constant interest and encouragement, and to Sir Rudolph Peters, F.R.S., for many discussions and much advice. This work was supported by grants from the Agricultural Research Council and the Graham Research Fund, University of London. One of us (C.H.G.) is indebted to the Commonwealth Scientific and Industrial Research Organization of Australia for a Studentship during the tenure of which this study was carried out.

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Observations on some Nutritional Factors that Influence the Lipotropic Activity of Methionine

BY A. E. HARPER* AND D. A. BENTON

Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, U.S.A.

(Received 13 September 1955)

The discovery that fatty infiltration of the liver is a sign, not only of a choline deficiency, but also of certain amino acid deficiencies (Singal, Hazan, Sydenstricker & Littlejohn, 1953; Harper, Monson, Benton & Elvehjem, 1953; Cole & Scott, 1954) emphasized the need for a re-investigation of the lipotropic action of protein. Subsequently it was shown, in experiments in which there was only one variable, that two distinct lipotropic effects of protein can be demonstrated (Harper, Monson, Benton, Winje & Elvehjem, 1954*c*; Harper, Benton, Winje & Elvehjem, 1954*b*). In animals fed on diets deficient in choline, the methionine contained in the dietary protein serves as a precursor for the synthesis of choline (which was previously well known) and, in animals fed on diets low in protein, additional amounts of well-balanced proteins prevent partial deficiencies or imbalances of essential amino acids which cause an accumulation of liver fat. These observations, which explained many of the inconsistencies in earlier studies on the lipotropic action of protein, have been confirmed and similar conclusions have been drawn by Lucas & Ridout (1955). The papers of Harper, Benton, Winje & Elvehjem (1954*a*), Lucas & Ridout (1955) and Best, Hartroft, Lucas & Ridout (1955) contain references to much of the early literature. The two types of fatty infiltration can also be differentiated histologically (Shils &

Stewart, 1954; Nino-Herrera, Harper & Elvehjem, 1954; Cole & Scott, 1954; Best *et al.* 1955).

Subsequently, when these effects were being studied in greater detail, an anomalous and complicating situation was encountered. It had been shown previously (Singal *et al.* 1953; Harper *et al.* 1953) that a supplement of threonine, gelatin or casein decreased the deposition of liver fat in rats receiving a diet low in protein (casein) and containing choline. Threonine and casein were found to have similar though less pronounced effects when the choline in the diet was replaced by methionine (Harper *et al.* 1954*b*), but gelatin, surprisingly, was without effect under these conditions. The original object of the investigation reported in this paper was to explain this unexpected observation; the end result, however, has been a study of some factors that influence the lipotropic activity of methionine.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain, 21 days old and weighing 40–50 g., were used throughout this investigation. Each group consisted of six rats maintained in individual, suspended cages with $\frac{1}{2}$ in. mesh screen bottoms. The average initial weights for the groups within each experiment did not differ by more than 1 g. The rats were fed *ad lib.* and were weighed weekly.

The percentage composition of the basal diet was as follows: casein, 9.0; sucrose, 82.0; corn oil, 5.0; salts (Hegsted, Mills, Elvehjem & Hart, 1941), 4.0. Vitamins were included to provide (in mg./100 g. of ration): thiamine

* Present address: Department of Biochemistry, University of Cambridge.

hydrochloride, 0.5; riboflavin, 0.5; nicotinic acid, 1.0; calcium pantothenate, 2.0; pyridoxine hydrochloride, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B₁₂, 0.002; inositol, 10.0. Each rat was given orally at the beginning of the experiment and each week thereafter 2 drops of halibut-liver oil diluted with corn oil and fortified to provide: α -tocopherol, 4.0 mg.; 2-methyl-1:4-naphthoquinone, 0.04 mg.; vitamin A, 400 i.u.; vitamin D, 4 i.u. Ascorbic acid (50 mg./kg.) was added to minimize the destruction of thiamine (Kandutsch & Baumann, 1953), and all rations were refrigerated. All changes in the basal diet, as indicated in the tables, were compensated by adjusting the percentage of sucrose.

At the end of the experimental period each rat was stunned and decapitated, and the liver was removed for the determination of fat. The livers were weighed and homogenized in a glass homogenizer. The homogenates were dried in an oven at 100° and the dry weight was determined. The fat content was determined as total ether extract of the dried and ground liver.

RESULTS

The results presented in the tables are representative of some twenty experiments. In general, the values given are averages for representative groups

have discussed this problem, which is not uncommon in studies on the deposition of liver fat, and have suggested, as a solution, the averaging of relatively larger numbers of individual values. Although such averages may be relied on to indicate trends, the possibility that certain exceptions may be of significance cannot be dismissed. Therefore exceptions to the average, generally typical values are discussed in the text.

The original observation, that a supplement of gelatin did not cause a decrease in the deposition of liver fat in rats receiving a diet containing 9% of casein, and in which the choline had been replaced by methionine at the level recommended by Treadwell (1948) for maximum lipotropic activity, was thought to be exceptional. However, similar results were obtained in several replicates of the original experiment, as indicated by the values presented in Table 1. In each case in Expt. 1, in which 0.15% of choline chloride was included in the diets, the fat content of the liver was within the normal range when gelatin was provided, and these values were highly reproducible from experiment to experiment. On the other hand, in Expt. 2, in

Table 1. *Effect of gelatin on the deposition of liver fat in rats receiving 9% of casein with or without choline*

Each of the diets indicated below was supplemented with 0.1% of DL-tryptophan and was fed for 2 weeks.

Expt.	Group	Supplements of basal diet			Rate of gain (g./week)	Liver fat	
		DL-methionine (%)	Choline chloride (%)	Gelatin (%)		Dry wt. (%)	Wet wt. (%)
1	1	0.3	0.15	—	15.9 ± 0.9	32.2 ± 2.0	10.8 ± 0.8
	2	0.3	0.15	6	24.6 ± 1.7	17.2 ± 1.1	4.9 ± 0.3
	3	0.3	0.15	12	25.4 ± 2.9	14.3 ± 0.5	4.1 ± 0.2
	4	0.3	0.15	18	26.6 ± 1.6	12.6 ± 1.3	3.4 ± 0.4
2	5	1.0	—	—	13.9 ± 1.1	31.6 ± 2.3	10.5 ± 1.0
	6	1.0	—	6	28.6 ± 1.0	36.5 ± 2.5	12.3 ± 1.2
	7	1.0	—	12	28.3 ± 1.3	35.5 ± 1.8	11.8 ± 0.9
	8	1.0	—	18	28.0 ± 1.2	23.4 ± 1.2	7.1 ± 0.4

of six rats, and in each case the standard error of the mean is included. In a few cases, however, in which considerable variability was noted from experiment to experiment, the values represent averages for two or three experimental groups. Previously, when diets containing choline were used in a study of the lipotropic effects of proteins and amino acids (Harper *et al.* 1954c), relative values of liver fat of comparable groups were consistent, although considerable variability was observed between experiments. In the present investigation, in which the choline in the diet was replaced by additional methionine, there were a few cases in which relative values for comparable groups varied somewhat from experiment to experiment. Best, Lucas, Ridout & Patterson (1950) and Best, Ridout, Patterson & Lucas (1951)

which the diets contained an additional 0.7% of DL-methionine instead of 0.15% of choline chloride, the accumulation of liver fat was not decreased by supplements of either 6 or 12% of gelatin. Actually, the average values for these groups are somewhat, but probably not significantly, higher than the control value. In a few instances, however, much more definite increases were observed. Only when 18% of gelatin was included in this type of diet was a value below that of the control group obtained.

All the diets used in the first two experiments were supplemented with 0.1% of DL-tryptophan, thus the growth rates of the groups that received gelatin were considerably greater than those of the groups that did not. This suggested that the stimulation of growth might have caused an increase in

the requirement for methionine for protein synthesis, and that the accumulation of liver fat might be attributable to an insufficient supply of this amino acid for choline synthesis.

The results presented in Table 2 show that this was not the case. When tryptophan was omitted from the diet (groups 9 and 10) the rate of growth of the group receiving gelatin was only slightly greater than that of the control group, yet the fat content of the liver was not decreased and was similar to that of group 12 which received tryptophan.

seen from the results presented in Table 3, choline chloride (0.15%) was completely effective in preventing an abnormal accumulation of liver fat and betaine hydrochloride (0.45%), while not as effective as choline under the conditions of this experiment, was considerably more effective than an additional 0.7% of DL-methionine. Neither aminoethanol (0.1%) nor glycine (1.5%) enhanced the lipotropic activity of methionine.

As glycine, which causes a decrease in the deposition of liver fat in rats receiving low-protein diets containing choline (Harper *et al.* 1954c;

Table 2. *Effect of gelatin on the deposition of liver fat in rats fed on low-protein (9% casein) diets containing different levels of tryptophan and methionine*

The diets indicated below were fed for 2 weeks.

Expt.	Group	Supplements of basal diet				Rate of gain (g./week)	Liver fat	
		Gelatin (%)	DL- Methionine (%)	DL- Tryptophan (%)	Choline chloride (%)		Dry wt. (%)	Wet wt. (%)
3	9	—	1.0	—	—	15.8±0.9	33.6±2.4	11.1±1.1
	10	12	1.0	—	—	19.6±1.5	31.7±2.7	10.4±1.1
	11	—	1.0	0.1	—	14.8±1.4	29.2±2.0	9.1±0.8
	12	12	1.0	0.1	—	29.7±1.5	33.2±1.1	10.2±0.4
	13	12	1.0	0.1	0.15	30.2±1.5	15.7±1.0	4.6±0.3
4	14	12	1.0	0.1	—	26.8±1.2	38.9±2.0	13.5±1.0
	15	12	1.5	0.1	—	21.8±0.8	41.3±3.3	14.7±1.8
	16	12	2.0	0.1	—	17.2±0.8	44.6±4.7	16.8±2.8
	17	12	2.5	0.1	—	16.6±1.5	43.4±1.6	14.8±0.7

Table 3. *Effect of certain substances known to be involved in the synthesis of choline on the deposition of liver fat in rats receiving 9% of casein and 12% of gelatin*

Each of the diets indicated below was supplemented with 0.1% of DL-tryptophan and was fed for 2 weeks.

Expt.	Group	Supplements of basal diet				Rate of gain (g./week)	Liver fat	
		Gelatin (%)	DL- Methionine (%)	Choline chloride (%)	Supplement		Dry wt. (%)	Wet wt. (%)
5	18	12	0.3	—	—	27.5±1.2	47.8±3.6	20.0±1.7
	19	12	0.3	0.15	—	27.5±1.1	14.8±0.8	4.1±0.2
	20	12	0.3	—	0.45% betaine HCl	26.5±0.8	23.9±2.2	7.4±0.8
	21	12	1.0	—	—	25.5±1.5	41.3±2.8	14.9±1.4
	22	12	1.0	—	0.1% aminoethanol	27.0±1.7	39.6±3.9	14.6±1.9
23	12	1.0	—	—	1.5% glycine	22.0±1.2	42.1±1.9	15.4±1.0

When the methionine content of the diet was increased (Expt. 4) substantially, even up to a level that retarded growth, the fat content of the liver was not decreased. It was therefore evident that the accumulation of liver fat could be attributed neither to the rapid growth rate nor to an insufficient supply of methionine in the diet.

In subsequent experiments the effects of a number of substances known to play a role in the synthesis of choline were compared. As can be

Benton, Harper & Elvehjem, 1955), was ineffective in the present study; as glycine, arginine and guanidinoacetic acid have been shown to counteract to some extent the toxic effects of a dietary excess of methionine (McKittrick, 1947; Roth, Allison & Milch, 1950; Hardin & Hove, 1951); and, as gelatin contains relatively large amounts of glycine and arginine (Block & Bolling, 1951); these two amino acids, in amounts equivalent to those contained in either 6 or 12% of

gelatin, were tested for their effect on the deposition of liver fat in rats fed on low-protein (9% casein) diets containing additional methionine in place of choline. It can be seen from the results presented in Table 4, which are average values for experiments for which the control values were similar, that there was a decrease in the accumulation of fat in the livers of rats receiving 9% of casein and 1% of methionine when 0.36% of DL-threonine was included in the diet (group 24 versus group 29). The average fat content for the groups receiving threonine was not as low as that observed in previous experiments (Harper *et al.* 1954*b*); however, in those experiments the values for the control groups were also considerably lower. The values for groups receiving threonine were less variable when the experiments were continued for 3-4 weeks.

The addition to the diet of 1.0% of L-arginine hydrochloride, in either the presence or the absence of supplementary threonine, was without effect on the deposition of liver fat. On the other hand, there was some increase in the deposition of liver fat when 3.0% of glycine was added to the diet, and a still further increase when both arginine and glycine were added. The greatest accumulation of liver fat was found in the groups that received 2.3% of guanidinoacetic acid. The average content of liver fat for each of the groups that received 0.36% of DL-threonine with glycine or arginine and glycine was less than that for the comparable group that did not receive additional threonine. The guanidinoacetic acid apparently created a severe choline deficiency, as demonstrated by Stetten & Grail (1942), which masked entirely any effect of threonine (Harper *et al.* 1954*c*).

The effect of glycine in increasing the deposition of liver fat under the conditions of this experiment could not be demonstrated consistently, but in no instance was there any evidence, in several unreported experiments as well as in those included in Table 4, that glycine caused a decrease in the accumulation of liver fat. An increase in the deposition of liver fat was observed in each of the experiments in which a combination of arginine and glycine was fed. The results were similar whether the levels of arginine and glycine were equivalent to those in 6 or 12% of gelatin.

In order to examine further the effect of the level of casein and the effect of amino acid supplements on the lipotropic activity of methionine, several experiments were undertaken, of which the results presented in Table 5 are representative.

It is evident that, when the casein content of the diet was either 20% or 40%, and the total amount of methionine in the diet was approximately 1.3%, the content of liver fat was very little, if at all, affected by the presence of gelatin, of glycine, or

Table 4. *Effect of arginine, glycine and guanidinoacetic acid on fat deposition in the livers of rats fed on diets containing 9% of casein*
Each of the diets indicated below was supplemented with 0.1% of DL-tryptophan and was fed for 2 weeks.

Expt.	Group	Supplements of basal diet					No. of rats	Rate of gain (g./week)	Liver fat	
		DL-Methionine (%)	DL-Threonine (%)	L-Arginine HCl (%)	Glycine (%)	Guanidinoacetic acid (%)			Dry wt. (%)	Wet wt. (%)
6	24	1.0	—	—	—	—	18	13.8±0.4	36.0±1.8	12.8±0.8
	25	1.0	—	1.0	—	—	12	12.0±0.7	38.9±2.3	13.9±1.2
	26	1.0	—	—	3.0	—	12	12.3±0.5	43.4±2.1	16.2±1.3
	27	1.0	—	1.0	3.0	—	18	14.4±0.5	49.5±1.9	19.3±1.1
	28	1.0	—	—	—	2.3	6	12.0±1.0	64.4±1.3	29.8±1.0
	29	1.0	0.36	—	—	—	18	18.9±0.5	26.0±1.5	8.3±0.6
	30	1.0	0.36	1.0	—	—	12	18.3±0.9	26.0±1.1	8.2±0.4
	31	1.0	0.36	—	3.0	—	12	18.1±0.8	33.6±3.3	11.8±1.5
	32	1.0	0.36	1.0	3.0	—	18	19.6±0.5	40.8±2.3	15.1±1.2
	33	1.0	0.36	—	—	2.3	6	14.5±1.7	68.2±1.1	34.3±0.9

of arginine and glycine. In these 2-week experiments choline always gave some reduction in the deposition of liver fat beyond that obtained with methionine.

When a combination of the amino acids that are most limiting in a diet containing 9 % of casein and 12 % of gelatin were provided (Expt. 8) some decrease in the deposition of liver fat occurred. An equal effect was obtained when threonine alone was added. The values obtained in these experiments with higher levels of casein and with amino acid supplements were approximately the same as that for the group that received 18 % of gelatin (Table 1, group 8). The extra 6 % of gelatin would provide the equivalent of 0.18 % of DL-threonine.

In view of the observation that 0.45 % of betaine hydrochloride was more effective than 1.0 % of DL-methionine under the conditions of an earlier experiment (Table 3), and in view of the satisfactory responses to betaine reported by Best *et al.* (1950), who used a similar diet, betaine and methionine were compared at what were thought to be optimum levels in two experiments. The results presented in Table 6 are representative. When the diet contained 9 % of casein and 12 % of gelatin, 0.6 % of betaine hydrochloride was more effective than 1.0 % of DL-methionine, as had been found earlier, but the combination of betaine and methionine was less effective than betaine alone. When the diet contained 18 % of casein, 0.7 % of DL-methionine or 0.6 % of betaine hydrochloride was equally effective, but again the combination of the two was less effective. The poor growth observed in the group that received betaine but no methionine with 9 % of casein and 12 % of gelatin is attributed to a methionine deficiency, for it can be seen that when a small amount of DL-methionine (0.3 %) was included in this diet growth was greatly improved. These experiments were of 3 weeks' duration and the values throughout were somewhat lower than those observed previously in 2-week experiments (Table 3), but the greater effectiveness of betaine was apparent in both cases.

Since the liver fat values generally were lower when the experiments were extended from 2 to 3 weeks, it was considered important to determine whether the effect of gelatin was transitory. From the results presented in Table 7 it is evident that rats receiving methionine in place of choline, either with 9 % of casein and 12 % of gelatin or with 18 % of casein, exhibited a relatively high liver-fat content after 1 week, and that this decreased gradually to an approximately normal value over a period of 4-5 weeks with both types of diet. It is also evident that at each interval the value for the group receiving 9 % of casein and gelatin was higher than that for the group receiving 18 % of casein. These 4- to 5-week experiments

Table 7. Effect of time on deposition of liver fat in rats receiving methionine in place of choline

Expt.	Group	Level of casein (%)	Supplements of basal diet				Weeks on expt.	Rate of gain (g./week)	Liver fat	
			Gelatin (%)	DL-Tryptophan (%)	DL-Methionine (%)				Dry wt. (%)	Wet wt. (%)
10	55	9	12	0.1	1.0		1	19.8 ± 1.8	43.0 ± 2.1	16.2 ± 1.2
	56	9	12	0.1	1.0		2	27.5 ± 1.1	35.7 ± 1.8	12.7 ± 1.0
	57	9	12	0.1	1.0		3	33.1 ± 1.2	26.3 ± 2.4	8.8 ± 1.1
	58	9	12	0.1	1.0		4	34.1 ± 1.0	14.5 ± 1.7	4.3 ± 0.4
	59	9	12	0.1	1.0		5	35.6 ± 1.0	10.1 ± 0.8	3.0 ± 0.3
11	60	18	—	—	0.7		1	24.8 ± 1.7	30.0 ± 2.4	9.2 ± 1.1
	61	18	—	—	0.7		2	30.3 ± 1.0	22.2 ± 2.2	6.5 ± 0.7
	62	18	—	—	0.7		3	34.2 ± 1.0	18.8 ± 1.5	5.6 ± 0.5
	63	18	—	—	0.7		4	36.6 ± 0.7	12.8 ± 1.0	3.7 ± 0.3
	64	18	—	—	0.7		5	35.9 ± 1.3	8.9 ± 0.6	3.0 ± 0.2

represent the only instance in this Laboratory in which the liver-fat content of rats receiving methionine as the only source of methyl groups in the diet has been found to be as low as that obtained with choline.

DISCUSSION

Both gelatin and glycine, in amounts which cause a reduction in the deposition of liver fat in rats receiving diets containing 9% of casein and choline for 2-3 weeks (Singal *et al.* 1953; Harper *et al.* 1953; Benton *et al.* 1955) were without effect when the choline in the diet was replaced by 1.0% of DL-methionine. This effect cannot be attributed to a deficiency of either methionine or aminoethanol, nor to the more rapid growth rate of the rats receiving the additional gelatin (Tables 2 and 3). In the former case (Harper *et al.* 1953, 1954a) the accumulation of liver fat could be attributed to a deficiency or imbalance of certain dietary amino acids. In the latter, however, the diet was deficient primarily in choline and, since the inclusion of choline with gelatin resulted in normal values for liver fat, it has been assumed that the fatty infiltration under these conditions indicates that insufficient choline is being synthesized.

If the accumulation of liver fat under the conditions of these experiments is used as a measure of choline synthesis, it becomes apparent that the rate of synthesis of choline from methionine in the young rat is affected by at least two dietary factors: the adequacy of the dietary protein with respect to its content of essential amino acids, and the glycine and arginine content of the diet.

The significance of the first factor is evident from the decrease in the deposition of liver fat that occurred when the diet containing 9% of casein and 12% of gelatin was supplemented with threonine, when the casein content of the diet was increased, and when the gelatin content was increased to 18%. Beveridge, Lucas & O'Grady (1944) also found less accumulation of liver fat when the gelatin in their diet was replaced by casein. That the system affected by threonine in this case is different from that affected in the previous studies (Singal *et al.* 1953; Harper *et al.* 1953) is evident from the fact that in the latter case choline had to be present before an effect of threonine could be demonstrated, whereas in the present study, in which diets containing much more protein were used, no effect of threonine could be demonstrated when choline was provided. It is of interest that this effect on the deposition of liver fat was observed in rats that were growing well and that showed no growth response to the threonine supplement.

The effect of arginine and glycine in modifying the lipotropic activity of methionine is not un-

expected in view of the observations of Stetten & Grail (1942) that the methyl acceptor guanidinoacetic acid increased the severity of a choline deficiency and those of Roth *et al.* (1950), Hardin & Hove (1951) and Cohn & Berg (1951) that the toxicity of an excess of methionine was decreased by including arginine or glycine or both or guanidinoacetic acid in the diet. Apparently the methyl group of methionine is used to convert excessive amounts of guanidinoacetic acid or high levels of arginine and glycine into creatine (Stetten & Grail, 1942), and this may cause a substantial decrease in the amount of methionine available for choline synthesis. If the supply of choline in the diet is inadequate, an increase in the deposition of liver fat would be expected (see Table 4). These observations may account for the report by Salmon (1950) that gelatin increases the requirement of the rat for methionine. This effect of an excess of arginine and glycine was less apparent when threonine was added to the diet containing 9% of casein (Table 4), and was not at all evident when the diet contained 20% or 40% of casein (Table 5), suggesting that arginine and glycine are more readily metabolized via pathways other than creatine formation when the dietary protein is adequate.

Although an excess of methionine would be expected to overcome the effect of an excess of arginine and glycine (Hardin & Hove, 1951), it did not in these short-term experiments (Table 2). This, and the observation that betaine was more effective than methionine in the 2- to 3-week experiments in which diets containing gelatin were used, require further investigation, particularly since the methyl groups of betaine are presumably metabolized via methionine. It is possible that both of these observations are related to the known toxic action of an excess of dietary methionine (see Harper, Benton & Elvehjem, 1955 for references) of which an increase in the deposition of liver fat may be another manifestation (Table 6; Roth *et al.* 1950). The exact level at which methionine becomes toxic is uncertain and is influenced by a number of factors (Treadwell, 1948; Wretling & Rose, 1950; Grau & Kamei, 1950; DeBey, Snell & Baumann, 1952; Baron & Allison, 1954). The level of 1.2-1.3% generally used in this study undoubtedly approaches the toxic range but no evidence of a significant retardation of growth was obtained with this level in either the present study or that of Treadwell (1948).

The failure to obtain a decrease in the deposition of liver fat in 2- to 3-week experiments when gelatin was added to a diet containing 9% of casein and 1% of methionine can apparently be attributed in the main to the inadequacy of the supply of threonine in the diet containing casein

and gelatin and to the high levels of glycine and arginine provided by the gelatin (compare groups 24 and 32, Table 4, with groups 5 and 7, Table 1). These factors are obviously of importance only in short experiments (3-4 weeks) because after 4 weeks the liver-fat content of rats receiving 9 % of casein and 12 % of gelatin is in the normal range.

It would appear that some time is required for the young rat, even when fed on an adequate diet (Table 7), to adapt itself to the use of methionine instead of choline. This suggests that the capacity of the rat to synthesize choline increases as the rat matures. The time required for such an adaptation is apparently modified by the adequacy of the dietary protein (Table 7). Since the protein requirement decreases as the animal matures and its growth rate falls off, a diet that is inadequate for the very young animal may be perfectly adequate later on. The observation of Beveridge, Lucas & O'Grady (1945), that the liver-fat content of rats receiving gelatin supplemented with essential amino acids was higher than that of rats receiving casein, may be attributed to the high arginine and glycine content of the diets that contained gelatin and to the fact that their experiments were of only 3 weeks' duration.

These observations emphasize the caution that must be exercised in attempting to compare quantitatively the effectiveness of choline and methionine as lipotropic agents. Distinctly different values would be obtained if the comparisons were made after 1, 2, 3 or 4 weeks in experiments begun with weanling rats; and at each interval the results would differ with different types of diets. This may account for some of the differences of opinion about the amount of methionine required for maximum lipotropic activity.

The results of this study emphasize once again the importance of the balance of amino acids in the diet on the deposition of liver fat, and it is now evident that at least three distinct effects of the non-sulphur-containing amino acids can be demonstrated. Beveridge *et al.* (1945) and Treadwell, Tidwell & Gast (1944) demonstrated that if a hypolipotropic diet were made more adequate with respect to essential amino acids an increase in growth and food consumption could cause an increase in liver-fat deposition. The observations of Singal *et al.* (1953), Harper *et al.* (1953) and Cole & Scott (1954) showed that deficiencies of threonine, lysine and tryptophan could cause an accumulation of liver fat through a mechanism distinctly different from that of the well-known lipotropic factors, and it does not seem improbable that some other individual amino acid deficiencies may have similar effects. Finally, it is evident that the levels of arginine and glycine and of threonine may influence the lipotropic activity of methionine even in

relatively high protein diets. Dick, Hall, Sydenstricker, McCollum & Bowles (1952) and Lucas & Ridout (1955) have suggested that some fatty livers may represent a non-specific effect of protein deficiency rather than a specific lack of any one essential amino acid, but this is surely another way of saying that when the diet is deficient in several factors the effects of the individual deficiencies cannot readily be distinguished.

SUMMARY

1. Some factors that influence the lipotropic activity of methionine in the young rat have been investigated.

2. In experiments of less than 4 weeks' duration, fatty infiltration of the liver was evident in young rats when the choline in diets that permitted a satisfactory rate of growth was replaced by methionine.

3. The extent of the fatty infiltration was greater when the diet contained 9 % of casein and 12 % of gelatin than when it contained 18 % or more of casein.

4. The greater fatty infiltration observed in rats receiving the diet containing gelatin has been attributed to the low content of threonine and the high content of arginine and glycine in gelatin.

5. No abnormal accumulation of liver fat was found in rats fed on either diet for more than 4 weeks, which suggests that the capacity of the young rat to synthesize choline increases as it matures and that the effect of gelatin is of significance only during the period of early growth, when relatively high-protein diets containing additional methionine in place of choline are fed.

6. The significance of these factors in the investigation of the lipotropic activity of methionine has been discussed.

The authors wish to express their appreciation to Dr C. A. Elvehjem for many helpful discussions during the course of this work. They wish to thank Miss Marie Winje, M.Sc., for her assistance with some of the experiments.

This work is published with the approval of the Director of the Wisconsin Agricultural Experiment Station and was supported in part by grants from the National Livestock and Meat Board, Chicago, Ill., and the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. The crystalline vitamins were kindly provided by Merck and Co., Rahway, New Jersey.

A preliminary report of some of this work was presented by one of us (A.E.H.) before the University of Toronto Physiological Society, 16 November 1954.

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The Fatty Acid Composition of Indian Turtle Fat

BY S. P. PATHAK AND L. M. DEY

Department of Industrial Chemistry, Banaras Hindu University, India

(Received 20 September 1955)

As very little is known about the composition of the fats of amphibians, particularly of tropical waters, the present study on the fatty acid composition of the body fat of an Indian turtle was undertaken.

EXPERIMENTAL

The sample of body fat was from a turtle (*Erismochelonia imbricata*) of marine origin, caught in the Bay of Bengal (east coast of Madras State). It was kindly supplied by the Superintendent, Fisheries Technological Station, Kozhikode. The fat was refined and freed of phosphatides by the usual methods. It was then hydrolysed, and the mixed fatty acids thus obtained were resolved into three groups of

simpler mixtures of fatty acids by the lead salt-ethanol method (Hilditch, 1947) followed by further resolution of the soluble fraction by the lithium salt-acetone method (Tsujimoto, 1920; Tsujimoto & Kimura, 1923). Each group of the fatty acids was separately converted into the methyl esters, taking the usual precautions as recommended by Bjarnason & Meara (1944). The esters were then subjected to fractional distillation under high vacuum (0.1 mm. Hg) through an efficient electrically heated and packed column (Longenecker, 1937). The compositions of the subfractions were calculated from their respective iodine values and saponification equivalents according to the method recommended by Hilditch (1947). The mean unsaturations, as given in parentheses in Table 3, were determined as usual by interpolation and extrapolation

Table 1. Separation of the acids of turtle fat by the lead salt-ethanol and lithium salt-acetone methods

Group	Description	Wt.		Iodine value
		(g.)	(%)	
A	Lead salt-ethanol-insoluble	51.2	31.8	12.5
B	Lead salt-ethanol-soluble but lithium salt-acetone-insoluble	73.7	45.8	104.4
C	Lithium salt-acetone-soluble	36.0	22.4	166.8