ica, in press; C. R. Peters, thesis, University of California, Los Angeles (1975).
9. J. L. Kavanau and C. E. Rischer, *Science* **161**, 1256 (1968); in preparation.
10. J. L. Kavanau and J. Ramos, *Science* **169**, 780 (1970).
11. J. L. Kavanau, in *Systems Analysis in Ecology*, K. E. F. Watt, Ed. (Academic Press, New York, 1966), pp. 99–146.
12. For practical purposes the twilight periods (Table 1) were standardized to the 45 minutes preceding sunrise and following sunset.
13. See J. R. Napier and P. H. Napier [*A Handbook of Living Primates* (Academic Press, New York, 1967)] and (*8*) for citations and discussion.
14. E. Bünning, *Photochem. Photobiol.* **9**, 219 (1969); H. G. Erkert, *Z. Vgl. Physiol.* **64**, 37 (1969).
15. Current parallel telemetric field studies of several rodent species by L. Shields, from this laboratory, suggest the generalization that times of onset and cessation of activity (or bouts of activity) tend to center on the most abruptly occurring light changes that the animals experience, whether these be deep twilights or merely local sunset and sunrise. In this connection, Pariente (*3*) noted that most returns of the Malagasy lemur, *Phaner furcifer*, to the nest took place at a time when the rate of increase of dawn illuminance was maximal.
16. E. Dodt, *Vision Res.* 7, 509 (1967); D. I. Hamasaki, *J. Comp. Neurol.* **130**, 163 (1967); C. Pedler, *Int. Rev. Gen. Exp. Zool.* **4**, 219 (1969); L. R. Wolin and L. C. Massopust, Jr., in *Advances in Primatology*, vol. 1, *The Primate Brain*, C. R. Noback and W. Montagna Eds. (Appleton-Century-Crofts, New York, 1970), pp. 1–27; R. G. Murray, A. E. Jones, A. Murray, *Anat. Rec.* **175**, 673 (1973).
17. In this connection, Pariente (*3*) noted that *Phaner furcifer* in the wild become highly active at just the time when human vision is shifting from light-adapted to dark-adapted, and he noted that "it is conceivable" that the mechanism for discriminating a specific illuminance level "would call for a duplex cone-rod system."
18. Similar tentative behavior has been noted in Malagasy lemurs (*3, 19*). Thus, *Lepilemur mustelinus leucopus* are said to habitually extend their heads out of their shelters to see whether the light conditions are favorable.
19. P. Charles-Dominique and C. M. Hladik, *Terre Vie* **25**, 3 (1971).
20. G. L. Walls, *The Vertebrate Eye* (Hafner, New York, 1963).
21. Supported by NSF grant GB 27533 to J.L.K. We thank Dr. R. Edgerton and the Los Angeles Zoo for the loan of animals.

15 September 1975; revised 28 October 1975

Breast Cancer Patients: Substance in Blood Causing Acceleration of Erythrocyte Sedimentation Rate

Abstract. *The frequency distribution curve of the erythrocyte sedimentation rates obtained from a population of 3523 normal females was compared with the distribution found in 544 patients with benign breast disease and that for 385 patients with carcinoma of the breast. No significant difference was found between the normal female population and patients with benign lesions of the breast. This was in contrast to the distorted distribution curve of erythrocyte sedimentation rates exhibited by the population of patients having malignant breast lesions. An unknown substance present in the blood plasma of the cancer patients appears to be responsible for the abnormal sedimentation rate phenomenon.*

This report describes the presence of an unknown substance in the blood of patients with breast cancer that is not exhibited by large populations of either normal females or patients with a variety of benign breast lesions. The existence of this component is inferred and detected through its influence in causing a significant acceleration in the erythrocyte sedimentation rate (ESR) of specific cancer patients. The ESR differences were established through a comparison of the frequency distribution of ESR data obtained from several thousand individuals.

The data of this report are not offered in their present form for clinical application to either diagnosis or prognosis of mammary carcinoma. Of more relevance to the cancer process are the physiological implications of an unknown substance in the plasma of patients having early malignant breast lesions. Inasmuch as this substance was not detected in a population of patients having benign breast nodules that were clinically indistinguishable from the malignant lesions, its characterization is pertinent in furthering an understanding of specific physiological aspects of neoplasia.

Double-blind ESR tests were run on large populations of normal females and various patients examined in a cancer clinic (*1*). Most of those exhibiting breast nodules and other suspected malignant lesions were admitted to the hospital for further tests and possible surgery. Reported here are the analyses of the comparative ESR findings for the patients that were diagnosed following biopsy or surgery as having either benign or malignant breast lesions. The generous size of both the normal and patient populations examined permitted the resulting data to be analyzed as ESR frequency distribution curves of the normal and disease categories rather than as simple averages of the individual values found in each patient group. This type of analysis provides a more sensitive and reliable basis for detecting significant ESR differences between sizable populations.

The ESR baseline data for normal females were obtained from a population of presumed healthy women who voluntarily submitted themselves for a standard physical and hematological examination at a cancer detection clinic (*1*). This "normal" ESR distribution curve was derived from a population of 3523 women of various ages, and is depicted by the shaded areas in Figs. 1 and 2. The only consecutive blood samples that were not included in the composition of this "normal" curve were those from the small number of individuals whose examination revealed a suspected malignancy or other serious pathologies, and who thus were referred to the hospital for further examination. Such sample discards represented less than 1 percent of the women examined (*1*).

The two shaded areas of Figs. 1 and 2 show the relatively minor differences in the distribution of ESR values with and without hematocrit correction. The theoretical purpose of such correction of the observed ESR rate lies in the potential influence of the blood hematocrit, or percentage of packed erythrocytes, upon the sedimentation rate of the red blood cells. All other factors being equal, the higher the percentage of erythrocytes in the blood, the slower the ESR. With conspicuous anemia the ESR thus tends to be faster, while it is slower in patients with polycythemia. Correction of the observed ESR for hematocrit differences therefore tends to nullify this variable, yielding ESR values that are unaffected by alterations in blood viscosity and related physical factors associated with tendencies toward polycythemia or anemia (*2*).

It follows that if there were a systematic difference in the hematocrits of any of the normal or patient populations tested, its effect would be exhibited in the uncorrected ESR curves and remedied in the corrected ESR values. This is not a significant factor in any of the groups examined (Figs. 1 and 2), and the accelerated ESR values observed in the patients with malignant breast lesions cannot therefore be ascribed to anemia or other systematic hematocrit alterations (*3*).

The similarity of the distribution of both the corrected and uncorrected ESR values of women having benign breast lesions to those of women in the normal population is shown in Fig. 1. The benign lesions were detected by clinical examination and could not be distinguished with certainty, at that point, from carcinoma. The final diagnosis was established by surgical removal or biopsy of the lesion or nodule and microscopic examination by experienced pathologists (*4*). The following breast lesions were among those diagnosed as benign: cysts, abscesses, mastitis, duct papilloma, fibroadenoma, fat necrosis, lipoma, and fibrocystic tumors. Patients with these conditions were combined to produce the ESR frequency distribution curve in Fig. 1.

The ESR data obtained from the patients who were subsequently shown to have some variety of breast carcinoma are depicted in Fig. 2. There was a significant shift in the ESR values to the right of the normal distribution curve, which demonstrates that a sizable percentage of the patients with mammary carcinoma had higher ESR values. These differences are

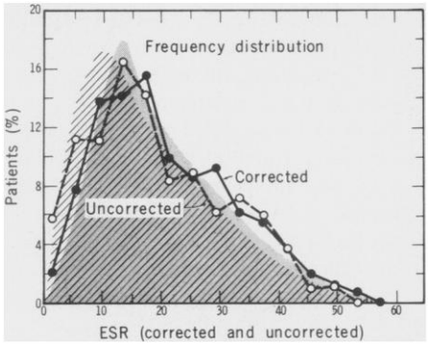

Fig. 1. Similarity of the frequency distribution of erythrocyte sedimentation rates (ESR) of 544 patients with benign breast lesions, as compared with a population of 3523 normal females. The shaded areas represent the normal population while the connected points show the ESR distribution found in patients with benign breast lesions. The area depicted by the diagonal lines represents the frequency distribution of the raw ESR data of the normal population without any correction for the individual hematocrit values, while the shaded area shows the minimal consequences of correcting the ESR values for the minor hematocrit fluctuations observed with normal females. The connected open circles represent the uncorrected values, while the closed circles are corrected ESR values for patients with benign breast lesions.

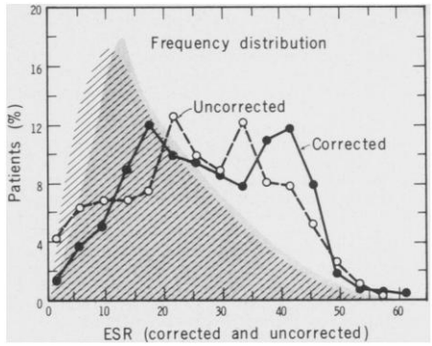

Fig. 2. Demonstration of the significant shift toward higher values of the ESR of a population of 385 patients with malignant lesions of the breast, as compared with a population of 3523 normal females. Hematocrit-corrected and uncorrected ESR values are plotted to demonstrate that the ESR alterations observed in the cancer patients cannot be ascribed to anemia or other systematic alterations in the blood hematocrits or to erythrocyte concentrations. The shaded and lined areas represent the hematocrit-corrected and uncorrected normal population distribution as described in Fig. 1. The closed and open circles depict the corrected and uncorrected ESR frequency distributions of the blood samples from the patients with breast cancer.

highly significant ($P < .001$). A double peaking of this distribution curve was seen in both the corrected and uncorrected ESR data. The significance of this bimodal distribution is not known, although it is tempting to speculate that the two peaks represent either separate stages, or separate categories, of malignant breast disease, such as pre- and postmenopausal varieties.

These data were obtained from untreated patients, and the ESR values were determined from blood samples taken during the initial clinical examination (*1*). As with the benign lesion group, the cancer patients were treated surgically, and their diagnoses established by standard pathological analysis of submitted breast tissue and adjacent nodes (*4*).

Although it is not possible to establish how long, on the average, the breast cancers had been present at the time the blood samples were obtained, in general these patients had early disease, since they had reported to the clinic individually or had been referred by a physician following the initial discovery of a breast nodule. Correlation studies on the abnormal segment of the ESR population with the size of the primary nodules, or the number of positive lymph nodes, have not been undertaken.

Determination of ESR's has been useful in the diagnosis and monitoring of some pathological conditions such as rheumatic fever and tuberculosis; however, when ESR tests have been applied to cancer patients in general, the variabilities observed have usually been more confusing than helpful (*5*). However, when a large population of patients with a specific type of cancer is compared with adequate populations of healthy individuals and patients having comparable benign conditions, a more quantitative analysis is possible by utilizing ESR frequency distribution curves. The results of such analyses are more easily interpreted. This is particularly obvious when specific neoplastic diseases are examined rather than cancer in general.

There are also substances in the blood of patients with a variety of nonneoplastic diseases that are capable of altering ESR behavior (*5*). However, it has not been firmly established whether any common factors are involved or whether there is a wide assortment of specific metabolic products or abnormal proteins, differing in each disease, that are capable of altering the erythrocyte sedimentation rate. In view of the incomplete knowledge concerning the mechanisms involved in the alteration of ESR, it would be useful to know the nature of the individual factors associated with each neoplastic disease, as well as those associated with tuberculosis, rheumatic fever, pregnancy, and other nonmalignant conditions.

Alterations of plasma protein species in malignancy have been studied by many investigators. Among the various blood changes, increases in plasma fibrinogen were found to occur in a significant number of cancer cases (*6*). A high percentage of plasma fibrin has also been found in conditions in which foreign proteins enter the blood stream, such as cancer and a variety of other diseases, including infections (*6*). Thus, the question arises as to whether the ESR is altered because of specific foreign proteins produced by the malignancies, or by a secondary accumulation of fibrin or fibrinogen. Both categories of substances are capable of increasing ESR. Of possible relevance is the reported presence of glycoproteins of high molecular weight on the surface of mammary carcinoma cells, which may be released into the circulation. Possible correlation between this shedding process and tumor metastases has been suggested (*7*). Since similar molecules are capable of enhancing ESR, these separate observations may be related. Whatever may be responsible, the data reported here establish that in the case of breast lesions, ESR enhancement occurs in association with malignancy but not in analogous benign conditions.

In the case of cancer, it would be instructive to characterize and compare the low and high ESR plasmas by using acrylamide gel electrophoresis or other sensitive procedures in an effort to isolate the plasma components associated with high sedimentation rates. It has been shown, for example, that the acrylamide disc patterns obtained with plasma from patients with various cancer types differ from each other and from the plasma patterns of normal individuals (*8*). The reintroduction of such isolated materials into normal blood samples, with a concomitant induction of elevated ESR, would remove much of the mystery that presently surrounds ESR phenomena in the disease process.

These ESR data (i) establish the presence of either an abnormal substance, or possibly an abnormal concentration of some normal component, in the blood of a significant number of breast cancer patients; and (ii) indicate that this substance or condition is not demonstrable in a large population of normal females or, more provocatively, in a large population of patients with benign breast lesions. Additional clues for rational approaches to cancer therapy or diagnosis might be found if the relationship of the enhanced erythrocyte sedimentation phenomenon to neoplasia were clarified.

VERNON RILEY
Pacific Northwest Research Foundation and Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

References and Notes

1. The 3523 normal blood samples were obtained from women of various ages, living in New York City and environs, who voluntarily came to an outpatient cancer detection center for a standard physical examination. For some women it was a first visit, while for others it was their prescheduled annual examination. Blood samples were taken by venipuncture using standard heparin-treated Vacu-

tainer tubes. Hematocrits and ESR values were determined by procedures developed at Memorial Sloan-Kettering Cancer Center (*9*). The cancer detection clinic did not accept patients presenting any specific or general health complaints. Such individuals were referred to the adjoining Memorial Center outpatient clinic for comprehensive examination and further appropriate medical action. Blood samples obtained from patients with breast lesions were tested daily for ESR and other parameters, along with other blood samples taken from all patients examined in the medical center. Thus, identification of the patients and matching of their diagnosis and ESR occurred days or weeks later, following hospitalization and biopsy or surgery. The breast cases were later segregated from the other patients, and their specific diagnoses were tabulated and correlated with the "blind" ESR findings. All diagnoses were established through standard pathological procedures.

2. M. M. Wintrobe, in *Clinical Hematology* (Lea & Febiger, Philadelphia, ed. 6, 1967), pp. 354–365.
3. Aside from the blood viscosity effect associated with abnormal hematocrits or with sickle cell anemia and impaired rouleau formation, evidence indicates that the factors responsible for determining red cell sedimentation behavior ordinarily reside in the plasma and not in the erythrocytes. This has been demonstrated by separating and interchanging the plasma and red cells from normal and abnormal blood samples. Normal red cells have an accelerated ESR in plasma from blood with a high ESR, while the red cells from abnormal blood sediment normally in plasma from healthy subjects. This was first established 250 years ago by Hewson [cited in (*2*), p. 355] and confirmed in our laboratory (*10*), as well as by others [R. Fahreus, *Acta Med. Scand.* **55**, 1 (1921)].
4. All patients tested in this study were examined in one of the several specialty clinics of Memorial Sloan-Kettering Cancer Center; initial blood samples were taken as part of the examination. Where the possibility of a malignancy existed, the patients were scheduled for biopsy or surgery. The patients in this study were all assigned to the Memorial Center Breast Service. Final pathological diagnoses, made on paraffin sections of the removed tissue by the Memorial Center pathology department, provided the data employed in these studies.
5. H. L. Bolen, *Am. J. Surg.* **63**, 316 (1944); M. W. Ropes, *J. Clin. Invest.* **18**, 791 (1939); M. A. Peyman, *Br. J. Cancer* **16**, 56 (1962).
6. J. C. Broom, *J. Lab. Clin. Med.* **22**, 998 (1937); K. Meyer, E. Hahnel, R. R. Feiner, *Proc. Soc. Exp. Biol. Med.* **58**, 36 (1945); C. M. Gordon and J. R. Wardley, *Biochem. J.* **37**, 393 (1943); W. J. Dieckman and C. R. Wegner, *Arch. Intern. Med.* **53**, 353 (1934); S. N. Lima and J. W. Brown, *Proc. Soc. Exp. Biol. Med.* **35**, 598 (1937); G. Toennies, *Cancer Res.* **7**, 198 (1947); J. Hardwicke and J. R. Squire, *Clin. Sci.* **11**, 335 (1952); M. D. Rourke and A. C. Ernstene, *J. Clin. Invest.* **8**, 545 (1930).
7. U. Kim, A. Baumler, C. Carruthers, K. Bielat, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1012 (19→ A. G. Cooper, J. F. Codington, M. C. Brown, *ibid.* **71**, 1224 (1974).
8. A. L. Tarnoky, in *Chromatographic and Electrophoretic Techniques*, vol. 2, *Zone Electrophoresis*, I. Smith, Ed. (Wiley-Interscience, New York, 1968), pp. 396–397.
9. V. Riley and W. C. Valles, *Proc. Exp. Biol. Med.* **91**, 341 (1956).
10. V. Riley, in preparation.
11. I am indebted to C. C. Stock, R. W. Houde, E. Day, C. P. Rhoads, R. Pelligra, W. Ragelson, and H. A. Abrahams for valuable aid in these studies. Partially supported by Sloan-Kettering Institute for Cancer Research, Memorial Hospital for Cancer and Allied Disease, National Cancer Institute single instrument grant, American Cancer Society, Leukemia Research Foundation, and NIH grant CA 12188.

4 August 1975

Microtubule Assembly and the Intracellular Transport of Secretory Granules in Pancreatic Islets

Abstract. *Polymerized and depolymerized tubulin were measured in pancreatic islets under various physiological and pharmacological conditions. Variations in insulin release from islets of fed or fasted rats were accompanied by concomitant changes in tubulin polymerization. Glucose induced the formation of microtubules in vitro independent of extracellular calcium. Total and polymerized tubulin content were decreased by fasting and restored by glucose feeding.*

Microtubules have been implicated in various secretory processes occurring via exocytosis (*1, 2*), but the mechanism of their involvement remains obscure. Most of these reports are based on the observation that agents which disrupt the microtubules, such as colchicine and vinblastine, inhibit the specific secretory activity under study. On the basis of the observation that colchicine inhibits the second phase of glucose-induced insulin release, Lacy proposed that the vectorial transport of insulin granules to the cell membrane occurs along a microtubular-microfilamentous network (*1, 3*). In analogy to other microtubule-dependent movements (*4*), it seemed possible that the second and sustained phase of insulin secretion may be regulated, at least in part, by the degree of tubulin polymerization. To test this possibility, a method has been developed in our laboratory to measure the cellular pools of polymerized (that is, microtubules) and depolymerized tubulin (*5*). The sensitivity of this technique permits measurement of as little as 40 ng of tubulin, an amount present in about ten isolated rat islets.

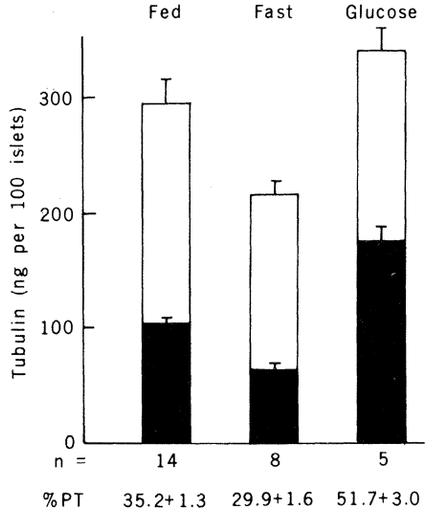

Fig. 1. Total tubulin (open columns) and polymerized tubulin (closed columns), measured in islets from fed, 72-hour fasted and dextrose fed rats. The degree of tubulin polymerization (percent PT) represents polymerized tubulin (SN-2)/total tubulin (SN-1 + SN-2). All values represent means ± the standard error of the mean; the significance of differences was measured by Student's *t*-test.

After isolation (*6*) or incubation under varying experimental conditions, 200 rat islets were homogenized in 125 μl of a microtubule-stabilizing solution (MTS) (*7*) and centrifuged at 8500*g* for 10 minutes at room temperature. Depolymerized tubulin was assayed in duplicate 25- μl portions of the supernatant fraction (SN-1) by a modification of a previously described colchicine binding assay (*8*) with 0.12 nmole of colchicine containing 5 nc of ^{3}H-labeled colchicine and incubating for 150 minutes at 37°C. The pellet (PP-1), containing the precipitated microtubules, was resuspended in 75 μl of ice-cold microtubule depolymerizing solution (TS) (*7*), and centrifuged at 8500*g* for 10 minutes at 4°C; and the tubulin activity was assayed in duplicate in the resultant supernatant fraction (SN-2). Colchicine binding activity was found exclusively in SN-1 and SN-2 and coeluted with ^{125}I-labeled tubulin (*9*) on a Bio-Gel A-5m column. No detectable colchicine binding activity was observed in the second precipitate (PP-2) when resuspended in 75 μl of TS. Furthermore, when sections of PP-1 and PP-2 were examined by electron microscopy, microtubules were readily apparent in PP-1, and disappeared completely after exposure to the TS solution. When islets were exposed to 4°C for 20 minutes prior to homogenization in MTS, colchicine binding activity of SN-1 was significantly increased and that of SN-2 was decreased, an indication of the lability of islet microtubules at low temperature comparable to that reported in other systems (*10*).

The total tubulin content of islets obtained from fed rats averaged 295.3 ± 19.5 ng of tubulin per 100 islets of which 35.2 ± 1.3 percent (104.16 ± 3.85 ng per 100 islets) was in the polymerized form (Fig. 1). In rats fasted for 72 hours, total tubulin decreased about 27 percent (217 ± 13 ng per 100 islets; $P < .02$), and polymerized tubulin decreased to an even greater extent (64.55 ± 3.74 ng per 100 islets; $P < .01$). When rats were maintained exclusively on a solution of 30 percent dextrose drinking water in 0.2 percent saline, total tubulin increased slightly but not significantly, whereas polymerized tubulin increased approximately 70 percent (175.82 ± 11.39 ng per 100 islets; $P < .001$). These changes in microtubule content parallel comparable alterations in the insulin secretory response to glucose observed either in vivo or with isolated rat islets under similar conditions (*11, 12*).

To determine the role of glucose in modulating the polymerization of tubulin, islets