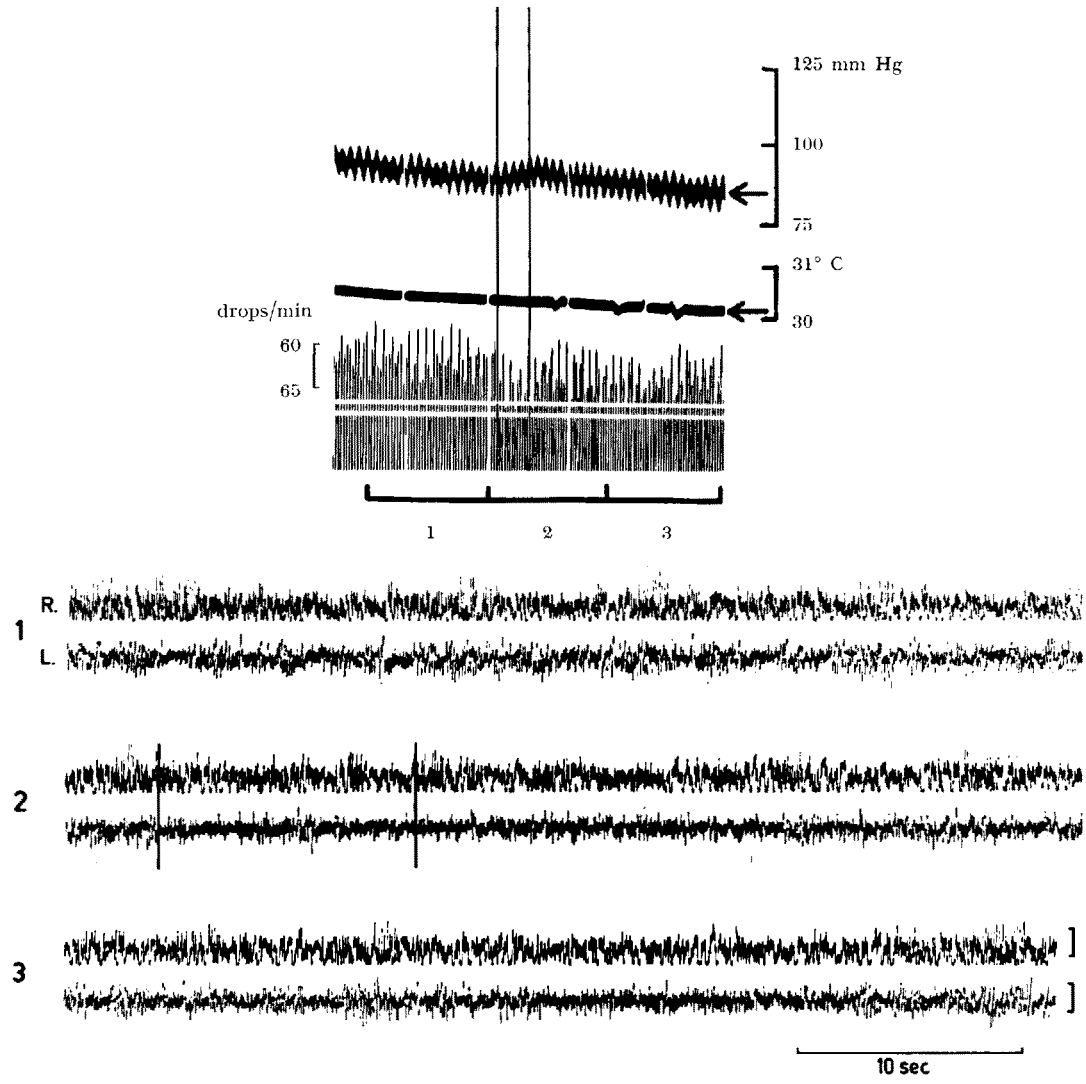


Fig. 2. – Cat, *encéphale isolé* preparation. *Upper diagram:* Blood pressure, ear skin temperature and cerebral blood flow (*Cf.* Fig. 1). *Lower diagram:* EEG tracings from right (*R*) and left (*L*) cruciate region during periods marked 1 to 3 below upper diagram. Between two vertical lines injection of 40 μg/kg of LSD-25 into the left carotid artery. Note activation pattern in EEG-record from left hemisphere.

Zusammenfassung

Es wurde gezeigt, dass LSD-25 (*d*-Lysergsäure-diäthylamid) in Dosen von 30 bis 100 γ/kg Körpergewicht bei der nichtnarkotisierten Katze (*encéphale isolé*) meistens eine Erhöhung des zerebralen Gefässwiderstandes herbeiführte. In die Carotis injiziert, gab LSD-25 in denselben Dosen nach vorübergehender, halbseitiger Aktivierung eine Depression des Elektroenzephalogrammes. Kleinere Dosen gaben im EEG eine Aktivierung oder waren ohne Wirkung.

Relationship Between Methionine and Aromatic Amino Acids in *Escherichia Coli*

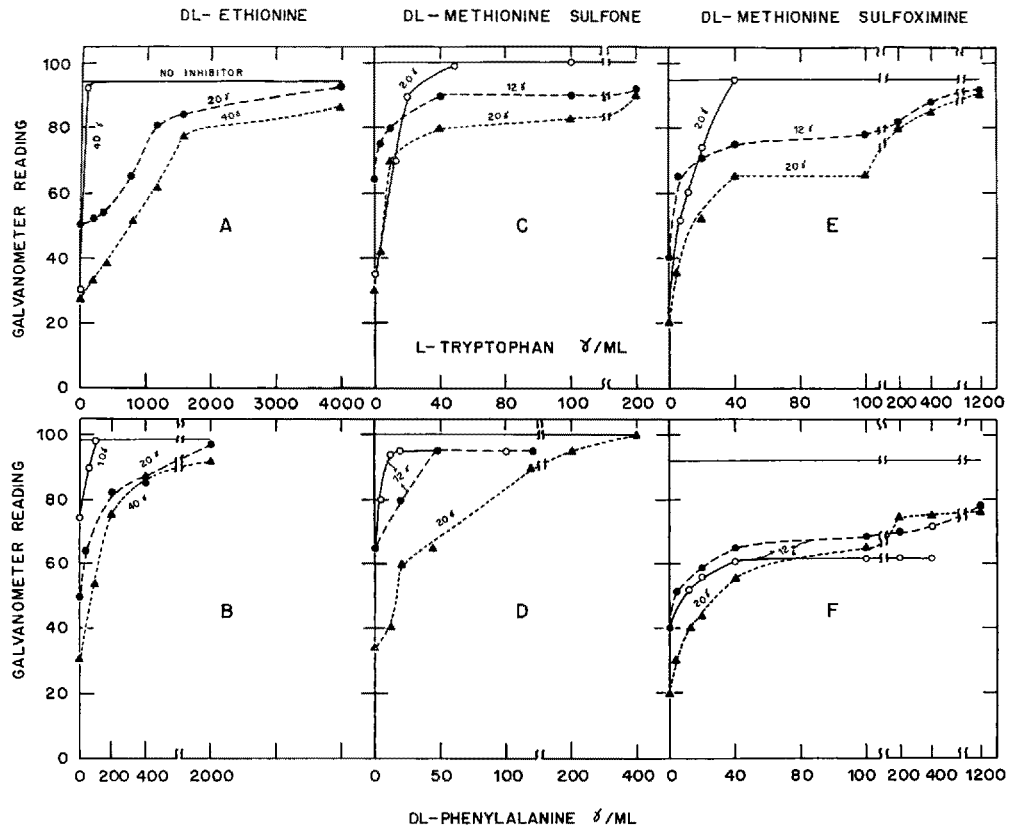
It was previously reported from this laboratory[1] that the inhibition of *Escherichia coli* by chloromycetin, aureomycin, terramycin and 5-fluorotryptophan, could be alleviated, within limits, by methionine, and by tryptophan, phenylalanine and tyrosine. This appeared to indicate that there is a relationship between the aromatic amino acids and methionine. To confirm this relationship, the problem was approached from the opposite angle, i.e., to see whether the aromatic amino acids play a role in the metabolism of methionine.

On the basis of extensive work carried out in recent years, it has generally been considered that the methionine analogues, ethionine, methionine sulfone, methionine sulfoximine and methoxinine are specific antagonists of methionine metabolism both in bacteria and animals (for a general review see [2]).

The present work with *E. coli* shows, however, that the inhibition caused by the antimetabolites of methionine can be reversed not only by methionine, but by tryptophan, phenylalanine and somewhat by tyrosine

[1] E. D. Bergmann, S. Sicher, and B. E. Volcani, Bull. Res. Council Israel *4*, 19 (1954).

[2] G. J. Martin, *Biological Antagonism* (The Blakiston Co. Inc., New York 1951), p. 118.



Reversal of DL-ethionine, DL-methionine sulfone and DL-methionine sulfoximine inhibition of the growth of *E. coli* by DL-methionine, L-tryptophan, DL-phenylalanine and L-tyrosine. Incubated 17–20 h at 37°C. The figures above the curve represent γ/ml of the inhibitor. *A, C, E* ○——○ methionine; other curves tryptophan. *B, D, F* ○——○ tyrosine; other curves phenylalanine.

as well. This substantiates our previous results. The interpretation, however, of this relationship is still obscure.

Inhibition analyses were carried out with *E. coli* (ATCC 9637) in a basal medium[3] as previously described[4]. The various sterile, glass-filtered, inhibitors were added aseptically to the autoclaved medium. Cultures were incubated at 37°C for 17 to 20 h, and growth turbidity was measured with the Klett-Summerson photoelectric colorimeter using a No. 64 red filter. All experiments were carried out in duplicate.

The inhibitory concentrations of the various compounds and the amounts of the amino acids required to reverse the inhibition were determined from dose-response curves. The antibacterial indices (γ/ml of inhibitor *versus* γ/ml of metabolite) for different levels of inhibition were obtained from the corresponding growth curves.

DL-ethionine was obtained from Bios Laboratories, Inc.; DL-methionine sulfone, DL-methionine sulfoximine and DL-methionine sulfoxide were products of California Foundation for Biochemical Research. DL-methoxinine was a gift from Dr. J. P. ENGLISH.

As shown in the Figure the inhibition caused by ethionine, methionine sulfone, and methionine sulfoximine is reversed, by methionine, L-tryptophan or DL-phenylalanine; methionine was most effective. The reversal by tyrosine was confined to the lower inhibitory levels; e.g., with ethionine, above 75% of full growth, with methionine sulfone above 65% growth (in this case it was equally as active as methionine) and with methionine sulfoximine, tyrosine alleviated the inhibition only by 20% in all the inhibitory levels regardless of tyrosine concentration. The antibacterial indices are given in the Table.

Antibacterial indices of *E. coli* inhibited by DL-ethionine, DL-methionine sulfone or DL-methionine sulfoximine, reversed by DL-methionine, L-tryptophan and DL-phenylalanine.

Antagonist	γ/ml	% Full growth	Antibacterial indices at 50% growth		
			DL-Methionine	L-Tryptophan	DL-Phenylalanine
DL-Ethionine	40	30	80	0·05	0·4
	200	20	166	0·10	0·2
DL-Methionine sulfone	12	65	4·8*	6*	2·4*
	20	35	1·5*	2*	0·3*
DL-Methionine sulfoximine	12	40	0·3	3	3·0
	16	30	0·8	2	1·6

* At 70% full growth.

With DL-methoxinine, the complete inhibition (800 γ/ml) was reversed to full growth by L-tryptophan (2000 γ/ml) or DL-phenylalanine (2000 γ/ml). The effect of tyrosine was not tested, because only a small amount of methoxinine was available.

[3] B. D. DAVIS and E. S. MINGIOLI, J. Bacteriol. *60*, 17 (1950).

[4] B. E. VOLCANI, S. SICHER, E. D. BERGMANN, and H. BENDAS, J. biol. Chem. *207*, 411 (1954).

The inhibition caused by DL-methionine sulfoxide, an antimetabolite of glutamic acid in *L. arabinousus*[5], is not counteracted even at 75% of full growth (10 γ/ml) by methionine (400 γ/ml), L-tryptophan or DL-phenylalanine (4000 γ/ml).

These results led Dr. M. FELDMAN of this Institute, to study the effect of tryptophan on the inhibitory action of ethionine during chick morphogenesis, full details of which will be published elsewhere. It has previously been shown[6] that ethionine shows two types of inhibitory effects: (1) it retards the rate of differentiation of the embryo; (2) it causes malformation, mainly of the neural tissue. In the present study it was found that methionine overcomes, almost completely, both effects; L-tryptophan however, showed very little effect on ethionine-malformation, but abolished to a great extent the retarded differentiation. This indicates that the two processes are related to different amino acids metabolism.

In the light of these experiments, it is important to determine whether the relation between methionine and the aromatic amino acids exists in mammalia also. If it does, then effects which have been attributed to methionine inhibition alone must be re-examined to see whether the aromatic amino acids are involved as well.

We are greatly indebted to Dr. M. FELDMAN for permission to describe his results prior to publication, and to Dr. J. P. ENGLISH for the DL-methoxinine.

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The Weizmann Institute of Science, Rehovoth, Israel, April 15, 1956.

Zusammenfassung

Wachstumshemmung von *Escherichia coli*, durch Äthionin, Methioninsulfon, Methioninsulfoximin und Methoxinin verursacht, wird neben Methionin auch durch Tryptophan, Phenylalanin und in gewissem Umfang durch Tyrosin aufgehoben, was auf einen Zusammenhang im Stoffwechsel von Methionin und den aromatischen Aminosäuren hinweist.

[5] H. WAELSCH, P. OWADES, H. K. MILLER, and E. BOREK, J. biol. Chem. *166*, 273 (1946).

[6] M. FELDMAN and C. H. WADDINGTON, J. Embryol. exp. Morphol. *3*, 44 (1955).

The Apparent Expansion of the Inulin Space in the Nephrectomized Dog

As has been reported by GAUDINO *et al.*[1], in man and in the dog inulin given by the constant infusion technique is distributed evenly throughout the total extracellular space, is not metabolized in the organism, does not enter the intracellular space and is eliminated completely and exculsively through the kidneys. GAUDINO and LEVITT[2], as well as RAISZ *et al.*[3], have reported that in the dog the apparent inulin space expands after nephrectomy. FINKENSTAEDT *et al.*[4], HAMBURGER and MATHÉ[5], and also EPSTEIN *et al.*[6], have stated recently that in the nephrectomized dog and anuric man alike, the plasma concentration after a single injection of inulin decreased steadily, simulating thereby an expansion of extracellular space. Other possible explanations are: uptake of inulin by cells ,breakdown in the organism or elimination through some extrarenal route.

We have studied the apparent inulin space in nephrectomized dogs. Immediately after nephrectomy a single injection of inulin was administered and its level in plasma was determined daily until the animal died. The inulin preparation used was nearly 100 per cent yeast-resistant and about 90 per cent alkali-resistant. The solution injected and each daily sample of plasma were analysed partly after treatment with yeast, according to HARRISON[7], and partly after heating in the presence of alkali as described by LITTLE[8]. By dividing the injected quantity of inulin by plasma concentration, the apparent inulin space is calculated and is expressed as percentage of preoperative body weight. The means of our data are tabulated (with standard error and number of cases). As can be seen, the apparent distribution space continues to expand until spontaneous death occurs. (Statistical analysis was computed by FISHER's *t*-test[9].) There is no difference in distribution between the yeast-resistant and alkali-resistant inulins.

In an attempt to decide whether we are dealing with a real expansion of the extracellular space, after-loading experiments were carried out. Two techniques were used: in one group inulin was injected immediately after nephrectomy and at 3 to 5 days distribution was determined after injecting an other dose. In the other group the first dose of inulin was administered at 3 to 5 days following nephrectomy. As the two types of after-loading yielded comparable results, the means for both groups are presented together. In the after-loading experiments, the inulin space value corresponds with that for days 1 and 2 and is significantly smaller than the inulin space for the corresponding day, as determined on the basis of inulin injected on day 1.

Thus, in our experiments the true extracellular space did not increase in the nephrectomized dog and the apparent expansion of the inulin space (i.e. decrease in plasma concentration) was due either to a metabolisation or/and to an extrarenal elimination or/and to cellular storage of inulin. Since JANCSÓ[10] succeeded in demonstrating by histological methods cellular storage of inulin in reticuloendotholial cells of connective tissue and liver of mice, the latter possibility seems to be the most likely.

A detailed account of our data is to be published in Acta Physiologica Hungarica.

P. BÁLINT and I. FORGÁCS

Physiological Institute of the Medical University, Budapest, July 25, 1956.

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[2] M. GAUDINO and M. F. LEVITT, Amer. J. Physiol. *157*, 387 (1949).

[3] L. G. RAISZ, M. K. YOUNG, and I. T. STINSON, Amer. J. Physiol. *174*, 72 (1953).

[4] J. T. FINKENSTAEDT, M. P. O'MEARA, and J. P. MERRILL, J. clin. Invest. *32*, 209 (1953).

[5] J. HAMBURGER and G. MATHÉ, *Fluid Balance in Anuria*, in *The Kidney*, Ciba Symposium (Churchill, London 1954), p. 288.

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[10] N. JANCSÓ, *Speicherung* (Akadémiai Kiadó, Budapest 1955).