

## Maintenance of Pregnancy in Protein-Deficient Rats with Dietary Supplements of Methionine<sup>1</sup> (34494)

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Rats placed on a protein-free diet during gestation fail to maintain pregnancy unless given exogenous gonadotropins (1, 2), ovarian steroids (3-6), adrenocorticoids or ACTH (7), or transitory supplemental protein (8, 9). Pregnancy is maintained also when essential crystalline amino acids are the sole source of protein in the diet (10).

The deletion from the diet of certain essential amino acids, including methionine (11), tryptophane (12), or lysine (13), interferes with reproductive function in nonpregnant female rats, although the absence of either phenylalanine (14), leucine (15), or histidine (16), apparently has little effect on female reproductive organs. In pregnant rats, likewise, omission of methionine (11) or tryptophane (17) from the diet leads to reproductive failure, and the addition of 4% methionine to a 20% casein diet results in subnormal fetal and placental weights (18).

In this investigation a different approach to the study of reproductive function has been utilized—that of supplementing the protein-free diet with a single crystalline amino acid in an attempt to maintain pregnancy.

**Materials and Methods.** Adult virgin Long-Evans rats, 75-85 days of age and weighing 175-215 g were bred with normal adult males. Mating was considered positive when spermatozoa were found in the vaginal smear, and the day of finding sperm was considered as day zero of pregnancy. Mated females were distributed among experimental groups having similar average body weights. Beginning on day zero each rat was placed in an individual cage and fed a purified protein-free diet supplemented with *dl*-methionine or

*l*-cystine *ad libitum* for the duration of pregnancy. Control rats received unsupplemented protein-free diet.<sup>2</sup> All rats received distilled drinking water *ad libitum*. The cages were provided with wire mesh floors to prevent coprophagia. Vaginal smears were examined daily, and the animals were weighed regularly. All rats were sacrificed on day 21 of pregnancy. At autopsy the uterus was examined for implantation sites, the ovaries for corpora lutea, and the ovaries and living young were weighed and fixed in Bouin's fluid. An additional group of rats of the same age and body weight was not mated.

**Results and Discussion.** Table I shows the effect of supplementing the protein-free diet with four levels of methionine. Control rats receiving unsupplemented protein-free diet had early vaginal erythrocytes and all failed to maintain pregnancy. Resorbing implantation sites were very small, indicative of very early embryonic resorption. In contrast, pregnancy was maintained in 30% of animals which received dietary supplements of either 0.2% or 0.4% methionine. At either higher or lower levels of methionine supplement reproductive improvement was less marked.

Despite the absence of all but one of the essential dietary amino acids, pregnancy was maintained in 6 of 20 rats which received either 0.2% or 0.4% methionine. The percentage of methionine-supplemented rats with

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<sup>2</sup> The protein-free diet was complete in all known essential dietary nutrients except protein. In the supplemented diets a single crystalline amino acid was added to the diet, replacing an equivalent amount of sucrose. Fat-soluble vitamins were supplied weekly. Composition of the protein-free diet and of the fat-soluble vitamin supplement was reported previously (27).

TABLE I. Effect of Methionine Supplements to Pregnant Rats on a Protein-Free Diet.

Supplement (g/kg diet)	No. rats bred	Weight change during gesta- tion (%)	Vaginal RBC (day)	Living litters <sup>a</sup> (%)	Implantation sites/rat		Average fetus wt (g)
					Total no.	Living no.	
0	13	-24.6	10.4	0	9.8	0	—
1	10	-23.3	10.6	20	10.6	1.1	2.5
2	10	-17.8	11.1	30	8.9	2.7	2.8
4	10	-19.6	10.7	30	8.9	1.9	3.1
8	10	-22.4	10.6	10	9.7	0.9	2.5

<sup>a</sup> Percentage of rats with living embryos at autopsy, day 21 of pregnancy. Implantation sites were present in all rats.

litters, when compared with controls, was statistically significant ( $p < .01$ ).

The primary deficiency at low casein levels appears to be that of labile methyl groups (20). To test whether the beneficial effect of methionine supplementation in this study was due to the methyl or to the sulfhydryl group, a group of animals was fed the protein-free diet supplemented with 0.4% cystine. Twenty per cent of the animals in this group maintained pregnancy to term, indicating that the sulfhydryl group, rather than the methyl group, was the important moiety in maintaining pregnancy. This was not unexpected since, in these experiments, choline, also a potent methyl donor, was supplied in sufficient amounts in the diet.

The daily injection of 20 mg methionine into immature protein-deficient female rats resulted in a significant increase in weight of ovaries plus uterus after 10–14 days (19). To test whether a similar gonadotropic effect might have occurred in adult rats fed methionine-supplemented diet, an experiment was conducted in nonpregnant animals. Sixteen animals which had had regular 4-day estrous cycles were fed either protein-free diet or the diet supplemented with 0.2–0.4% methionine. Vaginal smears were examined daily. The animals were sacrificed after they had attained anestrus. Vaginal cornification was last observed  $10.9 \pm 1.3$  days after institution of the diet in the protein-deficient group, and  $11.5 \pm 1.3$  days in the supplemented group. Although the supplemented animals had slightly heavier ovaries and uteri when autop-

sied after 20 days on the diet (101 days of age), the differences were not significant. These data indicated that gonadotropin secretion by the anterior pituitary was no less severely inhibited by the methionine supplemented diet than by the protein-free diet.

The methionine supplement may have had a direct effect upon the developing blastocyst and/or the uterine endometrium. Methionine stimulates the growth of rabbit blastocysts (21), and blastocysts rapidly incorporate S-35 methionine injected into pregnant mice (22). In day 5 implantation sites in mice there was a localized uptake of S-35 methionine by the decidua (23). It remains to be determined, however, whether methionine will improve uterine decidualization of pseudopregnant rats.

Leung *et al.* (24) measured plasma concentration of essential amino acids and found that, in male rats fed protein-free diet only, the level of methionine was much lower than that of control animals over a 7-day period. Small amounts of dietary methionine may have significantly improved plasma concentration in protein-deficient rats, for methionine was as rapidly absorbed from the intestine in protein-deficient rats as in controls for up to 24 days (25). This would support the suggestion of Udupa *et al.* (26), based on the improvement of wound healing by methionine supplementation of protein-free diet, that the effect of methionine is to increase the efficiency of metabolism of proteins derived from the body's own tissues.

*Summary.* The supplementation of protein-free diets with 0.2% to 0.4% methionine significantly improved reproductive performance in pregnant rats. It is suggested that the effect of the amino acid supplement was to provide a more beneficial environment for ovum implantation by increasing the efficiency of protein metabolism and perhaps by facilitating decidualization of the uterine endometrium.

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