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.

At present he is using this arrested growth as a base line, adding nutrients one by one and determining which will renew bone growth.

We have not as yet tried the single-food-choice technique on larger animals, such as the dog, cat, or monkey. Some nutritional observations made in 1816 by Magendie (*2*) suggest that it may work quite as well on dogs and within much the same time limits. Magendie found that dogs kept on an exclusive diet of butter or sugar or gelatin lived 30–36 days, which is about the same length of time that rats live on these foodstuffs alone.

In summary, a method of prolonged partial starvation has been described which provides ideal preparation for dissection of spinal, peripheral, and autonomic nerves of the rat; also for the differentiation between glandular and fatty tissue.

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Fibrinolytic Activity of Purified Thrombin[1]

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Highly purified thrombin at a concentration of 9,000 units per ml will completely dissolve a 0.5% fibrin clot at 37.5° C within a 90-min period. Evidence that thrombin is responsible for dissolution of the clots is based upon the degree of purification of thrombin preparations, failure of prothrombin from which the thrombin was derived to lyse fibrin clots, equal ability of thrombin prepared by two different methods of activation to lyse fibrin, relative lack of inhibition of fibrinolysis in the presence of antifibrinolysin (antiplasmin) or soybean antitrypsin, and heat inactivation of thrombin which parallels the decrement in lytic activity.

Reagents used in fibrinolytic tests consisted of fibrinogen substrate, prothrombin or thrombin buffered with imidazole, and in some experiments antifibrinolysin or soybean antitrypsin. Fibrinogen, prepared from bovine plasma by the freeze–thaw technique (*21*) was free of demonstrable fibrinolysin (plasmin) and profibrinolysin (plasminogen). Prothrombin was prepared from bovine plasma by techniques which have been described (*20, 22*). These products had specific prothrombin activity values ranging from 1,200 to 1,400 units[3] per mg of dry weight. Thrombin was prepared from prothrombin by activation with citrate or thromboplastin (*18, 20, 22*). Reactants in the lytic tests were buffered at pH 7.2 with 1% imidazole. At this concentration imidazole has been found to be strongly bacteriostatic (*6*).

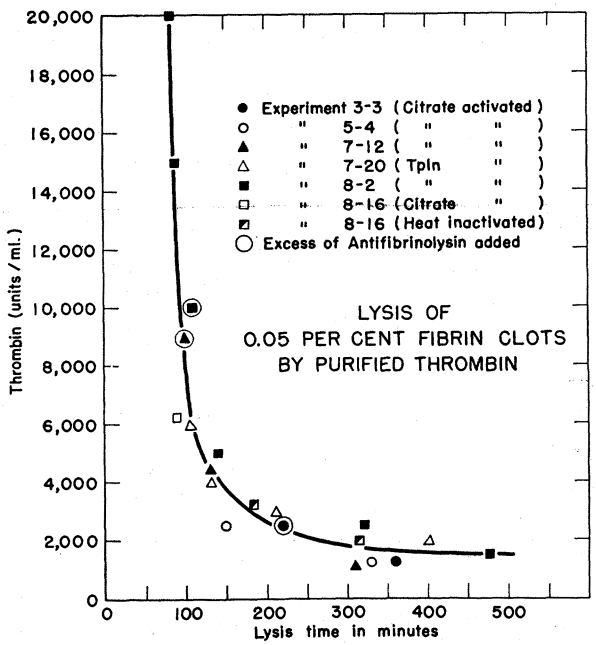

FIG. 1.

All tests for fibrinolytic activity were carried out at 37.5° C. Fibrin clots were formed in test tubes approximately 7 mm inside diam and 75 mm in length. To 0.2 ml of a 0.1% fibrinogen solution was added an equal volume of the substance to be tested. Lysis was considered to be complete when material in the tube flowed freely as the tube was tipped with the open end at an angle of 10° below the horizontal. At the point of complete lysis the solutions became clear and nonviscous. After the lysed solutions had stood for varying periods of time a flocculent precipitate appeared. The nature of this precipitate has not been investigated.

The plot on the graph shown in Fig. 1 results in deviation from the smoothed curve within the limits of error of the analytical methods used. Each experiment, indicated by a distinctive symbol, represents a single thrombin preparation which was used in its most concentrated form and in one or more dilutions.

All prothrombin preparations were checked for fibrinogenolytic and fibrinolytic activity prior to conversion to thrombin. Fibrinogen solutions containing prothrombin were clotted solidly by addition of thrombin after 72 hr of incubation. Similar solutions, to which a trace of thrombin was added at the start of incubation, clotted but did not lyse. These results indicate that prothrombin does not have a measurable lytic action on either fibrinogen or fibrin.

Since prothrombin preparations from which thrombins were derived had no fibrinolytic activity, it is evident that the lytic property appeared as a result of prothrombin activation. Two methods of activation were used.

[1] Aided by a grant from the U. S. Public Health Service, National Institutes of Health.

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[3] The unit of prothrombin and the unit of thrombin used in this work are those described by Ware and Seegers (*24*).