TABLE I.
Tissue Concentration of Radioactivity in Terms of Micrograms of Methadone* per Gram.

Rat No.	Time after inj., min.	Cerebrum		Plasma		Adrenals		Liver	
		per g water	per g dry tissue	per g water	per g dry tissue	per g water	per g dry tissue	per g water	per g dry tissue
16	10	0	0	0.4	4.9	0	0	2.4	5.4
21	10	0	0	0.05	5.5	11.2	16.9	4.1	9.5
17	20	2.3	8.3	0.9	10.5	16.0	42.8	18.8	49.2
22	20	6.0	20.0	2.6	27.9	39.7	68.4	10.9	25.7
18	30	0	0	0.9	9.8	22.9	26.4	20.1	47.5
23	30	3.3	11.9	1.2	14.5	27.4	51.5	13.4	33.6
19	40	3.6	13.0	2.0	24.9	48.5	93.0	25.5	60.7
24	40	2.5	8.6	2.5	30.0	45.0	96.5	30.8	79.4

induced by an initial high concentration of the drug in the brain.

In contrast to the cerebrum, both the liver and adrenals showed a marked increase in concentration with time; the rate of increase in the adrenals was more pronounced. These differences, obtained even when calculated on the basis of tissue water, suggest a definite mechanism for accumulation in these tissues.

Summary. Based on the determination of radioactivity for periods of 10, 20, 30, and 40 minutes following a subcutaneous injection of 10 mg/kg of C^{-14} labelled methadone HBr into rats, it was found that:

(a) Methadone* makes its appearance in the blood within 10 minutes, and is confined to the plasma.

(b) There was no activity in the cerebrum before 10 minutes, and even thereafter the concentration is very low. The rate of accumulation roughly parallels that of the plasma indicating no special affinity of the brain for the drug.

(c) The adrenals and liver show a marked ability to accumulate methadone* at a rate in excess of that shown by either the cerebrum or plasma.

(d) There was no activity in the sciatic nerve within the period studied.

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A Nervous Syndrome Produced with Phenylalanine and Methionine.* (17428)

L. V. HANKES AND C. A. ELVEHJEM

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

During studies on the metabolic interrelationships between threonine, phenylalanine, tryptophan and niacin, it was noted that animals fed a niacin-tryptophan deficient ration supplemented with 0.208% DL-phenylalanine and 0.2% DL-methionine for a period of 12 weeks developed an interesting nervous syndrome. The symptoms include an arched back with extended legs in a rigid condition. The head oscillates vertically almost continuously, and the rate of movement increases until the animal apparently becomes fatigued. After a short rest period, this movement reoccurs. The animal may show a slow rotary motion of the body and periodically it may move backwards at a rapid rate. Death re-

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sults 36 to 50 hours after the onset of the symptoms. Blood pressure measurements on one animal with very severe symptoms gave normal values. Autopsy of all animals failed to reveal any gross abnormalities in the structure of any of the organs; however, the stomachs of some were very slightly bloated.

The animals fed niacin or tryptophan in the ratio in addition to the added DL-phenylalanine and DL-methionine were normal. The rations supplemented with 0.2% L-cystine plus 0.208% DL-phenylalanine instead of 0.2% DL-methionine plus 0.208% phenylalanine did not produce this syndrome. Even though DL-threonine is generally much more active as a growth inhibitor in the 9% casein ration, it did not produce these symptoms, when either L-cystine or DL-methionine was added to the ration. Apparently there is a definite relationship between methionine and phenylalanine which is responsible for the nervous syndrome.

An experiment was designed (Table I) to determine what levels of methionine and phenylalanine would be required to shorten the period for onset of the syndrome, and what compounds could be used to cure it. The composition of the basal ration was the same as that used in previous studies.[1] The combinations of 0.208% DL-phenylalanine plus 0.2% DL-methionine, 0.208% DL-phenylalanine plus 0.4% DL-methionine and 0.416% DL-phenylalanine plus 0.4% DL-methionine all appeared to produce the nervous syndrome in the same period of time. The administration of niacin or tryptophan by stomach tube, the injection of niacin, or the feeding of tryptophan solution orally by dropper, after the animals exhibited severe nervous symptoms, did not produce a cure. On the other hand if the level of casein in the ration was doubled at the onset of the early symptoms of the syndrome, the animals quickly recovered. It is important that niacin or tryptophan protected the animals when given from the beginning, but they were inactive when given after the syndrome had reached a definite stage.

TABLE I. Response of Individual Rats to Various Supplements Administered After Onset of Nervous Syndrome.

Supplement	Time required for symptoms to appear, wk	Survival time after appearance of symptoms, hr	Treatment
0.208% DL-Phenylalanine + 0.2% DL-methionine	12	56	No treatment.
"	12.5	50	100 mg DL-tryptophan in 25% alcohol sol. fed (ml containing 20 mg) by stomach tube 48 hr. 1 ml fed every 8 hr.
"	7	24	No treatment.
"	9	60	12.5 mg niacin in 25% alcohol sol. fed (ml containing 12.5 mg) by stomach tube. After 24 hr period, 8 mg niacin (8 mg/ml) in aqueous sol. inj.
"	11	—	18% casein ration fed; complete recovery followed.
" + 0.4%	3	24	No treatment.
"	8	36	No treatment. Blood pressure normal (125 mm). Brain removed for pathological studies.
"	11	—	18% casein ration fed; complete recovery followed.
0.416% "	9	36	25 mg DL-tryptophan in 25% alcohol fed by dropper 48 hr. At autopsy all tissues appeared normal.
"	11	—	18% casein ration fed; complete recovery followed.
"	—	—	The rat removed at end of 13 wk.

[1] Hankes, L. V., Henderson, L. M., Brickson, W. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1948, **174**, 873.

The apparent inability of the animals to control their movements indicated that the cells in certain areas of the brain may have been affected. The levels of methionine and phenylalanine in the rations may have altered the ratio of amino acids in the brain cells[2] to such an extent that permanent changes in the enzyme or metabolic systems of the cells took place. Furthermore, the observation that the symptoms were not reversible by feeding or injecting niacin would substantiate this conclusion, since this compound prevented the onset of the nervous symptoms, when it was present in the ration. A gross examination of the brains of several animals did not reveal any abnormalities.

It is believed that this is the first time a syndrome of this nature has been observed in experiments with rations containing an imbalance of amino acids. There are known human diseases such as cystinuria,[3,4] alkaptonuria,[5] tyrosinosis,[6] and phenylpyruvic oligophrenia,[7,8] which have been related to inborn errors of amino acid metabolism. In these diseases normal metabolic processes involving phenylalanine and tyrosine are either partially or completely inhibited, and the disorders are generally recognized by the persistent excretion of abnormal urinary constituents.[9,10]

When our results are considered in the light of faulty amino acid metabolism, it is feasible that the effects observed were due partially to a limiting ability of the animal to metabolize phenylalanine. It is also possible that the causative agent in this syndrome was the D isomer of this amino acid, since it has been observed that the growth of rats was reduced drastically when this isomer was supplemented at low levels in a 9% casein ration.[1]

Summary. Animals fed a niacin-tryptophan deficient ration supplemented with 0.208% DL-phenylalanine and 0.2% DL-methionine for a period of 12 weeks developed a nervous syndrome. Generous quantities of tryptophan or niacin in the ration protected the animals from this syndrome, but neither compound cured the symptoms once they had progressed beyond a given stage.

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A Modified Diphenylamine Procedure for Fructose or Inulin Determination. (17429)

DORIS ROLF, A. SURTSHIN AND H. L. WHITE*

From the Department of Physiology and the Division of Gerontology, Washington University School of Medicine, St. Louis, Mo.

The use of inulin clearance as a measure of glomerular filtration rate has prompted the appearance of a number of methods for the determination of inulin or fructose, the majority of which are based either on the Seliwanoff reaction[1] or the reaction with

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