TABLE I. Succinic Dehydrogenase (SDN)/DNA Ratio and RNA/DNA Ratio (See Text) for Supraoptic Nucleus (SON) and Anterior Area (AA) with Results of t-Test with P = <.05 Considered Significant.

		Normal	Dehydrated	P	% Increase
SDH/DNA	SON	1.91 ± .68	2.58 ± .85	>.30	35
RNA/DNA	SON	2.23 ± .85	4.42 ±1.26	<.05	98
SDH/DNA	AA	2.66 ± .55	6.41 ± .35	<.001	140
RNA/DNA	AA	2.35 ±1.11	9.25 ± .61	<.001	293

the results. Using DNA as a base line for tissue amount the ratio of SDH and RNA was calculated for each group and the average of these is given in the Table. A t-test was applied and a P value of less than 0.05 was considered as significant. From the Table it may be seen that while SDH increased 35% in the dehydrated animals this was found to be not statistically significant. It would appear, therefore, that the Krebs cycle does not play an important role in production of the antidiuretic hormone by the SON. RNA was significantly increased as might be expected. The surprising finding, however, is that a very large increase in both SDH and RNA occurred in the AA. Since other hypothalamic nuclei were not analyzed nor other brain regions it is not known whether this increase is peculiar to the AA. Why dehydration should increase activity in these neurons is not clear.

Summary. A quantitative method that combines an analysis for succinic dehydrogenase activity (SDH) and ribonucleic acid (RNA) content in nerve cells was applied to the supraoptic nucleus (SON) and the anterior area (AA) of the hypothalamus of normal and dehydrated rats. Dehydration resulted in a significant increase in RNA in the SON but not in SDH. In the AA both SDH and RNA increased significantly.

We thank Carolyn Dansie for her technical assistance.

1. Edström, J.-E., Eichner, D., Schor, N., *Regional Neurochemistry,* Pergamon Press, New York, 1961, p274.

2. Kivalo, E., Rinne, U. K., Makela, S. K., *Ann. Med. Exp. Fenn.,* 1959, v37, 28.

3. Arvy, L., *Neurosecretion,* H. Heller, & R. B. Clark, Eds., Academic Press, New York, 1962, p215.

4. Nachlas, M. M., Kwan-Chung Tson, de Souza, E., Chao-Shing Cheng, Seligman, A. M., *J. Histochem.,* 1957, v5, 420.

5. Scott, J. F., Fraccastoro, A. P., Taft, E. B., *J. Histochem. and Cytochem.,* 1956, v4, 1.

Received June 7, 1964. P.S.E.B.M., 1964, v117.

Nitrogen Balance in Radiation Chimeras.* (29527)

W. H. MCARTHUR, C. C. CONGDON, AND A. L. KRETCHMAR

Knoxville College, Knoxville, Tenn., Biology Division, Oak Ridge National Laboratory, and Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.

Mice exposed to lethal doses of total-body irradiation can recover if they are given normal bone marrow cells after irradiation(1). If the injected cells are genetically different from the irradiated host, the animals may subsequently die because of a secondary syndrome not related to the acute effects of irradiation. This syndrome is presumed to be a consequence of immunological reaction between the grafted cells and the host animal. It has been suggested that secondary disease is associated with a "metabolic starvation" (2) because food intake of mice given homologous marrow is adequate and comparable to that of normal or isologous marrow-treated mice. We have previously reported results

* Research sponsored by U. S. Atomic Energy Commission under contract with Knoxville College, Union Carbide Corp., and Oak Ridge Institute of Nuclear Studies.

that confirmed the similarity of food intake among normal, and isologous or homologous marrow-treated, irradiated mice(3), and showed that mice of these 3 groups had comparable degrees of positive nitrogen balance despite loss of body weight among the animals given homologous marrow. We report here the results of more recent experiments, carried through 90 days, which show long-range positive nitrogen balance but no gain of body weight among homologous irradiation chimeras.

Materials and methods. A metabolic experiment was done on each of 24 mice. Eight mice were unirradiated, uninjected controls; 8 were given 900 r total-body X-irradiation, and then injected intravenously with 40×10^6 isologous bone marrow cells; and 8 were similarly irradiated but given 40×10^6 homologous bone marrow cells. X-ray control animals, not given bone marrow, showed weight loss, negative nitrogen balance, and death usually before the 10th day after irradiation and, therefore, were not included in the analysis. Each animal was then placed in a 600 ml beaker with a wire-screen platform which was held about 2 inches above the bottom of the beaker and which surrounded a specially constructed feeder. The feeder was spring-loaded so that the food pellets were held against a wire retainer, at the top of the feeder, through which the animal had access to the food. The design was such that the mouse could only separate small pieces of the pellet while eating and this minimized dropping of food particles into the bottom of the cage where they would be included in the excreta. A few ml of dilute (0.5 N) H_2SO_4 were placed in the bottom of the beaker so that feces and urine would be immediately acidified and covered to prevent loss of nitrogen as ammonia.

No attempt was made to trap ammonia in the gas phase (as might be liberated by bacterial decomposition of urine on the wire screen of the cage floor or conceivably be present in exhaled air from the animal). Although some ammonia in the gas phase would doubtless diffuse to the surface of the acid in the floor of the cage and be trapped, it is likely that some loss could occur by diffusion or by convection from air currents upward and thus escape collection. It is possible that a systematic positive error is present in our data due to such loss; however, such an error can reasonably be expected to be similar among all groups and should not affect conclusions based on comparisons of one group to another.

A wire-mesh screen, through which a water tube extended into the top of the beaker, covered the assembly. The animal was left undisturbed in this "metabolism cage" for 48 hours, when it was removed, rapidly weighed on a torsion balance to the nearest 0.1 g, and placed in a clean metabolism cage. The cage from which the animal had been removed was then rinsed down with dilute H_2SO_4, the rinsed wire-screen floor and feeder were removed and the combined acid, urine, and feces mixture rinsed into a specimen bottle. Food was removed from the feeder, weighed, and "food eaten" determined by difference from the known weight of the pellets placed in the feeder at the beginning of the period. Any food that dropped into the bottom of the beaker would thus be included as food eaten but its nitrogen content would be included as part of the nitrogen of the excreta and would not be lost in the nitrogen balance calculation. Each experiment was carried through 45 consecutive periods for a total of 90 days with measurements of animal weight, food intake, and nitrogen balance at the end of each period.

In the analysis of the data, the first 5 periods were ignored since during this time weight loss and recovery occur due to the irradiation alone and our principal interest relates to the metabolism of the mice after chimerism is established. In addition, most normal mice will lose weight for the first 1 to 3 periods under the conditions of the caging but then go into positive nitrogen balance and show continuous slow gain in body weight during the remainder of the experiment. We wished to ignore this initial "caging effect." At the end of the metabolic experiments, a regression line was fit to the body-weight data, and the slope of this line, averaged for all animals in each of the 3 treatment groups, is entered in Tables I and

TABLE I. Average Weight Gain and Nitrogen Balance of Normal Mice and of Mice Given 900 r Total-Body Irradiation and Isologous or Homologous Bone Marrow Cells. Experiments carried through only 30 days.

Group	Wt gain, g/day	Nitrogen balance,* mg/hr
Normal	.06	.55
Isologous	.06	.70
Homologous	.02	.73

* Calculated on the assumption that there was 35.6 mg nitrogen per gram food pellet (manufacturer's value). Our analyses ranged from 36.0 to 33.6 with mean 34.7 mg/g. All nitrogen-balance calculations are therefore subject to a systematic positive error which does not, however, affect the comparison among groups.

II as weight gain. The mean nitrogen balance and its standard error were computed for each metabolic experiment, and the average of these means for the 3 groups is entered as nitrogen balance. Two metabolic experiments in the isologous and 2 in the homologous marrow-treated groups were incomplete because the animals died, and the incompleted data from these were omitted in the final analysis. Two metabolic experiments in each of the 3 groups were limited to 30 days and the results are separately recorded in Table I. The variance was so large that the differences among means were not statistically significant. The differences were in the same direction as for the more complete data of Table II, however, so we have recorded the results separately without showing the standard error. Table II is a summary of 14 experiments (6 normal and 4 for each of the marrow-treated groups). The correlation between food intake and nitrogen balance was computed for all metabolic experiments and the results summarized in Table III.

The details of the bone marrow treatment have been reported previously(4,5). Irradiation was with a constant potential X-ray machine, 250 kilovolts, 15 ma. Inherent filtration was 1 mm Al; half-value layer, 0.5 mm Cu; target-object distance 60 cm; dose rate in air approximately 157 r/min.

Male mice 10-14 weeks old were used. The irradiated recipient animals were ($C_{57}L/_{cum}$ $\times$ $A/He/_{cum}$)F_1 hybrids. The donors of homologous bone marrow were ($101/_{cum}$ $\times$ $C_3H/_{Auf./cum}$)F_1 mice. The dose of irradiation was 900 r total body, and 40 $\times$ 10^6 isologous or homologous cells were injected intravenously on the day of irradiation.

Nitrogen content of each specimen was determined with a Technicon Autodigester and Autoanalyzer(6).

Results. During the first 30 days (Table I) both isologous and homologous marrow-treated groups were in positive nitrogen balance and retained more nitrogen than did normal control animals. The weight gain of isologous marrow-treated animals was the same as that of normal mice. Animals given homologous marrow gained little or no weight.

The data summarized in Table II show that animals treated with isologous marrow maintain a normal rate of gain in body weight through 90 days but retain significantly more nitrogen than normal animals. The animals given homologous marrow fail to gain weight but retain twice as much nitrogen as normal mice. Both marrow-treated groups show the same degree of increased positive nitrogen balance.

In Fig. 1 body weight and nitrogen balance data are plotted from 4 experiments. The curves are not intended to illustrate typical results but were selected to show that even the homologous chimeras with prolonged (Fig. 1*d*) or cyclical (Fig. 1*c*) secondary weight loss maintain positive nitrogen balance throughout periods of loss in body weight. Indeed, some of the data (Fig. 1*c*) suggest that nitrogen balance becomes more positive during cyclical periods of loss of body weight.

The correlation between nitrogen balance and food intake (Table III) did not change

TABLE II. Average Weight Gain and Nitrogen Balance for the Period 10-90 Days of Normal Mice and of Mice Given 900 r Total-Body Irradiation and Isologous or Homologous Bone Marrow Cells.

Group	Wt gain, g/day	Nitrogen balance,† mg/hr
Normal	.06 $\pm$ .009	.34 $\pm$ .08
Isologous	.05 $\pm$ .011	.67 $\pm$ .09
Homologous	.009 $\pm$ .011	.72 $\pm$ .09

* $\pm$ Standard error of mean.
† See Table I nitrogen-balance calculations.

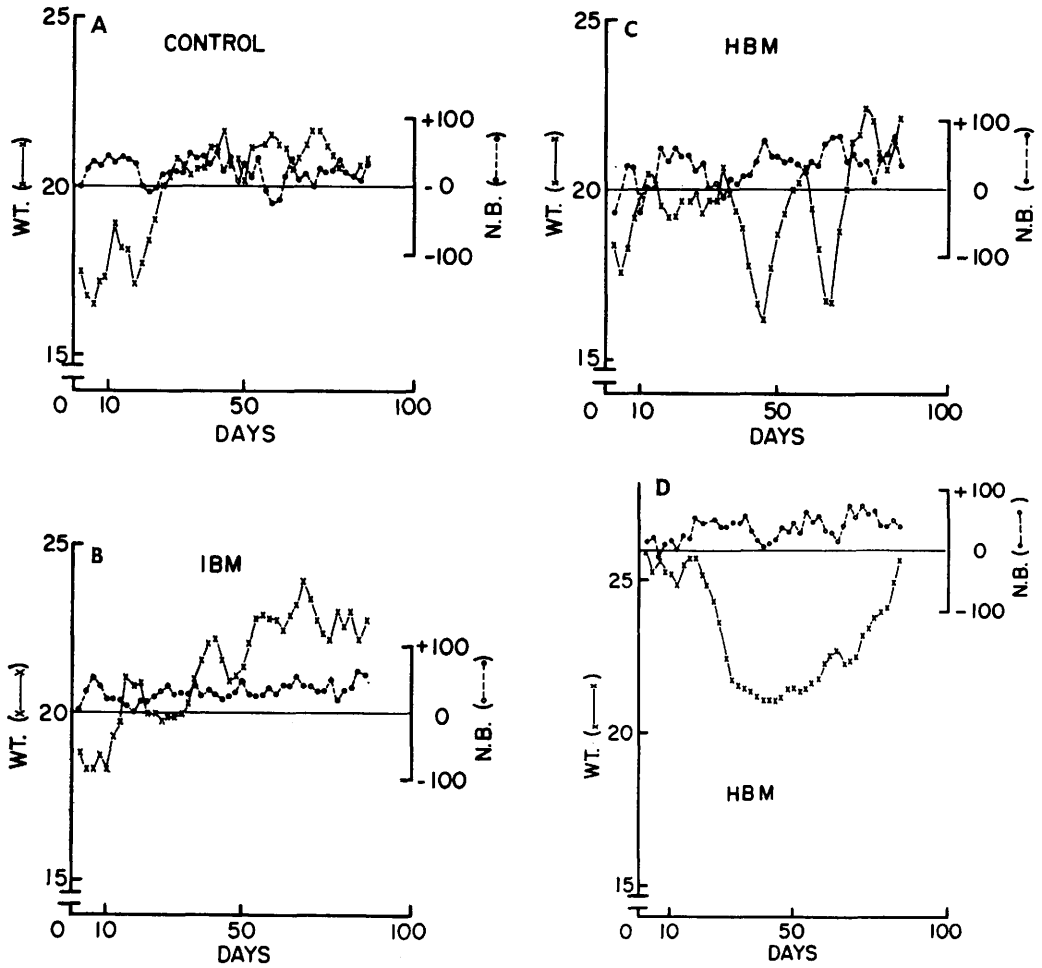

FIG. 1. Plot of body weight, in grams, (×——×) and nitrogen balance, in mg per 48 hr, (○-----○) of mice after total-body X-irradiation and treatment with bone marrow. (*a*) Unirradiated control not given bone marrow cells. (*b*) Irradiated (900 r) treated with 40×10^6 isologous bone marrow cells. (*c*) Irradiated (900 r) treated with 40×10^6 homologous bone marrow cells showing cyclic body weight changes. (*d*) showing prolonged loss in body weight.

significantly during the 90 days of these experiments.

Discussion. The normal correlation between nitrogen balance and food intake in irradiation chimeras indicates that no significant alteration in digestion and absorption processes was present in these animals. Diarrhea is frequently(7) part of the secondary disease syndrome. Our chimeras were evidently exhibiting a relatively mild secondary disease since they did not show the marked weight loss often seen, but the results show that diarrhea is not a fundamental feature of the syndrome. The metabolic alteration in secondary disease is clearly more subtle than effects at the level of the overall physiologic processes of digestion, absorption, and assimilation of foodstuffs.

We presume that the elevated positive balance in irradiated animals given isologous

TABLE III. Correlation* Between Nitrogen Balance and Food Intake in Normal Mice and in Mice Given 900 r Total-Body Irradiation and Isologous or Homologous Bone Marrow Cells.

	Time after treatment	
	10-30 days	30-90 days
Normal	.56	.63
Isologous	.59	.60
Homologous	.66	.68

* Coefficient of correlation.

marrow is the sum of the nitrogen retained for growth, as in the normal mice, and the nitrogen required to complete repair of the damage induced by the lethal irradiation. Despite this elevated retention of nitrogen, which in animals given isologous marrow is sufficient for both growth and repair, the mice treated with homologous cells are unable to grow normally. We speculate that nitrogen is directed into some abnormal compartment or compartments in animals with secondary disease and that this is related in some way to the immunologic conflict between grafted homologous cells and the host animals. If nitrogen in these abnormal forms is retained with less water, glycogen, and solutes than in normal tissues (muscle, for example, contains approximately 75% water, 20% protein, and 5% glycogen and solutes), then the nitrogen balance can be strongly positive even with loss or no gain in gross body weight. This retention of nitrogen in homologous chimeras is of sufficient degree that it should be possible to identify the supposed abnormal compartments and experiments along these lines are in progress. Suu *et al*(5,8) have reported considerable increases in lysozyme activity of some of the tissues of irradiated, marrow-treated mice, notably in bone marrow, kidney, and spleen; this increase was especially marked in animals given homologous cells. Abnormal components in electrophoretic patterns of the proteins of lymphoid tissue have been reported(9). It seems doubtful that a combination of these 2 effects could account for all of the extra nitrogen retained by homologous chimeras and it may be that our results on nitrogen balance are the result of a combination of many relatively small effects in several tissues.

Another possible explanation is suggested by the work of Hager *et al*(10) who have demonstrated antigen-antibody complexes on homograft and host cells during rejection of renal homografts in dogs. If this binding of antibody to cell surface occurs in the irradiation chimera, considerable protein could be synthesized and bound in the tissues of the host as antigen-antibody complex. This process might explain how during periods of increased graft-*vs*-host reaction (as suggested by loss of body weight, Fig. 1*c*) the nitrogen balance may become more positive than during relatively more quiescent periods during which some recovery of weight can occur.

Summary. Positive nitrogen balance was observed in mice given 900 r total-body X-irradiation and then treated by injection of living bone marrow cells. The average level was about twice that of normal controls for 90 days after irradiation and bone marrow treatment. Mice given isologous cells showed a normal rate of gain in body weight. However, mice given homologous cells failed to gain weight despite the same degree of positive nitrogen balance.

We gratefully acknowledge the help of Dr. D. Gosslee, Mathematics Division, Oak Ridge National Laboratory, in analysis of the data.

1. Congdon, C. C., in Tocantins, L. M., ed., *Progress in Hematology,* New York, Grune, 1959, v2, 21.

2. Urso, I. S., Congdon, C. C., Owen, R. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 395.

3. McArthur, W. H., Kretchmar, A. L., Congdon, C. C., *Blood,* 1963, v22, 503.

4. Kretchmar, A. L., Congdon, C. C., *Am. J. Physiol.,* 1961, v200, 102.

5. Suu, V-I., Congdon, C. C., Kretchmar, A. L., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 481.

6. Ferrari, A., *Ann. N. Y. Acad. Sci.,* 1960, v87, 792.

7. DeVries, M. J., Vos, O., *J. Nat. Cancer Inst.,* 1959, v23, 1403.

8. Suu, V-T., Congdon, C. C., Kretchmar, A. L., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 825.

9. Schwarzmann, V., Mathé, G., Amiel, J. L., *Nature,* 1961, v189, 1025.

10. Hager, E., Reagan, W., DuPuy, M., Wallach, D., *Sixth International Homotransplantation Conference, N. Y. Acad. Sci.,* in press.

Received June 11, 1964. P.S.E.B.M., 1964, v117.