acetyl chloride (*4*). A thermometer was placed in the tube and the solution was heated to boiling. The resulting yellow solution was then allowed to cool in the atmosphere while stirring gently, and the temperature noted at the moment when the solution turned blue.

The critical thermochromic temperature was unaffected by considerable variation in base concentration. Such a large excess is present relative to the indicator components that its concentration remains practically constant regardless of the extent of the chelate formation. The mole ratios of ferric chloride : propyl gallate : various bases were 1 : 1.5 : 28 to 58.

The aliphatic amines and 2-aminopyridine were strong enough bases to keep the bromobenzene solution of the indicator blue at its boiling point. Attempts to use solvents possessing higher boiling points and/or greater intrinsic acidities to extend the range to strong bases proved futile. However, in view of the wide variation in stability constants of various chelates, it should be possible to find different chelate-solvent combinations for the estimation of other ranges of basicity and acidity.

References and Notes

1. A term coined to indicate change of color with solvent.
2. S. Soloway and A. Lipschitz, *Anal. Chem.* **24**, 898 (1952); S. Soloway and S. Wilen, *ibid.* **24**, 979 (1952); S. Soloway and P. Rosen, *ibid.* **25**, 595 (1953).
3. The detailed presentation of these data, their meaning, and their use as a basis for classifying organic compounds will be the subject of a future publication.
4. The utility of this indicator for the determination of water in alcohols will be described in a later paper. The determination depends on the observation that the addition of water to an alcoholic solution of this indicator causes a color change from yellow or green to blue. This change results from the fact that water functions as a base in dilute alcoholic solution.

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Action of Cortisone on Disseminated Tumor Cells after Removal of the Primary Growth

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It has been reported by several workers (*1*) that repeated injections of cortisone give rise to an increased metastatic spread of a variety of transplanted and induced tumors. This finding has been disputed by Kaliss using a transplanted tumor (*2*). From the sum of evidence available it would seem clear that cortisone does have an effect in metastasis, but that it is one that may well operate only with specific tumors and not with others.

In attempting to analyze this phenomenon Pomeroy (*3*) has found that cortisone will increase the number of "takes" following an intravenous injection of a suspension of transplantable tumor cells. This would seem to indicate that cortisone exerts an action on the phase of metastasis occurring after vascular penetration, and that it might well be primarily concerned with the growth of cells that have already been disseminated systemically; under these conditions it would be expected that different tumors might well respond differently. The use of the intravenous injection technique for the study of metastasis introduces several additional factors not seen when spontaneous metastasis occurs, such as the introduction of many dead as well as living cells, and the introduction of material from another host. In the present experiments (*4*) use has been made of a technique of implantation into the tail, subsequent complete removal of the tumor, and study of cells disseminated previous to excision.

The tumor used was a transplantable carcinoma originally from the bladder epithelium of a C57 black mouse, designated as T150, and subsequently carried in the C57 black strain. The tumor was ground in saline, and the suspension diluted to provide a concentration of 10^7 cells/cm^3; 0.05 ml of this suspension was injected subcutaneously into the tail with a 22-gage needle. The injection was directed caudally to avoid spread toward the base of the tail. Under these conditions a viable tumor growth appears on the 14th to 19th day following injection, reaches a size of 1 cm in diameter in 2 days and then spreads rapidly, invading the subcutaneous tissue of the caudal region of the body. There are three possible mishaps that may occur with this procedure: (i) in some 10 percent of cases the tumor may fail to grow at all; (ii) in some 8 percent of this material the tumor grew diffusely down the tail, instead of as a localized mass; (iii) in scattered instances the injection was made directly into the tail vein, noted at first by an absence of resistance to the plunger of the syringe, and often by distress and even death of the animal. All these occurrences were taken as indications to discard these particular animals.

Tumor T150 is a spontaneously metastasizing neoplasm, inducing multiple metastases in the lungs that have been described elsewhere in detail (*5*). When the growth in the tail has reached a diameter of 1 cm some tumor emboli are released, and preliminary studies have revealed that a small number of metastatic lesions are noted if the tail is amputated at this stage.

For the present experiment 78 C57 black mice from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. were used. They were housed in plastic cages and fed Rockland mouse diet with water ad libitum. The mice were implanted with the tumor in the tail as previously described, and after the appearance of the growth were divided into pairs having tumors of the same size and duration of growth. When the tumors had reached a size of 1 cm the tail was amputated as near the root as possible, and of the pair, one animal was then maintained as a control

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

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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