic	1.7	0.6	0.4	0.1	0.7	0.3
Palmitic	25.2	24.1	14.8	9.9	16.6	13.1
Stearic	11.6	12.2	7.1	4.7	9.0	7.4
Arachidic	0.0	0.0	0.2	0.3	0.0	0.0
Glycerides in fat:						
Oleic	58.4	54.3	56.7	64.6	40.4	39.8
Linolic	1.2	7.1	19.5	19.7	31.9	38.3
Linolenic	0.0	0.0	0.0	0.0	0.02	0.5
Arachidonic	0.02	0.06	0.12	0.05	0.08	0.05
Myristic	1.8	0.7	0.4	0.1	0.8	0.3
Palmitic	26.5	25.2	15.5	10.4	17.4	14.5
Stearic	12.2	12.8	7.5	4.9	9.4	8.0
Arachidic	0.0	0.0	0.2	0.3	0.0	0.0

feeding. The melting point of pure arachidic acid is reported as 77°C. but repeated crystallization of the fractions failed to raise the melting point to this figure. However, a mixed melting point

with an impure arachidic acid obtained for the purpose was 73.6 indicating the presence of the acid in the lard. It is probable that there was lignoceric acid mixed with the arachidic acid although none could be separated by fractional crystallization from alcohol.

Although the occurrence of myristic acid has been questioned by Amberger and Wiesehahn (4) it was satisfactorily identified in all the six samples. Its presence was proved by mixed melting point determinations in which mixtures containing myristic acid melted at 45–46°C. This is lower than for any mixture of palmitic and stearic acids and higher than for any mixture of lauric and myristic acids. Lauric acid was not found although its presence in lard has been reported by Lewkowitsch (5) and others. It may, of course, occur when the feed contains the acid.

In comparing the fatty acid composition of the various samples, that of Sample 1 is particularly noteworthy since it is largely the product of synthesis from carbohydrate. The hogs had access to brewers' rice and tankage in separate compartments of a self-feeder and ate only 4 pounds of tankage to 333 pounds of rice for each 100 pounds of gain. The result was a low protein intake with a surplus of carbohydrate for fat synthesis. The small amount of oil ingested may have supplied some linolic and oleic acid to the adipose tissue but the fatty acids are almost entirely the result of synthesis. Oleic acid predominates and occurs in an amount comparable to that in corn and in peanut-fed hogs. The low amount of linolic acid and absence of linolenic acid accounts for the hardness of the lard. Myristic acid was present in greater amounts than in any other sample while palmitic and stearic acids occurred in amounts comparable to that in the second sample.

The course of fattening in corn-fed hogs which has already been reported (6) needs little further mention. The increase in linolic acid, probably derived from corn oil, was evidently responsible for the slightly softer fat over that of rice-fed hogs.

The close resemblance in the composition of lards from peanut- and soybean-fed hogs to the respective plant oils was mentioned in a previous paper (1). With more complete data available, a better comparison between the plant oils and the lards is now possible. The data compiled in Table IV show the composition of peanut, soy bean, and also corn oils. The small differences in the peanut oils derived from the Virginia variety as compared to the Spanish

variety of peanuts are unlikely to be reflected in the lards to any appreciable extent since the supply of ingested peanut oil from the feeding of whole peanuts is greatly in excess of body demands for storage. Virginia peanuts were fed the pigs of Lot 3 for 80 days while Spanish peanuts were fed Lot 4 for 277 days. In addition, due to the lighter starting weight, the latter lot derived a much greater proportion of the total adipose fat from peanuts than the

TABLE IV.

Composition of Plant Oils.

	Peanut oil.*		Soy bean oil.†	Corn oil.‡
	Virginia.	Spanish.		
Saponification value	187.8	182.2	189.5	187.3
Iodine No.	94.8	90.1	128.0	117.2
Acetyl value	9.5	8.7	17.0	10.0
Refractive index, 40°C.§	1.4625	1.4625	1.4660	1.4642
Unsaturated acids, *per cent.*	78.7	74.6	83.5	82.5
Saturated " " "	16.4	20.6	11.5	11.2
Oleic glyceride	60.6	52.9	33.4	45.4
Linolic "	21.6	24.7	51.6	40.9
Linolenic "			2.3	
Myristic "				
Palmitic "	6.3	8.2	6.8	7.7
Stearic "	4.9	6.2	4.4	3.5
Arachidic "	3.3	4.0	?	?
Lignoceric "	2.6	3.1	?	?

*See bibliography (7).

†See bibliography (8).

‡See bibliography (9).

§The refractive index for peanut oil is taken from Table IV in the preceding article (1). The values on the other oils were calculated to 40°C. from values quoted in the original reference at 20°C.

Lot 3 pigs. It is thought that this is the explanation of the higher percentage of oleic acid and the lower percentage of saturated acids in Sample 4 over that in Sample 3. Compared to the composition of the oil of the Spanish variety of peanuts, the lard (Sample 4) shows a higher percentage of the oleic acid glyceride, a lower percentage of linolic acid glyceride, and a slightly wider ratio of palmitic to stearic acid glyceride. The most significant difference noted in the peanut lards is the low amount of arachidic acid

present, especially when the supply of arachidic acid plus lignoceric acid in the oil was greater than that of stearic acid. These high melting point saturated acids evidently failed to be absorbed or were changed to other acids in the animal body. The small amounts of myristic acid were evidently products of synthesis or of transformation from other fatty acids in the peanut lards as well as in the other samples since none are reported in any of the oils. The full amounts of all the other fatty acids can be accounted for in the ingested peanut oil.

The composition of the lard in soy bean feeding is also largely controlled by the composition of soy bean oil. The most apparent difference between the lard and the oil seems to lie in the lower proportion of linolic acid in the lard. The samples actually show a much higher per cent of glycerides of the saturated acids and only a few per cent more oleic acid glyceride than the oil. Sample 5 is little different from peanut lard in degree of unsaturation but Sample 6 shows the effect of the increase in linolic acid and may be classed as a more oily lard than is obtained in peanut feeding.

Although 2.3 per cent linolenic glyceride is reported in soy bean oil, only 0.5 per cent was recovered in the lard of Sample 6. There is little doubt but that this acid is readily absorbed by the intestinal tract but since it is also easily oxidized, the low amount found may be due to different reasons than in the case of arachidic acid with its high melting point.

The proportions between the various saturated acids are much more constant for the six samples than between the unsaturated acids. Indeed, it appears significant that the proportion of palmitic acid to stearic acid was approximately the ratio of 2:1 for all samples. Even in the case of the peanut and soy bean lards there was some selective action in favor of palmitic acid, while with the low fat rations, the ratio was very evidently controlled by the synthetic powers of the body.

The high amount of oleic acid in all samples, not only in Samples 1 and 2 where it had to be synthesized but in the others as well, indicates that it ranks ahead of palmitic and stearic acids as a normal constituent of lard.

The rise in the linolic acid from 1.2 per cent to 18.8 per cent in Samples 1 to 4 furnished a close measure of increasing softness of these samples. However, in the case of Samples 5 and 6 the

decided increase in the linolic acid content is partly offset by the decrease in oleic acid.

SUMMARY.

A complete separation of the fatty acids was made on six samples of fat obtained from as many lots of hogs fed rations varying in fat content.

The fatty acids occurring in all samples were oleic, linolic, arachidonic, myristic, palmitic, and stearic. Palmitic acid and stearic acid occurred in a ratio of 2:1.

The feeding of soy beans caused the deposition of small quantities of linolenic acid, while the feeding of peanuts led to the deposition of arachidic acid. The oils of these two feeds have a pronounced effect on the composition of the lard. A greater likeness was noted between peanut oil and "peanut lard" than between soy bean oil and "soy bean lard."

The fat formed on a ration of brewers' rice and tankage which contained less than 1 per cent fat was very hard. The glycerides of oleic, palmitic, and stearic acids composed over 97 per cent of the fat.

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