

The inhibition caused by DL-methionine sulfoxide, an antimetabolite of glutamic acid in *L. arabinosus*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 shown⁶ 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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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 exclusively through the kidneys. GAUDINO and LEVITT², as well as RAISZ *et al.*³, have reported that in the dog the apparent inulin space expands after nephrectomy. FIN-

KENSTAEDT *et al.*⁴, HAMBURGER and MATHÉ⁵, 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 HARRISON⁷, and partly after heating in the presence of alkali as described by LITTLE⁸. 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*-test⁹.) 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 metabolism or/and to an extrarenal elimination or/and to cellular storage of inulin. Since JANCÓS¹⁰ succeeded in demonstrating by histological methods cellular storage of inulin in reticuloendothelial 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*.

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

¹ M. GAUDINO, I. L. SCHWARTZ, and M. F. LEVITT, Proc. Soc. exp. Biol. Med. 68, 507 (1948). – M. GAUDINO and M. F. LEVITT, Amer. J. Physiol. 157, 387 (1949).

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.

⁶ F. H. EPSTEIN, C. R. KLEEMAN, M. E. RUBINI, and E. LANDIN, Amer. J. Physiol. 182, 553 (1955).

⁷ H. E. HARRISON, Proc. Soc. exp. Biol. Med. 49, 109 (1942).

⁸ J. M. LITTLE, J. biol. Chem. 180, 747 (1949).

⁹ P. A. FISHER, *Statistical Methods for Research Workers*, 10th ed. (Oliver and Boyd, London 1946).

¹⁰ N. JANCÓS, *Speicherung* (Akadémiai Kiadó, Budapest 1955).

	1th day	2nd day	3rd day	4th day	5th day
Inulin space (yeast-resistant)	17.7 ± 3.2 n = 18	22.6 ± 4.1 n = 18	27.0 ± 4.7 n = 15	33.2 ± 4.9 n = 8	38.0 ± 8.8 n = 3
P	< 0.001 < 0.01 < 0.02 > 0.05				
Inulin space (alkali-resistant)	17.2 ± 2.1 n = 19	23.4 ± 4.2 n = 19	27.8 ± 5.3 n = 15	36.9 ± 11.4 n = 7	41.1 ± 11.7 n = 4
P	< 0.001 < 0.01 < 0.05 > 0.05				
Inulin space after-loaded (yeast-resistant)	—	—	19.6 ± 2.6 n = 7	19.7 ± 1.5 n = 5	23.3 n = 2
P	< 0.01 ¹ < 0.001 ¹ < 0.001 ¹ < 0.001 ¹				
Inulin space after-loaded (alkali-resistant)	—	—	17.5 ± 2.5 n = 8	20.2 ± 4.5 n = 5	20.0 n = 1
P	< 0.01 ¹ < 0.02 ¹ < 0.02 ¹ < 0.02 ¹				

¹ Against non-after-loaded series of the same day.

² Against non-after-loaded series of the first day.

Zusammenfassung

Der Verteilungsraum des Inulins beim nephrektomierten Hund am Operationstag wurde bei 17% des Körpergewichts gefunden. In den nächsten Tagen verminderte sich die Seruminulinkonzentration fortlaufend, unter scheinbarer Vergrößerung des Verteilungsraumes. Am 3. bis 5. Tag wurden einige Tiere erneut oder zum ersten Mal mit Inulin belastet, und es konnte festgestellt werden, dass keine wirkliche Vergrößerung des Verteilungsraumes eintritt. Für die Verminderung der Seruminulinkonzentration wird auf Grund von JANCÓS Untersuchungen eine intrazelluläre Speicherung als wahrscheinlich angenommen.

Co-enzyme A Content in the American Cockroach (*Periplaneta americana* L.) and the Housefly (*Musca domestica* L.)

In view of the importance assumed in the last few years by CoA as regulator of many metabolic mechanisms¹, and also in relation with earlier work by the present authors² on the insecticidal action of iodo-, bromo-, and chloroacetic acids (halogen-containing alkylating agents), whose toxic action develops by the alkylation of -SH groups (CoA contains an -SH group, to which its activity is closely bound), it was decided to estimate this co-enzyme in insects.

Adult males of the American cockroach (*Periplaneta americana*) and adult females of the housefly (*Musca domestica*) were used as biological material. CoA estimation was carried out as follows: 1 g of material is kept in the bottom of a test tube in a boiling water bath for 10 min, and then transferred to a cooled glass mortar at 0° and ground up with quartz sand after addition of 5 ml of iced water. The extract is left at 0° for 15 min and then centrifuged in a refrigerated centrifuge. 0.5 ml of extract prepared in this way are estimated for CoA by

¹ G. FORNAINI and E. MARINELLO, *Giorn. Biochim.* 3, 97 (1954).
² S. BETTINI and M. BOCCACCI, *Riv. Parass.* 13, 165 (1952); R. C. Ist. Sup. Sanità 17, 188 (1954); *Riv. Parass.* 16, 13 (1955). – S. BETTINI, M. BOCCACCI, and C. ROSSI, *Riv. Parass.* 16, 103 (1955).

the 'acetylation of sulfanilamide' method, as reported by NOVELLI³.

Some of the coxal muscles (red muscles) of *P. americana* contain considerably higher quantities of succinic dehydrogenase than other muscles (white muscles) also of the coxa, as the authors have previously shown⁴; furthermore, like succinic dehydrogenase, CoA is bound to the oxidative metabolism of carbohydrates. For these reasons, it was also decided to look for differences in the CoA content of the two types of muscle. The nomenclature of the muscles tested is reported in an earlier paper⁴.

Table I.—CoA content in units/g of fresh material (*P. americana*)

Whole coxae	White coxal muscles	Red coxal muscles
11.4	6.1	28.0

It may be seen from Table I that the CoA content of the white coxal muscles is about 1/5 that of the red coxal muscles. This finding, and the fact that the red muscles contain considerably larger quantities of succinic dehydrogenase and cytochrome⁵ than the white muscles, together confirm the hypothesis that the oxidative metabolic functions are more developed in the red muscles.

In *M. domestica*, practically equal values were obtained by estimation of CoA in the whole insect and the thorax alone. The curve of CoA content against age was also studied in whole flies.

Table II.—CoA content in units/g of fresh material (whole flies) against age in *M. domestica*

Age in days	1	3	6	9	12
CoA content	2.3	5.1	5.5	7.7	6.5

It will be seen from Table II that the CoA content in *M. domestica* increases with age up to the 9th day. A similar curve had already been met with for the increase in the average diameter of the muscle sarcosomes of the

³ D. NOVELLI, in *Methods of Biochemical Analysis*, Vol. II (Interscience Publishers, New York 1955), p. 201.
⁴ S. BETTINI and M. BOCCACCI, *R. C. Ist. Sup. Sanità* 17, 188 (1954).
⁵ V. B. WIGGLESWORTH, *The Principles of Insect Physiology* (Methuen, 1950), p. 410.