ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF
DENMARK FINLAND NORWAY AND SWEDEN

Vol. 7
Fasc. 1

THERAPY PHYSICS BIOLOGY

1968
February

TISSUE OXYGEN TENSION OF CERVIX CANCER

Comparison of effects of breathing a carbon dioxide mixture and pure oxygen

by

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The influence of varied inspired oxygen on malignant tissue radiosensitivity has been the subject of considerable research, speculation, and clinical investigation in numerous radiotherapy centers. It is quite well documented that many types of human cancer tend to outgrow their blood supply with consequent development of areas of hypoxic but viable cancer cells. These would presumably be less radiosensitive than the well oxygenated surrounding normal tissues. Attempts have been made to alter this situation by administration of 100 % oxygen prior to irradiation at atmospheric or elevated pressure. Since the use of oxygen under high pressure is more time-consuming and hazardous to the patient, we have chosen to investigate the use of oxygen at atmospheric pressure in patients with carcinoma of the cervix. The present study was designed to determine the influence of adding carbon dioxide to the inspired oxygen on

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tissue oxygen tension in the cervix cancers as determined by polarographic measurement.

Several investigators have compared the use of a mixture of 5 % CO_2 and 95 % O_2 to 100 % O_2 during irradiation of animal tumors. In experiments with spontaneous mammary adenocarcinomas in mice, DuSault (1963) found a higher cure rate following fractionated radiation treatment with the animals on a mixture of 5 % CO_2 and 95 % O_2 than with 100 % O_2. The proportion of mice with impalpable tumor two months after irradiation was nearly as great after treatment with this mixture at atmospheric pressure as with oxygen at three atmospheres. Inch, McCredie & Kruuv (1966) treated two types of mammary carcinomas in mice, an 'isotransplant' which had been carried serially for many generations in C_3H mice, and an 'isoimplant' from a spontaneous C_3H mammary tumor to first and second generation offspring of the same strain. Only in the latter did the 5 % CO_2 mixture give a 'cure rate' (impalpable at 100 days) greater than 100 % oxygen.

Assuming that these results could be explained through an increase in tumor blood circulation, a search for a possible CO_2 effect on the oxygen tension of human cancers seemed indicated. We have examined the oxygen tension in cancer of the uterine cervix by polarography in a large series of patients, and have continuously recorded changes in pO_2 with the patients breathing pure oxygen at one atmosphere's pressure. In an unselected group of these patients we have compared the effects of breathing 100 % O_2 with those of breathing a mixture of 5 % CO_2 and 95 % O_2.

Material and Methods

The *material* comprised 37 patients with carcinoma of the uterine cervix in clinical stage II (36 had squamous cell carcinoma, and one had an adenocarcinoma). Some of the patients were examined two or three times during the early phase of external irradiation, resulting in 55 different examinations.

The comparisons to be analyzed were carried out both before and during radiation treatment. As radiation-induced changes in the tumor tissue could possibly influence the oxygen and carbon dioxide responses, the conditions at the time of the examinations are described, and the results analyzed separately.

Treatment commenced for all the patients with a series of 12 betatron fractions to external pelvic fields, and during this period the present investigation included three special examinations. Irradiations from 31 and 33 MeV betatrons were used, 200 R (Victoreen) skin dose per fraction, each fraction being delivered in about 10 minutes. Five to six fractions were given per week.

The first examination was made before treatment, the second examination

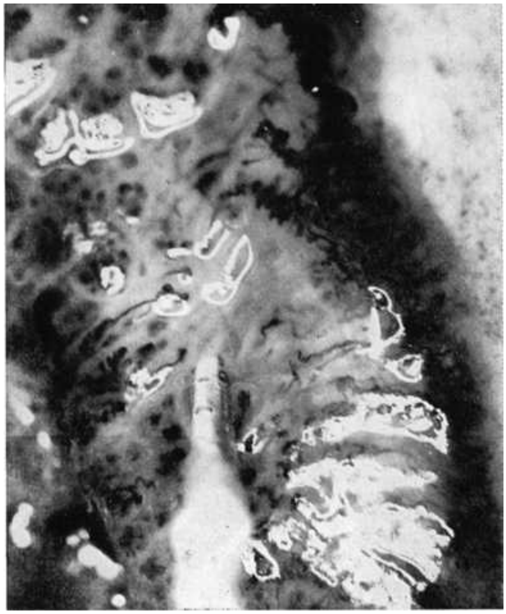


Fig. 1. Electrode in position in tumor tissue. Colpophotograph according to the method of KOLLER (1964). ×10.

after 775 R (Victoreen) delivered in 5 fractions and 6 days, the third examination after 1 700 R (Victoreen) delivered in 11 fractions and 15 days. These average doses have been based upon the isodose charts and a mean estimated tumor depth.

The *oxygen tension* was measured directly in tumor tissue by means of bare platinum micro-electrodes of 250 μ diameter. (The electrodes were kindly furnished by Mr Paul V. Gabel of the Albert Einstein College of Medicine, New York, N. Y., U. S. A.)

The electrodes were placed superficially in the tumors guided by a Zeiss colposcope at 10 to 15 times magnification (Fig. 1). This enabled the examiner to see blood vessels down to capillary size distinctly and choose an avascular area between vessels for placement of the electrodes. As previously described, the distance between these superficial capillaries in cervix cancer is often greater than 350 μ (KOLSTAD 1964). The chance of hitting blood vessels below the tumor surface was diminished by the very superficial position of the electrodes. Active bleeding was seldom observed, either at the time of insertion or during the recording, when the electrodes were controlled regularly by inspection.

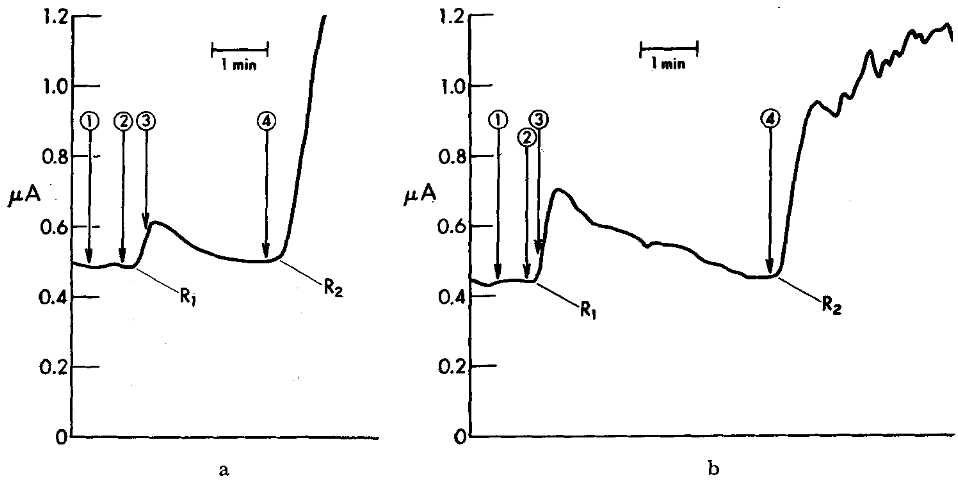


Fig. 2. Determination of response times by continuous recording of the polarographic current. The symbols indicate: 1—the patient breathes air; 2—inhalation of 5 % CO_2 and 95 % O_2 starts; R_1—initial deflection from the control level; distance between 2 and R_1 = response time; 3—air breathing is resumed, and when the original air breathing level has been reached, at 4—100 % O_2 is administered; R_2—new point of deflection from control level.

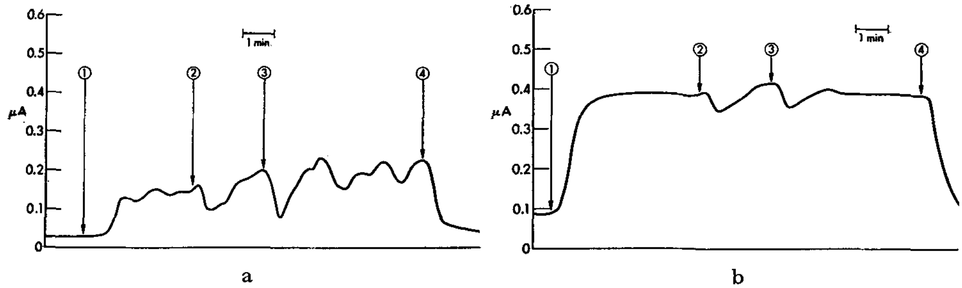
a) Code No. 50, first examination. In this case, both response times were equal, each measuring 9 seconds.

b) Code No. 51, second examination. The response time for the CO_2 mixture was 12 seconds and for the 100 % O_2 it was 9 seconds.

The vagina was kept open by a self-retracting Cusco speculum, and the electrodes were kept in position in the tumor during the whole examination. A Polariter PO4 (Radiometer, Copenhagen) was used for recording the polarographic current and the silver-coated Cusco speculum was utilized as reference electrode. By a sweeping voltage curve with the electrode in tissue, a plateau was noted to occur between — 0.3 and — 0.6 volts. The comparisons of maximum oxygen tensions were made with a fixed voltage of — 0.4 V. Since bare platinum electrodes cannot be calibrated in absolute units of oxygen tension, change in tissue pO_2 are reported in units of microampères (μ A).

Experimental procedure. A platinum electrode was inserted in the tumor tissue and polarized until a steady state current had been recorded. The gases to be compared, 100 % O_2 and the mixture of 5 % CO_2 and 95 % O_2, were then administered alternately at atmospheric pressure through an ordinary anesthesia face mask. A Ruben valve prevented rebreathing of expired air. The patient was instructed, and the mask was applied tightly to the face.

The special examinations were time-consuming, often lasting up to three



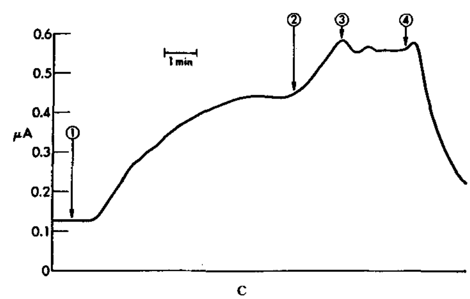


Fig. 3. Determination of plateau levels. Circled numbers indicate: 1—the patient starts to breathe 100 % oxygen and continues to do so until a new current plateau has been reached; 2—inhalation of a mixture of 5 % CO_2 and 95 % O_2 is substituted, changing the masks as quickly as possible; 3—100 % oxygen administration is resumed; 4—the patient breathes air, and the polarographic current returns to the control value.

a) Code No. 93, first examination. The new current plateau was reached at 0.14 μ A. The new plateau level reached with the CO_2 mixture was 0.20 μ A; the rise after resuming oxygen breathing may represent a residual effect of the CO_2. The curve demonstrates a CO_2 effect by the rapid increase in pO_2 from the plateau level during oxygen administration.

b) Code No. 96, third examination. After radiation therapy, the plateau levels of the polarographic current on air and oxygen breathing were higher than at the first examination. A brief administration of the 5 % CO_2 mixture (at 2) resulted in a slight increase in pO_2 from 0.38 μ A to 0.41 μ A, which is not a significant change.

c) Code No. 85, third examination. After administration of oxygen there was again a high plateau current (0.44 μ A). An increase to 0.56 μ A followed the period of breathing the 5 % CO_2 mixture. This is the maximal difference observed after radiation therapy and suggests a CO_2 effect.

quarters of an hour (colpophotography generally preceding the pO_2 measurements). The patients were very cooperative, but not to tire them unnecessarily, certain time limits were observed. Further, we did not want the patients to experience any untoward effects of carbon dioxide breathing, and therefore made them breathe the CO_2 mixture for only a few minutes at a time. This determined the sequence of gas breathing to be found in the examples of records and the tables of results. Concern for the patients precluded an ideal experimental set-up.

Two parameters were compared: 'response time' and 'plateau level'.

Response time is the time (in seconds) from mask application to the first detectable deviation of the recorded polarographic current from the control value. The initial deflection from the control level was generally quite distinct, and to ensure reproducible conditions the mask was always applied immediately prior to inspiration. If the response to both O_2 and the CO_2—O_2 mixture was ill-defined, the records were omitted from comparison. The response time thus represents the lung-uterus circulation time plus the intratumor circulation and diffusion time.

Plateau level represents the maximum increase in current following the first five to ten minutes of oxygen breathing.

All comparisons have been paired, meaning that each patient breathed both gases alternately during the same examination, under essentially the same conditions. The second response time was determined only after the control value of polarographic current, on room air, had been reached. For plateau levels, the masks with different gases were changed as quickly as possible to make the 'dip' in pO_2 as small as possible. The sequence of events is illustrated in the examples of Figs 2 and 3.

Wilcoxon's test for pair differences was used, the probabilities relating to the two-tailed test (Documenta Geigy Scientific Tables 1962).

Results

Response time. Comparisons of response time at the first, second and third examinations are shown in Tables 1, 2 and 3, respectively. No difference was found in any of the groups between the response time of the two gases. If all the response times are compared without relation to treatment, there is still no difference between the gases, suggesting that any small difference between the individual examinations most likely has been due to chance.

Plateau level. Comparisons of the first and third examinations are shown in Tables 4 and 5. Before radiation treatment the tumor oxygen tension attained a higher plateau when the CO_2 mixture was administered to the patient than when pure oxygen was given.

The difference found for the first examination could not be confirmed for the third. It should be noted, however, that the plateau levels on oxygen found at the third examinations were generally much higher than those at the first.

In the comparisons of Tables 4 and 5, the CO_2 mixture was administered after the pure oxygen. In a number of patients, oxygen was administered a second time after the few minutes of the CO_2 mixture, with no difference observed in the plateau response.

Discussion

Our results indicate that the inhalation of a mixture of 5 % CO_2 and 95 % O_2 may result in slightly higher tumor oxygen tension in cervix cancer before radiation treatment than the inhalation of 100 % O_2, at one atmosphere's

Tables 1 to 3

Comparison of response times at the 1st, 2nd and 3rd examinations — Horizontally, the response times are arranged in true examination order, vertically the arrangement is according to Wilcoxon's test for paired comparisons — CO_2 indicates the 5 % carbon dioxide mixture and O_2 the pure oxygen

Code number	Response time in seconds (in order of application)			Wilcoxon's test between CO_2 first and O_2 second		
	CO_2	O_2	CO_2	Diff.	Rank number	
Table 1 — First examination						
50	9	9	—	0		
56	21	21	—	0		
43	15	17	—	2	1	
B-a	12	9	—	3		2
B-b	18	21	15	3	3	
57	6	9	—	3	4	
41	24	21	21	3		5
53	45	39	—	6		6
42	33	42	15	9	7	
52	0	15	—	15	8	
				$T_2 = 13$ $n = 8$ $2\alpha > .10$ No difference		
Table 2 — Second examination						
38		51	33			
48	—	15	18			
39	15	15	—	0		
51	12	9	—	3		1
43	18	12	12	6		2
44	18	12	—	6		3
40	21	10	10	11		4
52	14	0	—	14		5
				$T_1 = 15$ $n = 5$ $2\alpha = .10$ Series too small for conclusion		
Table 3 — Third examination						
38	15	15		0		
39	24	24		0		
52	18	18		0		
42	27	30		3	1	
35	15	10		5		2
43	30	36		6	3	
40	18	12		6		4
48	3	9		6	5	
47	21	30		9	6	
				$T_2 = 6$ $n = 6$ $2\alpha > .10$ No difference		

Tables 4 and 5

Comparisons of plateau levels — Arrangement as for tables 1 to 3

Code number	Baseline current (air breathing) $\mu A \times 100$	Plateau level $\mu A \times 100$		Plateau level increase over baseline in %		Wilcoxon's test		
		100 % O_2	5 % CO_2 95 % O_2	100 % O_2	5 % CO_2 95 % O_2	% Diff.	Rank number	
Table 4 — First examination								
90	14	19	19	136	136	0		
105	10	18	17	180	170	10		1
94	3	23	22	766	733	38		2.5
111	3	4	5	133	166	33	2.5	
110	5	13	15	260	300	40	4	
104	1	3	4	300	400	100	5	
101	1	11	13	1 100	1 300	200	6	
103	1	25	27	2 500	2 700	200	7	
93	3	14	20	467	667	200	8	
96	1	13	17	1 300	1 700	400	9	
						$T_2 = 3.5$ n = 9 $.05 > 2\alpha > .02$ Significant difference		
Table 5 — Third examination (after irradiation)								
84	20	44	43	220	215	5		1
83	7	11	12	157	172	15	2	
89	6	16	18	267	300	33	3	
96	9	38	41	422	456	34	4	
87	20	67	76	335	380	45	5	
86	13	39	32	300	246	54		6
88	11	22	15	200	136	64		7
90	14	36	45	257	322	65	8	
94	11	54	45	491	409	82		9
85	13	44	56	338	432	94	10	
						$T_2 = 23$ n = 10 $2\alpha > .10$ No difference		

pressure. Since the inspired gas contained 5 % by volume less oxygen, the opposite effect could be expected if the hypercapnea was without effect. This effect of CO_2 on tumor oxygen tension could not be observed during radiation treatment. No difference was found in the response time between the two gases.

Carbon dioxide acts as a respiratory stimulant. The effect on the circulation is complex. There is increased cardiac output. The direct effect on blood vessels is dilatation, whereas the indirect effect is activation of the sympathetic nervous system. Peripherally local vasodilatation appears to be stronger than the nervous vasoconstriction (GOODMAN & GILMAN 1965). There is evidence that carbon dioxide also acts directly upon tumor vessels of transplantable mouse hepatomas (KLIGERMAN & HENEL 1961) but the response is unpredictable. Another factor to consider is that carbon dioxide produces a shift to the right in the oxyhemoglobin dissociation curve, the most important result probably being a better oxygen yield to the tissues.

These considerations suggest that CO_2 might increase oxygen tension in peripheral tissues, and possibly also in tumors. Increased tumor oxygen tension is also the most likely explanation of the better results of radiotherapy in animals reported by DUSAULT (1963) and by INCH, MCCREDIE & KRUUV (1966). The latter investigators noted an effect in poorly vascularized isoimplants but not in well vascularized isotransplants.

Our results may be of some help in differentiating the mechanisms whereby hypercapnea might increase tumor oxygen. Since the measurement of lung-tumor circulation time, indicated by the 'response time', remains unchanged, increased cardiac output does not seem to be a significant mechanism. Peripheral vasodilatation might give a real increase in tissue oxygen tension, or an apparent increase due to lessened distance from the measuring electrode to the capillary wall. An increase in the plateau reading of pO_2 with the CO_2 mixture was observed only in the unirradiated cancers. This suggests that if the tumor vessels are already maximally dilated by the radiation effect, similar to the radiation erythema of skin, there will be no response to other vasodilating agents. The higher plateau level of tumor pO_2 on oxygen after radiation therapy would support this assumption. A similar rise in average pO_2 in tumor following external radiation therapy has been observed with the patient breathing air in a much larger series of cases (to be published elsewhere). Since the plateau reading is higher after radiation, both with air and oxygen breathing, a vasodilating effect of CO_2 may be present but more difficult to demonstrate, and possibly of less significance. A rise in pO_2 due to a shift in the oxyhemoglobin dissociation curve would not be expected to show any relation to radiation treatment, and may be assumed to be of less importance.

Since the CO_2 mixture was administered after O_2, a conditioning effect of pure O_2 cannot be ruled out. As previously stated, oxygen was administered a second time after the few minutes of the CO_2 mixture in several patients, with no difference observed in the plateau response. To really test this possibility, however, would have required more prolonged administration of the CO_2 mixture before O_2, which was not done because of concern for the patients.

Previous studies of cervix cancer have demonstrated that hypoxic foci are probably present (KOLSTAD 1964). Since the range of low pO_2 which causes reduced radiosensitivity but not cell death is very limited, any slight increase in tumor pO_2 may theoretically be of importance (DESCHNER & GRAY 1959). Whether this can be achieved by administration of 100 % O_2 at atmospheric pressure is presently being tested in a clinical trial at the Norwegian Radium Hospital. The present study suggests that a mixture of 5 % CO_2 and 95 % O_2 may give better tumor oxygenation than pure oxygen, if administered at the beginning of radiotherapy.

SUMMARY

Clinical tests were made in 37 patients with carcinoma of the uterine cervix to investigate the effect on tumor oxygen tension of a mixture containing 5 % CO_2 and 95 % O_2 as compared to the effect of 100 % O_2, inhaled at one atmosphere's pressure. Oxygen tension was measured polarographically in tumor tissue. Before the radiation treatment, the CO_2 mixture resulted in slightly higher maximum oxygen tension than the breathing of pure O_2. After a series of fractionated external betatron treatments, this difference had disappeared, which would seem to indicate that the observed increase in tumor oxygen tension associated with the addition of CO_2 be due primarily to vasodilatation of tumor vessels.

ZUSAMMENFASSUNG

Klinische Versuche wurden an 37 Patientinnen mit Karzinom des Cervix uteri vorgenommen, um die Wirkung am Sauerstoffdruck des Tumorgewebes von einer Mischung enthaltend 5 % CO_2 und 95 % O_2 im Vergleich mit der Wirkung von 100 % O_2, inhaliert beim Druck von 1 Atmosphäre, zu untersuchen. Der Sauerstoffdruck wurde polarographisch im Tumorgewebe gemessen. Bevor der Bestrahlungstherapie bewirkte die CO_2-Mischung einen unbedeutend höheren maximalen Sauerstoffdruck als die Inhalation von reinem Sauerstoff. Nach einer Reihe von externen Betatronbestrahlungen verschwand dieser Unterschied, was vermuten liess, dass die mit der Zugabe von CO_2 beobachtete Zunahme im Tumor-Sauerstoffdruck vor allem der Dilatation der Blutgefässe des Tumors zuzuschreiben sei.

RÉSUMÉ

Des tests cliniques ont été pratiqués sur 37 malades atteintes de cancer du col de l'utérus pour comparer les effets sur la tension d'oxygène de la tumeur d'un mélange contenant 5 % de CO_2 et 95 % d'O_2 et les effets de l'oxygène pur inhalé à la pression d'une atmosphère. La tension d'oxygène a été mesurée polarographiquement dans le tissu tumoral. Avant le traitement par les radiations, le mélange contenant du CO_2 donnait des tensions maximales d'oxygène un peu supérieures à celles que donnait l'inhalation d'oxygène pur. Après une série de traitements externes fractionnés par le bétatron, cette différence disparaît, ce qui fait penser que l'augmentation de la tension d'oxygène de la tumeur liée à l'addition de CO_2 soit due essentiellement à la dilatation des vaisseaux de la tumeur.

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