NATIONAL COTTONSEED PRODUCTS ASSOCIATION

Compilation of total points off from accepted average plus tolerance and final official grades on the thirty samples of cottonseed co-operative work, 1933–34.

Chemist Number	Points Off from Accepted Average Plus Tolerance							Official Grade
	Oil	Ammonia	F. F. Ac.	Moisture	Errors	Rules	Total	
1	1.10	0.24	1.10	0.00	0.00	0.00	2.44	95.12
2	1.00	0.18	0.40	0.00	0.30	0.40	2.28	95.44
3	0.30	0.24	0.70	0.00	0.00	0.40	1.64	96.72
5	0.80	0.10	1.20	0.00	0.00	0.40	2.50	95.00
6	3.70	0.98	3.40	0.25	0.60	0.00	8.93	82.14
7	0.70	0.26	1.00	0.05	0.60	0.00	2.61	94.78
8	2.20	0.38	2.70	0.80	0.00	0.00	6.08	87.84
9	2.20	0.52	4.20	0.05	0.60	0.40	7.97	84.06
10	1.30	0.28	1.50	0.10	0.10	0.00	3.28	93.44
*11	4.40	1.82	3.70	0.00	0.00	0.00	9.92	79.66
12	3.10	0.46	1.10	0.55	0.20	0.00	5.41	89.18
13	2.40	0.40	1.10	0.10	0.00	0.00	4.00	92.00
14	0.60	0.16	0.10	0.30	0.00	0.00	1.16	97.68
15	2.60	0.50	0.70	0.45	0.00	0.00	4.25	91.50
16	1.60	0.86	1.30	0.30	0.00	0.00	4.06	91.88
17	2.90	0.28	1.50	0.85	0.50	0.40	6.43	87.14
18	1.30	0.74	2.70	0.30	0.00	0.00	5.04	89.92
19	0.50	0.42	2.30	0.05	0.00	0.00	3.27	93.46
20	1.40	0.16	0.20	0.15	0.30	0.00	2.21	95.58
21	2.60	1.14	1.60	0.15	0.40	0.00	5.89	88.22
22	0.10	0.50	1.90	0.00	0.00	0.00	2.50	95.00
23	1.80	0.06	0.40	0.05	0.00	0.00	2.31	95.38
24	1.20	0.10	1.10	0.00	0.00	0.00	2.40	95.20
25	3.10	0.52	0.80	0.00	0.30	0.00	4.72	90.56
26	2.60	0.58	0.80	0.30	0.10	0.40	4.78	90.44
27	3.30	0.26	3.80	0.20	0.30	0.00	7.86	84.28
28	0.90	1.06	1.30	0.00	0.00	0.00	3.26	93.48
29	2.10	0.60	0.60	0.05	0.00	0.00	3.35	93.30
30	1.30	0.10	2.60	0.05	0.60	0.00	4.65	90.70
31	0.60	0.36	1.50	0.20	0.00	0.40	3.06	93.88
32	4.30	0.68	3.10	0.40	0.50	0.40	9.38	81.24
33	1.30	0.40	1.20	0.15	0.00	0.40	3.45	93.10
34	0.70	0.10	0.60	0.00	0.00	0.00	1.40	97.20
35	1.10	0.62	2.50	0.00	0.00	0.00	4.22	91.56
36	0.60	0.14	0.10	0.00	0.00	0.00	0.84	98.32
37	2.20	0.14	1.50	0.10	0.10	0.00	4.04	91.92
38	3.20	0.26	1.80	0.90	0.30	0.40	6.86	86.28
39	1.20	0.02	1.40	0.50	0.00	0.00	3.12	93.76
40	1.30	0.02	0.30	0.45	0.50	0.00	2.57	94.86
42	0.40	0.14	1.50	0.15	0.00	0.00	2.19	95.62
43	2.00	0.76	0.90	1.85	0.00	0.40	5.91	88.18
†45	1.90	0.12	1.80	0.50	0.00	0.40	4.72	89.20
‡46	0.40	0.12	1.50	0.10	1.40	0.40	3.92	76.48
Average	1.76	0.42	1.53	0.25	0.15	0.11	4.22	91.56

*Based on 19 samples. †On 25 samples. ‡On 10 samples.

There are no perfect records on oil, ammonia and free fatty acids.

	Off on only one sample	Off on only two samples
Oil	Number 22	Numbers 2–14
Ammonia	Numbers 23–39–40	Numbers 14–34
Free fatty acids	Numbers 2–14–23–36–40	Number 20

Classification of collaborators on basis of season grades:

Grade	Numbers	Total
95–100	1–2–3–5–14–20–22–23–24–34–36–42	12
90– 95	7–10–13–15–16–19–25–26–28–29–30–31–33–35–37–39–40	17
85– 90	8–12–17–18–21–38–43–45	8
80– 85	6–9–27–32	4
Below 80	11–46	2

SOME BIOCHEMICAL ASPECTS OF FATS. PART I

By William E. Anderson and Harold H. Williams*

(FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, YALE UNIVERSITY, NEW HAVEN, CONN.)

A discussion of the biochemical aspects of lipids must necessarily include the components which resist the action of alkali—in other words, the unsaponifiable material. A few years ago Drummond (1) remarked:

"From the standpoint of the biochemist, the study of the unsaponifiable constituents of the natural oils and fats is a veritable El Dorado for those prepared to undertake the very difficult task of attacking the problems that await solution in this field of research."

The diversity of substances present in the unsaponifiable portion of the mixture commonly called fats is indicated by the presence of hydrocarbons, alcohols, carotinoids, vitamins, and sterols. Whereas most of the animal and vegetable fats consist almost entirely of glycerides and a small percentage of unsaponifiable matter, the liver oils of some sharks contain as high as 90 per cent of unsaponifiable lipid. On the other hand, Chibnall and Channon (2) found the ether extract of the leaf cell cytoplasm of spring cabbage to contain only about 27 per cent of fatty acids.

Since the discovery of carotene and ergosterol as precursors of vitamin A and D, respectively, we recognize that all unsaponifiable components may be of the utmost biological significance. However, it is beyond the scope of this discussion to deal with each substance individually. The unsaponifiable lipid is a common constituent of protoplasm. The various components of the unsaponifiable fraction, however, are not common to all living matter. Some are peculiar to the plant, others to the animal kingdom; sterols on the other hand, are common to plant and animals alike, being, respectively, represented by phytosterols and cholesterol. This discussion will be confined to some of the biochemical aspects of cholesterol since this substance is today occupying an important place in biochemical investigation.

The ubiquitous occurrence of this substance in the structure of the animal cell is indicative of its im-

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*Sterling Fellow, 1934-35.

portance to living processes. It is an ironic fact that our knowledge concerning the actual role of cholesterol in vital phenomena is still very limited in spite of extensive investigation.

The discovery of cholesterol was made by Poulletier de la Salle (3) when he extracted it from biliary calculi in 1758. It was not until 1814, however, that its true nature as an unsaponifiable lipid was established by Chevreul (3) who named it cholesterine. Chemically it is characterized by remarkable stability. Windaus (4) has shown that it is an unsaturated secondary alcohol of the formula $C_{27}H_{46}O$.

Originally it was believed that cholesterol was derived from the plant sterols of the food, inasmuch as the animal body was considered incapable of the high order of synthesis required for such complex compounds. Ingested cholesterol is absorbed by the alimentary tract, but in this respect the metabolism of the sterol in herbivora is apparently quite different from that in carnivora or omnivora. Gardner and co-workers (5) have demonstrated that in the ox, sheep, horse, rabbit and hippopotamus, cholesterol is never excreted in the feces of healthy animals; cholesterol excreted in the bile, including that which arises from the breakdown of red blood cells, is completely reabsorbed, demonstrating the so-called "cholesterol cycle." Gardner concludes that this substance is strictly conserved in the metabolism of herbivora, and any waste in such animals must be replaced by synthesis.

The early work attempting to demonstrate the absorption of plant sterols into the animal body and their subsequent conversion to cholesterol gave inconclusive results. Schoenheimer (6) with the aid of others was able to show that the intestinal wall of a variety of species is impervious to plant sterols which, when fed in known amounts, are excreted quantitatively in the feces; in the tissues they act as foreign bodies. Cholesterol, on the other hand, is quite readily absorbed.

It has long been known that toxic effects are produced in herbivora when they ingest cholesterol, which is not a normal constituent of their dietary. In 1912, Anitschkow (7) produced atherosis—a condition similar to human athero-sclerosis—in rabbits by feeding this substance. It is quite conceivable that were plant sterols absorbed and converted to cholesterol, such toxic actions would long ago have exterminated the herbivora. The absorption studies definitely explained this misconception.

Early balance studies suggested that the animal cell was able to synthesize cholesterol, a fact confirmed later. Schoenheimer and others have definitely demonstrated that cholesterol is an animal product. They (8) have further shown that the body can destroy cholesterol. This destruction, together with the existing excretory mechanism, appears to be the means by which the carnivora and omnivora protect themselves from the toxic effects of this substance as compared to the herbivora. No one has ever produced in carnivora and omnivora such changes as occur in rabbits, by feeding cholesterol. As an interesting correlation in the evolution of a bodily defense mechanism, it can be pointed out that cholesterol is a constant constituent of the dietary of the carnivore and omnivore, whereas it is foreign to that of the herbivore.

The physiological significance of cholesterol is still a mystery. It exists in the body in two forms: as the free or alcoholic condition, and as the ester combined with the higher fatty acids. The red blood cells and the brain contain practically no ester cholesterol. Other tissues of the body exhibit an entirely different picture. The blood plasma contains 65 to 75 per cent of the cholesterol in the ester form. Organs such as the white blood cells, adrenals, kidneys, heart, and liver contain varying proportions, from one-tenth to one-half as ester. Mueller (9) has suggested the probability that the two forms serve quite distinct functions. Recent work has tended to enhance rather than negate this belief. Free cholesterol is known to act, at least in vivo, as a neutralizing substance to a number of different poisons such as saponin, tetanolysin, and as a hemolytic agent of the pneumococcus. Keyes (10) suggested that cholesterol is antagonistic to lecithin which is a hemolytic agent in large quantities. This is important in the light of the high cholesterol content of the red corpuscles which, according to Bloor (11), are active in the formation of lecithin. Bloor and MacPherson (12) also have found low cholesterol values of blood in anemia. Windaus (13) has shown the strong inhibitory action of cholesterol towards hemolytic substances such as the unsaturated fatty acids. Cholesterol exerts a neutralizing and inhibiting action of the lipolytic enzymes, and thus may help regulate the rate of lipid digestion within the cell; it aids the other cell lipids in giving to cells their power of holding large quantities of water without losing their peculiar semi-fluid character, and without dissolving. This sterol also forms a large part of the wool fat known as lanolin, and as a constituent of the sebum of the skin, protects the epidermal structures.

Mueller (14) has shown that the cholesterol of the newly laid hen's egg is practically all in a free condition and that during the period of incubation this condition obtains until about the thirteenth day, from which time there is a gradual esterification until, at the time of hatching, over 40 per cent of the cholesterol is present in the form of ester. Hanes (15) believes that the cholesterol ester ordinarily functions as a detoxifying agent in the following way: the lecithin is broken down in the latter stages of embryonic development to furnish phosphorus for calcification, and the toxic fatty acids thus set free are combined with the free cholesterol to form harmless cholesterol esters. Free cholesterol is a part of Mayer's (16) lipid "élément constant," and remains an essential part of the living structure of the protoplasm along with the phospholipids and proteins until the death of the animal.

The distribution and occurrence of free cholesterol in the body apparently bears no relation to the fat metabolism, whereas the ester fraction appears to function in this respect. Heuck and Wacher (17) believe it plays an important role in fat metabolism since they found that cholesterol added to the food appeared in the blood stream combined as esters with fatty acids; also that the artificial enrichment of the diet in cholesterol not only led to a hypercholesterolemia, but to an increase in the fatty acid fraction of the blood lipids. Bloor (18) has noted a constant relationship between the total lipids, lecithin, and cholesterol. He has repeatedly suggested that cholesterol has some important function in late fat metabolism. According to Shope (19) blood serum from calves at birth, before having received colostrum, contains cholesterol only in the free form, and that in very small quantities. Soon after nursing for the first time, cholesterol ester appears in the blood serum and the total cholesterol begins to increase.

Bloor (20) asserts that the fatty

acids combined with cholesterol in beef, pig and dog blood are more highly unsaturated than those occurring in the other lipid constituents of the blood. This same result has been demonstrated in the blood of lactating cows by Schaible (21); and Maynard and McCay (22) have shown that during lactation the changes in cholesterol parallel those occurring in the other blood lipids. The observations of Williams and Maynard (23) indicate that the blood cholesterol changes during lactation, aside from the influence of the mammary gland upon the blood lipid level, result from the difference in the level of lipid intake rather than from the absorption of specific types of fats. These changes occur mostly in the ester fraction, the free cholesterol changing very little, if any.

Mueller (24) has shown that cholesterol is absorbed by the animal body from the intestinal tract by way of the thoracic duct. Irrespective of whether it is fed in the free condition or in the form of esters of the higher fatty acids, it is always found to occur as a mixture of about one part free to three or four parts ester by the time it reaches the chyle. Changes in the plasma cholesterol content of both man and animals can be brought about by sufficiently prolonged feeding of low or high cholesterol containing diets. There appears to be no relation between the level of plasma cholesterol and the amount ingested. The ratio of free to ester is frequently observed to be disturbed. In the words of Gardner (5):

"These peculiar changes cannot be explained by the influx of cholesterol absorbed from the alimentary canal, but are evidence of an active endogenous metabolism, in which cholesterol takes part, during digestion. Apart from the changes first mentioned, the only physiological condition in which the value of the plasma cholesterol is altered markedly is in pregnancy. In this condition, from about the twentieth week, the free cholesterol increases to a maximum which it reaches about the thirtieth week, and concurrently the ester cholesterol falls to a minimum. From this time the amount of free cholesterol decreases and the ester increases till an almost normal ratio of free and ester cholesterol is reached before or near parturition."

From the biochemical standpoint, free cholesterol appears to have a quite distinct role from that of the ester. It seems to function as an essential constituent of the living structure of protoplasm. The cholesterol ester apparently acts as a transport agent in fat metabolism and its formation in certain instances appears to be the result of the detoxification of the unsaturated fatty acids. Further speculation as to the possible role of cholesterol in normal metabolism results in a chimera.

Singularly, cholesterol has as much interest for the pathologist as for the biochemist and physiologist. However, its situation in abnormal cellular function is even more obscure than in normal metabolism. Hypocholesterolemia occurs at the height of the fever in a variety of febrile and infective conditions. It is also reported in hyperactive thyroid cases and in certain types of anemia. Hypercholesterolemia occurs in certain typs of nephritis (particularly where there is diffuse tissue degeneration and edema), and also in hypothyroidism, biliary stasis, and diabetes. Biliary stasis appears to be merely the obstruction of one of the three normal pathways of cholesterol excretion. In this connection Whipple's (25) statement on the source of bile cholesterol may be quoted:

"It is suggested by various writers that bile cholesterol results from the secretion of the biliary tract epithelium, from liver parenchyma degeneration, from red cell disintegration, from general tissue wear and tear, from food cholesterol, from the adrenals, and other glands of internal secretion, etc."

He further remarks that:

"Nobody has as yet suggested that it is derived from the wear and tear of the cerebral cortex under the stress of environmental conditions!"

The diabetic fluctuations of blood cholesterol appear to be associated with the deranged fat metabolism. Blood cholesterol determinations in cases of diabetes serve as a valuable prognostic tool in the hands of clinicians. The changes occurring in the blood cholesterol in pathological conditions appear to be the effect rather than cause; they occur as a result of tissue degeneration, faulty fat metabolism, and obstruction to an excretory outlet.

The early work of a number of investigators (26, 27, 28) demonstrated the production of fatty livers in rabbits by feeding cholesterol. Recently other groups of workers (29, 30, 31, 32) have made similar observations using rats and other animals. The results of these researches have apparently opened up a new direction of interest in the study of cholesterol metabolism. The daily ingestion of 100 milligrams or more of cholesterol produces fatty livers in the experimental animals. The weight of the liver is greater in terms of body weight, the increased weight being due partly at least, if not wholly, to the increased fat content. The excess lipid is composed of neutral fat and cholesterol esters, the phospholipids remaining practically unchanged. Best and co-workers (30) have observed that the feeding of small amounts of choline along with the cholesterol prevents the liver from becoming fatty. Choline appears to antagonize the deposit of neutral fat and retards the accumulation of the cholesterol esters. Chanutin and Ludewig (32) have studied the lipid content of other organs, such as heart, brain, blood, and kidneys after cholesterol ingestion and have observed no changes.

Williams, Anderson, and Mendel (33) have studied this problem using relatively purified diets. The main interest has been in the possible role of cholesterol in intermediary fat metabolism. Diets rich and poor in fat as devised by Anderson and Mendel (34) were fed to control groups of rats, whereas the same diets to which 1 per cent of cholesterol was added were furnished to experimental animals. A further control of the experiments was accomplished by allowing each rat to ingest the equivalent of 3,000 calories of the respective diets.

Regarding the outcome of these experiments, it was observed that the lipid content of the livers of the control animals was similar on diets either rich or poor in fat; on the other hand, when cholesterol was added to the ration, the liver weights of the rats ingesting the high fat diets increased 20 to 30 per cent in terms of body weight. This increase was not evident with the low fat diet. Apparently the cholesterol was not absorbed. Addition of 1 per cent butter oil to the carbohydrate ration has increased the absorption, but still not to the extent obtained with the high fat diets. A study of the adrenals reveals no effect of the cholesterol ingestion on their cholesterol content. However, the adrenal weights were greater in the animals receiving the rich fat ration than in those receiving the diet poor in fat. Moehlig (35) has previously made a similar observation. Some evidence was obtained that cholesterol ingestion increased the ester fraction of the blood cholesterol, an observation in line with the view that the esters aid in fat transport. A study of the lipid composition of the fatty livers reveals a large increase in neutral fat and a marked increase in cholesterol esters; on the other hand, the phospholipid content appears unchanged. Iodine values of the different lipid fractions show that cholesterol probably exerts a saturating effect upon the fatty acids. The only apparent conclusion from these data, at present, is that cholesterol is in some way concerned with fat metabolism, especially where the liver is involved.

In summary, we can still state that the role of cholesterol in the vital process remains a mystery which thirty years of research have failed to solve. That it has a function in cell life can hardly be denied. It confronts us from the epidermis to the inmost bodily protoplasm; from the cerebral cortex to the pedal capillaries. The renewed activity in studying this problem at the present time is a reasonable indication that new light will soon be shed on the function of this important biochemical compound.

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THE METHOD OF

SOYBEAN OIL EXTRACTION

AS DEVELOPED AT THE EDISON INSTITUTE OF TECHNOLOGY*

By H. JOYCE
Edison Institute, Dearborn, Michigan

The Edison Institute of Technology was founded in 1929, by Mr. Ford in honor of Thomas A. Edison. The purpose of the Institute is to train students along the lines that Edison pursued in his work and to give them an opportunity to study farm products for industrial possibilities.

In Mr. Ford's Early American Village, in Dearborn, the Edison Institute has its research laboratory which is large enough for semi-plant production; the raw materials for carrying out experiments come from an experimental farm. The students deal with the practical problems and some of the products of the Institute have been used in the Ford car.

After working on practically every principal farm crop grown in the United States we finally chose the soybean for complete development because of its versatility. It not only has a wide use in industry but possesses a high nutritional value. It is complete enough to sustain life for a good length of time. The soybean is a summer leguminous annual, the beans resembling navy beans in size. The Orient and the United States produce 94 per cent of the world's crop, 14 per cent being grown in the United States. The bean contains 17 to 20 per cent oil, 35 to 40 per cent protein and from 33 to 35 per cent carbohydrates which are digestible. It is rich in minerals and contains vitamin A, B, and D and has an alkaline ash. The soybean may be grown wherever corn or cotton is grown, taking from 70 to 120 days to mature.

After a series of experiments with the soybeans it was found that the meal could be utilized in the manufacture of plastic material and the oil in making enamels for the car. The meal, to be satisfactory for use, in the plastic, however, had to have an oil content of 2 per cent or lower. We decided to develop an extractor because the previous methods of extraction did not remove the oil to this extent with sufficient speed and economy. The batch system of extracting the oil is not continuous, and the time required for extraction is excessive. The pressing method is not continuous and the pressed meal contains too high an oil content to warrant its use. The expeller method is continuous but the meal also contained too high an oil content to be used.

We wanted to build an extractor suitable for our needs and one that the farmer could afford to buy and operate. It must be compact, operate continuously, and would remove 95 to 98 per cent of the oil.

The work was started in the laboratory using a small Soxhlet apparatus and, after observing its action, a large apparatus was built that would handle 20 pounds of meal. This method of extraction

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