Literature Cited

(1) Aggazzoti, A., *Clin. Vet.* **7,** 54 (1910).
(2) Andres, J., *Schweiz. Arch.* **70,** 225 (1928).
(3) Auernheimer, O., *Z. Fleisch- u. Milchhyg.* **20,** 393 (1910).
(4) Barcroft, J., McAnally, R. A., Phillipson, A. T., *J. Exptl. Biol.* **20,** 120 (1944).
(5) Bechdel, S. I., Eckles, C. H., Palmer, L. S., *J. Dairy Sci.* **9,** 409 (1926).
(6) Blaxter, K. L., Hutcheson, M. K., Robertson, J. M., Wilson, A. L., *Brit. J. Nutrition* **6,** i (1952).
(7) Carroll, E. J., Hungate, R. E., *Appl. Microbiol.* **2,** 205 (1954).
(8) Davenport, E., Ill. Agr. Expt. Sta. Bull. **46** (1897).
(9) Flatt, W. P., Ph.D. thesis, Cornell University, Ithaca, N. Y., 1955.
(10) Grossman, J. D., *J. Am. Vet. Med. Assoc.* **114,** 416 (1949).
(11) Hastings, E. G., *Bact. Rev.* **8,** 235 (1944).
(12) Herman, H. A., Missouri Agr. Expt. Sta. Research Bull. **245** (1936).
(13) Kesler, E. M., Ronning, M., Knodt, C. B., *J. Animal Sci.* **10,** 969 (1951).
(14) Lagerlof, N., *Skand. Vet. Tidskr.* **19,** 253 (1929).
(15) McCandlish, A. C., *J. Dairy Sci.* **6,** 347 (1923).
(16) Magee, H. E., *J. Exptl. Biol.* **9,** 409 (1932).
(17) Mangold, E., "Handbuch der Ernährung und des Stoffwechsels der Landwirtschaftlichen Nutztiere," vol. **2,** Verlag Julius Springer, Berlin, 1929.
(18) Marshall, S. P., Arnold, P. T. D., Becker, R. B., *J. Dairy Sci.* **33,** 379 (1950).
(19) Mead, S. W., Regan, W. M., *Ibid.*, **14,** 283 (1931).
(20) Reid, J. T., *Ibid.*, **36,** 955 (1953).
(21) Sanborn, J. W., Utah Agr. Expt. Sta. Bull. **21** (1893).
(22) Savage, E. S., McCay, C. M., *J. Dairy Sci.* **25,** 595 (1942).
(23) Sisson, S., Grossman, J. D., "Anatomy of the Domestic Animals," W. B. Saunders Co., Philadelphia, Pa., 1938.
(24) Turk, K. L., Burke, J. D., Correll Extension Bull. **761** (1949).
(25) Trautmann, A., *Arch. Tierernähr. und Tierzucht* **7,** 400 (1932).
(26) *Ibid.*, p. 421.
(27) *Ibid.*, **9,** 575 (1933).
(28) Warner, R. G., Bernholdt, H. F., Grippin, C. H., Loosli, J. K., *J. Dairy Sci.* **36,** 599 (1953).
(29) Wiese, A. C., Johnson, B. C., Mitchell, H. H., Nevens, W. B., *J. Nutrition* **33,** 263 (1947).
(30) Wise, G. H., Petersen, W. E., Gullickson, T. W., *J. Dairy Sci.* **22,** 559 (1939).

Received October 17, 1955. Accepted April 27, 1956.

DIETARY ENERGY REQUIREMENTS

Effects of Caloric Intake on Nitrogen Balance and Organ Composition of Adult Rats

HAROLD L. ROSENTHAL and JAMES B. ALLISON

Division of Biochemistry, Department of Pathology, Rochester General Hospital, Rochester, N. Y., and Bureau of Biological Research, Rutgers University, State University of New Jersey, New Brunswick, N. J.

Restriction in caloric intake, with or without protein in the diet, resulted in depletion in body fat and body protein and an increase in body water in adult rats. The increase in water was particularly marked in rats fed a restricted caloric intake in the presence of dietary casein. Nitrogen balance also decreased as calories were reduced, increased catabolic activity being a first response to such a restriction. The data were interpreted to mean that the animal could adapt to maintain essential tissues through shifts in metabolism, although continued deprivation in caloric intake resulted in marked loss in tissue nitrogen. There could be an optimum caloric intake for each protein intake. Differential changes of body tissues after caloric restriction demonstrated that some organs were more labile than others.

The energy requirements and the maintenance of essential tissue protein constituents are of first importance to the living system. A restriction in caloric intake results in certain metabolic adjustments that are directed toward correction of the deficit in energy intake. At the same time, essential anabolic functions are continued. Thus Allison, Anderson, and Seeley (*4, 5*) found that a marked restriction in caloric intake in dogs could prevent attainment of nitrogen equilibrium with retention of dietary nitrogen at low nitrogen intakes. Further studies by Rosenthal and Allison (*18*) and Rosenthal (*17*) suggested the following sequence of response to a caloric restriction in dogs. With sufficient fat and labile protein stores, reduced calories result in increased catabolism of labile protein stores, thus raising the excretion of urinary urea nitrogen. The retention of dietary nitrogen, as measured by the nitrogen balance index, was not altered. Upon depletion of fat and labile protein stores, the over-all catabolic activity of the animal was reduced, thereby conserving body nitrogen and reducing energy requirements. Eventually, however, the animal was forced to dip into the tissue reserves to such an extent that the nitrogen balance index was reduced and the body was badly depleted of tissue proteins.

These studies, together with numerous others (*6, 7, 11, 14*), emphasize that the response to a caloric restriction is a function of the physiological state of the animal as well as the diet. It is possible that there is an optimum caloric intake for each protein intake, the perfect balance between protein and calories resulting in an adequate development of body mass.

The following experiments were undertaken, therefore, to study more fully the responses in rats to caloric restriction, measured not only in terms of nitrogen balance but also in terms of changes that occur in various organs of the body.

Materials and Methods

Adult male rats of the Long-Evans strain were used when 5 to 6 months of age, and weighing 350 grams. These animals had, therefore, a goodly store of fat and protein reserves. The animals were fed a semisynthetic diet compounded as follows (percentages): casein 5.0, lard 3.3, sucrose 12.5, dextrose 12.5, dextrin 12.5, salt mixture 1.0, agar 1.4, and water 51.8. Vitamin supplements as milligrams per kilogram of wet weight of diet were: choline chloride 420, inositol 42, riboflavin 1.3, thiamine hydrochloride 0.8, ascorbic acid 1.7, and pyridoxine hydrochloride 0.6; 17 each of calcium pathothenate, niacin, *p*-aminobenzoic acid, and α-tocopherol; and 0.08 each of 2-methyl-1,4-naphthoquinone, biotin, and folic acid. Cod liver oil (4.2 grams) supplied 7500 U.S.P. units of vitamin A and 750 U.S.P. units of vitamin D. The agar diet made it possible more accurately to prepare diets, estimate uneaten food, and reduce scatter. This basic diet supplied 200 calories per 100 grams of wet diet. Diets of low caloric density were prepared by substituting water for carbohydrate calories. The protein-free diets were compounded by substituting dextrin carbohydrate for an equivalent amount of protein. The low fat content of the diet also made it possible to lower the caloric density without altering the total intake of protein, fat, or nondigestible matter. All diets were fed on the basis of calories per day per kilogram of initial body weight. Water was available *ad libitum*.

The rats were placed in metabolism cages in pairs for measurement of daily food intake and daily urine and feces collections. Each group contained six to 16 animals totaling 74 animals in all. They were equilibrated on each of the various diets for 2 days prior to starting collections. The rats were weighed at the start of the experiments and at the end of each collection period. Prior to termination of the experiment, four animals died. Urine collections were made in acid, stored at 6° C., and pooled in 4- or 6-day periods for nitrogen determination. Feces were stored at 6° C., pooled into 4- or 6-day periods, homogenized in acid, and analyzed for nitrogen. On completion of the experiment, red cell counts, hematocrit, and hemoglobin (as acid hematin) were determined in the usual manner. Plasma proteins were analyzed by the method of Howe (*9*) as modified by Robinson, Price, and Hogden (*15*). The fresh weight of the liver, kidneys, adrenals, testes, spleen, heart, and seminal vesicles (after the removal of seminal fluid) were recorded. All organs were dried to constant weight at 95° C. The dried liver, kidneys, and heart were ground to uniform consistency and analyzed for total nitrogen. The dried ground livers were analyzed for fat content by the method of Hsaio (*10*). Total body analyses of some animals were made after removal of hair with clippers, by drying the carcass to constant weight for determination of water content and Soxhlet extraction of the dried carcass with ether for determination of body fat. Nitrogen determinations were made on the ground, dry, fat-free residue. Protein concentrations were calculated from micro-Kjeldahl nitrogen values by use of the factor 6.25.

Results and Discussion

Body weight was maintained with 43 cal. per rat per day for rats fed diets supplying 250 mg. of casein nitrogen per rat per day. When the caloric intake of the diet was increased to 74 cal. per rat per day, the rats increased 24% in weight during the 36 days. A decrease in caloric intake to 18 cal. per rat per day resulted in a 33% loss in weight in 36 days. Throughout the experimental period the animals consumed all of the diet offered them and it was observed that the severely restricted rats resorted to coprophagy.

Food intake decreased rapidly during the first 2 weeks in rats fed protein-free diets supplying 76 cal. per rat per day. During the remaining 3 weeks, the groups offered 76 cal. per rat per day consumed approximately the same amount as the rats fed 46 cal. per rat per day. Both groups lost 22.5% of their body weight during the experiment. Rats fed 19 cal. per rat per day consumed all food offered and lost 29.6% of their body weight.

Nitrogen Balance The excretion of urinary nitrogen increased and nitrogen balance decreased as the caloric intake was lowered in rats fed diets containing a constant amount of protein. These changes in nitrogen balance with caloric intake are illustrated

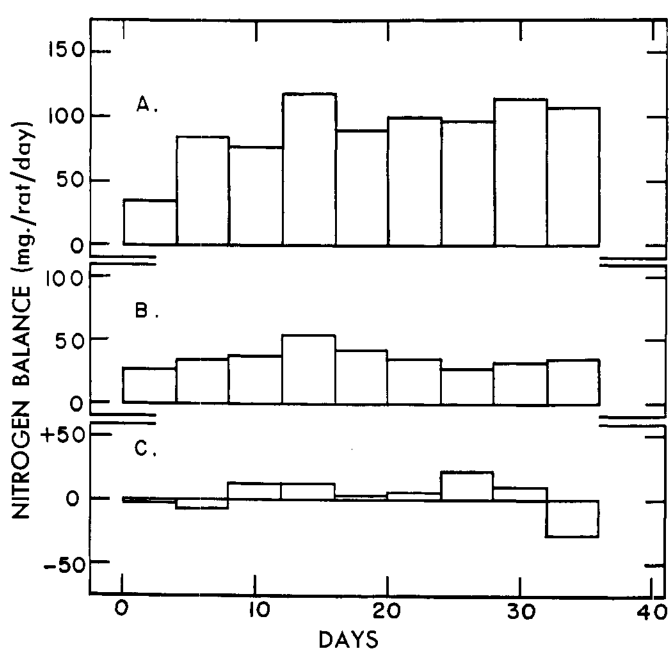

Figure 1. Nitrogen balance *vs.* days fed diets containing constant casein at different caloric intakes

A. 74 calories per rat per day (15 animals)
B. 43 calories per rat per day (16 animals)
C. 18 calories per rat per day (15 animals)

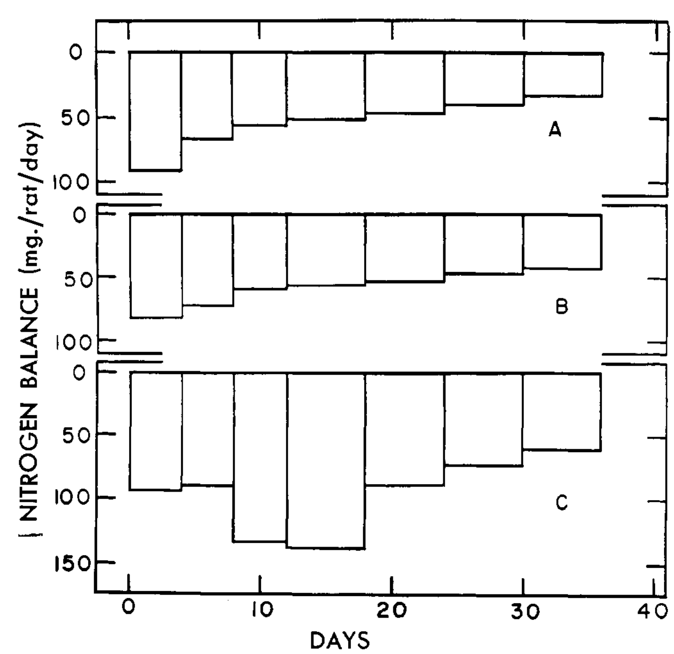

Figure 2. Nitrogen balance *vs.* days fed protein-free diets at different caloric intakes

A. 76 calories per rat per day (14 animals)
B. 46 calories per rat per day (12 animals)
C. 19 calories per rat per day (14 animals)

Table I. Tissue Composition of Rats Fed a Constant Casein Diet at Various Caloric Intakes for 36 Days

Caloric intake, cal./rat/day	74	43	18
No. of animals	7	8	7
Body weight, g.	459	355	242
Plasma albumin, g./100 ml.	3.01 ± 0.09[a]	3.39 ± 0.15[a]	2.51 ± 0.12[a]
Plasma globulin, g./100 ml.	3.25 ± 0.17	3.01 ± 0.15	3.58 ± 0.15
Albumin/globulin	0.93	1.13	0.70
Adrenal (2)			
Weight, mg.	36.8 ± 2.4	34.8 ± 2.3	35.8 ± 3.8
Mg./100 g. body wt.	8.0 ± 0.6	9.9 ± 0.6	14.9 ± 0.8
Testes (2)			
Weight, g.	3.04 ± 0.08	2.91 ± 0.10	2.62 ± 0.12
G./100 g. body wt.	0.67 ± 0.03	0.83 ± 0.05	1.03 ± 0.05
Moisture, %	86.2 ± 0.17	86.7 ± 0.81	86.7 ± 0.02
Spleen			
Weight, g.	1.13 ± 0.09	0.74 ± 0.04	0.59 ± 0.09
G./100 g. body wt.	0.25 ± 0.02	0.21 ± 0.02	0.24 ± 0.03
Moisture, %	77.3 ± 0.6	76.8 ± 0.4	75.9 ± 0.6
Seminal vesicles			
Weight, g.	0.75 ± 0.06	0.64 ± 0.06	0.34 ± 0.05
G./100 g. body wt.	0.16 ± 0.01	0.18 ± 0.02	0.14 ± 0.02

[a] Standard error of mean.

in Figure 1 and may be interpreted as follows. With 74 cal. per rat per day, *A*, the animal gained well in nitrogen, attaining a positive balance of 100 mg. per day. With reduction of the calories to 43 cal. per rat per day, *B*, the gain in nitrogen was lowered, as represented by a positive balance of 50 mg. per day. A further restriction to 18 cal. per rat per day, *C*, reduced the nitrogen balance to near equilibrium, a condition where the over-all catabolic activity of the labile protein stores was probably reduced to conserve nitrogen. Protein-depleted animals may retain nitrogen at much lower caloric intakes than do normal animals (*3*, *17*, *18*). Eventually, however, the animals lost the reserve stores and drifted into marked negative balance.

The nitrogen balance of rats fed protein-free diets did not remain constant, as shown in Figure 2. For rats fed 76 or 46 cal. per rat per day, the loss of body nitrogen (nitrogen balance) decreased in a regular fashion. However, in rats fed 19 cal. per rat per day, there was a marked increase in the excretion of body nitrogen between days 8 to 18 of the experimental period. Following this loss, the excretion of nitrogen decreased in a manner similar to that of the other groups. The marked loss of nitrogen in rats fed a low caloric intake (days 8 to 18) is interpreted to indicate changes in physiological state and adjustment of the caloric and protein economy of the animal, changes similar to those discussed for Figure 1, *C*.

Blood The erythrocyte count (8,600,000), hematocrit (48.2%), hemoglobin (13.5%), and nonprotein nitrogen (22 mg. per 100 ml. of blood) were not altered by any of the dietary regimes containing protein. Total plasma proteins and albumin and globulin concentrations were similar in rats fed 74 or 43 cal. per rat per day. In rats restricted to 18 cal. per rat per day, however, the albumin-globulin (A/G) ratio was somewhat depressed, owing to decreased plasma albumin levels. The average erythrocyte count (8,000,000), hematocrit (41.8%), hemoglobin (13.5%), and nonprotein nitrogen (20 mg. per 100 ml.) were essentially normal in animals fed the protein-free diets. The raiding of labile protein stores to maintain blood cytology has been described in the dog by Robscheit-Robbins and Whipple (*16*) and McCoy and coworkers (*13*). Total plasma proteins and A/G ratio were depressed to 0.70 to 0.80 in all groups fed protein-free diets (compare data in Tables I and II).

The total wet weight of the adrenals remained essentially constant in rats fed a constant amount of nitrogen with various caloric intakes, but the relative weight increased as the body weight decreased (Table I). The weight of the spleen and seminal vesicles decreased with the fall in body weight. The testes also decreased as body weight was lost, but the reduction was not proportional to body weight. These changes were not due to shifts in tissue water, since water contents were similar for each of the groups.

The wet weight of the adrenals, testes, and seminal vesicles remained essentially constant for rats fed protein-free diets at different caloric intakes. Because the body weights of all groups are similar, organ weight per unit of body weight is also similar (Table II).

Liver, heart, and kidney weight decreased as the caloric intake (and body weight) was lowered in animals fed a constant casein intake (Table III). Although liver weight was altered proportionately with body weight, the heart and kidney weights became relatively larger as the weight of the animals became smaller. The total amount of protein in the livers decreased with the weight of the organ and the percentage of protein was similar (19.9% of wet weight) for the rats fed 47- and 18-calorie intakes. In animals fed 74 cal. per rat per day, however, a small but significant increase in liver fat diluted the protein concentration to 18.9%. When the liver protein was calculated in relation to body weight of the animal, the ratio was found to remain constant. For the heart and kidney, relative organ weight and protein concentration increased as body weight (and caloric intake) decreased.

The total and relative weights of the liver decreased with increasing caloric deficiency in animals fed the protein-free diet (Table IV). The progressive decrease in moisture content of the liver as the caloric intake was lowered is

Table II. Tissue Composition of Rats Fed Protein-Free Diet at Different Caloric Intakes for 36 Days

Caloric intake, cal./rat/day	76	46	19
No. of animals	6	4	6
Weight, g.	273	289	282
Plasma albumin, g./100 ml.	2.05 ± 0.08[a]	2.29 ± 0.06[a]	2.27 ± 0.12[a]
Plasma globulin, g./100 ml.	2.59 ± 0.08	2.70 ± 0.10	3.26 ± 0.20
Albumin/globulin	0.79	0.85	0.70
Adrenal (2)			
Weight, mg.	26.2 ± 1.8	34.5 ± 3.1	32.1 ± 3.6
Mg./100 g. body wt.	9.7 ± 0.5	11.9 ± 0.4	11.3 ± 0.9
Testes (2)			
Weight, g.	2.47 ± 0.14	2.74 ± 0.29	2.40 ± 0.27
G./100 g. body wt.	0.91 ± 0.04	0.94 ± 0.06	0.97 ± 0.07
Moisture, %	86.8 ± 0.2	85.7 ± 0.8	84.2 ± 1.4
Spleen			
Weight, g.	0.44 ± 0.03	0.52 ± 0.05	0.63 ± 0.06
G./100 g. body wt.	0.16 ± 0.01	0.18 ± 0.01	0.22 ± 0.01
Moisture, %	79.0 ± 0.6	78.6 ± 0.5	77.3 ± 0.3
Seminal vesicles			
Weight, g.	0.32 ± 0.02	0.36 ± 0.07	0.32 ± 0.04
G./100 g. body wt.	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01

[a] Standard error of mean.

associated with an accumulation of liver fat in rats fed excessive calories. The loss of liver protein among the three groups of rats indicates that dietary protein is of critical importance and this limiting factor is not affected by concomitant restrictions of caloric intake. The composition of the heart was not altered by changes of caloric intake under conditions of protein-free feeding. Similar results were apparent for the kidney.

Total body analyses demonstrated that caloric restriction in the presence of protein intake resulted in a marked depletion of fat stores and the decrease in fat was associated with an increased percentage of water (Table V). On a fat-free basis, however, the percentage of water was constant and equivalent to approximately 72.5%. The total carcass protein was lower in rats fed 18 cal. per rat per day, although the percentage of body protein in the severely restricted rats was greater than in animals receiving 43 cal. per rat per day. The increased concentration of protein in the carcasses of severely restricted rats was probably due to the reduction of body fat. In fact, when calculated on a fat-free basis, the percentage of protein was constant and equal to about 20.5%. Similarly, total body analyses demonstrated that rats fed 46 cal. per rat per day of protein-free diet contained more fat and less water than rats fed 19 cal. per rat per day. The total quantity of carcass protein was lower in rats fed 19 cal. per rat per day of the protein-deficient diet, but the concentration of body protein was greater than in rats fed the higher caloric intake. On a fat-free basis, the percentage of protein was independent of caloric intake and the percentage of water was higher in the animals fed 46 calories per day. One might expect increased extracellular water and fatty liver to develop in animals fed a protein-deficient diet with a high caloric intake (*2*).

These experiments demonstrate that rats may be maintained in positive nitrogen balance when subjected to a dietary caloric deficiency, although the reduction of nitrogen balance that follows a systematic lowered caloric intake is associated with a loss of labile protein stores. This gradual reduction of nitrogen balance represents a characteristic response to caloric restriction which has been described recently by Rosenthal and Allison (*18*) in dogs and by Calloway and Spector (*7*) in rats. The animals did not remain static on restricted diets but adapted through shifts in metabolism, possibly to maintain essential tissues in a steady state commensurate with the quantity and quality of dietary nutrients. Thus, animals fed a markedly restricted caloric intake remained in positive nitrogen balance until body energy stores, in the form of adipose tissue, were depleted. The data were interpreted to mean that the catabolic activity of nitrogen was reduced upon depletion in protein stores. The retention of nitrogen and marked loss of body fat in rats fed restricted caloric intakes indicated that body energy pools

Table III. Tissue Composition of Rats Fed a Constant Casein Diet at Various Caloric Intakes for 36 Days

Caloric intake, cal./rat/day	74	43	18
No. of animals	7	8	7
Body weight, g.	459	355	242
Liver			
Weight, g.	12.19 ± 0.47[a]	9.34 ± 0.52[a]	6.38 ± 0.65[a]
G./100 g. body wt.	2.66 ± 0.07	2.63 ± 0.05	2.61 ± 0.16
Moisture, %	71.1 ± 0.4	70.8 ± 0.4	72.3 ± 0.3
Fat, % of dry wt.	32.6 ± 0.9	28.2 ± 0.9	28.6 ± 1.8
Protein, g.	2.30 ± 0.11	1.86 ± 0.10	1.27 ± 0.14
G./100 g. body wt.	0.50	0.52	0.52
Heart			
Weight, g.	1.08 ± 0.02	0.93 ± 0.03	0.75 ± 0.04
G./100 g. body wt.	0.24 ± 0.01	0.27 ± 0.01	0.32 ± 0.01
Moisture, %	78.6 ± 0.3	78.6 ± 0.3	78.9 ± 0.3
Protein, g.	0.158 ± 0.004	0.136 ± 0.004	0.112 ± 0.007
G./100 g. body wt.	0.034	0.038	0.046
Kidney (2)			
Weight, g.	2.65 ± 0.04	2.26 ± 0.07	1.90 ± 0.14
G./100 g. body wt.	0.58 ± 0.02	0.65 ± 0.02	0.78 ± 0.03
Moisture, %	77.1 ± 0.4	77.4 ± 0.4	78.1 ± 0.6
Protein, g.	0.389 ± 0.011	0.337 ± 0.011	0.278 ± 0.018
G./100 g. body wt.	0.085	0.095	0.115

[a] Standard error of mean.

Table IV. Tissue Composition of Rats Fed Protein-Free Diet at Various Caloric Intakes for 36 Days

Caloric intake, cal./rat/day	76	46	19
No. of animals	6	4	6
Body weight, g.	273	289	282
Liver			
Weight, g.	10.05 ± 0.57[a]	9.10 ± 0.55[a]	7.46 ± 0.71[a]
G./100 g. body wt.	3.67 ± 0.09	3.15 ± 0.14	2.62 ± 0.15
Moisture, %	64.6 ± 0.9	68.5 ± 2.3	73.0 ± 0.4
Fat, % of dry wt.	56.9 ± 2.0	45.7 ± 5.4	28.8 ± 2.2
Protein, g.	1.14 ± 0.06	1.25 ± 0.08	1.20 ± 0.05
G./100 g. body wt.	0.42	0.43	0.43
Heart			
Weight, g.	0.72 ± 0.04	0.78 ± 0.04	0.79 ± 0.04
G./100 g. body wt.	0.26 ± 0.01	0.27 ± 0.01	0.29 ± 0.01
Moisture, %	78.5 ± 0.2	78.1 ± 0.1	77.8 ± 0.3
Protein, g.	0.109 ± 0.006	0.122 ± 0.006	0.126 ± 0.005
G./100 g. body wt.	0.040	0.042	0.045
Kidney (2)			
Weight, g.	1.73 ± 0.08	2.17 ± 0.18	1.97 ± 0.12
G./100 g. body wt.	0.63 ± 0.01	0.75 ± 0.01	0.70 ± 0.02
Moisture, %	77.3 ± 0.3	77.4 ± 2.1	76.9 ± 0.2
Protein, g.	0.260 ± 0.013	0.316 ± 0.023	0.305 ± 0.061
G./100 g. body wt.	0.095	0.104	0.108

[a] Standard error of mean.

Table V. Body Composition of Rats Fed Diets of Variable Caloric Intake for 36 Days

	Casein		Protein-Free	
Caloric intake, cal./rat/day	43	18	46	19
No. of animals	8	8	8	8
Initial weight, g.	360	364	379	349
Final weight, g.	346	243	292	229
Protein				
G.	57.3	48.8	50.9	44.7
G./100 g. body wt.	16.5	20.1	17.4	19.5
G./100 g. fat-free body wt.	20.2	20.4	20.8	20.6
Moisture				
G./100 g. body wt.	58.4	70.4	60.6	64.2
G./100 g. fat-free body wt.	72.2	72.7	72.5	67.8
Lipides				
G.	66.2	8.1	47.9	11.7
G./100 g. body wt.	19.1	3.3	16.4	5.1

may supply a good deal of the daily caloric requirement (*1*, *8*, *12*). The animal then rapidly drifted into negative balance with concomitant increased excretion of urinary nitrogen and destruction of body protein to yield much needed energy. This rapid loss in body protein represents the ultimate response to a caloric deficit, and in the present experiments occurred after the animals had been on a restricted diet for 32 days. The differential changes of body tissues, however, after a period of caloric restriction demonstrates the lability of some organs and the stability of others to deprivation in calories.

Literature Cited

(1) Addis, T., Poo, L. J., Lew, W., *J. Biol. Chem.* **115,** 111 (1936).
(2) Allison, J. B., J. Agr. Food Chem. **1,** 71 (1953).
(3) Allison, J. B., *V^me^ Congr. Intern. Médicine Tropicale et Paludism* **2,** 465 (1954).
(4) Allison, J. B., Anderson, J. A., *J. Nutrition* **29,** 413 (1945).
(5) Allison, J. B., Anderson, J. A., Seeley, R. D., *Ann. N. Y. Acad. Sci.* **47,** 245 (1946).
(6) Calloway, D. H., Spector, H., *Am. J. Clin. Nutrition* **2,** 405 (1954).
(7) Calloway, D. H., Spector, H., *J. Nutrition* **56,** 533 (1955).
(8) Elman, R., Heifetz, C. J., *J. Exptl. Med.* **73,** 417 (1941).
(9) Howe, P. E., *J. Biol. Chem.* **49,** 109 (1921).
(10) Hsaio, S. C., *Science* **107,** 24 (1948).
(11) Johnson, R. M., Deuel, H. J., Jr., Morehouse, M. G., Mehl, J. W., *J. Nutrition* **33,** 371 (1947).
(12) Kosterlitz, H. W., Campbell, R W., *Nutrition Abstr. Revs.* **15,** 1 (1945-46).
(13) McCoy, J. R., Allison, J. B., Crossley, M. L., Wannemacher, R. W., Jr., *Am. J. Vet. Research,* in press.
(14) Munro, H. N., *Physiol. Rev.* **31,** 449 (1951).
(15) Robinson, H. W., Price, J. W., Hogden, C. G., *J. Biol. Chem.* **120,** 481 (1937).
(16) Robscheit-Robbins, F. S., Whipple, G. H., *J. Exptl. Med.* **89,** 339 (1949).
(17) Rosenthal, H. L., *J. Nutrition* **48,** 243 (1952).
(18) Rosenthal, H. L., Allison, J. B., *Ibid.*, **44,** 423 (1951).

Received for review October 3, 1955. Accepted March 5, 1956. Presented in part before the Division of Biological Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

FEED ANTIOXIDANTS

Inhibitory Effect of Feed Grade Diphenyl-*p*-phenylenediamine (DPPD) on Parturition in Rats

BERNARD L. OSER and MONA OSER

Food Research Laboratories, Inc., 48-14 Thirty-Third St., Long Island City 1, N. Y.

Feed grade diphenyl-*p*-phenylenediamine (DPPD), at dietary levels only several times in excess of those used for the prevention of encephalomalacia in chicks, induces prolonged gestation in rats, associated with high mortality both of overdeveloped pups at birth and of the mothers during or after parturition.

The demonstration that chick encephalomalacia resulted from a deficit of vitamin E relative to the unsaturated fatty acids in the ration (*1*), directed attention to the protective effect of antioxidants on tocopherols, which are themselves antioxidants against rancidity and vitamin A oxidation. Diphenyl-*p*-phenylenediamine (DPPD) has proved to be the most effective of the antioxidants studied (*4*, *5*) and, until recently, without objection from the Food and Drug Administration, it has been used extensively in poultry rations. Though it is an aromatic amine, its low solubility, as well as results of toxicological feeding tests, have militated against the possibility of hazard from this use. It should be emphasized that the commercial grades of DPPD contain from 5 to 10% of unidentified impurities.

Subsequent studies designed to establish the quantitative relationship between minimal tocopherol levels and concentrations of DPPD required for protection have revealed a toxic manifestation in pregnant rats—viz., delay or complete failure of parturition with attendant mortality of both the mother and litter. This effect has been observed in both synthetic and natural type diets. The following experiment will illustrate the nature of the phenomenon and the levels of DPPD required to induce it.

Five groups of 10 healthy female rats were selected from the Food Research Laboratories' breeding colony, each having previously produced and weaned a normal litter. They were placed in mating cages and fed *ad libitum* the following diet (Table I). This diet was estimated to provide 18.6 mg. of α-tocopherol per 100 grams from natural sources, of which (cf. footnotes to table) 12 mg. were added in the form of distilled mixed tocopherols, N. F. (Distillation Products Industries, Rochester, N. Y.). DPPD [Good-rite DPPD feed grade antioxidant (95% minimum *N,N'*-diphenyl-*p*-phenylenediamine) a product of the B. F. Goodrich Co.] was added at four levels, 0.025, 0.10, 0.40, and 1.60%, the lowest being about twice the concentration employed in practical broiler rations. It was incorporated into the vegetable fat before mixing in the final diet.

After 2 weeks on this diet, vaginal smears were made daily, and at proestrus one breeder male of known fertility was placed in the cage with the female. The male was allowed to remain for not less than 17 nor more than 24 hours—i.e., through stages I and II of the estrus cycle (*2*). Examinations were made for the vaginal plug and the smears were examined for the presence of spermatozoa. In addition smears were made daily to confirm cessation of the cycle. When a mating was unsuccessful, another male was provided at the next proestrus period. Records were kept of body weight, duration of pregnancy, number and weight of pups cast or found *in utero*, and mortality up to the end of a normal gestation and lactation period. The results of this experiment are shown in Table II.

All females were initially fertile, although in some cases more than one mating was necessary for conception to