

The British Journal of Cancer

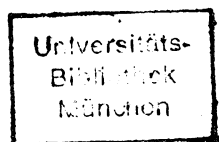
EDITORIAL BOARD

	L. G. Lajtha <i>Chairman</i>	
P. Alexander	J. F. Fowler	M. Moore
A. C. Allison	B. W. Fox	A. M. Neville
N. M. Bleehen	D. A. G. Galton	J. Paul
P. Brookes	D. G. Harnden	B. R. Pullan
E. H. Cooper	J. S. Jenkins	B. R. Rabin
D. Crowther	N. H. Kemp	F. J. C. Roe
A. R. Currie	J. Knowelden	M. Vessey
A. P. M. Forrest	N. A. Mitchison	R. A. Weiss

Associate Editor: A. J. Bateman

Volume 36, 1977

H. K. LEWIS & Co. Ltd., LONDON



Copyright © 1977
Made and printed in Great Britain for
H. K. Lewis & Co. Ltd., by
Adlard & Son Ltd., Bartholomew Press, Dorking

CONTENTS

No. 1—July

	PAGE
CURRIE, G. A. AND HEDLEY, D. W.—Monocytes and Macrophages in Malignant Melanoma. I. Peripheral Blood Macrophage Precursors.	1
MURRAY, E., MCCARTHY, W. H. AND HERSEY, P.—Blocking Factors Against Leucocyte-dependent Melanoma Antibody in the Sera of Melanoma Patients	7
LAMPERT, I. A., JONES, P. D. E., SADLER, T. E., AND CASTRO, J. E.—Intravascular Coagulation Resulting from Intravenous Injection of <i>C. Parvum</i> in Mice	15
HEWITT, H. B. AND BLAKE, E. R.—Facilitation of Nodal Metastasis From a Non-immunogenic Murine Carcinoma by Previous Whole-body Irradiation of Tumour Recipients	23
MANTOVANI, A., EVANS, R. AND ALEXANDER, P.—Non-specific Cytotoxicity of Spleen Cells in Mice Bearing Transplanted Chemically Induced Fibrosarcomas	35
BOMFORD, R. AND MORENO, C.—Mechanism of the Anti-tumour Effect of Glucans and Fructosans and a Comparison with <i>C. Parvum</i>	41
BARA, J., MALAREWICZ, A., LOISILLIER, F. AND BURTIN, P.—Antigens Common to Human Ovarian Mucinous Cyst Fluid and Gastric Mucosa	49
PAYNE, S. V., SMITH, J. L., JONES, D. B. AND WRIGHT, D. H.—Lymphocyte Markers in Non-Hodgkin's Lymphomas	57
PAPADOPOULOU, D., LEVY, S., CHAMAILLARD, L., BEESAU, O., HUBERT-HABART, M. AND MARKOVITS, P.—Hamster Cells, Untreated and Treated with Chemical Carcinogens, Maintained <i>In vitro</i> for 2½ Years	65
WEBB, T.—Sulphated Acid Mucopolysaccharides in SV40-Transformed Human Cells from Normal and Mucopolysaccharidosis Patients	72
ALALAWI, F. A. AND CHAPMAN, I. V.—Combined Effects of Bleomycin and X-rays on DNA Synthesis in Asynchronous Ehrlich Ascites Cells in Suspension	78
STEPHENS, T. C., PEACOCK, J. H. AND STEEL, G. G.—Cell Survival in B16 Melanoma after Treatment with Combinations of Cytotoxic Agents: Lack of Potentiation	84
VAN DEN BRENK, H. A. S., CROWE, M. C. AND STONE, M. G.—Reactions of the Tumour Bed to Lethally Irradiated Tumour Cells, and the Révész Effect	94
STANLEY, J. A., SHIPLEY, W. U. AND STEEL, G. G.—Influence of Tumour Size on Hypoxic Fraction and Therapeutic Sensitivity of Lewis Lung Tumour	105
BRATT, H. AND HATHAWAY, D. E.—Fate of Methyl Methacrylate in Rats	114
SMITH, I. E., PECKHAM, M. J., MCELWAIN, T. J., GAZET, J.-C. AND AUSTIN, D. E.—Hodgkin's Disease in Children	120

July—(cont.)

	PAGE
SIMARAK, S., DE JONG, U. N. (dec'd), BRESLOW, N., DAHL, C. J., RUCKPHAOPUNT, K., SCHEELINGS, P. AND MACLENNAN, R.—Cancer of the Oral Cavity, Pharynx/Larynx and Lung in North Thailand: Case-control Study and Analysis of Cigar Smoke	130
SOERIPTO, JENSEN, O. M. AND MUIR, C. S.—Cancer in Yogyakarta, Indonesia: Relative Frequencies	141
<i>Letter to the Editor</i>	
AJAYI, O. O. AND OKPAKO, D. T.—Prostaglandin-like Substances in Burkitt Lymphoma Tissue	149
BOOK REVIEWS	151

No. 2—August

VAN BEEK, W. P., EMMELOT, P. AND HOMBURG, C.—Comparison of Cell Surface Glycoprotein of Rat Hepatomas and Embryonic Rat Liver	157
GUY, D., LATNER, A. L. AND TURNER, G. A.—Radioiodination Studies of Tumour Cell-surface Proteins After Different Disaggregation Procedures	166
MAYER, A. M. S., BASOMBRIO, M. A. AND PASQUALINI, C. D.—Enhanced Growth of Syngeneic Moloney Sarcoma with Decreased Immunity in the Regressors	173
KARPAS, A., SANDLER, R. M. AND THORBURN, R. J.—Null Cell Properties of a Lymphoid Cell Line from a Child with Acute Lymphoblastic Leukaemia	177
WADDELL, A. W., BIRD, C. C. AND CURRIE, A. R.—Effect of Methylprednisolone on the Nucleoside Metabolism of a Human Lymphoblastoid Cell Line	187
NISHIZUMI, M., ALBERT, R. E., BURNS, F. J. AND BILGER, L.—Hepatic Cell Loss and Proliferation Induced by N-2-Fluorenylacetamide, Diethylnitrosamine, and Aflatoxin B ₁ in Relation to Hepatoma Induction	192
SHELDON, P. W. AND HILL, S. A.—Further Investigations of the Effects of the Hypoxic-cell Radiosensitizer, Ro-07-0582, on Local Control of a Mouse Tumour	198
HOUGHTON, P. J., HOUGHTON, J. A. AND TAYLOR, D. M.—Effects of Cytotoxic Agents on TdR Incorporation and Growth Delay in Human Colonic Tumour Xenografts	206
DYSON, P. AND HEPPLESTON, A. G.—Cell Kinetics of Urethane-induced Murine Pulmonary Adenomas. III. Implications of the Disparity Between the Rates of Entry into DNA Synthesis and into Mitosis	215
MOORE, J. V. AND DIXON, B.—Metastasis of a Transplantable Mammary Tumour in Rats Treated with Cyclophosphamide and/or Irradiation	221
KROHN, K. A., DENARDO, S. J., WHEELER, D. W. AND DENARDO, G. L.—I-Fibrinogen as an Oncophilic Radiodiagnostic Agent: Distribution Kinetics in Tumour-bearing Mice	227
GOLDSMITH, M., KOUTCHER, J. A. AND DAMADIAN, R.—NMR in Cancer XII: Application of the NMR Malignancy Index to Human Lung Tumours	235

August—(cont.)

	PAGE
GOSALVEZ, M., DIAZ-GIL, J., COLOMA, J. AND SALGANICOFF, L.—Spectral and Metabolic Characteristics of Mitochondrial Fractions from Rotenone-induced Tumours	243
BALL, J., FREEDMAN, L. AND SISSONS, H. A.—Malignant Round-cell Tumours of Bone. An Analytical Histological Study from the Cancer Research Campaign's Bone Tumour Panel	254
<i>Clinical Reports</i>	
HIMELSTEIN-BRAW, R., PETERS, H. AND FABER, M.—Influence of Irradiation and Chemotherapy on the Ovaries of Children with Abdominal Tumours	269
McELWAIN, T. J., TOY, J., SMITH, E., PECKHAM, M. J. AND AUSTIN, D. E.—A Combination of Chlorambucil, Vinblastine, Procarbazine and Prednisolone for Treatment of Hodgkin's Disease	276
<i>Short Communications</i>	
WATSON, J. V. AND TAYLOR, I. W.—Cell Cycle Analysis <i>In vitro</i> Using Flow Cytofluorimetry After Synchronization	281
CHIU, B., HAUSE, L., ROTHWELL, D., KOETHE, S. AND STRAUMFJORD, J.—Effects of Encephalitogenic Factor on Lymphocytic Electrophoretic Mobility for Cancer Patients and Controls	288
BOOK REVIEWS	291

No. 3—September

FALCÃO, R. P., SONIS, S. T., MACLENNAN, I. C. M., CHASSOUX, D., DAVIES, A. J. S. AND MUNRO, T. R.—Assessment of Drug Sensitivity of Human Leukaemic Myeloblasts. I. Labelling Human Myeloblasts with ¹²⁵ IUdR for Survival Studies in Mice	297
SONIS, S. T., FALCÃO, R. P. AND MACLENNAN, I. C. M.—Assessment of Drug Sensitivity of Human Leukaemic Myeloblasts. II. The Toxic Effects of Cytosine Arabinoside on ¹²⁵ IUdR-labelled Human Leukaemic Myeloblasts in Mice	307
STEPHENS, T. C. AND PEACOCK, J. H.—Tumour Volume Response, Initial Cell Kill and Cellular Repopulation in B16 Melanoma Treated with Cyclophosphamide and 1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea	313
DENEKAMP, J.—Early and Late Radiation Reactions in Mouse Feet	322
OTU, A. A., RUSSELL, R. J., WILKINSON, P. C. AND WHITE, R. G.—Alterations of Mononuclear Phagocyte Function Induced by Lewis Lung Carcinoma in C57BL Mice	330
ROBINSON, E., BARTAL, A., HONIGMAN, J. AND COHEN, Y.—A Preliminary Study of Intravenous Methanol Extraction Residue of BCG in Advanced Cancer	341
HANCOCK, B. W., BRUCE, L., DUNSMORE, I. R., MILFORD WARD, A. AND RICHMOND J.—Follow-up Studies on the Immune Status of Patients with Hodgkin's Disease after Splenectomy and Treatment, in Relapse and Remission	347
HAWKINS, R. A., HILL, A., FREEDMAN, B., GORE, S. M., ROBERTS, M. M. AND FORREST, A. P. M.—Reproducibility of Measurements of Oestrogen Receptor Concentration Breast in Cancer	355

September—(cont.)

	PAGE
PRIOR, P. AND WATERHOUSE, J. A. H.—Second Primary Cancers in Patients with Tumours of the Salivary Glands	362
POCKLINGTON, T. AND FOSTER, M. A.—Electron Spin Resonance of Caeruloplasmin and Iron Transferrin in Blood of Patients with Various Malignant Diseases	369
KENNEDY, J. R., YANG, T.-J. AND ALLEN, P. L.—Canine Transmissible Venereal Sarcoma: Electron Microscopic Changes with Time after Transplantation	375
<i>Short Communications</i>	
PETERS, L. J., MASON, K. A. AND MCBRIDE, W. H.—Pitfalls in the Use of the Lung Colony Assay to Assess T-cell Function in Irradiated Mice	386
TAKABE, Y., MIYAMOTO, T., WATANABE, M. AND TERASIMA, T.—Synergism of X-rays and Bleomycin on Ehrlich Ascites Tumour Cells	391
<i>Letters to the Editor</i>	
WATSON, J. V.—Fluorescence Calibration in Flow Cytofluorimetry	396
ECANOW, B., GOLD, B. H. AND SADOVE, M.—The Role of Inert Foreign Bodies in the Pathogenesis of Cancer	397
YANIV, A. AND EYLAN, E.—Iododeoxyuridine Activation of Oncornavirus-like Particles of Hamster Origin	397
B.A.C.R. 18th ANNUAL GENERAL MEETING	400
BOOK REVIEWS	432
MEETING ANNOUNCEMENT	434

No. 4—October

KARPAS, A.—A Humoral Cytotoxic Substance Produced by a Human Killer Cell Line	437
MORGAN, G., MCCARTHY, W. H. AND HERSEY, P.—Detection of Carcinoembryonic-like Antigen on Melanoma Cells by Leucocyte-dependent-antibody Assays	446
MANTOVANI, A., POLENTARUTTI, N., ALESSANDRI, G., VECCHI, A., GIULIANI, F. AND SPREAFICO, F.—Activation of K Cells in Mice with Transplanted Tumours Differing in Immunogenicity and Metastasizing Capacity	453
KAY, A. B. AND MCVIE, J. G.—Monocyte Chemotaxis in Bronchial Carcinoma and Cigarette Smokers	461
SEARLE, C. E. AND JONES, E. L.—Effects of Repeated Applications of Two Semi-permanent Hair Dyes to the Skin of A and DBA _f Mice	467
REZNIK, G. AND MOHR, U.—Effect of Di-Isopropanolnitrosamine in European Hamsters	479
BRANCA, M. AND NICOLETTI, L.—The Effect of Cyclophosphamide on MSV-H Oncogenesis	487
TAYLOR, I. W. AND BLEEHEEN, N. M.—Interaction of ICRF 159 with Radiation, and its Effect on Sub-lethal and Potentially Lethal Radiation Damage <i>In vitro</i>	493
KNEALE, G. W. AND STEWART, A. M.—Age Variation in the Cancer Risks from Foetal Irradiation	501

October—(cont).

	PAGE
PRICE, C. H. G. AND JEFFREE, G. M.—Incidence of Bone Sarcoma in S.W. England, 1946–1974, in Relation to Age, Sex, Tumour Site and Histology	511
<i>Short Communication</i>	
UEYAMA, Y., MORITA, K., KONDO, Y., SATO, N., ASANO, S., OHSAWA, N., SAKURAI, M., NAGUMO, F., IJIMA, K. AND TAMAOKI, N.—Direct and Serial Transplantation of a Ph ¹ + ve Human Myeloblastoid Tumour into Nude Mice	523
BOOK REVIEWS	528

No. 5—November

FORRESTER, J. A., DANDO, P. M., SMITH, W. J. AND TURBERVILLE, C.—Failure to Confirm the Macrophage Electrophoretic Mobility Test in Cancer	537
ARVILOMMI, H., DALE, M. M., DESAI, H. N., MONGAR, J. L. AND RICHARDSON, M.—Failure to Obtain Positive MEM Tests in either Cell-mediated Immune Conditions in the Guinea-pig or in Human Cancer	545
PETTINGALE, K. W., MERRETT, T. G. AND TEE, D. E. H.—Prognostic Value of Serum Levels of Immunoglobulins (IgG, IgA, IgM, IgE) in Breast Cancer. A Preliminary Study	550
STYLES, J. A.—A Method for Detecting Carcinogenic Organic Chemicals using Mammalian Cells in Culture	558
ASHBY, J., STYLES, J. A. AND ANDERSON, D.—Selection of an <i>In vitro</i> Carcinogenicity Test for Derivatives of the Carcinogen Hexamethylphosphoramide	564
HAMADA, Satoshi AND HAMADA, Sachiko—Localization of Carcinoembryonic Antigen in Medullary Thyroid Carcinoma by Immunofluorescent Techniques	572
AHERNE, W. A., CAMPLEJOHN, R. S., AL-WISWASY, M., FORD, D. AND KELLERER, A. M.—Assessment of Inherent Fluctuations of Mitotic and Labelling Indices of Human Tumours	577
WEBB, T. AND HARDING, M.—Chromosome Complement and SV40 Transformation of Cells from Patients Susceptible to Malignant Disease	583
WATSON, J. V. AND CHAMBERS, S. H.—Fluorescence Discrimination between Diploids Cells on their RNA Content: A Possible Distinction between Clonogenic and Non-clonogenic Cells	592
ATKINS, D., IBBOTSON, K. J., HILLIER, K., HUNT, N. H., HAMMONDS, J. C. AND MARTIN, T. J.—Secretion of Prostaglandins as Bone-resorbing Agents by Renal Cortical Carcinoma in Culture	601
AHERNE, G. W., PIALI, E. M. AND MARKS, V.—Development and Application of a Radioimmunoassay for Methotrexate	608
STEEL, G. G., ADAMS, K. AND STEPHENS, T. C.—Clonogenic Assays in the B16 Melanoma: Response to Cyclophosphamide	618
MACLENNAN, I. C. M., PETO, J. AND KAY, H. E. M.—Analysis of Treatment in Childhood Leukamia. V. Advantage of Reduced Chemotherapy During and Immediately After Cranial Irradiation	625

November—(cont.)

	PAGE
JUSSAWALLA, D. J. AND JAIN, D. K.—Breast Cancer and Religion in Greater Bombay Women: An Epidemiological Study of 2130 Women Over a 9-Year Period	634
<i>Short Communications</i>	
LIOTTA, L. A., GATTOZZI, C., KLEINERMAN, J. AND SAIDEL, G.—Reduction of Tumour Cell Entry into Vessels by BCG-activated Macrophages	639
TWENTYMAN, P. R.—An Artefact in Clonogenic Assays of Bleomycin Cytotoxicity	642
BOOK REVIEWS	645
MEETING ANNOUNCEMENTS	648

No. 6—December

STEEL, G. G. AND ADAMS, K.—Enhancement by Cytotoxic Agents of Artificial Pulmonary Metastasis	653
GRDINA, D. J., HITTELMAN, W. N., WHITE, R. A. AND MEISTRICH, M. L.—Relevance of Density, Size and DNA Content of Tumour Cells to the Lung Colony Assay	659
GUDEWICZ, P. W. AND SABA, T. M.—Inhibition of Phagocytosis and Glucose Metabolism of Alveolar Macrophages during Pulmonary Tumour Growth	670
BRYANT, G. M., SOHAL, R. S., ARGUS, M. F. AND ARCOS, J. C.—Ultrastructural and Metabolic Determinants of Resistance to Azo-dye and Susceptibility to Nitrosamine Carcinogenesis of the Guinea-pig	678
HORTON, L., FOX, C., CORRIN, B. AND SÓNKSEN, P. H.—Streptozotocin-induced Renal Tumours in Rats	692
McKENZIE, C. G., EVANS, I. M. A., HILLYARD, C. J., HILL, P., CARTER, S., TAN, M. K. AND MACINTYRE, I.—Biochemical Marker in Bronchial Carcinoma	700
BAUER, H. W. AND AX, W.—Detection of Sensitized Human Blood Lymphocytes by Agglutination with Basic Peptides: A Possible Test for Malignant Disease	708
NG, W. S., NG, M. H., HO, H. C. AND LAMELIN, J. P.— <i>In vitro</i> Immune Responses to PPD, Extracts from Raji Cells and Nasopharyngeal Carcinoma Biopsies in NPC Leucocytes	713
JAMASBI, R. J. AND NETTESHEIM, P.—Non-immunological Enhancement of Tumour Transplantability in X-Irradiated Host Animals	723
BIRCHALL, J. P., OWEN, J. J. T. AND OWEN, B. S.—Analysis of Heteroantisera to Cells from Human Malignant Effusions by Immunofluorescence and Protein A Binding	730
ORBACH-ARBOUYS, S., LHERITIER, J., ALLOUCHE M. AND POUILLART, P.—Intense Tumour Cell Destruction by Syngeneic Mice: Role of Macrophages, Complement Activation and Tumour Cell Factors	743

December—(cont.)

THATCHER, N., PALMER, M. K., GASIUNAS, N. AND CROWTHER, D.—Lymphocyte Function and Responses to Chemoimmunotherapy in Patients with Metastatic Melanoma	751
BLECHER, T. E. AND BISBY, R. H.—Mononuclear Cell-membrane "Fluidity": A Study in Some Haematological Malignancies	763
REES, J. K. H., SANDLER, R. M., CHALLENGER, J. AND HAYHOE, F. G. J.—Treatment of Acute Myeloid Leukaemia with a Triple Cytotoxic Regime: DAT	770
JONES, P. D. E., SADLER, T. E. AND CASTRO, J. E.—Effect of <i>Corynebacterium Parvum</i> on Peripheral Blood Platelets	777
TURGMAN, J., MODAN, B., SHILON, M., RAPPAPORT, Y. AND SHANON, E.—Nasopharyngeal Cancer in a Total Population: Selected Clinical and Epidemiological Aspects	783
ADAMI, H. O., RIMSTEN, A., STENKVIST, B. AND VEGELIUS, J.—Influence of Height, Weight and Obesity on Risk of Breast Cancer in an Unselected Swedish Population	787
CAMERON, H. M. AND WARWICK, G. P.—Primary Cancer of the Liver in Kenyan Children	793
BERAL, V., RAMCHARAN, S. AND FARIS, R.—Malignant Melanoma and Oral Contraceptive Use Among Women in California	804
<i>Short Communications</i>	
TAKAKU, F., YAMANAKA, T. AND HASHIMOTO, Y.—Usefulness of the SCM Test in the Diagnosis of Gastric Cancer	810
BARKLA, D. H. AND TUTTON, P. J. M.—Cytotoxicity of Cyproheptadine and Methysergide to Chemically Induced Carcinomas of Rat Colon	814
<i>Letters to the Editor</i>	
GRATTAROLA, R. AND JONES, M. K.—Concentration of Testosterone Glucuronide in Urine from Women with Breast Tumours	818
BOOK REVIEWS	819
ANNOUNCEMENT	821
MEETING ANNOUNCEMENTS	822

ASSESSMENT OF INHERENT FLUCTUATIONS OF MITOTIC AND LABELLING INDICES OF HUMAN TUMOURS

W. A. AHERNE, R. S. CAMPLEJOHN, M. AL-WISWASY, D. FORD
AND A. M. KELLERER

*From the Department of Pathology, RVI, Newcastle upon Tyne and
Institut für Medizinische Strahlenkunde der Universität Würzburg, Germany*

Received 24 April 1977 Accepted 27 June 1977

Summary.—A method is presented to evaluate the influence of statistical errors and inherent variation on the determination of mitotic and labelling indices of human tumours. In most of the experiments reported here, sufficient cells were counted to yield a statistical error which is small in comparison to the inherent differences in the proliferative indices, both between different sites in the same tumour and between different tumours of the same histological type. These inherent fluctuations are, therefore, a critical factor in cell kinetic studies of human tumours.

THE study of cell population kinetics ideally requires tissue samples which are large, or multiple, or both. Generally, this does not pose a serious problem when the subject of research is an experimental tumour population. But the goal of most tumour cell population studies is a better understanding of human cancer, and there is no realistic substitute for investigating *human* tumours as they occur in the patient, whenever this is feasible. Naturally, clinical research must be subordinated to a proper concern for the comfort of an ailing patient. It follows that the question of statistical accuracy must be considered very carefully; we must make the best of the limited samples available. In such circumstances the urge to strain after conclusions is strong, and accordingly the need to be aware of covert statistical errors is more essential than usual. In this paper we examine different sources of statistical uncertainty in two important parameters of cell population kinetics, namely the labelling and mitotic indices.

MATERIAL

The material comprises 5 cases of advanced, resected nephroblastoma (Wilms' tumour)

on which multiple mitotic indices were determined; 6 cases of early invasive but operable mammary carcinoma, on which both mitotic and labelling indices were determined at multiple sites (both types of mammary carcinoma were received as excision biopsies for rapid diagnosis); 2 cases of metastatic axillary lymph node deposits of mammary carcinoma, on which were determined the mitotic index and the labelling index both at multiple sites; and 2 cases of colorectal carcinoma on which the mitotic index was found, again at multiple sites.

METHODS

The mitotic index was determined on spatially distinct blocks in the cases of nephroblastoma and colorectal carcinoma, and retrospectively from stored histological sections in the case of mammary carcinoma except those which were also sampled for labelling, where again spatially distinct blocks were chosen. After primary fixation in formol-saline followed by secondary fixation in mercuric chloride (except for labelled blocks, which were fixed in Carnoy's fluid) sections were cut and stained by standard methods.

The labelling index was determined by culturing 1-mm³ fragments of tumour in 3 ml of Waymouth's medium (Wellcome) supplemented by 15% foetal bovine serum

(Flow Laboratories) and with [^3H]-thymidine (Radiochemical Centre, Amersham) at a concentration of $10\ \mu\text{Ci/ml}$. The culture was maintained for 30 min, after which the fragments were fixed for a short period in Carnoy's fluid and finally sectioned at $3\ \mu\text{m}$. The sections were dipped for autoradiography in Ilford K2 emulsion and exposed for 2 weeks.

STATISTICAL ANALYSIS

Mitotic and labelling indices (I_M and I_S , respectively) are estimated from experimentally determined proportions, $p = r/n$, where r is the number of cells which, in the one case, are in mitosis and, in the other, are labelled, and n is the total number of observed cells. The quantity r is subject to binomial statistics; accordingly, the variance of p is equal to $\langle p \rangle (1 - \langle p \rangle)/n$, where the mean $\langle p \rangle$ of p is equal to I_M or I_S^* . Since, in the cases which will be considered, I_M and I_S are of the order 0.005–0.05, the distributions actually reduce to Poisson distributions and the variance of p can be set equal to $\langle p \rangle/n$. Whenever r is sufficiently large (*e.g.* > 10) the situation is further simplified; and the distribution of p is approximately normal, with variance $\langle p \rangle/n$.

These considerations apply to the ideal case where samples are taken from the same site in the same tumour. In practice the standard deviation of the observed ratio r/n will be increased, as we shall show, due to differences in I_M or I_S either (a) between samples taken from different tumours, or (b) between samples taken from different sites in the same tumour. In other words, the actual standard deviation of experimentally determined values p is due not only to the sampling error of finite cell counts, but also to inherent variations in I_M and I_S , within tumours and between tumours. Our study is concerned with an assessment of the relative importance of these three sources of error: finite number of observed cells, fluctuations within tumour, and fluctuations between tumours.

The procedure applied is, in essence a comparison of the variances of observed values for I_M and I_S with their putative variances

($\langle p \rangle/n$) resulting from the finite cell count. As a first step, one can ask whether the variances are significantly larger than the putative variances. If they are, one can go further and estimate, from the discrepancy between observed variance and putative variance, the magnitude of the inherent variations of I_M or I_S .

Under the null assumption that the samples are homogeneous, one finds that the sum of the ratios of the actual to the putative deviations is distributed as χ^2 with $N - 1$ degrees of freedom (Snedecor and Cochran, 1971):

$$\chi^2 = \sum_{i=1}^N (p_i - p)^2 / p/n_i \quad (1)$$

$$= \frac{1}{p} \sum_{i=1}^N p_i r_i - \sum_{i=1}^N r_i$$

Where the samples can have different sizes n_i , and where $p = \Sigma r_i / \Sigma n_i$. In the following sections, experimental results will be compared with the theoretical distribution of χ^2 .

In those cases where the observed χ^2 is significantly larger than expected, the contribution of the two different types of statistical fluctuation can be estimated. This is done in the following way.

Let σ_{obs} be the observed standard deviation in a group of N samples

$$\sigma_{\text{obs}} = \left[\sum_{i=1}^N (p_i - \bar{p})^2 \right]^{1/2} (N - 1)^{-1/2}$$

$$= \left[\sum_{i=1}^N p_i^2 - 1/N \left(\sum_{i=1}^N p_i \right)^2 \right]^{1/2} \quad (2)$$

$$\times (N - 1)^{-1/2}$$

where p_i are the observed values and \bar{p} is their average. Since the inherent fluctuations and the fluctuations due to the finite cell count are independent, one can assume that the observed variance σ_{obs}^2 is the sum of the inherent variance σ^2 of I_M or I_S and the putative variance σ_p^2 which is due to the finite cell count.

$$\sigma_{\text{obs}}^2 = \sigma^2 + \sigma_p^2 \quad (3)$$

The putative variance for an individual observed value is, as has been stated, $\langle p \rangle/n_i$. As an estimate of $\langle p \rangle$, one has to

* The symbol $\langle p \rangle$ is used for the expectation value of p (*i.e.* the index) at a particular site in the tumour, while the more commonly used symbol \bar{p} is reserved for the average of the observed values p_i at different sites (see Equation (2)).

use the observed value p_i , and one therefore obtains the following estimate of σ_p^2 for a group of samples:

$$\sigma_p^2 = \frac{1}{N} \sum_{i=1}^N \frac{p_i}{n_i} = \frac{1}{N} \sum_{i=1}^N \frac{p_i^2}{r_i} \quad (4)$$

From Equations (2), (3), and (4) one obtains the estimate of the inherent standard deviation σ of I_M or I_S :

$$\begin{aligned} \sigma^2 &= \sigma_{\text{obs}}^2 - \sigma_p^2 \\ &= \frac{\sum_{i=1}^N p_i^2 - \frac{1}{N} \left(\sum_{i=1}^N p_i \right)^2}{N-1} - \frac{1}{N} \sum_{i=1}^N \frac{p_i^2}{r_i} \end{aligned} \quad (5)$$

In the next section these relations will be applied to the experimental observations.

RESULTS

Discussion of the analysis in a selected example

The analysis will be illustrated in detail as applied to the mitotic index in nephroblastoma. It will then suffice to present a table summarizing the results for the other tumours.

In each of 5 tumours 5 separate samples (histological blocks) were evaluated. Accordingly one may consider two different questions. First, we ask whether the observed fluctuations within individual tumours agree with the fluctuations which are expected due to sampling error in the finite number of cells observed, or whether they exceed these putative fluctuations. Secondly, we compare the observed fluctuations with the putative fluctuations for the *totality* of the results from all 5 tumours. In this second analysis a difference between the observed and the putative fluctuations represents the influence not only of variations in the mitotic index within tumours, but also of variations between tumours.

In order to analyse the fluctuations between different samples (histological blocks) within the same tumour, one

must apply Equation (1) separately for each tumour, and then sum the results for all 5 tumours. The quantity χ^2 will be written with the index w to indicate that it relates to variations from block to block within the same tumour:

$$\chi_w^2 = \sum_{j=1}^M \left(\frac{1}{p_j} \sum_{i=1}^{N_j} p_{i,j} \cdot r_{i,j} - \sum_{i=1}^{N_j} r_{i,j} \right) \quad (6)$$

In this formula i is the number of the block; j is the number of the tumour; M is the number of tumours; N_j is the number of blocks observed in the j th tumour. The quantities p_j , $p_{i,j}$, and $r_{i,j}$ are the values of p , p_i , r_i (see Equation (1)) for the individual tumour. The number of degrees of freedom is equal to the total number of blocks minus M .

The procedure is illustrated by Table I, which shows the mitotic index, the raw data on which the index was based, and the results of the χ^2 analysis in 5 separate sites (blocks) in each of 5 nephroblastomas. We see that the within-tumour χ_w^2 is 311 with 20 degrees of freedom. The probability of the purely chance occurrence of such a high value of χ^2 is < 0.001 ; one therefore concludes that the fluctuations in mitotic index from block to block are real.

We now consider the second aspect, and analyse the fluctuations over all blocks pooled from all 5 nephroblastomas. In this case one applies equation (1) for the totality of data where p is the total observed mean. The index j , which refers to the number of the tumour, can be omitted and the sum extends over the total number, $N = 25$, of blocks. The resulting quantity will be written with the index b to indicate that it refers to fluctuations not only within tumours but also between tumours:

$$\chi_b^2 = \frac{1}{p} \sum_{i=1}^N p_i r_i - \sum_{i=1}^N r_i \quad (7)$$

The number of degrees of freedom is equal to $N - 1$.

TABLE I.—*Mitotic Index, I_M (%), in Sections from Blocks taken at 5 Separate Sites in 5 Cases of Advanced (> 500 g) Nephroblastoma. Number of Mitoses Counted is Shown in Brackets*

Tumour	Block 1	2	3	4	5	I_M (%) \pm s.e.
N1	0.50 (225)	0.63 (291)	0.68 (244)	0.71 (305)	1.03 (365)	0.71 ± 0.09
N2	0.61 (318)	0.66 (305)	0.69 (292)	0.72 (328)	0.79 (290)	0.69 ± 0.03
N3	0.72 (301)	0.86 (407)	0.97 (424)	1.05 (494)	1.43 (662)	1.01 ± 0.12
N4	0.78 (425)	1.00 (418)	1.03 (444)	1.09 (494)	1.14 (494)	1.01 ± 0.06
N5	1.06 (325)	1.08 (526)	1.10 (526)	1.36 (532)	1.50 (525)	1.22 ± 0.09
Total:						0.93 ± 0.11

Fluctuations within tumours:

$$\chi_w^2 = 311; \text{d.f. } 20; P < 0.001.$$

$$\sigma_{\text{obs}} = 0.19\%; \sigma_p = 0.05\%; \sigma = 0.18\%.$$

Fluctuations between tumours:

$$\chi_b^2 = 782; \text{d.f. } 24; P < 0.001.$$

$$\sigma_{\text{obs}} = 0.27\%; \sigma_p = 0.05\%; \sigma = 0.26\%.$$

Parallel labelling studies were not made on these blocks, but studies on 3 comparable tumours have been reported by Camplejohn and Ahern (1974); these gave $I_s = 34.5\%$, 15.8% and 22.1% .

The resulting value is $\chi_b^2 = 782$ with 24 degrees of freedom. The probability of a random value as large as this is far below 0.001. The variations of the mitotic index between different tumours are even larger than those within tumours.

In the last column of the table, the mean of the observations is given for each tumour together with its standard error. The latter is equal to $\sigma_{\text{obs}}/\sqrt{N}$ (see Equation (2)). From these values one can already estimate the systematic fluctuations of I_M between tumours. It is, however, of interest to assess the fluctuations within tumours and between tumours quantitatively by using Equations (2), (4) and (5).

If one analyses the 5 tumours separately one obtains for each tumour the 3 values σ_{obs}^2 , σ_p^2 and σ^2 . The averages of these values for the 5 tumours are calculated and the resulting standard deviations (σ_{obs} , σ_p , and σ) are given in Table I. For the observed standard deviation one obtains $\sigma_{\text{obs}} = 0.19\%$. The putative standard deviation due to the finite cell count is $\sigma_p = 0.05\%$. The estimated standard deviation of the actual fluctua-

tions of I_M between blocks in the same tumour is $\sigma = 0.18\%$.

For convenience, both the values of I_M and of the standard deviations are given in % of the total cell number. One must note that the standard deviations are not given as per cent of the index I_M .

One finds that σ in this case substantially exceeds the putative fluctuations σ_p due to the finite cell count. It follows that the cell count could have been reduced in this experiment with very little loss of statistical accuracy. In order to improve the accuracy one would have to examine more blocks per tumour.

If one pools all samples from the 5 different tumours, one obtains an observed standard deviation of the samples from their common mean, $I_M = 0.93\%$, which has the value $\sigma_{\text{obs}} = 0.27\%$. The putative standard deviation due to the finite cell count remains unchanged at 0.05% , and the estimated inherent standard deviation of I_M in the various blocks from the common mean has the value $\sigma = 0.26\%$. It is therefore substantially larger than the fluctuations within tumours.

TABLE II.—*Summary of Results for Mammary Carcinoma (Primary and Metastatic) and for Primary Colorectal Carcinoma*

(a) Mitotic index

Tumour type	Number of tumours	Number of sites per tumour	Mean mitotic index for group of tumours (I_M)	Fluctuations within tumours	Fluctuations between tumours
Primary invasive mammary carcinoma	5	5	(251)* 0.4%	$\chi_w^2 = 27$; d.f. 19; $0.1 < P < 0.2$ $\sigma_{\text{obs}} = 0.16\%$; $\sigma_p = 0.13\%$; $\sigma = 0.08\%$	$\chi_b^2 = 81$; d.f. 23; $P < 0.001$ $\sigma_{\text{obs}} = 0.22\%$; $\sigma_p = 0.13\%$; $\sigma = 0.18\%$
Metastatic mammary carcinoma (lymph node deposits)	2	7-9	(275) 0.86%	$\chi_w^2 = 28$; d.f. 14; $P < 0.04$ $\sigma_{\text{obs}} = 0.34\%$; $\sigma_p = 0.21\%$; $\sigma = 0.26\%$	$\chi_b^2 = 103$; d.f. 15; $P < 0.001$ $\sigma_{\text{obs}} = 0.53\%$; $\sigma_p = 0.21\%$; $\sigma = 0.41\%$
Primary colorectal carcinoma	2	10	(908) 0.91%	$\chi_w^2 = 70$; d.f. 18; $P < 0.001$ $\sigma_{\text{obs}} = 0.26\%$; $\sigma_p = 0.13\%$; $\sigma = 0.22\%$	$\chi_b^2 = 71$; d.f. 19; $P < 0.001$ $\sigma_{\text{obs}} = 0.26\%$; $\sigma_p = 0.13\%$; $\sigma = 0.22\%$

(b) Labelling index

Tumour type	Number of tumours	Number of sites per tumour	Mean labelling index for group of tumours (I_s)	Fluctuations within tumours	Fluctuations between tumours
Primary invasive mammary carcinoma	4	5	(626) 2.45%	$\chi_w^2 = 120$; d.f. 16; $P < 0.001$ $\sigma_{\text{obs}} = 1.3\%$; $\sigma_p = 0.5\%$; $\sigma = 1.2\%$	$\chi_b^2 = 349$; d.f. 19; $P < 0.001$ $\sigma_{\text{obs}} = 1.9\%$; $\sigma_p = 0.5\%$; $\sigma = 1.8\%$
Metastatic mammary carcinoma (lymph node deposits)	2	6-9	(2199) 8.0%	$\chi_w^2 = 122$; d.f. 13; $P < 0.001$ $\sigma_{\text{obs}} = 2.6\%$; $\sigma_p = 0.7\%$; $\sigma = 2.5\%$	$\chi_b^2 = 265$; d.f. 14; $P < 0.001$ $\sigma_{\text{obs}} = 3.1\%$; $\sigma_p = 0.7\%$; $\sigma = 3.0\%$

* () Figure in brackets represents the total number of mitoses or labelled cells recorded for each group of tumours.

The results for the remaining cases are summarized in Table II. It is evident that the values of χ^2 , with one exception, are significantly different from the values expected as a result of sampling error. The exception is the set of results for the within-tumour fluctuation of the mitotic index in primary mammary carcinoma (Table II). In this case only few mitoses were observed. The standard deviation σ_p due to the finite cell count is therefore so large that it masks the inherent fluctuations of I_M . An estimated value of the inherent standard deviation $\sigma = 0.08\%$ is nevertheless given. Since this estimate is subject to considerable uncertainty, it is set in brackets.

The variation over axillary metastatic mammary carcinoma in two cases was also examined, these being the only suitable cases in our material now that radical mastectomy is less common. In this case, quite significant contributions of the systematic variations are found; the results are also given in Table II.

The mitotic index in the metastatic deposits of mammary carcinoma was significantly greater than the mitotic index in the primary tumours.

Mammary carcinoma is the only tumour whose labelling index we were able to estimate and analyse in this study.

COMMENT

At least in colorectal carcinoma and nephroblastoma, there are real differences in mitotic activity from site to site within each tumour and between tumours of indistinguishable histological type. The same may be true of the labelling index in mammary carcinoma.

In mammary carcinomas the mitotic counts were too low to permit accurate

assessment of the inherent variations in I_M . There is, however, no indication that inherent fluctuations are absent in this case.

The majority of cytokinetic measures used to characterize cell populations are based on proportions of mitoses (or metaphases) and proportions of labelled cells. In the light of our findings it appears difficult to obtain tissue samples which represent tumours unequivocally, at least in man. We have no reason to suppose that the tumours we studied are exceptional. Indeed, large variations in proliferative indices, in both human and animal tumours, have been reported by a number of authors. It has been shown that proliferative activity at a site depends upon a variety of factors such as proximity to a blood vessel or peripheral or central position (*e.g.* Hermens and Barendsen, 1967; Shirakawa *et al.*, 1970; Tannock, 1968). The aim of the present paper is to provide a method of assessing the relative magnitude of inherent and statistical variations. It is hoped that this technique could prove useful in the planning and evaluation of cell kinetic studies.

REFERENCES

- CAMPLEJOHN, R. S. & AHERNE, W. A. (1974) *In vitro* Labelling of Childhood Cancers with Tritiated Thymidine. *Br. J. Cancer*, **29**, 487.
- HERMENS, A. P. & BARENDSEN, G. W. (1967) Cellular Proliferation in an Experimental Rhabdomyosarcoma in the Rat. *Eur. J. Cancer*, **3**, 361.
- SHIRAKAWA, S., LUCE, J. K., TANNOCK, I. & FREI, E. (1970) Cell Proliferation in Human Melanoma. *J. clin. Invest.*, **49**, 1188.
- SNEDECOR, G. W. & COCHRAN, W. G. (1971) *Statistical Methods*. Ames: Iowa State University Press.
- TANNOCK, I. F. (1968) The Relation between Cell Proliferation and the Vascular System in a Transplanted Mouse Mammary Tumour. *Br. J. Cancer*, **22**, 258.