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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(Introduced by C. P. Rhoads.)

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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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1 Drew, A. H., *Brit. J. Exp. Path.*, 1922, **3**, 20.

2 Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, **13**, 495.

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.

TABLE I.
The Effect of Varying the Aqueous Humor/Chicken Serum Ratio in the Supernatant of Roller-Tube Cultures of Sarcoma 180. Data represent average of 2 or more experiments. A.H. = Aqueous Humor; C.P. = Chicken Plasma; C.S. = Chicken Serum; 37°C.

Clot	Supernatant concentration of AH to C.S.	Lysis		Growth	
		24 hr	72 hr	24 hr	72 hr
AH/CP = 1:2	3:1	0	2.0	2.5	4.5
,,	2:1	0	1.0	2.0	4.5
,,	1:1	0	0	3.0	4.5
,,	1:2	0	0	1.5	4.0
,,	1:3	0	0	2.5	4.5

TABLE II.
Growth of Sarcoma 180 in the Absence of Serum. Data from one experiment but are representative of additional duplicate trials. 37°C.

Clot	Supernatant (drops)		Lysis 48 hr	Growth 48 hr
	Salt solution	Chicken embryo extract		
Chicken plasma	10	0	0	3.5
,, ,,	9	1	0	4.5
,, ,,	8	2	0	4.0
,, ,,	7	3	0	4.5
,, ,,	6	4	0	4.5

faction although they have been unsuccessful in extracting such a component from tissue.

In our experiments, mouse Sarcoma 180 was grown in roller tubes in a chicken plasma clot with a supernatant ordinarily composed of a balanced salt solution,[6] mammalian serum and chicken embryo extract (one part minced whole embryo to one part balanced salt solution). In such a preparation the clot was partly liquefied within 24-48 hours. This liquefaction sometimes continued until the tissue fragment was entirely surrounded by a ring of liquefaction. Our investigation was concerned with attempts to control such liquefaction and to examine the nature of the mechanism involved by altering the medium in which the tumor is customarily grown.

Material and Methods. The ability of various tumors to grow in the anterior chamber of the rabbit's eye suggested the use of aqueous humor in tissue culture. In our first experiments, the aqueous humors of rabbit, sheep or steer were incorporated in the plasma clot. The effect of modifying the serum content of the supernatant nutrient medium was also examined. Preliminary experiments were carried out in Maximow slides in which hanging drop cultures were set up with various ratios of aqueous humor and chicken plasma in the clot. Subsequent experiments were carried out in roller tube cultures following the technique of Gey and Gey.[6] Tissue fragments were oriented in glass culture tubes and then covered with a layer of chicken plasma. After clotting of the plasma was complete, the supernatant fluid was added and the tubes were incubated at 37°C in a rotor. Seven to 12-day-old mouse Sarcoma 180 tumors, carried in CFW mice, were used in all the experiments. The degree of lysis was graded by dividing the perimeter of the tumor fragment into four quadrants, each with a value of one. Thus, lysis in one quadrant was assigned a grade of 1; 2 quadrants a grade of 2, etc. The amount of growth was graded in a similar manner. A grade of 4 indicated a complete fringe of growth around the fragment. When the width of growth was equal to the diameter of the fragment, a grade of 5 was assigned.

Results. In Maximow slide hanging drop preparations in which aqueous humor was incorporated in chicken plasma clots no lysis of the clot appeared over a 4-day period, al-

[6] Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, **27**, 45.

though there was extensive tissue growth. The most vigorous growth was obtained with concentrations of aqueous humor to chicken plasma of 1:1 and 1:2. Normal growth of the tissue for a period exceeding 4 days has not been successful, the cells becoming extremely granular and rounded, and eventually disintegrating.

All subsequent experiments were carried out in roller tubes. In these preparations lysis of the aqueous humor/chicken plasma clots occurred when a supernatant fluid containing mammalian serum was present. By varying the constituents of the supernatant, it was possible to block this lytic action. If the ratio of aqueous humor to chicken serum was 1:1 or 1:2 in the supernatant fluid, no lysis occurred. Further experiments (Table I) showed that the chicken serum was the responsible factor in preventing lysis. Aqueous humor did not inhibit lysis. On the contrary, if present in high enough concentration, it promoted fibrinolysis.

Table I shows that as the concentration of aqueous humor decreased and the concentration of chicken serum increased, the amount of lysis decreased. At an aqueous humor/chicken serum ratio of 1:1 in the supernatant, lysis was completely absent and growth was apparently unaffected. With higher concentrations of chicken serum in the supernatant, lysis was similarly absent although growth appeared to be adversely affected.

When all sera were omitted from the supernatant fluid, leaving only the balanced salt solution and chicken embryo extract, no lysis of the chicken plasma clot occurred, although there was good growth. The addition of chicken embryo extract did not stimulate lytic activity even though more extensive growth occurred. These data are presented in Table II.

Table III summarizes the roller tube experiments in which the supernatant medium was altered in attempts to prevent lysis of the clot. In these experiments a clot composed solely of chicken plasma was employed.

It will be noted that the presence of avian serum in the supernatants is correlated with the occurrence of little or no lysis, while the presence of mammalian sera resulted in a significant degree of lysis. The addition of sufficient amounts of chicken serum (replacing an equal volume of the balanced salt solution) to the mammalian sera resulted in loss of lytic activity, rabbit serum being an exception—at least at the concentrations tested.

TABLE III.

Plasma Clot Lysis at 48 Hours in Roller Tube Cultures of Mouse Sarcoma 180 Cells in the Presence of Various Sera. 37°C. C.P. = Chicken plasma; H.S. = Horse serum; C.S. = Chicken serum; R.S. = Rabbit serum; Hu.S. = Human serum.

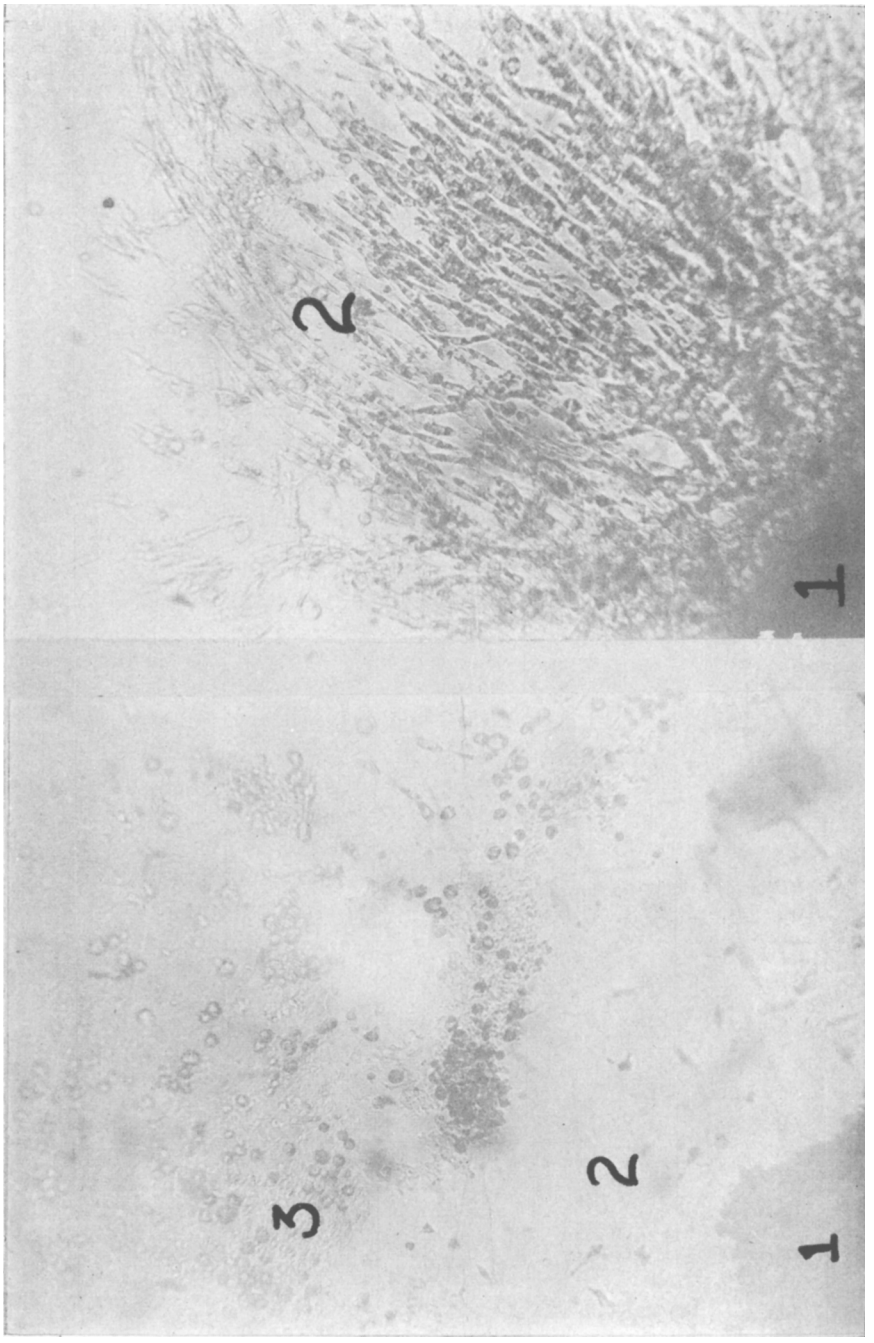
Clot	Supernatant*	Lysis	Growth
C.P.	Chicken serum	0	4.5
"	Duck serum	0	4.5
"	Horse serum	2.0	5.0
"	H.S. + C.S. (2:3)	0	5.0
"	Rabbit serum	2.0	4.5
"	R.S. + C.S. (2:3)	2.0	5.0
"	Human serum	4.0	4.5
"	Hu.S. + C.S. (2:3)	2.5	4.5
	Hu.S. + C.S. (2:4)	1.0	4.5

* Includes a balanced salt solution and chicken embryo extract.

With human serum in the supernatant, the liquefaction at 72 hours was so complete around the fragment that it was occasionally washed away, leaving an empty space surrounded by a ring of cells. The use of serum previously heated at 56°C for 3 hours resulted in the complete absence of lysis and rapid growth of the cultures. Fig. 1 shows the effect of heating the serum.

Discussion. Inasmuch as omitting serum, using heterologous serum, or heating the serum affects the amount of liquefaction, it seems probable that serum contains a factor essential to the lytic mechanism. Since the area of liquefaction is invariably contiguous with the tumor fragment, it appears that the tissue also contributes to the lytic mechanism. The experimental data presented are in harmony with blood enzyme studies[7] in which it has been suggested that an active proteolytic enzyme is produced as the result of an interaction of a cell activator (streptokinase) and the profibrinolysin normally present in plasma and serum. According to this theory heat in-

7 Christensen, L. R., *J. Gen. Physiol.*, 1945, **28**, 363.



A B

FIG. 1.

72-hour growth of Sarcoma 180 using unheated and heated human serum in the supernatant. Living cultures in roller tubes, × 80.
A—unheated human serum; 1—original explant; 2—lysed area; 3—moribund cells.
B—heated human serum; 1—original explant; 2—extensive outgrowth of Sarcoma 180 cells.

activation of the serum containing profibrinolysin or the omission of serum from the culture medium explains the lack of lytic activity in such cases. The different degrees of lysis occurring when various sera are used may be due to differences in the amount of profibrinolysin present in the different sera. Correspondingly, mammalian aqueous humor can be considered as a dilute mammalian serum which contains only profibrinolysin.

When mammalian Sarcoma 180 is grown in the presence of avian serum, the absence of lysis in this case may be due to (1) lack of sufficient profibrinolysin in the serum, or (2) the inability of mammalian tissue to activate avian profibrinolysin. Since the Rous chicken sarcoma causes extensive liquefaction of chicken plasma clots,[8] it appears that chicken plasma does contain profibrinolysin. It is more likely, therefore, that mammalian tissue is unable to activate avian profibrinolysin. The inhibitory effect of chicken serum on the lytic activity of various mammalian sera may be explained by assuming that the profibrinolysin of the chicken serum combines with the activator from the mammalian tumor Sarcoma 180 to form an inactive complex:

Kinase (Mammalian tissue) + Profibrinolysin (Mammalian serum) ⟶ Fibrinolysin (Active)

Kinase (Mammalian tissue) + Profibrinolysin (Avian serum) ⟶ Kinase-Profibrinolysin (Inactive)

Thus, if sufficient avian serum is added to mammalian serum in the presence of mammalian cells, it effectively removes the mammalian kinase through formation of the inactive complex and prevents activation of the mammalian profibrinolysin and subsequent lysis of the fibrin.

This hypothesis is further supported by the data presented in Table IV. Chicken serum previously heated to 56°C for 3 hours was added to a mammalian tissue preparation with a supernatant containing mammalian serum. No inhibition of the lytic process was observed.

The absence of lysis in preparations in which heated human serum was used exclusively in the supernatant might be explained by postulating the activation of inhibitory substances due to prolonged heating at 56°C. However, the addition of heated human serum to supernatants containing only normal human serum did not result in any significant effect on the lytic process. This indicates that the heated human serum did not contain an inhibitor, and that the absence of lysis in tubes in which the supernatant contained only heated serum is best explained on the basis of destruction of a proenzyme or enzyme concerned with fibrinolysis.

TABLE IV.

Effect of Heated Serum on Lysis in the Presence of Unheated Human Serum. Each datum represents duplicate determinations. C.P. = Chicken plasma; C.S. = Chicken serum; Hu.S. = Human serum. Roller tube cultures, 37°C.

Clot	Supernatant*	48 hr Lysis	48 hr Growth
C.P.	Hu.S. + C.S. (2:4)	1.0	4.5
,,	,, + heated C.S. (2:4)	3.5	4.5
,,	,, + (2)	3.5	4.0
,,	,, + (6)	3.5	4.0
,,	,, + heated Hu.S. (2:2)	4.0	4.0
,,	,, + ,, ,, (2:4)	3.0	4.0

* Includes a balanced salt solution and chicken embryo extract.

Fischer,[8] working with the Rous chicken sarcoma, which liquefies chicken plasma clot, was able to grow this tumor without liquefaction in a clot made from rabbit plasma. In this case it may be that the reciprocal event occurs: avian tissue kinase forms an inactive complex with mammalian profibrinolysin. Fischer further found that on the addition of fresh chicken serum to the rabbit plasma, lysis of the clot occurred; whereas chicken serum heated at 56°C for 3-4 hours produced no such action. Since chicken tumor cells caused liquefaction of the clot in the presence of chicken plasma or chicken serum but not in the presence of rabbit plasma, Fischer concluded that lysis occurred only in the presence of homologous plasma or serum. It is not clear how exclusive his use of the term "homologous" is intended to be. We have shown that differences in sera from other mammalian orders are not sufficient to prevent lysis by mouse Sarcoma 180. Lambert and Hanes[9] demonstrated that when rat and mouse sarcoma cells were grown in the plasma of various animals, liquefaction occurred in the presence of the plasma of guinea pig, rabbit, dog and human (the latter producing the greatest amount of liquefaction). In goat plasma there was neither growth nor lysis. Although there was good growth in pigeon plasma, the authors imply

[8] Fisher, A., *Nature*, 1946, **157**, 442.

[9] Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, **14**, 129.

that there was no lysis.

The anti-lytic effect of chicken plasma used in culturing mammalian tumors has been established by other investigators. Gey and Gey[6] have reported that the use of a large amount of chicken plasma helped to prevent early liquefaction, but sometimes delayed growth. A strain of sarcoma cells derived from a dibenzanthracene mouse tumor was cultured *in vitro* for more than two years by Jacoby,[10] with almost no liquefaction of the chicken plasma clot. The supernatant consisted of chicken serum, chicken embryo juice and Tyrode solution. Lewis and Strong,[11] in a survey of over 50 different spontaneous mouse tumors in tissue culture, found that the cultures rapidly liquefied the clot when it was composed of mouse plasma or a mixture of mouse and chicken plasma. In chicken plasma, however, such liquefaction did not occur.

In assigning the profibrinolysis to the serum and the kinase to the cells we are following the current ideas of Christensen and McLeod.[7,12] Our data are not such as to eliminate the inverse possibility: profibrinolysin in the tissue activated by a blood kinase. We cannot assume that there are differences only in the amount of profibrinolysin of various animal sera. There may be differences in the amounts of activator released by the cells of animals of different orders, since Fleisher and Loeb,[4] and Astrup and Permin[5] have reported differences in the lytic activity of tissues from various animals.

Summary. 1. Mouse Sarcoma 180 cells, in the presence of mammalian serum, cause lysis of chicken plasma clots. 2. Mammalian aqueous humor has slight influence on the fibrinolytic process. 3. When Sarcoma 180 is grown in the presence of avian serum, no lysis occurs. If a sufficient quantity of chicken serum is added to a supernatant fluid containing mammalian serum there is a decrease in the amount of clot lysis. 4. Lysis of the plasma clot by Sarcoma 180 does not occur when mammalian serum previously heated to 56°C for 3 hours is used, or when all serum is omitted from the supernatant. 5. The different degrees of lysis obtained when sera from various mammalian orders are used may be due to differences in the amount of profibrinolysin contained in the various sera. 6. There are two factors necessary for the lytic mechanism, tentatively classified as a profibrinolysin from the serum, and an activator of the profibrinolysin derived from the tissue.

10 Jacoby, F., *Nature*, 1943, **152**, 299.

11 Lewis, M. R., and Strong, L. C., *Am. J. Cancer*, 1934, **20**, 72.

12 Christensen, L. R., and McLeod, C. M., *J. Gen. Physiol.*, 1945, **28**, 559.

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Dichotomy Between Hypophyseal Content and Amount of Circulating Gonadotrophins During Starvation.*

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The amounts of gonadotrophin contained within the pituitary gland have often been assumed to parallel the amount produced and released into the blood stream. Since the gonadotrophic hormone content of the hypophysis must be the resultant of the amounts produced and the amounts released, they do not necessarily parallel each other. It is the purpose of this investigation to assess the effect of acute starvation on pituitary gonadotrophic hormone content and its release into the circulation.

The effective amount of circulating gonad-

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