A New Cytological and Histological Fixing Fluid

Earl H. Newcomer

Department of Botany,
University of Connecticut, Storrs

In the preparation of chromosomes for microscopic examination by the smear or squash technique, the Carnoy fixation[1] or one of its modifications is perhaps the most widely used by cytologists. It has, however, a number of disadvantages. The mixture should be freshly or at least recently made up before use; tissues cannot be stored in it for more than a few weeks; and, if it contains chloroform, the Feulgen reaction is inhibited with time. The frequent practice of storing materials in 70% ethanol after Carnoy fixation is, in our hands, only a desperate resort made necessary by the exigencies of the season or available research time and its use results in a rapid and steady deterioration of the stainability of the cells. As a result, the cytologist is the victim of a hectic cycle of feast and famine for research and teaching materials, and his choice of materials and prosecution of research are often seriously circumscribed.

This problem recently became acute with us during a study of avian chromosomes. Since only freshly killed material gave adequate staining reactions, the sacrifice of expensive birds at a frequency demanded by technical considerations might well have made the project too expensive for completion. An earlier effort to solve this problem by changing the staining combination (*1*), although an improvement, still left us with a chloroform-containing fixative which inhibited the Feulgen reaction and hardened the tissues after prolonged storage.

For these reasons a series of experiments combining a number of nonpolar or weakly polar organic compounds with propionic acid was run, using avian testes and 8-day-chick embryos as test materials for fixation. By an empirical process of examination, recombination, and elimination, the following formula (by volume) emerged: 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part ether (petroleum), 1 part acetone, and 1 part dioxane.

This mixture is stable and can be made up in stock solution for use when needed, and it produces superb results. We have been using it in our laboratory for the past year in teaching and research and have found it eminently satisfactory for both plant and animal tissues. It has been used for avian testes, 40-hr and 8-day whole-chick embryos, the gonads of 4 mammals, grasshopper testes, and the following plant materials: *Tradescantia* and cucurbit microsporocytes, and root-tips of *Allium, Lilium,* and *Narcissis.* All the above materials, after storage in the fixative in the refrigerator at 4° C for periods of 5–11 months, are still staining sharply with propionic carmine and the Feulgen stain.

The time of hydrolysis for Feulgen in normal HCl at 60° C is 10 min for plant materials and 20 min for the animal tissues after prolonged storage.

With animal tissues the fixative is replaced once after 12–24 hr with fresh fluid, after which all materials are stored in the refrigerator until needed. These tissues can be either smeared directly or prepared for embedding in paraffin and the sectioning technique by replacing the fixative with absolute alcohol which is followed by xylol and paraffin. Thus all stages of dehydration save the one are eliminated, and we believe the fixation image is better than those usually produced by the conventional fluids containing salts of heavy metals. Furthermore, no bleaching is required prior to staining.

In preparation for hydrolysis for the Feulgen reaction, the tissues are washed for 1–2 hr, hydrolyzed, rinsed in distilled water, stained 2–3 hr in Feulgen, and smeared directly from Feulgen on the slide in a drop of 45% acetic acid. To make the Feulgen smear permanent, the slide is heated, pressure is applied on the coverslip, and the slide is immediately inverted in a Petri dish and covered with absolute alcohol to which fast green may be added for counterstain. When the coverslip comes off, it is immediately reunited to the slide with diaphane or euparal. The results, in our hands, are equivalent to any of the more complicated Feulgen procedures usually recommended in the literature. The same procedure may be used for making permanent preparations of propionic carmine smears.

[1] The original Carnoy fluid apparently consisted (by volume) of 6 parts absolute ethanol, 3 parts chloroform, and 1 part glacial acetic acid. Most investigators transpose the proportions between chloroform and acetic acid or omit the chloroform entirely.

Reference

1. NEWCOMER, E. H. *Stain Technol.,* **27**, 205 (1952).

Manuscript received March 17, 1953.

Comparative Effects of Total Body Radiation, Nitrogen Mustard, and Triethylene Melamine on the Hematopoietic System of Terminal Cancer Patients[1]

R. Kenneth Loeffler,[2] Vincent P. Collins,[2] and George A. Hyman

Departments of Radiotherapy and Pathology,
Francis Delafield Hospital,
Columbia-Presbyterian Medical Center, New York City

Nitrogen mustard and allied agents have come to be considered as alternative agents for radiotherapy in the treatment of several forms of cancer. Comparable clinical regression of disease and similar histologic changes have led to the use of the term "radio-

[1] Supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-302.

[2] Present address: Department of Radiology, Baylor University, Texas Medical Center, Houston, Texas.

mimetic." Where both agents are of known effectiveness, nitrogen mustard is usually preferred for generalized disease because the dose of total body radiation is believed to be limited to an ineffective level by radiation sickness and bone marrow depression. At the same time, the anticipated nausea, vomiting, and profound hematologic changes which follow vigorous chemotherapy are considered justifiable, and recovery is known to be reasonably certain. Comparably severe effects have not been produced by doses ordinarily employed in total body radiation. It seems possible that tolerance and recovery from radiation have been underestimated. A study was therefore undertaken to compare the effects of nitrogen mustard and allied agents with the effects of increasing doses of total body radiation.

All patients had advanced cancer and were considered suitable candidates for chemotherapy. One patient was given 5 mg of methylbis (2-chloroethyl)-amine.(HN_2) intravenously, one was given 5 mg of triethylene melamine (TEM) orally, and two received 50 r total body radiation. The x-ray factors were two million volts peak potential, target-skin distance 360 cm, filter 4 mm of lead, half value layer 6.6 mm of lead, air dose 50 r, skin dose 55 r, depth dose at 10 cm—41 r, and depth dose at 20 cm—23 r. In a second group, two patients were given 10 mg of HN_2, two were given 10 mg of TEM, and one received 100 r total body radiation under the same conditions as the previous group. When the patient receiving 100 r total body radiation failed to show any appreciable hematologic depression as a result of this treatment, one further patient was given 150 r total body radiation.

All patients studied received the following laboratory examinations before treatment: red blood count, hemoglobin, white blood count, differential count, reticulocyte count, platelet count, gastric analysis, bone marrow aspiration biopsy, plasma protein determination, serum bilirubin determination, plasma iron concentration, plasma iron turnover rate using Fe^{59} as a tracer, and red cell uptake of injected Fe^{59}. The iron studies were carried out because of the work of Huff, Hennessy *et al.* (*1, 2*). This demonstrated an effect of total body irradiation on the turnover of plasma iron and the utilization of this iron in the formation of red blood cells which appeared to be quantitatively related to the dose delivered.

These tests were repeated as frequently as was feasible following treatment. All patients were in a terminal state and it was not expected that the treatment would appreciably change the course of their diseases. None of the ten patients studied showed a deterioration in his condition that could be ascribed to the treatment given although several expired within a relatively short time thereafter.

Both chemotherapy and radiotherapy were given as single doses or exposures to eliminate the time factor. It is generally appreciated that higher doses are tolerated if radiation is given over a period of time (*3, 4*). The demonstration of higher tolerance levels of total body radiation may well prove of value in the management of diseases currently being treated routinely with nitrogen mustard and its derivatives.

All patients receiving nitrogen mustard or TEM developed malaise of varying severity, but none of the patients receiving radiation mentioned any changes in

TABLE 1

Patient	Disease	Treatment	RBC (millions)	Hb (grams %)	WBC (thousands)	Differential	Platelets (thousands)
W. B.	Carcinoma of lung	50 r	4.3 ā rx* N. C.	12 ā rx N. C.	6–8 ā rx N. C.	N. C.†	230–260 ā rx N. C.
J. F.	Carcinoma of colon	50 r	3.0 ā rx N. C.	9 ā rx N. C.	9–11 ā rx N. C.	N. C.	270 ā rx 90 at 2–3 wk
D. R.	Carcinoma of scalp	100 r	4.9 ā rx 4.0 at 6 wk	14 ā rx 11 at 6 wk	7–9 ā rx N. C.	N. C.	250 ā rx N. C.
R. F.	Carcinoma of sigmoid colon	150 r	3.4 ā rx N. C.	9 ā rx N. C.	7–9 ā rx 5 at 5 wk	N. C.	200–210 ā rx 100–130 at 4–5 wk
G. S.	Carcinoma of cervix uteri	5 mg HN_2	4.0 ā rx 3.0 at 16 days	9 ā rx 6 at 16 days	15 ā rx 8 at 3 wk	N. C.	300 ā rx N. C.
J. P.	Carcinoma of lip	10 mg HN_2	4.0 ā rx 3.3 at 31 days	12 ā rx 10 at 31 days	7–9 ā rx 5 at 18 days	N. C.	175–200 ā rx N. C.
M. B.	Carcinoma of vulva	10 mg HN_2	3.6 ā rx 3.1 at 23 days	10 ā rx 7 at 23 days	8–15 ā rx 7 at 23–30 days	12% lymph. ā rx 28% lymph. at 23–30 days	300–330 ā rx N. C.
J. A. H.	Carcinoma of prostate	5 mg TEM	4.2 ā rx 3.7 at 6 days	12 ā rx 10 at 6 days	15 ā rx N. C.	N. C.	210–240 ā rx N. C.
A. M.	Carcinoma of lung	10 mg TEM	4.5 ā rx 3.4 at 36 days	13 ā rx 9 at 36 days	11 ā rx 5 at 28 days	14% lymph. ā rx 5% lymph. at 7 days	170–200 ā rx N. C.
J. E. H.	Glioblastoma multiforme	10 mg TEM	5.1 ā rx N. C.	15 ā rx N. C.	9–11 ā rx N. C.	N. C.	180–210 ā rx N. C.

* ā rx before treatment.
† N. C. no significant change.

TABLE 1 (*Continued*)

Patient	Marrow aspiration	Other	Iron turnover rate (half-time in hr)	Plasma iron concentration (μg %)	Period of follow-up
W. B.	Hypoplasia ā rx* N. C.	N. C.†	1.0 ā rx 0.5 at 3 hr 1.1 at 7 days	65 ā rx 41 at 3 hr 40 at 7 days	5 wk died
J. F.	Normal ā rx N. C.	Free HCl: 55° ā rx 0° at 1 day 41° at 7 days	0.4 ā rx 0.5 at 3 hr 0.7 at 7 days	49 ā rx 43 at 3 hr 58 at 7 days	5 wk died
D. R.	Normal ā rx Hypoactive at 7 days	N. C.	2.0 ā rx 1.1 at 1 day 1.5 at 7 days	68 ā rx 112 at 7 days 89 at 51 days, 18 at 66 days	4 months living
R. F.	Normal ā rx Hypoactive at 7 days Normal at 36 days	Icterus: 2–5 ā rx 14 at 4 days 2–4 at 8 days	0.7 ā rx 0.7 at 1 day 7 days and 35 days	150 ā rx 75–85 at 1–23 days 150 at 29 days	3 months living
G. S.	Normal ā rx Greatly decreased at 21 days	N. C.	0.8 ā rx 0.8 at 1 day 3.0 at 8 days	93 ā rx 73 at 1 day, 59 at 8 days 45 at 17 days, 27 at 24 days	Deteriorating at 27 days signed out
J. P.	Normal ā rx N. C.	N. C.	1.1 ā rx 1.2 at 1 day 0.9 at 7 days 0.8 at 35 days	85 ā rx 75–100 at 1–36 days 38 at 44 days	4 months living
M. B.	Normal ā rx Increased cellularity at 15 days	N. C.	1.0 ā rx 1.1 at 1 day 0.6 at 8 days	85 ā rx 72 at 8 days 33 at 23 days, 85 at 38 days	4 months living
J. A. H.	Normal ā rx N. C.	N. C.	1.2 ā rx 1.2 at 1 day 1.2 at 7 days	123 ā rx 121 at 1 day 83 at 7 days	7 days signed out
A. M.	Normal ā rx N. C.	N. C.	0.7 ā rx 0.6 at 1 day 0.5 at 7 days 0.6 at 36 days	85 ā rx 55–65 at 4–30 days 47 at 36 days	3 months living
J. E. H.	Not done	Not done	1.1 ā rx 0.9 at 1 day 0.6 at 8 days	123 ā rx 82 at 1 day 115 at 8 days	11 days died

* ā rx before treatment.
† N. C. no significant change.

symptoms. One patient stated (J.F.) that he felt much better for 2 days following the treatment.

The data is summarized in Table 1. Except for the few changes mentioned specifically, there were no alterations in plasma proteins, serum bilirubin, reticulocytes, or gastric acidity.

References

1. Huff, R. L., *et al. J. Clin. Invest.*, **29**, 1041 (1950).
2. Hennessy, T. G., and Huff, R. L. *Proc. Soc. Exptl. Biol. Med.*, **73**, 436 (1950).
3. Strandqvist, M. *Acta Radiol.*, Suppl. LV (1944).
4. Paterson, R. *The Treatment of Malignant Disease by Radium and X-rays.* London: Edward Arnold, 36–40 (1949).

Manuscript received February 6, 1953.

Aural Detection of Grain Infested Internally with Insects

R. E. Adams, J. E. Wolfe, Max Milner, and J. A. Shellenberger

Department of Electrical Engineering and *Department of Flour and Feed Milling Industries, Kansas State College, Manhattan*

The detection of internal or hidden insect infestation in grains by electronic means was suggested before modern electronic techniques were available (*1*), but no useful developments of this kind have occurred. Our study of this application, which is one of a continuing series of investigations at this station aimed at developing inspection techniques for internally infested grain (2–5), has indicated that it may have considerable practical utility and, in addition, potentialities as a research tool for the insect physiologist concerned with the movement and feeding habits of insects which infest grain kernels internally.

The techniques and equipment are simple. The only major equipment requirement, in addition to a low noise level audio amplifier, a suitable microphone, and a loudspeaker, is a soundproof box. Our best results have been obtained with a concrete box having walls