that Ca removed from the fetal blood to support skeletal mineralization is rapidly replenished without seriously threatening the steady state Ca concentrations in fetal or maternal bloods.

Summary. Transfer of Ca from mother to fetus concurrent with transfer from fetus to mother was studied in 3 pregnant rhesus monkeys. Ca^{45} was injected into a fetal interplacental vessel simultaneously with intravenous injection of Ca^{47} into the anesthetized mother. Samples of maternal blood (MB), fetal blood (FB), and amniotic fluid (AF) were assayed for Ca^{45}, Ca^{47} and non-radioactive Ca. Initial loss of Ca^{47} from MB was matched by rapid initial appearance in FB ($t_{½} < 6$ min) and somewhat slower entry into AF. Similarly, Ca^{45} appeared in MB at a rate almost equal to the initial rate of loss from FB. The quantity of Ca crossing the placenta daily was at least 6-10 times that required for fetal skeletal growth.

We are grateful for expert assistance given by Dr. R. A. Bashore, Dr. R. A. Chez and Miss E. James during the surgical operations and are indebted to G. V. Alexander and L. McAnulty for spectrographic analyses.

1. Pecher, C., Pecher, J., Proc. Soc. Exp. Biol. and Med., 1941, v46, 91.

2. Comar, C. L., Ann. N. Y. Acad. Sci., 1956, v64, 281.

3. Wasserman, R. M., Comar, C. L., Nold, M. M., Langermann, F. W., Am. J. Physiol., 1957, v189, 91.

4. Plumlee, M. P., Hansard, S. L., Comar, C. L., Beeson, W. M., ibid., 1952, v171, 678.

5. MacDonald, N. S., Hutchinson, D. L., Hepler, M., Radiation Research, 1962, v17, 752.

6. Friedman, E. A., Gray, M. J., Hutchinson, D. L., Plentl, A. A., J. Clin. Invest., 1959, v38, 961.

7. Hutchinson, D. L., Gray, M. J., Plentl, A. A., Alvarez, H., Caldeyro-Barcia, R., Kaplan, B., Lind, J., ibid., 1959, v38, 971.

8. Bender, M. A., Science, 1955, v122, 156.

9. Ramsey, E. M., Am. J. Obstet. and Gynec., 1962, v84, 1649.

10. Sheppard, C. W., Basic Principles of the Tracer Method, Wiley & Sons, New York, 1962, p40.

11. Thomas, R. O., Litovitz, T. A., Rubin, M. I., Geschickter, C. F., Am. J. Physiol., 1952, v169, 568.

12. Armstrong, W. D., Johnson, J. A., Singer, L., Lienke, R. I., Premer, M. L., ibid., 1952, v171, 641.

13. Yamashita, T., J. Japanese Obstet. and Gynecol. Soc., 1959, v6, 1, (Biol. Abstr., 1960, v40, 1154).

14. Pickering, D. E., Kontaxis, N. E., J. Endocrinol., 1961, v23, 267.

15. Kelly, H. J., Sloan, R. E., Hoffman, W., Saunders, C., Human Biology, 1951, v23, 61.

16. Flexner, L. B., Pohl, H. A., Am. J. Physiol., 1941, v132, 344.

Received January 25, 1965. P.S.E.B.M., 1965, v119.

Effect of Wheat Germ Lipase on Human Cells Transformed *in vitro* By Simian Virus 40. (30216)

ROSLYN WALLACE AND A. W. MOYER (Introduced H. R. Cox)
Lederle Laboratories, Pearl River, N. Y.

In recent years, increased attention has been directed to surface changes as an important feature in malignant transformation. The work of Coman, Abercrombie, Ambrose and Easty has established the existence of major differences in the surface structure of normal and neoplastic cells(1-9). The striking lack of adhesiveness, of contact inhibition, *i.e.*, inhibition of movement on contact, and of organized cell arrangements which characterize most tumor cells are thought to be due to alterations in surface structure of the external membrane during transformation of a normal to a neoplastic cell(5). The finding of Ambrose *et al*(9) that wheat germ lipase (WGL) acts selectively on the growth of tumor cells in culture when compared with homologous normal cells, and other work on cell interactions with this enzyme(8,10-12), were further attempts to define the nature of this surface change. Changes in the surface membrane have also been observed in cells transformed by viruses, and are believed to be of importance in viral carcinogenesis(13),

Recent reports of transformation of human cells *in vitro* by simian virus 40 (SV40)(14, 15) have provided an analogous process in primate cells to that observed with cells and tumor viruses from chickens and rodents. It appeared of interest to determine whether selective inhibition of tumor cells by WGL is extended to cells transformed by viruses, and whether human cells transformed *in vitro* by SV40 are specifically affected. Experiments to test this possibility are described here.

Materials and methods. Tissue cultures used were:

Led-130(16), a strain of human diploid lung fibroblasts capable of limited growth *in vitro* and similar to strains established by Hayflick and Moorhead(17).

SV-TR, Led-130 cells transformed by infection with SV40, and characterized by cellular alterations similar to those described (14,15,18). These transformed cells have enhanced growth properties and longevity, but are incapable of continuous growth *in vitro*; they invariably enter a period of decline, usually ending in death of the cultures(16, 19). Growth on glass consists of a random network of loosely connected, irregularly shaped, rather plump cells. SV40 infectivity titers of culture fluids range from 10^4 to 10^7 $TCID_{50}$/0.1 ml.

T_1, T_2, T_3 lines, separately derived from SV-TR cultures following a period of growth decline. These lines appear stable and autonomous, *i.e.,* capable of continuous growth in culture, and are presently in their 83rd, 76th or 45th passage, respectively, following viral transformation. T_2 and T_3 are morphologically similar to SV-TR cells, while T_1 cells are polygonal and tend to form compact, apparently fused mosaic colonies on glass, resembling epithelial cells in growth pattern. This epithelial structure is consistent with that seen in histological sections of T_1 implants growing in the hamster cheek pouch.

T_1 has higher plating efficiencies and growth rates in culture than T_2 and T_3 lines. Although infectious SV40 could not be demonstrated in fluids from T_1 and T_2 cultures soon after their emergence as autonomous lines, cell homogenates of these cultures reveal infectivity when inoculated directly on Cercopithecus monkey kidney cell layers. Infectivity titers of fluids from T_3 cultures have not diminished after transformation, and are comparable to those from cultures of SV-TR cells.

All stock cultures were propagated in 8-oz Neutraglas bottles in Lapagt medium(20) by procedures previously reported(16).

Virus used was SV40, strain 776, obtained from the late Dr. Joseph Smadel, Division of Biologics Standards, Nat. Inst. of Health. Methods of viral propagation and titration for infectivity were as previously described (16).

WGL, lot 5548, was a preparation purchased from Worthington Biochemical Corp., Freehold, N. J. This enzyme was dissolved at a concentration of 4 mg/ml in Hanks' solution lacking sodium bicarbonate (glucosol) (21), and was sterilized by passage through a 0.45 μ Millipore filter.*

Portions of the filtered solution were inactivated according to the procedures of Aub (12), by heating at 60°C for 15 minutes. When heated samples were centrifuged, and the supernate was assayed for esterase activity on triacetin, no enzyme activity could be measured.

Fresh or heated solutions of WGL were added directly to cultures to give final concentrations of 0.02 to 0.004% in the culture medium.

Inhibition of cell growth was measured by plating dilute cell suspension in the presence or absence of the enzyme, and counting the number of macroscopical colonies formed after incubation for 10 to 14 days. Two or three plates were used for each test. Plating techniques have been described elsewhere (16). In some experiments, cells were also cultured in 16 $\times$ 150 mm tubes for comparable periods, and estimates of total cell growth were determined by enumeration of cell nuclei(22). Individual counts on 3 to 4 tubes were averaged for each determination.

The effect of WGL on the transformation process was tested in replicate bottle cultures containing approximately 1 $\times$ 10^6 Led-130

* Millipore Corp., Bedford, Mass.

TABLE I. Effect of Wheat-Germ Lipase on Plating of 10^4 Human Fibroblast Cells of Led-130 Strain and of Substrains Transformed by SV40.

Cells	No. of passages in tissue culture: Total	No. of passages in tissue culture: After transformation	No. of macroscopic colonies per plate at 10 days: Control	No. of macroscopic colonies per plate at 10 days: Plus 0.01% wheat-germ lipase
Led-130	19	—	69, 65	60, 65
SV-TR	32	12	101, 99	46, 48
T_2	37	22	98, 95	7, 9
T_1	46	36	210, 195	232, 202

TABLE II. Effect of Wheat-Germ Lipase on Growth of 5×10^4 Human Fibroblast Cells of Led-130 Strain and of Substrains Transformed by SV40.

Cells	No. of passages in tissue culture: Total	No. of passages in tissue culture: After transformation	Growth index*: Control	Growth index*: Plus 0.01% wheat-germ lipase
Led-130	12	—	20.0	20.0
SV-TR	32	14	10.5	6.9
T_2	40	25	3.2	1.9
T_1	50	40	13.1	10.8

* $\frac{\text{Avg No. of nuclei per culture at 10 days}}{\text{Avg No. of nuclei per culture at 0 days}}$.

cells in their 31st tissue culture passage. Medium was removed from one-half the cultures and replaced with 10 ml of SV40-infected fluid having a titer of 10^8 $TCID_{50}$/0.1 ml. Following incubation for 2.5 hours at 37°C, infected bottles were each rinsed twice with 10 ml of glucosol in order to remove unabsorbed virus. Cultures were replenished with 15 ml of medium, and WGL solution was added to bottles in infected and control groups, to provide a final concentration of 0.01% in the culture medium. Bottles were incubated at 37°C and subcultured as required. Culture fluids were collected at intervals during the observation period and titrated for SV40 infectivity. At each renewal of fluid medium, cultures were replenished with WGL.

Results. When 10^4 cells of diploid fibroblast strain Led-130 were plated in the presence of WGL at 0.01% concentration, little to no inhibition of colony formation occurred. However, strong inhibition of SV-TR, T_2 and T_3 cells was observed in WGL at this concentration, and inhibition was reflected both in number and in size of colonies formed (Tables I & III; Fig. 1 & 2). The increase in total number of cells was also little reduced in Led-130 cultures by low concentrations of WGL, whereas marked reduction in total growth of transformed cells was observed (Tables II, III & IV).

TABLE IV. Effect of Fresh and of Heated Wheat-Germ Lipase on Growth of Human Fibroblast Cells of Led-130 Strain and of Substrains Transformed by SV40.

Cells	No. of passages in tissue culture: Total	No. of passages in tissue culture: After transformation	Growth index*: Control	Growth index*: Plus 0.01% wheat-germ lipase: Fresh	Growth index*: Plus 0.01% wheat-germ lipase: Heated
Led-130	12	—	30.5	25.0	20.4
SV-TR	35	7	4.0	1.4	1.4
T_2	82	67	5.3	1.2	1.7

* $\frac{\text{Avg No. of nuclei per culture at 7 days}}{\text{Avg No. of nuclei per culture at 0 days}}$.

TABLE III. Effect of Wheat-Germ Lipase on Growth of Human Fibroblast Cells of Led-130 Strain and of Substrains Transformed by SV40, After 14 Days of Incubation.

Cells	No. of passages in tissue culture: Total	No. of passages in tissue culture: After transformation	No. of cells plated	No. of macroscopic colonies per plate: Control	No. of macroscopic colonies per plate: Plus 0.01% wheat-germ lipase	Avg No. of nuclei per culture ($\times 10^3$): Control	Avg No. of nuclei per culture ($\times 10^3$): Plus 0.01% wheat-germ lipase
Led-130	28	—	10^4	15, 18	12, 14	10	8
T_3	61	41	5×10^3	133, —	85, 90	611	75
			1×10^3	69, 75	10, 15	—	—
T_2	76	61	5×10^3	120, 135	15, 9	769	38
			1×10^3	73, 80	3, 5	—	—
T_1	88	77	1×10^3	201, 190	110, 102	593	382
			5×10^2	79, 88	22, 30	—	—

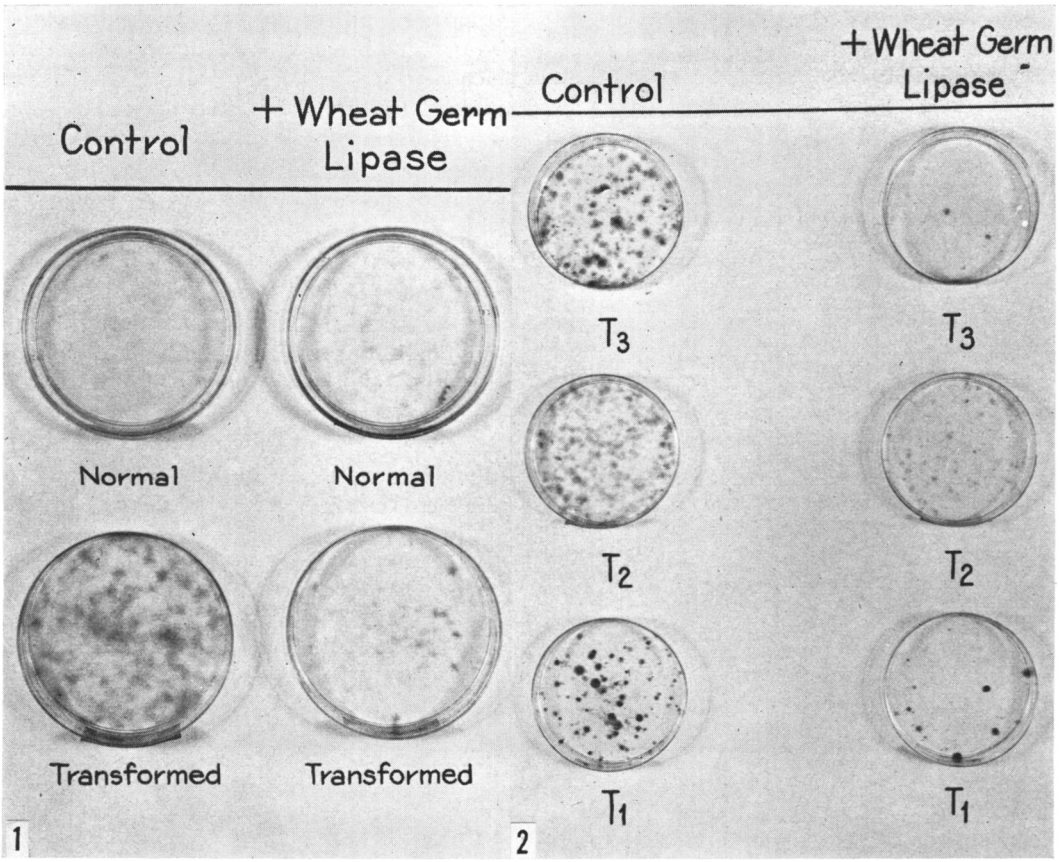

FIG. 1. Effect of 0.01% wheat-germ lipase on growth of 1×10^6 human fibroblast cells of Led-130 strain (normal) and of SV-TR substrain (transformed by SV40) after 10 days of incubation. Leishman's stain.

FIG. 2. Effect of 0.01% wheat-germ lipase on growth of SV40 transformed cell lines T_1, T_2 and T_3 after 14 days of incubation. Leishman's stain.

Although the plating ability of 10^4 T_1 cells was not reduced in medium containing WGL, an inhibitory effect of the enzyme could be demonstrated when fewer cells (5×10^2 to 1×10^3) were plated (Tables I & III). As the T_1 line has a somewhat higher plating efficiency than the other transformed cells used in this study, it at first appeared that larger surviving cell concentrations might neutralize the inhibitory effect of low levels of WGL. That this is not the case can be seen from results summarized in Tables II and III. Table II shows that with inocula of 5×10^4 cells per culture tube, all transformed cell lines were inhibited by WGL, as reflected in values for total cell counts per culture. Also, as data in Table III show, cultures containing the fewest surviving cells after 14 days were the unaffected normal fibroblasts, Led-130, while T_1, T_2, and T_3 lines, having larger and mutually comparable surviving populations, again revealed varying degrees of growth inhibition by WGL.

In all tests so far performed, WGL inhibits T_1 cells less than it inhibits SV-TR, T_2 or T_3 cells. This observation may be of interest in view of the morphological dissimilarity of T_1 cells and the other cell lines tested. To determine whether the presence of WGL in medium of SV40-infected Led-130 cultures could inhibit cell transformation by the virus, aging cultures in 31st passage were selected and treated as described in *Materials and methods*. After approximately 31 *in vitro* passages, in which cultures are subdivided 1:3 at each passage, Led-130 fibroblasts enter their char-

acteristic senescent phase(17) and survive one or two further passages before mitosis ceases and death occurs. These aging cultures with low growth potential apparently provide a selective advantage in favor of rapidly growing transformed cells. Transformation after infection with SV40 occurs earlier(23,24), and can be recognized by the appearance of proliferating centers of transformed cells.

In the experiment described here, the changes preceding transformation (appearance of giant cells, cellular destruction, lowered pH of fluid medium) occurred in all infected cultures, with or without WGL, at 2 weeks and one passage after infection. During the next 6 days, macroscopically dense centers of transformed cells developed; 22 such centers were counted in infected control cultures and 25 centers counted in infected cultures containing WGL. Uninfected cultures remained sparsely populated with senescent cells and diminishing growth rates. Thus the presence of 0.01% WGL, which inhibits the growth of SV40-transformed cells, failed to delay or inhibit the formation of transformed cell centers.

Transformed cells developing in cultures containing WGL appeared to be viable and to multiply as well as transformed cells in infected controls into the 3rd passage, approximately 4 weeks after transformation. At this time, severe cell clumping and degenerating cells were noted in transformed cultures with WGL, and these cultures did not survive the 4th passage after infection. Cell clumping was never observed in transformed cultures of infected controls, although characteristic nuclear abnormalities and cell destruction accompanied by slowing growth(16) developed in the 6th passage, approximately 2.5 months after infection. All cultures were terminated after a total observation period of 3 months. Virus titers during this period ranged from 10^4 to $10^{6.5}$ $TCID_{50}$/0.1 ml in all infected cultures, evidently unaffected by WGL in the culture medium. The enzyme produced no deleterious effect on uninfected control cultures, but appeared rather beneficial in that those with WGL contained more viable cells and survived one further passage than uninfected controls without WGL.

It was of interest to know whether cells transformed in the presence of WGL were altered in sensitivity to the enzyme. Cells were collected by trypsin-dispersal from both groups of infected cultures following one tissue culture passage after transformation. 1×10^6 cells of each suspension were plated in the presence and absence of WGL at 0.01%, cultured for 10 days, and the number of macroscopical colonies on each plate counted. Results showed some reduction of colony formation by control transformed cells in medium containing WGL. On the other hand, cells transformed in the presence of WGL had about equal plating efficiencies in medium with or without WGL, and the efficiency approximated that of control transformed cells plated in absence of WGL. Under these conditions, it would appear that cells recently transformed in the presence of the enzyme acquire an increased plating efficiency. If this is true, the effect seems to be transient, as these transformed cells growing in WGL medium later reveal reduced growth capability and changes leading to early death.

Specific cell membrane interaction with the cytotoxic fraction from WGL, as well as its cellular uptake and tumor-specific agglutinating effect, have been found independent of aliesterase activity of the preparation used (11,12). Whether inactivated WGL would also inhibit the growth of SV40-transformed cells remained to be determined. To test this possibility, fresh or heat-inactivated solutions of WGL were added to culture tubes containing 2×10^4 Led-130, SV-TR or T_2 cells. These cultures were incubated at 37°C for 7 days and then prepared for enumeration of cell nuclei. The results presented in Table IV indicate that the inhibitory action of WGL on SV40-transformed cells is not destroyed at temperatures which inactivate its enzymatic specificity for triacetin.

Discussion. In reported instances where isolated normal and tumor cells of comparable derivation have been tested, tumor cells proved to be more sensitive to the cytotoxic effect of WGL(9,10,12). In this study, cells transformed *in vitro* by a virus were also selectively inhibited by this enzyme. Moreover, as human cells transformed by SV40

were used for this work, these cells may be revealed as having one further property in common with the neoplastic cell(25). Previous studies in this laboratory indicate that although SV-TR cells have some properties of cultured human tumor-cell lines, they lack (but may acquire) others, such as potentially unlimited growth *in vitro* and an ability to multiply as free cell suspensions in agitated fluid medium. Jensen, who tested similar cultures for homotransplantability, obtained positive results only with transformed cells maintained in culture 373 days or longer, and suggested a progression toward increased neoplastic properties on further propagation of such cells *in vitro*(25). The marked loss of mutual adhesiveness characteristic of transformed cells is, however, a property acquired early in transformation and one of the first notable signs of cell alteration. In this report, sensitivity to WGL was demonstrated in cells from cultures in their first passage as well as in later passages after viral transformation, and may well coincide with early surface changes reflected by loss of adhesiveness. It is tempting to think that a similar phenomenon may exist in the findings of Coman(5), who measured a loss of mutual adhesiveness in rabbit epidermal cells as early as one hour after contact with a carcinogen and proposed that surface change may be an essential and necessary step in carcinogenesis.

The mode of action of WGL as a cytotoxic substance is unclear, and was not investigated in this study. Aub(12) did not find the tumor specific factor in 2 other enzymes with lipolytic activity, namely, phospholipase and porcine pancreatic lipase; nor was the tumor specific effect associated with aliesterase activity(11,12). The active factor is apparently a large molecule and it has been suggested that it is a mucopolysaccharide(12).

A Hale-positive coat has been demonstrated on the surfaces of certain ascitic tumor cells, and the substance responsible for the staining reaction is sialomucin, an acid mucopolysaccharide which can be removed by treatment with neuraminidase(26). Hamster tumor cells transformed by polyoma virus also have Hale-positive coats, and an inverse relationship between intensity of the Hale reaction and degree of contact inhibition has been observed(27). As this reaction was abolished by neuraminidase, it was concluded that an increase in sialomucin on the cell surface is responsible for loss of contact inhibition by these cells. That complexes of neuraminic acid and polysaccharide or protein may not be implicated as cell-surface reactive sites for WGL is suggested however, by the work of Cormack *et al*(10,11). These investigators followed cell uptake by normal and tumor cells of a fluorescein-labeled cytotoxic fraction from WGL. An affinity of the enzyme for the cell membrane was demonstrated, and although labeled fraction was observed in cytoplasmic droplets pinocytosed in both normal and tumor cells, fusion of droplets to form large degenerative vacuoles occurred only in tumor cells. The pattern of these interactions was not altered by pretreating the cells with neuraminidase.

In this laboratory, staining of normal diploid fibroblasts Led-130 and of SV40-transformed substrains with Mowry's modification of the Hale procedure(28) revealed some positive staining by both normal and transformed cells, with possibly more positive areas on SV-TR cells than others. No quantitative correlation appeared to exist between Hale-positive reactions of the various cell lines and their degree of inhibition by WGL.

In view of these and other findings, further efforts to elucidate the nature of the tumor-specific action of WGL, as well as studies to determine its effect on cancer cell growth *in vivo*, seem warranted.

Summary. Selective inhibition by preparations of wheat-germ lipase (WGL) was demonstrated for human cells transformed *in vitro* by SV40. This inhibition was reflected in the number and size of colonies formed, as well as in total cell growth, in a medium containing 0.01% of WGL. The presence of WGL in the medium of SV40-infected human diploid cells failed, however, to delay or inhibit the formation of transformed cell centers. Cells transformed in medium containing WGL appeared to multiply no less than control transformed cells during the first 4 weeks after transformation. They then be-

gan to clump, and to reveal reduced growth capabilities which led to early death of the cultures. The inhibitory action of WGL on transformed cells was not destroyed by heating the preparation at 60°C, a procedure which inactivated its esterase activity for triacetin.

The authors are indebted to Mrs. Susan Kulina for excellent technical assistance, to Dr. Erwin Jungherr for staining and examining cultured cells, and to Mrs. Esther Chasan for aid in preparation of the manuscript.

1. Coman, D. R., Cancer Res., 1944, v4, 625.
2. Coman, D. R., Anderson, T. F., ibid., 1955, v15, 541.
3. Ambrose, E. J., James, A. M., Lowick, J. H. B., Nature (London), 1956, v177, 576.
4. Abercrombie, M., Heaysman, J. E. M., Korthauser, H. M., Exp. Cell Res., 1957, v13, 276.
5. Coman, D. R., Cancer Res., 1960, v20, 1202.
6. Easty, G. C., Easty, D. M., Ambrose, E. J., Exp. Cell Res., 1960, v19, 539.
7. Abercrombie, M., Ambrose, E. J., Cancer Res., 1962, v22, 525.
8. Easty, G. C., Easty, D. M., Ambrose, E. J., Exp. Cell Res., 1960, v19, 539.
9. Ambrose, E. J., Dudgeon, J. A., Easty, D. M., Easty, G. C., ibid., 1961, v24, 220.
10. Cormack, D. H., Easty, G. C., Ambrose, E. J., Nature (London), 1961, v190, 1207.
11. Cormack, D. H., Ambrose, E. J., Exp. Cell Res., 1963, v31, 566.
12. Aub, J. C., Tieslan, C., Lankester, A., Proc. Nat. Acad. Sci. (U. S.), 1963, v50, 613.
13. Vogt, P. K., Cancer Res., 1963, v23, 1519.
14. Koprowski, H., Pontén, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., Saksela, E., J. Cell. Comp. Physiol., 1962, v59, 281.
15. Shein, H., Enders, J. F., Proc. Nat. Acad. Sci. (U. S.), 1962, v48, 1164.
16. Moyer, A. W., Wallace, R., Cox, H. R., J. Nat. Cancer Inst., 1964, v33, 227.
17. Hayflick, L., Moorhead, P. S., Exp. Cell Res., 1961, v25, 585.
18. Pontén, J., Jensen, F., Koprowski, H., J. Cell. Comp. Physiol., 1963, v61, 145.
19. Shein, H. M., Enders, J. F., Palmer, L., Grogan, E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 618.
20. Wallace, R., ibid., 1964, v116, 990.
21. Hanks, J. H., Wallace, R. E., ibid., 1949, v71, 196.
22. Sanford, K. K., Earle, W. R., Evans, V. J., Waltz, H. K., Shannon, J. E., J. Nat. Cancer Inst., 1951, v11, 773.
23. Jensen, F., Koprowski, H., Pontén, J. A., Proc. Nat. Acad. Sci. (U.S.), 1963, v50, 343.
24. Todaro, G. J., Walman, S. R., Green, H., J. Cell. Comp. Physiol., 1963, v62, 257.
25. Jensen, F., Koprowski, H., Pagano, J., Pontén, J., Ravdin, R., J. Nat. Cancer Inst., 1964, v32, 917.
26. Gasic, G., Gasic, T., Nature (London), 1962, v196, 170.
27. Defendi, V., Gasic, G., J. Cell. Comp. Physiol., 1963, v62, 23.
28. Mowry, R. W., Lab. Invest., 1958, v7, 566.

Received February 15, 1965. P.S.E.B.M., 1965, v119.

Association of Factors XI and XII with Blood Platelets.* (30217)

HERBERT I. HOROWITZ AND MITSU M. FUJIMOTO (Introduced by Aaron J. Marcus)
Departments of Medicine and Laboratories, Bronx-Lebanon Hospital Center, New York City

The concept that physiological clotting occurs in close relationship to the platelet surface(1,2) has been supported by evidence that many of the coagulation factors are absorbed by platelets. This evidence has recently been reviewed(1,3,4). The degree to which the clotting factors adhere to the platelet surface varies widely. Some factor V(5, 6) and fibrinogen(6,7) persist on the platelet despite many washes, whereas factor VIII can be removed by several washes(3,5), and prothrombin as well as factors VII, IX and X are even more readily removed(3). Detailed information about the presence of factors XI and XII on platelets has been lacking. Caen and Haas(9) found that normal platelets increased the consumption of prothrombin in recalcified factor XI-deficient plasma. Gormsen(10) demonstrated that thrice-washed normal platelets corrected the

* Supported by U. S. P. H. S. Grant HE 07844.