A STUDY OF THE ACTION OF METHIONINE HOMOLOGUES AT CELLULAR LEVEL AND IN THE SYSTEMS OF ORGANIZED GROWTH

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IN THE course of previous researches the complex nature of the interference of ethionine with the metabolism of different living systems both in dynamic equilibrium phases and in growing ones, was studied. Much data was collected about the role played by sulphhydril amino acids in maintaining the normal biochemistry of bacteria, cells *in vitro*, and adult animals. The meaning of the metabolic effects was analysed as far as the phenomena of growth and cellular homeostasis are concerned. There are two ways, corresponding to the biochemical functions of sulphhydril amino acids, by which ethionine exerts its inhibitory action. In completely developed systems, where the protein turnover is reduced to the maintenance rate, ethionine acts against the processes of cell catabolism. The type of action is determined by the way ethionine is used. Thus, in the rat the steatogenous action usually prevails, while during intoxication from bromobenzene the detoxification mechanism is preferentially affected[1]. In rapidly growing systems, where the use of sulphhydril amino acids is required for the building of proteins, the action of ethionine is especially directed against the increase of mass of the systems examined, by a direct interference with protein synthesis,[2,3,4] and perhaps with mitotic mechanisms as well. Restriction of weight gain in chick embryos[5,6] and inhibition of multiplication of fibroblasts[7,8,9] and monkey cells cultured *in vitro*[10,2] as well as of bacteria and protozoa[11], shows that the antagonistic action is exerted not only at the level of the whole organism but also at strictly cellular levels, beyond any hormonal or nervous integration.

In a second series of researches the action of the antagonistic agent was thoroughly investigated through a separate examination of the different parts forming the ethionine molecule. For such a purpose an antagonistic non-methylated analogue of methionine—i.e. norleucine[12,13] — which exerts no action on lipid metabolism was first employed in tissue culture[14]. Later, a series of homocysteines substituted on the S-sulphhydril was prepared: D,L,DL-n-propyl and isopropylho-

mocysteine; D,L,DL-n-butyl and isobutylhomocysteine; DL-octylhomocysteine; D,L,DL-benzylhomocysteine.

Up to now the results with completely developed rat liver show that the antimetabolic action on intermediary metabolism—especially lipid metabolism—is lost by substituting homocysteine with longer side chains[15]. To clarify further the nature of the action exerted by the homocysteine derivatives on cellular and organized growth, we have used biological objects which proved highly useful for these tests, i.e. cells cultured *in vitro*, chick embryos and protozoa. The results are presented below.

TECHNIQUE

1. *Cultures in vitro*

A *HeLa* strain from the Rome Istituto Superiore di Sanità was employed. In order to obtain a higher uniformity of the biological material the strain was subjected to a new clonic isolation, after the technique of Puck and others[16].

The strain was maintained in Roux flasks, with monthly transplantation by trypsinization, on Eagle's semisynthetic medium containing 20% fresh calf serum sterilized through a Seitz filter, buffered at pH 7·2–7·5 with bicarbonate (phenol red indicator) and with the addition of penicillin and streptomycin each at final concentrations of 100 units per ml of medium. Under such conditions, the strain shows an indifinite transplantability and an excellent growth regularity. It covers the flask surface with a continuous cellular coat within 20-30 days producing 25 mg dry weight of cells.

Methionine homologues, dissolved in Hanks' liquid, were added to the medium at the very moment subcultures were prepared. Every four days the medium was renewed and cellular material was collected on the fifteenth day. Into some of the flasks we introduced sterile plates of mica on which cells grow in thin layers, thus allowing subsequent morphological examination of the elements. At the end of the culture period, the cellular layer was collected by trypsinization, centrifugation, and re-suspension in distilled water with 5% formol. Its weight was estimated after drying to constant weight. In this group of tests, the activities of the following substances—at the concentrations shown in Table I — were examined: L-,D,DL-methionine, DL-ethionine, L-,D-propylhomocysteine, L-D-isopropylhomocysteine, L-,D-butylhomocysteine, L-,D-isobutylhomocysteine. The results obtained were submitted to statistical analysis using the *t* test in most of the cases and analysis of variance with orthogonal polynomials.

2. *Chick Embryos*

White Leghorn eggs were employed, taken from the fifth to seventh incubation day, injected in the yolk with quantities varying from 20 to

60 mg per egg of the homologues under examination. In some tests the drug was administered through the chorio-allantoic membrane and in the air space. At different times after administration the embryos, removed from the eggs and freed from the membrane, were weighed both before and after drying to constant weight. Variations of growth have been estimated by means of Hamburger's tables, correlating the chronological age to the morphogenesis[17].

The changes in morphogenesis were studied microscopically in different sections of the embryo. The following homologues of methionine were examined in this group of tests from the point of view of their activities: L,D-propylhomocysteine: 30 mg per egg; L-isopropylhomocysteine: 20, 30, 60 mg per egg; D-isopropylhomocysteine: 30 mg per egg; L-isobutylhomocysteine: 30 mg per egg; L-benzylhomocysteine: 500 mg per egg. (This homologue is only slightly soluble in water. It was administered to the eggs by deposition in the chorio-allantoic membrane).

The action of optical isomers of methionine and ethionine was the subject of our previous papers which may be referred to for the technique and results[5,6].

3. *Protozoa*

A strain of *Tetrahymena pyriformis W.*, subjected to a previous clonic isolation was used. A semisynthetic medium sterilized by candle filter was employed for maintenance of the strain and study of the action of the homologues. Its composition is as follows; proteose-peptone 2 g, glucose 0·5 g, yeast extract 0·1 g, NaCl 0·1g, $Na_2HPO_4.12H_2O$ 0·1 g, bidistilled H_2O 100 ml.

The medium, in 5 ml quantities, was added to tubes placed horizontally to obtain a sufficiently oxygenated thin layer. The growth of the micro-organism was studied with regard to time in methionine (0·1%) and ethionine (0·1%) tests and on the 5th day after implantation with the propyl and butyl derivatives (1 per cent). The temperature of growth was 27 °C. Photometric evaluation was made at 660 mμ.

RESULTS

1. *Cultures in vitro*

(a). *Controls.* The growth of 34 cultures was intensive and regular in all the experimental groups. Cells in Eagle medium containing serum adhere to the flask glass during the first 5 hours from implantation. They have a granular, round shape. They are highly refractile and the first migration phenomena take place at the eighth to tenth hour. The cells produce 2–3 pseudopodia, acquire a starlike, laminar, highly transparent shape and on the second day of culture undergo mitosis. During the next 7–9 days the flask surface becomes covered with a continuous

layer of cells which adjust themselves in mutual contact in a polyhedral pattern. The nuclei are voluminous with one or more nucleoli. There are many giant cells. Mitosis is frequent. Cultures acidify the medium to a marked degree within 24–36 hr. With initial inoculi of 0·2–0·5 mg dry weight; the weight of cells on the 8th day of culture ranges from 2·04 to 3·5 mg, on the tenth day from 3·95 to 4·94 mg, on the fifteenth day from 6 to 8 mg. Thus, on the fifteenth day of culture the dry weight gain of material recovered reaches values ranging from 10 to 20 times the inoculum weight.

(b). *Methionine.* In cultures treated with L-methionine at concentrations of 0·025 and 0·05%, growth appears intensive and regular. With identical inoculi, the dry weights are greater than in the controls. Concentrations of 0·05% of the D-isomer can also be tolerated by the cells, while the racemic amino acid shows a partially inhibitory action on the growth process. All the features of the colonies, the microscopic morphology of the cells, the colour changes in the cultural medium with respect to time appear perfectly normal.

(c). *Ethionine.* Ethionine exerts a strong inhibitory action upon growth. A 0·1% concentration of the homologue not only cuts off the growth process, but does not permit survival of the cells which rapidly turn granular and round, and, having stuck to the glass for a short time, drop into the medium.

A 0·05% concentration still results in inhibition. The dry weights obtained on the fifteenth day of culture are only as large as the initial explant. The cells are refractile with picnotic nuclei, and are necrotic. A 0·025% concentration allows a restricted growth which is equal to 20–30% that of the controls.

Ethionine also shows a high toxicity in thoroughly developed cultures when added to controls on the fifteenth day of growth it produces a rapid decrease of cell population and the culture can no longer be transplanted.

(d). n-*Propylhomocysteine.* Concentrations of L-propylhomocysteine at 0·05% produce no change in the *HeLa* strain growth while equal concentrations of the D-isomer reduce growth to a degree that is statistically significant in comparison to both control cultures and to those treated with L-isomer.

With 0·1% concentrations, the growth reduction is greater with a statistical probability at the 5% and 1% limits. The difference between the dry weights of cultures treated with L and D propylhomocysteine in 0·1% concentration have no statistical significance. Concentrations of 0·5% are completely inhibitory.

(e). *Isopropylhomocysteine.* Addition of L-isopropylhomocysteine to Eagle medium results in net inhibition of growth. A total of 47 cultures were treated with the homologues at four different tests concentrations

TABLE I

Dry weights of cells cultured in Eagle medium in the presence of some homologues of methionine

(Mean values and their standard errors are recorded)

Exp. 1. Inoculum 0·31 mg/flash 15 Days — 20 cultures				Exp. 2 Inoc. 0·58 mg 15 Days — 25 cultures					Exp. 3 Inoc. 0·47 mg 15 Days — 15 cultures			Exp. 4 Inoc. 0·48 mg 15 Days — 11 cultures		
Controls	DL-Ethionine			Controls	L-Propylhomocyst.		D-Propylhomocyst		Controls	Propylhomocyst.		Controls	Iso-propylhomoc.	
	0·1%	0·05%	0·025%		0·3%	0·1%	0·3%	0·1%		0·05% L-	0·05% D-		0·6% L-	0·6% D-
7·470 ± 0·506	0·054 ± 0·022	0·506 ± 0·119	2·028 ± 0·127	6·296 ± 0·434	0·878 ± 0·076	4·970 ± 0·215	0·714 ± 0·059	3·450 ± 0·219	6·650 ± 0·307	6·420 ± 0·294	4·538 ± 0·252	6·380 ± 0·267	0·555 ± 0·106	0·023 ± 0·014

Exp. 5 Incc. 0·53 mg 15 Days — 17 cultures				Exp. 6 Incc. 0·53 mg 15 Days — 14 cultures				Exp. 7 Inoc. 0·27 mg 15 Days — 7 cultures		Exp. 8 Inoc. 0·15 mg 15 Days — 9 cultures		
D-isopropyl		Methionine		Controls	Isopropylhomocyst.		L-meth.	Controls	L-isopropyl. 0·1%	Controls	Isopropylhomocyst,	
0·33%	0·1%	0·05% D-	0·05% DL-		0·05% L-	0·05% D-	0·05%				0·025% L-	0·025% D-
0·095 ± 0·008	7·940 ± 0·573	7·750 ± 0·284	3·257 ± 0·428	8·086 ± 0·206	0·633 ± 0·121	8·864 ± 0·101	9·023 ± 0·380	4·420 ± 0·531	1·201 ± 0·073	5·350 ± 0·426	5·947 ± 0·083	4·970 ± 0·199

Exp. 9 Inoc. 0·19 mg 15 Days — 15 cultures			Exp. 10 Incc. 0·69 mg 10 Days — 28 cultures						Exp. 11 Inoc. 0·19 mg 8 Days — 15 cultures		
Controls	Butylhomocyst.		Controls	Isobutylhomocysteine					Controls	Butylhomocysteine	
	0·25% L-	0·25% D-		0·25% L-	0·25% D-	0·125% L-	0·125% D-	0·5% D-		0·125% L-	0·125% D-
6·872 ± 0·347	0·936 ± 0·074	1·000 ± 0·136	4·358 ± 0·243	1·380 ± 0·101	1·032 ± 0·126	1·998 ± 0·119	1·596 ± 0·140	0·643 ± 0·144	2·732 ± 0·255	2·226 ± 0·234	1·856 ± 0·103

which varied from 0·025 to 0·1% in the case of the L-isomer and from 0·025 to 0·33% in the case of the D-isomer of isopropylhomocysteine. The data obtained show that the highest L-isomer concentration tolerated by cells is the 0·025%, while the D-isomer is much less active. A 0·1% concentration is tolerated by cells whereas to obtain a total inhibition of mitosis and growth a 0·33% concentration is required.

The statistical comparison shown in Table II refers to test No. 6 (0·05% concentrations) and demonstrates the following: L,D-isopropylhomocysteine and L-methionine in 0·05% concentration modify the growth of the strain *in vitro*. Weight differences between normal cultures and cultures treated with L-methionine on one hand and cultures treated with both isomers of isopropylhomocysteine on the other hand are highly significant. The difference between weights of cultures treated with the L and D-isomer is highly significant while there is little significance in the difference between controls and L-methionine. Microscopic examination of inhibited cultures shows that the cells settle evenly on the wall of the Kolle flask in the course of the first 6–10 hours from implantation, but after 24 hr the cell population is composed of small groups of cells or of single, highly opaque, granular,

TABLE II

Statistical analysis of the data of the experiment No. 6 of Table I

Controls	L-Meth.	L-Isopropyl	D-Isopropyl
8·03	9·05	0·62	8·71
7·76	8·35	0·85	8·68
8·46	9·67	0·43	9·06
			9·16
			8·71
M 8·086	9·023	0·633	8·864
C 0·130	0·439	0·0445	0·0518

Variance analysis

		L.G.	W	F	F 1%	F 0·1%
S^2_T	156·203	13	12·62			
S^2_G	154·796	3	51·548	361	6·55	12·55
R	1·434	10	0·143			

Resolution

C	LM	L	D	
+1	+1	−1	−1	0
+1	−1	0	0	0
0	0	−1	+1	0
0	0	0	0	

LINES	L.G.	W	P
I	1	26·45	< 0·001
II	1	1·316	< 0·05
III	1	127·20	< 0·001

round shaped cells. In the controls, in contrast, the elements appear large and starlike with thin threads and with the appearance of transparent plates, mitosis is present here, although totally absent in the treated cultures (Fig. 1 and 2).

(f). n-*Butylhomocysteine*. Values obtained from 30 cultures, divided into two series, of which one was carried to the eighth day and the

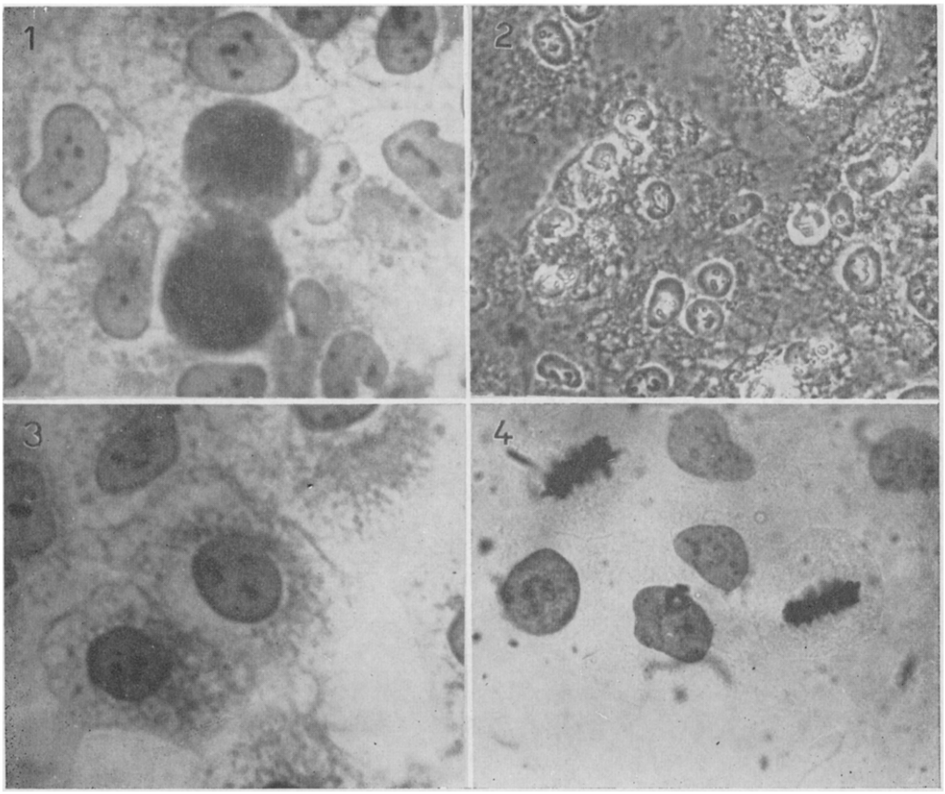

FIG. 1. *HeLa* cells cultured *in vitro* in Eagle medium for 15 days.
1, 3, 4 = magnification. × 1000
2 = magnification. × 400
(phase contrast on non-fixed material)

FIG. 2. *HeLa* cells cultured *in vitro* for 15 days in Eagle medium containing 0·1% of L-isopropylhomocysteine.
Magnification × 1000

other to the fifteenth, show that 0·25% concentrations result in total inhibition of the growth process while 0·125% concentrations are only slighly inhibitory. Statistically the difference between weight averages of control cultures and those treated with the L-isomer reach the limit of significance, while comparison between controls and D-isomer exceeds the significance threshold of 5% but does not reach the 1% level.

(g). iso-*Butylhomocysteine*. Values obtained from 28 cultures kept in a thermostat for 10 days show that concentrations from 0·125 to 0·250% exert a strong inhibition of the growth process. No significant differences are observed in the activity of the D and L-isomer. Microscopically, cultures seem to be formed by isolated colonies of a few star-shaped, granular refractile cells showing rare mitosis. Average values concerning 158 cultures divided into eleven tests are shown in Table I.

2. Chick Embryos

Propyl, butyl, and benzylhomocysteine, at the highest doses which could be administered because of solubility do not modify the development of the chick embryo.

Growth curves traced for these homologues with both fresh and dry weight values are essentially similar to those of control embroyos.

Curve shapes and their slopes, expressing the rates of the mass increase follow the exponential pattern peculiar to the growth of a normal embryo. In such groups, mortality reaches 10–20 per cent, as the result of injecting considerable amounts of fluid into the yolk. L-Isopropylhomocysteine however, is as inhibitory to embryonal development as ethionine[5].

Whereas 20 mg per egg allows a regular growth, 30 mg per egg appreciably reduces the embryo weight by the fourth day after administration, and 60 mg per egg diminishes the weight increase after the second day and brings it to a stop between the sixteenth and seventeenth incubation day. Final fresh and dry weights are a third and a fourth, respectively, of weights of controls and the ratio of dry weight to fresh weight — always lower than the characteristic control ratio — demonstrates a high degree of inhibition of the embryonal body.

These phenomena can also be observed when the homologue is administered through the allantoic membrane and in the air space. What distinguishes the activity of isopropylhomocysteine from that of ethionine is the lack of morphogenetic malformations peculiar to the latter. With the isopropyl homologue there is a complete lack of ocular lesions, hepatic steatosis or skin oedema.

Ossification processes are normal and so is the development of vertebral bodies, of lungs and kidneys.

TABLE III

Weights of chick embryos treated with L-*isopropylhomocysteine (each value represents the average of 5 data)*

Days of incubation	Controls			L-isopropylhomocysteine 60 mg/egg.								
				Yolk			Allantoide			Air space		
	Fresh weight (f.w.)	Dry weight (d.w.)	d.w./f.w.	f.w.	d.w.	d.w./f.w.	f.w.	d.w.	d.w./f.w.	f.w.	d.w.	d.w./f.w.
5	0·296	0·016	0·054									
6	0·411	0·023	0·056									
7	0·808	0·046	0·057	Injection			Injection			Injection		
8	1·396	0·090	0·064	—	—	—	—	—	—	—	—	—
9	1·599	0·107	0·067	0·849	0·052	0·061	0·855	0·055	0·064	0·929	0·060	0·065
10	2·613	0·184	0·070	—	—	—	—	—	—	—	—	—
11	3·110	0·239	0·077	—	—	—	—	—	—	—	—	—
12	6·417	0·539	0·084	2·166	0·145	0·067	2·872	0·201	0·070	2·970	0·224	0·075
13	7·334	0·716	0·098	—	—	—	—	—	—	—	—	—
14	8·502	0·938	0·110	3·535	0·287	0·081	4·436	0·387	0·087	4·474	0·401	0·090
15	11·515	1·450	0·126	—	—	—	—	—	—	—	—	—
16	14·690	2·150	0·146	6·756	0·168	0·091	9·743	1·207	0·124	8·320	0·705	0·085
17	17·520	2·933	0·167	—	—	—	—	—	—	—	—	—
18	—	—	—	6·974	0·639	0·092	9·696	1·356	0·140	100% Mortality		
19	24·355	4·693	0·193	100% Mortality								
20	—	—	—									
21	—	—	—									

Table III shows figures of both fresh and dry weights and the ratio dry weight to fresh weight. Each value is the average of the weights of five embryos.

In Fig. 3 are plotted the growth curves of the fresh weights obtained from both control embryos and embryos treated with some-substituted homocysteine (Fig. 3).

3. *Protozoa*

Homocysteines substituted with groups of molecular weight greater than C_2H_5 inhibit the growth of *Tetrahymena pyr W.*, although such inhibition is not specific, as shown by the fact that the same inhibitory degree occurs in the presence of corresponding amounts of methionine.

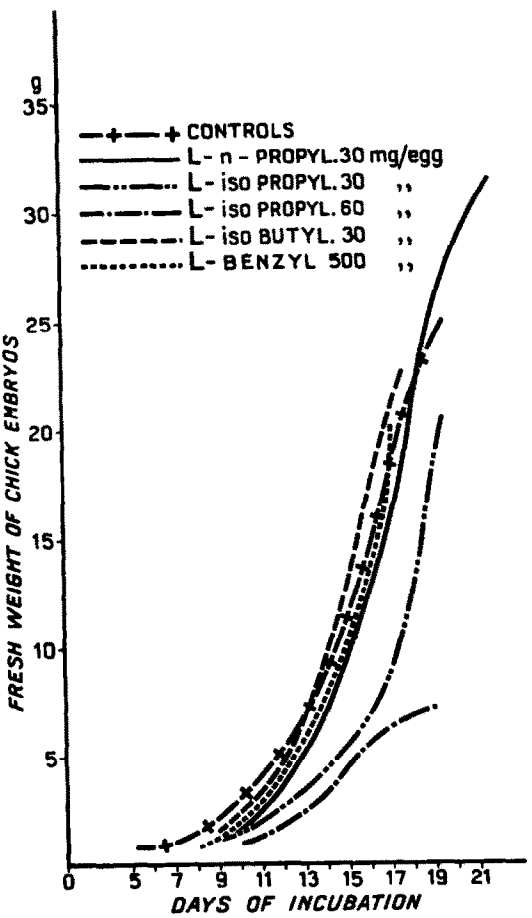

FIG. 3. Curves of growth with regard to development time of chick embryos treated with substituted homocysteines.
(Substances have been administered in the yolk with the exception of the benzyl-substituted homologue which has been placed on the chorio-allantoic membrane)

TABLE IV

Action of methionine and some substituted homocysteines on the growth of Tetrahymena pyr. W.

(Growth evaluation by optical density at 660 mμ)

Cultures	Experiment 1				Experiment 2				
	Controls	L-Meth.	Isopropylhomocyst. L-	Isopropylhomocyst. D-	Controls	DL-Meth	Isobutylhomocyst. L-	Isobutylhomocyst. D-	Isobutylhomocyst. DL-
1	0·410	0·250	0·230	0·450	0·310	0·400	0·090	0·230	0·170
2	0·500	0·320	0·200	0·400	0·340	0·180	0·220	0·220	0·140
3	0·600	0·300	0·220	0·350	0·400	0·180	0·200	0·210	0·140
4	0·660	0·320	0·240	0·330	0·230	0·210	0·130	0·080	0·140
5	0·580	0·320	0·210	0·320	0·280	0·170	0·120	0·130	0·130
6	0·560	0·210	0·300	0·330	0·420	0·240	0·260	0·190	0·140
7	0·560	0·250	0·280	0·330	0·420	0·160	0·170	0·170	0·130
8	0·400	0·220	0·180	0·320	0·340	0·150	0·300	0·160	0·150
9	0·540	0·250	0·260	0·400	0·400	0·250	0·070	0·200	0·200
10	0·500	0·240	0·320	0·330	0·440	0·150	0·200		0·200
11	0·520	0·290	0·250	0·450					
Average	0·530	0·270	0·245	0·365	0·348	0·209	0·176	0·170	0·154

Cultures	Experiment 3				Experiment 4			
	Controls	L-Meth.	Butylhomocyst. L-	Butylhomocyst. D-	Controls	Methionine L-	Methionine D-	Methionine DL-
1	0·330	0·150	0·280	0·510	0·450	0·130	0·160	0·130
2	0·360	0·330	0·220	0·450	0·420	0·110	0·160	0·090
3	0·540	0·320	0·240	0·800	0·520	0·130	0·200	0·090
4	0·480	0·360	0·220	0·800	0·420	0·130	0·170	0·090
5	0·540	0·190	0·260	0·610	0·320	0·130	0·250	0·090
6	0·700	0·180	0·230	0·610	0·420	0·150	0·190	0·190
7	0·450	0·200	0·220	0·450	0·350	0·130	0·270	0·120
8	0·520	0·200	0·200	0·760	0·520	0·160	0·180	0·130
9	0·490	0·250	0·170	0·540	0·620	0·130	0·330	0·140
10	0·540	0·320	0·230		0·540	0·140	0·170	0·130
11					0·500		0·260	0·140
12					0·580			0·210
Average	0·495	0·250	0·227	0·614	0·472	0·134	0·213	0·129

Differences between averages of the photometric extinctions of cultures treated with L-isomers and averages of extinctions of control cultures are all statistically significant, whereas differences between the average of extinctions of cultures treated with these homologues

and those of the cultures treated with the corresponding isomer of the natural amino acid are of no significance.

A comparison between averages of values obtained from cultures treated with laevo and dextro homologues shows that D-forms are less inhibitory than L-forms and the comparison between D-homologues and methionine shows that the natural amino acid is more inhibitory than the dextro compounds of its higher homologues.

The inhibition which is encountered with such compounds must therefore be regarded as an expression of an excess of the amino acid concentration in the culture medium.

Data concerning our experiments and the statistical analyses are shown in Tables IV and V, respectively.

TABLE V

Statistical analysis of the data of Table IV

		t exper.	GL	$t_{5\%}$	$t_{1\%}$
Isopropylhomocyst.	C — L	10·94	20	2·086	2·845
	L — L	1·45	20	2·086	2·845
	C — L	9·91	20	2·086	2·845
Isobutylhomocyst.	L — M	0·985	18	2·101	2·878
	C — M	3·860	18	2·101	2·878
	M — DL	2·106	18	2·101	2·878
Butylhomocyst.	L — M	0·957	18	2·101	2·878
	C — D	2·095	17	2·110	2·898
	C — L	7·860	18	2·101	2·870
	C — M	6·04	18	2·101	2·878

In contrast to the above, the data reported graphically in Fig. 4 show that the ethyl-substituted homologue ethionine, even in low concentration, exerts a strong inhibitory action on the growth of *Tetrahymena*.

Differences of growth between cultures with ethionine and methionine at a concentration of 0·1%, are significant after the second day of implantation, and on the sixth day the difference between the average values approach 50 per cent. Therefore, the inhibition found must be regardel as a specific one and not caused by an excessive concentration in the medium.

CONCLUSIONS

The results obtained from the study of S-substituted homocysteine permit the following conclusions:

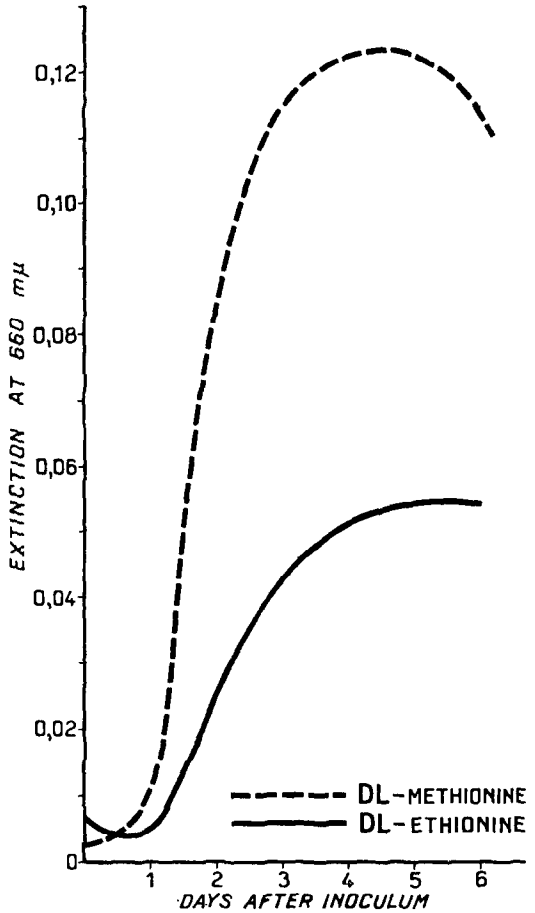

FIG. 4. Curves of growth of *Tetrahymena pry. W* in presence of 0·1% methionine and ethionine.
(Photometric evaluation has been made each day)

(1) The isolated cells of warm-blooded metazoa cultivated *in vitro* are sensitive — although to different degrees — to all the higher homologues of methionine. On these cells the action exerted by the L-isomers with branched chains of lower molecular weight is regularly observed. This speaks in favour of the homologues substituting themselves for the natural amino acid in the synthesis of proteins.

(2) On the chick embryo the L-isomer of isopropylhomocysteine is the only one to show inhibition of weight gain. Such action is similar to that of ethionine but differs from the latter by the fact that in the case of the isopropyl derivative lesions of morphogenesis and organogenesis are not detected.

(3) On ciliate protozoa the inhibitory action is not specific but is rather related to the amino acid nature of the homologues. In fact, an

identical growth-inhibiting action takes place in the presence of equimolecular quantities of methionine.

(4) On bacteria, as we demonstrated in previous studies using an *E. coli* strain, the highest activity occurs with the D-forms of the homologues, and is particularly marked with benzylhomocysteine. This seems to be due to changes in permeability rather than to an antogonism with methionine.

(5) Radicals with molecular weights greater than C_2H_5 substituted on the sulphhydryl group are not metabolically transferrable and do not interfere with the mechanisms of lipidogenesis and of lipid transport as in the case of methionine and ethionine.

In the course of earier studies we demonstrated that not one of these homologues possesses a steatogenous action.

Therefore, we come to the conclusion that a substitution of the methyl of methionine with groups having a molecular weight greater than the ethylic group abolishes, in the derivative compounds, the dismetabolic action of ethionine, while in some of them the growth inhibiting action is still maintained.

Relationships between structure and inhibitory activity appear to be rather complex. The action of substituted homocysteines are dependent not only on the length of the substituting chain, but also on the orientation of C atoms in it, on the optical activity of molecules and on the biological substrata for the growth in mass of the living substance, while the block in multiplication of bacteria and protozoa as produced also by D-homologues, makes us think of different and still unknown biochemical mechanisms.

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