and the respiratory quotient remains close to 0.7. The results obtained on the ligated depancreatized animals are presented in Table I. It will be noted that the basal R.Q.'s on the tissues of dogs 3

TABLE I.

Dog	Survival, days	Blood* glucose, mg %	No addition		Glucose		Lactic Acid	
			$-QO_2$†	R.Q.	$-QO_2$	R.Q.	$-QO_2$	R.Q.
					18.1	.74		
1	14	+385	17.0	.70	18.3	.72	19.3	.79
2	10	314	17.5	.74	19.0	.79	23.2	.85
					19.1	.85		
3	25	68	17.6	.82	19.6	.81	21.3	.86
					15.6	.88		
4	25	296	14.3	.85	15.5	.87	18.5	.93

*Blood glucose at time of sacrifice.
†—QO_2 = mm³ oxygen consumed per mg dog weight of tissue per hour.

and 4 were definitely above 0.7. In all the experiments, glucose produces a questionable rise in oxygen consumption and R.Q., while lactate causes a significant increase in both oxygen uptake and respiratory quotient. The level of the blood sugar at the time the kidney was excised did not influence the character of the result. Previous work on hypophysectomized depancreatized animals failed to reveal an elevated basal R.Q.[3] and the addition of glucose failed to stimulate metabolism but lactic acid was oxidized. Further experiments are now in progress to determine whether a larger series of kidneys excised from hypophysectomized depancreatized animals would yield results similar to those obtained from the depancreatized preparation with lumbo-adrenal veins ligated.

9911

Adrenin Content of Adrenals of Cats Subjected to Anoxia.

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Effects of anoxemia upon the rate of secretion of epinephrine from the adrenals are not entirely clear, possibly because of the different experimental methods utilized by various authors. Cannon[1]

[3] Fazekas, J., Campbell, E. H., Jr., and Himwich, H. E., *Am. J. Physiol.*, 1937, **118**, 297.

[1] Cannon, W. B., *Endocrinology and Metabolism*, New York, 1924, **2**, 174; Cannon, W. B., and Hoskins, R. G., *Am. J. Physiol.*, 1911-12, **29**, 274.

has reviewed the positive evidence for increased adrenin secretion during anoxia, while Stewart[2] has presented certain conflicting results. Most work in the literature is concerned with anoxia of brief duration. Grollman[3] states that the type of anoxemia is important, as that of carbon monoxide stimulates epinephrine secretion while that of anoxia may not. The common occurrence of sympathetic effects during anoxia suggests that there is a definite effect on adrenal activity. Hartman[4] has shown that anoxic mydriasis after excision of the superior cervical ganglion is prevented by adrenalectomy.

The present paper is concerned only with the amount of adrenin present in the adrenals of cats after prolonged treatment at low tensions of oxygen. This approach admittedly offers no direct evidence of altered rate of secretion of epinephrine during the anoxia, since the results may be alternatively explained as effects on the rate of formation of epinephrine. It does, however, offer data which may be of aid in interpreting results of secretion studies. Previous studies[5] of the effects of anesthesia on the adrenin content of the adrenals also suggested the importance of determining the effects of simple anoxia.

Forty cats were used, of which half furnished control observations. In all except one case (see table), the cats were paired to be of similar weight, and both animals of each pair were treated at the same time, one as a control and the other as an anoxic animal. All were kept in the laboratory under identical conditions for at least 3 days before use, and other precautions recommended by Elliott[6] were rigidly followed. The anoxia-treated cats were placed in a chamber, previously described,[7] where they remained for 3 hours at an oxygen tension of 53 mm Hg, corresponding to an altitude of approximately 28,000 ft. Excitement and mydriasis were noted chiefly at the beginning of the anoxia, and most cats were quiet or depressed within a few minutes at this oxygen tension.

Following anoxic treatment, the cats were transected just below the diaphragm by a single blow of a heavy meat cleaver. Although this technic may not entirely prevent postmortem liberation of epi-

2 Stewart, G. N., *Endocrinology and Metabolism*, New York, 1924, **2**, 127; Pearlman, I., and Vincent, S., *Endocrinol.*, 1919, **3**, 121.

3 Grollman, A., *The Adrenals*, Baltimore, 1936.

4 Hartman, F. A., *Endocrinology and Metabolism*, New York, 1924, **2**, 101.

5 Emerson, G. A., *Anesthesia and Analgesia*, 1938, **17**, 109.

6 Elliott, T. R., *J. Physiol.*, 1912, **44**, 374.

7 Van Liere, E. J., *Am. J. Physiol.*, 1927, **82**, 727.

nephrine from the adrenals, the circulation is interrupted so quickly that little epinephrine can be lost from the adrenals and surrounding tissue. Adrenin content of the adrenals was then determined by a method previously used.[5] This adaptation of the Moodey colorimetric method has been found to yield results closely paralleling those obtained by bioassay methods.[8] Control animals were treated similarly in all respects except that they were kept under normal conditions in the laboratory.

TABLE I.
Adrenin Content of Both Adrenals of Anoxemic Cats.

Controls			Anoxemic		
Body wt, kg	Total Adrenin, mg	Total Adrenin, mg/kg	Body wt, kg	Total Adrenin, mg	Total Adrenin, mg/kg
2.055	.500	.243	1.745	.120	.069
1.080	.325	.301	0.950	.308	.324
1.625	.300	.185	1.580	.160	.101
1.105	.295	.267	0.930	.275	.296
2.325	.600	.258	2.655	.290	.109
0.775	.250	.323	1.780	.280	.157
1.750	.373	.213	2.255	.106	.047
2.340	.336	.144	2.390	.158	.066
1.555	.184	.119	1.510	.162	.107
1.780	.485	.272	1.470	.375	.255
1.180	.420	.356	0.960	.115	.120
3.160	.650	.206	2.360	.340	.144
1.700	.400	.235	1.410	.155	.110
2.450	.500	.204	2.350	.480	.104
1.920	.535	.279	1.510	.323	.214
2.875	.618	.215	3.030	.451	.149
0.690	.465	.674	0.880	.225	.256
2.010	.510	.254	2.250	.445	.198
3.410	.730	.214	2.450	.548	.224
0.455*	.145	.319	1.955*	.250	.128
		Averages.			
1.812	.431	.264	1.821	.278	.159

*These 2 cats were used on different days; all others were paired and used as pairs on the same days.

Total Adrenin, mg/kg: *Standard error* for controls, ± 0.025; for anoxemic cats, ± 0.018. Difference between means, 0.105 ± 0.031. *Standard deviation of the series* for controls, ± 0.110; for anoxemic cats, ± 0.078.

Results are collected in Table I. The control and experimental groups were of almost the same average weight; this point is of some importance since the adrenin content is known to vary widely with the weight of the animals, particularly when it is expressed as a function of the weight. A significant difference between the means of mg per kg of total adrenin, in both adrenals, was found.

8 Moodey, C. R., *M.S. Thesis in Pharmacology, Univ. of Calif. Library,* 1936; Abreu, B. E., and Emerson, G. A., *Proc. W. Va. Acad. Sci.,* in press.

The results indicate, therefore, that an appreciable decrease in the adrenin content of the adrenals occurs in cats treated for a sufficiently long time at a very low oxygen tension. An additional cat subjected to an hour exposure at the same oxygen tension showed a value of 0.27 mg of adrenin per kg of body weight, which suggests that shorter exposure may not be effective; but since this value is within twice the standard deviation of the 3-hour series, the result cannot be considered significant. The possible effects from lighter degrees of anoxemia, for shorter periods, such as occur clinically during nitrous oxide anesthesia, need further investigation. Similarly, determinations of threshold degrees of anoxia and of time of exposure are beyond the scope of the present study.

Summary. Cats treated for 3 hours at an oxygen tension of 53 mm Hg show a 40% decrease in the adrenin content of their adrenals, as determined by an adaptation of the Moodey colorimetric technic.

9912 P

A Method for the Isolation of Pregnandiol from the Urine of Pregnant Mares.

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The isolation of pregnandiol from the urine of pregnant mares has been reported by Marker, *et al.*[1] A method for the isolation of pregnandiol from the urine of pregnant mares based on a method for the quantitative determination of pregnandiol in human pregnancy urine[2] is herewith described because of its relative simplicity and possible application in the study of the function of the corpus luteum of other animals.

The fresh urine* without the addition of preservative is incubated at 37°C for 96 hours. It is filtered with suction and the precipitate is collected and heated with 100 cc of 95% ethyl alcohol. The alcoholic mixture is filtered with suction. This filtrate is evaporated to

1 Marker, R. E., Kamm, O., Crooks, H. M., Jr., Oakwood, T. S., Lawson, E. J., and Wittle, E. L., *J. Am. Chem. Soc.*, 1937, **59**, 2297.

2 Weil, P. G., to be published.

* In this determination a liter of urine of the sixth month of pregnancy was used.