

suitable for the manometric estimation of cytochrome oxidase by the technique used by Walter (3), because the high phenol oxidase activity of these preparations masks other oxidative activity in the same system. Distinct cytochrome oxidase activity could be demonstrated manometrically if the homogenates were dialyzed thoroughly, or if the particulate cellular fraction (containing the cytochrome oxidase activity) was separated from the phenol oxidase by high-speed centrifugation.

Therefore, since a cytochrome oxidase is also present in sweet potatoes, and since the elicitation of increased gas exchange on the addition of phenolic substances does not seem to be a very critical basis for determining the nature of a terminal oxidase in living cells, it was of interest to re-examine the role played by phenol oxidase in sweet potato respiration. For this purpose, the effect of carbon monoxide on respiration was studied, as it had been shown earlier (6) that the carbon monoxide inhibition of sweet potato phenol oxidase activity was insensitive to light, whereas the carbon monoxide inhibition of sweet potato cytochrome oxidase was easily eliminated by light. The respiration of thin disks (7 mm diam, 0.5 mm thick) of root tissue in 0.05 M potassium phosphate buffer (pH 7.1) was measured at 25° C by standard manometric techniques (7) in various ratios of carbon monoxide or nitrogen to oxygen. The system was irradiated with light of about 300 ft-c incident on the manometer vessels.

In Table 1 are given values for oxygen consumption by sweet potato disks in several gas mixtures and in darkness and light. Respiration was strongly inhibited at the higher ratios of carbon monoxide to oxygen. It is evident that the inhibitions were completely eliminated by illumination except for a small amount at the highest CO/O₂ ratio. For comparison, the results with purified preparations of both sweet potato cytochrome oxidase and phenol oxidase under the same conditions are also presented.

Several varieties of sweet potatoes were examined, but no essential differences in results were noted that were due to difference in variety. Inasmuch as the

carbon monoxide inhibition of respiration was so sensitive to light, and the carbon monoxide inhibition of phenol oxidase activity was completely insensitive, it would seem that sweet potato phenol oxidase is precluded from consideration as a terminal oxidase of respiration under the conditions used here. Conversely, the participation of the cytochrome oxidase in respiration, although not entirely proved, is rendered likely by the similar reaction of respiration and of cytochrome oxidase activity to carbon monoxide inhibition and to illumination.

References

1. WALTER, E. M., and NELSON, J. M. *Arch. Biochem.*, **6**, 131 (1945).
2. RUDKIN, G. O., and NELSON, J. M. *J. Am. Chem. Soc.*, **69**, 1470 (1947).
3. WALTER, E. M. Diss., Columbia Univ., New York (1947).
4. NELSON, J. M. In W. McElroy and B. Glass (Eds.), *A Symposium on Copper Metabolism*. Baltimore: Johns Hopkins Univ. Press (1950).
5. WEBSTER, G. C. *Am. J. Bot.*, **39**, 739 (1952).
6. ———. Ph.D. thesis. Univ. Minnesota, Minneapolis (1952).
7. UMBREIT, W., BURRIS, R., and STAUFFER, J. *Manometric Techniques and Tissue Metabolism*. Minneapolis: Burgess Pub. Co. (1949).

Manuscript received September 8, 1952.

The Dependence of the Secondary Sex Ratio in Humans on the Age of the Father¹

Edward Novitski

Department of Zoology, University of Missouri, Columbia

That older mothers produce fewer male offspring than younger mothers is well known. This decrease in sex ratio with increasing age has given rise to a number of conjectures, some of which are based on embryological or gynecological considerations (1-3). From the genetic point of view, one might be inclined to wonder if possibly this decrease in sex ratio is, in fact, a function of the age of the father, with the above relationship being a simple consequence of the correlation between ages of spouses.

A simple statistical test has been made of data bearing on this point. From the analysis described below, it appears that this decrease in sex ratio is actually linearly related to the age of the father and is independent of the age of the mother.

The appropriate statistics giving the sex of each child at birth, with the ages of the mother and of the father, are given in the yearbooks of the U. S. Bureau of Vital Statistics for the years 1947, 1948, and 1949. The figures for the three years for the whites have been combined, including only those births for which the ages of both parents were known.

A multiple linear regression has been calculated for these data. Only those aspects of the calculations bearing on the general argument and the tests of significance will be presented here.

¹ This investigation was supported by a research grant from the National Cancer Institute, of the National Institutes of Health, USPHS.

TABLE 1

EFFECT OF CARBON MONOXIDE ON OXYGEN CONSUMPTION BY SWEET POTATO SLICES, BY PURIFIED PHENOL OXIDASE, AND PURIFIED CYTOCHROME OXIDASE FROM SWEET POTATO, AND THE EFFECT OF LIGHT ON THE OBSERVED INHIBITION

Experimental material	Gas ratio (in the liquid phase)	Percentage inhibition of oxygen consumption in a given ratio of CO/O ₂ as compared with a control in N ₂ /O ₂ of the same ratio	
		In the dark	In the light
Slices	3.1	25	0
“	6.8	46	0
“	14.4	67	12
Phenol oxidase	6.8	80	81
Cytochrome oxidase	6.8	68	0

Following the usual convention in approaching problems of multiple linear regression (4), the best fitting plane is defined as

$$Y = a + b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2),$$

where Y = calculated sex ratio for a given set of ages of parents, a = average sex ratio, b_1 = regression coefficient on the age of the father, b_2 = regression coefficient on the age of the mother, x_1 = age of the father, in units of five-year intervals, with the 25-29 age group defined as the 0 class, x_2 = age of the mother, defined similarly to x_1 , and $\bar{x}_1$, $\bar{x}_2$ = average ages of fathers and mothers, respectively, at the time of birth of children.

The calculation of a , b_1 , and b_2 gives the equation

$$Y = 0.5142367 - .000629 (x_1 - \bar{x}_1) - .000196 (x_2 - \bar{x}_2).$$

The standard errors of the constants are:

$$\begin{aligned} s_a &= .000164 \\ s_{b_1} &= .000173 \\ s_{b_2} &= .000202 \end{aligned}$$

The analysis of the sums of squares gives:

Item	χ^2	N
Regression	42.5	2
Remainder	84.8	87
Total	127.3	89

The ratio b_1/s_{b_1} equals 3.6, which corresponds to a P -value of less than .001. However, the ratio $b_2/s_{b_2} = 0.97$, with P -value of 0.3. Thus it appears that there is highly significant effect of the age of the male, but not of the age of the female.

Assuming that b_2 is in fact equal to zero, a simple linear regression may be calculated, which gives the following equation:

$$Y = 0.5142367 - .0007535 (x - \bar{x}_1).$$

The analysis of the sums of squares gives:

Item	χ^2	N
Regression	41.6	1
Remainder	5.6	8
Total	47.2	9

The low value of the remainder χ^2 , although adding very little to the evidence provided by the partial regression coefficients, shows excellent agreement of the data with the hypothesis that the sex ratio is adequately accounted for by simple linear regression on the age of the father.

E. R. Dempster, of the University of California, has analyzed the above data using the path coefficient method of Wright (5), from which it can be estimated that about 23% of the variance in sex ratio is due to a direct effect of the age of the father, 1.6% to a direct effect of the age of the mother, and 9% to a joint effect, the latter two, however, not being statistically significant.

A multiple regression has also been calculated for comparable data for the nonwhite population. The values of the constants are as follows:

$$\begin{aligned} a &= .50630 & s_a &= .00046 \\ b_1 &= -.00074 & s_{b_1} &= .00040 \\ b_2 &= +.00033 & s_{b_2} &= .00161 \end{aligned}$$

Neither of the coefficients differs significantly from zero. It should be pointed out, however, that the total number of nonwhite births for which information on parental ages was available for the three-year period was 1,157,994, whereas the white births amounted to 9,279,697. It does not seem unlikely that additional data will lead to the same conclusion for the nonwhite births as for the white births; i.e., that there is a decrease in sex ratio independent of the mothers' age but dependent on the fathers' age.

Finally, it must be noted that the over-all sex ratio, a , is significantly lower in the nonwhites than in the whites, in agreement with previous comparisons of this kind. This cannot be accounted for by a lower average age of nonwhites at the time of birth of offspring. The average age of white fathers is 0.588 (or slightly more than half of a 5-year unit above the midpoint of the 25-29 year age group) and of the nonwhite fathers, 0.564. This lower age could account for a smaller percentage, at most, of the observed difference.

The conclusion that the changing age of the male, rather than of the female, parent influences the sex ratio does not necessarily mean that a genetic mechanism must be involved. There might be conditions of, for instance, a physiological nature which, changing with the age of the male, would tend to shift the sex ratio. This analysis does indicate, however, that such forces would have to operate on the male sex predominantly, if not exclusively, and, contrarily, those explanations of the shift based on factors affecting the female sex would seem to be excluded. It might be pointed out, also, that this age effect could very well be responsible for other correlations that have been previously established. One such is the decreasing sex ratio with increasing birth order, studied by Cioceo (6). His data give the percentage of males among first-born as 0.5153, and among the fifth or higher order as 0.5124. An average age difference of fathers amounting to only about twenty years would be required to account for this difference. It would be instructive to analyze data giving simultaneously the sex of the child, birth order, and age of the father, if such data were available.

This shift in the sex ratio is superimposed on an average sex ratio which, as is well known, deviates significantly from the simple 1:1. Whether this average deviation is likewise primarily a function of the male sex is a highly controversial question. The simplest genetic considerations suggest that X- and Y-bearing spermatozoa should be produced in exactly equal numbers. It is conceivable, however, that this is not so; one hypothetical system which would allow for a certain amount of deviation has been described by the author (7). With the limited information available at present, this system is purely conjectural, and

consequently no detailed consideration will be given it here. It might be pointed out, however, that this mechanism, based primarily on the nonrandom disjunction of heteromorphic homologues in *Drosophila*, would adequately account for the age effect described here by attributing it to a decreasing level of crossing over with increasing age and would also account for interracial differences as a reflection of the degree of structural heterozygosity in those races.

References

1. GATES, R. R. *Human Genetics*. New York: Macmillan, chap. 20 (1946).
2. HUNTINGTON, E. *Season of Birth—Its Relation to Human Abilities*. New York: Wiley, chaps. 9, 10, 11 (1938).
3. STERN, C. *Principles of Human Genetics*. San Francisco: Freeman, chap. 20 (1950).
4. MATHER, K. *Statistical Analysis in Biology*. New York: Interscience, chap. 9 (1947).
5. WRIGHT, S. J. *Agr. Research*, **20**, 557 (1921).
6. CIOCCO, A. *Human Biol.*, **10**, 36 (1938).
7. NOVITSKI, E. *Genetics*, **36**, 267 (1951).

Manuscript received September 8, 1952.

Hibernation and Cortical Electrical Activity in the Woodchuck (*Marmota monax*)¹

Charles P. Lyman² and Paul O. Chatfield

Departments of Anatomy and Physiology, Harvard Medical School, Boston, Massachusetts

Previous work from these laboratories showed that spontaneous cortical electrical activity could not be recorded in the golden hamster arousing from hibernation until the cortical temperature had reached 19°–21° C (1). These studies, extended to the woodchuck (*Marmota monax*), reveal profound differences between this hibernator and the hamster in regard to the electrocorticogram and in the behavior of the animal during hibernation.

In the present experiment the electrocorticogram and the cortical temperature were recorded by means of a device that had been previously attached to the skull. This device was made throughout of stainless steel and consisted essentially of a plate 1 × 1 × 0.1 cm, which was drilled at each corner to receive screws and drilled and threaded in the center to receive the electrode and thermocouple carrier. The carrier was a short, L-shaped tube of 0.15 cm outside diameter, threaded on one end to screw into the central hole of the plate, and containing 2 silver electrodes and an iron-constantan thermocouple. The electrodes protruded slightly from the end of the threaded tube, and the inside of the tube was filled with polyethylene.³ All wires were coated with the same substance.

To attach the device, the skull of a woodchuck, which was hibernating in a cold room kept at 3°–7° C, was exposed under sterile conditions, and the plate fastened

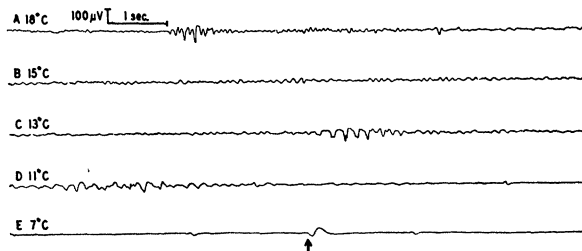


FIG. 1. Electrocorticograms of a hibernating woodchuck at various cortical temperatures as indicated. The arrow in record E indicates a cortical response evoked by noise. Other deflections in record E are artifacts from EKG. Calibration in record A applies to all.

to the skull by the screws. A hole was then drilled in the skull through the central opening of the plate and the carrier screwed in place so that the electrodes rested on the dura. The wires from the thermocouple and electrodes were forced through the subcutaneous tissue and brought out through the skin of the interscapular region. The incision in the skin of the head was then closed over the device. Cortical temperature was measured by a Micromax thermoelectric recorder. The operative procedure, which did not require anesthesia because the animal was hibernating, caused the animal to awaken gradually with a concurrent increase in body temperature. Five days later, when it was observed that the animal was re-entering hibernation, recording of cortical activity (on a Grass ink-writer) was started and continued intermittently for 18 days. During this period the animal's cortical temperature fluctuated between 6° and 35° C.

Since a cortical response could easily be evoked by auditory stimulation, even at cortical temperatures as low as 7° C, it was concluded that the electrodes had been placed on an auditory receiving area (Fig. 1 E). Slow, nondescript, spontaneous cortical activity could also be recorded at this temperature, although it was sporadic. At 11° C and above, spontaneous burst activity was apparent, and the components of the bursts increased in frequency as the temperature rose (Fig. 1 A–D). Adequate recordings at temperatures higher than 18° C were precluded by the large number of muscle artifacts caused by the tensing and movement of the animal.

These observations are reported at this time because Kayser and his co-workers (2, 3) have recently recorded spontaneous cortical activity in the hibernating and artificially cooled ground squirrel at deep body temperatures of 5°–6° C and have commented upon how different their results are from those described by us in the hamster, without suggesting that species differences might explain the discrepancy. Like the ground squirrel, the woodchuck shows spontaneous cortical electrical activity at body temperatures at which it is completely absent in the hamster. Furthermore, the woodchuck shows an evoked auditory cortical potential at temperatures at which the auditory nerve of the hamster does not conduct (4). The general pattern of hibernation in the woodchuck also differs

¹ This research was supported by a grant under Air Force Contract AF 33(038)-18133.

² Associate curator of mammals, Museum of Comparative Zoology, Harvard University, Cambridge, Mass.

³ Clay-Adams Co. Inc., New York.