Nucleotide Conversions in Various Tissues of Pregnant and Tumor-bearing Mice.* (20840)

HARRY G. ALBAUM AND PAUL A. ZAHL.
(With the technical assistance of A. N. Nowak, M. L. Elgart, and D. H. Suess.)
From the Biology Research Laboratory, Brooklyn College, N. Y., and the Haskins Laboratories, New York City.

Earlier(1,2) we reported that during the growth of implanted sarcoma 180 in mice there occurs simultaneously a decrease in blood adenosine triphosphate (ATP) and an increase in tumor tissue nucleotide. The only other of several experimental conditions tested, under which we observed a depression of blood nucleotide, was pregnancy. The present investigation is concerned, first, with the relationship between nucleotide level in the blood of pregnant mice at various gestational stages and that in embryo tissue; second, with the nucleotide levels in liver, spleen, and muscle of tumor-bearing animals, as well as in liver and spleen of pregnant mice. Since in our earlier study(2) we had found that the injection of 5-adenylic acid tended to correct the depressed ATP blood level in tumor-bearing mice, as well as the elevated nucleotide level within the tumor tissue, experiments were undertaken to ascertain the effect of injected 5-adenylic acid on the nucleotide level in spleen, liver, and tumor tissue of tumor-bearing mice, and in the spleen, liver, and embryo tissue of pregnant mice.

Materials and methods. White Swiss female mice of standard age were used throughout. In the case of the tumor experiments, the animals were implanted bilaterally with sarcoma 180, using methods now standard for this procedure. Tumors were removed 7 days after implantation. In the case of the pregnancy experiments, embryos—more correctly, fetuses—were removed at various stages of gestation; the tissue classified as "embryo" comprised the amnion, allantois, placenta, and fetus. The amniotic fluid was in each case discarded. The 16-day stage of pregnancy was arbitrarily selected for certain of the experiments on embryo tissue. The animals were killed by cervical fracture; then as quickly as possible, the tissues of interest were excised and dropped into liquid nitrogen. In the case of tumors, the central necrotic core of each growth was removed and discarded. Tissues were often taken from multiple donors of the same experimental class so as to yield a wet weight aliquot of about one g. For muscle assay, a rectangular section from the ventral abdominal musculature was used. The frozen tissues were pulverized and extracted once with a 5-ml aliquot of cold 10% trichloroacetic acid and twice with 2 ml aliquots of 5% trichloroacetic acid. Following centri-

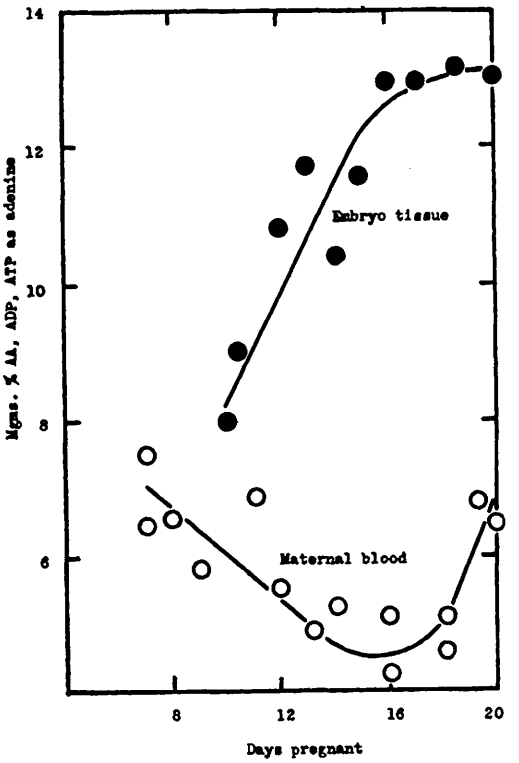

FIG. 1. Known nucleotide (AA, ADP, and ATP) in blood of mice at various stages of pregnancy, and in the embryo tissue.

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TABLE I. AA, ADP, ATP, and Total Purine Levels in Liver Tissue from Normal, Tumor-Bearing, and 16-Day Pregnant Mice, with and without Adenylic Acid Pre-Treatment. Mean values, expressed as mg % adenine, given along with their standard errors. I = animals inj. intrav., one hr before sacrifice, with 5 mg 5-adenylic acid in .25 ml water. U = No inj.

	Normal		Tumor-bearing		Pregnant	
	U	I	U	I	U	I
AA	12.6 ± 1.16	12.7 ± .89	12.2 ± .87	12.3 ± .80	11.2 ± 1.07	10.7 ± .49
ADP	5.6 ± 1.56	3.9 ± 1.03	4.0 ± .83	3.3 ± .80	2.5 ± 1.00	1.6 ± .69
ATP	14.1 ± 1.18	18.6 ± 1.19*	12.2 ± .83	14.7 ± .96*	7.7 ± .93†	10.3 ± .75*
Total purine	56.8 ± 1.21	61.9 ± 1.71*	54.5 ± .95	58.1 ±1.53*	48.0 ± 1.74†	54.3 ±1.75*

* Difference between means of uninj. (U) and adenylic acid inj. (I) significant (*i.e.*, diff. between means/S. $E._{diff}$ is 2 or more.

† Difference between means of experimental group and normal group significant.

$$S.E._{mean} = \frac{\sigma}{\sqrt{n}}; \quad S.E._{diff} = \sqrt{(S.E.)^2_{control} + (S.E.)^2_{experimental}}$$

fugation, the extracts were combined, neutralized, made to 10-ml volume, treated with .5 ml of 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), following methods described earlier (1). Total purine was determined spectrophotometrically from the 2600 Å reading.

Results. The combined adenine present as AA, ADP, and ATP in the mouse embryo is shown in Fig. 1, plotted as a function of pregnancy period. Also plotted are the data from our earlier publication(2) on the blood level of these nucleotides in pregnant mice. It is clear that decrease in blood nucleotide is related to an increase in embryo nucleotide. The nucleotide level in pregnancy blood begins a return to normal at about the 16th day of pregnancy, at which time the embryo level appears already to have reached a constant value. The rapid increase of embryo nucleotide up to about the 16th day of pregnancy may reflect the tissue differentiation pattern of fetal growth.

Table I shows the nucleotide levels in liver from normal, tumor-bearing, and pregnant mice. Although the ATP level in liver tissue from tumor-bearing animals tends to be lower than in normal animals, the difference between the means is not statistically significant, probably because of the relatively small number of determinations made and the extent of their variability. The ATP level in liver tissue from pregnant animals, on the other hand, undergoes an appreciable and very significant lowering. The total purine is depressed somewhat in the liver of tumor-bearing mice and, again, more markedly, in the liver of pregnant animals. In all cases, injection of adenylic acid raises both the ATP and the total purine levels in mice, regardless of experimental state, an effect recently demonstrated for rats(3).

Table II shows similar data for the spleen of normal, tumor-bearing, and pregnant mice.

TABLE II. AA, ADP, ATP, and Total Purine Levels in Spleen Tissue from Normal, Tumor-Bearing, and 16-Day Pregnant Mice, with and without Adenylic Acid Pre-Treatment. Mean values, expressed as mg % adenine, given along with their standard errors. Injection data identical to that of Table I.

	Normal		Tumor-bearing		Pregnant	
	U	I	U	I	U	I
AA	6.7 ± .93	5.5 ± .84	8.2 ± 1.04	6.7 ± .69	8.6 ± 1.09	5.2 ± 1.09
ADP	3.1 ± 1.24	3.4 ± 1.24	4.4 ± .93	4.5 ± 1.24	7.0 ± 2.96	2.9 ± 2.53
ATP	19.2 ± 1.21	22.8 ± .99*	14.2 ± 1.51†	17.4 ± 1.68	9.1 ± 2.38†	14.4 ± 1.98
Total purine	48.6 ± 1.20	52.0 ± 1.62	46.2 ± 1.3	48.0 ± 1.28	51.8 ± 1.55	52.0 ± 3.94

* and † See footnotes, Table I.

TABLE III. AA, ADP, ATP, and Total Purine Levels in Abdominal Muscle Tissue from Normal and Tumor-Bearing Mice, with and without Adenylic Acid Pre-Treatment. Mean values, expressed as mg % adenine, given along with their standard errors. Injection data identical to that of Table I.

	Normal		Tumor-bearing	
	U	I	U	I
AA	3.1 ± .25	3.3 ± .33	2.8 ± .30	2.9 ± .24
ADP	1.2 ± .26	.9 ± .53	1.8 ± .48	2.0 ± .64
ATP	54.7 ± 2.22	57.0 ± 2.09	49.5 ± 2.00	52.7 ± 1.93
Total purine	71.4 ± 1.19	72.0 ± 1.69	65.6 ± 1.77†	67.3 ± 1.31

† See footnote, Table I.

One observes a marked decrease in spleen ATP for both tumor-bearing and pregnant animals, but without an accompanying decrease in total purine, indicating shifts from the unidentifiable purine fraction. As in the case of liver, changes in the spleen of pregnant animals are more marked than those occurring in tumor-bearing animals. Injected adenylic acid tends to a limited extent to increase ATP and total purine in the spleen of mice from all the experimental groups.

Table III shows nucleotide levels in abdominal muscle from normal and tumor-bearing mice. Muscle tissue from pregnant mice was not studied in this connection. There is a slight decrease of total purine in muscle tissue from tumor-bearing animals, both with or without injected adenylic acid, but no significant changes are seen in the AA, ADP, or ATP levels in muscle from either normal or tumor-bearing mice. This is at variance with data reported in this connection for rats(3), where the injection of adenylic acid in large doses significantly elevated the ATP level in gastrocnemius muscle. This variance may be related to the adenylic acid dose used for rats having been 25 times greater than the dose we used for mice.

Table IV shows the effect of injected adenylic acid on the nucleotide levels in embryo tissue as well as in tumor tissue. It is clear that whereas injected adenylic acid significantly lowers the ATP level of tumor tissue, as previously reported(2), adenylic acid induces no significant change in the nucleotide level of embryos. This may mean that injected adenylic acid does not pass across the placental barrier.

Summary. In mice, a progressive lowering of blood nucleotide during pregnancy occurs simultaneously with an increase in nucleotide level for the embryos of such animals. Injection of adenylic acid into tumor-bearing mice produces a lowering of the nucleotide level in the tumor tissue, in contrast with no significant effect on the nucleotide level in embryo tissue. The liver and spleen undergo a lowering of tissue ATP as a function of pregnancy, and to a lesser extent as a function of tumor-growth. No significant alteration in muscle nucleotide is seen for mice bearing implanted tumors. The injection of adenylic acid elevates the ATP level in liver and spleen of normal, tumor-bearing, and pregnant animals, but, at least in the case of normal and tumor-bearing mice, has no effect on muscle nucleotide. Following adenylic acid injection, liver tissue from normal, tumor-bearing, and

TABLE IV. AA, ADP, ATP, and Total Purine Levels in Embryo Tissue from 16-Day Pregnant Mice, and in 7-Day Tumors (Mouse Sarcoma 180). Mean values, expressed as mg % adenine, given along with their standard errors. Injection data identical to that of Table I.

	Embryo		Tumor	
	U	I	U	I
AA	2.0 ± .18	1.8 ± .24	3.3 ± .52	3.2 ± .45
ADP	1.3 ± .30	1.0 ± .21	.8 ± .34	3.2 ± 1.04
ATP	9.0 ± .56	9.0 ± .56	11.2 ± .73	8.4 ± .88*
Total purine	28.5 ± .51	25.8 ± .77	35.4 ± 1.11	32.6 ± 1.42

* See footnote, Table I.

pregnant mice shows an increase in total purine, whereas the spleen does not.

1. Zahl, Paul A., and Albaum, Harry G., PROC. SOC. EXP. BIOL. AND MED., 1952, v77, 388.

2. Albaum, Harry G., and Zahl, Paul A., *ibid.*, 1953, v82, 337.

3. Albaum, Harry G., Pankin, David, and Harvill, Eduard, *ibid.*, 1953, v84, 529.

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Pharmacology of Smooth Muscle Valve in Renal Portal Circulation of Birds.* (20841)

BARBARA R. RENNICK AND HECTOR GANDIA. (Introduced by Gordon K. Moe.)

From the Department of Physiology, State University of New York, Upstate Medical Center, Syracuse, N. Y.

The avian renal portal system, first described by Jacobson in 1822(1) was not proved functional until 1948 by Sperber(2). In the bird the external iliac vein (EIV) communicates with the internal iliac vein which drains into the renal parenchyma. The EIV also connects directly to the renal vein (RV) by a large venous shunt. Interposed at the point of communication from EIV to RV is a valve-like structure described by Spanner (3) which is of variable shape in various species of birds but which is composed of smooth muscle, and usually has a conical shape with single or multiple apical openings. When contracted, the valve would tend to divert iliac vein blood through the kidney and when relaxed, blood would pass directly into the renal vein. Sperber described the position and appearance of the valve (Fig. 1) and presumed that it regulated blood flow to the renal tubules. Sperber clearly illustrated the functional significance of the renal portal system in the chicken by demonstrating that phenol red injected into one leg appeared in excess in the urine of the ipsilateral kidney.

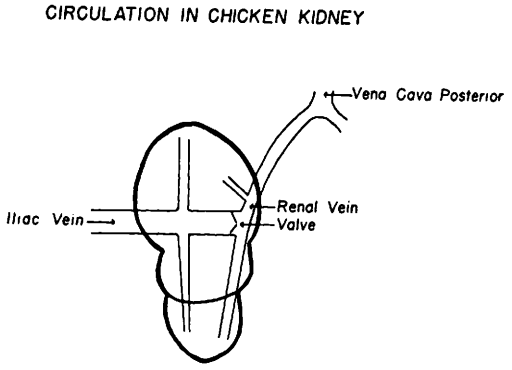

FIG. 1. Renal-portal circulation in chicken kidney.

The present report deals with the response of this valve to autonomic drugs as determined by direct recording of the response of the isolated structure suspended in a smooth muscle bath, and as estimated by the excess excretion of para-aminohippurate by one kidney when this substance was injected into the ipsilateral leg or iliac vein.

Method. In vitro. Valves were dissected from veins of freshly killed turkeys† weighing about 25 lb. The valve was suspended in oxygenated Tyrode's solution at 37.5°C in an Anderson muscle bath and attached to a light weight lever recording on a smoked drum. The bath volume was 30 ml. *PAH uptake* by chicken kidney slices was determined by the method of Cross and Taggart(4) with one modification. The final PAH concentration was 0.0013 M. Slice to medium ratios were calculated on a wet weight basis. *In vivo.* Hens were anesthetized with sodium barbital, 200 mg/kg intravenously and infused with 1 ml per minute of glucose-saline solution. Urine was collected separately thru plastic cups‡ sewed over the cloacal orifice of each ureter and the recovery of PAH in the urine

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