Recently, Berger, Slein, Colowick & Cori (1946) and Kunitz & McDonald (1946) have shown that the hexokinase reaction proceeds with mannose at about half the rate with *d*-glucose. This may account for the longer time taken to reach the maximum rate in the fermentation by yeast juice at 25° of mannose as compared with glucose (cf. Fig. 1). The equality of the two maxima is best explained by the previous observation that at low phosphate concentration (0.040M) the reaction

controls the over-all rate of hexose fermentation by dried yeast (Gottschalk, 1946).

It would thus appear that the observed differences between the fermentation of *d*-mannose and *d*-glucose by yeast cells and by yeast preparations over the range of temperature tested (0–38°) may be due to: (1) the slower rate at which mannose penetrates the cell-membrane, and/or (2) the slower rate at which mannose reacts with hexokinase, and/or (3) the instability of mannose-6-phosphate isomerase at temperatures above 28°.

SUMMARY

1. In contrast to that of *d*-glucose, the temperature coefficient of *d*-mannose fermentation by yeast cells is dependent on substrate concentration; it increases considerably with decreasing mannose concentration. With yeast extract as fermenting agent no such difference between mannose and glucose is observed. It would thus appear that the cell membrane is responsible for this phenomenon.

2. The maximum rates of mannose and glucose fermentation at 25° by yeast extract are the same provided that during the preparation of the yeast extract the temperature does not exceed 28°; with higher temperatures mannose is fermented markedly slower than glucose. Taken with previous findings of Jephcott & Robison (1934) this result suggests the existence in yeast of two aldo-hexose phosphate isomerases, the mannose-6-phosphate isomerase being more readily destroyed at 28–38° than the glucose-6-phosphate isomerase.

3. Experiments at 10° afford evidence that both α - and β -*d*-mannopyranose are fermented without preliminary interconversion by mutarotation.

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The Effect of Diet on the Glutathione Content of the Liver

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It has been shown recently (Glynn, Himsworth & Neuberg, 1945) that white rats kept on a diet containing all the amino-acids occurring in proteins with the exception of methionine and cystine, develop after varying lengths of time massive necrosis of the liver. Although it is not quite certain that the dietary deficiency of sulphur-containing amino-acids was the sole cause of the liver lesions observed, the results suggested that the proneness of the rats to develop hepatic necrosis was speci-

fically associated with a lack of cystine, or its potential precursor methionine, in the amino-acid mixture. It seemed likely that the absence of these amino-acids from the diet would produce a reduction in the relative concentration of the non-protein sulphur in the tissues. Thus Lee & Lewis (1934) found in rats, which had been reared on a diet relatively deficient in cystine, low sulphur values in protein-free extracts of liver, kidney and muscle; these authors also observed that the ratio of

nitrogen to sulphur in such extracts was very much higher than normal, indicating a relatively greater loss of sulphur than of nitrogen. It was noted that the composition of the proteins of these tissues was not altered. In dogs also, protein depletion is associated with an increase of the ratio of total nitrogen to total sulphur in the liver (Miller & Whipple, 1942). Moreover, the results obtained by French workers (Randoïn & Fabre, 1927, 1931; Binet & Weller, 1934, 1935; Marenzi & Braier, 1934) with the not very specific iodometric method, indicated that a definite decrease of the glutathione content of many organs, especially of the liver and kidney, is produced by starvation or administration of a diet poor in cystine or methionine. It was therefore proposed to investigate in some detail the effect of the diet and particularly of changes in the amounts of cystine and methionine supplied, on the glutathione content of the tissues. It was thus hoped to establish an association between variations in the chemical composition of the liver and the onset of hepatic necrosis.

In the course of this work the wider aspects of the problem became apparent. It was pointed out by one of us (Neuberger, 1946) in a discussion on the metabolism of amino-acids, that the clinical picture produced by dietary deficiencies of the various essential amino-acids is on the whole remarkably uniform. This is to be expected in so far as a deficiency leads only to an interference with the synthesis of proteins. Since the amino-acid composition of any particular species of protein molecules is fixed, the utilization of amino-acids for protein production is interdependent. In certain cases the body may adjust itself to a deficiency in a particular amino-acid by synthesizing proteins relatively poor in the missing compound at the expense of other proteins. This has indeed been found for cystine deficiency which leads to a decrease in the cystine content of hair (Lightbody & Lewis, 1929). The morphological changes observed indicated that the proteins poor in sulphur were relatively increased by the dietary deficiency (Smuts, Mitchell & Hamilton, 1932). However, it may be expected that such an effect will be rare; it does not indeed, even in the case quoted, extend to the proteins of tissues like liver and muscle. With low-molecular metabolites which are produced from essential amino-acids such an interdependence does not exist and a deficiency may therefore lead to a decrease of the concentration of the substance in the tissues. Specific lesions may therefore be expected to be associated with chemical changes in body constituents other than proteins. Lack of sulphur-containing amino-acids in the diet appears to produce effects which are not only more severe than those observed in deficiencies of other amino-acids, but the symptoms also seem to be qualitatively of

a different character. It is to be expected therefore, that this difference would be reflected in a change of the relative composition of the body, particularly with regard to the non-protein constituents of the tissues.

EXPERIMENTAL

Young black and white or Wistar albino rats usually weighing 60–90 g. were used.

Diet

Unless otherwise stated the animals were given a stock diet which supported good growth (about 2.7 g./day) and contained 18–20% protein, mainly derived from dried milk and wholemeal flour. The low-protein diet had the following composition: salt mixture (no. 2, *U.S. Pharmacopoeia*, 1942) 4.5%, fat (arachis oil and cod-liver oil) 12%, starch 83.5%. This diet was adequate except in protein of which the only source was 100 mg. of dried yeast which was added as a daily supplement to provide certain vitamins. The protein content of this yeast was 40–50% and the daily intake of protein (40–50 mg.) was therefore very small. Amino-acid supplements were mixed with the diet which was given in such amounts as to ensure complete consumption. Diets containing 10% of cystine or methionine consisted of a mixture of the amino-acid and the stock diet and were fed *ad libitum*. The 60% casein diet consisted of: casein 60%, salt mixture 5%, fat (arachis oil and cod-liver oil) 12%, starch 18%, dried yeast 5%, and was also fed *ad libitum*. Details of the diets in which the nitrogen was supplied by a mixture of pure amino-acids may be found in the papers of Neuberger & Webster (1946, 1947).

On the low protein diet the animals steadily lost weight at the rate of about 1.3 g./day and the diets containing 10% of cystine and methionine also caused a rapid loss of weight. The diet containing 60% casein supported growth to about the same extent as the stock diet.

Estimation of glutathione

The animals were killed some 2 hr. after removal of food, either by asphyxiation with coal gas or by a blow on the head. After puncturing the heart, the tissues were removed as rapidly as possible, washed free of blood and sliced into 2% salicylsulphonic acid contained in a weighed 'Potter homogenizer' tube. The whole was then kept in an ice-salt mixture until it was reweighed, thawed, homogenized and finally filtered. The extracts were then used for glutathione estimation for which three methods were applied.

Results were expressed in terms of mg./100 g. of tissue and in the case of the liver also in terms of mg./100 g. of body weight. This latter expression to some extent eliminates the variations due to change in liver weight.

(1) *Iodometric method.* This was an adaptation of the method of Fujita & Numata (1938) in which the interfering ascorbic acid was removed by addition of excess of a solution of 2:6-dichlorophenol-indophenol (0.025%, freshly prepared) and back titration of the excess with a standard ascorbic acid solution (0.0025% in 2% metaphosphoric acid, also freshly prepared) (cf. Kennaway, Kennaway & Warren, 1944). The glutathione was then titrated at 0–5° with a standard solution of potassium iodate (0.0005N in 2% salicylsulphonic acid) in the presence of potassium iodide (final concentration 0.5%). Recovery experiments suggested that this modified method is reliable.

(2) *Colorimetric method* (Fujita & Numata, 1939). The intensity of the absorption of the solution was measured in the Spekker absorptiometer using the Ilford Spectrum green filter. Contrary to the report of Herrmann & Moses (1945) we found that delay between the addition of nitroprusside and ammonia caused a marked reduction in the sensitivity of the method and the two additions were therefore made immediately before the reading was taken. With this precaution the method gave accurate and reproducible results with pure glutathione solutions above a concentration of 0.01%.

(3) *Manometric method*. This method was devised by Woodward (1935) and depends on the coglyoxalase action of glutathione. Here it was found advisable to store the yeast glyoxalase preparation in evacuated ampoules at 0° in order to preserve the activity effectively.

Comparison of methods

Table 1 contains averages of a number of results obtained by the three methods. The agreement is quite good with normal livers, though the values obtained by the iodometric method are somewhat high, and in cases where the liver contained only small amounts of glutathione the difference became significant. Under such conditions the colorimetric method was also found unsuitable owing to the occurrence of an inflexion in the calibration curve of absorptiometer readings.

Table 1. *Comparison of the different methods used for the estimation of glutathione*

(The figures given are averages of a number of estimations. Each individual sample was assayed by three methods. Values are given in mg. of glutathione/100 g. of tissue.)

Tissue	No. of estimations	Method of estimation		
		Iodometric	Manometric	Nitroprusside
Liver { Normal	16	220	183	196
{ Low	7	76	54	—
Kidney	10	234	74	97
Lung	4	88	63	—
Intestine	6	129	81	—

The discrepancy between the manometric and iodometric methods was much more serious in the case of the kidney. It was thought that this may be due to the presence of an enzyme which rapidly liberates cysteine from glutathione. Such an enzyme has been found in rat kidney (Schroeder & Woodward, 1937), and we have observed that cysteine takes up more than one equivalent of iodine under the conditions of our titrations. In order to eliminate the effect of such an enzyme the treatment of the kidney was carried out as rapidly as possible, the tissue often being minced and frozen in salicylsulphonic acid within a minute of opening the abdomen. In spite of this, however, the discrepancy was not resolved. It is doubtful, therefore, whether the difference between the results obtained by the two methods is completely explained by the presence of cysteine but we have not investigated this matter further.

In the case of lung and intestine, results obtained by the manometric method were invariably lower than those obtained by the iodometric method.

The manometric method appears to be the most reliable because of its specificity. Aspartathione and isogluthathione are the only compounds so far discovered which are capable of simulating glutathione as coglyoxalase (Behrens, 1941) and these are not known to occur in the body. Unless otherwise stated, the results quoted in this paper were obtained by the manometric method. In no case was the determination of oxidized glutathione attempted. This omission was considered justified on the grounds that previous workers have found little or none of it in the tissues (Binet & Weller, 1935; Dohan & Woodward, 1939).

RESULTS

Glutathione content of tissues from normal animals. Values for the glutathione content of the liver of animals maintained on the stock diet are given in Table 2. The results show a wide variation as indicated by the standard deviation. There is, however, no significant difference between the two strains of rats or between the two sexes. Data for certain other tissues are given in Table 3. The

figures are averages of a number of determinations; for the kidney the manometric method was used, whilst for other tissues the iodometric method was employed. These results also showed some scatter but not to the same degree as those for the liver and the average figures agree with those reported recently in the literature by other workers (for references see Herrmann & Moses, 1945).

The effect of protein deficiency and fasting on the glutathione content of rat tissues. After being transferred to the low protein diet the animals lost weight and the glutathione content of the livers of both strains fell, as can be seen from Table 2. The initial fall was very rapid, the change after 1 day being quite marked. The values then decreased more gradually to reach, after about 10 days, a more or less steady level of some 2 mg./100 g. body weight. To test the significance of these changes the figures for both albino and black and white rats

Table 2. *Effect of fasting and dietary protein deficiency on the concentration of glutathione in the liver*(Figures in the last column represent the mean $\pm$ the standard deviation.)

Strain of rats	No. of rats	Dietary regimen	Period on diet (days)	Liver weight (g./100 g. of body weight)	Liver glutathione	
					(mg./100 g. of liver)	(mg./100 g. of body weight)
Black and white	8	Stock	—	4.40	208	9.20 $\pm$ 4.32
	6	Fasting	24 hr.	4.30	231	10.20 $\pm$ 1.41
		"	48 hr.			
					Combined	9.64 $\pm$ 3.40
	5	Low protein	1	4.12	151	5.96 $\pm$ 1.31
	5	"	4-5	5.20	110	5.30 $\pm$ 1.49
	3	"	14	4.00	44	1.41
	3	"	16-17	4.80	45	2.14
	1	"	23	3.06	67	2.05
Albino	9	Stock	—	5.42	174	9.16 $\pm$ 3.02
	2	Fasting	24 hr.	3.78	160	5.84
	1	"	72 hr.	2.02	140	2.83
	2	Low protein	1	4.15	130	5.40
	4	"	3-4	3.86	52	2.00
	2	"	19	4.25	55	2.34

Table 3. *Effects of various diets on the glutathione contents of several tissues*

(The values are expressed in terms of mg. of glutathione/100 g. of tissue. Figures in parenthesis give the number of experiments from which each mean is calculated.)

Tissue	Dietary regimen					
	Stock diet		Low protein diet given for			Low protein diet + supplement of cystine or methionine
	Mean	Range of values	1 day	3-5 days	20 days	
Kidney	77 (10)	60-102	—	71 (5)	56 (2)	97 (6)
Muscle	50 (7)	40- 66	64 (2)	34 (2)	37 (1)	42 (4)
Lung	102 (7)	79-133	113 (5)	100 (5)	86 (2)	115 (3)
Intestine	121 (4)	114-132	119 (2)	115 (4)	—	140 (5)

were collected into four groups, those for rats on normal stock diet (20 animals, group A), and those for rats given low protein diet for 1 day (7 animals, group B), 3-5 days (9 animals, group C) and 14-23 days (10 animals, group D). The *t* values were calculated by the method of Fisher (1925) and found to be as follows for the differences between the mean values of group A and groups B, C and D respectively: 2.39, 4.03, 5.44. The difference after 1 day was therefore significant ($P < 0.05$) and the other differences highly significant ($P < 0.01$). The response of other tissues to a low protein diet has not been thoroughly investigated but the results presented in Table 3 suggest that any change which takes place in these tissues is of a less drastic and more gradual nature.

In contrast to the above result, fasting for 24 or 48 hr. had little or no effect on the glutathione content of the livers of black and white rats though there was considerable loss of body weight. The albino rats seemed to be somewhat more sensitive in this respect.

The effect of addition of cystine or methionine to a low protein diet on the glutathione content of rat livers. To determine whether cystine or methionine would affect the depletion of liver glutathione produced by the low protein diet, these amino-acids were administered with this diet in daily amounts of 30 and 37 mg./rat respectively. The results in Table 4 show that, when given from the beginning of the low protein period, these supplements were very effective and the levels of glutathione found in the liver appeared to be higher than the normal values. Similarly such supplements rapidly restored the liver glutathione content after depletion caused by varying periods of low protein diet. This latter effect was shown to be transitory, however. Thus, when the rats were fed on the low protein diet for 21 days and then given cystine or methionine for 2 days and returned to the low protein diet for a further 1 day before being killed, the liver glutathione had once more fallen to a very low level. The liver glutathione contents in four such experiments were (in terms of mg./100 g. body weight)

Table 4. *Effect of addition of methionine or cystine to a low protein diet on the glutathione content of the liver*

(Cystine was given in a dose of 30 mg./day and methionine in a dose of 37 mg./day. Each value represents one animal.)

Strain of rat	Period on diet (days)		Nature of supplement	Liver wt. (g./100 g. body wt.)	Liver glutathione	
	1st period low protein diet	2nd period low protein diet + supplement			(mg./100 g. liver)	(mg./100 g. body wt.)
Black and white	0	6	Cystine	6.20	220	13.64
				5.80	260	15.10
	0	7	Methionine	5.84	345	20.15
				5.48	320	17.50
	4	1	Cystine	5.80	250	14.50
				5.60	312	17.50
	4	2	Cystine	5.40	280	15.12
				6.00	271	16.26
	4	7	Cystine	5.74	255	14.64
	4	1	Methionine	6.10	187	11.40
	4	2	Methionine	5.95	235	14.00
	4	7	Methionine	5.80	255	14.79
	21	0	—	3.06	66	2.02
	21	1	Cystine	3.75	282	10.60
	21	1	Methionine	4.65	312	14.51
Albino	19	0	—	4.25	45	1.92
				4.26	65	2.76
	14	1	Cystine	5.20	130	6.76
	14	2	Cystine	4.75	162	7.70
	14	5	Cystine	4.80	350	16.80
	14	1	Methionine	4.66	145	6.75
	14	2	Methionine	5.15	280	14.42
	14	5	Methionine	5.35	380	20.33

2.43 and 2.74 for cystine supplements and 3.03 and 4.34 for methionine supplements respectively. The results in the last column of Table 3 show that when either cystine or methionine was added to the low protein diet the glutathione content of other tissues was well maintained.

Experiments with pure amino-acid diets. The specificity of the relation between the dietary intake of sulphur-containing amino-acids and the liver glutathione was established more firmly by experiments in which the nitrogen of the diet was supplied in the form of mixtures of pure amino-acids. The results of these experiments are shown in Table 5. The full diet supported good growth and maintained a normal concentration of glutathione in the liver. When both cystine and methionine were omitted for periods varying between 35 and 42 days, the glutathione content of the liver was reduced in four animals out of six to nearly the same level as that produced by prolonged protein deficiency. In two rats, however, the values found were very much higher than expected. With cystine alone the glutathione content of the liver remained within normal limits. In contrast, the omission of histidine, another essential amino-acid, from the mixture for 34 days had little effect on the glutathione content of the liver. The two low values, in terms of mg./100 g. body weight, are probably

explained by the great reduction in liver weight relative to body weight observed in these animals.

Table 5. *Variation of the glutathione content of the livers of rats fed on diets in which the nitrogen was in the form of mixtures of pure amino-acids*

(The amino-acid mixture was either complete (A), or devoid of both cystine and methionine (B), cystine (C), methionine (D) or histidine (E).)

Rat strain	Diet	Liver glutathione	
		(mg./100 g. of liver)	(mg./100 g. of body weight)
Black and white	B	60	3.30
		63	2.90
		49	2.49
	C	200	7.13
		250	11.80
Albino	A	152	7.99
		125	5.35
		40	2.18
	D	115	6.18
		250	15.60
		315	17.20
	E	260	9.30
		150	6.14
		220	7.63
		160	5.61

The effect of diets rich in cystine or methionine on the glutathione content of rat livers. Diets containing 10% of cystine or methionine did not produce a spectacular increase in the glutathione content of the liver in spite of daily intakes of 500–1000 mg. of cystine and 250–500 mg. methionine respectively. The results in Table 6 suggest that an increase over the normal values takes place initially but eventually the glutathione concentration appears to be normal or even slightly reduced. Consumption of a diet containing 60% of casein for 5 days appeared to have little effect.

glutathione to dietary changes, are in keeping with their results. This lability causes a certain amount of difficulty in assessing the results of glutathione estimation. The wide variations encountered, even in normal animals, suggest that a single determination gives only a momentary glimpse of a rapidly changing situation.

The experiments with both low protein and pure amino-acid diets lead to the conclusion that the glutathione content of the liver depends entirely on the availability of cystine or methionine in the diet and not at all on the supply of other essential

Table 6. *Effect of diets rich in cystine or methionine on the glutathione content of the livers of albino rats*

(The figures in the final column are based on the results obtained by the manometric method.)

Diet	Period on diet (days)	Liver glutathione (mg./100 g. liver)			(mg./100 g. body weight)
		Iodometric method	Colorimetric method	Manometric method	
10% cystine	1	578	264	250	11.20
	1	600	—	250	15.00
	2	525	267	225	11.90
	3	334	—	230	12.11
	5	—	157	162	5.55
10% methionine	1	275	—	145	9.00
	2	392	400	370	17.30
	3	339	—	170	7.41
60% casein	5	—	216	212	9.02
	5	—	281	285	15.60

In the experiments in which excessive amounts of cystine were given the results obtained by the iodometric method were much higher than those indicated by either of the other two methods. The better agreement shown between the manometric and colorimetric methods suggests that glutathione is the only thiol compound present, and as cystine takes up very little iodine under the conditions of our experiments, the occurrence of the high reducing value is difficult to explain. This matter has not been investigated further.

DISCUSSION

Though glutathione has only been isolated from a few tissues, it is generally thought to occur in most cells, in varying concentrations. The function of this tripeptide is not definitely known, but its biological importance is probably associated with its SH group. Waelsch & Rittenberg (1941, 1942), using isotopic nitrogen, showed that glutathione is rapidly broken down and resynthesized in both intestine and liver; these authors calculated that half of the glutathione of the liver was replaced in 2–4 hr. Our findings, which indicate a very marked and quick response of the concentration of liver

amino-acids. Thus administration of cystine to rats kept on a practically protein-free diet completely prevented the decrease of glutathione in the liver found on the basal diet alone, and similarly the loss caused by prolonged depletion was quickly made good by the addition of either cystine or methionine to this basal diet. The body can apparently supply the other two components of the tripeptide, viz. glutamic acid and glycine, fairly easily; this is also shown by the observation that administration of these non-essential amino-acids together with cystine causes an increase in the glutathione content of the liver which was not higher than that found with cystine alone.

Though variations in the glutathione content of the liver produced by changes of the diet are wide, they occur only within certain upper and lower limits. Thus a very marked increase in the intake of cystine or methionine has only a slight effect and the same also applies to an increase of proteins in the diet as a whole. Conversely, even in prolonged deficiency, glutathione never disappears from the liver, and indeed the average values found after a depletion of 19–23 days are not lower than those observed after a period of 14 days. It is likely that a steady state is established in which glutathione

is synthesized at a reduced rate in the liver from amino-acids obtained from the break-down of tissue proteins. Such a dynamic equilibrium between the sulphur-containing amino-acids present in proteins and the cystine in the glutathione of the liver may also explain the relatively high values of liver glutathione found in some animals on amino-acid diets deficient in methionine and cystine. In starvation, proteins are utilized to supply caloric requirements and are broken down more rapidly than in specific protein deficiency. It is likely therefore that in starvation larger quantities of cystine and methionine are available for glutathione synthesis and this probably explains our finding that fasting for a period of 24–48 hr. had little effect on the glutathione content of the livers of black and white rats. Binet & Weller (1935) subjected guinea-pigs to prolonged fasting (8 days) and found only a slight drop in the liver glutathione.

Our data, though not very extensive on this point, indicate that the glutathione content of other organs is not influenced by dietary variations to the same extent as that of the liver. Glutathione appears to share this lability with other constituents of the liver cell such as glycogen and the labile protein (Kosterlitz, 1944; Harrison & Long, 1945) and ribonucleic acid fraction (Davidson & Waymouth, 1944) of liver cytoplasm. It is doubtful, however, whether the decrease in the glutathione content of the liver is the only change in the relative composition of the body produced by a dietary deficiency of cystine and methionine. Thus the loss of non-protein sulphur found by Lee & Lewis (1934) in muscle and liver cannot be accounted for by glutathione alone and this applies also to the findings of Miller & Whipple (1942) in dogs. It is likely therefore that the concentration of some other sulphur-containing compound, present in many tissues in the non-protein fraction, is reduced in dietary deficiency.

The original purpose of this investigation was to find out whether the acute massive necrosis of the liver observed in rats reared on diets containing only traces of cystine and methionine is caused by a change in the composition of the liver with respect to sulphur. Dent (1947) has shown that the relative amounts of cystine and methionine in the proteins of the liver are not reduced, even in animals in which hepatic necrosis is observed. The results of this investigation show that a diet which may produce liver lesions also reduces considerably the glutathione content of the liver and probably also the concentration of other low-molecular sulphur-containing compounds. However, in the present

experiments, hepatic necrosis was produced neither in the albino nor in the black and white rats, though the animals lost nearly half their original body weight during the period of deficiency. It is therefore impossible to state whether the onset of acute necrosis is associated with a further decrease or virtual disappearance of glutathione in the liver. It is doubtful, and in our opinion unlikely, that a decrease in the concentration of glutathione or any other sulphur-containing compound in the liver is by itself responsible for liver lesions. It is more likely that a toxic agent of an infectious nature, such as a virus, or a substance which arises in the normal metabolism of the animal, is involved. A deficient diet is likely to make the animal more susceptible to such a toxic agent and this effect may be associated with a reduction of the glutathione of the liver. The increased sensitivity to various toxic substances of animals kept on diets in which the intake of sulphur-containing amino-acid is the limiting factor has been demonstrated by several workers (Miller & Whipple, 1942; Heppel, Highman & Porterfield, 1946). The fact that the liver is the organ involved in these toxic effects may be connected with the marked lability of the liver glutathione in response to dietary changes.

SUMMARY

1. A diet low in protein produced in rats an almost instantaneous and marked reduction in the liver glutathione content which after about 14 days reached a constant level corresponding to 20–30 % of the normal value. This fall was prevented by the addition of cystine or methionine without a corresponding increase of the intake of other amino-acids. Administration of cystine or methionine also restored very quickly to normal low glutathione values produced by a deficiency. Other organs were not greatly affected.

2. An increase in the intake of cystine or methionine to a high level did not produce a marked effect on the glutathione content of the liver as measured by a colorimetric or manometric method, though a considerable increase was indicated with the iodometric method. Starvation appeared to affect the glutathione content of the liver to a lesser extent than protein deficiency.

3. Similar results were obtained with diets in which nitrogen was supplied in the form of pure amino-acids. It is concluded that the glutathione content of the liver depends solely on the intake of cystine or methionine. The bearing of these results on the dietary production of hepatic necrosis is discussed.

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The Folic Acid Group of Growth Factors

1. THE COMPLEXITY OF GROWTH FACTORS FOR *STREPTOCOCCUS LACTIS* R

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Considerable confusion has arisen with regard to the various preparations which have been reported as having activity for *Streptococcus lactis* R. In the main this has been due to a desire on the part of many workers to over-simplify the question by claiming that preparations obtained by different methods from a variety of sources contain identical substances. The complexity of the subject was obvious, however, as soon as it became possible to compare the activity of the liver fractions of Bohonos, Hutchings & Peterson (1941) and of Stokstad (1943), with a yeast-derived sample obtained by the last worker, and one isolated from fermentation residues by Bohonos, Hutchings, Slobodkin & Stokstad (1944). Comparative data for these preparations, and for other active materials whose preparation and properties have since been reported, are given in Table 1.

Recently Johnson (1946) published data for *Lactobacillus casei* and *Strep. lactis* R, claiming to show that folic acid, Vitamin B₆ and the *L. casei*

factor (both synthetic, i.e. pteroyl-glutamic acid and natural, i.e. the preparation from liver) are identical, a conclusion which does not appear to be completely justified in view of the data of Table 1. Since synergism has been observed between various fractions isolated from liver (Chattaway, Dolby & Happold, 1944), it was decided to examine the effect of the simultaneous addition of pairs of various growth factor preparations to culture media, using *Strep. lactis* R as test organism. This organism was chosen in preference to *L. casei* because, using a medium based on an acid-hydrolysate of casein as generally employed in these laboratories, it has not been found possible to stimulate good growth of *L. casei* by the sole addition of one of the 'folic acid group' of factors. Simultaneous addition of a pair of growth-factor preparations, separately apparently identical as tested by the growth response of *Strep. lactis* R, might indicate differences in composition, whether the individual growth-factor preparations were single or complex