showed percentage weight losses of 15.7, 34.4 and 23.6, respectively. Organ weights, except for the stomach, returned nearly to normal on the 4th and 5th day. The significantly greater weight loss in the small intestine was believed to reflect greater sensitivity of this part to ionizing radiation. It was pointed out that these weight changes were not due to dehydration or edema of the tissues or to the lowered food intake alone, but were in a large part due to specific effects of radiation. 2. The stomach showed the greatest variation in contents after irradiation, with a marked increase occurring during the first 72 hours which was believed to be associated with delayed emptying of the stomach. During this same period the small intestine and large intestine showed slightly reduced contents which became liquid and foul-smelling in character. From the 3rd to 4th days through the 7th day there was a marked increase in contents in all parts above the control range with a change to the normal semi-solid state. It was believed that this change was the reflection of compensatory increased food consumption associated with improved gastrointestinal function.

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Certain Nucleotide Relationships in Normal and in Tumor-Bearing Mice.* (20110)

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We have reported that male mice bearing implanted sarcoma 180 undergo a significant lowering in blood adenosine triphosphate during the interval of tumor growth(1). The first part of the present investigation was concerned with ascertaining whether similar alterations in blood nucleotide appear in female mice during the estrous period as compared with the diestrous, or during pregnancy when extensive mitotic activity occurs which may be similar to that of tumor growth. At the same time, analyses were performed on the blood of

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animals fed with thyroid or with thiouracil to determine whether the marked alterations in tissue metabolism induced by these agents affect the nucleotide pattern of the blood.

In addition, it appeared desirable to determine whether the lowered adenosine triphosphate level occurring in the blood during tumor growth is related to the nucleotide levels in the tumor tissue itself. Another point appeared to merit investigation: one of us(2) had reported that the injection of 5-adenylate into human subjects elevated the adenosine triphosphate of the blood. The question arose as to whether similar increases could be produced in mice, especially in the blood of tumor-bearing mice, as well as in the tumor tissue of such mice.

Materials and methods. White Swiss female mice of 20-22 g were used throughout, except for the experiments on pregnant mice where body weights were undetermined. The state of the estrous cycle was ascertained by the use of a standard vaginal smear method. For the metabolism experiments, mice were fed for periods of between 13 and 23 days on Rockland diet containing 2% desiccated thyroid or 0.5% thiouracil powder. In the case of the tumor experiments, mice were implanted bilaterally with sarcoma 180, using methods now standard for this procedure. The removal of blood, the preparation of filtrates, and the measurement of total purine and known nucleotides was carried out as before(1). For tumor removal, the animal was killed by cervical fracture; then as quickly as possible the tumor tissue was excised, the central necrotic core of each tumor mass removed and discarded, and the remaining viable tissue dropped into liquid nitrogen. Enough tumor tissue was taken, often from multiple donors of the same experimental class, to yield an aliquot of about 1 g of tissue. The frozen fragments were pulverized and extracted once with a 5 ml aliquot of cold 10% trichloracetic acid and twice with 2 ml aliquots of 5% trichloracetic acid. The extracts, following centrifugation, were combined, neutralized, made to a 10 ml volume, treated with 0.5 ml 25% barium acetate and 4 volumes of ethyl alcohol, and placed in the cold room overnight. The barium precipitates were collected by centrifugation, dissolved in dilute HCl, freed of barium with Na_2SO_4, neutralized, made to volume and assayed enzymatically for adenine due to adenylic acid (AA), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) in a fashion similar to that used for blood.

TABLE I. Known Blood Nucleotide (Combined AA, ADP, and ATP) and Total Blood Purine for Normal Male Mice, Female Mice in Estrus, Diestrus, Pregnancy, and after Feeding on Diet Containing Thyroid and Thiouracil. The values are means and their stand. errors, expressed as mg % adenine. Also shown are number of determinations (n) used in calculating the means and stand. errors.

Condition	n	Known nucleotide	Total purine
Males, normal	39	7.0 ± .20	13.3 ± .27
Females, estrus	21	6.8 ± .17	13.4 ± .28
" diestrus	24	7.0 ± .13	13.5 ± .28
" pregnant	27	5.8 ± .22	12.5 ± .27
Thyroid fed	13	7.2 ± .27	14.3 ± .32
Thiouracil fed	13	7.5 ± .20	14.1 ± .29

TABLE II. AA, ADP, and ATP, and Total Purine Content of Normal Blood and Blood from Tumor-Bearing Mice, Sacrificed at Various Intervals after Intravenous Injection of AA as the Sodium Salt. Shown in the last column is the change in proportion of known nucleotide as a function of the total purine. Values expressed as mg % adenine.

Condition, mg AA	hr	ATP	ADP	AA	Sum (AA, ADP, ATP)	Total purine	% known nucleotide of total purine
Normal (control)		6.8	.4	.0	7.2	13.2	54.6
4	½	10.0	.2	.5	10.7	15.1	70.8
4	1	8.5	.7	.5	9.7	14.0	69.2
4	3	6.8	.0	.0	6.8	12.4	53.2
4	4	7.1	.3	.1	7.5	13.9	53.9
8	½	12.5	.6	.3	13.4	19.2	69.7
7-day tumor		5.0	.3	.3	5.6	11.5	48.7
4	1	7.9	.5	.4	8.8	13.4	65.7
4	4	4.4	.0	.4	4.8	10.6	45.3

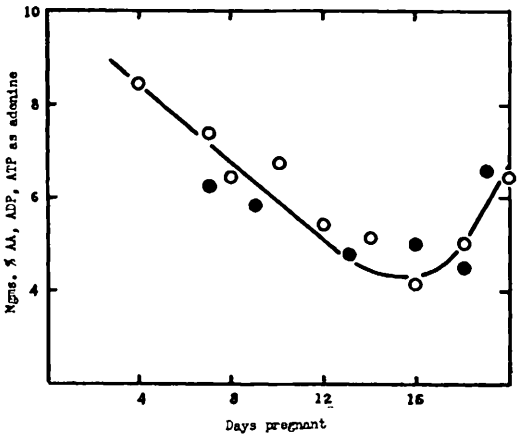

FIG. 1. Known nucleotide (AA, ADP, and ATP) in blood of mice at various stages of pregnancy. Two sets of assays indicated.

Results and discussion. In Table I are presented the mean values along with their standard errors of combined known blood nucleotides (AA, ADP, ATP) and the total purine for female mice during estrus, diestrus, pregnancy, and for animals fed on a diet containing thyroid or thiouracil. Also shown as the first entry in Table I are data taken from our earlier publication(1) on the blood of normal males. As we had found earlier, the major part of the adenine in the known nucleotide fraction is present as ATP (see control line in Table II). Statistical analysis reveals that the blood values shown in Table I do not (except for those of pregnant animals) differ significantly from the values obtained earlier for normal males. The values for pregnant mice compared with females in either estrus or diestrus show a significant reduction in known nucleotide (the difference between the means exceeds their standard errors by more than 4 times), as well as in total purine ($d/S.E._m$—2.6). The animals of the pregnancy group ranged from 4 to 19 days of term. Examination of the individual data shows that the greatest reduction in known blood nucleotide occurs at about the 16th day of pregnancy. The pertinent data are plotted in Fig. 1, representing 2 separate runs made several months apart. It is clear from these results that depression in blood nucleotide is not specific to the presence of sarcoma 180 in the animal. The presence of a growing fetus brings about a similar alteration, although not as marked a one. In the pregnant animal, the level begins to return to normal after about the 16th day (5 days before parturition), whereas in the tumor-bearing animal, the level does not begin to rise until the time when involution and resorption of the tumor often begin (about the 12th day after implantation).

In Fig. 2 are shown data on the known nucleotide content (AA, ADP, ATP) of tumor tissue expressed as mg of adenine/100 g of tissue. The figures alongside each point represent the number of separate analyses performed. Also plotted are the data from our earlier publication(1) on the blood level of these nucleotides in mice in which tumors are growing. It is clear that the decrease in blood nucleotide is related to the increase in concen-

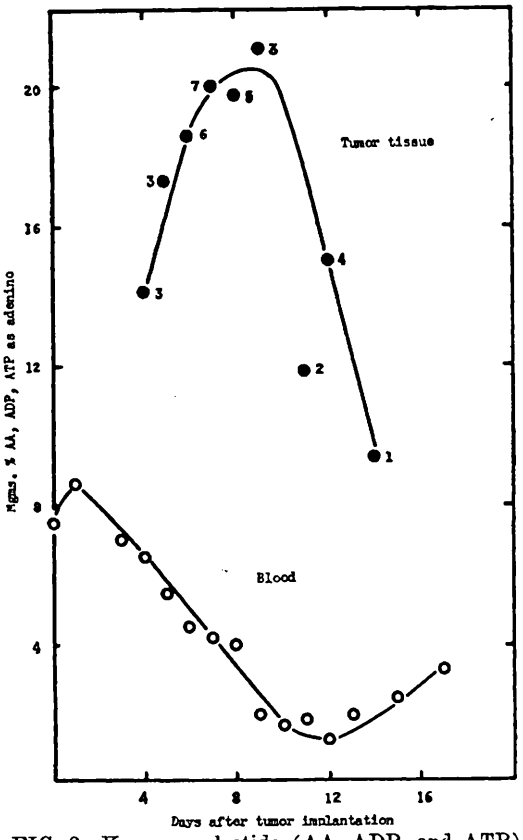

FIG. 2. Known nucleotide (AA, ADP, and ATP) in blood and in tumor tissue of mice at various stages following tumor implantation. Figures in upper curve represent No. of separate analyses performed.

TABLE III. AA, ADP, and ATP, and Total Purine in 7-8 Day Tumors, following Intravenous Injection of 4 mg of AA. Animals sacrificed 1 hr after inj. Values expressed as mg % adenine.

Animal No.	AA	ADP	ATP	Total purine
		Controls		
E62	3.7	.0	11.6	38.6
63	4.2	.9	7.4	32.0
67	3.0	4.8	7.9	33.7
80	3.3	.5	13.0	33.3
82	3.4	3.2	8.3	32.9
85	2.3	.0	8.5	25.8
86	2.2	2.3	7.8	27.2
Mean	3.1	1.7	9.2	31.9
		AA injected		
E64	2.5	3.5	6.9	27.3
65	-1.6	6.3	4.2	26.2
66	2.2	6.7	5.2	31.0
81	1.8	1.9	2.3	17.4
83	2.3	2.9	9.9	28.5
84	.9	1.2	3.3	15.0
Mean	1.9	3.8	5.3	24.2

tration of tumor nucleotide, and that the blood level does not begin to rise again until its concentration in the tumor has decreased appreciably. Tumor nucleotide reaches its highest concentration at about the 8th day after implantation. This corresponds to the period when the proportion of viable cells in the tumor is at a maximum(3).

The next part of the study was concerned with the effect of injected adenylic acid on the blood nucleotide level of normal and tumor-bearing mice. In normal humans, the injection of 20 mg of 5-adenylic acid intramuscularly produces a transient increase in the blood ATP level without any appreciable increase in the total purine(2). Intravenous injection of AA was not performed on human subjects. In the mouse experiments reported here, 4 (and in one case 8) mg doses of AA as the sodium salt in aqueous solution† were injected intravenously. Animals were sacrificed at the end of 30 minutes, one, 3, and 4 hours, and the blood assayed for AA, ADP, ATP, and total purine. The results are shown in Table II. It is clear that the injection of AA produces a marked elevation in the blood ATP of normal mice. This increase persists for about an hour and then the level returns to normal. The increase in human blood ATP after intramuscular injection of AA is not usually seen until one hour after injection. At the 4 mg dose, it is interesting to point out that for normal mice the increase in ATP is greater than that which occurs in the total purine, offering additional evidence that injected AA causes, at least in part, a shift in the purine reservoir in the direction of ATP. This is also shown by the figures in the 6th column of Table II, where the percent of known nucleotide, as a function of total purine, is shown to rise and then return to normal by the end of 3 hours. Similar results have been obtained for the blood of tumor-bearing animals. These data are shown in the lower section of Table II.

In a preliminary series of experiments on tumor-bearing animals from which blood had been taken for the analyses described above and which were injected intravenously with 4 mg of AA, low values for known nucleotides in the tumor tissue were observed. The greatest effect appeared to be on tumors which had been implanted for about 7-8 days. Tumor tissue analyses, accordingly, were carried out mainly on such animals, both with and without injected adenylic acid. The results, shown in Table III, indicate that whereas injected AA elevates the level of blood ATP, it lowers the ATP level in the tumor tissue. It appears also to lower the AA and the total purine level in the tumor, but to raise the ADP. The lowered AA and ATP and the elevated ADP levels seem to suggest an effect of the injected nucleotide on the myokinase system:

$$2\text{ADP} \underset{}{\overset{\text{myokinase}}{\rightleftharpoons}} \text{AA} + \text{ATP}$$

Summary. 1. Data are presented on the blood nucleotide level of mice in estrus, diestrus, in pregnancy, and after thyroid and thiouracil feeding. Of these conditions, only pregnancy produces a significant lowering of the nucleotide fraction containing AA, ADP, and ATP. This decrease is similar to that observed in mice bearing sarcoma 180. In pregnant mice the blood level begins to return to normal after the 16th day of pregnancy. 2. During tumor growth, as blood nucleotide

† The adenylic acid used was a gift from the Ernst Bischoff Co. It is marketed under the trade name of My-B-Den.

decreases, tumor nucleotide increases, reaching a maximum at about the 8th day after implantation, followed by a rapid fall. This fall appears to be correlated with the tendency (beginning about the 10th day of tumor growth) for the blood nucleotide level in tumor-bearing mice to return to normal. 3. The intravenous injection of adenylic acid into normal mice causes a transitory rise in blood ATP. With a dose of 4 mg, this increase is in excess of that in the total purine. A similar increase in blood ATP occurs after the injection of AA into the blood stream of tumor-bearing animals. The tumors of such injected animals show a lowered level of AA, ATP, and total purine, but an elevated level of ADP as compared with uninjected controls.

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Single Pyramidal Fiber Responses to Electrical Stimulation.* (20111)

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In an earlier study of the responses of spinal motor neurones following selective activation of pyramidal fibers, an explanation of the findings was based upon the assumption that the pyramidal fibers responded once to each electrical pulse and that there was numerical agreement between the number of pyramidal shocks and the number of impulses initiated in each of the responding fibers(1). Two deviations from the numerical equivalence were considered possible. Some pyramidal fibers may have behaved like certain fibers of peripheral sensory nerves and responded repetitively to single shocks of long duration(2,3). Other pyramidal fibers, due to their presumed longer refractory periods, may have been unable to follow the higher stimulation frequencies, with the resulting occurrence of a response deficit.

It was the purpose of this study to test this assumption by recording responses from single pyramidal fibers activated by pulses of the parameters used previously.

Methods. The experiments have been performed upon cats and macaques in surgical anesthesia as a result of the administration of diallylbarbituric acid in urethane solution. In some instances movement was prevented through the use of d-tubocurarine coupled with artificial ventilation at a level roughly equivalent to the level of ventilation established by the preparation before curarization. The dorsal surface of the medulla oblongata was exposed through an enlarged foramen magnum and the spinal cord was exposed by laminectomy at the desired level. *Stimulating pulses* were controllable in all dimensions and were triggered by the oscillograph sweep generator. The rectangular pulses were applied to the preparation through the mediation of a Grass Stimulus Isolation Unit which slowed the rise and decay times to a degree insignificant for the purposes of this study and which permitted the maintenance of a steady voltage at the stimulating electrodes throughout the duration of the pulse. The current through the stimulating electrodes was measured with the aid of a monitoring oscillograph. The stimulating pulses were applied to the preparation through two forms of stimulating electrodes. Orthodromically conducted volleys were initiated at the level of the pyramid through concentric bipolar electrodes oriented stereotactically. Antidromically conducted volleys were initiated at appropriate cord

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