Table 1. Incidence of pulmonary metastases in C57 black mice after removal of the primary growth. The animals were paired (one control and one cortisone-treated) according to the time of appearance and size of the primary growth.

Treatment	No. of animals	No. of animals with metastases	Total number of metastases (gross count)
Controls	38	18	56
Cortisone*	40	31	193

* 0.5 mg of cortisone for 5 days following removal of the primary growth.

and the other received either 4 or 5 injections of cortisone on alternate days. The cortisone (Cortisone Acetate—Upjohn) was administered as 0.5 mg in normal saline dose. All the mice were sacrificed at 14 to 16 days following amputation of the tail. The lungs were removed and all metastases counted with the dissecting microscope, the lungs then being fixed in formalin and sectioned for histological study.

The results are shown in Table 1, where it can be seen that of 38 control mice, not treated with cortisone, 20 were completely free of metastases, whereas in the case of the 40 mice treated with cortisone only 3 did not have metastases. Counts of the total numbers of individual metastases revealed an even greater difference, the control group having 56 and the mice treated with cortisone having a total of 196 macroscopic lesions. The time at which the primary tumor reached a size of 1 cm had no influence on the number of metastases; it can be seen from Table 2 that the behavior of tumors arising on the 14th day was the same as for those arising on the 20th day. The metastases in the cortisone-treated animals were consistently larger than those in the control group. In the latter the lesions ranged in size from 0.5 to 3.0 mm, 60 percent being between 1.0 and 1.5 mm in diameter, whereas in the cortisone treated group they ranged from 1 to 5 mm, 80 percent being between 2 and 3 mm in diameter.

The results of this experiment would seem to confirm the view suggested by Pomeroy that cortisone has an action in stimulating metastasis that acts, at least in part, on the phase of the process that occurs after vascular dissemination. This action seems to be analogous to the action of cortisone in prolonging the life of incompatible grafts of normal tissues in the rabbit (*6*) and in the heterotransplantation of tumors (*7*). In keeping with suggestions proposed to explain these other actions of cortisone, it appears most acceptable to consider this action as one mediated through the host.

Table 2. Incidence of pulmonary metastases in C57 BL/6 mice in relationship to the time of appearance of the primary growth after implantation in the tail.

Time of appearance (days)	14	15	16	17	18	19	20
No. of animals	8	14	12	15	14	8	7
No. of animals without metastases	3	5	4	5	6	3	3
No. of metastases	25	40	41	46	40	28	29

It is of considerable interest to note that the animals in these experiments contained numbers of latent tumor cells present after the original tumor had been eradicated, some of which grew only after an additional stimulus was provided. Apart from the implications of this experiment in relationship to the action of cortisone, it would seem that this technique has much to recommend it both for the study of metastasis, and particularly as a tool for the screening of chemotherapeutic agents for cancer. The mouse containing disseminated tumor cells capable of growing into metastases provides a situation quite analogous with the human being following the surgical extirpation of a tumor. Conceivably some agents may be capable of destroying these isolated cells even though they are incapable of attacking an established tumor mass.

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Effect of Hyperventilation on the Human Electroretinogram

Mathew Alpern, J. Faris, P. Eskildsen, P. Garnett

Pacific University, Forest Grove, Oregon

In a previous paper (*1*) a theory was presented that related the rather marked effects of changes in the blood pCO_2 on certain psychophysical visual thresholds (*2*) to processes within the visual cells. Since then experiments on animals (*3*) suggest that the *a*- and *b*-waves of the electroretinogram (E.R.G.) are responses of the visual cells. Dodt (*4*) has shown that hyperventilation (H.V.) is accompanied by changes in the human E.R.G. response to multiple light flashes. Because of the high frequency of the light stimulus in

his experiments it is not possible to deduce from them the way in which the various components of the E.R.G. are changed during H.V. It therefore seemed important to determine whether variation in blood pCO_2 would be associated with changes in the *a*- and *b*-waves of the E.R.G. response to a single flash of light.

Experiments (*5*) were made on two young dark-adapted observers at moderate and high intensities (0.011×10^5 and 1.1×10^5 trolands (*6*) respectively) of stimulus flash (81 msec duration) and under conditions of normal breathing and rather marked H.V. (*7*) in an apparatus previously described (*8*).

The results of these experiments are summarized in Table 1. At moderate intensities, when no *a*-wave appeared in the E.R.G., H.V. was associated with a significant increase in the size of the *b*-wave. At high intensities, H.V. was associated with a significant decrease in the size of the *a*-wave as well as an increase in the size of the *b*-wave. The two components of the *a*-wave (identified here as a_1 and a_2 respectively) described by Armington *et al.* (*9*) were differentially affected during H.V. However, during H.V. the *a*-wave sometimes disappeared completely in certain responses, but this did not occur each time the light was flashed. All of these effects were reversible once normal breathing was resumed. Figure 1 shows data from typical experiments at the high stimulus intensity.

During H.V. the human E.R.G., which at high intensities of stimulus flash is an I-E.R.G. (*10*) assumes the characteristic shape of an E-E.R.G. such as is obtained by our moderate intensity of stimulus flash.

These data suggest then that changes in blood pCO_2 may affect physiological processes within the eye, perhaps at the photoreceptor level. The conversion in the human eye under conditions of H.V. from an I to an E retina suggests that H.V. reduces the magnitude of the PIII component of Granit (*11*). This interpretation implies that H.V. should produce a change in any effect related to the PIII component of the E.R.G. For example, it has been suggested that alpha adaptation (*12*) and meta contrast (*13*) are related to the PIII component. If so, then it seems reasonable to expect rather markedly different results from these experiments when carried out under conditions of H.V.

Table 1. Electroretinogram response to a light flash (81 msec duration) during normal breathing and hyperventilation (two observers).

Retinal illuminance (trolands)	Wave	Normal breathing* (μv)	H.V.* (μv)	Difference	Statistical confidence level
1.1×10^3	b	131 ± 20	174 ± 15	+43	0.01
1.1×10^5	a	109 ± 15	80 ± 28	−29	.01
1.1×10^5	a_1	68 ± 11	62 ± 19	− 6	.2
1.1×10^5	a_2	43 ± 8	19 ± 12	−24	.01
1.1×10^5	b	188 ± 22	216 ± 20	+28	.01

* Mean ± one standard deviation computed from at least 26 responses in each case.

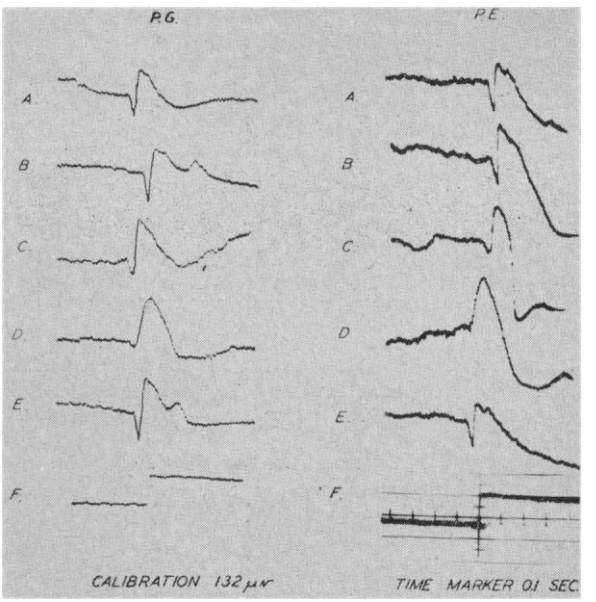

Fig. 1. Effect of hyperventilation on E.R.G. of dark-adapted human being. *P.G.: A,* control response before onset of H.V.; *B,* 1 min after start of H.V.; *C,* 2 min after start of H.V.; *D,* 3.7 min after start of H.V.; *E,* control response after cessation of H.V. and observer reported loss of all subjective symptoms. *P.E.: A,* same as *A* for P.G.; *B,* 2 min after start of H.V.; *C,* 3 min after start of H.V.; *D,* 6 min after start of H.V.; *E,* same as *E* for P.G.

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5. Assisted by grants from the Tektronix Foundation and Theta Chapter, Omega Epsilon Phi Fraternity.
6. The troland is a unit of intensity of light at the retina, equal to the illuminance received per square millimeter of pupillary area from a surface having a luminance of one candle per square meter.
7. No measurement of blood *p*H was made. The characteristic symptoms of H.V. appeared usually after 1 min. The intensity of H.V. was approx. equal to that in previous experiments (*1*) in which the blood *p*H rose about 0.11 units.
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