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Abstracts Of Recent Publications Of The Professional Staff Of The Henry Ford Hospital And The Edsel B. Ford Institute For Medical Research

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ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL
STAFF OF THE HENRY FORD HOSPITAL AND THE
EDEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

INTERACTIONS OF HYDROCORTISONE AND THYROTROPIN ON THYROID SECRETION OF INTACT DOGS. N. B. ACKERMAN, R. W. SMITH, JR. AND J. M. MILLER. *Metabolism* 10:27, 1961.

The effects of cortisol on the release of hormones from the thyroid gland of dogs were studied by serial measurements of PBI¹³¹ levels in blood from the thyroid vein. Infusion of 100 to 150 mg. of cortisol for one to three hours produced no appreciable change in levels of thyroid vein PBI¹³¹. Three hours of TSH infusion (0.2 to 20 units) rapidly accelerated PBI¹³¹ release during and after the infusion. When cortisol was administered simultaneously with TSH, the usual accelerated release of thyroid hormone was inhibited. This depressive action of cortisol was brief and disappeared as plasma corticoid levels decreased after the cortisol infusion was stopped. When TSH administration was maintained after stopping the cortisol infusion, a secondary increase of thyroid vein PBI¹³¹ resulted. In animals with renal arteries ligated bilaterally, cortisol produced no comparable modification of TSH effect on thyroid secretion. These findings suggest that at least one possible effect of cortisol on thyroid function may be to increase TSH inactivation or sequestration by the kidneys.

LEG ULCERS IN RHEUMATOID ARTHRITIS: USE OF PRESSURE-GRADIENT THERAPY, HIGH-PROTEIN DIET AND OTHER MEASURES PRODUCES SUCCESSFUL RESULTS. J. BENINSON AND D. C. ENSIGN. *J.A.M.A.* 175:437, 1961.

Our study of 9 patients with leg ulcerations complicating rheumatoid arthritis showed that pressure-gradient therapy was successful. The therapy involved "pressure-sandwich" dressings with use of a pressure-gradient technique to heal the ulcer, and which, if correctly applied, eliminated the associated intractable pain. A diet high in protein was stressed which shortened the healing time, and the healed state of the ulcer was maintained by using custom-tailored pressure-gradient supports, measurements for which were determined by the physician. When necessary, standard metatarsal bars attached to the outside of the shoe were prescribed to correct pes planus deformity. Moderate walking exercise in these supports was advised to control edema. A morphologic classification of the ulceration for prognostic purposes was developed.

SIX YEARS OF PRESSURE-GRADIENT THERAPY; A GENERAL SURVEY. J. BENINSON. *Angiology* 12:38, 1961.

The various usage to which pressure-gradient therapy has been put to date have been mentioned and anticipated usages suggested. Doubtlessly, other applications are inevitable. Perhaps they will one day be employed by man in space flight. Obviously much research will be necessary to elucidate the nature of the changes induced by pressure-gradient therapy in all the tissues involved. We are indebted to the now

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deceased Conrad Jobst for his contributions to the treatment of people afflicted with leg ulcers and edematous limbs.

MODIFIED NECK DISSECTION FOR THYROID CARCINOMA. M. A. BLOCK AND J. M. MILLER. *Am. J. Surg.* 101:349, 1961.

The presence of palpable cervical lymphadenopathy in patients with carcinoma of the thyroid nearly always indicates the presence of metastases to the nodes. A radical neck dissection is indicated in the surgical treatment of most of these patients who are otherwise operable (primary lesion can be removed). In our experience at least one-third of patients with carcinoma of the thyroid but without palpable cervical lymphadenopathy do have metastases to the cervical nodes. Whether or not cervical node dissections should be used for these patients is, therefore, not decisively established. However, a modified neck dissection is considered justified in the surgical treatment of many of this group of patients and better results are suggested if it is done. A modified neck dissection has been defined as a dissection which is identical to the classical radical neck dissection except for the preservation of the sternocleidomastoid muscle and the submaxillary gland area. This includes removal of the nodes adjacent to the thyroid and pre-supposes complete removal of the primary lesion.

PERORAL JEJUNAL BIOPSY IN A PATIENT WITH DIABETIC DIARRHEA: A CASE REPORT. T. BRIDWELL AND F. W. WHITEHOUSE. *Diabetes* 10:58, 1961.

The "diarrhea of diabetes" was first described in 1936 by Bargaen et. al. It is characterized by frequent, watery stools usually nocturnal in occurrence. Intermittency of symptoms and fecal incontinence further delineate this form of diarrhea. It is generally associated with diabetes of long duration which has been inadequately controlled. An associated peripheral neuropathy is common, and, indeed, it is accepted that diabetic diarrhea is merely another manifestation of the neuropathy of diabetes. Diabetic diarrhea usually is classified along with the neurogenic bladder, impotency, and postural hypotension as a form of "autonomic neuropathy." The jejunal mucosal pattern in a patient with diabetic diarrhea is reported herein.

MYELOMATOSIS WITH XANTHOMATOSIS. B. FRAME, M. R. PACTER AND R. K. NIXON. *Ann. Int. Med.* 54:134, 1961.

A case is presented in which myelomatosis was associated with hyperlipidemia and skin xanthomata. Discussion of the deranged lipid and protein fractions in the patient's serum is related to previously recorded serum lipid changes in multiple myeloma. It is possible that, in certain cases of myeloma, the plasma cells may directly produce abnormal lipid fractions, or that a sufficiently abnormal lipid-binding protein is secreted to account for the serum lipid aberrations in the disease.

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*STUDY ON THE DETERMINATION OF HEAVY WATER IN PLASMA OR URINE. O. H. GAEBLER AND H. C. CHOITZ. Clin. Chem. 6:549, 1960.

A technic has been described for determining the excess of deuterium oxide in plasma or urine, by directly measuring 19:18 ratios in vapor from these fluids with a mass spectrometer of suitable design. Although the 19:18 ratio for water varied with sample size and other experimental conditions, the increment in ratio per unit concentration of added D₂O remained quite uniform. A virtually linear relationship between the increment in ratio and the concentration of added D₂O also facilitated checking the calibration. The time required for flushing out deuterium when a Pyrex inlet system was used could be minimized by carrying out both the calibrations.

AIR EMBOLISM. T. N. JAMES. Am. Heart J. 61:423, 1961.

The entrance of air into the circulatory system may or may not be fatal, depending among other things on the volume of air injected and the rate of injection. It has been estimated that at the same rate of injection the minimal lethal volume of air entering the right side of the heart is seventy times that entering the left side. Sudden death after the entrance of air into the left side of the heart is due to occlusion of the coronary arteries; death also follows occlusion of the cerebral arteries by the same volume of air, but in dogs this occurs only after many hours. So long as the heart can maintain normal arterial perfusion pressure, air will be pushed through the capillaries into the venous circulation. That air under normal systemic perfusion pressure will readily pass a capillary barrier is not generally appreciated.

ANATOMY OF THE HEART: NORMAL AND PATHOLOGIC, AS RELATED TO CARDIAC FUNCTION. T. N. JAMES. In, American College of Chest Physicians: Clinical Cardiopulmonary Physiology, ed. by Burgess L. Gordon. Ed. 2, New York, Grune & Stratton, 1960, p. 8.

Although a knowledge of anatomy of the heart is a prerequisite in the considerations of the pathology and physiology, such information has assumed perhaps greatest significance in cardiac surgery. Thus, with the birth of cardiac surgery there has been a resurgence of interest in the meticulous descriptions of Vesalius, Vieussens, Spalteholz and others. This rediscovery of old facts will be of benefit to all of us. In the present review of the normal and pathologic anatomy of the heart, emphasis is directed to features that are of particular clinical significance. For example, the section on coronary artery anatomy is expanded because of the clinical interest in coronary disease; the more detailed considerations are omitted due to availability in standard references on gross anatomy. It should be mentioned, however, that certain features which currently seem most important may assume lesser roles in the future, owing to advances in medical knowledge. This scholarly article describes pericardium, myocardium, endocardium, cardiac valves, conduction centers and pathways, innervation, coronary arteries, veins of the heart, and lymphatic system of the heart, each in normalcy and disease.

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CORONARY ANATOMY FOR THE PRACTICING INTERNIST. T. N. JAMES. Minnesota Med. 43:847, 1960.

If knowledge of the exact location of a coronary occlusion is not important, then an enormous amount of present effort for the production roentgenograms of the coronary arteries is being misdirected. It is of course important, but not all the reasons are fully appreciated. An acute coronary occlusion is not, like Gertrude Stein's rose, just an acute coronary occlusion. It behaves differently depending on where the subsequent myocardial infarction occurs. Consideration of just a few simple points of human coronary anatomy can help explain this difference in behavior. Although many myocardial infarctions (perhaps the majority) are streaky or lamellar or diffuse, enough of them involve specific localized areas to warrant their consideration. Of these discrete infarcts, there are three groups, each of which is due to occlusion of one of the three main coronary arteries: Anteroseptal infarcts are due to occlusion of the left anterior descending coronary artery. High lateral infarcts are usually due to occlusion of the left circumflex artery. Posterior infarcts are due to right coronary artery occlusion. Mixed infarcts are better understood if the three basic infarcts above are remembered. *Anteroseptal infarcts* are the commonest of these three. The primary electrocardiographic changes are in leads I, aVL, and V2 to V4. Other factors being equal, more proximal occlusions of the left anterior descending artery produce larger infarcts. *Lateral infarcts* involve the obtuse margin of the left ventricle, usually more toward the base than apex of the heart. Electrocardiographically the primary changes are in leads I, aVL, and HV3 to HV5 (high leads); the conventional precordial V leads are conspicuously normal or show only the edge of the infarct. *Posterior infarcts* are the most interesting from the standpoint of coronary anatomy. Since 90 per cent of humans supply the crux of the heart (where most "pure" posterior infarcts occur) via the right coronary artery, it follows that 90 per cent of these infarcts will be due to right coronary occlusion.

PHYSIOLOGY AND MORPHOLOGY OF BLOOD COAGULATION IN POLYCYTHEMIA VERA. T. N. JAMES, S. A. JOHNSON AND R. W. MONTGOMERY. J. Appl. Physiol. 15:1049, 1960.

The observation that red cells fall out of the clotted blood in polycythemia vera has been documented previously. This phenomenon appears in all patients with initial thrombocytosis but continues to occur even when the red cell mass and platelet numbers come under control. The only known coagulation defect is a pronounced reduction of platelet factor₃ in the platelets. In this investigation, platelet-plasma clots from normal individuals and polycythemia vera patients were sectioned and stained at various intervals during coagulation and clot retraction. Great disorganization of the platelet-fibrin network was observed in the polycythemia vera clots when compared with the normal clots, and it was apparent that the red cells could easily slip through the openings in the network.

PRIMARY HYPERPARATHYROIDISM: A DIAGNOSTIC CHALLENGE. W. C. MIEHER, JR., V. THIBAudeau AND B. FRAME. Arch. Int. Med. 107:361, 1961.

A series of 21 proven cases of primary hyperparathyroidism diagnosed between

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1931 and 1959 is reported. From an analysis of the cases reported and a review of the recent literature, the various clinical syndromes presented by hyperparathyroidism are discussed and the laboratory and x-ray aids to diagnosis are reviewed. The authors are of the opinion that the key to the more frequent diagnosis of hyperparathyroidism involves the screening by serum calcium and phosphorus determinations of patients with the following conditions: (1) Urinary calculi or renal calcinosis. (2) Recurrent or chronic urinary tract infection; unexplained hematuria and azotemia. (3) Demineralizing or osteolytic skeletal disease. (4) Symptoms of peptic ulcer, with or without x-ray evidence of ulcer. (4) Pancreatitis and/or x-ray evidence of pancreatic calcinosis. (6) Recurrent or persistent vomiting of unexplained cause. (7) Sustained hypertension especially with renal damage. (8) Unexplained behavioral changes in previously well-adjusted persons. (9) Unexplained polydipsia and polyuria. (10) Patients previously operated upon for parathyroid adenoma (should probably be checked yearly). (11) Patients with symptoms of islet-cell or pituitary adenomas and (12) Family history of hyperparathyroidism.

***ASSAY OF THE DPNH DEHYDROGENASE OF THE RESPIRATORY CHAIN IN PARTICULATE AND SOLUBLE PREPARATIONS.** S. MINAKAMI, R. L. RINGLER AND T. P. SINGER. *Biochem. & Biophys. Res. Comm.* 3:423, 1960.

This paper describes the assay conditions developed for following the activity of the DPNH dehydrogenase of the respiratory chain during purification. An assay has been elaborated which measures the full activity of the enzyme in organized sub-cellular systems as well as in the soluble, purified state. By means of this assay, as described in the companion paper, it has been possible to extract in soluble form for the first time the enzyme in question, using heart mitochondria as a source. Judicious use of this assay enables one to localize exactly the site of action of numerous metabolic inhibitors of interest, such as barbiturates.

MYXEDEMA NEUROPATHY AND MYOPATHY: A CLINICAL AND PATHOLOGIC STUDY. S. N. NICKEL, B. FRAME, J. BEBIN, W. W. TOURTELLOTTE, J. A. PARKER AND B. R. HUGHES. *Neurology* 11:125, 1961.

Manifestations suggesting neurologic and muscular impairment were sought in 25 patients with primary myxedema. Objective sensory and motor dysfunctions with decreased peripheral sensation and proximal muscle weakness were significant findings in this study. The pathologic alterations in nerve and muscle consisted of mucinous deposits in the endo- and perineurium as well as in the endo- and perimysium. The myelin sheaths and axis cylinders were altered. Both structure and staining changes were present in muscle fibers. The serum studies revealed a normal total protein concentration; however, separation of the various protein fractions revealed significantly decreased albumin and increased beta and gamma globulin. The increased gamma globulin was confirmed by an immunochemical technic. The total serum cholesterol and total serum lipoproteins were elevated significantly. Furthermore, the alpha-2 globulin lipoprotein was significantly elevated. Serum glycoprotein values were normal. The spinal fluid ex-

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amination revealed increased total protein and gamma globulin fraction. Myxedema neuropathy and myxedema myopathy are terms suggested to define the clinical and pathologic picture seen in patients in this study.

EFFECTS ON BEHAVIOR AND CONSCIOUSNESS OBTAINED THROUGH STIMULATION OF THE RETICULAR FORMATION OF THE MACACCA MULATTA. L. D. PROCTOR, R. S. KNIGHTON, J. A. CHURCHILL AND J. BEBIN. First International Congress of Neurological Sciences (1957). London, Pergamon Press, 1959, v. 5 p. 180.

Further observations have been set forth, showing the effects of stimulation of the reticular formation in the macacca mulatta. Stimulation of widely spaced electrodes in the reticular formation in areas between the anterior hypothalamic nucleus and the reticular substance at the junction of the mid-brain and pons has produced in one instance hemiballismic movements and in another 'genital response.' Impairment of consciousness, as measured by impairment of the animals' efficiency in performing a psychometric test, was shown to occur in varying the placement and parameters of stimulation within these areas. Two other animals in which electrodes were placed outside of the reticular formation (caudate nucleus, pulvinar of the thalamus, in the transverse fissure above the collicular plate and in the transverse fissure immediately above the stratum zonale of the dorsal thalamus), showed only motor components as a result of stimulation using voltages up to ten times that used in stimulation of the reticular formation.

INTERRELATION OF TESTS OF INTESTINAL ABSORPTION. J. A. RINALDO, JR. Gastroenterology 40:86, 1961.

Tests useful in the assessment of intestinal absorption include measurement of the amount of ingested *d*-xylose in the urine, fasting serum carotene concentration, and fecal fat. The present investigation examines certain aspects of these tests, including the interrelation among them, the relation between the clinical severity of a disease of malabsorption and the variation of each test, and the determination of the most efficient application of the tests as a group. We measured fasting serum carotene concentration, 4-hour urine *d*-xylose excretion, and 72-hour fecal fat excretion in two groups of patients. The first group included functional patients having diarrhea but excreting a normal amount of fecal fat. The second group consisted of patients having steatorrhea resulting from nontropical sprue or exocrine pancreatic insufficiency or partial gastrectomy. Each patient was graded according to the clinical severity of his disease, and the grade obtained was compared to the test result. Fecal fat excretion was the only test that showed consistent progressive abnormality as the clinical severity increased, but then for only sprue and pancreatic insufficiency. A correlation was found between serum carotene concentration and fecal fat excretion, but this correlation was not sufficiently close to make serum carotene determination a useful predictor of severity of steatorrhea.

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THE VARIATION IN CLINICAL RESPONSE TO MARPLAN WITH DURATION OF ILLNESS. R. R. SCHOPBACH. *Am. J. Psychiat.* 117:746, 1961.

This paper indicates that the effects of hydrazines upon depressions vary with the duration of the illness. A lowered serotonin metabolism in depression is also suggested. Routinely 30 mg. Marplan per day was given either in one dose or divided to spread the psychologic effect. Of the 28 patients only 6 were suffering from an acute depression. Of these 6 acute depressions 2 cleared within 1-2 days, making it doubtful that the drug was the responsible curative agent. The results in the group of 22 more chronically disturbed depressed patients were less gratifying. Thus, as with all therapies, Marplan is more effective in acute depressions but is still of definite value in those of longer duration.

*ISOLATION AND PROPERTIES OF THE DPNH DEHYDROGENASE OF THE RESPIRATORY CHAIN FROM HEART MITOCHONDRIA. R. L. RINGLER, S. MINAKAMI AND T. P. SINGER. *Biochem. & Biophys. Res. Comm.* 3:417, 1960.

This paper reports the first successful solubilization and partial purification of what is perhaps the most important oxidizing enzyme in all higher forms of life: the one responsible for the reoxidation of reduced DPN by way of the respiratory chain. This enzyme links the oxidation of some 95% of the oxidative events which result in energy conservation in the cell to the system of oxidative phosphorylation, which traps and transforms this energy into ATP, the primary chemical fuel for all synthetic processes and work performed by living cells. The paper describes various properties of the enzyme, such as substrate specificity, action of inhibitors, absorption spectrum, turnover number, etc.

*ON THE MULTIPLICITY OF LACTIC DEHYDROGENASES IN YEAST. T. P. SINGER, E. B. KEARNEY, C. GREGOLIN, E. BOERI AND M. RIPPA. *Biochem. & Biophys. Res. Comm.* 3:428, 1960.

This paper is a brief summary of the results of a collaborative investigation by the Department of Human Physiology of the University of Ferrara (Italy) and the Enzyme Division of this institute. It is concerned with several problems, the most fundamental one of which is the demonstration that an oxidative enzyme, specific for the D(-) configuration of lactate (D-*a*-hydroxy acid dehydrogenase), present only in *anaerobic* yeast, is not a precursor of the L (+) specific lactic dehydrogenase of aerobic cells (the so-called cytochrome b_2), contrary to repeated claims in the literature. This conclusion was based on a comparison of the enzymatic and chemical properties of the two isolated proteins, on the effect of growth conditions on the formation of the two enzymes, and on a study of mutant cells. The paper further reports the discovery of two new lactic dehydrogenases (which were later isolated in Detroit) and also demonstrates that, unlike generally believed, alcohol is not the only normal product of yeast fermentation but that, as in animal tissues, considerable amounts of lactate accumulate during anaerobic glycolysis.

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HEALING COMPLICATIONS WITH PLASTIC ARTERIAL IMPLANTS. R. F. SMITH AND D. E. SZILAGYI. *Arch. Surg.* 82:14, 1961.

The purpose of this study was to recount the incidence, causes, and treatment of healing complications (wound infections, anastomotic false aneurysms, and suture line hemorrhages) in 343 reconstructive arterial operations using plastic arterial substitutes (in 90 percent of the cases elastic woven Dacron prostheses). There were 3 superficial and 11 deep wound infections. Among the cases of deep wound infection 6 serious hemorrhages occurred, and in 3 fatal septicemia developed; in most of these cases the infected prostheses had to be removed. The etiological factors in the 32 false aneurysms, 7 of which ruptured, were in order of frequency: diseased arterial wall at the site of anastomosis, defective suturing, defective prosthesis, and deep wound infection; excision with reconstructive repair was unsuccessful in over half of the cases.

ARTERIOPLASTY FOR SEVERE ISCHEMIA OF THE LOWER LIMBS: INDICATIONS AND RESULTS. D. E. SZILAGYI, R. F. SMITH AND A. ESPHANI. *Surgery* 49:242, 1961.

The use of grafting procedures in patients with severe ischemia of the lower limbs caused by occlusive arteriopathy is clearly indicated whenever the possibility of success exists and the patient's general condition permits the intervention. The minimum technical requirement for femoropopliteal grafting in these cases is the presence of an open popliteal arterial stem with at least one functioning infrapopliteal branch. The minimum technical requirement for aortoiliac grafting is a functional common femoral arterial segment or the existence of an anatomic situation in the popliteal area described above. A successful operation will always completely rehabilitate the patient. The duration of the rehabilitation is not known but it is probably at least 2 years in two-thirds of the cases. An unsuccessful operation may precipitate amputation but, under the operative indications described, such an amputation either is inevitable or merely means the loss of a functionless limb.

THE BIOLOGIC FATE OF HUMAN HOMOLOGOUS ARTERIAL GRAFTS. D. E. SZILAGYI. *Minerva Cardioangiologica Europea* 8:55, 1960.

We wish to report very briefly the observations we made in course of the study of the structural alterations that take place in human homologous arterial grafts after implantation. The study was based on an experience of 350 surgical procedures utilizing such grafts and performed between January 1952 and July 1957. In spite of the excellence of their technical qualities and the brilliant early results they have generally yielded, from a long-range point of view both the human aorta and the human femoral artery suffer from serious deficiencies as homologous vascular substitutes; the femoral artery, in particular, seems intrinsically poorly suited to serve as homograft. Consequently, in our practice the use of homologous arterial grafts has been completely abandoned.

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GROIN TO CALF BY-PASS GRAFT IN THE TREATMENT OF FEMORO-POPLITEAL ARTERIAL OCCLUSIVE DISEASE. D. E. SZILAGYI AND L. S. FALLIS. *Minerva Cardioangiologica Europea* 8:150, 1960.

The cause of the late failures after grafting procedures for the correction of femoro-popliteal arterial occlusive lesions are very briefly reviewed, and the point is made that the large majority of these failures have in the past been due to incomplete reconstruction of the arterial tree. A surgical technique is described that allows the insertion of a long femoro-popliteal bypass in an uninterrupted technical sequence. Bridging, in this manner, a longer segment of diseased artery and thus achieving a more nearly complete circumvention of the occlusive process, this operation promises to reduce the late failure rate of grafting operations for occlusive disease of the femoro-popliteal area.

MALIGNANT MEDIASTINAL TERATOMA WITH BONE METASTASES: A CASE REPORT. A. W. TEMPLETON. *Radiology* 76:245, 1961.

A teratoma is a true neoplasm, composed of multiple tissues foreign to the part of the body in which it arises and possessing the power of progressive growth. According to its behavior or structure, it may be benign or malignant. Clinically, malignant mediastinal teratomas are seen predominantly in males. The average age of occurrence is twenty-seven years. After onset of symptoms, the average duration of life in Schlumberger's series was five and a half months. Presenting complaints included cough, chest pain, dyspnea, hemoptysis, and engorgement of the neck veins. An unusual case of malignant mediastinal teratoma with proved metastases to the lumbar spine has been presented.

CLINICAL EVALUATION OF CANCER CHEMOTHERAPY WITH 5-FLUOROURACIL. V. K. VAITKEVICIUS, M. J. BRENNAN, V. L. BECKETT, J. E. KELLY AND R. W. TALLEY. *Cancer* 14:131, 1961.

5-FU was able to induce objective tumor regressions in a variety of tumors although commonly only at the expense of severe toxicity. Vigorous supportive therapy, combined with early treatment of complicating infections, reduced the hazards of therapy sufficiently so that in far advanced cases of otherwise untreatable breast and primary hepatocellular carcinomas 5-FU became a clinically useful agent. In the treatment of ovarian carcinoma, the results obtained by 5-FU therapy are comparable to those obtained with radiomimetic agents. Because of the more unpleasant symptomatic effects of 5-FU treatment, radiomimetic drugs are probably preferable. 5-FU has a narrow therapeutic range. Toxicity is regularly produced with effective doses but is manageable. The spectrum of tumors undergoing clinically valuable regressions is narrow but does include such common tumors as carcinoma of the breast, ovary, and large bowel as well as hepatocellular carcinoma.

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FREEZE-PRESERVATION OF ENZYMATICALLY PREPARED TUMOR CELL SUSPENSIONS AND THEIR USE IN TUMOR TRANSPLANTATION. V. K. VAITKEVICIUS, W. L. SIMPSON AND M. J. BRENNAN. *Cancer Res.* 21:187, 1961.

Methods currently in use for preparation of cell suspensions for tissue culture are useful for tumor transplantation work and permit quantitative assays of factors affecting transplantation. Enzymatic digestion yields higher numbers of viable cells than the commonly used mechanical method of squeezing cells through metal meshes. Cell suspensions prepared by enzymatic digestion can be stored at low temperatures for prolonged periods of time without changing their transplantability. Tumors requiring only small inoculum sizes for transplantation can be successfully freeze-preserved by virtually any method of tissue freezing currently in use. The less "virulent" tumors studied require protection by adequate suspension media with added glycerol for successful preservation and may be injured by enzymatic digestion prior to freezing. The elimination of stroma in such cell suspensions allows more adequate quantitation of the transplanted tumor cells and decreases the variability in the latent periods and in the percentages of successful transplantations. The best over-all preservation of mouse tumor cells for transplantation appeared to follow enzymatic isolation, suspension in the Eagle-Earle solution and glycerol, followed by addition of serum before freezing. The possible applicability of these technics to work with human tumors is now being tested.



