TABLE III. Calculation of Ratio of Strontium to Calcium Moving from Blood to Bone of Rats. (D = Sr/Ca ratio in diet.)

Group		1	2	3	Ref.
Sr/Ca in bone at steady state	(B)	.57 D	.45 D	.27 D	(5)
" " blood " " "	(P)	.46 D	.43 D	.21 D	(5)
Accretion rate of calcium	(A)	.17	.17	.17	(11)
Resorption " " "	(R)	.13	.13	.13	(11)
Sr/Ca leaving bone	(L)	1.3	1.3	1.3	
" entering "	(E)	1.5	1.3	1.6	

Note: Equation for E derived as follows:

$$\frac{\text{Sr in bone}}{\text{Ca in bone}} = \frac{\text{Rate of Sr entry} - \text{Rate of Sr leaving}}{\text{Rate of Ca entry} - \text{Rate of Ca leaving}} = \frac{(A)(E)(P) - (R)(L)(B)}{A - R} = B,$$

$$\text{and } E = \frac{B(A - R + RL)}{(A)(P)}.$$

rat by peritoneal lavage. From comparisons of strontium/calcium ratios in lavage solutions and bones of nephrectomized rats it is estimated that strontium was preferentially released from bone by a factor of 1.3 over calcium. 2) It was calculated that strontium preferentially entered bone from blood by a factor of 1.3 to 1.6. The differential removal of the 2 elements from the intact rat was shown to be due mainly to renal function. Eight hours of peritoneal lavage removed about 5% of the Sr* that had been deposited for 24 hours but only about 0.6% of that deposited for 8 days.

1. Libby, W. F., *Proc. Nat. Acad. Sci. U. S.,* 1956, v42, 365.

2. Kulp, J. L., Eckelmann, W. R., and Schulert, A. R., *Science,* 1957, v125, 219.

3. Comar, C. L., Whitney, I. B., and Lengemann, F. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 232.

4. Singer, L., Maqsood, M., Medlen, A. B., and Comar, C. L., *Arch. Biochem. and Biophysics,* 1957, v66, 404.

5. Comar, C. L., Wasserman, R. H., and Nold, M. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 859.

6. Comar, C. L., and Wasserman, R. H., in: Progress in Nuclear Energy, Series VI, v1, 1956, *Biological Sciences,* Pergamon Press Ltd., London.

7. Lengemann, F. W., PROC. SOC. EXP. BIOL. AND AND MED., 1957, v94, 64.

8. Comar, C. L., *Radioisotopes in Biology and Agriculture.* McGraw-Hill Book Co., New York, 1955.

9. Talmage, R. V., and Elliot, J. R., *Endocrinology,* 1956, v59, 27.

10. MacDonald, N. S., Noyes, P., and Lorick, P. C., *Amer. J. Physiol.,* 1957, v188, 131.

11. Bauer, G. C. H., Carlsson, A., and Lindquist, B., *Kungl. Fysiografiska Sallskapets I Lund Forhandlingar,* 1955, v25, 3.

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Effects of Testosterone, Sesame Oil, and Castration on Tissue Respiration of Male Rats Exposed to Cold. (23239)

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Addition of testosterone *in vitro* to tissue slices is reported to have either an augmenting(1) or a depressing(2,3) effect on rate of oxygen uptake of tissue slices depending upon concentration. Smith, Kochakian and Fondal(4) report a decrease in rate of oxygen uptake of tissue slices and homogenates of liver and kidney from rabbits, rats and guinea

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TABLE I. Oxygen Uptake of Kidney Slices Recorded at 15 Min. Intervals between 30 and 105 Min. Incubation Period.

Incubation time		45	60	75	90	105	S.D. of line	Dev. of 75 min. value
Control	O	13	12	10	11	10		
	C	13	12	12	11	10	.66	
Control—	O	16	18	11	17	15		
Test. *in vitro**	C	17	16	—	15	14	1.75	−4.50‡
Castrate control	O	20	16	19	20	16		
	C	18	18	18	18	18	1.66	
Castrate control—	O	18	20	18	21	15		
Test. *in vitro**	C	19	18	—	17	16	1.85	− .75
Oil—23°C	O	14	15	11	12	10		
	C	14	14	—	13	—	.78	−2.50†
2°C	O	13	13	10	13	10		
	C	13	13	—	13	—	.53	−3.00†
T.O.—23°C	O	15	14	25	38	20		
2°C	O	17	17	14	12	16		
	C	17	17	—	—	17	1.47	−5.25†
				Castrated				
Oil—23°C	O	17	16	14	16	14		
	C	17	16	—	15	14	.54	−1.30‡
2°C	O	15	13	11	14	14		
	C	14	14	—	13	13	.93	−2.70†
T.P.—23°C	O	16	15	12	13	15		
	C	15	15	—	14	14	.84	−2.40‡
2°C	O	16	18	12	18	16		
	C	17	17	—	17	17	−.93	−5.00†

* Testosterone added *in vitro* after 1 hr incubation to all groups except control and castrated control.

† Significantly different from calculated line at 1% level.

‡ *Idem*, at 5% level.

O—observed value; C—calculated value.

pigs, when testosterone is added to the incubation medium. Dirscherl and Hauptmann (1) demonstrated a stimulating effect of testosterone in low concentrations in contrast to a depressing effect reported by other investigators.

This experiment was performed to determine the effect of testosterone added *in vitro* upon respiration of tissues from cold exposed rats. Testosterone was added to tissue slices from castrated and intact animals, previously treated with testosterone propionate or sesame oil, and exposed to a low ambient temperature for 50 days.

Methods. Oxygen uptake of approximately 90 samples each of liver, kidney and brain slices from male Sprague-Dawley rats was determined in the Warburg constant volume respirometer. Tissues were obtained from animals treated as follows. Rats were treated daily with either 1 mg testosterone propionate in 0.1 ml sesame oil, or sesame oil alone for 50 days. Half of the animals were maintained at room temperature and half were kept at 2 ± 2°C for the same periods and treated similarly. Half of the rats maintained at each temperature were castrated at 30 days and all treatments started at 60 days of age. Rats in cold were kept in individual wire cages without nesting material; those at room temperature were housed 5-10 animals/cage. Food and water were given *ad libitum*. The animals were killed by cervical luxation. Samples of liver, kidney and brain were removed and transferred to cold Krebs-Ringer-Phosphate (K-R-P) solution (pH 7.41) within 2 minutes after death. Tissues were sliced in a Stadie-Riggs microtome, weighed to 0.1 mg and placed in Warburg flasks containing 2 ml of cold K-R-P. Flasks were kept in ice until the flask-manometer systems were transferred to water bath at 38 ± 0.01°C. After

TABLE II. Oxygen Uptake of Brain Slices Recorded at 15 Min. Intervals between 30 and 105 Min. Incubation Period.

Incubation time		45	60	75	90	105	S.D. of line	Dev. of 75 min. value
Control	O	11	7	6	4	4		
	C	10	9	7	5	4	1.38	
Control—	O	8	9	4	7	6		
Test. *in vitro**	C	9	8	—	6	6	.75	−2.50†
Castrate control	O	12	8	9	11	5		
	C	12	11	9	8	7	1.85	
Castrate control—	O	14	11	11	11	8		
Test. *in vitro**	C	14	13	—	10	8	1.00	− .25
Oil—23°C	O	9	6	2	8	5		
	C	9	7	—	6	5	.84	−4.40†
2°C	O	11	7	7	8	5		
	C	11	10	—	8	7	1.25	−2.60‡
T.P.—23°C	O	11	10	13	24	9		
2°C	O	12	13	5	8	5		
	C	14	12	—	9	7	1.41	−5.40†
				Castrated				
Oil—23°C	O	14	12	8	10	8		
	C	14	13	—	9	7	.75	−2.90†
2°C	O	11	11	4	9	5		
	C	12	11	—	9	9	2.62	−6.20‡
T.P.—23°C	O	13	10	7	9	9		
	C	13	12	—	9	7	1.41	−3.50‡
2°C	O	12	10	5	11	8		
	C	13	11	—	8	6	1.85	−4.50‡

* Testosterone added *in vitro* after 1 hr incubation to all groups except control and castrated control.

† Significantly different from calculated line at 1% level.

‡ *Idem*, at 5% level.

O—observed value; C—calculated value.

15 minutes equilibration, manometers were read at 15-minute intervals during 2 hours incubation. Testosterone (0.2 ml) was tipped into the reaction chamber from sidearms after one hour. Testosterone was dissolved in dehydrated ethyl alcohol and diluted to 10% immediately before use. Final concentration was 2 mM. Tissues of intact and castrated controls were divided into 2 groups. Testosterone was added *in vitro* to tissues from 1 group of intact and 1 of castrated rats, whereas the remainder of tissues were permitted to respire for 2 hours with no addition of testosterone. Although values were obtained at 15 minute intervals during the entire period, the data presented in Tables I and II are for incubation time between 30 and 105 minutes. This procedure was followed to demonstrate changes immediately after addition of testosterone. A straight line curve was calculated by the method of "least squares" using the 15 minute values. For this calculation the value for the interval immediately following addition of testosterone (75 minute value) was not incorporated. To ascertain whether testosterone had significantly altered oxygen uptake the deviation of this value (75 minute value) was determined and compared to the deviation of other values on the line.

Results. No significant change in rate of oxygen uptake appeared in liver slices from any of the animals regardless of whether testosterone was added.

Effects of testosterone in vitro *on tissue respiration of control rats.* The following data are illustrated in Tables I and II and Fig. 1. The control group maintained at room temperature (23 ± 2°C) and given no treatment either *in vivo* or *in vitro* had a con-

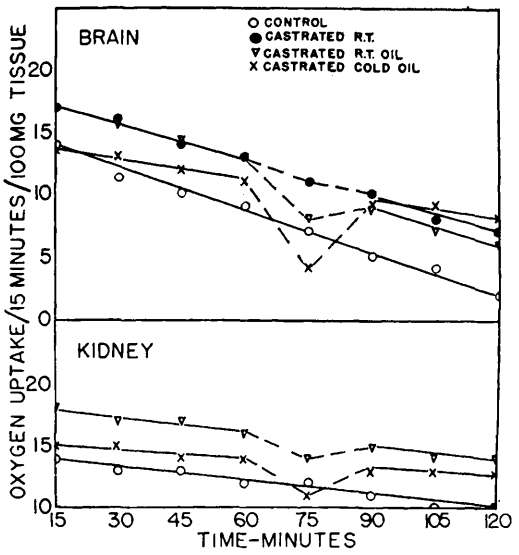

FIG. 1. Curves are calculated as "best fit" lines by the method of least squares; the 75 min. values are observed values. R. T., room temperature; oil, Sesame oil.

stant rate of oxygen uptake with no change in the 75 minute values for either kidney or brain tissue. Oxygen uptake of kidney and brain slices from controls maintained at room temperature and receiving no treatment *in vivo,* but with testosterone added *in vitro* showed a significant decrease in the 75 minute value. The oxygen uptake curves, both calculated and observed, for kidney and brain slices, from castrated rats kept at room temperature with no treatment either *in vivo* or *in vitro,* were not changed at any point. This was to be expected. Tissue slices from castrated rats maintained at room temperature showed no change in rate of oxygen uptake when testosterone was added *in vitro.*

Effects of testosterone propionate and sesame oil in vivo *and testosterone* in vitro *on tissue respiration.* Rats at room temperature treated with sesame oil showed a significant difference in rate of oxygen uptake after *in vitro* addition of testosterone to kidney and brain slices. A corresponding group exposed to cold (2 ± 2°C) also showed a significant decrease in rate of oxygen uptake after *in vitro* addition of testosterone. These decreases were significant at 1 and 5% levels. Intact animals treated *in vivo* with testosterone propionate and housed at room temperature showed a significant increase in rate of oxygen uptake from kidney and brain slices. However, testosterone propionate administered *in vivo* during exposure of rats to cold resulted in a highly significant depression of oxygen uptake in both kidney and brain slices after *in vitro* addition of testosterone. Intact animals treated *in vivo* with sesame oil and kept at room temperature show a significant decrease in rate of oxygen uptake after *in vitro* addition of testosterone. Animals treated in the same way except exposed to cold gave similar results. This applies to kidney and brain slices. Castrated rats treated with testosterone propionate *in vivo* either at room temperature or exposed to cold gave results of similar pattern to groups treated with sesame oil. This applies to both kidney and brain slices.

Discussion. The failure of testosterone to alter oxygen uptake of liver slices was probably due to low concentration of hormone used in these experiments. However, depression of oxygen uptake by kidney and brain slices from intact animals following addition of testosterone *in vitro* is in agreement with previous reports(2,3) as is failure to note any effect on rate of oxygen uptake following *in vitro* addition of testosterone to tissue from castrated male rats(3).

Castration induces a change in response of tissue to testosterone propionate, sesame oil and cold. Treatment *in vivo* with testosterone propionate or sesame oil restores the capacity of tissues to respond to *in vitro* addition of testosterone; however restoration of this response by sesame oil is limited. Tissue from untreated castrated rats did not respond. The decrease in rate of oxygen uptake following addition *in vitro* of testosterone to kidney and brain slices from castrated rats treated with oil and exposed to either room temperature or cold indicates an action for sesame oil similar to that of testosterone propionate. It appears that both sesame oil and testosterone propionate may have sensitized the tissue in such a manner that depression of oxygen uptake resulted after addition of testosterone *in vitro.* Physiological responses to sesame oil have been reported by others. Crafts(5) reports a de-

crease in weight of prostate and seminal vesicles from animals treated with androsterone dissolved in sesame oil or sesame oil alone. Tobin(6) found sesame oil prolonged survival of pregnant rats adrenalectomized after onset of pregnancy. He concluded that under these conditions sesame oil is not an inert vehicle. Our results, while not dealing with the same responses, indicate that sesame oil may be physiologically active(7).

Tissue slices from intact rats treated daily with either testosterone propionate or sesame oil responded to *in vitro* addition of testosterone by a decrease in rate of oxygen uptake with one exception. This increase was found in tissues from intact animals treated with testosterone propionate and maintained at room temperature. No explanation is offered.

Liver metabolism of steroids is complex, therefore, it is probable that the liver can metabolize a greater number of compounds than can other tissues(8) without a significant alteration in respiration. Kochakian (9) has presented evidence that kidney and liver do react differently in the metabolism of androgens.

Our results indicate that treatment with sesame oil *in vivo* restores the capacity of tissues from castrated male rats to respond to testosterone *in vitro*. Exposure to cold enhances this action of sesame oil.

Summary. 1. The effect of addition of testosterone (2mM) *in vitro* to liver, kidney and brain slices from intact and castrated rats treated with testosterone propionate and sesame oil *in vivo* and exposed to cold ($2 \pm 2°C$) was studied in the Warburg constant volume respirometer. 2. No significant change was found in rate of oxygen uptake of liver slices with any treatment. These results with liver reflect a more rapid rate of destruction of testosterone. Kidney and brain slices from intact animals show a decrease in rate of oxygen uptake after addition of testosterone *in vitro*. Kidney and brain slices from untreated castrated rats kept at room temperature exhibit no change in rate of oxygen uptake after addition of testosterone *in vitro*. 3. Tissue from castrated rats treated with sesame oil and maintained at room temperature or in cold show a decrease similar to tissues from intact animals following addition of testosterone *in vitro*. 4. All changes were temporary and inhibitory, except in the case of animals treated with testosterone propionate *in vivo* at room temperature.

1. Dirscherl, W., and Hauptmann, H., *Biochem. Z.*, 1950, v320, 199.

2. Kochakian, C. D., *Ann. N. Y. Acad. Sci.*, 1951, v54, 534.

3. Eisenberg, E., Gordan, G. S., and Elliott, H. W., *Science,* 1949, v109, 337.

4. Smith, T. C., Kochakian, C. D., and Fondal, E., *Am. J. Physiol.*, 1953, v174, 247.

5. Crafts, R. C., *Endocrinol.*, 1942 v31, 142.

6. Tobin, G. E., *ibid.*, 1941, v28, 419.

7. Calhoon, T. N., and Angerer, C. A., *ibid.*, 1950, v46, 327.

8. Dorfman, R. I., and Shipley, R. A., In *Androgens: Biochemistry, Physiology and Clinical Significance,* Wiley and Sons, N. Y., 1956.

9. Kochakian, C. D., and Stidworthy, G., *J. Biochem.*, 1954, v210, 933.

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