THE RÔLE OF THE LIVER IN THE SYNTHESIS OF FATTY ACIDS FROM CARBOHYDRATE*

VIRGINIA C. DICKERSON, JAY TEPPERMAN, AND C. N. H. LONG

Little is known about the chemical reactions involved in the conversion of carbohydrate to fatty acids. Hypothetical schemes have been abundant, and have included aldol condensation of acetaldehyde (Magnus-Levy[23]), aldol condensation of acetaldehyde with pyruvic acid (Smedley,[30] Smedley and Lubrzynska[31]), direct condensation of hexoses (Fischer[10]), and other possibilities (Hilditch[15]). The lack of experimental data upon which a rational synthetic scheme might be constructed is not surprising in view of the prevailing uncertainty concerning the very site of the reaction.

The studies of Hoffmann and Wertheimer,[16] Hausberger and Neuenschwander-Lemmer,[13] Ruska and Oestreicher,[29] Henle and Szpingier,[14] and, more recently, those of Tuerkischer and Wertheimer[36] and of Mirski[25] have clearly established the fact that, at least in some circumstances, adipose tissue itself is capable of converting carbohydrate to fatty acids. The work of Henle and Szpingier and of Mirski is especially noteworthy, for these investigators devised *in vitro* systems for the study of the reaction.

The evidence in support of hepatic synthesis of new fat can be considered only presumptive, although some authorities (Longenecker[22] and Hilditch[15]) believe the liver tissue to be capable of effecting the conversion. This view is shared by Gavin and McHenry,[11] who found large concentrations of fatty acids in the livers of rats which were synthesizing fatty acids from carbohydrate. In addition, Waelsch and his co-workers[37] analyzed the livers of animals which were given a high-carbohydrate diet and deuterium oxide in their drinking water and found high concentrations of deuterium-containing (i.e., newly synthesized) fatty acids. However, these chronic experiments on intact animals do not constitute

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absolute proof that the synthesis is performed by the liver since interchanges of fatty acids between liver and fat depots are known to occur readily.

The studies of Tepperman, Brobeck, and Long[34] suggest that the conversion of carbohydrate to fat may take place both in the liver and in extrahepatic tissues. These workers compared rats which were forced to eat their entire daily ration in one hour with control animals which were allowed access to food throughout the day and night. Since the trained animals exhibited a mean Respiratory Quotient (R.Q.) of 1.22 after the administration of glucose, whereas the untrained ones similarly treated showed R.Q.'s, of about 1.05, it was concluded that one effect of dietary training was an augmentation of the rate of fatty acid synthesis from carbohydrate. Studies of the R.Q. of insulin-treated eviscerate preparations of trained and untrained animals showed a persistent difference in R.Q. even in the functional absence of the liver, but this difference was not so striking as it had been in the intact animals. The possibility that the effect of dietary conditioning might persist in the isolated liver, and that the conversion of carbohydrate to fat might be demonstrated both *in vivo* and *in vitro* by the technic of comparing trained rats with untrained ones, led to the experimental work which is to be reported here.

Materials and methods

Diet and training.

Male rats of the Sprague-Dawley strain were used in this study, except where otherwise noted. Their weight at the beginning of dietary training was between 185 and 220 grams. All control animals were fed pellets of Purina Fox Chow *ad libitum*. The length of time required for the control animals to consume their daily ration was not determined accurately, but a few observations revealed that most of the diet was taken during a period of from 10 to 14 hours.

Rats were trained to eat their day's quota of food during a one-hour feeding period in each day, usually from 8:30 to 9:30 A. M. The original diet consisted of commercial sucrose, 70 per cent; commercial casein, 15 per cent; brewers' yeast, 10 per cent; Osborne-Mendel's salt mixture, 5 per cent; cod-liver oil supplement twice weekly. On this dietary régime trained animals exhibited the types of growth curves shown in Figures 1 and 2, in which are plotted the mean 23-hour fasted weights of five groups of animals during their conditioning period. The individual weight curves in any one group

described remarkably parallel courses. Group A, 11 Yale female rats on the sucrose diet, showed an initial sharp weight loss, levelling off within 10 days. After the 14th day there was a slow gain in weight, but on the 25th day of training the mean weight of the animals was still below the initial level. Group D, consisting of 16 Sprague-Dawley male rats on the same diet, started at the same initial weight and exhibited the same loss during the first 4 days. From this point on they gained weight steadily, and they achieved

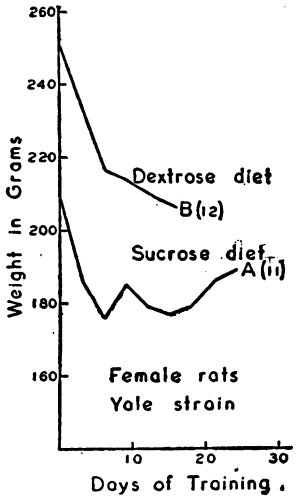

FIG. 1. The course of dietary training in female rats. All animals were fed for one hour each day.

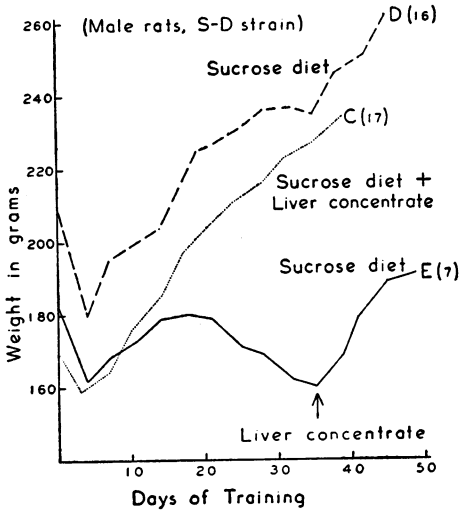

FIG. 2. The course of training in male rats fed for one hour each day. See text for further details.

their initial weight by the 15th day. During the following 30 days a progressive increase in weight occurred. This form of weight record was found to be typical of about two-thirds of the male rats which were trained on the original diet; the remaining male animals showed weight curves like that of Group E. By the 15th day the weights of these animals had returned almost to the initial level, but beginning on about the 20th day there was a steady loss. The addition of approximately 0.5 per cent of dried liver concentrate (Eli Lilly and Co., 1 U.S.P. unit/12.75 gm.) to their diet on the 35th day of training caused a sharp upward inflection of the growth curve, and this weight gain continued until the animals were sacrificed for experimental purposes. Although the mechanisms involved in this effect of added liver concentrate were not clear, it appeared wise to incorporate the concentrate in subsequent diet mixtures in order to prevent the E type of weight curve (see curve C). To insure a more uniform intake of fat-soluble vitamins and essential fatty acids, 1 per cent of cod-liver oil was added to the diet.

An attempt was made to train a group of 12 female rats of the Yale strain on a diet in which the sucrose was replaced isocalorically by commercial dextrose (Cerelose). The result of this experiment is shown in curve B. The animals continued to lose weight until the 16th day, at which time they were given other food. The reason for the lack of effectiveness of the dextrose diet in this type of dietary training may, perhaps, be related to the comparative superiority of sucrose as a fat-former (Feyder[9]).

Analytical methods.

Total fatty acids were determined by a modification of the Stoddard and Drury[32] method. Liver lipoids were extracted and saponified, the fatty acids precipitated, and, after separation by filtration, titrated with dilute alkali. "Fat-free" filter paper subjected to a 4-hour extraction in boiling alcohol was used throughout. The mean recovery of known amounts of palmitic, oleic, and linoleic acids by this method was 99.4 ± 0.33 per cent.

The Yasuda[41] modification of the Rosenmund and Kuhnhenn[28] method was used in determining the halogen uptake of the liver fatty acids. This method utilizes a solution of pyridine sulfate dibromide in glacial acetic acid as the halogenating agent. Since fatty acids were determined as microequivalents, degree of unsaturation was expressed by means of the "iodine ratio," which can be defined as the number of microequivalents of halogen which can be taken up by one microequivalent of fatty acid. Thus, the iodine ratio of oleic acid is 2.0, of linoleic acid, 4.0, and so on. The observed uptake of halogen in practise was actually in excess of the degree of unsaturation of the fatty acids, for cholesterol, an unsaturated compound having an iodine number of 69, was also present in the tissue analyzed. In the work to be presented here, each liver acted as its own control, and changes in iodine ratio, rather than the absolute iodine ratio, were of interest. Therefore, no attempt was made to correct the observed iodine ratios for cholesterol. The method was tested on known mixtures of fatty acids and was found to give accurate and readily reproducible results.

I. *Changes in Degree of Unsaturation of Liver Fatty Acids in Vivo.*

The purpose of this phase of the study was to discover what changes, if any, occur in the total liver fatty acids and their iodine ratios after the administration of various sugars to trained and untrained rats. The rat liver is so constructed that it is possible to ligate and excise one lobe without causing any appreciable hemorrhage. Therefore, if one lobe is removed for analysis at the time of administration of the sugar and the remainder after any specified interval, each liver can be made to serve as its own control. This general procedure was employed in the experiments to be described.

The 24-hour fasted rats were anesthetized with Nembutal, the right median lobe of the liver was exposed, ligated, and removed through a midline incision, and the appropriate test solution administered by the route indicated. The sample was chilled and the incision in the abdominal wall closed with interrupted sutures. Analyses were begun as soon as three animals had been prepared in the manner described. Each lobe of liver was cut with a razor blade into fragments weighing from 25 to 50 mg. and duplicate or triplicate analyses were made on about 175 mg. of tissue for estimation of total fatty acids and on from 350 to 400 mg. of tissue for determination of degree of unsaturation of fatty acids. Wet weight/dry weight ratios of the tissues were also made. At the end of the appropriate time interval, the animal was again anesthetized and a second liver sample, the left lateral lobe, was removed. This was treated in all respects the same as the initial sample, and the animal was then sacrificed.

It was found that neither total fatty acids per gram of dry tissue nor the iodine ratio of the fatty acids changes when the samples were wrapped in filter paper, moistened with 0.9 per cent saline, and allowed to remain in a chilled glass chamber for 45 minutes. Repeated tests showed that the variation among lobes of the same liver is no greater than that among samples taken from the same lobe, and it was therefore assumed that the composition of the right median lobe with respect to wet/dry weight ratio, total fatty acids, and iodine ratio was an accurate index of the initial composition of the left lateral lobe.

When the mean initial fatty acid content of the livers of all trained animals used in this part of the study was compared with the initial mean for the untrained rats, a striking difference appeared. The value for 51 trained rats was 373 ± 2.56 microequivalents per gram of dry liver, while that for 45 untrained ones was 414 ± 3.93. Statistical analysis of the data showed this difference to be highly significant. These figures, when converted to grams of tripalmitin per 100 grams of wet liver, became 10.00 for the trained and 11.34 for the untrained animals.

When the changes in total fatty acid content of the livers of trained and untrained rats were compared, no consistent pattern of response was found in either group, and, in fact, there was often a great variation in both the direction and magnitude of the change

in any one group of experiments. It was fully apparent that evidence based on changes in liver fatty acid content could be used neither to support nor refute the hypothesis that the liver is a site of fat synthesis. Similarly, it was concluded that the observed changes in wet weight/dry weight ratios could not be interpreted as being due necessarily to the experimental treatment of the animals, since, inevitably, there were variations in the degree of moistness of the filter paper in the storage chamber.

However, the iodine ratios of the liver fatty acids of trained and untrained animals were found to be practically identical. The mean initial iodine ratio of the liver fatty acids of 36 trained rats was 3.52±0.014, while that of 36 untrained animals was 3.49± 0.013, indicating that the average degree of unsaturation was the same in the two cases. In addition, the variations in iodine ratio following the administration of test solutions either orally or subcutaneously were found to be remarkably consistent. Since no difference in response was found after 1.5, 3, or 6 hours, all of the terminal values were grouped. When this was done it was found that the administration of water to trained and untrained animals produced no significant change in degree of unsaturation of the liver fatty acids. The administration of 30 per cent glucose solution (2 ml./100 gm. body weight) to untrained rats was also without statistically significant effect. In the trained animals, on the other hand, the administration of glucose caused a highly significant decrease in iodine ratio, i.e., from 3.51± 0.024 to 3.14±0.029. Unlike glucose, the administration of sucrose resulted in an apparent decrease in the degree of unsaturation of liver fatty acids in both untrained and trained rats; the magnitude of the change in the untrained animals, though significant in itself,

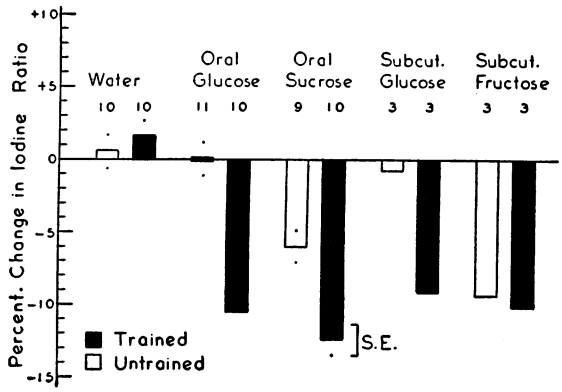

FIG. 3. Per cent change in halogen ratio of liver lipids in trained and untrained animals. Numbers over columns signify the number of rats in each group.

was not so great as that seen in trained rats after either glucose or sucrose.

Since sucrose yields, on hydrolysis, equal parts of glucose and fructose, and since the former, when administered orally, had been shown to be without effect on the iodine ratio of liver fatty acids, small groups of trained and untrained rats were injected subcutaneously with solutions of glucose and fructose. Again, glucose caused no change in the untrained rats, but lowered the mean halogen ratio from 3.51 to 3.19 in animals which had been trained on the sucrose diet. Fructose, however, caused an equivalent lowering in the two groups of animals. The changes in degree of unsaturation of liver lipids are summarized in Figure 3.

II. *The Respiratory Metabolism of Liver Slices in Vitro.*

Manometric method and media

The respiratory metabolism of liver slices *in vitro* was investigated by means of the Summerson[33] differential method. Slightly modified Dixon-Keilin[6] vessels were used, and the volume of each pair of vessels was equalized by adjustment of the size of the glass rods used to displace the alkali. The members of each pair contained equal weights of tissue as well as equal volumes of medium and acid. All experiments were carried out for one hour at 37.8° C. with a shaking speed of from 80 to 84 per minute.

Six different media were used in the experiments to be described:

(1) Bicarbonate-buffered physiological salt solution (Krebs and Henseleit[18]).

(2) "High calcium" medium, a phosphate-buffered Krebs-Henseleit salt solution in which the concentration of stock calcium chloride solution was increased to 783 mg. per 100 ml., the potassium diacid phosphate omitted, and the bicarbonate replaced by 5 ml. of a phosphate-buffer mixture (Krebs[17]). This solution was supersaturated with respect to calcium phosphate, so that a white precipitate began to settle out almost immediately. It contained about 10.5 mg. of calcium and approximately 150 mg. of phosphate ion per 100 ml.

(3) "Low calcium" medium, in which the stock calcium chloride solution contained but 564 mg. of $CaCl_2.2H_2O$ per 100 ml. The final concentration of calcium was therefore reduced to 8 mg. per 100 ml.

(4) "Low phosphate" medium, in which the final concentration of phosphate was about 50 mg. per 100 ml.

(5) "Normal" rat serum, frozen in 13 ml. portions and thawed immediately before being used.

(6) "Neutralized" rat serum, prepared by the procedure described by Warren.[38] Normal serum was acidified with hydrochloric acid, the carbon

dioxide was removed by evacuation, and the serum was frozen in 12 ml. portions. Before it was used each newly thawed portion was treated with 1.2 ml. of m./15 phosphate-buffer (pH 7.4).

In most instances the liver tissue was obtained from rats which had been fasted for 24 hours. The animal was anesthetized with Nembutal, the liver was removed and rinsed in phosphate-buffered physiological salt solution, blotted with filter paper, and chilled. Slices approximately 0.4 mm. thick and 6 mm. square were cut by hand according to the method of Deutsch.[4] Samples of 100 mg. were quickly weighed on a torsion balance and placed in the prepared Dixon-Keilin vessels. Triplicate samples weighing from 76 to 120 mg. were taken for determination of the wet weight/dry weight ratio. Values for Qo_2 calculated on the basis of initial dry weight are much lower than are those obtained by using final dry weight, a widely prevalent technic. However, values for Qo_2 obtained in the Summerson vessels were identical with those found by Wilhelmi,[40] who used the conventional single-vessel type of Warburg manometer and calculated his results on the basis of initial dry weight.

When the experimental work on the respiratory exchanges of liver slices *in vitro* was begun, bicarbonate-buffered physiological salt solution was used as the medium. In spite of repeated attempts, however, consistent results could not be obtained with this medium. The incidence of leakage of gas from the vessels was high, and, further, the addition of pyruvate to the slices resulted in R.Q.'s no higher than 0.77, although it is well-known that liver slices in the presence of this substance regularly yield R.Q.'s of about 1.2 (Elliott, Greig, and Benoy[8]). Subsequent experiments showed that carbon dioxide absorption was often incomplete in bicarbonate-buffered preparations.

With the high calcium medium, which may be taken as typical of all the phosphate-buffered media and "neutralized" serum, readily reproducible data were obtained. Therefore, the use of bicarbonate-buffer solutions was abandoned, and all of the results herein reported were obtained in phosphate-buffered media. The addition of M/50 pyruvate to liver slices in the high calcium solution resulted in R.Q.'s of from 1.18 to 1.20, while the oxygen consumption of the slices increased about 30 per cent over the no-substrate level. When leakage occurred, the fact that the result was due to an artefact was usually clearly apparent, and the experiment was discarded. Occasionally, however, the validity of individual measurements was merely questionable and it was therefore neces-

sary to adopt an arbitrary basis for the exclusion of data. All experiments in which, in the absence of obvious gas leakage, the duplicate determinations of oxygen consumption failed to agree within 10 per cent were discarded unless the R.Q.'s agreed within 10 per cent. In practise, it was necessary to exclude very few experiments on this basis, and most of the duplicate samples showed excellent agreement.

The addition of glucose to liver slices from untrained rats failed to cause a statistically significant change in R.Q. In the high calcium medium, slices from nine livers exhibited a mean R.Q. of 0.55±S.E. 0.020 without added substrate and 0.50±0.022 in the presence of 400 mg. per cent of glucose. The behavior of liver slices from trained rats in technically comparable experiments was in striking contrast to that of tissue taken from untrained animals. The mean R.Q. of liver slices from nine "trained" rats rose from 0.52 ± 0.019 to 0.72 ± 0.015 upon the addition of glucose, an increase of unquestionable statistical significance. These results are shown graphically in Figure 4.

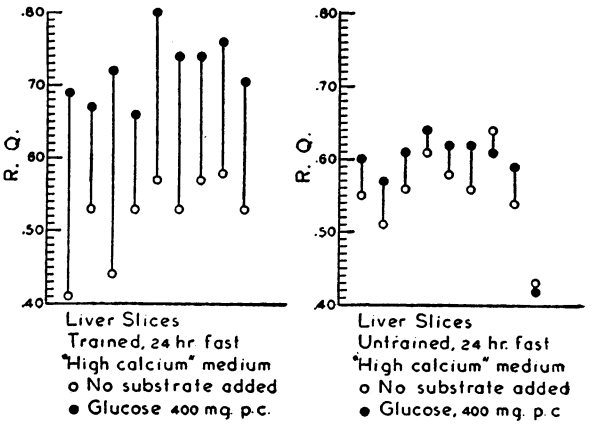

FIG. 4. The effect of added glucose upon the respiratory quotient of liver slices. Each line represents the mean of duplicate determinations on the same liver.

The results of 13 groups of experiments which involved different combinations of media, dietary training, and substrate, are shown graphically in Figure 5. It will be seen that there was only a small difference in R.Q. increment between liver slices taken from trained and untrained animals and suspended in low calcium medium (Fig. 5, groups 3 and 4); statistical analysis of the increment in both groups showed that the rise with substrate in both groups was of no significance. Slices from trained animals studied in the low phosphate medium responded as similar preparations had done in

high calcium (Fig. 5, groups 2 and 5). Slices observed in un-neutralized rat serum (Fig. 5, groups 6 and 7) showed a difference in R.Q. increment which was less than half as great as that seen in neutralized serum (Fig. 5, groups 8 and 9). Unlike glucose, fructose brought about an increase in the R.Q. of liver slices from both trained and untrained rats (Fig. 5, groups 10, 11, 12, and 13). The amount of increase was about equal in the two groups, and was the same in two different media.

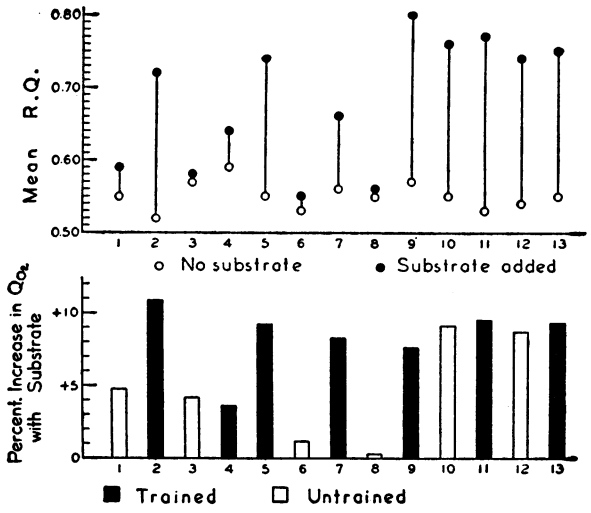

FIG. 5. Respiratory exchange of liver slices in vitro. Key as follows:

Group	*No. of animals*	*Training*	*Medium and substrate*
1	9	untrained	high calcium, glucose
2	9	trained	high calcium, glucose
3	3	untrained	low calcium, glucose
4	7	trained	low calcium, glucose
5	3	trained	low phosphate, glucose
6	3	untrained	normal serum, glucose
7	3	trained	normal serum, glucose
8	3	untrained	neutralized serum, glucose
9	4	trained	neutralized serum, glucose
10	4	untrained	high calcium, fructose
11	4	trained	high calcium, fructose
12	4	untrained	low calcium, fructose
13	3	trained	low calcium, fructose

Since the R.Q. is the ratio of carbon dioxide production to oxygen consumption, it is of importance to know whether the observed increases in R.Q. were due to increases in Q_{CO_2} or to decreases in Q_{O_2}. It was found that whenever the R.Q. rose, *both* the Q_{O_2} and Q_{CO_2} increased, the latter, of course, to a greater extent than the former. The per cent increases in Q_{O_2} are shown graphically in Figure 5. The difference between the increases in Q_{O_2} observed in liver slices from trained and untrained rats was found to be highly significant (Fig. 5, groups 1 and 2). Although all of the experimental data were not subjected to statistical analysis, there was a tendency for the Q_{O_2} to rise when the increment in R.Q. was large (see Fig. 5).

The effect on the R.Q. and Q_{O_2} of amorphous insulin (1 unit

and 0.05 units per ml.) was investigated in liver slices from three trained rats. In all cases the medium contained 400 mg. per cent of glucose. The insulin did not appear to increase either R.Q. or Qo_2 in both the high calcium medium and neutralized serum.

Liver slices from two trained rats were suspended in the high calcium medium and the respiratory exchange was determined with no substrate and in the presence of 1.6 per cent of purified glycogen. It was found that the effect of glycogen was approximately the same as that of glucose in the same medium, for the Qo_2 increased by about 10 per cent and the mean R.Q. rose from 0.51 to 0.69.

Discussion

The observation that the mean total fatty acid content of the livers of untrained rats fasted for 24 hours is about 11 per cent greater than that of trained ones suggests that, as a part of the adaptation incident to training, the latter animals may have better-developed fat transport mechanisms as well as an enhanced ability to convert carbohydrate to fatty acids. In this connection, it is pertinent to note that all of the trained animals contained much less depot fat than did the untrained ones, and that, undoubtedly, this was due to the fact that the process of training is accompanied by a certain degree of chronic underfeeding. The similarity of the initial iodine ratios of the liver fatty acids of trained and untrained rats suggests that the mechanisms involved in the distribution of fatty acids from the liver to the periphery are, very likely, equally selective in the two groups of animals.

In any animal, the fatty acid content of the liver depends upon a number of factors, including absorption of fat, synthesis of fatty acids from non-fat sources, utilization, mobilization of fat from the depots to the liver, and transport of fat from the liver to extra-hepatic tissues. Therefore, the failure to demonstrate any significant increase in total liver fatty acids in no way lessens the possibility that fatty acid synthesis from carbohydrate may have occurred in some of the circumstances reported here. Indeed, it is possible that when carbohydrate is converted to fat in the liver, the removal of fat from that organ may be stimulated. The fact that, under certain conditions, a fall in the iodine ratio of the liver fatty acids occurred while the total fatty acid content remained relatively constant suggests that this may be so.

It is generally accepted, especially in view of the findings of Schoenheimer and his co-workers (Bernhard and Schoenheimer,[2, 3] Rittenberg and Schoenheimer[27]), that the animal organism synthesizes from carbohydrate fatty acids which are saturated or which contain only one double bond. Therefore, the observation that the iodine ratio of liver fatty acids falls in untrained rats given sucrose or fructose, and in trained animals after glucose, sucrose, or fructose, indicates that fatty acid synthesis can take place in the liver under the conditions stated. The possibility that fatty acids formed in extrahepatic sites may have been transported to the liver cannot be disproved by the data presented here, but it is difficult to imagine that such a mobilization would occur in animals which were well supplied with carbohydrate. From these experiments it cannot be inferred that the liver of the untrained animal is incapable of forming fatty acids from glucose, but only that the rate of this conversion is too slow to be detected by the methods described. In the absence of any information concerning the nature and quantity of the fatty acids removed from the liver during the experimental period, it is impossible to use changes in saturation of fatty acids in calculating the amount of fatty acids synthesized from carbohydrate during absorption.

The respiratory quotients which were observed in the absence of added substrate in liver slices from trained and untrained rats were low, but were of the same magnitude as the quotients found by several other groups of investigators. Elliott and Baker,[7] using a Krebs bicarbonate medium, observed R.Q.'s of 0.46 to 0.53 in liver slices from normal fasted rats, while Elliott, Greig, and Benoy[8] found quotients as low as 0.34. The R.Q.'s reported by Bach and Holmes[1] vary between 0.48 and 0.57. All of these investigators used the Dixon-Keilin differential manometric technic.

It has often been observed that neither the R.Q. nor the Q_{O_2} of liver slices from normal fasted rats increases appreciably upon the addition of glucose to the medium. Wigglesworth,[39] Quastel and Wheatley,[26] Dickens and Greville,[5] Elliott and Baker,[7] and Elliott, Grieg, and Benoy[8] have all contributed evidence which strengthens this impression. In the present investigation it was again found that added glucose has little effect upon the respiratory exchange of liver slices from fasted, untrained rats. There was a slight increase in both R.Q. and Q_{O_2} which may have been due, in

part, to better maintenance of the initial respiratory rate (Laser[19]). In the face of this body of evidence, the effect of glucose on the respiratory exchange of livers of trained rats is especially noteworthy. Not only was there a much more pronounced tendency for the Q_{O_2} to increase, but a striking rise in the R.Q. occurred in all but one medium. It is true that the R.Q. never exceeded or even approached unity, yet the probability remains that the rise was due to a synthesis of fatty acids from glucose. Inasmuch as the over-all R.Q. of any tissue or living organism is a vector of the forces which simultaneously tend to raise and lower it, it is difficult to conceive of fatty acid formation from glucose proceeding at such a prodigious rate that it would elevate the quotient of liver slices (whose intrinsic quotient is very low) far over unity. Since the isolated liver slice has no mechanisms for storing fat which may be newly synthesized, a mass-action effect may tend progressively to slow the conversion, and therefore inhibit the tissue from performing the synthesis at its maximal capacity.

When fructose was added to liver slices, the R.Q. showed a pronounced rise without regard to the antecedent dietary training of the animals from which the livers were obtained. Dickens and Greville[5] also observed that fructose, but not glucose, increases the R.Q. of normal liver slices *in vitro*. The extent of the rise was about the same as that observed in the "trained" liver slices in the presence of glucose, and again it may have been due to the conversion of the monosaccharide to fat. This observation articulates well with the data on changes in saturation of liver fatty acids presented above. The probability that fat is synthesized more readily from fructose than from glucose in the intact rat has been shown by Feyder,[9] and it seems likely that this may also be the case in isolated tissues. The fact that the effect of fructose was no greater in liver slices from trained than in those from untrained rats suggests that dietary training does not influence the rate of conversion of this sugar to fat. The tendency of oxygen consumption of the slices to increase in association with large increments in R.Q. may be an index of increased expenditure of energy to provide for the "up hill" nature of the conversion of carbohydrate to fatty acids.

Further evidence, which offers strong support to the hypothesis that the increases in R.Q. were due to fatty acid synthesis, has been obtained in a few experiments in which the fatty acid content of

liver slices incubated in different media has been determined. Slices from a single "trained" liver, suspended in a neutralized serum containing 400 mg. per cent of glucose, oxygenated and shaken at 37.8° C. for three hours, showed an apparent increase in total fatty acids of about 8 per cent. Under similar circumstances, slices from an "untrained" liver showed a change in fatty acids of less than +2 per cent, while in the absence of added glucose there was a tendency toward a decrease in fatty acid content in both types of liver. Results of a similar nature were obtained in one "trained" and one "untrained" liver when the high calcium medium was used, but in the low calcium medium, in which liver slices from trained rats failed to show a significant increase in R.Q. on the addition of substrate, no increase in fatty acids could be demonstrated in the livers of three trained animals. These experiments are few and of a preliminary nature, but they are completely in agreement with the hypothesis that the observed increases in R.Q. represent new fatty acid formation.

The concentration of glucose used in these experiments was selected because it was hoped that this relatively large amount of sugar would act as a driving force on the reactions concerned in fatty acid synthesis. It is not, however, a necessarily "unphysiological" concentration, for both Tsai and Yi[35] and Giragossintz and Olmsted[12] observed blood sugar concentrations of over 300 mg. per cent in the portal venous blood of cats during intestinal absorption of glucose.

That one of the conditions favorable to the *in vitro* synthesis of fatty acids from carbohydrate is a phosphate concentration of about 50 mg. per 100 ml. is shown by a comparison of the respiratory metabolism of "trained" liver slices in the high calcium, low phosphate, and low calcium media. In the last of these, which contained about 8 mg. of calcium and about 150 mg. of phosphate per 100 ml., the R.Q. increased only slightly upon the addition of glucose, whereas in the other two media a striking increase was observed. The high calcium medium was made up to contain the same amount of phosphate and 10.5 mg. per cent of calcium, but precipitation of phosphates reduced the concentration of that ion to about 45 mg. per 100 ml., and decreased the concentration of calcium ion to an unknown, but probably very low, level. On the other hand, the low phosphate medium contained 10.5 mg. of calcium and 50 mg.

of phosphate per 100 ml. Since the addition of glucose to "trained" liver slices caused increases in the R.Q. in both the high calcium and low phosphate media, the failure of the quotient to rise in the low calcium medium may have been due to the high phosphate content. Therefore, if the observed increases in R.Q. were due to fatty acid synthesis, the conversion of carbohydrate to fatty acids by liver slices *in vitro* may well be dependent, in part, upon the phosphate concentration of the medium. This impression is confirmed by a comparison of the results obtained with un-neutralized rat blood serum and phosphate-buffered serum.

These observations are consistent with the suggestion of Hausberger and Neuenschwander-Lemmer[13] that phosphorylation is concerned in the conversion of carbohydrate to fatty acids. If this is the case, it is conceivable that phosphate concentration influences the activity of the mechanisms responsible for the phosphorylation of glucose. It was found in two experiments with "trained" liver slices that the increase in R.Q. caused by the addition of glycogen was no greater than that caused by glucose. This point is deserving of further investigation in livers from both trained and untrained animals. It is known (Lipmann[20]) that in glycogenolysis the first introduction of phosphate is accomplished without a change in free energy. On the other hand, the primary phosphorylation of glucose involves the expenditure of free energy. It is possible, therefore, that although the R.Q. of "untrained" livers failed to rise in response to glucose, evidence of fatty acid synthesis from glycogen may be obtained. This would point to the probability that one of the principal effects of the dietary training of the rats is to increase the rate of phosphorylation of glucose.

When fructose was added to "trained" or "untrained" liver slices in either high or low calcium medium, the R.Q. rose significantly, and to about the same extent. If this increase in R.Q. can be interpreted as signifying fatty acid synthesis, it is apparent that the conversion of fructose to fat is independent of either dietary training or wide variations in the phosphate concentration of the medium, providing the latter is 45 mg. per 100 ml. or more. Unlike the situation with respect to glucose, the rate of phosphorylation of fructose does not appear to be a limiting factor in fatty acid formation. Moreover, since extracts of various tissues, including liver, readily convert fructose-6-phosphate to glucose-6-phos-

phate (Lohmann,[21] Meyerhof[24]), the course of the reactions that take place after the preliminary phosphorylation is probably the same whether glucose or fructose is being converted to fatty acids. These considerations supply further support for the hypothesis that one of the most crucial phases of the biochemical adaptation to the type of dietary training we have described may be associated with those enzyme systems which are involved in the primary phosphorylation of glucose.

Summary

The livers of rats trained to eat their ration in one hour daily have been studied *in vivo* and *in vitro* for evidences of fatty acid synthesis from glucose and fructose. Livers of rats with normal eating habits have been examined under the same conditions. The experimental data support the hypothesis that fatty acid synthesis from carbohydrate can occur in the liver.

Although the livers of 45 untrained, fasted rats contained about 11 per cent more of fatty acids than did those of 51 trained animals, the halogen uptake of the fatty acids was identical in the two cases. The changes in halogen uptake of the liver lipids in the two groups of animals following the administration of glucose revealed a striking increase in the degree of unsaturation in the "trained" livers, whereas the "untrained" ones showed no change. Fructose produced just as marked a change in iodine ratio in both "trained" and "untrained" livers as did glucose.

Liver slices from trained and untrained fasted rats showed no difference in R.Q. or in oxygen uptake. The R.Q. of liver slices from trained rats increased by about 40 per cent upon the addition of glucose or fructose. Liver slices from untrained animals showed a similar response to fructose, but the addition of glucose was without appreciable effect on the R.Q. The oxygen consumption of the liver slices tended to increase whenever the R.Q. increased in response to the addition of sugar to the medium.

The rise in R.Q. in glucose-containing media was found to be favored by a phosphate concentration of about 50 mg. per cent. The corresponding rise in R.Q. caused by the addition of fructose to liver slices from trained or untrained rats was equally great at phosphate concentrations of 150 and 50 mg. per cent.

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