Fats in Relation to Caloric Efficiency^{**}

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FAT has several unique properties when used as a dietary component. Besides being a carrier of the fat soluble vitaming Λ , D, and **F** and and the fat soluble vitamins A , D , and E and providing essential fatty acids, it serves a very important purpose in conferring satiety upon the diet. The importance of fat has been further emphasized in recent years, chiefly because it has been in very short supply.

These problems have created much additional interest in fat metabolism in general, and our laboratory has been concerned for some time with the question of efficiency of utilization of diets of varying fat content. Although much is known concerning the functions of fat, it has been regarded, from a practical standpoint, as being interchangeable with carbohydrate on a metabolizable energy basis, and diets in which the fat is varied are usually prepared by changing the content of these two dietary components. It is by this method that the various diets used in our experiments were prepared. This obviously involves' a study of the interrelations of fat and carbohydrate, and it is logical to believe that utilization can be determined most reliably when these components constitute part of a normal diet.

The experimental subjects in this work have been either weanling or essentially mature male albino rats, the weanling rats being used in the body balance experiments and mature animals in all other work.

The rations used were compounded to contain various amounts of fat from 2 to 30% by weight of the ration with carbohydrate being interchanged for fat on a caloric basis. The protein and mineral contents were essentially the same in all rations. During the course of the work suggestions were received to increase the choline, pyridoxine, or pantothenic acid of the rations to make certain that these had not been at border-line levels of intake. In order to accumulate more data upon which to base conclusions and to satisfy the suggestions above, additional investigations were made in the same manner as previously but with greatly increased intakes of 10 of the vitamins. Results were unaffected by the new vitamin level, indicating that these nutrients were furnished in optimal amounts throughout the experiment. The vitamin intake was identical in all rations of any given experiment.

Basic components of the diet were 4% salt mixture, 2% corn oil, snfficient protein mixture of high quality proteins to constitute a $22%$ level in the diet, carbohydrate mixture, and lard, with the carbohydrate and lard being interchanged to adjust diets to desired fat level and to render all diets cquicalorie.

By separate analyses of the carbohydrate, fat, and protein mixtures and the use of simple algebra, it was possible to compound rations of given fat pereentagcs and which could be fed on an isoealoric basis. For instance, one gram of the low fat diet would be exactly equal in energy and protein content to a lesser weight of the high fat diet. The diets were fed to supply identical amounts of protein and energy.

Three methods of experimentation were followed, all measuring the heat production by an indirect approach, namely, a) the body balance method, b) the Haldane respiratory quotient procedure, and e) the carbon-nitrogen balance method.

The body balance method, as developcd in this laboratory, provides for normal freedom of movement of the animal with quantitative feed allowance and collection of excreta. A single measurement of the heat production was made for a 70-day period hy subtraction of the energy of the excreta and of the body gain from the gross energy of the food. The desirable points in the use of this method are that a) the heat is measured over a very long period of time and b) dimunition rather than magnification of experimental error prevails.

The body gain in this procedure is determined by difference between analysis of eontrol animals sacrificed at the start of the experiment and analysis of the experimental animals which are killed at the end of the experiment. The bodies were dried in vacuum desiccators, extracted in large Soxhlet extractors with ether, and ground first through a large Wiley mill and then through a burr-type or Excelsior mill. The ground samples were very finely divided, and excellent sampling was possible.

In the determinations of heat increments or energy expense of utilization of diets the well-known Haldane respiratory quotient proeedure was used. As a part of this experimental method the excreta were collected for a period of at least 8 days after first establishing the animals on a constant feed intake of one of the diets to be tested. The long collection periods were used in order that the error resulting from irregularities of excretion would be minimized and to allow time for the animals to become thoroughly adjusted to the experimental conditions.

An important part of this procedure for the determination of heat increments was the use of a maintenance plane of nutrition as a base value instead of the fasting heat production. The heat increment was determined by this method as the increase in heat production between a maintenance and a supermaintenanee plane of nutrition of the same diet. The technique of using a fasting heat production is further involved by the dynamic effect of the catabolism of body tissue.

Dynamic effeets determined by relating the fasting heat production in which no food is involved, to that resulting from the ingestion of a single foodstuff, can hardly be expected to represent what occurs when this food is incorporated in a nutritionally "wellbalanced" diet.

The last method to be described for the indirect measurement of heat is the carbon-nitrogen balance procedure. This has been used since the time of Volt and needs little comment except one point which can be made that will doubtless do much to establish its

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Fat content of diet	Gross energy intake	Energy intake			Metaboliz-	Energy output			Energy
		Protein	Carbohy- drate	Fat	able	In feces	In urine	As heat	retained
-25	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.
	2854 2854 2854 2854	799 799 799 799	1912 1713 1456 657	143 342 599 1398	2601 2605 2610 2615	128 126 121 123	125 123 123 116	2195 2165 2154 2155	406 440 456 460

TABLE 1 Partition of Average Daily Intake of Energy Per Rat During 70 Days¹

By body balance procedure.

accuracy to one unfamiliar with this field. In the use of the large respiration calorimeter at The Pennsylvania State College this method of calculation of heat production has always been used and comparisons made with the heat directly determined. Covering a period of years and many experiments, the average agreement in heat production between these two methods, one direct the other indirect, has differed by less than 1% .

The respiration apparatus used in this method was constructed of sheet copper with water seal and of sufficient size to permit the usual type individual rat eage to be placed in it (1). The reason for using this different type apparatus was that respiration measurements could be made for 2-3 days continuously under conditions of normal voluntary activity. The heat production thus measured was very representative of normal conditions.

Three experiments have been conducted by the body balance procedure—the first of these with 4 groups of 10 rats each with equicaloric consumption of diets of 2, 5, 10 or 30% fat (2) . The animals were selected from 10 litters, one rat from each litter being given one of the 4 diets. A modification of the paired-feeding method was used with the amount of feed given to each rat of any given litter being determined by the individual of the four which ate the smallest amount of food.

The acceptability of the diets increased as the fat content increased with only 3 refusals of feed during 70 days by the rats on the 30% fat diet as compared to 32 refusals by the rats receiving the 2% fat diet.

The rats on the four diets of 2, 5, 10, and 30% fat gained on an average 171 gm., 183 gm., 188 gm., and 191 gm., respectively, during the 10 weeks of the experiment. The rats on the 30% diet thus gained an average of 20 gm. more than their litter-mates on the 2% fat diet.

Figures relating to the partition of feed nitrogen are not given although they show a reasonable degree of significance in the experiment. However, in the other extensive experiments to be discussed here, the fat content of the diet was without significant influence on the nitrogen retention, warranting the general conclusion that the fat content of the diets, as studied, was without positive influence on the utilization of nitrogen.

It is interesting to note in connection with the discussion of nitrogen metabolism that this type of experiment permits the computation of recovery of feed nitrogen in the urine, feces, and body gain. The average figure for recovery in these experiments was about 98.5% of the feed nitrogen.

The complete energy balance is given in Table 1, and from this it is seen that only slight differences in loss of energy in urine and feces occurred while a greater difference was manifested in heat production.

The odds that the heat production, computed by Student's method, from the 30% fat diet was less than from the 2% fat diet were 59 to 1; but the difference in energy retained was of greater significance, the odds being 253 to 1 that the amount of energy retained from the 30% fat diet was greater than that retained from the 2% fat diet. With respect to the utilization of energy the increased efficiency of the 5% fat diet as compared with the 2% fat diet was much greater than that of the 30% fat diet as compared with the 5% fat diet.

A second experiment was conducted as nearly alike this one as possible except that a much higher level of 10 of the vitamins was given than formerly. Fat levels of 2, 10, and 30% were compared with the 5% level being dropped (3) . As the results of this experiment are very similar to the one just described, further tables presenting the data will be unnecessary. The discussion will therefore be made on the basis of the data shown in Table 1.

Statistically significant results were obtained for greater body gains of fat and energy, and decrease in heat production, in the order of increasing fat contents of the diets. The significance of this decrease in heat production is expressed by odds of 1,999 to 1, and the increase in energy retention by odds of 98 to 1, in the order of increasing fat contents of the diets.

In a third experiment, using the body balance proeedure, a low (2%) and a high (30%) fat diet were compared in which the protein content was reduced from the usual 22% level to 7% (4). This was done to determine if the level of protein in the diet had any influence on the manner in which the energy was more efficiently utilized in the high fat diets.

Two groups of weanling rats of 12 each, selected as litter-mate pairs, were fed by the paired feeding method for 70 days in the usual manner. Results with this work added further confirmation to the original conclusions with regard to heat production and gain of energy. The rats on the high fat diet gained on the average 14% more energy in the form of fat than the group on the low fat diet. The total gain of energy was 10.5% greater in the rats on the high fat diet.

Although the significance of some of these figures is less than when the higher level of protein was fed, the results are qualitatively in complete agreement with the other work. As the food intake was less on the low protein diets, differences are naturally less pronounced.

The second type of experimentation undertaken had as its aim the measurements of heat increments (dynamic effects) of the diets of different fat content. In previous work by Forbes and Swift (5) it was shown that the lowest heat increments of combinations of any of the three main classes of food, carbohydrate, protein, and fat, were obtained from beef protein and lard and from cerelose and lard when these supplements were superimposed on a complete diet. The least efficient combinations, as indicated by maximum heat increments, was the one containing no fat, which was composed of cerelose and beef protein. These observations aroused an interest in the relation of fat to heat increments, and this was naturally included as an integral part of the program of study outlined.

In this first study 4 groups of 12 essentially mature male rats were used, these being selected from 12 litters with a rat from each litter receiving one of the 4 fat diets to be tested, either 2, 5, 10, or 30% fat (6) . The rats were all the same age, 205 days old, when first used in the respiration chambers. The heat production was measured for each rat for each dietary treatment, either maintenance or supermaintenance, during a 7-hour period on two consecutive days. Muscular activity was practically nil. As indicated earlier, the heat increment was determined as the difference in heat production between the same rats on two planes of nutrition when consuming the same diet.

The metabolizable energy values of the daily food were virtually equal for the four diets at either level of food intake (Table 2). However, the heat production or energy expense of utilization at both planes of nutrition, diminished in the increasing order of the fat contents of the diets. The decreases in heat increments were in the same order (Table 2) and were equivalent to 7.29, 6.39, 5.78, and 4.10 Cal. for the 2, 5, 10, and 30% fat diets, respectively. These values amounted to 36, 31, 29, and $20\%,$ respectively, of the gross energy of the feed given above the maintenance plane for the 2, 5, 10, and 30% fat diets.

This entire experiment was run a second time to see if the increased level of vitamin intake had any influence on results obtained. This point was not only answered in the negative, but other data were obtained, adding further confirmation to earlier findings. The second experiment was conducted throughout in the same manner as the first except that the 5% fat diet was dropped and only 3 diets were used (7). Again the heat production decreased markedly at both planes of nutrition as the fat content of the diet was increased. The heat increments were likewise very similar to those obtained in the first experiments.

During the course of this work it became evident that the amount of energy expended in heat production, other than dynamic effect, varied with the intake of fat and was of importance in explaining the differences in the total heat productions obtained. As the work reported has not shown any appreciable difference in metabolizable energy on the different levels of fat intake, the heat productions must therefore contain different expenditures of energy in the form of activity as this represents the only remaining outlet for the use of energy which has not been measured. A full explanation of the energy balances on the different fat diets will obviously include a study of this activity.

TABLE 3

¹ Measurement of heat made by the carbon-nitrogen balance method.

The evaluation of activity in terms of energy presents a very difficult problem, and an indirect approach was made to its measurement by determining the total heat production under conditions of normal cage activity and again with activity restricted to a minimum, all other imposed conditions being the same. The difference was considered to represent the energy expenditure associated with activity.

In this experiment 2 groups of mature rats, 12 animals in each group, were selected on the basis of uniformity of weight and age to be fed diets of low and high fat, 2 and 30%, respectively. The feed intake was maintained constant and on an equicaloric basis throughout with exereta collection periods of 8 days' duration preceding the respiration measurements. The carbon-nitrogen balance procedure was used with the respiration chambers made of sheet copper, permitting the animals the same freedom of movement during the time when the heat production was being measured as normally.

Two complete periods were run after which the diets were reversed, and two more excreta collection and respiration periods were completed. In other words, each rat, with a few exceptions due to refusal of feed, had two periods on the low fat and two on the high fat diet.

The information on energy exchange for the first and last periods is given in Table 3. On account of the similarity of the data two periods only need to

¹ Heat measurements made by Haldane respiratory quotient procedure.

be given as representative of the entire experiment. Conelnsions are drawn obviously in consideration of all 4 periods.

The daily heat productions were about the same for both diets in each of the four periods although the trend in three of the four periods was for the rats on the low fat diet to have a slightly higher heat production than those on the high fat diet.

This uniformity in heat production may be somewhat surprising since the heat production as measured by the body balance procedure in growing rats was greater on the low fat diet than on the high fat diet. The one big difference between these two experiments is in the age of the experimental animals, and consequently their physiological status is very different. In the young animals growth is one of the most urgent demands made upon the available energy. In the mature animals the demand for growth is no longer present, and the energy intake is dissipated almost entirely in heat production and in loss in visible exereta.

In view of the results of this experiment which show essentially the same heat production as well as metabolizable energy on a high and a low fat intake and in view of the earlier work in which it was found that the dynamic effect was less on the high fat diet, at least under conditions of experimentation imposed, it may be concluded that in the present experiment more heat remained to be expended in the various forms of activity on the high fat diets.

As a part of this conclusion is drawn from data in two separate experiments conducted at different times and on different animals, it was decided to eonduet an experiment in which the heat production was measured on consecutive days in the same animals with activity restricted in the one case, and with normal cage activity in the other. The results obtained from this work could, therefore, not he subjected to the criticism mentioned.

The procedure decided upon was to measure the heat production under restricted activity with the IIaldane apparatus for periods of 7 hours and to measure the heat production under unrestricted activity with the carbon-nitrogen balance method of the experiment just discussed.

Two groups of 10 rats were used in this study consuming either the low or high fat diet, the usual metabolism data being obtained.

The heat production as measured with activity either restricted or unrestrieted follows closely the pattern of previous experiments and thereby adds further confirmation to these findings. The total heat produetions with activity unlimited were not affeeted to any appreciable extent by the level of fat intake but the heat productions, as determined with activity restricted, were significantly different. As the heat increment decreased with increase in fat content of the diet, the activity must therefore increase in the same proportion to produce a similar total heat production. The important point is that a numher of variable factors such as different animals. time of year, experimental routine, etc., did not signifieantly influence the results obtained. By following the technique of having the respiration measurements made with the same animals on consecutive days, there ean be little doubt of the part that activity plays in the total heat production as measured by this procedure. It is recognized that one possibility remains which could influenec the respiration measurements with the llahlane method since the period of observation does not extend over a full day and any change in metabolism during a time other than when the measurements were being made would not be reflected in the final results.

Further work has been done with this idea in mind to inelude a thorough investigation of the fluctuations in heat production throughout the day on both the low and high level of fat intake with 12 mature rats on each diet. First, the hourly curve of total heat production, with normal aetivity ineluded, was studied for 2 complete days using the earbonnitrogen balance procedure. This was done by stopping the ventilation of the respiration chambers every 3 hours, long enough to weigh the absorption tubes. The eurve of heat production obtained is presented in Figure 1. From this it is apparent that the two diets, fed on an isocaloric basis, produced heat at quite different rates at different times of the day. On the low fat diet there was an increase in heat produetion after food was given either in the morning or evening. It is to be noted that the daily allowance of food was divided into two equal portions and fed exaetly at 12-hour intervals as indicated in Figure 1 (S A.M. and 8 P.M.). The increase in heat during the morning seems to be largely dynamic effect since it did not start until the food was given, while in the evening the increase started slowly between 4 P.M. and 7 P.M. This increase is obviously due to activity of the rats.

With the Iow fat diet the peak of dynamic effect and extra aetivity which occurred following the meal was always reached within two hours after the meal was consumed.

A comparison of the amount of activity during the day and night is elearly demonstrated by the animals on either diet. There appears to be little activity in the rats on the high fat diet during the day, but after 4 P.M. the increase is evident with this continning throughout the night. Ingestion of the high fat diet had little demonstrable effect on the metabolic rate. A general similarity is noted in this work to that reported by Herring and Brody (8) on the diurnal metabolic rhythm of the rat.

It is of particular interest and concern in this *work* that the heat produetion of the mature rats on the high fat diet was greater at two times during the day than in the rats on the low fat diet, these times being between 4 and 7 P.M. and 4 and 7 A.M. Although the heat increments have been measured with activity controlled to a minimum, the question arises at once as to whether the effectiveness of this eontrol has been sufficiently good to prevent similar variations in these measurements.

This point was checked by choosing one of the times for the determination of heat increments when the total heat production was greater on the high fat diet than on the low fat diet. The period of observation *began* at 1 P.M. and continued until 7:30 P.M. Again the heat increment was determined as the difference in heat production between a maintenance and a supermaintenance plane of nutrition on the same animals with activity at a minimum. Twelve rats were used on both the low and high level of fat intake. This type of problem has been of coneern in planning metabolism work and much thought has been given to it in the past.

With the heat increments being determined late in the afternoon and early evening, the fundamental findings of the former work were again satisfactorily verified. The magnitude of the differences in heat increments was not as great as formerly, but they were still highly significant.

Effieieney of utilization of the metabolizable energy of the two diets is expressed by odds of $9,600$ to 1 **thai** the high fat diet was more effieiently utilized than the 2% fat diet at the supermaintenance plane of nutrition.

Although not directly an energy conversion problem, the investigations of Maynard and eo-workers are of interest (9, 10, 11, and 12). Over a period of years they have studied the role of fat in the ration of the dairy eow and report that a eertain amount is necessary in the ration for optimum milk production. When a low fat ration was fed, there was a decrease in milk volume while a ration of higher fat content usually produeed increased amounts of milk of higher fat eontent. Maynard, MeCay, Williams, and Madsen (13) concluded that a concentrate containing 4% fat ix satisfactory if fed with good roughage and if supplied at the rate of 1 pound for every 3 to $3\frac{1}{2}$ pounds of milk. In more recent work at the same institution, however, Lucas and Loosli (14) were unable to demonstrate a direct dependenee of milk production on dietary fat.

The practical importance of dietary fat is perhaps best realized by comparisons in the earlier work between the 2 and 5% levels of fat intake as this range includes many of our livestock feeds. The increased efficiency of utilization of energy of the 5% fat diet as compared with the 2% fat diet was much greater than that of the 30% fat diet as compared with the **5%** fat diet.

The solvent method of extraction of oils from various feed concentrates is also of eoneern since this method results in a product of extremely low fat content.

Summary

The efficiency of utilization of diets containing amounts of fat varying from 2 to 30% has been investigated in a series of metabolism studies using male albino rats as experimental subjects.

All diets were compounded in a manner to make them equiealorie and to contain the same amounts of protein, vitamins, and minerals.

Statistically significant results were obtained for body gains of fat and energy, and decrease in heat production, in the order of increasing fat eontent of the diets in two 70-day body balance experiments in eomparisons made between litter-mate animals.

The energy expense of utilization (heat inerement) of diets containing from 2 to 30% fat varied from 36 to 16% , respectively, of the gross energy of the diets as determined in two experiments involving the IIaldane respiratory quotient procedure. The fat content of the diet had little effect on nitrogen utilization, caused slight decreases in metabolizable energy, but was responsible for larger dcreases in heat produetion as the level of fat in the diet increased. The fat therefore conferred economy of utilization of food energy. The time of day which the heat increments were measured wax eliminated as a possible factor in the determination of results.

Decreasing the protein content of the diets from the usual 22% level to 7% did not change previous results and again indicated a better utilization of the high fat diets as determined by the body balance method. Increased weight gains, including increased gains of fat and energy, and decreased heat production were associated with the high fat diet.

The portion of the heat production due to normal voluntary activity was found to vary in a reciprocal manner with the heat increment—the lower heat increment of the high fat diets being associated with more activity.

A study was made of the variations in heat production and activity throughout the day.

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Some Physical and Chemical Properties of Extraction Naphthas*

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LTHOUGH the physical and chemical properties of extraction naphthas have been covered in considerable detail in previous papers $(1, 2)$, the question of evaporation losses, per cent oil remaining in the meal, and other plant performances as related to the solvent are being discussed rather frequently by both technical and non-technical personnel in an effort to account for extraction plant results. Accordingly, the following data are being presented with the view toward providing information on several of the commercially available light petroleum naphthas of the hexane type in order that the characteristics of this type extraction solvent can be better understood.

It has been known for many years that the various petroleum naphthas, including even the highly refined and comparatively narrow boiling range pentane, hexane, and heptane fractions that were introduced to the oil and fat industry in 1930, were composed of a number of different chemical compounds. Also, it long has been known that each of these hydrocarbon compounds, even though closely related to the others, has a somewhat different boiling point, specific gravity, solvent action, and other properties. Furthermore, it long has been known in the petroleum industry that it is difficult to assign percentage figures for the several naphtha constituents even though many of the naphtha fractions are composed of a half dozen or more different saturated hydrocarbon compounds as well as small amounts of unsaturated hydrocarbon and sulphur compounds. In fact, it only has been in comparatively recent years that laboratory analytical procedures have been devised which permit the assignment of fairly accurate figures to percentage composition of light petroleum naphtha fractions; that is, separating the several naphtha constituents one from the other is still a difficult feat to perform, not only from a manufacturing standpoint but also from a laboratory analytical standpoint. Based on past experience, different data for percentage composition of the commercial hexane type naphthas in Table I probably would be reported if they were analyzed some six months from now when using a modification of the presently employed method or

some other method. These things should be kept in mind constantly by anyone attempting to correlate extraction plant results with any given naphtha.

An extensive investigation into the matter of pure hydrocarbons from petroleum was conducted in 1943 by John Griswold, C. V. Van Berg, and J. E. Kaseh (3) of the University of Texas, who utilized selective solvents and chemical reactions as well as effective fractionation in their analytical technique for determining the composition of a commercial hexane fraction, namely Skellysolve-B. However, the data shown in Table I for chemical composition of several commercial hexane type naphthas were obtained (4) by the method developed by Gooding, Adams, and Rall and described in U.S. Bureau of Mines "Report PCR 44-009." In this method of analysis use is made of densities and refractive indices for the mercury "g" line and the sodium "D" line, which are determined at 68°F, for the various effectively fractionated portions of the hydrocarbon mixture. A reflux ratio of 30 to 1 was used in a perforated-plate type glass column having 55 theoretical and 80 actual plates in order to obtain the narrow boiling range fractions for the various measurements.

Although the A.S.T.M. D.86-46 method ordinarily is used for determining boiling range of rubber solvent naphtha, mineral spirits, motor gasoline, and other comparatively wide boiling range naphtha fractions, the A.S.T.M. D.216-40 method for distillation of natural gasoline was used for determining the data in Table II, since it has been found to be more satisfactory for analyzing the rather volatile narrow boiling range fractions of the pentane, hexane, and heptane type. Volumetrie "recovery, residue, and loss" data in these distillations are not shown since it is considered that they have little if any meaning from a practical standpoint in extraction plant operations. For example, during the last stages of distillation, the compounds that make up the solvent are subjected to higher temperatures than those encountered in a typical extraction plant operation—local temperatures that perhaps go as high as 900°F., because of super heating-with the result that cracking and general decomposition take place with formation of compounds that did not exist in the original solvent.

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