

OXYGEN CONSUMPTION OF ADRENALECTOMIZED AND RENAL DAMAGED FROGS^{1,2}

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The frog, both as a representative of poikilotherms and as experimental material for adrenocortical investigations, possesses certain advantages over homoiotherms for studies involving penetration rates of known molecular-volume vital dyes and of radioactive physiological ions through its integument (Angerer, 1942), of water into and from the whole organism (Angerer and Angerer, 1949; Angerer, 1950) and into and from skeletal muscles (Angerer and Angerer, 1950), and for studies treating of skin bio-electric potentials (Williams and Angerer, 1950). Recently success has been attained with injections of adrenocortical extract (ACE) into otherwise normal frogs with regard to metabolism (Calhoun and Angerer, 1951) and skin bio-electric potentials (Williams and Angerer, 1952). In the light of these studies and of the paucity of data treating of the metabolism of normal frogs, it was considered desirable (a) to determine for the first time the rates of oxygen consumption at various intervals of time after renal damage and (b) after adrenalectomy, with the view of extending the study of adrenocortical function to the poikilotherms, and (c) to obtain data for the oxygen consumption of another species of frogs by a different respirometric technique.

METHOD

Unfed male frogs, *Rana pipiens*, of winter (late autumn and winter) and early spring (before spawning) stock, weighing between 20 and 30 gm. and showing testicular fat at autopsy, were divided into 3 major groups (12–26 frogs/group): (1) adrenalectomized, both “paired” (curve A', fig. 1) and “unpaired” (curve A), (2) renal damaged (sham-operated control, curve R), and (3) unoperated (normal control, curve N) frogs. The method of adrenalectomy, of renal damage, of postoperative care on sphagnum moss, and of determining adrenocortical insufficiency has been described elsewhere (Angerer and Angerer, 1949; Angerer, 1950). “Paired” animals (included in groups 1 and 2) served as their own controls prior to adrenalectomy; “unpaired” frogs had their controls (unoperated and renal damage) randomly and simultaneously selected from the same stock as those designated for a given operative procedure.

Oxygen consumption was determined by direct-reading volumeters. The accuracy of this technique is comparable to that of the Winkler method (Calhoun and Angerer, 1950, 1953). The frogs were gradually acclimated (17–21 hr.) to the temperature of the waterbath to prevent possible thermal stimulation (Angerer, 1940). The frogs were permitted to sit in a given volume of tapwater sufficient to moisten their integuments. The volumeters were oscillated through a distance of 3 cm., at a frequency of 110 cycles/min. and at a bath temperature of $30 \pm 0.01^\circ \text{C}$. After a 15-minute equilibration period, readings were made immediately at 15-minute intervals for at least one hour. All readings were corrected to standard pressure and unit body weight. The results were expressed as ml. oxygen consumed/gm. of body weight (wet)/hr.

¹Aided by grants from the Comly-Coleman Research Fund, College of Medicine, and from the Graduate School, The Ohio State University.

²Presented at the Columbus meetings, American Physiological Society: Amer. Jour. Physiol. 163: 737. 1950.

RESULTS AND DISCUSSION

Normal frogs. The mean oxygen consumption for all (38) frogs studied is 0.175 ± 0.003 (standard error) ml./gm./hr. at 30° C. There is little fluctuation in this mean over 5 successive days as is indicated by the low value for its standard error. Curve N, figure 1, serves not only for purpose of reference when the percent

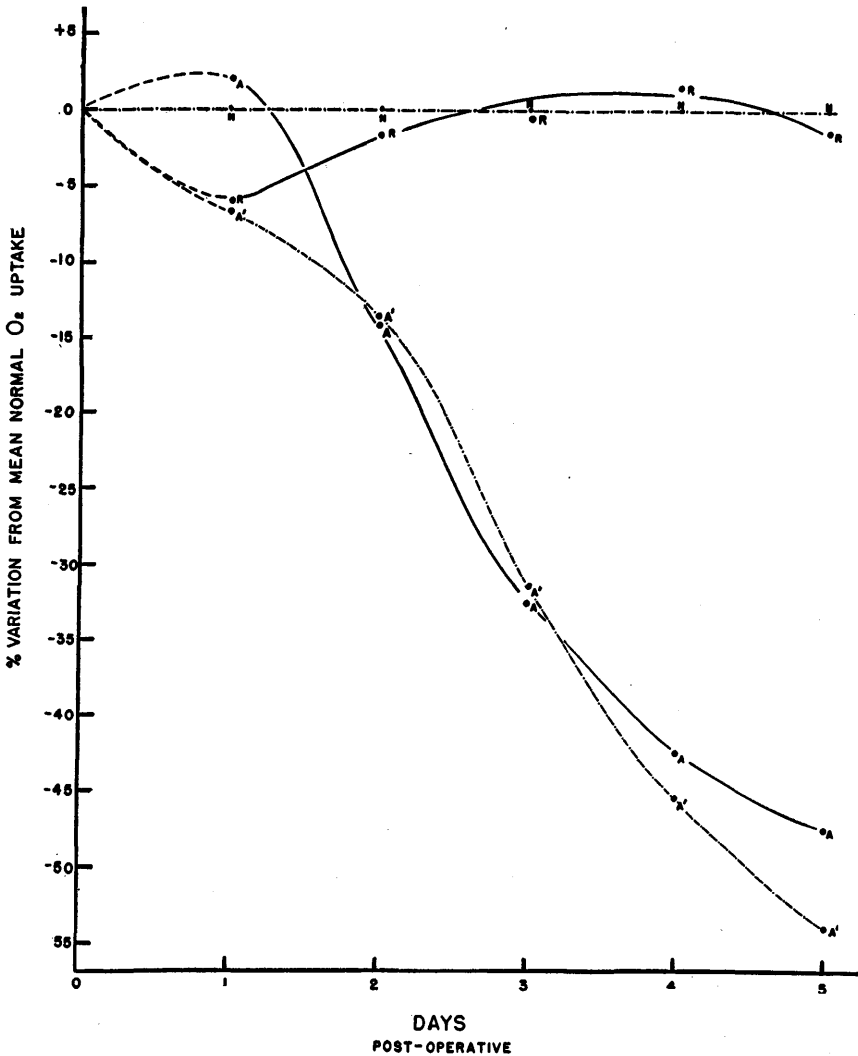


FIGURE 1. The percent variation in mean oxygen uptake (ml./gm./hr.) for renal damaged (R) and for "paired" (A') and "unpaired" (A) adrenalectomized frogs compared with normal (N) frogs (ordinates) is plotted against their postoperative time in days (abscissae). "Basal" winter and spring frogs were studied at 30° C. ("Paired" indicates each frog served as its own control.)

variation in mean oxygen uptake (ordinate) is plotted against postoperative time in days (abscissa) but also indicates, incidentally, the mean oxygen uptake (0.175 ml./gm./hr.) of all normal frogs during a period of 5 successive days. When winter and spring frogs are studied, under the stated experimental conditions,

the means are 0.183 ± 0.006 and 0.165 ± 0.006 ml. O_2 /gm./hr., respectively. The difference (-9.8%) between these values is not significant ($P > 0.05$, Student's method); therefore, the mean for all stocks is used in subsequent comparisons. The lack of significance between the means for our winter and spring stocks is probably due to all frogs being kept under routine laboratory conditions, as previously outlined, and in a "basal" state, in that the frogs were unfed and subsisted on their fat depots. The values hitherto reported (Loewy, 1926) for 2 frog species, studied at $20^\circ C$, are 0.080 and 0.260 ml. O_2 /gm./hr. for winter and April *R. temporaria* and 0.100 and 0.210 ml. O_2 /gm./hr. for winter and April *R. fusca*, respectively. Our mean of 0.175 ml. O_2 /gm./hr. at $30^\circ C$, when corrected to $20^\circ C$, on the assumption that the Q_{10} is ca. 2.3, gives a value identical with that which is found for winter *R. temporaria*. April frogs, to be sure, would have a higher metabolic rate due to their feeding, increased physical activity and incipient spawning. Indeed, Krogh (1904) found the highest oxygen uptake occurs during the spawning season. Krogh was interested in another phase of frog metabolism and, owing to experimental necessity, forcibly confined his animals. Even so, he obtained values of ca. 0.108 and 0.59-0.113 ml. O_2 /gm./hr. at $20^\circ C$ for winter *R. fusca* and *R. esculenta*, respectively.

It is interesting to note that Calhoun and Angerer (1955) using the same apparatus, frog stock and experimental technique, except for the omission of shaking of the volumeters during the course of the experiment, (cf. their results, 1953, p. 242) obtained a mean of 0.156 ml. O_2 /gm./hr. at $30^\circ C$. This may represent a truer "basal" value for *R. pipiens*, though the value is approximately at the limit of experimental error and its statistical significance is questioned.

Renal damaged frogs. The daily mean values for the oxygen consumption of 12 renal damaged frogs is presented in curve R, figure 1. After an initial decrease of 6.8 percent at the end of the first postoperative day, the data show little variation thereafter with time when compared with normal frogs (curve N). There is no statistical significance between these values (curve R) and those for normal frogs (curve N). All subsequent comparisons, unless otherwise stated, are made with unoperated frogs. These controls are used in place of renal damaged frogs in order that data from "paired" and "unpaired" adrenalectomized frogs may be compared. There is little reason to believe that sham operations with their concomitant renal damage would alter the oxygen uptake of amphibious and aquatic animals as long as the regenerative area is relatively small compared to the total respiring tissue mass. Furthermore, the amount of renal damage arising, of necessity, as a result of adrenal ablation, is apparently without injurious effects to the frog (Angerer, 1950).

Adrenalectomized frogs. Figure 1 gives data for the daily mean rates of oxygen consumption for "paired" (curve A¹) and "unpaired" (curve A) adrenalectomized frogs compared with their controls (curve N). While 71 percent of the "paired" frogs show a decrease in mean oxygen consumption at the end of the first postoperative day, a statistically significant decrease (ca. 13%, $P < 0.05$) is observed by the end of the second postoperative day. Thereafter the values decrease until the end of the fifth day when a mean of 0.095 ml./gm./hr. is obtained. This value is ca. 49 percent below that for the mean initial (zero) time or for the renal damaged frogs at the end of a corresponding postoperative interval.

A comparison of data for "paired" (curve A¹) and "unpaired" (curve A) adrenalectomized frogs shows that both sets of data fall along essentially sigmoid curves. Whether the curve form is significant, or fortuitous, is problematical. At best, it suggests many reactions are occurring simultaneously. To our knowledge a comparable treatment of data for warm blooded animals does not exist. Secondly, there is a consistently greater decrease in mean oxygen uptake by the end of the fifth postoperative day for both winter (10%) and spring (12%) "paired" than for "unpaired" adrenalectomized frogs. Little variation is observed between

winter (0.101 ml. O_2 /gm./hr. and spring 0.089 ml.) "unpaired" adrenalectomized frogs by the end of the fifth postoperative day. Thirdly, the death point for a majority (ca. 65%) of the adrenalectomized frogs under prevailing experimental conditions occurs by the end of the fifth day, whereas normally it is between the seventh and twelfth postoperative day (Angerer, 1950). The last two items are probably to be interpreted as due to the stress induced in handling and in subjecting the frogs to the relatively high environmental temperature. These two stressing agents would increase the demand for unavailable adrenocortical hormone(s) (Hartman and Brownell, 1949). A decrease in metabolism following adrenalectomy has been noted for several homoiotherms: cats (Aub, Forman and Bright, 1922), rabbits (Marine and Baumann, 1922), rats (Carr and Beck, 1927), and so on. The chief difference is the extent of the metabolic depression between homoiotherms (ca. 25%) and poikilotherms (ca. 50%).

SUMMARY

The oxygen consumption of 20–30 gm. male frogs, *R. pipiens*, was studied by direct-reading volumeters. All frogs studied may be placed in 3 major groups: *A*, unoperated (normal control, curve N, fig. 1); *B*, renal damaged (sham operated control curve R); *C*, "paired" (curve A') and "unpaired" (curve A) adrenalectomized frogs (see "method").

1. The mean oxygen consumption for winter and spring frogs under "basal" conditions is 0.175 ± 0.003 ml./gm./hr. at 30° C; no statistical difference is observed between winter and spring stocks under the experimental conditions employed.

2. There is no significant difference between the mean values for oxygen uptake for excessive renal damaged and normal frogs (cf. curves R and N).

3. Adrenalectomized frogs show no significant change in oxygen consumption at the end of the first postoperative day compared with unoperated or renal damaged frogs. Thereafter, the means for both "paired" (curve A') and "unpaired" (curve A) adrenalectomized frogs fall along a sigmoid curve (fig. 1). On the fifth postoperative day a mean of 0.095 ml. O_2 /gm./hr. is obtained. The figure represents a decrease of 46 percent below that of the controls.

4. The stress of repeated handling and of subjecting adrenalectomized frogs to relatively high environmental temperatures depresses both their metabolism and viability (cf. curves A' and A).

ACKNOWLEDGMENTS

We wish to thank Dr. Thomas B. Calhoun, formerly of this laboratory and now of the Department of Physiology, Medical College of South Carolina, Charleston, for his help to M. C. M. in conjunction with the use of the respirometers.

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