

EFFECT OF ADRENOCORTICAL EXTRACT AND RELATIVELY SMALL DOSES OF SESAME OIL ON THE OXYGEN CONSUMPTION OF GOLDFISH¹

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Sesame oil has been shown (Pollia, 1937; Bruce and Tobin, 1940; Clausen, 1940; Spurr and Kochakian, 1940; Crafts, 1942; Calhoun and Angerer, 1950a) to produce a variety of effects in animals, despite the fact that it and other carrying agents have been considered more or less biologically inert (Brown, Wilder and Schwartz, 1944). It has been reported (Calhoun and Angerer, 1950a) that heavy doses of sesame oil (0.0036 ml./gm. body weight injected 3×/week for 8 to 10 weeks inclusive) decrease the rate of oxygen consumption of goldfish by ca. 37 percent when compared with controls, and that adrenocortical extract (ACE) injected simultaneously with sesame oil "protects" these animals from this effect. The total dosage of sesame oil previously administered (Calhoun and Angerer, 1950a) was relatively large, being equivalent to 252 ml./70 kg. However, it is comparable in magnitude to dosages previously reported (Pollia, 1937; Bruce and Tobin, 1940; Clausen, 1940; Spurr and Kochakian, 1940; Crafts, 1942). This study was undertaken for the following reasons: to determine (1) whether a smaller (1/20) volume, *i. e.*, a more clinical dose, of sesame oil than hitherto reported produces a metabolic disturbance; (2) whether ACE antagonizes the foregoing disturbance; and (3) whether the effect of ACE on the metabolism of normal poikilotherms can elucidate the confused literature existing for homoiotherms.

METHODS

The common goldfish, *Carassius auratus*, was used as the experimental animal in the hope that a poikilothermic vertebrate might produce reactions otherwise obscured by the more efficient regulatory mechanisms, *e.g.*, temperature and endocrine, existing in homoiotherms. The fish were obtained, maintained and rendered in a "basal" state as previously described (Calhoun and Angerer, 1950a).

All determinations of oxygen consumption were made by direct reading volumeters (Calhoun and Angerer, 1953). This method has the same order of experimental accuracy as obtained by the Winkler method (Calhoun and Angerer, 1950a; Calhoun, 1953). Determinations of oxygen consumption were made at 15 min. intervals for a period of at least 1 hr. The rate of oxygen consumption was calculated on the basis of ml./gm. (wet) of fish/hr. at 30.00±0.01°C. The extremely small injections of sesame oil and of ACE were accomplished by means of a micro-syringe constructed according to the general specifications described by Woodrow (1949).

RESULTS

Sesame oil. Determinations on 12 different "normal" fish gave a mean value of 0.296±0.115 (standard deviation) ml. O₂ consumed/gm./hr., at 30°C (zero week, line 1, table 1). These fish were then injected intramuscularly with 0.00018 ml. of sesame oil/gm. of body weight 3×/week during 7 consecutive weeks. In table 1 there are found the various mean values for oxygen uptake (column 4)

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with their respective standard deviations (column 5) which were obtained at the end of the week indicated (column 1) after the initial injection. At the close of the second week there was an apparent transient increase of *ca.* 9 percent in the rate of oxygen consumption. This was immediately followed by a sharp decrease until the end of the seventh week when the value was *ca.* 37 percent below the initial (zero time) value.

Sesame oil plus ACE. The mean oxygen consumption (control) was determined for 12 different normal goldfish, and it gave 0.397 ± 0.130 ml. of O_2 consumed/gm./hr. These fish were then injected intramuscularly 3 \times /week for 7 consecutive weeks with 0.00018 ml. of sesame oil plus 0.00036 ml. of Upjohn's Adrenal Cortical Extract/gm. of body weight. The rates of oxygen consumption of individual fish were determined at the close of each of 7 consecutive weeks. The means of these data are presented in paired form with their respective controls (sesame oil injected fish) in table 1. Although the initial rates of oxygen consumption of the 2 groups of animals gave different values (*cf.* 0.296 and 0.397 ml. O_2 consumed/gm./hr.) they were not significantly different ($P=0.08$, Student's method). Analysis of these data (table 1) shows that at the end of the second week the oxygen consumption had decreased *ca.* 35 percent compared with the initial week, and remained near this value for the next 5 wk. A comparison of the mean oxygen uptake of the ACE-oil-treated group with the oil-treated (control) group for any given week (table 1, Column 8) indicates significant decreases at the end of 2, 3 and 6 wk. of *ca.* 52 percent ($P<0.01$), 44 percent ($P<0.01$) and 30 percent ($P<0.02$), respectively.

Sesame oil plus ACE on body weight (table 2). The rates of gain in mean body weight of these 2 groups of animals fail to exhibit the linearity which was obtained for a similar experiment involving larger volumes of sesame oil (Calhoon and Angerer, 1950a). At the end of 7 wk. as compared with zero week the mean increase in body weight of fish given ACE plus sesame oil (table 2) is *ca.* 13 percent while in the same interval, the mean increase for fish given sesame oil alone is *ca.* 11 percent (table 2). The difference between these mean values at the end of the seventh week is not statistically significant ($P>0.20$).

DISCUSSION

The effects of small doses of sesame oil on the total oxygen consumption of goldfish (table 1) is open to the same interpretation as was suggested for the effects of large doses, *i. e.*, they cause liver damage (Calhoon and Angerer, 1950a). There was, as expected, a time difference: "large" doses (0.0036 ml./gm.) of sesame oil produced *ca.* 30 percent metabolic depression at the end of 1 wk. (Calhoon and Angerer, 1950a), while "small" doses (0.00018 ml./gm.) produced the same degree of depression, but only after 5 to 6 wk. This may be explained as due to the cumulative effect of the oil (Brown, Wilder and Schwartz, 1944). Indeed, the amount of oil accumulating as a result of "small" doses at the end of 5 to 6 wk., assuming 50 percent loss (Brown, Wilder and Schwartz, 1944), is the same as for 1 "large" dose given at the end of 1 wk. (Calhoon and Angerer, 1950a). Thus whether the oil is given in small doses over a long period of time, or in large doses over a short period of time, the same critical level is reached, producing the same degree of depression (*ca.* 25-35%) in total oxygen consumption.

While "small" doses of sesame oil have little effect on the total oxygen consumption of goldfish for the first 3 wk, the same quantity of sesame oil plus ACE (table 1) depresses the oxygen consumption of these fish. The difference between these 2 sets of mean values at the end of 2 and 3 wk. is *ca.* -52 percent ($P<0.01$) and -44 percent ($P<0.01$), respectively, and must be interpreted as due to the action of ACE on oxygen consumption. Investigations with frogs show relatively the same degree of depression in oxygen consumption when ACE is given alone (Calhoon and Angerer, 1955). These results, indicating a decrease in oxygen

TABLE 1
Sesame oil plus ACE on total oxygen consumption

Week	Treatment	No. of Fish	Mean ml./O ₂ /gm./hr.	Std. Dev.	Weekly Comparison with Own zero week		Weekly Comparison of Both Groups***	
					% Diff.	P-Value**	Using Oils as Control % Diff.	P-Value**
0	oil	12	0.296	0.115		
	" +ACE	12	0.397	0.130	+34.1	0.08
1	"	12	0.297	0.062	0.3		
	" +ACE	12	0.355	0.087	-10.6	0.40	-14.5	0.20
2	"	10	0.322	0.076	+ 8.8	0.60		
	" +ACE	10	0.257	0.044	-35.3	0.01	-51.9	0.01
3	"	10	0.298	0.043	0.1		
	" +ACE	10	0.268	0.052	-32.5	0.01	-43.9	0.01
4	"	10	0.233	0.106	-21.3	0.20		
	" +ACE	10	0.312	0.090	-21.4	0.10	-9.9	0.60
5	"	10	0.234	0.069	-21.0	0.20		
	" +ACE	4*	0.253	0.079	-36.3	0.10	-35.0	0.07
6	"	10	0.245	0.055	-17.2	0.20		
	" +ACE	10	0.273	0.067	-31.2	0.02	-29.8	0.02
7	"	10	0.186	0.032	-37.2	0.01		
	" +ACE	10	0.280	0.094	-29.5	0.01	- 3.0	0.90

*Only 4 fish run this day because of unavoidable circumstances.

**Probability of there being no difference between experiment and control values.

***Since there is no statistically significant difference between the means of the 2 groups on the initial week, the apparent mean difference (0.101) was added to the difference between the 2 points at each successive week's analysis.

consumption of normal fish treated with ACE, tend to agree with those in the literature on rabbits (Marine and Baumann, 1922) and dogs (Franke, 1932) but to disagree with those which give either an increase for dogs (Koehler and Hastings, 1929; Hoffman and Talesnick, 1948) or no change for dogs (Harrop, Pffner, Weinstein and Swingle, 1931; Missiuro, Dill and Edwards, 1938), cats (Harrop, Pffner, Weinstein and Swingle, 1931) and humans (Hitchcock, Grubbs, and Hartman, 1938).

It is suggested that the metabolic depression observed in these cold blooded animals is due to a direct action of ACE on tissue metabolism. This is in line with previous reports to the effect that various adrenocortical fractions depress the QO_2 values of brain and liver slices (Tipton, 1939; Gordon, Bentinck and Eisenberg, 1951), the oxidation of glucose by tissues (Long, Katzin, and Fry, 1940; Evans, 1941), the activities of various dehydrogenases in brain slices (Gordon, Bentinck and Eisenberg, 1951) and the actions of various other enzymes (D amino-acid oxidase, tyrosinase, urease, transaminase and ascorbic acid oxidase) in kidney slices (Umbreit, 1951; Hayano, and Dorfman, 1951). However, it is possible that the observed depression in oxygen consumption of goldfish, as well as of frogs (Calhoon and Angerer, 1955), may involve a dysfunction of the pituitary, since a pituitary-adrenal cortex relationship is known to exist at least for the frog (Atwell, 1935, 1937). This aspect of the problem must await further development.

It is suggested that the difference observed between this and a previous report (Calhoon and Angerer, 1950a) is interpretable on the basis that in the latter study, sesame oil *per se* produced a "stress"; while this metabolic "stress" was nullified in the presence of adrenocortical extract. However, in the present report, during the first 2 wk. no metabolic disturbance was produced by the injected sesame oil and the ACE was free to produce its inhibitory effect on total oxygen consumption. It is felt that this is especially true, since it is anticipated that were the present study carried on for a longer time the mean difference between the two sets of data for oxygen uptake (column 4, table 1) would decrease to zero and subsequently diverge in a manner similar to that represented by the curves previously published (Calhoon and Angerer, 1950a) on the effects of large doses of sesame oil alone, and together with ACE.

There is a progressive increase with time in body weight for the sesame injected fish. At the end of 7 wk. *ca.* 11 percent increase occurs when compared with zero week. When ACE is injected simultaneously with oil the body weights tend to oscillate around the respective controls. There is no trend to show any acceleratory effect on body weight nor any degree of linearity between dosage and time as was observed for "large" doses of sesame oil (Calhoon and Angerer, 1950a).

SUMMARY

1. Goldfish injected intramuscularly with 0.00018 ml. of sesame oil/gm. body weight $3\times$ /week for 7 wk. show relatively no change in oxygen consumption during the first 2 or 3 weeks (table 1). Thereafter, a progressive decrement is attained. The data indicate a continuing decrease were the experiments carried on for a longer time.

2. Fish injected with 0.00018 ml. of sesame oil simultaneously with 0.00036 ml. of ACE/gm. body weight $3\times$ /week for 7 wk. give *ca.* -35 percent in mean oxygen consumption at the end of 2 weeks with respect to the controls. This value tends to be maintained thereafter for the duration of the experiment.

3. When ACE and oil are injected simultaneously and the body weights are determined on a weekly basis, their mean values tend to oscillate about the respective controls. This is unlike the effect of "large" doses of ACE and sesame oil.

TABLE 2
Sesame oil plus ACE on mean body weight

Week	Treatment	No. of Fish	Mean Body Wt.	Std. Dev.	Weekly Comparison with Own zero week		Weekly Comparison of Both Groups Using Oils as Control	
					% Diff.	P-Value**	% Diff.	P-Value**
0	oil	12	6.3	2.8	-23.8	0.10
	" +ACE	12	4.8	1.0		
1	"	12	6.6	2.7	+ 4.8	0.80	-28.8	0.02
	" +ACE	12	4.7	1.0	- 2.1	0.80		
2	"	10	6.4	1.8	+ 1.6	0.30	-18.8	0.10
	" +ACE	10	5.2	1.0	+ 8.3	0.30		
3	"	10	6.4	1.7	+ 1.6	0.90	-18.8	0.10
	" +ACE	10	5.2	1.1	+ 8.3	0.40		
4	"	10	7.5	1.8	+19.0	0.20	-33.3	0.01
	" +ACE	10	5.0	1.2	+ 4.2	0.60		
5	"	10	6.9	1.6	+ 9.5	0.50	-14.5	0.10
	" +ACE	4*	5.9	0.9	+22.9	0.05		
6	"	10	6.7	1.8	+ 6.3	0.60	-22.4	0.05
	" +ACE	10	5.2	1.2	+ 8.3	0.40		
7	"	10	7.0	1.8	+11.1	0.40	-22.9	0.02
	" +ACE	10	5.4	1.1	+12.5	0.20		

*Only 4 fish run this day because of unavoidable circumstances.

**Probability of there being no difference between experiments and control values.

4. The conclusions suggested from these results and from their comparison with a former study (Calhoon and Angerer, 1950a) using "large" doses of sesame oil are: (a) above a critical dosage, either single or cumulative, sesame oil depresses total oxygen consumption of goldfish; (b) ACE given simultaneously with sesame oil antagonizes (inhibits) the metabolic depressant action induced by the oil alone; (c) when given in excess of this amount it causes *ca.* -37 percent in total oxygen consumption with respect to the controls, which is interpreted as due to the action of ACE *per se*.

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