MATERIAL AND METHODS

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Pregnancy is an ideal physiological condition of gradual adaptation to different changes in bodily functions to fulfill the demand of growing fetus. Respiratory system is the most sensitive and vulnerable system amongst others to variation in body status. Therefore, study of pulmonary function tests is very much beneficial and holds a key position in clinical investigations contemporarily. Regular assessment of pulmonary functions by finding lung volumes, capacities and respiratory efficiency tests during gestational period would enable a clinician to manage mother's physical health; consequently fetal health is indirectly taken care of.

Formal officiating agendum was carried out for the study. Authorization for the study was procured from institutional authorities of corresponding academic departments viz. Head, Department of Physiology and Obstetrics and Gynecology, Medical College and Shree Sayaji General Hospital, Vadodara; Dean, Medical College and Superintendent, SSG Hospital, Vadodara. The ethics for the research were strictly followed for carrying out the study. Patients consent was taken before incorporating them in the study sample.

The *ambience of the laboratory setting* for assessing pulmonary functions of the subjects is considered to be most important factor in relation to subjective analysis of pulmonary function tests. The milieu of the lab was quiet, peaceful and anxiolytic with room temperature varying between 28^0 C to 35^0 C, minimum humidity and normal aqueous water vapor pressure. To avoid and to minimize the effect of irritating particles of drugs and dust in air, room location was selected in such a way that its atmosphere was pleasing with natural surroundings and hygienic conditions were maintained. Lab location and decor was given importance to make the subject comfortable while assessing her pulmonary functions during gestational period.

The pulmonary function tests are group of tests where many instruments and equipments are required. Hence, respiratory laboratory was set up especially for study purpose. Weighing machine along with height measuring scale, thermometer, barometer, hygrometer, stopwatch, measuring tape, sphygmomanometer, stethoscope, Hutchinson's spirometer, Ludwig's kymograph, Douglas bag, gasometer, mercury manometer etc. were the scientific instruments set in the lab.

Techniques used for data collection were first standardized before initiating the study. All techniques used in this study were traditional in nature rather than sophisticated as they were simple and are being followed universally and comprehensive globally, especially in India. Traditional equipments were used to avoid the problems arising from the voltage fluctuations that affected the sensitivity of the equipment, shortage of recording paper, etc. The equipments required though easy to handle in any condition and environment without significant error were heavy and hence mini respiratory laboratory was set up in Obstetric and Gynecology Department. The pulmonary functions were carried at the place of regular check up. This was done to avoid inconvenience faced by pregnant women and moreover such instruments could not be carried regularly in OPD as and when subjects were studied. To avoid the dyspnoea due to physical exertion respiratory lab for pulmonary assessment was set up at the ground floor in Obstetrics and Gynecology ward

Design of study and preference of the subjects – Two hundred female subjects were randomly taken for the horizontal – latitudinal study groups, viz. – controls, first, second and third trimester according to the gestational period.

Young healthy normal nonpregnant women of childbearing age were incidentally selected for control group. They were from amongst the hospital and college staff and also from those who accompanied the experimental subjects.

The experimental sample comprised of second or third gravid. Pregnant status of experimental group in first trimester was confirmed by immunological latex pregnancy test and by subjective history of last date of menstrual cycle and complains of morning sickness, queasiness, vomiting and heartburn. The gynecologist carried out clinical examination that included routine gynecological examination, pulse rate, blood pressure, etc. The proforma stating the history, investigations and clinical examination carried out was completed for each subject (Annexure I).

Making a choice of the subjects was primarily on the basis of their cooperation, consent and comprehension level. In each group, fifty subjects were included for the study. All the subjects were chosen with in the age range of 25 – 35 years and of middle socioeconomic status. Subjects having more than 10 deciliter of hemoglobin were considered in the sample. All the investigations and tests were performed at fixed time of the day that is morning to evade possible diurnal variations.

During assessment of pulmonary functions, information regarding smoking habits, tobacco chewing, respiratory infection or related disorder of pulmonary system, chronic cardiovascular disorder and any allergic manifestation were sought for. Data of only those who were nonsmokers and had no respiratory allergy, respiratory or cardiovascular problem was taken into deliberation for study purpose. Thus it was ensured as intimated above that all subjects in the study were normal at time of testing.

Required anthropometric measures as height, weight and chest circumference was noted. Routine examination of urine for protein and sugar was done along with hemoglobin estimation to avoid any discrepancy or deviation in pulmonary functions, as these factors are known to affect pulmonary functions.

The purpose and methods involved were briefly stated to the subjects of control group and to the subjects of experimental group who visited the antenatal clinic of obstetric and gynecology department or out patient department (indoor or outdoor patient). All the subjects were instructed to come in morning for pulmonary function tests with light breakfast. All the parameters of pulmonary function tests were measured in standing posture after giving full instructions and guidelines for performing the various tests. The method was demonstrated to them to avoid repetition of the procedure that causes discomfort. All the readings were taken after giving sufficient rest to subjects in between to avoid the effect of fatigue. The subjects were required to make at least three consecutive satisfactory efforts for each parameter and optimum performance from the three efforts was considered for analysis.

Pulmonary function tests were conducted by measuring the lung volumes, capacities and respiratory efficiency tests were executed with help of Hutchinson's spirometer (capacity – 9 liters), Ludwig's kymograph and mercury manometer.

Lung volumes were noted by displacement of bell of cylindrical shape inverted over a tube that forms the core of water filled external cylinder. With the help of calibrated pulley having pointer, displacement of the bell was recorded in liters. A writing point was fixed to the counterweight of bell for recording the graph on smoked paper fixed on the variable speed Ludwig's kymograph. Ludwig's kymograph was used where recording of graph was essential. It is electrically operated having speed gear for only two speeds – fast and slow. The recorded graph on smoked paper is fixed by fixative. After the graph dries, values of lung volumes and indices were calculated.

Before recording the spirogram the vertical axis was scaled to give volume in liters by raising or lowering the bell. Raising the bell from lowered position moved the pulley. With each liter reading a point was marked on vertical line with the writing point. In such a way 1 liter was found to be equivalent to 4.8 cms. The horizontal axis was scaled for time variable that depends on the speed of kymograph. On time axis 1 second was equivalent to 5.9 cms at fast speed

The writing device and the counterweight were adjusted in such a way that it offered no resistance or least resistance to the movement of air to and from the subject's lung. Leveling the spirometer on the base also did this. Water level in the spirometer was maintained throughout the study that was three inches below the rim of internal metal tube (core) of outer cylinder. This was done to prevent leakage of air volume and gave smooth and free movement of bell. Spirometer was used with a large mouthpiece pressed against face to cover mouth and nose together to prevent leakage of expired or inspired air in atmosphere. Before assessing lung volumes for each subject the stale air trapped in spirometer was expelled by raising and lowering the bell five to ten times. The mouthpiece was washed thoroughly and sterilized.

Respiratory rate was obtained simply by inspection of breathing pattern (thoracoabdominal pattern).

Tidal volume, expiratory reserve volume, inspiratory reserve volume, vital capacity and inspiratory capacity were measured by the Hutchinson's spirometer.

TV is the volume of air that is breathed out or in during normal single respiration cycle without any conscious effort. For measuring TV the subject was instructed to expire or inspire normally without any conscious effort in spirometer.

Expiratory reserve volume is the maximal volume of air that can be expired forcefully from the normal resting expiratory level. ERV was obtained by asking subject to inspire and expire normally the atmospheric air and then forcefully expire in the spirometer after normal expiration. For getting TV and ERV the pointer was kept on zero reading of pulley.

Following respiratory parameters were carried out in the present study.

S N	Parameters	Abbreviation	Unit
1	Respiratory rate	f	per minute
2	Tidal volume	TV	ml
3	Respiratory minute volume	RMV	L / min
4	Expiratory reserve volume	ERV	ml
5	Inspiratory reserve volume	IRV	ml
6	Inspiratory capacity	IC	ml
7	Vital capacity	VC	ml
8	Maximum voluntary ventilation	MVV	L / min
9	Respiratory reserve	RR	ml
10	Breathing reserve ratio	BRR	%
11	Forced expiratory vital capacity	FEVC	ml
12	Forced expiratory volume in 0.75 second	$FEV_{0.75\%}$	%
13	Forced expiratory volume in 1.00 second	$FEV_{1.00\%}$	%
14	Forced inspiratory vital capacity	FIVC	ml
15	Forced inspiratory volume in 0.75 second	$FIV_{0.75\%}$	%
16	Forced inspiratory volume in 1.00 second	$FIV_{1.00\%}$	
17	$FIV_{0.75\%}$ / $FEV_{0.75\%}$	-	
18	$FIV_{1.00\%}$ / $FEV_{1.00\%}$	-	
19	Maximum expiratory flow rate	MEFR	
20	Maximum inspiratory flow rate	MIFR	L / min
21	MIFR / MEFR	-	-
22	Maximum mid expiratory flow rate	MMEFR	L / min
23	Maximum mid inspiratory flow rate	MMIFR	L / min
24	MMIFR / MMEFR	-	
25	Breath holding test	BHT	Sec
26	Maximum expiratory effort test	MET	mm Hg
27	40 mm Hg Endurance test	ET	Sec
28	Hemoglobin	Hb	gm% (dl)
29	Heart rate	HR	beats/min
30	Systolic blood pressure	SBP	mm Hg
31	Diastolic blood pressure	DBP	mm Hg

To measure volumes during inspiratory phases the pointer of pulley was kept at 4 liters mark that raised the bell ensuring the air is inspired from the bell instead of atmosphere. The difference between initial and final position of pointer gave the measure of required volume. Inspiratory reserve volume is maximal volume of air inspired forcefully with efforts after normal resting inspiration. This was attained by asking the subject to expire and inspire normally in atmosphere and then forcefully inspire from the spirometer at the end of normal inspiration. Inspiratory capacity is the maximum volume of air that can be inspired from resting normal expiratory level. To get this the subject had to maximally inspire from the spirometer after normal expiration. IC in other terms can be expressed as sum of TV and IRV.

Vital capacity is the volume of air that can be expelled out after maximal and forceful inspiration or volume that can be inhaled maximally after forceful expiration, thus giving the expiratory VC or inspiratory VC respectively. VC obtained by spirometer was the expiratory VC wherein the subject was asked to breathe in maximally and forcefully the atmospheric air and then with all efforts expire into the spirometer.

Maximum voluntary ventilation is the volume of air that can be breathed per minute by maximal voluntary efforts. This was determined by using high flow respiratory valve, Douglas bag and gasometer. High flow respiratory valve is one way valve that prevents the back flow of air and allows the air to move in towards Douglas bag. Douglas bag is made of impervious flexible sheet that can be folded and unfolded to decrease and increase the volume depending on the subjects' performance. It has two openings – one for fitting the mouthpiece and other for connecting with gasometer. The mouth of this bag is fitted with unidirectional valve. Gasometer is hollow from inside and is filled with water. Working principle of gasometer is such that when air is forced in it pressure inside gasometer increases causing turbulence in water. This turbulence brings the movement of pointer on dial that gives the measure of volume collected. There are three small dials on face of gasometer that are calibrated in one, ten and hundred cubic feet. Before collecting air in bag the side tube of the bag connected to the gasometer is clamped. Initially all the pointers of the dials of gasometer are kept at zero reading. Subject was asked to breathe deeply, forcefully, maximally and rapidly in the Douglas bag through the high flow unidirectional respiratory valve for 15 seconds. After this, connecting tube is unclamped and the volume of air collected in Douglas bag is forced in gasometer by folding the bag gradually to push air. This gives MVV in cubic feet per 15 seconds. To convert MVV in L / min the volume of air collected (Y) is multiplied by

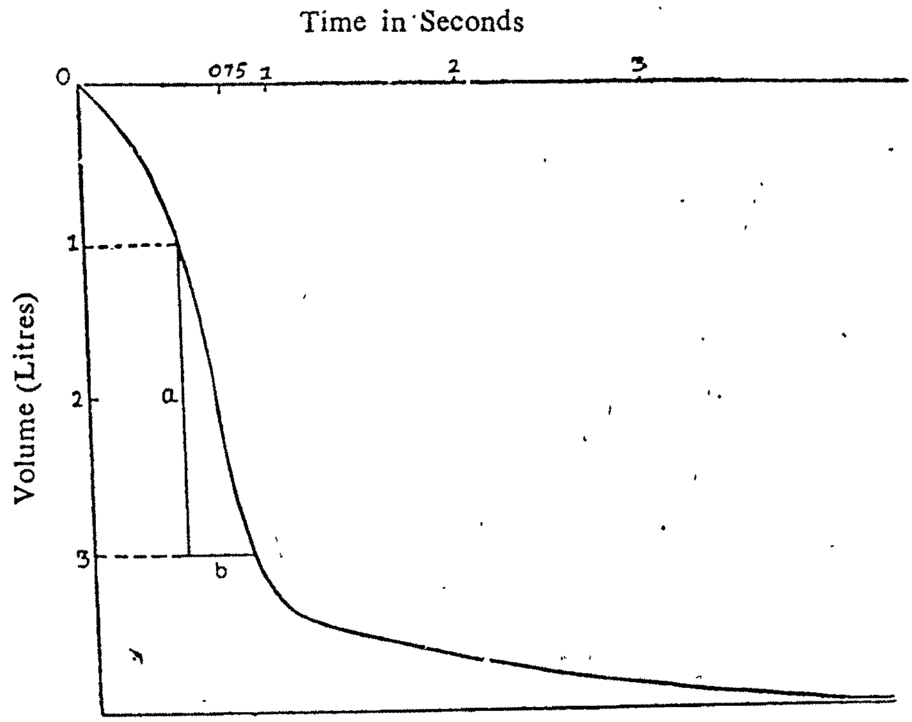

Fig. 5 Graph Showing MMEFR=a/b

Scale X axis 1 Second=5.9 cms.
Y axis 1 Litres=4.8 cms.

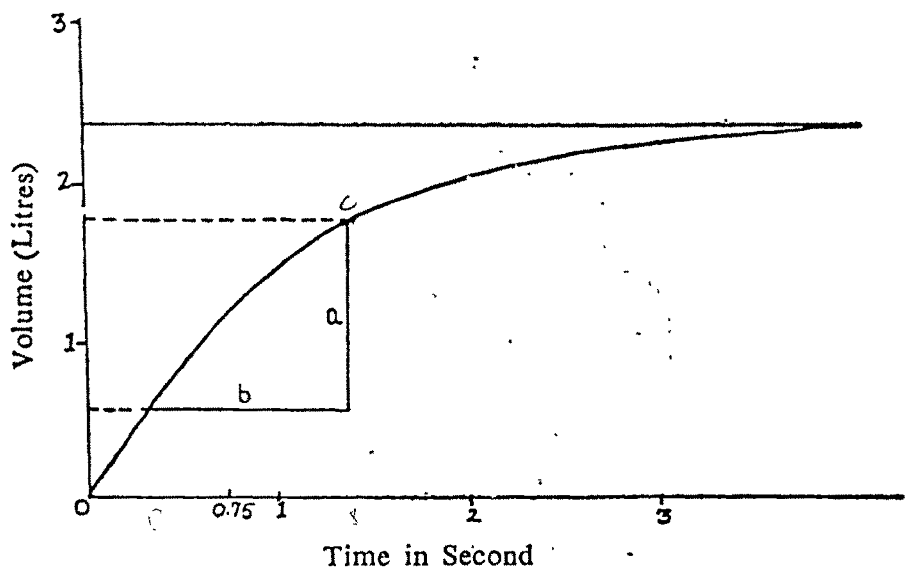

Fig. 6 Graph Showing MMIFR=a/b

Scale X axis 1 Second=5.9 cms.
Y axis 1 Litre=4.8 cms.

conversion factor 27 (to convert cubic feet in liters) and 4 (to convert sec in min). Hence MVV (L / min) = Y x 27 x 4.

Forced vital capacity with inspiration and expiration as mentioned above were recorded graphically with help of Ludwig's kymograph at the speed of 5.9 cms / sec. For forced expiratory vital capacity subject was instructed to inspire maximally followed by rapid, forceful and maximal expiratory effort into spirometer while for forced inspiratory vital capacity subject was instructed to expire maximally followed by rapid, forceful and maximal inspiratory effort. FEVC and FIVC at 0.75 and1.00 seconds were calculated from the graph obtained for FEVC and FIVC (fig 1 & 2). FEV and FIV for mentioned duration were expressed as percentage of FEVC and FIVC. Ratio of FIV to FEV for 0.75 and 1.00 second was calculated to find status of pulmons.

Maximum expiratory flow rate and maximum inspiratory flow rate were obtained from the graph of FEVC and FIVC. The flow rates that is MEFR and MIFR were measured from the graph (fig 3 & 4) by discarding the initial 200 ml of volume and then noting the time taken (b) to exhale or inhale one liter of air volume (a – that is from 200 to 1200 ml). The flow rate (a / b) is ratio of one liter of air breathed to the time taken for the same. Ratio of MIFR and MEFR was computed to determine the airflow status in obstructive or restrictive state.

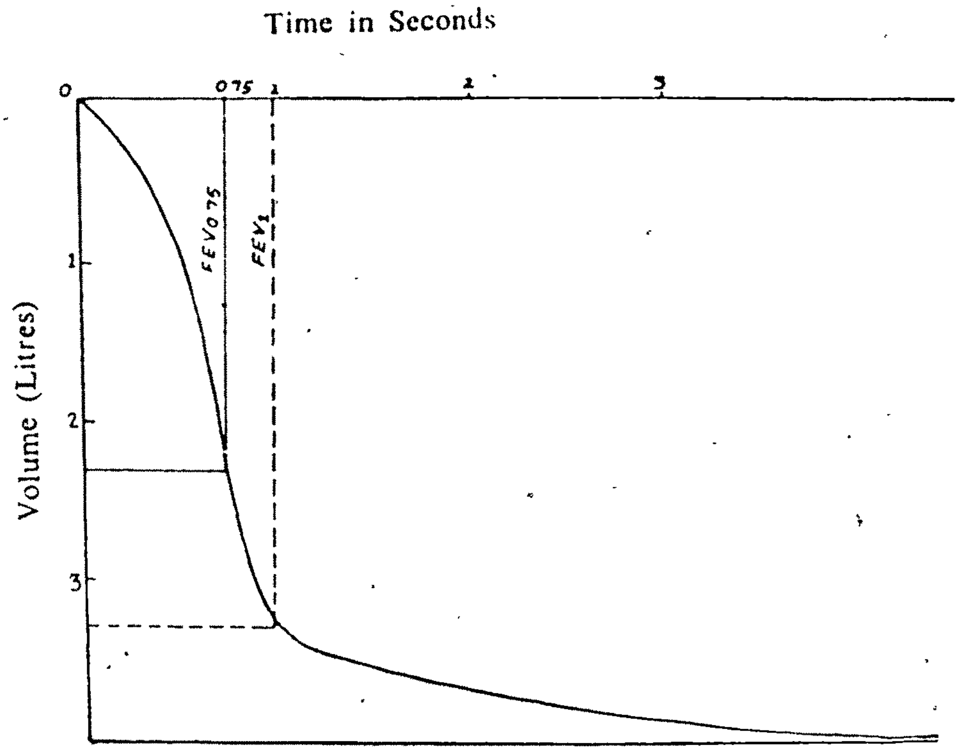

Fig. 1 Graph Showing $FEV_{0.75}$ & FEV_1

Scale X axis 1 second=5.9 cms.
Y axis 1 Litre=4.8 cms.

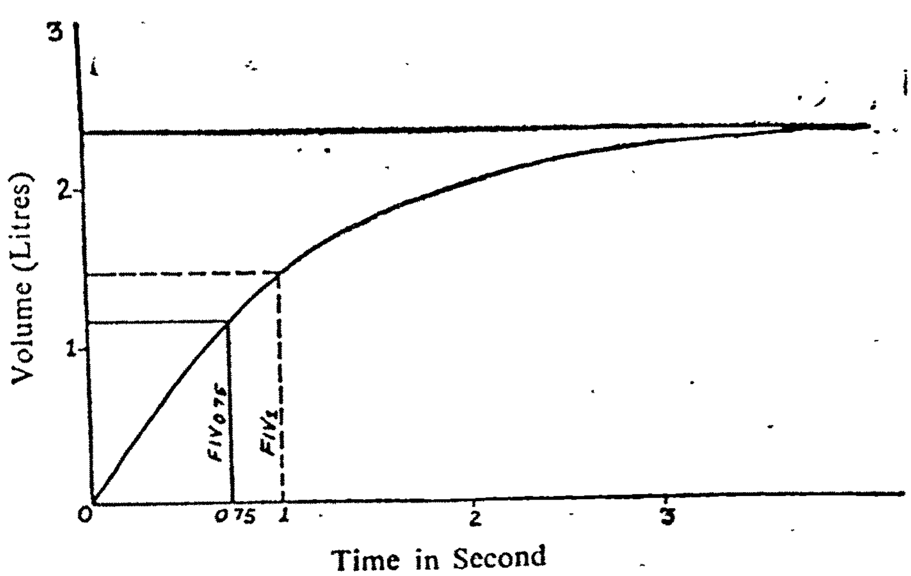

Fig. 2 Graph Showing $FIV_{0.75}$ & FIV_1

Scale X axis 1 second=5.9 cms.
Y axis 1 Litre=4.8 cms.

The maximum mid expiratory flow rate and maximum mid inspiratory flow rate (fig 5 & 6) were also obtained from the graph of FEVC and FIVC. These flow rates that is MMEFR and MMIFR (a / b) were determined by locating points on the volume time curves corresponding to 25% and 75% of FEVC and FIVC respectively (not considering the first and last 25% of FEVC and FIVC). This gives mid 50% of volume (a). Time taken (b) to expire or inspire mid 50% volume was noted. Thus flow rate (a / b) represents the average rate of airflow over the mid portion of forced vital capacity

Respiratory minute volume is the volume of air breathed in one minute in normal resting basal conditions. The product of tidal volume and respiratory rate gives the respiratory minute volume. Respiratory reserve is volume of air breathed with efforts apart from the resting condition in one minute and was calculated by subtracting respiratory minute volume from maximum voluntary ventilation. Breathing reserve ratio was found by taking the percentage of respiratory reserve to maximum voluntary ventilation.

Respiratory efficiency tests measured by mercury manometer were the 40-mm Hg endurance test and maximum expiratory effort test (expiratory pressure test). Asking the subject to raise the mercury level up to 40-mm Hg and hold it with efforts did endurance test. The time to maintain the 40

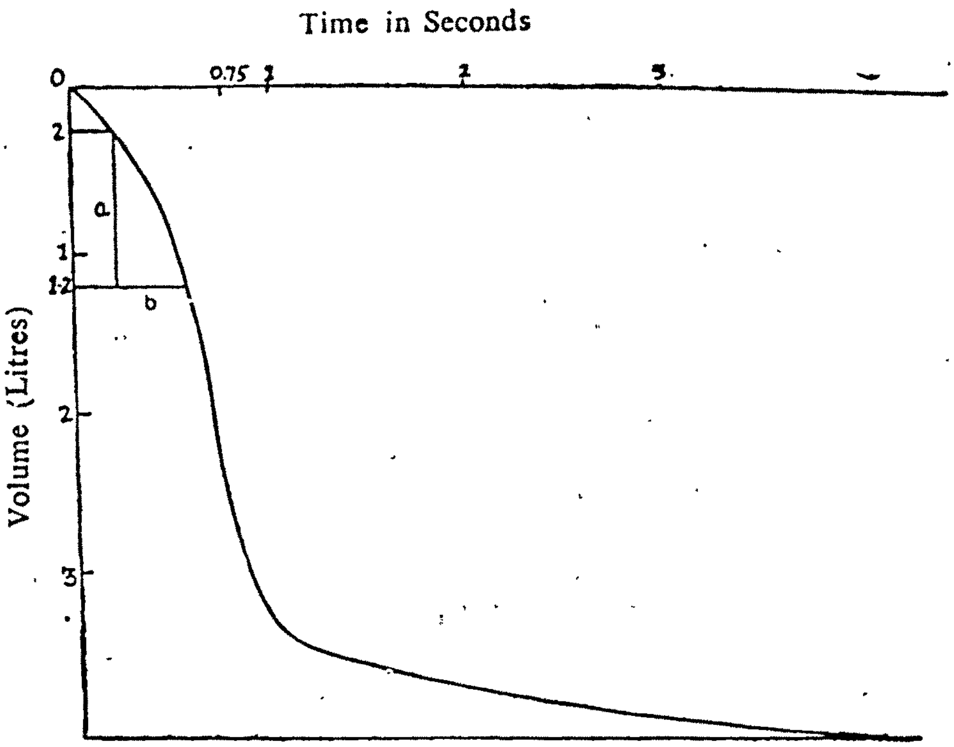

Fig. 3 Graph Showing MEFR=a/b

Scale X axis 1 Second=5.9 cms.
Y axis 1 Litre=4.8 cms.

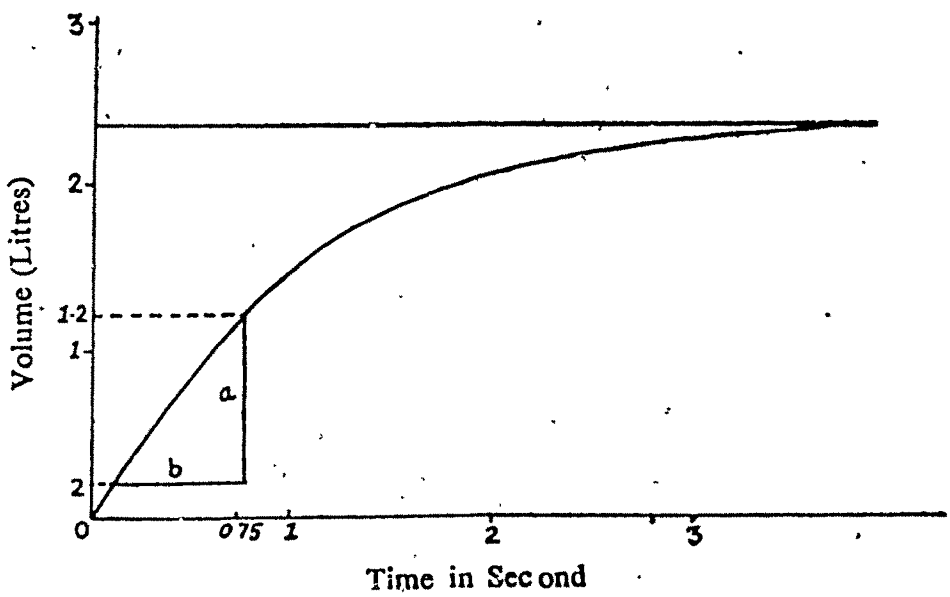

Fig. 4 Graph Showing MIFR=a/b

Scale X axis 1 Second=5.9 cms.
Y axis 1 Litre=4.8 cms.

mm Hg level was recorded by stopwatch. MET was obtained by instructing the subject to raise the mercury level in the manometer as high as possible through forceful expiration in mercury manometer tube with maximum efforts. Breath holding test – closing both nostrils and mouth after deep inspiration and the time taken to hold the breath was noted by stopwatch in seconds.

All the respiratory volumes were expressed in terms of body temperature pressure and saturated with water vapor (Annexure II).

Finally data of only forty subjects in each group was taken for statistical analysis as the performance of the subjects was not up to the mark due to their inadequate cognizance of the instructions given during testing that led to erroneous results of pulmonary functions. The results of all four groups were compared with each other and statistically analyzed. The significant values were expressed in terms of 'P' value by applying student 't' test. The changes in parameters in terms of increase or decrease were expressed as percentage within the groups.