Sydenstricker, Schmidt and Hall[3] have reported the development of cataracts in rats on a histidine-deficient diet. The study reported here was occasioned by the discovery of a well-developed cataract in a rat fed on a diet deficient in phenylalanine.

Methods. The rats used were from a Wistar strain. When 25-28 days of age they were placed in individual cages and given experimental diet and water *ad libitum*. The phenylalanine-deficient diet and a similar control diet used were those described by Sydenstricker, Hall, Bowles and Schmidt.[4] The development of cataracts was studied in 15 rats from 5 litters which were fed the phenylalanine-deficient diet. Seven rats from the same litters were fed the control diet. Three times weekly the eyes were examined for lenticular abnormalities with the biomicroscope after dilating the pupil with 0.5% solution of atropine sulphate in physiological saline solution. As soon as possible after the death of each deficient rat the lenses were removed, immersed in saline, and examined under a dissecting microscope.

Three deficient rats with cataracts of varying degrees of development were changed to the control diet so that any resulting regression of the lenticular changes could be observed.

Results. Definite lenticular changes were observed in all but 2 of the 15 rats in from 17 to 33 days (mean, 22 days). These changes continued to develop, resulting in a progressive lessening of the transparency of the lenses until at the time of death, after 20-53 days on the diet, various degrees of opacity were observed. The earliest change was a slight haziness of the lens substance. Next the lens star became visible. This was followed by a separation of the superficial fibers, and sometimes by a rough granular appearance of the epithelial layer. No changes in the lens capsule were observed. Finally, in the posterior portion of the lens a dense central opacity developed, eventually filling the central portion of the lens and leaving only a narrow peripheral portion comparatively clear.

Changing the deficient rats to the control diet resulted in a reversal of only the more superficial changes in the lens.

In none of the control rats were any lenticular changes observed.

Summary. The lenticular changes observed to result from phenylalanine-deficiency in the rat were diffuse haziness, opacity of the star, progressive separation of the fibers, granular changes in the epithelial layer and the development of dense central cataracts. Only the more superficial changes in the lenses were found to be reversed by adding *dl*-phenylalanine to the diet.

[3] Sydenstricker, V. P., Schmidt, H. L., Jr., and Hall, W. K., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 59.

[4] Sydenstricker, V. P., Hall, W. K., Bowles, L. L., and Schmidt, H. L., Jr., *J. Nutrition*, 1947, **34**, 481.

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Effect of Podophyllin on Tumor Cells in Tissue Culture.*

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Podophyllin, N.F. is a mixture of substances derived from the roots and rhizomes of the mandrake plant by hot ethanol extraction and subsequent precipitation in dilute HCl. Various preparations of the mandrake plant have been in use for at least a hundred years as laxatives and more recently as supposed

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liver stimulants.

The constituent chiefly responsible for the drastic laxative action of podophyllin is podophyllotoxin.[1] This is a highly toxic compound which causes mucosal inflammation and gastroenteritis. It likewise involves the nervous system resulting (in the cat) in disturbance of coordination of the posterior extremities, rapidly increasing weakness, increased respiratory rate, violent colonic cramps and death in coma.[2,3] The parenteral administration of podophyllin to chickens likewise resulted in nervous system involvement and cytological examination revealed damage or complete degeneration of the cerebellar Purkinje cells and other nervous elements.[4]

Kaplan[5] demonstrated that a suspension of podophyllin in oil was highly effective against *Condylomata acuminata*. These results were confirmed and extended by King and Sullivan[6,7] and by Sullivan and Blanchard.[8] These investigators likewise noted the similarity of podophyllin to colchicine in its effects on venereal warts. Sullivan and Wechsler[9] have shown that saturated aqueous solutions of podophyllin block mitosis in the root tips of *Allium cepa*. In very dilute solutions both podophyllin and podophyllotoxin destroy the mitotic spindles of cleaving *Asterias* and *Arbacia* eggs, as discovered recently by Corman.[10]

The effects of podophyllin on *Condylomata acuminata* and *Allium* made this compound of interest to us as a possible therapeutic agent in the treatment of cancer. Our interest was increased by the incidental use in tissue culture of placental serum from a patient previously treated with podophyllin for *Condylomata acuminata*. This placental serum caused severe damage to mouse tumor cells growing in roller tubes without affecting normal cells growing in the same tubes.

Material and Methods. It was considered desirable to design a method for the detection of materials of possible value in the chemotherapy of cancer which take advantage of the tissue culture techniques for mammalian cells. Since we planned to test a large number of materials it was necessary that the test be one which gave a maximum of information within a short time. The roller tube technique of Gey and Gey[11] was consequently adopted. In this procedure thin-walled pyrex glass tubes (150 mm x 15 mm) are used. Each tube contains 6 pieces of normal embryonic mouse skin arranged in a row along the bottom third of the tube and a row of 6 mouse tumor fragments similarly arranged on the opposite wall of the tube. Occasionally a third row of 6 pieces of a second tumor is placed in the tube. The mouse tumors used have been in-strain transmittable mouse sarcoma L946 from C-57 mice and lung tumor MA387 from AK mice.

The tissue fragments are held in place with a thin layer of chicken plasma clot. The nutrient medium totaling one ml consists of 0.4 ml Gey's solution, 0.2 ml chick embryo extract, 0.1 ml human placental serum and 0.3 ml horse serum. The tubes are closed with sterile rubber stoppers and incubated at 37°C in a rotating drum.

Our customary procedure is to incubate the tubes for 24 hours, then examine and grade the cells that have grown or migrated from each explant. Nutrient medium to the volume of 0.1 ml is withdrawn from the tube and the test material in a volume of 0.1 ml is then added to the nutrient medium. The tubes are incubated for another 24 hours, and the growing cells again graded.

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2 Viehover, A., and Mack, H., *J. Am. Pharm. Assn.*, 1938, **27**, 632.

3 Chenoweth, M. B., Hunt, C. C., and Philips, F. S., 1947, personal communication.

4 MacCardle, R. C., and Perrault, A., *Fourth Int. Cancer Cong.*, 1947, abstract.

5 Kaplan, I. W., *New Orleans Med. and Surg. J.*, 1942, **94**, 388.

6 King, L. S., and Sullivan, M., *Science*, 1946, **104**, 244.

7 Sullivan, M., and King, L. S., *Arch. Derm. Syph.*, 1947, **56**, 30.

8 Sullivan, M., and Blanchard, D., *Bull. Johns Hopkins Hosp.*, 1947, **81**, 65.

9 Sullivan, B. J., and Wechsler, H. I., *Science*, 1947, **105**, 433.

10 Corman, I., *Biol. Bull.*, 1947, in press.

11 Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, **27**, 45.

The tumor tissue is removed at this time and implanted into susceptible mice. The normal tissue is grown for 4 days longer in fresh normal nutrient medium and again graded.

In grading the outgrowths of cells from the original tissue fragments at 24 hours, 48 hours, and again at 6 days, all visible microscopic changes that can be adequately observed in living cells are examined. The following points are noted and a grade of 0 to 4 assigned to all except "growth" for which the score runs from 0 to 6; 1) "growth," 2) inhibition of lysis of the plasma clot, 3) number of abnormally rounded cells, 4) severity of cell rounding, 5) number of cells with granulated cytoplasm, 6) intensity of cytoplasmic granulation, and 7) extent of cell disintegration.

The scores for 3, 4, 5, 6, and 7 are added and by subtracting similarly calculated control values, a damage score for tumor cells termed the "Selective Index" is derived. This is represented by the formula—

$$SI = \left[(T^{E}_{48} - T^{E}_{24}) - (N^{E}_{48} - N^{E}_{24}) \right] - \left[(T^{C}_{48} - T^{C}_{24}) - (N^{C}_{48} - N^{C}_{24}) \right]$$

where T represents tumor tissue, N represents normal embryonic mouse skin, the superscript E represents experimental tube, the superscript C represents control tube and the subscripts 48 and 24 refer to the composite damage grades at those hours.

The Selective Index plus the scores for "growth" and inhibition of lysis of the plasma clot are useful in assessing the effects of chemotherapeutic candidates. The comparative effects on normal and tumor tissue of a large series of materials can be easily compared on this basis. The term "growth" here means both cell migration and cell proliferation and actually represents simply the increase around the original explant in the area which is covered by cells originating in the explant.

Selective Indices of 0 to 10 are considered negative, 10 to 30 are considered significant and over 30 highly significant. Less than 10% of all materials thus far tested in this manner have given significant differential effects. A number of substances that have been used more or less extensively in the treatment of human cancer have given negative results in this test. These include various nitrogen mustards, heptaldehyde, urethane, Fowler's solution, benzene and stilbamidine.

The podophyllin in these experiments was precipitated from a 95% ethanol solution in 10% gum acacia and subsequently diluted in 0.87% NaCl to the desired concentrations.

Experimental. Table I shows the results of a representative experiment with podophyllin. In this experiment each tube contains 6 pieces each of normal fetal skin, L946 and MA387. The figures presented each represent the sum of the scores for 6 tissue fragments. In this experiment the terms ($T^{48}_{C} - T^{24}_{C}$) and ($N^{48}_{C} - N^{24}_{C}$) were zero in parallel control preparations and ($N^{48}_{E} - N^{24}_{E}$) can be seen to be zero from Table I. The Selective Index was calculated therefore simply by subtracting the 24-hour tumor damage scores from the 48-hour tumor damage scores and averaging the results from the 2 tubes.

It is seen that podophyllin is significantly more damaging *in vitro* to mouse tumors L946 and MA387 than to normal embryonic mouse tissue including fibroblastic and epithelial elements.

Under the influence of podophyllin the tumor cells, which are typically spindle shaped and have long slender cytoplasmic processes, become rounded or lobose in form. Migration of the cells from the explant appears to be repressed. Normal fibroblast cells show the same changes but only at much higher concentrations of podophyllin. The tumor cells (unstained) which normally spread out in a loose interlacing sheet, coalesce into a dense and closely packed sheet or ring around the original explant. Isolated cells in the plasma clot appear rounded and smaller, as though some dehydration had occurred. The nucleus disappears from sight leaving only granular cytoplasm. There is a striking change in the normally fine cytoplasmic granulation. The fine granules are replaced by larger granules which aggregate in clumps throughout the cytoplasm. The non-granular portion of the cytoplasm loses its fine structure and assumes a clear hyaline appearance. Although few

TABLE I.
Screening Test—Podophyllin, 12 mg/l; N, Normal Fetal Mouse Skin; L, Sarcoma, L946; M, Lung Tumor MA387.

	Tube No. 1			Tube No. 2		
	N	L	M	N	L	M
	24-hr data					
Growth	12	22	16	18	29	15
Lysis inhibition	24	21	20	24	22	17
% rounding	0	1	0	0	1	0
Degree rounding	0	3	0	0	3	0
% granulation	24	24	24	24	24	24
Degree granulation	12	12	12	12	12	12
Disintegration	0	0	0	0	0	0
Total	36	40	36	36	40	36
	48 hr data					
Growth	14	25	19	18	24	19
Lysis inhibition	24	14	19	24	18	16
% rounding	0	24	20	0	24	20
Degree rounding	0	12	9	0	18	11
% granulation	24	24	24	24	24	24
Degree granulation	12	18	17	12	18	18
Disintegration	0	0	1	0	0	0
Total	36	78	71	36	84	73

Selective index of L946 = 41.
Selective index of MA387 = 36.

details of nuclear structure can be seen in the living cells, stained preparations indicate considerable nuclear derangement. The chromatin is represented by disorganized fragments in most cases and considerable degree of pycnosis in others. A slight amount of cellular disintegration often is manifest by the presence of cellular debris in the culture. Essentially the same phenomena occur in normal cells but only at higher concentrations of podophyllin.

Selective damage to the tumor cells as compared with normal cells is obtained with podophyllin over a concentration range of 0.08-20.0 mg/1. Within these concentrations the Selective Index varies from 10 to 45, with the highest scores occurring in the concentration range of 0.3-2.5 mg/1.

This picture of cellular damage and destruction is reversible under some conditions. When podophyllin concentrations of 5 mg/1 or less are employed, and the podophyllin removed after 24 hours' treatment by replacement with fresh normal medium, the normal cells may recover normal appearance and resume normal growth. Tumor cells, on the other hand, have not shown recovery unless the concentrations employed were 0.6 mg/1 or below.

This compares with results found with crude penicillin, from which normal fibroblasts quickly recovered whereas sarcoma cells died.[12]

The specific effects of podophyllin and its components on karyokinesis and cell division are of considerable interest, particularly in view of the fact that nuclear damage including abnormal mitosis has been produced in sarcoma L946 and lung tumor MA387 growing in mice. In these experiments extensive damage to mouse tumors *in vivo* was produced by the parenteral administration of podophyllin. Studies on cell division as well as investigations of the biochemical and carcinoclastic actions of this material are now in progress.

Tissue culture tests with podophyllotoxin indicate that this compound is not as effective as crude podophyllin in causing selective damage to tumor cells. Also, the concentration range within which these slight effects are obtained is quite narrow, in contrast to the wide effective concentration range of podo-

[12] Cornman, I., *J. Gen. Physiol.*, 1944, **28**, 113.

phyllin.

Conclusions. 1. Podophyllin exerts a selective damaging effect on mouse tumor cells in tissue culture over the concentration range 0.08-20.0 mg/l. 2. This damaging effect is more easily reversible in normal than in tumor cells. 3. Podophyllotoxin is not as effective as podophyllin in causing selective tumor damage. 4. *In vivo* studies with tumor-bearing mice confirm the selective tumor damaging effects of podophyllin which were first noted in tissue culture preparations.

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Experimental Alteration of the Ability of Tumor Cells to Lyse Plasma Clots *in vitro*.*

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One of the outstanding characteristics of tumor cells when grown in tissue culture is their ability to lyse the plasma clot in which they are embedded. This liquefaction of the clot has been mentioned by many investigators. Drew,[1] comparing cultures of normal and malignant tissues *in vitro,* noted that a 24-hour culture of mouse embryo heart tissue showed a ring of growth around the periphery of the original fragment, while a corresponding culture of mouse sarcoma showed a similar ring of growth separated from the original explant by a circular area of liquefaction. The growth of mouse sarcoma in rat plasma was described by Lambert and Hanes[2] as being "ring-form," since lysis of the clot allowed the contracting fibrin to retract from the original fragment, leaving the fragment situated on the periphery of the circle of cells like the setting in a signet ring. Carrel and Burrows[3] attributed their lack of success in the cultivation of human carcinoma to rapid liquefaction of the plasma clot.

It is important in the analysis of the nature of this lytic process to note that lysis of a plasma clot can be induced by normal tissue. Over 30 years ago, Fleisher and Loeb[4] examined the fibrinolytic effect of various tissue fragments surviving *in vitro* on the plasma of different animals. In general, it was found that mammalian tissues lysed clots formed from mammalian plasma, but had no lytic effect on chicken plasma clots. It was further reported that normal chicken tissues were completely ineffective in producing lysis of either mammalian or chicken plasma clots. The liquefying power of tissues usually decreased when a large amount of chicken plasma was added to the standard rabbit plasma clot.

Very recently, Astrup and Permin[5] reported that tissue slices from different mammals (ox, pig, rabbit and rat) produced lysis of the ox fibrin clots in which they were embedded, the degree of lysis depending on the organ and species of animal used. They, too, found that chicken tissues did not cause lysis of such clots. They suggested that a profibrinolysin in the plasma is activated by a cellular kinase, and thereupon causes lique-

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3 Carrel, A., and Burrows, M. T., *J. Exp. Med.*, 1911, **13**, 571.

4 Fleisher, M. S., and Loeb, L., *J. Biol. Chem.*, 1915, **21**, 477.

5 Astrup, T., and Permin, P. M., *Nature,* 1947, **159**, 681.