vision. It was suggested[5] that folic acid may function as a coenzyme in a system responsible for the synthesis of thymine or a thymine-like compound which is then utilized in the synthesis of nucleic acid. Further evidence of this relationship is to be seen in *Lactobacillus casei* which when grown in a folic acid deficient medium showed a marked decrease in desoxyribonucleic acid,[6] a universal constituent of cell nuclei.[7]

It is tempting to speculate that in the presence of aminopterin, a folic acid antagonist, the folic acid utilization in the organism may be interfered with, resulting in a reduction in nucleic acid synthesis and a marked retardation of the rate of cell division. These facts might serve to explain the growth inhibition obtained with the folic acid antagonists in certain of the leukemias[8,9] and in the transplantable mouse Sarcoma 180.[10]

Summary. Macroscopically and microscopically, oviducts of frogs receiving aminopterin (4-amino pteroylglutamic acid) failed to respond to the growth stimulating action of estradiol. However, a significantly greater number of mitotic figures were visible in these oviducts. Evidence is presented to suggest that the folic acid antagonists may exert their growth inhibitory action by interfering with folic acid utilization in the organism, with a consequent reduction in nucleic acid synthesis and a retardation of the rate of cell division.

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17225. Increased Metabolic Rate in Rats after X-Irradiation.

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Some metabolic reactions may be increased after exposure to total-body irradiation. Indirect evidence for increased metabolism in dogs 1-3 weeks after irradiation was presented by Prosser *et al.*:[1] (1) Weight loss was greater after irradiation than in non-irradiated dogs in which food intake was limited to amounts less than consumed by the irradiated animals. (2) Nitrogen excretion was maintained by irradiated dogs despite a decrease in food intake, and in the acute terminal period the nitrogen balance was negative. (3) The metabolic balance minus the water balance or metabolic index is given by [(food + water + weight loss) − (urine + feces)] − [(food water + water drunk) − (urine + feces water)]. This metabolic index is a measure of CO_2 exhaled plus water of metabolism and water of tissue breakdown. This quantity increased significantly in dogs during the first 2 weeks after irradiation and increased considerably during the acute terminal period.

As a check on the hypothesis that catabolism is increased after irradiation, basal metabolism has been measured in rats after total body exposure to x-rays.

Materials and methods. Forty-four albino rats, 24 males and 20 females, ranging in initial weight from 160 to 310 g, (average 206 g) were used. There was no apparent dif-

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1 Prosser, C. L., with contributions by Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., and Swift, M. N., *Radiology*, 1947, **49**, 299.

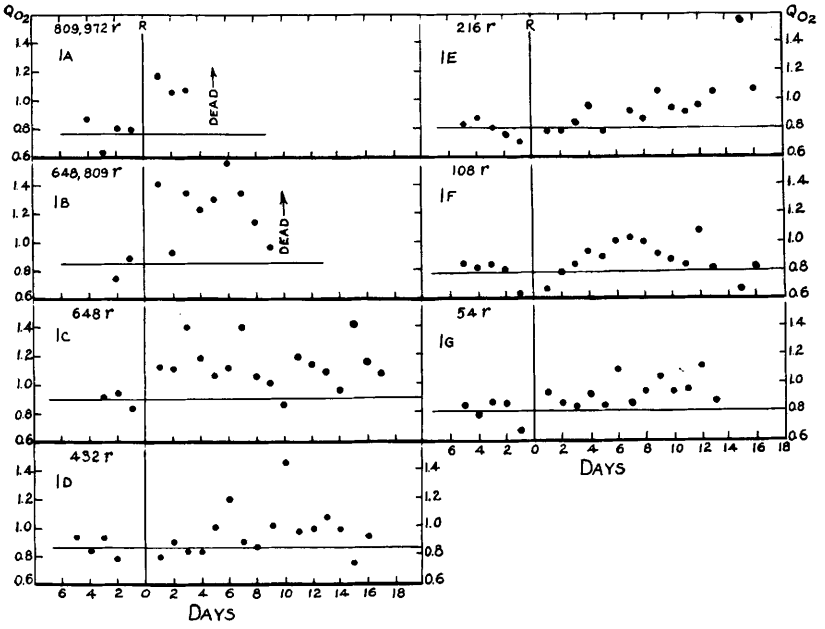

FIG. 1a-g.

Average oxygen consumption in ml O_2/g/hr of groups of rats irradiated at day R. 1a: 6 rats which died 3-5 days after exposure; 1b: 8 rats which died 8-10 days after exposure; 1c: 6 rats which survived potentially lethal doses; 1d: 4 rats exposed to 432 r. 1e: 5 at 210 r. 1f: 5 at 108 r. 1g: 4 at 54 r. Horizontal lines: Control average Q_{O_2} for each group.

ference in reaction as a function of size or sex. The postabsorptive oxygen consumption was measured daily between noon and 4 P.M.; animals were weighed after each metabolism measurement.

The equipment used for measurement of oxygen consumption was a modification of the apparatus described by Schwabe and Griffith.[2] This is a closed gas circuit consisting of a glass animal chamber, a circulating oil-immersed pump, CO_2 and H_2O absorbers, and two flasks arranged as water valves which maintain a constant pressure in the entire system. Oxygen is supplied through the pressure-maintaining flasks from a rubber bag. Volume differences in the first of the pressure flasks are transmitted to a water manometer from which a float writes on a kymograph. Movement of the animal was reflected in a steeper rise of the kymograph record. All measurements of Q_{O_2} were based on the least slope, (which corresponded to periods when the animal was at rest) and were expressed as ml of O_2 consumed per g body weight per hour. Animals were placed in the chamber for a half hour each day until they became accustomed to the apparatus. Then control measurements were made for several days prior to irradiation.

The rats were irradiated, 2 to 3 at a time, in a cell cut into a block of presswood. The dimensions of the block were 40 x 40 x 20 cm, those of the cell 18 x 18 x 10 cm. One half of the dose was administered from above, one half from below. Factors were: 200 K.V.p., no filter, T. D. of 55½ cm (to center of cell). The dosage distribution was determined by measurement with a Victoreen thimble chamber in a phantom of presswood equal in size to the irradiation block and cell. It was found that the dosage distribution was fairly uniform, the minimal dose (at the corners of the cell) being 83% of the maximal dose (in the center). The average tissue dose rate was 40.5 r per minute.

Experimental results. The animals have been divided into 4 groups, in a way that was

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TABLE I.
Oxygen Consumption in Contol Animals: Analysis of Variance.

	Sum of squared deviations	Degrees of freedom	Variance	Standard deviation
Daily variations	0.39	55	0.007	0.08
Var. between rats	0.35	17	0.021	0.14
Total variation about the general mean = 81	0.74	72	$\frac{\delta 1}{\delta 2}$ = 1.75;	P < 0.0027

suggested by a survey of the responses obtained. Of 20 rats which received doses of from 648 to 972 r, 6 died 3 to 5 days, 8 died 9-10 days after treatment, and the remaining 6 survived. The clustering of survival times around 2 values suggests 2 groups defined by survival times. That deaths occur at certain times after irradiation has been frequently observed; the clinical syndrome is different according to the time of death. The third group contains the rats which survived a potentially lethal dose. In the fourth group are animals treated with sublethal doses. The group averages of Qo_2 values are presented in Fig. 1.

Controls. Measurements of oxygen consumption before irradiation yielded an average of 0.83 ml/g/hr which is slightly higher than values given by Moses.[3] The variance was analyzed, following R. A. Fisher's procedure,[4] on the material obtained from 17 rats on each of which 4 to 5 control measurements had been made. The data are given in Table I which shows that the oxygen consumption of any single rat varies from day to day with a standard deviation of about 10% of the mean, and that differences between rats are higher than expected on the basis of random sampling. The excess standard deviation is about 15% of the mean.

Death in 3 to 5 days. Six of 12 rats which received 972 or 809 r developed severe diarrhea, lost weight rapidly, and died 3 to 5 days after exposure. Their Qo_2 was increased by about 35% within 24 hours after treatment (earlier measurements were not taken) (Fig. 1a). These animals lost an average of 17% of body weight by the time of death (Fig. 2a).

Death in 9 to 10 days. Six of the 12 rats irradiated with 809 or 972 r, and 2 out of 8 treated with 648 r, died 9 to 10 days after irradiation. No severe diarrhea occurred in this group. Their Qo_2 was elevated at the first measurement after exposure and remained—on an average—58% higher than the preirradiation level until the day before death, when it declined (Fig. 1b). Weight loss was not rapid in this group; it reached 17% at 5-6 days (Fig. 2b). Thereafter, there was little change in weight, some individuals showing a slight gain. Weight loss definitely did not mirror the course of the metabolic rate.

Survival after potentially lethal doses. Six of 8 rats which received 648 r survived beyond the time when acute death occurs. Each of these showed an increase in oxygen consumption. The average increase was 32% during the first 7 days. During the period of 8 to 10 days after the irradiation—at the time when the second group of non-survivors was dying—there was in some individuals a depression of the metabolic rate to only 10% above the preirradiation level. After this brief period, higher Qo_2 values (on the average 25% above normal) were again obtained. At 17 days, oxygen consumption was still elevated (Fig. 1c). The weight loss in this group reached about 7% on the fifth day. The weight remained at this level until about the ninth day, after which it rose toward the preirradiation level (Fig. 2c).

Sublethal doses. Doses of 432, 216, 108, and 54 r were administered to 4, 5, 5, and 4 rats respectively. With all 4 doses, the metabolic rate rose slightly (an average of 8%) for a 16-day period following irradiation. The difference is small but highly significant. It

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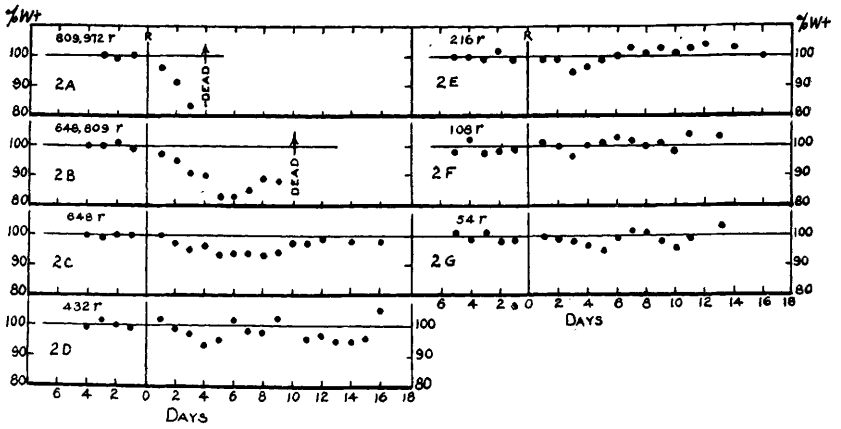

FIG. 2a-g.
Average percentage of body weight before and after irradiation at time R. For each group of rats 100% corresponds to the mean pre-irradiation weight. Doses as indicated.

is based on 74 tests before and 199 tests after irradiation, in a total of 18 rats. The standard deviation of this difference in metabolism between the rats before and after irradiation is about 0.014 or 1.7% of the normal metabolic rate. This is only one-fifth of the difference observed between pre- and postirradiation metabolism. An inspection of the curves (Fig. 1d to 1g) suggests that the rise of the metabolic rate occurs more slowly at these lower doses than in the 3 groups previously described in the lethal range, and that the response does not vary much over the dosage range from 54 to 432 r. Our material is not large enough to warrant any definite assertion beyond the general statement that the metabolic rate does increase after administration of small doses. Weight loss, if it did occur at all, was small and of short duration in the sublethal range (Fig. 2d to 2g).

Tests with nembutal. Eight of the experimental rats were lightly anaesthetized with 0.05 g nembutal per 100 g body weight each day before their metabolic rate was measured. The average Q_{O_2} of the nembutalized animals was 0.79 compared to the control value of 0.82 in 25 unanaesthetized animals tested at about the same time. The response of the metabolic rate to irradiation was about the same in both sets of animals.

Discussion. The preceding data show that total body irradiation produces increased oxygen consumption in rats. This reaction occurs over the whole dosage range tested, from 54 to 972 r. The increase is pronounced after lethal doses—whether or not the animal survives—and slight but still significant after sublethal doses. The rise occurs during the first postirradiation day after lethal doses; it seems to develop more slowly after sublethal doses.

The increased oxygen consumption is certainly not due to excessive feeding; irradiated animals eat less than normal. Neither is it due to increased activity, as shown by frequent observations of activity and by the nembutal tests. After high doses, the metabolic rate remains high during a time when the animals are recovering their normal weight, and after small doses the metabolic rate may go up without associated weight loss. Loss of body weight and increase in metabolic rate depend upon consumption of body substance, but there are probably several factors which influence only metabolic rate or body weight and some of these must be responsible for the observed lack of conformity between the two kinds of measurement.

The increase observed in metabolism of the rat after irradiation need not be interpreted as direct stimulation of oxidative enzyme systems. In fact, all available evidence suggests that the metabolic response may result from indirect action. Measurements in buffered saline of respiration of tissue slices from irradiated animals show either a depres-

sion or no change.[5] Similarly respiration is reduced in tissues and isolated enzymes irradiated *in vitro* (Barron *et al.*,[5] Dale[6] and others). Apparently the response of an intact animal where the tissues are bathed by the complex body fluids differs from the response of tissues in simpler media. The body fluids of irradiated animals contain products of tissue breakdown, and there is much evidence of toxemia during the acute radiation reaction. It is not likely that all the toxin is liberated at the time of irradiation. It is more probable that some cell injury remains latent for days and results, over an extensive period, in production of materials which have a specific dynamic action, reflected in elevated metabolism. It is of interest that thyroid metabolism, as indicated by the iodine turnover, is increased 2 to 3 days after irradiation.[7]

Summary. 1. Rats which died 3 to 5 days after total-body irradiation of 809 - 972 r showed an elevation of oxygen consumption of about 35% occurring within 24 hours.

2. Rats which died 9 to 10 days after doses of 648 to 972 r showed an increase in metabolism of about 58% and a decline prior to death.

3. Rats which survived doses of 648 r showed an average increase in oxygen consumption of 32% during the first week, and less pronounced increase extending over a period of at least 16 days after irradiation.

4. At doses from 54 to 432 r there was an increase in metabolism by about 8½%. The maximum oxygen consumption was reached later at sublethal rather than at lethal doses.

5. Increased metabolism did not coincide with weight loss either in time course or in dose dependence.

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17226. Differential Blood Counts on Rats During Shock Induced by Tourniquets.

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The hematological changes which occur during secondary shock have been reported by Cannon, Frazer, and Hooper,[1] and other workers as reviewed by Moon.[2] Among the changes reported are variations in the red and white cell counts, but the relative values of the different white cells in particular have been neglected. White and Dougherty[3] have shown that in instances of stimulus or stress there is a dissolution of lymphocytes. It is the purpose of this study to show the changes which occur in the percentages of lymphocytes and polymorphonuclear neutrophils during induced experimental shock. Through the course of the experiment, it was found that the changes in the percent of monocytes, basophils, and eosinophils were too small to be significant.

Materials and methods. Albino rats, Sherman strain, including both sexes, and ranging in weight from 98 to 292 g were used. Rubber bands, tightly wrapped, were placed at the highest possible point on both thighs. The rats were kept in a semi-conscious state with intraperitoneal injections of nembutal (Abbott). Blood was obtained by puncturing a tail vein and then subjected to the usual methods for determining gross and differential counts.

Preliminary experiments on 12 rats were

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[2] Moon, V. H., *Shock*, 1942, Lea and Febiger, Philadelphia, Pa.

[3] White, A., and Dougherty, J. F., *Ann. N. Y. Acad. Sci.*, 1946, **46**, 859.