extracellular titers. Intracellular infectivity reached a peak titer of 6.8 $\log_{10}$/0.1 ml at 48 hours after infection, after which it fell slightly and leveled off. Extracellular virus infectivity did not reach its peak titer of 5.8 $\log_{10}$/0.1 ml until 72 hours after infection, and remained stable until termination of the experiment. Rubella CF antigen was first detected 48 hours after infection and reached a maximum titer at 72 hours, followed by a sharp decrease. This decrease in titer was attributed to a probable stop in synthesis of the antigen by the infected cells, and thermal inactivation. Thermal and UV inactivation of virus infectivity followed the expression of a first order reaction. The CF antigen was thermo-labile at 34°C, and its inactivation was also exponential; the antigen was, however, stable to ultraviolet irradiation up to 30 minutes exposure.

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ATP Concentration and Localization of Sites of Epinephrine Induced Renal Artery Constriction.* (31144)

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Segmental constriction of the dog's renal artery in response to pharmacological doses of epinephrine has been described(1). This constriction in the main renal artery appeared predominantly in the zone of the first major bifurcation, occasionally in the mid portion, and less frequently in the proximal segment. Repeat studies in the same animal had demonstrated the reproducibility of this epinephrine induced constriction. Similarly, localized segmental constriction in response to pharmacological doses of epinephrine has been observed in the common, internal carotid and vertebral arteries. A narrowing of the post renal aortic segment has also been observed.

Histological studies of serial sections along the renal artery have revealed no morphological difference which could account for the observed zonal constriction. Consequently, studies of biochemical properties and functions of zonal segments of the renal artery have been initiated in an effort to see if any variation in these properties along the renal artery could be correlated with segmental renal artery constriction.

The intensity and prolonged time of the localized segmental constriction of the renal artery in response to epinephrine led us to hypothesize that such segments would require higher energy stores (adenosine triphosphate concentration—ATP) and/or higher ATP

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production capacity in order to sustain the more intense and prolonged muscular constriction than the adjacent segments of the artery. To test this hypothesis, ATP concentration determinations were made on different segments of the dog's renal artery. These results were then compared with the previously determined sites of localized constriction of the artery in response to pharmacological doses of epinephrine.

Methods. Control and post epinephrine renal angiographic studies, as previously described(1), were made of 14 normal mongrel dogs in order to determine the site of renal artery constriction in response to epinephrine. Several days later the animals were explored under pentobarbital anesthesia and one renal artery was carefully exposed.

Special precautions were used in preparation of the renal artery for ATP analyses. Every effort was made to assure maintenance of blood flow up to the very second of removal. The renal artery was exposed in such a manner that 2 pairs of scissors could be placed in position for rapid cutting of the artery—one adjacent to the aorta and one adjacent to the pelvis of the kidney where both branches of the artery would be cut with one closure of the blades. A mosquito clamp was attached to the peripheral connective tissue of the renal artery midway between the scissors. After preparation of the renal artery for cutting a 5-minute time period elapsed, prior to cutting, to permit the artery time to recover from the trauma due to manipulation. Both ends of the artery were cut simultaneously—with the remaining artery being clamped immediately after the cut—and the artery segment was rapidly transferred into a container of liquid propane (−190°C). The elapsed time from the cutting of the artery to immersion in liquid propane was usually less than 2 seconds.

The frozen renal artery was broken into 3 segments: Section 1—proximal segment, Section 2—includes the bifurcation, and Section 3—the 2 distal segments. The frozen artery sections were individually pulverized on a metal block (immersed in dry ice and absolute alcohol). The pulverized frozen tissue was placed in a tared, ice homogenizer, weighed and homogenized in ice cold 10% TCA (9 ml of 10% TCA per gram of tissue). The homogenate was diluted to a volume of 15 ml, neutralized with IN. KOH to pH 6.5-7.0 and diluted to a final volume of 20 ml. The ATP concentration of the preparation was measured by the firefly luminescence method(2), using firefly extract (Sigma FLE-50) and a Beckman DB spectrophotometer recorder as the light detection and recording equipment.

TABLE I. ATP Concentration in Renal Artery Segments.

Segment location	μg ATP / g tissue	(N)
Site of constriction when epinephrine was administered	115 ± 53*	(12)
Other 2 non-constriction segments when epinephrine was administered	53 ± 43	(22) P<<.001†

* Mean ± standard deviation.
† P is probability that the non-constrictive segments are the same as the constrictive segments.

Results. Of the 14 arteries studies which had both the epinephrine-angio studies and the segmental ATP concentration analyses, 10 had localized constriction at the bifurcation, 2 had constriction in the proximal segment and 2 showed rather uniform constriction along the entire artery.

A comparison of ATP concentration found in the constriction segment *vs* the non-constrictive segments is shown in Table I.

Table II shows relative ATP levels in the vessels manifesting different patterns of constriction. (Maximum value = 100%).

Even though the number of observations where constriction is not at the bifurcation are inadequate for statistical analysis, it is interesting to note that the maximum concentration of ATP is localized at the previously observed epinephrine induced constriction site.

The top photograph in Fig. 1 demonstrates well the homogeneous filling of the extra- and intra-arterial renal branches in the control period. The lower photograph is of the same animal 2 to 3 seconds following intravenous epinephrine and shows severe constriction at

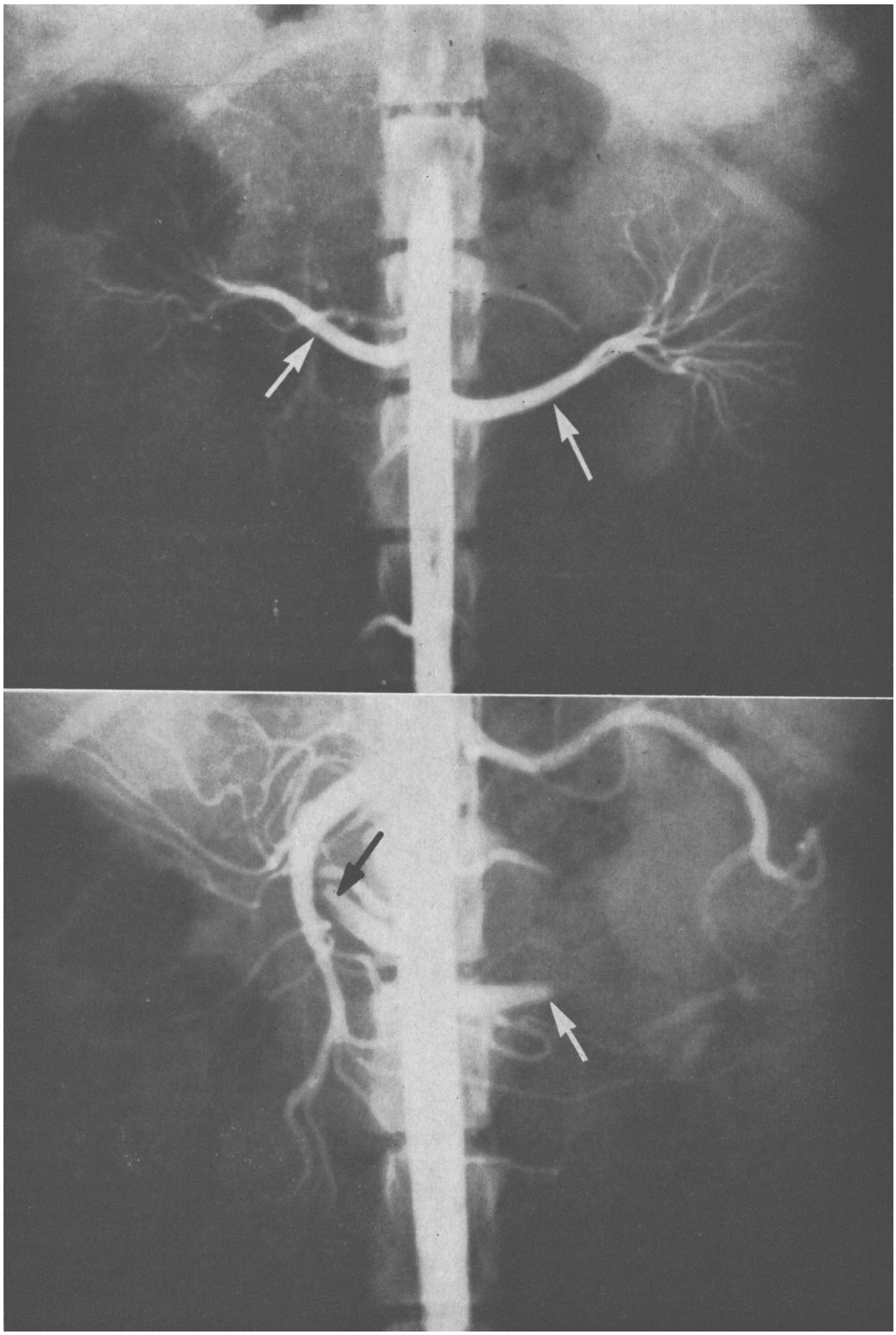

FIG. 1. Dog Exp. # 517. Upper picture is control angiogram and lower picture is angiogram post-epinephrine. Arrows identify the renal arteries.

TABLE II. Variation in ATP Concentration in Renal Artery Segments. (Average value of group where data from one animal is expressed as percent of the maximum ATP concentration found in the 3 segments in any one artery.)

	%	(N)	
1. Bifurcation constriction			
Maximum at bifurcation	100 ±49	(10)	
Other non-constriction segments	40.6±30	(18)	P<<.001*
2. Proximal constriction			
Maximum Sect. 1	100	(2)	
Other non-constrictive segments			
Sect. 2	73	(2)	
Sect. 3	18	(2)	
3. Generalized artery constriction			
Sect. 1	100	(2)	
Sect. 2	81	(2)	
Sect. 3	85.5	(2)	

* P is the probability that the non-constrictive segments in Group 1 are the same as the constrictive segments in Group 1.

the points of bifurcation of the right and left renal arteries as indicated by the arrows. The ATP concentration was maximal in this section and was 1.8 to 5 times greater, respectively, than the two adjacent sections.

Discussion. Neural, neurohumoral and pharmacologic stimuli of differing intensity or amounts may cause varying degrees of vascular constriction. Underlying mechanisms which may account for a vessel's specific response are yet to be fully defined.

Our initial study measured the ATP concentration in the walls of the renal arteries from their aortic origin through their first major bifurcation. The arbitrary division of the artery into 3 sections was partly for convenience, but also because these 3 areas appeared in initial experiments to be roughly the segments where constrictions did occur in response to epinephrine. Thus, in each experiment it was necessary to define the zonal response by angiography prior to analyzing the artery for ATP concentration.

The post epinephrine angiographic studies revealed 10 of the dogs primary renal artery response to be at the first major bifurcation zone. Two experiments demonstrated the response to be almost uniform along the entire artery and 2 in the proximal segment of the renal artery. These results correlate closely with our initial report of 13 experiments which noted 10 at the bifurcation, 2 medial and one proximal.

The results of our ATP measurements reveal that the region of the renal artery which shows the localized constriction when epinephrine is injected, normally has a higher level of energy stores (ATP) than does adjacent tissue. These results are the first evidence of biochemical differences along the renal artery which may be correlated with functional differences observed in the renal artery in response to epinephrine, even though no variation in morphology could be demonstrated. In a survey of literature we have found no information on ATP values for renal arteries (or smooth muscle) with which to make a comparison. It seems highly probable that these localized regions of constriction will, on further study, show other biochemical differences when compared with adjacent arterial segments.

Summary. 1. ATP concentration analyses were determined along the renal artery in a series of mongrel dogs that had previous post-epinephrine renal angiographic studies to identify the site of renal artery constriction. 2. Those segments of renal artery which exhibited post-epinephrine constriction were found to have higher ATP concentration than non-constrictive segments.

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