responding point of the agar layer of the "sluice." This process is continuous in the vertical direction, and it is not interrupted by arbitrarily chosen stepwise fractions. The advantages of continuous immunosedimentation analysis are similar to the advantages of immunoelectrophoresis by the method of Grabar and Williams [8] compared with separate analysis [9, 11] of individual stepwise fractions. In general, any two-dimensional fractionation — whether two-dimensional electrophoresis [10] or two-dimensional chromatography [2] — cannot be replaced simply by rechromatography, i.e, by chromatography (in a new system of solvents) of the separate fractions collected at the first stage. The new technique also can be used to investigate proteins with a very large molecular weight, for they are fractionated in an aqueous medium and not in gel.

The method is suitable for analysis of different protein mixtures; it has passed its test for analytical work on neonatal serum for investigation of the immune status.

By contrast to electrophoresis in polyacrylamide gel [10] in sodium dodecylsulfate, the method described above can be used to characterize, not protein subunits, but native, undegraded protein molecules.

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DISTORTIONS OF RESPIRATORY PARAMETERS DURING MUSCULAR WORK IN INVESTIGATIONS USING MASKS

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The study of external respiration during muscular activity is usually based on the spirographic principle. This technique requires a mouthpiece or a mask worn on the face, and the use of these appliances increases the dead space and the resistance to respiration. These two factors may distort parameters of respiration. The use of a mask is known to reduce the respiration rate [6, 7]. It has been suggested that the tidal volume (TV) is increased at the same time, but this has not been verified because of the lack of any maskless method of determining the pulmonary ventilation [9]. Changes in TV have been estimated principally purely on the basis of changes in the degree of resistance to respiration when using a mask [1-3, 8].

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TABLE 1. Distortions of Respiratory Parameters Due to Mask Methods during Physical Exertion

Series	Group of objects	Parameter	Running							
			during free breathing	during constrained breathing						
				No. of cases			limits of variations		mean shift	
				+	—	—	min.	max.	abs.	%
I	Male students	RR, cycles/min	19,6	6	17	1	—11	+23	—2,35	—12
		TV, ml	1372	20	4	—	—340	+652	+248	+17,9
		RMV, liters	23,90	13	11	—	—8,7	+9,7	+0,78	+3,3
	Female students	RR, cycles/min	26,6	19	20	3	—26	+9,5	—0,7	—2,6
		TV, ml	892	29	13	—	—622	+950	+89	+10
		RMV, liters	22,00	22	19	1	—13,8	+14,4	+1,13	+5,1
II	Male athletes	RR, cycles/min	37,5	—	16	—	—0,5	—12,5	—6,1	—16,3
		TV, ml	967	4	11	1	—82	+644	229	+23,7
		RMV, liters	37,89	6	10	—	—10	+19,6	+0,46	+1,23

TABLE 2. Changes in Respiratory Parameters with Alternation of Breathing Conditions during Physical Exertion

Series	Group of subjects	Parameter	Mean shifts during change in conditions of breathing			
			CB-FB		FB-CB	
			abs.	% of FB	abs.	% of FB
I	Male students	RR, cycles/min	+1,7	+9,2	—0,7	—3,8
		TV, ml	—220	—15,6	+185	+13,1
		RMV, liters	—1,31	—5,5	+1,93	+8,0
	Female students	RR, cycles/min	+1,2	+4,5	—0,2	—0,7
		TV, ml	—53	—5,8	+55	+6,0
		RMV, liters	+0,91	+3,9	+0,4	+17
II	Male athletes	RR, cycles/min	+7,7	+20,5	—5,4	—14,6
		TV, ml	—264	—27,3	+197	+20,3
		RMV, liters	+1,02	+2,8	+1,71	+4,7

Legend. CB) Running during constrained breathing through mouthpiece with tube; FB) running during free breathing.

The maskless method of dynamic radiorheopneumography (DRRPG), which the present writers have developed [4, 5], enables the distortions introduced by traditional mask methods when recording respiration to be estimated quantitatively and objectively. Two series of observations have been made by the use of this method.

In series I the data of DRRPG and the spirogram were recorded synchronously in 66 subjects (24 men and 42 women) aged 18-26 years during running on the spot for 5 min. To obtain true respiratory parameters during free breathing the spirograph was disconnected after running for 4 min and respiration was recorded by DRRPG only. These data were compared with the averaged values at the 3rd and 5th minutes, i.e., adjacent periods of running during constrained breathing.

In series II parameters of free and constrained breathing (through a mouthpiece with a tube 60 cm long and with an internal diameter of 30 mm) determined in four athletes aged 18-29 years were compared with data obtained during more strenuous exercise, namely during ing running at a gymnasium. Both frequency and volume parameters of respiration under both conditions were monitored by DRRPG. During running for 9-11 min, periods of free and constrained breathing, each lasting 2-3 min, alternated. An investigation of this kind was undertaken twice on each subject with an interval of 20-30 min, during which the athletes did their usual training routine.

The results were analyzed in two directions: 1) mean levels of respiratory parameters were assessed during breathing under different conditions, by using averaged values for each program; 2) changes due to transition from constrained to free breathing and vice versa were compared. Mean levels of respiratory parameters under the different conditions of breathing are given in Table 1. They show that in both series, in addition to the slowing of respiration observed by several investigators, the DRRPG technique also revealed a corresponding increase in the volume parameters. In men, both types of changes were more marked. During more strenuous physical exertion (series II) distortions under constrained breathing conditions increased significantly. In men, for instance, the respiration rate decreased on aver-

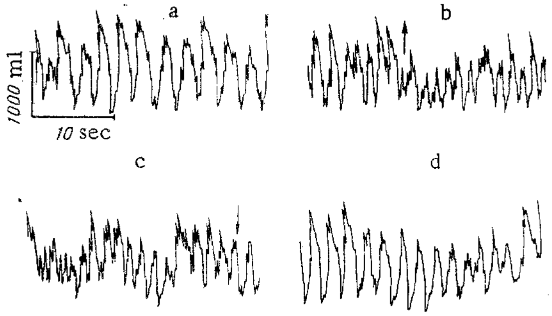

Fig. 1. Radiorheopneumogram during track running. Subject Sh., a man aged 29 years. Recording speed 2 mm/sec. Arrow pointing upward indicates beginning, downward — end of period of free breathing. a) Constrained breathing, b, c) free breathing, d) constrained breathing. Parameters corresponding to above periods: RR 19, 36, and 25 cycles/min, TV (mean values) 953, 664, and 1064 ml, RMV 18 and 11, 23 and 63, and 26 and 61 liters.

age by 16.3% compared with 12%; TV increased by 23.7% compared with 17.9%. One typical example illustrating changes in respiration rate (RR) and TV during running while breathing under the different conditions is illustrated in Fig. 1. Data for the respiratory minute volume are less precise and show only a slight tendency to increase. During even more strenuous exercise — running under more arduous circuit training conditions, according to reanalyzed data of previous observations not published in [5], distortions due to the mask were even greater: On average for nine subjects the decrease in RR was 20.4% and the increase in TV was 33.8%; a distinct increase in RMV also was discovered in this case, on average by 16.8%. Although individual variability was quite high, the results were mainly statistically significant (in series I for RR and TV in men $P < 0.02$ and 0.01, respectively, in women for TV $P < 0.05$; in series II for RR $P < 10^{-5}$, and for TV $P < 10^{-3}$. The tendency for pulmonary ventilation to increase when mask techniques were used during strenuous excerise was linked with the extra work of breathing, which was even more evident when breathing was constrained at high levels of RMV, when it required a corresponding expenditure of energy.

The greater distortions of the structure of breathing introduced by mask techniques in men than in women can be explained by the fact that the slower and deeper breathing which is characteristic of men more easily shows an additional shift in that direction than the rapid and more shallow breathing characteristic of women. The possibility therefore cannot be ruled out that data in the literature on the characteristics of external respiration during exercise, obtained by the use of mask techniques, if they are to be converted into true relationships require various corrections for men and for women.

Comparison of shifts in RR, TV, and RMV during the transition from constrained to free breathing and vice versa (Table 2) shows that in the first case the change in respiration is more substantial. The degree of these differences rises sharply with an increase in the intensity of muscular work. In series I they are nothing more than a tendency, but in series II the shifts during changes in the conditions of breathing in both directions are statistically highly significant (for RR and TV from $P < 0.02$ to $P < 10^{-5}$).

These results are of great interest. First, it can be postulated that during exertion the steady state of free breathing is more stable than the steady state of constrained breathing. Accordingly, the greatest distortions will be expected in cases when muscular work begins actually while breathing is constrained, and its working parameters are formed under those conditions. Second, distortion of respiration when mask methods are used becomes even more evident, for with these methods breathing is usually constrained from the very beginning of exercise.

Views on changes in respiration during muscular work based on the use of traditional mask methods thus require correction, with allowance for data obtained by the maskless technique, which itself introduces no distortions into the process being measured and does not hinder the act of breathing.

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