edema fluid/serum cholesterol ratio was 7%). Where lymphatic obstruction was the predominant factor in edema formation, the cholesterol content averaged 175.2 mg % (average edema fluid/serum cholesterol ratio was 58%). The average total cholesterol/protein nitrogen ratio in edema fluid is 0.26 ± 0.14.

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The Urea Complexes of Unsaturated Fatty Acids

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The phenomenon of urea complex formation with aliphatic straight chain compounds was discovered in 1940 by F. Bengen.[1] He showed that normal aliphatic compounds form complexes with urea by addition, whereas branched and cyclic compounds do not, thus allowing the separation of straight chain compounds from the others by complex formation. Bengen and Schlenk, in a preliminary report emphasizing that the complexes formed are of a type so far unknown (*1*), recently summarized research in this field during the intervening years. Zimmerschied *et al.* called attention to, confirmed, and extended the observations in the original patent application (*3, 4*). Schlenk reports in detail on the formation of urea complexes in relation to the shape of the organic molecule, their composition, their crystal structure, and their energy of formation (*2*).

These reports emphasize that to form urea complexes, straight chain molecules are required. It was of interest to learn what influence the *shape* of unbranched molecules has upon the urea addition. For this study the unsaturated fatty acids of the C_{18} series were chosen because the double bonds alter the shape of the molecules. In general, it was found that the unsaturated fatty acids form urea addition complexes also. The degree of unsaturation, the position of the double bonds, and *cis-trans* isomerism do not markedly influence the composition of the complexes, 14.0–14.5 moles urea per mole C_{18} acid.

A remarkable property of the complexes of the unsaturated acids is their resistance to autoxidation. This is illustrated in Fig. 1. In another experiment with larger quantities, the autoxidation of soybean fatty acids and their urea complexes was followed for several weeks by means of their peroxide contents (Table 1). From these experiments, performed in October, 1949, it is apparent that the unsaturated fatty acids are inaccessible to oxygen in the form of complexes. This is understandable from the crystal structure of urea addition complexes (*2*).

[1] German patent application O.Z. 12438 (March 18, 1940); Technical Oil Mission Reel 6 frames 263–270 in German, Reel 143 pages 135–139 in English.

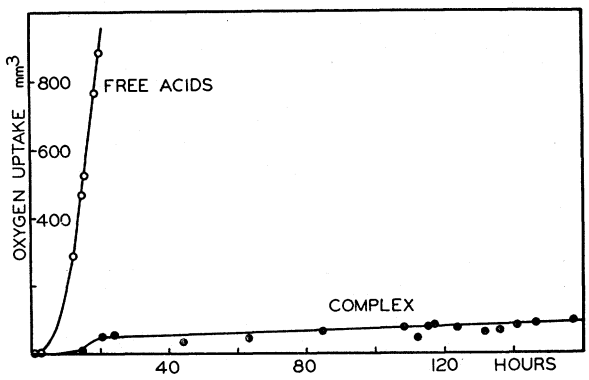

FIG. 1. Oxygen absorption of soybean fatty acid complexes and their freed acids in the Warburg respirometer at 37° under air. Samples: 400 mg complex, 90 mg freed acids.

TABLE 1

PEROXIDE VALUES OF SOYBEAN FATTY ACIDS AND THEIR COMPLEXES EXPOSED TO AIR AT ROOM TEMPERATURE

	Weeks			
	0	1	2	3
Free acids	1	82	193	260
Complex acids	1	..	6	3

TABLE 2

ENRICHMENT OF THE SATURATED AND UNSATURATED COMPONENTS OF FATTY ACID MIXTURES

Fatty acids		Urea	Complex acids		Non-complex acids	
Source	g	g	Yield, g	I.V.*	Yield, g	I.V.
Soybean, I.V. = 141 ...	100	30	9	56	81	162
Soybean, I.V. = 141 ...	100	100	37	88	56	180
Soybean, I.V. = 141 ...	100	200	67	119	27	191
Chinese tallow, I.V. = 19	51	100	27	6.5	18.5	38
Olive, I.V. = 80	50	15	5.5	54	36	93

* I.V. = Iodine value.

Although all normal saturated and unsaturated fatty acids thus far investigated form urea addition complexes, the yields under identical conditions vary widely. This can be caused by differences in the relationships:

$$x\ \text{Acid} + y\ \text{Urea} \rightleftharpoons \text{Acid}_x \cdot \text{Urea}_y$$

and can be used as a basis of separation of various types

of unbranched compounds. A series of experiments in which urea was added in amounts insufficient for total complex formation is summarized in Table 2. Under such conditions, saturated long chains combine with urea preferentially. In another experiment soybean oil fatty acids having iodine value 141 were separated into fractions having iodine values 86, 148, 181, and 200. In the same manner, other enrichments have been achieved. Autoxidized soybean fatty acids, peroxide value 33, were separated into fractions having peroxide values of 15 and 86. A mixture of lauric and stearic acids (50/50) having acid number 240 was fractionated to acid values of 207 (12/88) and 266 (83/17). Similar experiments have shown that urea complexes can be used for the separation of normal aliphatic compounds of different chemical character.

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An Ideal Preparation for Dissection of Spinal, Peripheral, and Autonomic Nerves of the Rat[1]

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In the normally nourished rat, the presence of large amounts of fat and bulky opaque muscle makes it very difficult or impossible to dissect out any except the largest nerves; furthermore, it is often difficult to distinguish glandular from fatty tissue. Ordinary starvation does not remedy this situation, since in the 3–5-day period that a rat survives without food, only a small amount of fat and very little muscle tissue is lost.

Only recently it was found that the method used in nutritional experiments carried on for many years in this laboratory with the so-called "single-food choice" diets (*3*) may provide ideal preparation for dissection of nerves and for differentiation between fat and glandular tissue in the rat. In the simplest form of these experiments, rats of a standard age and weight are kept on a diet limited to water and one foodstuff (for instance, dextrose, sucrose, olive oil, butter, casein, or lactalbumin) and the survival times are taken as a measure of the nutritional value of the foodstuff. On dextrose the rats live on the average 37 days—that is, 33 days longer than on no food at all. In a more complicated form of these experiments the rats have access to water and one foodstuff and also to a supporting substance: for instance to a single purified food, such as dextrose, and to a supporting substance such as thiamine. The increase in survival time over that obtained on the single food alone gives a measure of the part played by the supporting substance in the utilization of the foodstuffs. For example, on dextrose and with access to thiamine the rats live 76 days, over twice as long as on dextrose alone, thus giving a dramatic demonstration of the part played by thiamine in the utilization of dextrose (*4*). In slightly more complicated experiments the rats have access to a combination of foodstuffs, such as a solution of dextrose (15%) and alcohol (15%). On this diet the rats lived on the average 37 days, and with access also to a thiamine solution, 55 days.

Most of the specimens used for dissection of nerves and glands were rats that had been on the dextrose-alcohol-thiamine diet for 40–60 days. Specimens obtained with a diet of dextrose or sucrose, and thiamine (without alcohol) would have served just as well.

Of interest for the present purpose is the fact that on these single-food-choice diets the rats continue to live for a long period of time, lose weight at a slow rate, and after 40–60 days show no symptoms of nutritional deficiency except emaciation. Their teeth, skin, hair, and bones appear normal; none of the internal organs shows any lesions. However, the changes that result from emaciation make them ideal specimens for dissection. Not one trace of fat remains; most of the muscles are so thin and transparent that the underlying tissues may clearly be seen through them (for example, the lungs are visible through the muscle walls of the thorax); the cranial and sacral autonomic nerves, the sympathetic nerves and rami, and the spinal nerves stand out clearly without any obstruction; the glands of internal and external secretion are at least as large as in normal rats of the same size.

Special use of these prepared specimens has been made for the differentiation between true fat and tissue that often may be mistaken for fat. For example, the rat has deposits of so-called brown "fat" in several locations on the body—between the shoulder blades, retroperitoneally and retrothoracically along the spinal column, and near the salivary glands (*1*). In a normally nourished rat this brown fat can be distinguished from the surrounding fat, but often with some difficulty. In the partially starved rats the brown fat persists long after all regular fat has gone, and its dark red-brown color stands out sharply against the muscles. The response of this tissue to partial starvation is entirely different from that of regular fat, so that its designation as fat is probably a misnomer and its designation as a gland (hibernating?) may be more correct.

Glands of internal as well as of external secretion, such as the preputial glands that may be mistaken for fat, can also be clearly differentiated from fat by this method. The absence of fat surrounding the glands in these cases makes it possible to distinguish all the autonomic nerves that lead to them.

One of the most striking effects of the single food diet is the complete arrest of bone development. E. A. Park, who is making a study of the bones in these rats, states that the arrest is more complete than he has ever observed.

[1] Carried out under a grant from the Corn Industries Foundation, New York City.