AN EVALUATION OF THE RESPIRATORY CAPACITY OF HUMAN SPERMATOZOA

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Summary. The level of human sperm respiration in semen and in artificial media has been estimated by manometric and radio-chemical techniques. Our results show that the respiration is small, exhibiting rates that rarely exceed 1 to 2 $\mu l/10^8$ cells/hr. These low Zo_2 values and the limited sensitivity of the manometric apparatus stress the point that pooled specimens with high cell concentrations must be used in order to obtain reliable estimates of oxygen consumption. Attention is also drawn to the fact that measurements of oxygen uptake by cells in whole semen are further complicated by the high rates and variability of oxygen absorption by seminal plasma and that small errors in the estimation of this absorption can lead to substantial errors in estimates of cellular oxygen uptake. Although isotopic measurements of respiration are more sensitive than manometric techniques, some reserve is also required when interpreting results based on $^{14}CO_2$ evolution alone. Experiments with [U-^{14}C]glucose and [6-^{14}C]glucose indicate that the carbon atoms of glucose are not converted to CO_2 in an identical manner. The higher rates of $^{14}CO_2$ produced from the metabolism of uniformly labelled glucose suggest that a portion of the glucose molecule may be converted to CO_2 through non-oxidative pathways. In view of such a possibility, the ability of washed cells and whole semen to convert [6-^{14}C]glucose into labelled CO_2 provides less equivocal radiochemical evidence than that previously reported for true respiratory activity in human sperm cells. High phosphate concentrations inhibit the conversion of glucose to carbon dioxide. It is suggested that this may represent the presence of competing reactions for limiting intermediates involved in glycolytic and respiratory pathways. We have also described the use of the rapid Millipore filtration technique to follow glucose uptakes and incorporation into acid-insoluble (lipid) components of sperm cells. Uptake into acid-insoluble compounds is rapid and represents a substantial portion of the radio-active glucose that is retained by cells. The potential usefulness of this method to measure endogenous lipid turnover and the role of cellular lipid in endogenous respiration is also considered.

INTRODUCTION

The demonstration of cytochrome pigments in human sperm by Mann (1951) indicated that these cells possess a potential respiratory capacity. However, the extent to which this capacity is expressed has been a matter of controversy because of the conflicting results of manometric studies on oxygen uptake by semen and washed spermatozoa. In early studies with semen, Shettles (1940) reported a high respiratory rate with Zo_2 values ($\mu l/10^8$ spermatozoa/hr) between 6 and 10 and no oxygen uptake by the cell-free plasma. However, MacLeod (1941, 1943) observed a much smaller respiration by spermatozoa in a plasma-free medium with Zo_2 values between 1 and 2, which led him to suggest that oxygen uptake by semen is due solely to the seminal plasma. Ross, Miller & Kurzrok (1941) applied an average correction for plasma oxygen uptake and obtained Zo_2 values between 0 and 13. Davis & McCune (1950) reported an average Zo_2 of 11 for semen, but the values were not corrected for seminal plasma oxygen uptake. Using a calorimeter method, Rothschild (1960) concluded that human spermatozoa were capable of respiration but at a lower rate than bull spermatozoa. However, Williams & Hamner (1963) reported high Zo_2 values (>10) both for semen and for washed cell suspensions. On the other hand, Terner (1960) was unable to obtain any manometric readings with washed sperm suspensions but was able to demonstrate the capacity of washed cells and semen to oxidize labelled glucose, pyruvate and acetate. The conflicting reports cited above have led to a re-evaluation of the manometric method as applied to the measurement of human sperm respiration.

METHODS

Semen characteristics were determined by the methods that are standard in this laboratory (Freund, 1962, 1963); sperm counts were made in quadruplicate as described by Freund & Carol (1964). In some experiments pooled semen was used; in other experiments cells were separated from plasma by centrifugation (270 g for 10 min), washed twice with a balanced salts medium (see below), and resuspended in the medium. The motility of spermatozoa in semen, rated at 37° C, varied between 50 and 70%, while motilities after washing varied between 30 and 50%.

Two low phosphate media were used, since it has been noted (White, 1954) that human spermatozoa rapidly lose motility upon dilution with phosphate diluents. It has also been found (MacLeod & Freund, 1958; Freund & Mac-Leod, 1958; Rothschild, 1960) that dilution of human semen with 0·25 M-disodium phosphate or with a more complex phosphate buffer resulted in a reduction of metabolism and motility. Norman–Johnson-Solution 2 (N-J-2) (Norman, 1964) was used in some experiments since experience (Freund & Wiederman, 1966) has shown this medium to be superior for maintaining the motility of human spermatozoa. This is a citrate based medium containing 0·0734 M-sodium citrate; 0·0204 M-calcium chloride; 0·0018 M-magnesium chloride; 0·0024 M-sodium chloride; 0·0028 M-potassium chloride; and 0·0002 M-monosodium phosphate. The pH is 7·7 but the buffering capacity is low at physio-

logical pH. A more adequately buffered medium was used in other experiments; this medium contained 0·140 M-sodium chloride; 0·003 M-potassium chloride; 0·003 M-magnesium sulphate; 0·0042 M-disodium phosphate; and 0·00085 M-monosodium phosphate and the pH was 7·4. Sperm motility remained high (50 to 60%) after two washes with this medium. Both media were supplemented with glucose at a concentration dictated by the purpose of a particular experiment.

Manometric measurements

Single-arm Warburg manometers and horizontal capillary differential syringe manometers were used to measure oxygen uptake by raw semen and by washed cell suspensions. A discussion of the advantages of the latter apparatus has been reported (Peterson, Freund & Gilmont, 1967). For measurements with the single-arm manometer, seven experiments were run. Each experiment consisted of six determinations: three flasks containing semen and three flasks containing sperm-free seminal plasma, taken from the same pooled specimen. Sperm-free seminal plasma was prepared by centrifuging at 1500 g for 15 min. Each Warburg flask (15 ml capacity) contained 3·0 ml raw semen (or 3·0 ml sperm-free plasma), 0·3 ml White's phosphate, and penicillin. Centre wells contained 0·2 ml 10% KOH and a folded strip of filter paper. The temperature was 37° C and the gas phase was air. Four measurements of oxygen consumption by spermatozoa resuspended in seminal plasma were run with the differential manometer apparatus. The pooled specimen was centrifuged (270 g for 10 min) and the cells resuspended in a smaller volume of supernatant seminal plasma. The sperm-seminal plasma suspension (2·0 to 2·4 ml) was added to a Warburg flask (6 ml capacity) and an equal volume of sperm-free seminal plasma was added to the control arm flask. Centre wells in both flasks contained 0·1 ml 20% KOH and folded filter paper strips. Measurements were made at 37° C with air as gas phase. Experiments with washed cell suspensions were carried out identically; the control arms contained an equal volume of the suspending medium in place of the sperm suspension.

CO$_2$ measurements

^{14}CO$_2$ arising from [U-^{14}C]glucose and [6-^{14}C]glucose was collected using a gas train similar to that described by Baggioline & Bickel (1966). Cell suspensions were incubated in scintillation vials at 37° C with magnetic stirring. Vials were connected in series by plastic tubing through a cold trap into vials containing 3 ml 0·01 N-KOH. Vacuum lines were used to draw streams of water-saturated air over the cell suspensions and to carry evolved CO$_2$ into the collection vials. After the CO$_2$ was collected, the vials were dried at 110° C, the residue was dissolved in 0·3 ml distilled water, 10 ml Bray's scintillation fluid was added and vials were counted in a Nuclear Chicago liquid scintillation counter. Counting efficiencies varied between 60 and 70%. This collection procedure offers an advantage over that employed by Baggioline & Bickel since the ethanolamine-methanol absorbent used by these workers causes considerable quenching in either toluene or Bray's scintillation fluid; this quenching reduced counting efficiencies to below 20%. An important

TABLE 1

OXYGEN UPTAKE BY SEMEN AND SEMINAL PLASMA (VERTICAL MANOMETRY)

Experiment No.	Sperm count ($\times 10^8$) per flask	Initial motility (%)	Final motility* (%)	Treatment†	Oxygen uptake (μl) Minutes of incubation 30	60	90	120	150	180	Zo_2 ($\mu l/10^8$ sperm/hr) Hours 1	2	3	Mean
1	2·59	60	50	Semen	15·37	31·88	41·27	49·00	57·78	65·82	1·06	1·38	1·08	1·17
				Seminal plasma	14·05	29·12	36·23	42·66	49·28	56·69				
2	1·94	70	50	Semen	16·00	30·81	41·24	48·33	58·34	65·96	0·52	2·99	1·28	1·60
				Seminal plasma	17·59	29·81	36·03	41·53	48·77	56·68				
3	2·78	70	40	Semen	21·43	36·39	44·95	54·45	60·93	70·64	0·68	−0·26	−3·15	−0·91
				Seminal plasma	18·58	34·50	47·66	53·28	64·42	78·25				
4	1·92	60	40	Semen	12·30	27·28	38·78	47·27	52·46	57·36	2·76	0·45	0·36	1·19
				Seminal plasma	8·97	21·99	33·12	41·12	45·04	50·52				
5	2·69	70	40	Semen	22·08	36·40	47·98	57·49	64·07	72·72	2·64	1·32	1·01	1·66
				Seminal plasma	17·25	29·30	39·19	46·84	51·72	59·36				
6	2·99	60	50	Semen	20·08	40·13	53·59	62·98	72·15	79·67	2·70	4·08	3·36	3·38
				Seminal plasma	17·72	32·09	40·45	42·80	47·17	49·50				
7	2·48	70	50	Semen	17·56	28·82	37·69	48·44	56·16	63·89	1·56	1·46	0·89	1·30
				Seminal plasma	16·09	24·96	26·42	40·95	47·17	54·19				
Mean values ± standard errors				Semen	17·83 ±1·35	33·10 ±1·76	43·64 ±2·13	52·57 ±2·25	60·27 ±2·41	68·01 ±2·69	1·70 ±0·37	1·63 ±0·56	0·69 ±0·73	1·34 ±0·47
				Seminal plasma	15·75 ±1·26	28·82 ±1·59	37·01 ±2·48	44·16 ±1·70	50·51 ±2·45	57·88 ±3·64				

* After 3-hr incubation at 37° C.
† 3·0 ml semen or seminal plasma plus 0·3 ml White's phosphate/flask.

advantage of the gas train method was that background counts in controls (isotope in absence of cells) were reduced to zero. Experience indicated that high background counts are difficult to avoid using Warburg flasks in which alkali-soaked filter paper strips are placed in centre wells to absorb labelled carbon dioxide evolved. An assay of $^{14}CO_2$ production from the metabolism of [U-^{14}C]glucose carried out in triplicate with the gas train from the same cell suspension gave a standard deviation which was 5% of the mean.

Measurement of cellular uptake of radio-active glucose

The time course of radio-active glucose uptake was measured by rapid Millipore filtration (Britten, Roberts & French, 1955); 0·4-ml aliquots of washed cells suspensions, incubated in the presence of the radio-active substrate, were removed and the cells collected on membrane filters (Millipore type HA, 0·8 μ pore size, 25 mm diam). The filters were washed with three 3-ml volumes of medium before being placed in scintillation vials, dried and counted. Uptake into acid-insoluble compounds was determined by adding 0·4-ml aliquots of cell suspension to tubes containing an equal volume of cold 10% trichloracetic acid. After 30 min, the acid-insoluble material was collected on Millipore filters and dried in scintillation vials at 100° C for 15 min. Bray's scintillation fluid (10 ml) was added to the vial which was then counted.

Lactate determination

Lactic acid, produced by washed cell suspensions metabolizing glucose, was determined enzymatically with lactic dehydrogenase and diphosphopyridine nucleotide. These determinations were made on duplicate aliquots of medium from which cells had been removed by Millipore filtration.

RESULTS

Manometric measurements

The results of measurements of oxygen consumption by spermatozoa suspended in seminal plasma, as determined by both vertical single-arm manometry and horizontal capillary differential manometry, are shown in Tables 1 and 2, respectively. The Z_{O_2} values indicate that cellular oxygen

TABLE 2

DIFFERENTIAL MANOMETRIC MEASUREMENTS OF OXYGEN CONSUMPTION BY HUMAN SPERM-
ATOZOA SUSPENDED IN SEMINAL PLASMA AT 37° C

Temperature	Experiment no.	Sperm count ($\times 10^8$) per flask	Initial motility (%)	Z_{O_2}			
					Hours		Mean
				1	2	3	
37° C	1	0·90	60	2·33	1·00	1·00	1·44
	2	1·75	60	−1·65	2·40	2·80	1·18
	2	1·50	60	0·670	5·20	4·13	3·33
	3	2·58	50	−0·78	−0·78	−0·78	−0·78
Mean values				0·14	1·96	1·79	1·29

consumption by semen is small with an hourly average value of 1·3 for the 3-hr period. The standard errors of the mean, however, are large which may reflect metabolic differences in the pooled specimens (see discussion below). The standard error is probably also increased as a result of corrections that must be made due to the large amount of oxygen taken up by seminal plasma. The data in Table 1 emphasize that high cell counts are required to obtain reproducible manometric estimates of oxygen consumption. Since the cell count in single ejaculates seldom exceeds 10^8 cells (Freund, 1962, 1963; Freund & Carol, 1964), a consideration of the Zo_2 values reported here shows that such individual specimens would, on the average, consume less than 4 to 5 μl of oxygen over a 3-hr period. This level of oxygen uptake is too low to be reproducibly estimated by manometric apparatus in current use.

TABLE 3

DIFFERENTIAL MANOMETRIC MEASUREMENTS OF OXYGEN CONSUMPTION BY WASHED HUMAN SPERMATOZOA SUSPENDED IN NORMAN–JOHNSON-SOLUTION 2

| Temperature	Experiment no.	Sperm count ($\times 10^8$) per flask	Initial motility (%)	Zo_2			
					Hours		Mean
				1	2	3	
25° C	1	0·56	30	4·46	2·32	2·14	2·97
	2	0·62	40	−0·80	−0·80	−0·80	−0·80
	2	0·62	40	1·45	0·48	−1·13	0·27
Mean values				1·70	0·67	0·07	0·81
37° C	1	0·90	30	1·11	1·22	2·67	1·67
	1	0·90	30	−0·89	3·22	3·22	1·85
	2	0·44	10	9·32	0·68	0·91	3·64
	3	1·10	40	3·09	5·55	3·63	4·09
	4	2·0	30	3·50	—	—	3·50
	4	0·24	30	−5·00	−1·25	0·00	−2·08
	5	1·00	40	1·20	3·10	2·90	2·40
	6	1·25	35	−3·20	0·32	—	−1·44
Mean values				1·14	1·83	2·22	1·70

It was of further interest to compare these results with whole semen with manometric measurements of oxygen consumption by washed cells. The results of these measurements made at 25° C and 37° C are shown in Table 3. At 25° C oxygen uptake was negligible and at 37° C average Zo_2 values were only slightly higher than those determined with whole semen. Although the variability in these measurements is larger, the average Zo_2 values suggest a respiratory capacity for washed cells which is not significantly different from cells suspended in seminal plasma.

Tracer experiments

Text-fig. 1 plots the time course of $^{14}CO_2$ evolution resulting from meta-bolism of [U-^{14}C]glucose by spermatozoa in Norman–Johnson-Solution 2.

Several considerations indicated that the CO_2 is a product of sperm metabolism and not of bacterial contamination. First, the sigmoid curve is more simply interpretable as due to decline in metabolic activity of ageing spermatozoa rather than to respiratory activity of bacteria (exponential kinetics would be expected in the latter case). Second, we have tested a number of specimens for bacteria by plating on nutrient agar. In no case did bacteria exceed 10^3 per ml which is too low to account for the CO_2 produced. Finally, control experiments, in which isotope was added to cell-free media, showed that the suspending media contained no detectable CO_2-producing activity.

Table 4 summarizes results of four experiments, using [U-^{14}C]glucose as substrate, in which pooled sperm samples obtained on different days were used.
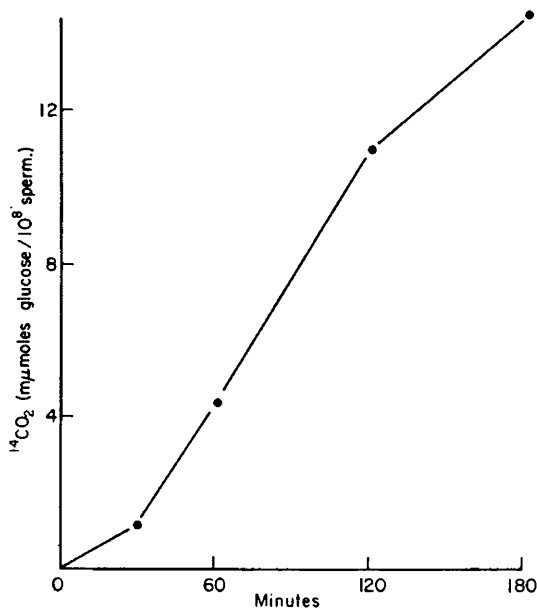
TEXT-FIG. 1. Time course of $^{14}CO_2$ production from [U-^{14}C]glucose by human spermatozoa suspended in Norman–Johnson-Solution 2. Incubation flasks contained 10^{-3} M [U-^{14}C]glucose (275,000 disintegrations/min/μmole) and 0.53×10^8 cells in a volume of 3 ml at pH 7·7 and 37° C. The ordinate expresses the amount of labelled glucose converted to $CO_2/10^8$ cells.

CO_2 production varied over a three- to four-fold range. This variation appears to be related to metabolic differences in the pooled specimens and not to experimental error. When measurements were made on the same pooled specimen, results were more reproducible and, as shown in Text-fig. 2, CO_2 production was not significantly affected by a ten-fold change in cell concentration.

The rate of CO_2 production by human spermatozoa is small (Terner, 1960, 1965). Considering the amount of lactate produced (about 1 μmole/10^8 cells/hr; Peterson & Freund, 1968), it can be estimated that less than 1% of the glucose metabolized is converted to CO_2 which reflects the predominance of

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TABLE 4

$^{14}CO_2$ PRODUCTION FROM $[U-^{14}C]$GLUCOSE $(10^{-3}M)$
BY HUMAN SPERMATOZOA SUSPENDED IN NORMAN–
JOHNSON-SOLUTION 2

Experiment no.	Sperm count $(\times 10^8)$ per vial	$^{14}CO_2$ $(m\mu moles\ glucose/10^8\ cells/hr)$*
1	0·40	8·1
2	0·35	13·4
3	0·53	4·7
4	0·30	2·8
Mean value		7·25

* Specific activity $= 2\cdot 7 \times 10^5$ disintegrations/min/μmole.
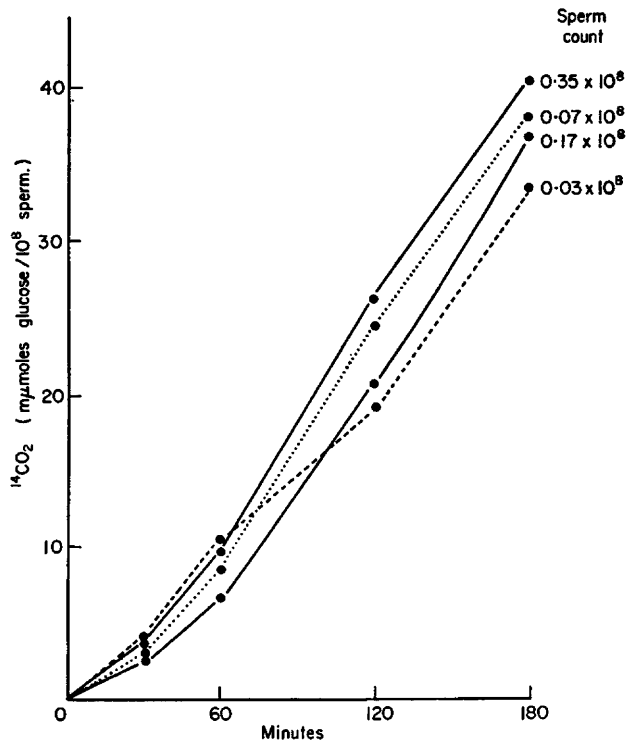
TEXT-FIG. 2. Effect of cell concentration on $^{14}CO_2$ production from $[U-^{14}C]$glucose by human spermatozoa. Incubation conditions were the same as indicated in Text-fig. 1. Each flask contained cells from the same pooled specimen which were washed and diluted to the indicated concentrations in Norman–Johnson-Solution 2.

glycolytic pathways. Assuming a respiratory coefficient of 1, the rate of CO_2 production obtained (7·25 mμmoles/glucose/10^8 cells/hr) would correspond to a Zo_2 of 0·97. However, if rates of $^{14}CO_2$ production from [U-^{14}C]glucose and [6-^{14}C]glucose are compared, estimates of respiratory CO_2 obtained with randomly labelled glucose may be high. This is demonstrated in Text-fig. 3 which shows $^{14}CO_2$ production from each isotope added to a washed cell suspension from the same pooled specimen. The suspension containing uniformly labelled glucose generated $^{14}CO_2$ at more than twice the rate of that
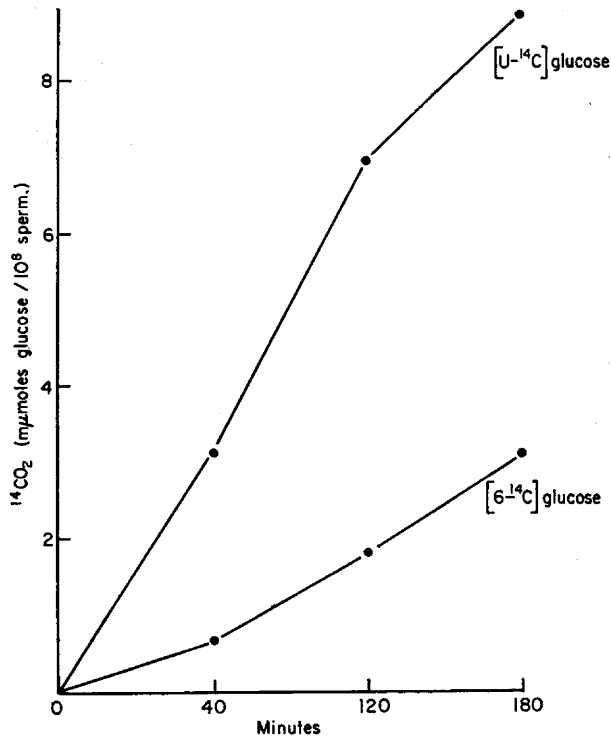
TEXT-FIG. 3. Comparison of the rates of $^{14}CO_2$ formation from [U-^{14}C]glucose and [6-^{14}C]glucose by washed human sperm suspensions. Flasks contained 0·74 × 10^8 cells in buffered salts medium and 10^{-3} M glucose. Specific activities were 275,000 disintegrations/min/μmole [U-^{14}C]glucose and 102,400 disintegrations/min/μmole [6-^{14}C]-glucose.

containing glucose labelled only in the carbon 6 position. This difference could be due to the presence of alternate pathways of CO_2 production such as pyruvate dismutation which occurs in bull spermatozoa (Terner, 1959). This reaction generates CO_2 by a non-oxidative mechanism involving carbon atoms 3 and 4 and apparently occurs under both anaerobic and aerobic conditions. Although this reaction in human spermatozoa must still be demonstrated, it would interfere with estimates of respiratory activity based solely on experiments with randomly labelled glucose. The conversion of [6-^{14}C]glucose to $^{14}CO_2$ by washed human sperm cells, however, provides less equivocal radiochemical

evidence than that previously reported that human spermatozoa are capable of true respiratory activity. Since it is not possible to estimate the extent to which each of the remaining carbon atoms of glucose is oxidized by the respiratory process, the data in Text-fig. 3 undoubtedly underestimate the actual rate of sperm respiration. Further, the radiochemical assay does not account for any endogenous respiratory activity which may also contribute to the overall rate.
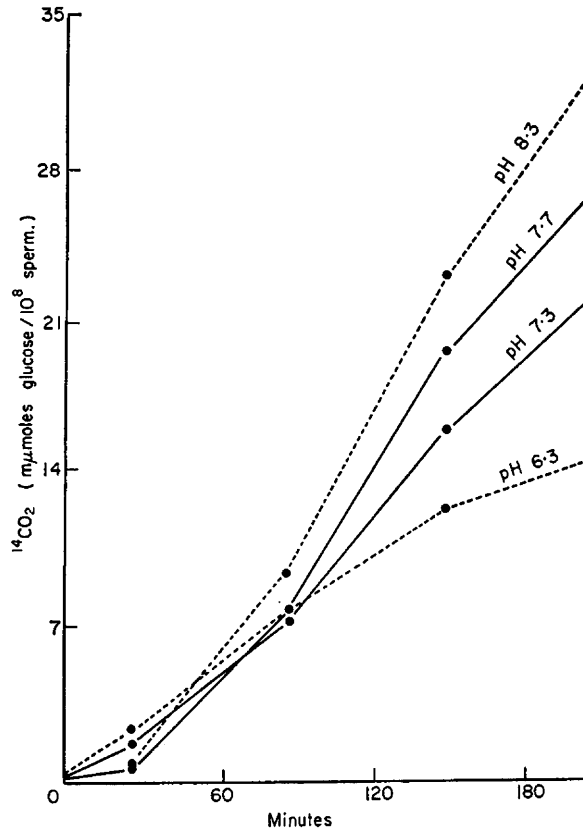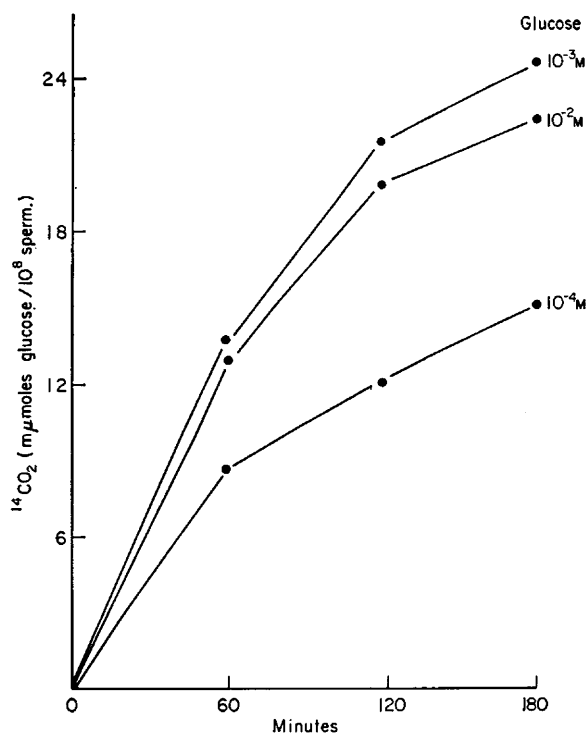

TEXT-FIG. 4. Effect of pH on $^{14}CO_2$ production from [U-^{14}C]glucose by human spermatozoa suspended in Norman–Johnson-Solution 2. Cells were suspended in medium adjusted to the indicated pH values by the addition of dilute (0·005 N) NaOH or HCl. Each flask contained $0·40 \times 10^8$ cells; other conditions were the same as in Text-fig. 1.

Factors affecting $^{14}CO_2$ production from [U-^{14}C]glucose

$^{14}CO_2$ production from uniformly labelled glucose is affected by changes in hydrogen ion concentration (Text-fig. 4). The results were obtained by suspending aliquots of the pooled and washed cell suspension in Norman–Johnson-Solution 2 in which pH was adjusted by addition of dilute HCl or NaOH. The rate of $^{14}CO_2$ evolution was maximal at the highest pH (8·3) but declined nearly two-fold when pH was reduced to 6·3. It has been noted (Blackshaw & Emmens, 1951) that human sperm motility is enhanced in moderately alkaline media

and it is possible that such increases in motility could be related to stimulation in respiratory activity.

The rate of labelled CO_2 production is also affected by changes in the concentration of glucose (Text-fig. 5). $^{14}CO_2$ formation is maximum at a glucose concentration of 10^{-3} M. A ten-fold further increase in concentration did not increase respiration but instead slightly decreased the rate. Other experiments have shown that lactate production from glucose also does not increase above a glucose concentration of 10^{-3} M. These results might suggest that the oxidative process is limited by the glycolytic rate at high ($>10^{-3}$ M) glucose concentrations. However, when dinitrophenol is added to these cell
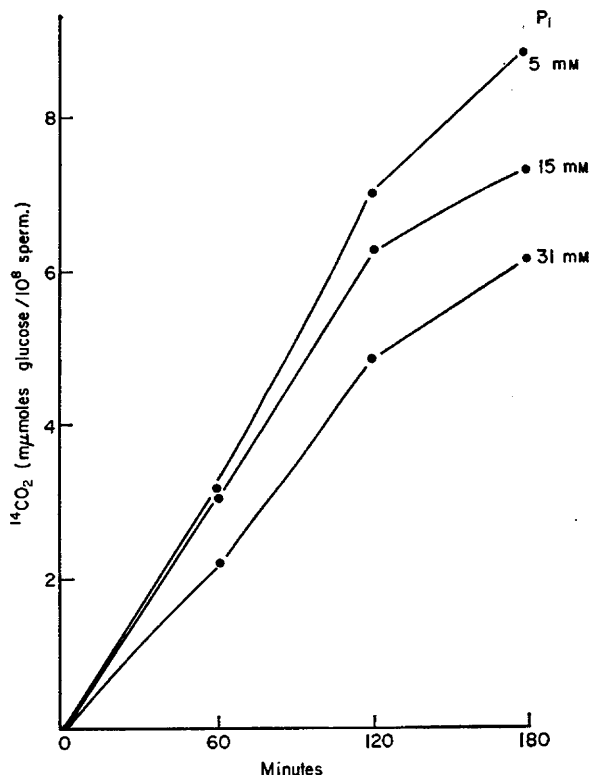

TEXT-FIG. 5. Effect of glucose concentration on the rate of $^{14}CO_2$ production from [U-^{14}C]glucose by human spermatozoa. Flasks contained 0.47×10^8 cells in salts medium buffered at pH 7·4. [U-^{14}C]Glucose was added at the indicated concentrations. Specific activities were 5×10^6 disintegrations/min/μmole (10^{-4} M), 5×10^5 disintegrations/min/μmole (10^{-3} M) and 5×10^4 disintegrations/min/μmole (10^{-2} M).

suspensions, glucose oxidation is almost doubled while the glycolytic rate is only slightly increased (10%) (Peterson & Freund, 1968). This would indicate that oxidation is limited by phosphate acceptors rather than the availability of glycolytic end products at saturating glucose concentrations. We attempted to test this possibility by determining the effects of added adenine nucleotides and inorganic phosphate on labelled glucose oxidation. ADP and 5′AMP, added at 1 mM had little effect on $^{14}CO_2$ production while high concentrations of inorganic phosphate inhibited the rate (Text-fig. 6). All three compounds

had little or no effect on the glycolytic rate, but it is conceivable that these results reflect in part the inability of the charged nucleotides to penetrate the cell membrane. It may be possible to obtain more conclusive results by using procedures which alter membrane permeability before adding test substances.

We have also initiated experiments designed to determine the metabolic fate of glucose added to sperm cell suspensions. Most of the glucose is metabolized to lactate with only a small fraction being converted to carbon dioxide but there is also a fraction that is retained by cells. This is shown in Text-fig. 7 where total glucose taken up by washed cell suspensions and glucose converted

TEXT-FIG. 6. Effect of inorganic phosphate concentration on the rate of $^{14}CO_2$ production from [U-^{14}C]glucose by human sperm cells. Flasks contained 0.74×10^8 cells suspended in phosphate buffered medium. Isomolarity was maintained in each flask by the subtraction of osmolar equivalents of NaCl for each increment in phosphate ion concentration. The concentration of glucose was 10^{-3} M (275,000 disintegrations/min/μmole).

to acid-insoluble cellular compounds has been followed, using the technique of rapid Millipore filtration. The difference in the two curves represents uptake into acid-soluble compounds. The figure shows that uptake into the acid-insoluble pool increased slowly and reached a maximum after 60 min. This suggests a relatively slow turnover of a portion of the compounds making up the pool. Thereafter, radio-activity in the soluble pool declined and after

120 min most of the radio-activity retained by cells was incorporated into acid-insoluble material. Since only a small portion of the exogenous substrate was utilized, the decrease in total glucose uptake indicates an increased leakage of pool constituents out of the cell. This may be related to the decline in motility during a particular experiment. Uptake into acid-insoluble material began almost immediately and continued to increase throughout the experiment. At the end of the 1st hour, accumulation into acid-insoluble material represented nearly one third of total radio-activity. Since protein and nucleic acid synthesis is negligible, most of the insoluble material is presumably lipid. This is supported by experiments which showed that labelled glucose can be converted by human spermatozoa into the glycerol moiety of cellular glycerides (Terner, 1966). Our experiments suggest that such incorporation is very rapid and that a considerable portion of the glucose retained by cells is channelled
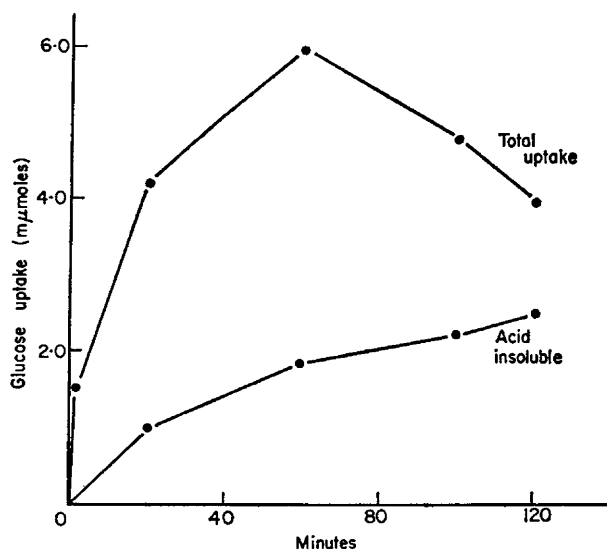
Text-fig. 7. Total uptake and uptake into acid-insoluble compounds of [U-^{14}C]glucose by human sperm cells. Flasks contained $1 \cdot 10 \times 10^8$ cells in buffered salts medium and 10^{-3} M glucose (445,000 disintegrations/min/μmole). Total uptake and uptake into acid-insoluble compounds were followed by the rapid Millipore filtration technique (Methods).

into this pathway. In a separate experiment, it was observed that acid-insoluble material formed continued to increase as glucose concentration was increased from 10^{-4} M to 10^{-2} M. At higher glycolytic rates, larger amounts of carbohydrates are converted to lipid which may serve as a source of energy in less nutritional environments. The experiment shown in Text-fig. 7 gives no information about the turnover of acid-insoluble (lipid) material. Uptake into lipid compounds may represent re-synthesis of lipid lost to catabolic processes or a net accumulation of cellular lipid. The rapid filtration technique, however, provides a method to test these alternatives.

DISCUSSION

In this study both manometric and radiochemical techniques have been used to estimate respiration by human spermatozoa suspended in seminal plasma and in artificial media. Respiration is low and rates rarely exceed 1 to 2 $\mu l/10^8$ cells/hr. This work confirms the manometric observations by MacLeod (1941, 1943) who was unable to detect an appreciable oxygen consumption by spermatozoa suspended in artificial media. The usefulness of the manometric method for measurement of oxygen uptake by human semen depends on an accurate estimate of uptake by the seminal plasma and, because respiration rates are small, the use of pooled specimens with high cell concentrations. Oxygen uptake by plasma is not only large (representing from 80 to 90% of total uptake by semen) but also varies from specimen to specimen. Variations in oxygen uptake by seminal plasma and by spermatozoa might be expected from known day-to-day differences in semen quality in pooled and individual specimens. Changes in sperm concentration, motility, morphology and seminal plasma volume have been shown in specimens from the same individual on different days (Freund, 1962). It is not unlikely, therefore, that such specimens will also show variation in metabolic activity. Obviously, similar variation will occur in pooled specimens (as has been shown by Freund & Wiederman, 1966) since such specimens are often obtained from different donors on different days.

The high Zo_2 values for human semen reported by Shettles (1940) are the result of failing to correct for plasma oxygen uptake. This also seems true of the results reported by Davis & McCune (1950), where plasma oxygen uptake was determined in only two specimens. Since uptake in these specimens was appreciably smaller than the average uptake by semen, the authors attributed most of the uptake by semen to sperm respiration. This is hardly justifiable in view of the variation in plasma volume and plasma oxygen uptake that occurs among different specimens. Clearly erroneous Zo_2 values will also result if an average value for plasma uptake is subtracted from total oxygen consumption. This appears to have been done by Williams & Hamner (1963) and may explain the unusually high sperm respiration rates reported.

There appears to be little difference in respiratory rates of spermatozoa in semen and washed cells suspended in Norman–Johnson-Solution 2 as determined by manometric measurements, and the rate of conversion of labelled glucose to CO_2. The average conversion rate of uniformly-labelled glucose to carbon dioxide (7·25 mμmoles/10^8 cells/hr) falls between the two markedly different values reported earlier by Terner (1965) for similar experiments (19·6 mμmoles/10^8 cells/hr, 1960; 3·82 mμmoles/10^8 cells/hr) 1965). These values correspond to a range of CO_2 production between 0·5 and 1·8 μl $CO_2/10^8$ cells/hr and, assuming a respiratory quotient of unity, are in good agreement with the manometric estimates of oxygen consumption. The lower rate of CO_2 formation from [6-^{14}C]glucose, however, suggests that a portion of the labelled CO_2 arising from the metabolism of uniformly labelled glucose may be due to non-respiratory pathways. This observation together with a lack of knowledge concerning the contribution of endogenous metabolism to CO_2

production places a degree of uncertainty as to how closely labelled CO_2 formation is related to oxygen uptake. The results obtained with $[6-^{14}C]$-glucose suggest a minimum rate of respiration and these isotope experiments support the conclusion that the respiration of washed cells is small and not significantly different from the respiration of spermatozoa in semen. Questions regarding the reason for this small respiration remain to be answered. Evidence has recently been presented (Nevo, 1966) that human spermatozoa respire in the absence of exogenous substrate and that this endogenous respiration supports motility. The mitochondrial apparatus may therefore serve in those circumstances where exogenous hexose is unavailable or in low supply (for example, sperm passage through the female reproductive tract). Presumably, lipid reserves support this respiration.

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