which is to the greatest extent accounted for by a slight rise of the carbohydrate containing alpha globulin. No evidence was obtained that the carbohydrate content of this small increment differs from that of the normal alpha globulin. In contrast the marked rise of gamma globulin is associated with an only very small increase of gamma globulin associated carbohydrates. This suggests that the excess gamma globulin in experimental tuberculosis differs from the normal gamma globulin by a very low carbohydrate content and suggests a different nature and possibly a different origin of this increment. The serum protein-bound lipids are not altered in experimental tuberculosis.

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Increased Metabolism in Fat-Deficiency: Relation to Dietary Fat.* (22827)

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Elevation of metabolic rate is consistently observed in rats fed a diet devoid of fat. As early as 1931, Wesson and Burr made reference to increased basal and carbohydrate assimilatory rates(1), findings which were confirmed and extended by Burr and Beber(2). It has been pointed out that disturbance in energy balance as indicated by increase in basal oxygen consumption is probably the earliest and most fundamental manifestation of the fat-deficiency syndrome, being recorded within 7-14 days after the start of the experimental diet in rats(3,4).

The manifestations of fat-deficiency are corrected by the administration of linoleic acid in amounts too small to exert an influence as a metabolite (*i.e.* "fat as fat *per se*"), indicating the specific role of this fatty acid as an essential substance(5). It is the purpose of this presentation to report the effect on oxygen consumption and body weight of

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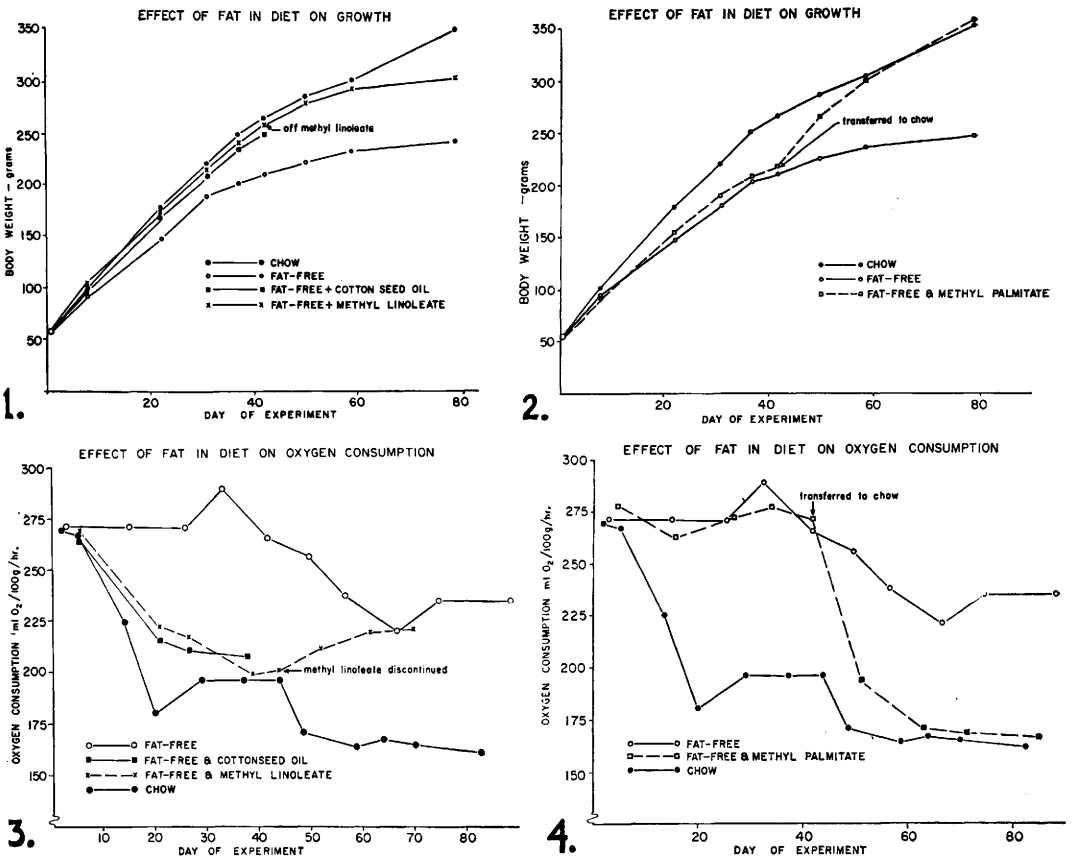

FIG. 1-4.

small amounts of saturated and unsaturated fatty acid preparations.

Materials and methods. Rats of the Holtzman strain were placed on experimental diets at weaning. Group I (12 rats) received the fat-free diet regularly used in this laboratory (3). Group II (12 animals) received the same fat-free diet supplemented by the daily administration for 6 weeks of 100 mg methyl linoleate‡ directly into the mouth via syringe. Tocopherol was added to the methyl linoleate for antioxidant effect in ratio of 1 to 100. It became necessary to discontinue this supplement after 6 weeks because of unexpected unavailability. Group III (12 rats) was fed the same fat-free diet similarly supplemented by daily administration of 100 mg methyl palmitate. Original plans for transfer from the palmitate to methyl linoleate were impossible, and the animals were transferred to laboratory chow.§ Group IV (12 rats) received the same fat-free diet similarly supplemented daily for 40 days with 200 mg cottonseed oil‖ which contains approximately 50% linoleic acid. Group V (15 rats) was fed laboratory chow. The animals were weighed weekly and observed for development of dermatitis. Oxygen consumption was determined weekly on 5 animals from each group according to a method previously described(3), modified by addition of a circulating pump. All determinations were carried out in an air-conditioned room with animals in a fasting, resting state; duplicate prolonged tracings were made after the animal had become motionless. The duration of the experiment was 11 weeks.

Results. The effects of various diets on growth are depicted in Fig. 1 and 2. It will

‡ Obtained from Hormel Institute, Austin, Minn.

§ Purina.

‖ Wesson.

be noted that supplementation with methyl linoleate resulted in gain entirely comparable to that achieved by animals fed laboratory chow, whereas an equal amount of saturated fatty acid (methyl palmitate) resulted in growth identical to that produced by feeding the fat-free diet alone. Following discontinuation of methyl linoleate, growth continued unaltered for approximately 20 days, after which it decelerated so that at 80 days, the mean weight of this group was significantly lower than that of chow-fed animals. In contrast, transfer of the methyl palmitate group to chow resulted in a pronounced acceleration in weight gain, which reached the level of chow-fed animals within a period of 20 days.

The basal oxygen consumption rates in the group receiving linoleate and cottonseed oil supplements were not significantly different from that of chow-fed animals (Fig. 3). Following withdrawal of methyl linoleate, oxygen consumption returned gradually to the level of fat-free controls. Comparison of these findings with those shown in Fig. 4 reveals that supplementation of fat-free diet with methyl palmitate produced no change in oxygen consumption rate. Following transfer to laboratory chow, a pronounced decrease was noted after 10 days and within 20 days the rate was identical with that of the chow-fed animals.

Although characteristic dermatitis developed in fat-free animals after approximately 9 weeks, no such changes were observed in any other groups. In the group in which supplementation with methyl linoleate was withdrawn after 6 weeks, no skin changes were observed during the remainder of the experiment although significant alterations in oxygen consumption and weight gain were observed.

Discussion. The amount of methyl linoleate administered in these experiments constituted the equivalent of approximately 1.5% of the total daily caloric intake as fat. It seems clear that the effects on oxygen consumption and body weight cannot be attributed to the simple caloric contribution of linoleate but to the provision of a specifically required substance. This conclusion is supported by the failure of a calorically equal amount of methyl palmitate to influence either oxygen consumption or weight. Although absorbability of tripalmitin from the gastrointestinal tract is known to be poor(6), that of palmitic acid appears to be great enough(7) to substantiate the use of methyl palmitate as an experimental control.

Supplementation with cottonseed oil to provide 3% of the calories as fat resulted in changes essentially identical to those following methyl linoleate alone, indicating that the effects were due to the linoleic acid content. Deuel(8) has shown that the increase in weight of male rats receiving 50 mg methyl linoleate daily approached that found in animals receiving 53% of the calories as cottonseed oil and has suggested that larger doses, 100 mg or more, might maintain weight comparable to rats receiving the high fat diet.

Recently, Hansen and coworkers(9) have reported that infants fed to satiety with skim milk (1% calories as fat and 0.04% as linoleic acid) consumed more calories per kg of weight gain than did infants ingesting a standard milk mixture providing 38% of the calories as fat and 0.9% as linoleic acid. When the skim milk diet was supplemented daily with an amount of methyl linoleate equivalent to 1% of the calories, the daily caloric consumption per kg body weight decreased 20-30% without affecting weight gain or activity. These findings suggest that fat-deprivation increases metabolism in human infants as in rats, with resultant increased caloric requirements and intake. The implications of these observations on principles of infant feeding are obvious, particularly in view of the popularity of low-fat milk mixtures in clinical practice.

Summary. Disturbance in energy metabolism, as indicated by increased basal oxygen consumption and growth retardation, is prevented when rats receiving a fat-free diet are given daily supplements of methyl linoleate (100 mg) or cottonseed oil (200 mg). However, similar supplementation with a saturated fatty acid (methyl palmitate, 100 mg) in no way modifies the development of the fat-deficiency syndrome.

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Improved Procedures for Preparation of Tissues for Chemical Analyses.* (22828)

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Many years of work on histochemical characterization of various tissues have required different technics for their preparation for precise chemical analyses. Previously, some soft tissues were minced and aliquots of wet minced tissue were weighed for all analyses: skeletal muscle(1,2), brain(3) and heart(4); some tissues were minced, dried and smashed in a special apparatus for all analyses: liver (5,6), skin(7,8), kidney(9), cartilage(10,11).

The method described here has many advantages: it is saving in time and effort while producing material excellent for sampling; it permits a uniform routine for all soft and hard tissues, especially cartilage; and it provides for numbers of tissues being taken from the same animal simultaneously and handled more satisfactorily.

Procedure. Tissues are taken from anesthetized animals, wiped with dry gauze and dropped quickly into ground glass-stoppered jars. After being cooled in a refrigerator to minimize water loss, the tissues that have visible connective tissue or fat are placed on a tile and adherent structures removed. The tissues are then dropped into glass-stoppered weighing bottles and again cooled before mincing with points of scissors. Tissues such as liver and brain that do not have visible fat or connective tissue are simply cooled and minced. Skin after being trimmed is cut into strips 8×10 cm. Samples are allowed to come to room temperature, and then weighed into vycor crucibles in aliquots depending upon amount of wet tissue available. These samples can range to 10 g of wet tissue. It is preferable to weigh 2 or more samples of the same tissue to check determinations for water and fat. The crucibles are next placed in 100°C thermostatically controlled oven for 48 hours, after which they are cooled in a desiccator over activated alumina oxide and then weighed. After this, the dried tissues are covered with dry ethyl ether and placed in another desiccator containing sufficient ethyl ether to cover bottom of desiccator to about one inch. After remaining in this ether desiccator for 24 hours, the ether layer over tissue containing extracted fat, is removed with Pasteur pipet and new dry ethyl ether added. This process is repeated at least 3 times. After removal of last ether extraction, the crucibles are placed in 100°C oven for approximately 5 hours, then cooled in a desiccator over activated alumina oxide and weighed. This gives a dry, fat-free solid which can be kept in a desiccator until ready to be ground and analyzed.

Grinding the dry tissue. The mills used for grinding dry tissues were either a Zassen-

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