glucosamine curves by showing greater absorption in the 400-480 mμ range. This difference diminishes when the polysaccharide is partly freed from protein.

Hemolysis, if marked, introduces a positive error in the method. The color produced by the carbohydrate tryptophane reaction obeys the Lambert-Beers law within the limits investigated.

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Oxygen Uptake of Human Placental Tissue as Affected by Selected Substrates and Drugs.*

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Very few human tissues other than the placenta are readily available for *in vitro* studies. Despite this fact, little is known about the influence of metabolites or of pharmacologic agents upon this tissue. Any knowledge that may be gained about the behavior of this complex organ, furthermore, may assist in understanding some unsolved problems of human reproduction. We have selected for study the substrates commonly used for the *in vitro* study of tissue metabolism, and those drugs most frequently employed in obstetrics.

Early reports upon the oxygen uptake of human placental tissue[1-4] gave very low Q_{O_2} values, perhaps attributable to their methods. Loesser[5] studied the oxygen consumption, anaerobic and aerobic glycolysis and in placentas two months of age found values for Q_{O_2} of 5.0, $Q_L^{N_2}$ of 7.0 and $Q_L^{O_2}$ of 5.1. At term, these values were 1.4, 3.5, and 0.57, respectively. Wang and Hellman[6] demonstrated changes in placental Q_{O_2} with relation to age in a manner corresponding well with the histologic changes in the villi. The Q_{O_2} was found to decrease from 5.3 at the second month to 1.7 at term, and the addition of glucose to the medium did not alter this value. No reports have been found regarding the influence of any other agents upon this tissue.

Methods. Placentas were placed in an ice-packed container immediately following delivery. The tissue was prepared for respiration studies in a cold box at 5°C.[7] In choosing the experimental material necrotic, highly calcified, or traumatized areas were avoided. Thick sagittal slices were immersed in Ringer's solution and the villi teased free with dissecting needles. Samples of villi weighing from 200 to 250 mg were blotted on filter paper, weighed, and transferred to Warburg flasks. Conventional manometric methods were used throughout these studies. The medium consisted of 2.0 ml of Krebs-Ringer-phosphate solution (pH 7.3) modified to conform more closely to the composition of interstitial fluid.[8] The substrate was 0.2% glucose unless otherwise specified, and the gas phase was oxygen. The oxygen consumption is expressed as microliters of oxygen per milligram dry weight tissue per hour (Q_{O_2}). In testing the effect of drugs upon oxygen uptake, a control period of 80 minutes preceded the experimental

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period of 80 minutes, with readings obtained at 20-minute intervals. The "normal" Q_{O_2} for each placenta is the average Q_{O_2} of all flasks for the first 80 minutes.

Results. The substrates studied were dextrose, sodium pyruvate, sodium succinate, and hydroquinone. The mean Q_{O_2} of 29 placentas delivered within 2 weeks of the estimated date of confinement was found to be 1.92 ± a standard deviation of 0.373. The presence of dextrose (0.011 M) made no significant difference in the rate of oxygen uptake. The Q_{O_2} could be maintained for 5 hours with a decline of only 13%. Similar results were obtained with sodium pyruvate (0.045 M). The addition of sodium succinate (0.017 M), however, gives an initial Q_{O_2} of 3.6, with a decline of 34% in 2 hours and 66% in 4 hours. Increasing the succinate concentration to 0.2 M raised the initial Q_{O_2} to 7.3, with a decline of 23% in 2 hours. With hydroquinone in concentrations of 0.045 M, 0.091 M, 0.182 M, and 0.364 M, the initial Q_{O_2} values were 16.4, 20.8, and 25.4 respectively. In 2 hours, all 4 values had fallen 78%. For any substrate studied, the figures represent the means of at least 6 observations on 3 placentas. While both succinate and hydroquinone are oxidizable substrates, the maximal Q_{O_2} obtainable with the former is limited by the concentration of enzymes in the succinoxidase system, whereas the maximal rate obtainable with hydroquinone (after correction for auto-oxidation) is limited only by the cytochrome-cytochrome oxidase system.

The drugs selected for study were merperidine (Demerol), methadon (10820, amidone, Dolophine), morphine, Amytal, and scopolamine. Observations were also made on diethylstilbestrol and dinitrophenol. Their effects upon the oxygen uptake of placental tissue are summarized in tabular form.

It will be noted that merperidine, Amytal, and scopolamine have only a depressant action, whereas methadon has a stimulating action in the lower concentrations. Morphine has no influence upon oxygen uptake. Dinitrophenol has the usual marked stimulating effect, while diethylstilbestrol, in aqueous suspension, has the same depressant effect noted on homogenates of liver and pituitary[9] or upon cellular suspensions of rat brain.[10]

Discussion. These studies indicate that the behavior of placental tissue in the Warburg flask is similar in many respects to other mammalian tissues. Like muscle or kidney, placental tissues contain stored substrates, since neither glucose nor pyruvate increase the Q_{O_2} above the non-substrate value. In common with most tissues, placental Q_{O_2} in the presence of succinate or hydroquinone is dependent, within limits, upon substrate concentration and is much higher than that obtained with such carbohydrate substrates as glucose or pyruvate.

The actions of the drugs tested agree qualitatively with effects reported for brain and other tissues. If the mechanism of drug action is likewise similar, it may be assumed that merperidine and methadon inhibit respiration by their action on certain dehydrogenases involved in the oxidation of carbohydrates, whereas Amytal exerts its inhibitory action at a point between the flavoproteins and cytochromes. Comparisons of placenta with other tissues may be made by reference to the remarks in Table I.

With the exception of Amytal, it will be noted that the concentrations of drugs employed are far in excess of the probable therapeutic concentration. It would not be anticipated, therefore, that the employment of such agents in the usual amounts would affect the total oxygen consumption of the placenta *in vivo*. It should be pointed out, furthermore, that the influence of a drug upon placental respiration is not related to the influence which that same drug may have upon the respiratory centers of the fetus. Finally, it must be recognized that our present state of knowledge does not permit us to surmise what effects, if any, a stimulation, whether by succinate or dinitrophenol administration, or a moderate drug depression of oxygen uptake would have upon the multiple functions of placental transfer and secretion.

Summary. The rate of oxygen consumption of fresh human chorionic villi was meas-

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[10] Gordan, G. S., and Elliott, H. W., *Endocrin.*, 1947, **41**, 517.

TABLE I.
Effect of Drugs upon Oxygen Uptake of Human Placental Tissue *in vitro*.

Drug	*In vivo* conc.*	*In vitro* conc.	Maximal effect on Q_{O_2} (in % change from control value)	Comparison with other tissues
Morphine	2 x 10-6 M	2.5 x 10-4 M	0	As found for brain slices.[8]
		5.0 x 10-3 M	0	
Merperidine	1.8 x 10-5 M	2.5 x 10-4 M	—10	Approximately 10 times as sensitive to merperidine as brain slices.[8]
		5 x 10-4 M	—27	
		1 x 10-3 M	—43	
		7.5 x 10-3 M	—93	
Methadon	2 x 10-6 M	2.5 x 10-4 M	+22	Post stimulatory inhibition at low concentrations not as marked as with brain slices.[8] Similar to diaphragm.[11]
		5 x 10-4 M	+38	
		1 x 10-3 M	+12	
		2 x 10-3 M	—93	
Scopolamine	8 x 10-8 M	2.5 x 10-4 M	—11	Initial inhibition followed by partial recovery after 80 minutes.†
		5 x 10-3 M	—21	
		2 x 10-2 M	—56	
Amytal	5 x 10-5 M	1 x 10-4 M	0	Similar to effects of Amytal on brain slices.[12]
		2 x 10-4 M	— 9	
		4.5 x 10-4 M	—31	
		9 x 10-4 M	—63	
		1.8 x 10-3 M	—97	
Dinitrophenol	—	4 x 10-6 M	+56	Usual effect of dinitrophenol.
Stilbestrol	—	aq. suspension 4 mg/vessel	—88	As found for brain homogenates.[10]

* Estimated extracellular concentration after therapeutic dose.
† Data for tissues other than human placenta not available.

ured by conventional manometric methods. The addition of dextrose or pyruvate did not influence the Q_{O_2} whereas succinate and hydroquinone increased the rate in proportion to their concentrations.

The Q_{O_2} of human placental tissue is depressed by merperidine, Amytal, scopolamine and diethylstilbestrol and stimulated by dinitrophenol. Morphine had no effect, while methadon stimulated respiration in low concentrations and depressed it at higher levels. None of the drugs exerted an effect in therapeutic concentrations.

[11] Bachelor, A. L., and Elliott, H. W., *Fed. Proc.*, 1948, **7**, 203.

[12] Fuhrman, F. A., and Field, J., II, *J. Pharmacol.*, 1943, **77**, 392.