turing the fragile opthalmic venous plexus with tip of blood collection tube. The tube may be sealed and centrifuged for hematocrit determination and plasma separation for enzyme or other biochemical studies. Bleeding procedure for a mouse requires less than 30 seconds, and 100 mice can be bled and processed by a technician in about 1½ hours. Appropriate illustrations demonstrate technical details of procedure.

1. Pettit, Auguste. *Compt. Rend. de la Soc. de Biol.*, 1913, v74, 11.

2. Halpern, B. N., Pacaud, A., *Compt. Rend. des Seances de la Soc. de Biol.* 1951, v145², 1456.

3. Stone, H., *Science,* 1954, v119, 100.

4. Fuson, R. B., Rambo, O. N., *Transplantation Bull.*, 1957, v4, 147.

5. Old, L. J., Personal Communication and Demonstration.

6. Riley, V., Valles, W. C., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 341.

7. Riley, V., Wroblewski, F., *Science,* 1960, v132, 151.

8. Riley, V., *et. al., Science,* 1960, v132, 544.

Received March 7, 1960. P.S.E.B.M., 1960, v104.

Rapidity of Breakdown of Nucleotides in Different Tissues.* (25976)

CHARLES BISHOP, DAVID M. RANKINE, MORTON B. KLEIN AND JOHN H. TALBOTT†
(Introduced by Fred R. Griffith, Jr.)
Dept. of Medicine, University of Buffalo School of Medicine, and Buffalo General Hospital, N. Y.

Mammalian blood can be incubated *in vitro* for several hours without affecting distribution and amounts of nucleotides in the formed elements of the blood. This has allowed us to perform incorporation studies under essentially *in vivo* conditions. In attempting to perform similar incorporation studies on other mammalian tissues we discovered that after incubation, the major nucleotide, ATP, had largely disappeared and that AMP was now present in considerable quantities. The present paper describes our attempt to determine *in vivo* distribution of nucleotides in certain tissues and the rapidity of change in this distribution after removal of the tissue from the animal.

Materials and methods. Dog muscle and dog and rabbit intestinal mucosa were originally selected because they might be expected to contain large amounts of nucleotides that would be turning over fairly rapidly. The muscle was minced with scissors while the mucosa was merely scraped off the intestine with a glass microscope slide. The nucleotides were extracted from either preparation with cold trichloroacetic acid (TCA) and fractionated on Dowex-1 formate columns according to our previous procedure for blood (1). In the first experiments a dog was given a lethal dose of Nembutal intravenously and the hind legs rapidly skinned. One portion of leg muscle was dropped into ice cold 10% TCA and minced with scissors. A second portion was excised and allowed to stand at room temperature for 20 minutes. It was then treated like the first. A third portion was minced with scissors in calcium-free Krebs-Ringer-Bicarbonate medium(2) containing 200 mg % glucose. Ice cold TCA was then added to the mince. A fourth portion was prepared like the third except that it was incubated 2 hours at 37° under 95%O_2–5%CO_2 before TCA was added. No attempt was made to weigh portions of muscle accurately, because this would have slowed down handling of the tissue and because only distribution of nucleotides was of interest at this point. A second type of experiment was designed to ascertain if nucleotides in intestinal mucosa were as labile upon incubation as nucleotides in dog muscle. For this purpose a dog was injected intravenously with lethal dose of Nembutal and a length of small intestine removed as quickly as possible. This was slit open, rinsed briefly with cold saline, and

* This work supported by grant from Nat. Inst. of Arthritis and Metab. Dis., N.I.H., Bethesda, Md.

† Present address: Am. Med. Assn., Chicago, Ill.

TABLE I. Adenine Nucleotide Distribution in Tissues.

	μmoles/tissue sample		
	AMP	ADP	ATP
A. Dog Muscle			
Minced immediately in cold TCA	.36	4.98	41.8
Stood 20 min., then minced in cold TCA	1.16	11.72	44.6
Minced in incubation medium, then TCA added	2.59	3.59	1.59
Minced in incubation medium and incubated 2 hr before TCA added	.31	3.52	.26
B. Dog Intestinal Mucosa			
Intestine slit and dropped into cold TCA	1.20	.34	.28
C. Rabbit Intestinal Mucosa			
Intestine slit and dropped into cold TCA	.87	.34	.51
Mucosa scraped off and dropped into cold TCA	1.00	.28	.32
Mucosa scraped into 10 ml of human plasma and incubated 2 hr before TCA added	.13	.08	.08
Idem, except 50 ml of plasma used	.07	.33	.32
TCA inj. into intestine *in vivo*	.39	.68	2.20

dropped into ice cold TCA. A third series was designed to verify lability of nucleotides in intestinal mucosa from another species. In the first experiment a rabbit was shot in the head and a piece of small intestine removed immediately. This was slit and dropped into cold TCA. At the same time, a second piece of intestine was removed and the mucosa scraped off and plunged into cold TCA. A third piece of intestine was scraped and the mucosa suspended in 10 ml of human plasma. A fourth piece was treated like the third except 50 ml of plasma were used. The third and fourth samples were incubated at 37° under 95%O_2–5%CO_2 for 2 hours and then added to cold TCA. Experiments with intestinal mucosa indicated that nucleotides from this tissue were extremely labile, and raised the question as to how one could extract these compounds to avoid deterioration. A possible solution was to inject TCA into living tissue. Accordingly a rabbit was anesthetized with Nembutal and the small intestine exteriorized through slit in the abdomen. A section of intestine was clamped at either end, care being exercised not to disturb blood circulation to this section. Into the lumen of this closed segment of intestine ice cold 10% TCA was rapidly injected. The instantaneous blanching of intestinal wall indicated that TCA had penetrated the mucosal layer.

Results are presented in the Table. From the first series it is apparent that in freshly excised dog muscle there was a relatively large quantity of ATP, some ADP, and very small amounts of AMP. This distribution is not unlike that found in freshly-drawn mammalian blood(1). The second experiment (A.2) suggested that, on standing, some of muscle ATP broke down to ADP and AMP. As is shown in A.3 mincing of dog muscle in incubation medium caused nearly complete loss of ATP. Exp. A.4 indicated that incubation did not lead to restoration of original nucleotide pattern. Dog muscle, like blood, contains other nucleotides than those recorded here, but these are ignored to simplify conclusions of these studies.

The experiment with dog intestinal mucosa (B.1) revealed that ATP was largely decomposed, giving rise to ADP and AMP.

Experiments with rabbit intestinal mucosa indicated the same sort of deterioration of the nucleotide pattern as seen in dog intestinal mucosa. In Exps. C.1 and C.2 tissue nucleotide patterns indicated a preponderance of AMP over ATP. Our experience with other tissue indicated this to be a grossly abnormal finding but at first we could not say with certainty that intestinal mucosa might not have an unusual nucleotide pattern. Our final results in C.5 reinforced our original assumption, *i.e.*, that ATP far exceeds ADP or AMP in *in vivo* conditions. Exps. C.3 and C.4 demonstrated that nucleotide pattern in intes-

tinal mucosa would not revert to normal under the incubation procedures used here.

In Exp. C.4 with 50 ml of plasma the ATP level was preserved at a slightly higher level than in Exp. C.3 in which only 10 ml of plasma were used. This suggests that although these incubation conditions were far from ideal, the extra plasma did have some beneficial effect, perhaps by supplying a nutrient or neutralizing some toxic substance.

Discussion. Our earlier studies showed that whole blood could be incubated for several hours with no loss of ATP. Extension of these studies to other tissues was uniformly unsuccessful. In dog muscle, mincing in incubation medium caused nucleotide breakdown. In dog or rabbit intestinal mucosa, the cells could not even be scraped off into any kind of medium without deterioration of the nucleotide pattern. Injecting trichloroacetic acid into the living tissue is similar to the study of Dietrich and Friedland(3) which reported *in vivo* injection of perchloric acid into rabbit intestine. Their AMP:ADP:ATP ratios of 0.10:0.31:1.28 were not greatly different from ours, but perhaps both results are only approximations to the true *in vivo* distribution.

Summary. Our experiments indicate that the nucleotides of different tissues vary greatly in lability. When dog muscle was removed rapidly and minced in ice cold trichloroacetic acid, its nucleotide pattern resembled that seen in fresh blood, *i.e.*, ATP greatly exceeded ADP or AMP. Delay in mincing the tissue caused some decrease in ATP with increases in ADP and AMP. On the other hand, dog or rabbit intestinal mucosa was so labile that usual procedure of removing tissue and transferring it immediately to cold trichloroacetic acid was much too slow. Injecting cold trichloroacetic acid into the live tissue was necessary to give a nucleotide pattern which resembled patterns seen in other tissues. Incubation under circumstances tried, were unsuccessful in restoring tissue nucleotide patterns. Extrapolation of foregoing results suggests that studies involving either distribution of nucleotides in tissues or determination of tissue nucleotides after *in vitro* incubation must indicate degree of lability of these tissue nucleotides.

1. Bishop, C., Rankine, D. M., Talbott, J. H., *J. Biol. Chem.*, 1959, v234, 1233.

2. Umbreit, N. W., Burris, R. H., Stauffer, J. F., (eds.), *Manometric Techniques*, 3rd Edit., Minneapolis, Burgess, 1957, p149.

3. Dietrich, L. S., Friedland, I. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 470.

Received May 13, 1960. P.S.E.B.M., 1960, v104.

Effect of Thyroidectomy on Development of Polyploid Nuclei in Rat Liver.*† (25977)

FRANK J. SWARTZ AND JAMES D. FORD, JR.‡ (Introduced by William B. Atkinson)
Dept. of Anatomy, University of Louisville, School of Medicine, Louisville, Ky.

The role of growth hormone in the appearance and maintenance of polyploid cells in normal liver was originally described by Helweg-Larsen(1) and Leuchtenberger *et al.*(2) and confirmed and extended by DiStefano and Diermeier(3) and Geschwind *et al.*(4). Recently, involvement of 2 other hormonal systems has become apparent. The gonadal hormones have been implicated as a result of a study of the effect of castration on polyploidization of rat liver(5) and, as indicated by Carriere in preliminary reports(6,7), thyroid hormone is also involved. The role of the latter has been further elaborated upon in recent experiments(8) on effects of thyroxin

* This work supported by U.S.P.H.S. grant.

† The authors are grateful to Dr. L. Lapham, Western Reserve Univ. for making available the photometer, and to E. Nagy and M. Henry for valuable technical assistance.

‡ Student Scholar of U.S.P.H.S. and Kentucky Heart Assn.