was carried out to specifically test the relationship of the state of mammary gland development at the time of the introduction of the milk agent to mammary carcinogenesis. The results would indicate that the relative non-susceptibility of older mice is not due to greater mammary gland development at the time of virus infection.

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Effects of Excess Dietary *dl*-Methionine and/or *l*-Arginine on Rats.*

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The addition of low concentrations of *dl*-methionine to a casein diet increases the nitrogen balance index in the rat while the addition of higher concentrations of the amino acid decreases the index and causes a marked loss in body weight.[1,2] Loss in body weight and inhibition in growth in animals receiving excess methionine has been reported from a number of laboratories.[3-5] Excess methionine could alter the need for other amino acids, for example, by increasing the demand for them in methylating reactions,[5,6] and by promoting the formation of one type of tissue protein at the expense of others. The question is raised as to whether or not the addition of other amino acids to the diet would reduce the drain on tissue sources which excess methionine could exert. The following experiments on rats were designed to answer this question in part.

Methods. Nitrogen balance indexes were determined using the diet and technics previously described for the dog.[7] The diet contained 12% casein (dry weight basis). Rats were pair-fed with those fed excess methionine (4.8%) where food intake was markedly restricted. The experiments were continued for a period of 20 days with urine and fecal collections throughout the period. At the end of the period the animals were autopsied and the livers and kidneys were dried at 95° C to constant weight. The dry livers and kidneys were analyzed for nitrogen by the micro-Kjeldahl procedure and were also analyzed for total fat. The urine was analyzed for creatinine and creatine by the Folin method.[8]

Results. The effects of relatively large addition of *l*-arginine and/or *dl*-methionine, to casein, on the nitrogen balance index and on the excretion of creatinine in the rat are recorded in Table I.

The average nitrogen balance index for casein was 0.81. The creatinine excretion on the casein diet was 28.3 mg/day/kg body weight. The addition of *l*-arginine to casein decreased the nitrogen balance index to 0.73 so that this amount of *l*-arginine did not improve the pattern of amino acids in the diet. Addition of excess *dl*-methionine to casein decreased the nitrogen balance index markedly so that the animal was in negative nitrogen balance and increased the excretion of creatinine significantly (34.3 mg), changes which

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TABLE I.
Nitrogen Balance Index and Creatinine Excretion in Rats. Average Data Obtained on 14 Animals.

Nitrogen source	Nitrogen balance index	Creatinine, mg/day/kg B.W.	Wt loss, g
Casein 12%	0.81	28.3 ± 1.2*	17 ± 2.1
12% casein + 1.7% arginine	0.73	31.2 ± 2.3	25 ± 5.1
12% " + 4.8% methionine	0.50	34.3 ± 0.9	59 ± 5.4
12% " + 1.7% arginine + 4.8% methionine	0.70	36.5 ± 0.7	35 ± 3.6

$$* e = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

TABLE II.
Effects of Feeding *dl*-Methionine and/or *l*-Arginine in 12% Casein Diet on Amount of Liver Nitrogen and Liver Fat in Rats. Average Data Obtained on 14 Animals.

Nitrogen source	g N/g liver*	Liver wt 100 g B.W.	g liver N/ 100 g B.W.	Liver* fat, g%
Control 12% casein	0.096 ± .002	3.24 ± .02	0.096 ± .002	19 ± 1
12% casein + 1.7% arginine	0.099 ± .005	3.03 ± .07	0.093 ± .002	18 ± 2
12% " + 4.8% methionine	0.112 ± .004	3.19 ± .06	0.110 ± .002	13 ± 1
12% " + 4.8% " + 1.7% arginine	0.106 ± .001	2.86 ± .07	0.103 ± .003	15 ± 1

* Dry basis.

TABLE III.
Effect of Feeding *dl*-Methionine and/or *l*-Arginine in a 12% Casein Diet on Weight of Kidney and on Total Kidney Nitrogen in Rats.

Nitrogen sources	H_2O %	Kidney wt, g/100 g B.W.	Total nitrogen, g/100 g B.W.
12% casein	74 ± 1.1	0.69 ± 0.02	0.021 ± 0.002
12% " + 1.7% arginine	76 ± 1.2	0.67 ± 0.02	0.019 ± 0.002
12% " + 4.8% methionine	76 ± 1.2	0.95 ± 0.04	0.027 ± 0.003
12% " + 1.7% arginine + 4.8% methionine	74 ± 1.1	0.79 ± 0.05	0.023 ± 0.002

are associated with loss in nitrogen stores. The addition of arginine to the casein-methionine mixture increased the nitrogen balance index (0.7) above that for *dl*-methionine and casein alone, the creatinine excretion remaining at a high level (36.5 mg/day/100 g body weight). The increase in excretion of creatinine associated with excess methionine and arginine may be the result of increased synthesis of creatine.[6]

The close correlation between nitrogen balance index and weight loss is illustrated also in Table I. These data demonstrate that the loss in weight was much higher in animals fed the excess methionine and that the presence of arginine with the excess *dl*-methionine prevented some of this loss.

The effects of feeding *dl*-methionine and/or *l*-arginine in the 12% casein diet on the amount of nitrogen in the liver and on liver fat are recorded in Table II.

The addition of *l*-arginine had little effect on the nitrogen of the liver but *dl*-methionine increased it, an increase which took place in the presence of a reduction in retention of nitrogen and a loss in body weight. The marked lipotropic effect of methionine is also shown by the reduction in the per cent of liver fat. Indeed the body fat is generally reduced below that found in the control animals, a reduction which contributed to the loss in body weight.

The addition of *dl*-methionine to the casein diet increased the size of the kidney while *l*-arginine alone had no effect (See Table III). The addition of *l*-arginine plus *dl*-methionine tended to increase the size of the kidneys but the increase was by no means as marked

as with *dl*-methionine alone, arginine having an antagonistic effect to methionine.

Summary. When excess *l*-arginine is added to casein, the nitrogen balance index is reduced slightly. An excess of *dl*-methionine on the other hand reduces the nitrogen balance index markedly and the excretion of creatinine is increased. When both *l*-arginine and *dl*-methionine are added to casein the nitrogen balance index is not reduced as much as it is in the presence of casein plus methionine, but the creatinine excretion is increased. The animals receiving 12% casein and pair-fed with the group receiving 12% casein plus 4.8% *dl*-methionine lose weight slightly over a 20-day period. Those receiving 12% casein plus 4.8% methionine lose weight markedly, a loss which is overcome in part by adding 1.7% *l*-arginine to the methionine diet. The decrease in weight in the presence of a large excess of methionine is associated with a loss in body nitrogen and fat. The addition of *dl*-methionine increases the amount of nitrogen in the liver and reduces the amount of fat. Feeding *dl*-methionine plus casein increases the size of the kidney, an effect which is antagonized by *l*-arginine.

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Influence of Protein Reserves on Nephrectomized and Renal Artery Ligated Dogs.*

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The contrasting symptomatology, speed of elevation of blood nonprotein nitrogen and survival period of animals, after bilateral ligation of the renal arteries and removal of the kidneys has been reported.[1]

Ligation of the arteries results in earlier and more severe evidence of toxicity, more rapid rise in blood nonprotein nitrogen and is more rapidly fatal. It is further of interest that the intravenous injection of renal extracts after bilateral nephrectomy tends to reproduce the effects of ligation of the arteries. In these circumstances toxicity and elevation of blood nonprotein nitrogen develop earlier and the duration of life is materially shortened. This evidence of association of accelerated nitrogen catabolism and toxicity is reinforced by the results of protein feeding to animals after removal of the kidneys[2] and also after substantial reduction of kidney function.[2,3] It points to a probable pathological nitrogen metabolism with production of toxic intermediates that represent some part of the augmented blood nonprotein nitrogen. Two factors seem to be concerned in the more rapid manifestations of toxicity and the rise in blood nonprotein nitrogen. They involve on the one hand some non-excretory renal function not yet known and on the other availability of tissue protein. The latter can be reasonably controlled by diet; this hypothesis is the basis of the experiments that follow.

Materials and Methods. Two groups of animals were given protein poor and protein rich diets respectively for protracted periods prior to the ligation of the renal arteries or the removal of the kidneys.

The daily ration of the protein poor diet per kilogram dog consisted of: Farina 15.0 g, Karo 2.5 g, Cod Liver Oil 0.4 g, Fresh yeast

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