culture system developed during these experiments may prove useful in the study of arthropod-borne virus transmission in the field. Further work is planned to explore applications of the method and its limitations. The major obstacle in the development of the CAS culture system was the incorporation of substances which would attract and stimulate mosquitoes to feed but were not harmful to cells or virus. Serum only partially fulfilled these requirements. Blood cells, which might have provided the additional needed feeding stimulus, were undesirable because of impaired visualization of viral plaques.

A variety of chemicals appears to attract arthropods or to stimulate them to feed. Hosoi(4) identified adenylic acid from ox blood cells as a feeding stimulant for *Culex pipiens*. He concluded that adenosine phosphates in blood cells were the main stimulus promoting gorging of the mosquito on a blood meal. Galun *et al*(5) observed that the effectiveness of the adenine nucleotides increased with each addition of a phosphate group. Adenosine tetraphosphate possessed the greatest activity and was closely followed by ATP. We determined that inclusion of ATP in the CAS culture provided an excellent blood cell substitute. Certain other biological compounds such as lysine(9,10) and steroids(11) have been reported to be attractive to female mosquitoes. The attractiveness of these substances in CAS cultures is being studied.

The findings presented here might be of potential use in another context. The Mediterranean fruit fly, *Ceratitis capitata*, was specifically eradicated from Florida through the combination of a synthetic attractant Trimedlue(12) and an insecticide. This suggests the possibility of a similar approach to the control of *A. aegypti* and other mosquito species.

Summary. A chick embryo cell culture containing adenosine triphosphate and covered with an animal-derived membrane was developed. Female *A. aegypti* fed upon these cultures readily. After exposure of the cultures to mosquitoes infected with Sindbis virus, plaques appeared within 72 hours and virus was recovered from the plaques. This culture system may be useful in the study of arbovirus ecology.

The authors gratefully acknowledge the technical assistance of Mrs. Alice M. Beach, Mrs. Judith M. Gibson, and Miss Ellen Lehtimaki.

1. Smithburn, K. C., Haddow, A. J., Lumsden, W. H. R., Ann. Trop. Med. Parasit., 1949, v43, 74.
2. Causey, O. R., Causey, C. E., Maroja, O. M., Macedo, D. H., Am. J. Trop. Med. Hyg., 1961, v10, 227.
3. Cockburn, T. A., Sooter, C. A., Langmuir, A. D., Am. J. Hyg., 1957, v65, 130.
4. Hosoi, T., J. Insect Physiol., 1959, v3, 191.
5. Galun, R., Avi-Dor, Y., Bar-Zeev, M., Science, 1963, v142, 1674.
6. Collins, W. E., Am. J. Hyg., 1963, v77, 109.
7. Earle, W. R., J. Nat. Cancer Inst., 1943, v4, 165.
8. Cooper, P. D., Virology, 1955, v1, 397.
9. Brown, A. W. A., Carmichael, A. G., J. Econ. Ent., 1961, v54, 317.
10. Lipsitz, E. Y., Brown, A. W. A., Bull. Ent. Res., 1964, v54, 675.
11. Roessler, P., N. J. Mosq. Ext. Assn. Proc., 1963, 250.
12. Jacobson, M., Beroza, M., Science, 1963, v140, 1367.

Received November 25, 1964. P.S.E.B.M., 1965, v118.

Serum Iron Levels in Patients with Malignant Disease.* (29956)

A. CLARK GRIFFIN, SEBRON C. DALE, DOROTHY WELLINGTON AND VIRGINIA C. WARD
University of Texas, M. D. Anderson Hospital and Tumor Institute, Departments of Medicine, Epidemiology, and Biochemistry, Houston

Iron and porphyrin metabolism may be affected considerably during the development of malignant neoplastic disease. Price(1) has reviewed the subject of anemia in cancer, and Nakahara and Fukuoka(2) have covered other aspects of tumor-host relations involving iron and porphyrin compounds. Several investiga-

*Supported by USPHS Grant No. C. A., 05831.

TABLE I. Serum Iron Levels Expressed in μg/100 ml.

Classification	Mean	Standard error of mean	95% Confidence limits†
Control (113)*	81.0	3.24	74.7–87.4
Malignant			
No metastases (160)	77.1	2.85	71.5–82.7
Regional metastases (97)	64.2	3.52	57.3–71.1
Widespread metastases (156)	47.8	2.44	43.0–52.6
Nonmalignant			
Inpatients (97)	83.0	5.50	72.2–93.8
Outpatients (56)	85.2	5.24	74.9–95.5
Tuberculous (40)	54.7	4.84	45.2–64.2

* No. of patients.

† Mean ± 1.96 standard error.

tors have reported a significant decrease in serum iron levels of tumor-bearing hosts(3-7), but there remains considerable doubt that a positive correlation does exist. The reduced serum iron levels which are often observed may be indirectly related to the malignant process. Many studies demonstrate that serum iron levels may be affected by certain environmental and physiological factors(3) and by pathological states, especially those associated with microbial etiology(3,8,9). Kampschmidt *et al*(10) have recently concluded that the depression in serum iron levels as well as other tumor-host effects may be attributed to the elaboration and release of substances by microorganisms associated with the tumorous growth.

Since interest in the possible effect of malignant growth on the host iron and porphyrin metabolism is widespread, the present study was undertaken to discover whether there is a positive correlation between serum iron levels and the stages of malignant development. Iron levels were determined on the serum of 413 cancer patients in this institution, 97 inpatients and 46 outpatients from a general hospital, and 40 tuberculous patients. For comparative purposes, the serum irons were also determined on 116 outpatients, in this institution, whose diagnostic tests for malignant disease had failed to reveal any disease process (Control, Series A).

Methods. Serum samples were obtained from patients with established histological diagnoses of malignant neoplastic disease and were arbitrarily classified as: a) widespread metastases, b) regional metastases, and c) no metastases. A subdivision of patients in each group was later made separating those with breast cancer from those with all sites except the breast. Serum samples in the groups with nonmalignant conditions were obtained from the general hospitals where they were examined. Patients who had received plasma or other transfusions, or whose iron levels would have obviously been affected were not used in the study. The blood samples were usually obtained in the morning from fasting patients and control group to minimize the effects of diurnal variation(3).

Serum irons were determined by the terpyridine procedure of Schade *et al*(11). Approximately one third of the samples were checked according to the method of Peters *et al*(12). The two procedures gave almost identical results. Accuracy and reproducibility were also checked against Hyland control sera and by repeated determination on the same patient samples. Iron-binding capacity was also determined on approximately 50 each of the controls and malignant sera. From the preliminary results, it was apparent that serum iron values would be more significant in the intended study.

Results. The mean serum iron levels for the control, malignant, and nonmalignant groups, with standard error and 95% confidence limits, are shown in Table 1. Values obtained for the control series were lower than the average literature values(3,13). These lower values may possibly be attributed to non-neoplastic disease processes, since individuals had been admitted for diagnostic study. The average serum iron readings of the control group, the 2 groups of patients with nonmalignant disease, and those with malignant disease but no metastases were similar. On the average, patients with regional

TABLE II. Serum Iron Studies, t-Test Results.

	Patients with cancer				Patients with nonmalignant disease	
	Metastases					
	Widespread	Regional	None	Control	Inpatient	Tuberculous
Patients with cancer						
Widespread metastases	—	$P < .001$	$P < .001$	$P < .001$	$P < .001$	NSD*
Regional metastases		—	$P < .01$	$P < .001$	$P < .01$	NSD
No metastases			—	NSD	NSD	$P < .001$
Control				—	NSD	$P < .001$
Patients with nonmalig. disease						
Inpatients					—	$P < .001$
Tuberculous						—

* No significant difference.

TABLE III. Serum Iron Studies, t-Test Results, Female Patients Only.

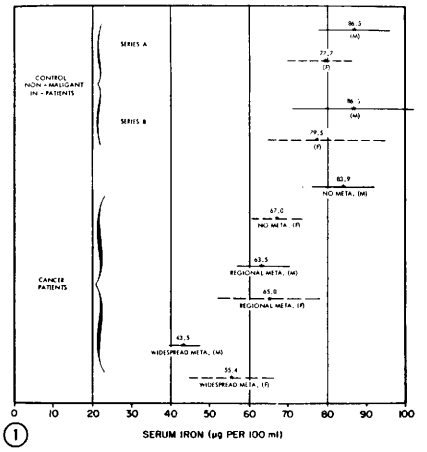
	Metastases			
	Widespread	Regional	None	Nonmalignant
		Non-breast cancer patients		
Widespread metastases	—	NSD*	$P < .01$	$P < .001$
Regional metastases		—	NSD	$P < .05$
No metastases			—	NSD
Nonmalignant				—
		Breast cancer patients		
Widespread metastases	—	NSD	NSD	NSD
Regional metastases		—	NSD	NSD
No metastases			—	NSD
Nonmalignant				—

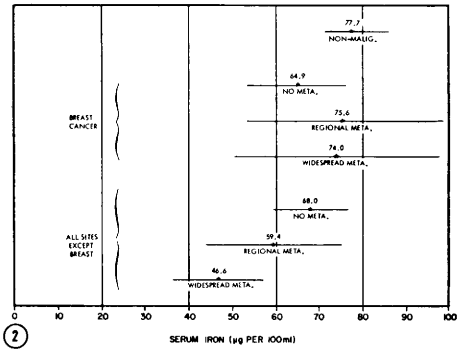
* No significant difference.

metastases had considerably lower levels and those with widespread metastases had the lowest readings of all. Generally, the tuberculous patients had serum iron readings which were between those of the patients with regional and those with widespread metastases. Using the standard t-test, significant differences at the 5% level or less were found between the control groups and patients with far advanced malignancies (Table II). From these results, it may be inferred that serum iron levels are related to the extent of metastatic involvement in the patient but are not a factor in determining a malignant condition.

No significant correlation between age and serum iron levels was found in the individuals included in this study. Serum iron level differences between the sexes are shown in Fig. 1. The t-test showed significant differences between iron levels in males and females in the categories of widespread metastases and of malignant growth with no metastases. The males had lower serum iron levels in the widespread metastases group and higher levels in the group with no metastases. These findings indicated that one or more types of malignant disease in the females deviated from the pattern of serum iron level in relation to extent of metastases that was clearly shown in the males, and thus might be obscuring the picture for the majority of female patients with malignant disease. The sample of female patients with malignant disease was subdivided by separating breast cancer patients from those with cancer of other sites (Fig. 2). Data from the "non-breast cancer" group followed the pattern shown in male patients, but for the breast cancer patients, the data are quite different. The statistical significance of the differences found in these patients is shown in Table III.

To confirm the statistical significances derived from the basic data, the Kolmogorov-Smirnov test for significant difference between two sample distributions was applied to all the groups and categories. This is a nonparametric test using the cumulative percentage distribution of patients at each blood





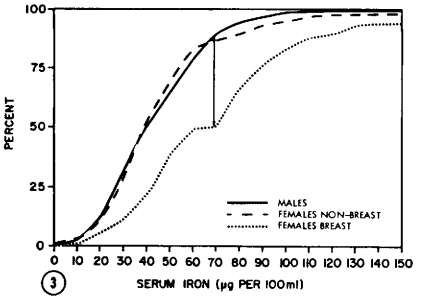


FIG. 1. Serum iron levels, group means and their 95% confidence limits. By sex and category.
FIG. 2. Serum iron levels, group means and their 95% confidence limit. Female patients. By site and category.
FIG. 3. Cumulative percentages of patients at each serum iron level. Widespread metastases. By sex and site.

serum iron level, and its advantage is that an assumption of normalcy in the population distributions is not required. This procedure confirmed the first results. Space does not permit showing all the graphs illustrating the various distributions and their pattern of differences. Fig. 3 illustrates the similarity in the widespread metastases category between serum iron levels of males and those females with cancer other than breast, and the marked difference in such levels between males and females with breast cancer. More than 75% of the males and of the females with "non-breast cancer" have blood serum iron levels of 70 and under, while only 50% of the breast cancer patients exhibit such levels.

Discussion. Cancer patients with no evidence of metastases had serum iron levels that approximated the control range, while average patients with advanced metastases had serum iron levels that were about half of the control value. In patients with some classes of tumors, the levels did not follow this trend. Patients with advanced breast tumors, while possessing a wide range of serum values, were not significantly different from the patients without metastases or from the controls. No explanation can be offered for this apparent difference between patients with advanced breast cancer and those with other advanced forms of cancer.

The sera obtained from patients with tuberculosis exhibited considerably reduced serum iron values. Generally, the values were between those observed in cancer patients with regional metastases and those with widespread metastases. This observation supports the general conception that a reduction in serum iron levels usually accompanies infections(3). Whether the reduced levels observed in the patients with advanced malignant disease are attributed to microorganisms in the tumorous growth, which Kampschmidt and associates had observed in experimental tumor systems (10), cannot be determined from the current data. The pathologists thought that most of the tumors from patients in this series did not have severe bacterial contamination. The serum iron levels of many in- and outpatients of a general hospital were similar to those of the control series. At present, it may be assumed only that there are nonspecific factors, common to some types of infection and malignant growth, which are capable of reducing serum iron levels. Further study will be required to elucidate the mechanism involved in such reported tumor-host effects as anemia, liver catalase depression, elaboration of toxo-

hormone, organ weight change, and changes in iron and porphyrin metabolism.

Summary. Serum iron levels were determined on individuals in the following categories (average value indicated in parenthesis): controls (81.0), cancer with no metastases (77.1), cancer with regional metastases (64.2), cancer with widespread metastases (47.8), nonmalignant disease (outpatients) (85.2), nonmalignant disease (inpatients) (83.0) and tuberculosis (54.7). Serum iron levels in patients with advanced breast cancer were not significantly different from the levels observed in cancer patients without metastases. The data indicate that the serum iron levels may be related to the extent of metastases, but that they are not a factor in determination of the presence of cancer *per se.*

We wish to acknowledge the valuable assistance and cooperation of Mr. Andrew Jitkoff, Jr., and Dr. Jack Abbott, Chief of Laboratories, The Methodist Hospital, and the assistance and advice of Dr. David Marrack, Department of Pathology, M. D. Anderson Hospital and Tumor Institute, in preparation of this manuscript.

1. Price, V. E., Greenfield, R. E., Adv. in Cancer Res., 1958, v5, 199.
2. Nakahara, W., Fukuoka, F., ibid., 1958, v5, 157.
3. Ramsay, W. N. M., Adv. in Clin. Chem., 1958, v1, 1.
4. Morriberon, D. J., Clinical Laboratorio (Zaragoza), 1962, v73, 422.
5. Kampschmidt, R. F., McCoy, T. A., Proc. Soc. Exp. Biol. and Med., 1960, v103, 869.
6. Otsuji, S., Dale, S., Griffin, A. C., submitted for publication.
7. Fredrikson, K. A., Acta Med. Scand., 1953, v146, 259.
8. Kawaguchi, O., Shikoku Acta Med., 1960, v16, 479.
9. Brendstrup, P., Acta Med. Scand., 1953, v145, 315.
10. Kampschmidt, R. F., Schultz, G., McKinzie, P., J. Nat. Cancer Inst., 1962, v28, 845.
11. Schade, A. L., Oyama, J., Reinhart, R. W., Miller, J. R., Proc. Soc. Exp. Biol. and Med., 1954, v87, 443.
12. Peters, T., Giovanniello, T. J., Apt, L., Ross, J. F., J. Lab. Clin. Med., 1956, v48, 280.
13. Fischer, D. S., Price, D. C., Clin. Chem., 1964, v10, 21.

Received November 30, 1964. P.S.E.B.M., 1965, v118.

20α-hydroxypregn-4-en-3-one, an Interfering Fluorogen in Assay of Corticosterone.* (29957)

GLORIA V. CALLARD, IAN P. CALLARD AND JAMES H. LEATHEM
Bureau of Biological Research, Rutgers, The State University, New Brunswick, N. J.

The quantitative measurement of serum corticosterone by fluorimetric techniques(1,2, 3) has been widely used as an estimate of adrenal function in rodents under various experimental conditions. The reliability of these methods is based on the assumption that the rat and mouse secrete no steroids which have fluorescence spectra similar to corticosterone under standard conditions in sulphuric acid. Interference from estrogen fluorescence is minimized in the extraction procedure(4).

Recently 20α-hydroxypregn-4-en-3-one was identified in rats in ovarian venous(5) and peripheral blood(6). Thus it seemed necessary to investigate the fluorescence properties of this steroid.

Materials and methods. All chemicals were of analytical reagent grade except for chloroform and methylene dichloride which were 'Chromatoquality.' 20α-hydroxypregn-4-en-3-one, its 20β isomer and corticosterone were obtained as gifts from Ciba (Basle), E. R. Squibb, and the National Institutes of Health Cancer Chemotherapy Section respectively. Fluorescence reagent was 55, 65, 75, 85 or 95% concentrated sulphuric acid in ethanol (v/v) or concentrated sulphuric acid alone. Fluorescence spectra were determined in an Aminco-Bowman Spectrofluorometer. All

* Supported by Grant AM-07333 from Nat. Inst. Health, U.S.P.H.S.