TABLE 2

EVIDENCE FOR THE PRESENCE OF DPN-ASE ON THE SURFACE OF THE RED CELL

Expt No.	Preparation (0.2 ml/flask)	Added materials	Nicotinamide (M)	$Q^{N_2+CO_2}_{lactate}$
6*a*	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	—	—	27.20
b	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	—	0.03	26.00
c	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Red cell suspension,* 0.05 ml	—	4.25
d	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Red cell suspension, 0.05 ml	0.03	24.20
e	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Unwashed stroma,† 0.05 ml	—	1.71
f	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Unwashed stroma, 0.05 ml	0.03	19.50
g	DPN alone $0.865 \times 10^{-4}M$	Red cell suspension, 0.05 ml	0.03	0.05

* Corresponding to 2.34 mg dry-cell residue per flask.

† Corresponding to 2.67 mg dry-cell residue per flask. The quantity of stroma used was about 10 times that represented by the quantity of cells in *.

the DPN except when an excess of nicotinamide is present (*4, 10–12*).

The evidence for the presence of DPN-ase on the cell surface is not inconsistent with the recent findings of McIlwain (*13*) that the DPN-ase in minced neural tissue preparations is associated with the cell debris.

One important implication of these observations is that DPN as such, cannot exist in the circulating plasma. Other workers (*14*), and the authors, have demonstrated that the coenzyme is not present in the plasma. Even though, as McIlwain (*15*) has shown, the reduced form of the coenzyme ($DPN \cdot H_2$) is not a substrate for DPN-ase, the existence of $DPN \cdot H_2$ in the plasma also is not possible since, in the presence of the plasma lactic dehydrogenase and pyruvate, it would be oxidized and thus be liable to rapid destruction by the red cells.

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Low-Temperature Sterilization of Organic Tissue by High-Voltage Cathode-Ray Irradiation[1, 2, 3]

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Recently a limited number of human tissue banks have been established to preserve blood vessels (*1*), bone (*2*), and cartilage (*3*), since in this way these substances can be made available for transplantation into humans whenever needed. It has been rather difficult, however, to keep these banks supplied with adequate amounts of sterile material since the tissues may often be contaminated before, during, or after removal from the body, at operation or autopsy, and as a result are not safe for transplantation. It is obvious that if a method could be found for sterilizing human tissue without denaturing it, this would be of great value.

The Surgical Research Laboratory of the Children's Medical Center became interested in this broad problem of organic tissue sterilization while attempting to sterilize blood vessels to insure a more constant supply of sterile vascular grafts for human use. In initial experiments attempts were made to decontaminate blood vessel segments with chemical antiseptics (*4*) and complex antibiotic combinations (*5*), but consistently satisfactory or adequate results were not obtained. In 1948 the Department of Food Technology at the Massachusetts Institute of Technology reported the marked bactericidal action of high-voltage cathode-ray irradiation in the sterilization of food (*6*). With the cooperation of John Trump at that institution, irradiation of intentionally contaminated blood vessel segments was carried out using a compact 3-mev electrostatic generator he designed (*7*), which produces high-voltage cathode rays that can penetrate organic material to a depth of 1.5 cm (*8*).

Initially, 125 blood vessel segments that had been

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obtained from dogs were heavily contaminated with a mixture of 24-hr pure cultures, of α-hemolytic streptococcus, β-hemolytic streptococcus, *Staphylococcus aureus, bacillus subtilus, monilia albicans, Escherichia coli, B. proteus,* and *B. pyocyaneus.* They were then sealed in individual polyethylene bags, frozen, and irradiated at carbon-dioxide ice temperature with from 1.5 million to 6.0 million roentgen equivalent physical units (*9*), thawed, and cultured for 7 days in beef heart broth. Of these specimens, only 3 grew out in culture; one had been treated with 2.0 million REP, another with 3.0 million REP, and the third with 5.0 million REP.

Following this, human aortic segments of larger caliber and wall thickness were collected at random, in an unsterile manner, from routine human autopsies that had been performed 6–36 hr post mortem by pathologists at the Massachusetts General Hospital, Peter Bent Brigham Hospital, and the Boston City Hospital. These were individually sealed in polyethylene bags and stored at −50° C. A total of 194 aortic segments was irradiated with 1.5–6.0 million REP. Ninety-four specimens were thawed, irradiated at room temperature, and then cultured; bacterial growth was obtained from three. The remaining 100 vessels were irradiated while still frozen (on dry ice at −80°), then thawed and cultured; two showed persistence of viable organisms. (Frozen but unirradiated controls were thawed, and all were positive when cultured.) These results showed the great effectiveness of cathode-ray irradiation in sterilizing tissue, and they suggested that the temperature of the contaminated tissue during the irradiation did not significantly alter the bactericidal effectiveness of the cathode rays.

Encouraged by these preliminary experiments, we irradiated 5 dog arterial segments at room temperature with 1.5 million REP, the dosage recommended for sterilizing foods. When these arterial grafts were transplanted into recipient animals they did not provide satisfactory vascular pathways, since in each case large occluding thrombi developed in the lumen of the graft and obstructed the blood flow.

Following this thirty-eight unsterile or intentionally contaminated frozen dog aortic segments were irradiated in a dry ice trough at −80° C with 1.5 or 2.0 million REP. Subsequently these blood vessels were transplanted into the abdominal aortas of dogs. In this group of animals there have been no large occluding thrombi, and there has been only one graft failure, that from dehiscence of a suture line because of infection. The remaining 37 vascular transplants appear to have retained their usefulness as grafts.

An additional 22 contaminated arterial grafts were irradiated at low temperatures, with higher dosages ranging from 3.0–6.0 million REP, and were then implanted as aortic grafts in recipient dogs; vessel wall destruction was encountered that became proportionately more marked as the dosage of irradiation was increased (*10*).

Of the 60 unsterile or intentionally contaminated grafts that were irradiated at low temperatures and implanted into animals, only 2 showed any evidence of infection. Apparently, low temperatures had succeeded in protecting vessel walls (in ranges of 1.5–2.0 million REP), but they had not significantly impaired the bactericidal effectiveness of this irradiation.

Recent studies have indicated that ionizing radiations may induce a great variety of chemical reactions, which in turn exert many different biological effects on organic tissues (*11*). The primary or direct action of irradiation on organic molecules is the production of ionization that causes them to undergo degradation, denaturation, or depolymerization. A secondary or indirect action of irradiation is caused by the ionization of intercellular and intracellular water molecules, which leads to the production of free radicals (hydrogen atoms and hydroxyl radicals), which have oxidizing and reducing capabilities (*12*). In the frozen state the diffusion rate of these free radicals is slowed down (*13*). In view of this, and from the results obtained in our experiments, it seems reasonable to assume that by maintaining grafts at low temperature during irradiation, one may succeed in blocking or minimizing those secondary reactions that are so prominent when organic tissue is irradiated at room temperature. In contrast, the degrading, depolymerizing, or denaturing direct action of ionizing irradiation on tissue appears to continue in spite of the frozen state of tissue during irradiation; this results in appreciable organic tissue destruction, which ultimately manifests itself, in the case of blood vessels, when they have been irradiated with dosages above 3.0 million REP.

Fortunately, the direct ionizing action of irradiation accounts for bacterial damage and destruction and is independent of temperature ranges, since it causes ionization in the genes and chromosomes of the microorganisms, which in turn gives lethal mutations or prevents reproduction (*11*).

The experimental findings in the irradiation of canine and human arterial segments already described lend support to these theories, since the microorganisms used to contaminate these arterial segments were destroyed in a very high percentage of cases, regardless of the protective effects of low temperature on the organic tissue.

From the facts at hand it appears that microorganisms are vastly more susceptible to the direct action of relatively low dosages of ionizing irradiation than are the cells that constitute vascular grafts. It is believed that the indirect or secondary action which takes place following the irradiation of the intercellular and intracellular water is not necessary to achieve the destruction of bacteria with a high degree of regularity.

It is apparent that at low temperatures one finds a zone of irradiation dosage which lies above that necessary to sterilize, but which is below that which will cause damage to, the tissues. When infected organic tissue is reduced to a low temperature and irradiated in this zone, bacteria are killed off with a high degree

of effectiveness, and the substance is still fit for use in medicine and surgery.

To date this method of low-temperature sterilization of vascular grafts has been employed in two humans with coarctation of the aorta, in whom the gap remaining in the aorta, after resection of the narrowed portion, could not be overcome by primary anastomosis. Frozen, irradiated aortic grafts (from human autopsy material) have been used in each case to bridge the aortic gap. These two patients have been followed 4 and 6 months postoperatively, and there has been complete relief of hypertension in each. It is believed that this represents the first time that any human organic substance sterilized by high-voltage cathode-ray irradiation at low temperatures has been sucessfully transferred from one human to another.

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Comments and Communications

Krebiozen[1]

KREBIOZEN is a term applied to an agent of unknown nature alleged to be useful in the treatment of malignant tumors. It is stated to have been discovered by Stevan Durovic, and to have been investigated for clinical activity by A. C. Ivy, head of the Department of Clinical Science, University of Illinois, in collaboration with others. A brochure concerning the agent and the experience with it was circulated as a presentation by Dr. Ivy at a meeting called by him in Chicago on March 26, 1951.[2]

Krebiozen is described as a white powder, soluble in water, mineral oil, and most organic solvents, prepared in an unspecified fashion from the serum of a horse treated in an unspecified way. It is certified as devoid of toxicity and is said to have been capable of restraining the growth of malignant neoplasms in an unstated number of dogs and cats.

The brochure describes the results of the use of Krebiozen in the treatment of 22 patients with various types of cancer. The patients can be divided into three groups on a chronological basis:

1) Seven patients treated during August, September, and October of 1941. Of these six are reported as dead.

2) Seven patients treated between January and June 1950. Two of these are reported as dead and five as living. Of the five, two are described as having early and advanced disease.

3) Eight patients treated between July and December 1950, of whom all are living. Of these, three are described as having early disease, one as having had the cancer removed surgically, and only two as having advanced lesions. In two the degree of extension is not specified.

One patient of Group 1 and three of Group 2, or four at most, of the total 14 individuals can be considered, on the basis of survival, as possibly showing evidence of control of the neoplasm, since eight of the 14 are dead, and two were not in an advanced stage. The period of observation was about a year.

Of the four patients (of the individuals included in the recently treated Group 3) who could, from the data, be regarded as having advanced cancer, the period of observation was only something over four months.

It is evident, therefore, that at the present time we cannot make any certain judgment that the claims on behalf of Krebiozen are valid.

Caution in cases like this is doubly indicated by the well-known fact that the history of the search for better means of cancer control is littered with the hidden wrecks of premature announcements based upon unwarranted conclusions. These cruel and irrevocable disappointments are due, uniformly, to three errors: (1) undue reliance on the subjective response of the patient; (2) unfamiliarity with the course of untreated cancer; (3) failure to require unequivocal objective evidence of an effect of the procedure on the cancer.

The unreliability of the subjective response of cancer patients in classic. Weil stated in 1915:

> It is a curious and interesting fact that almost every therapeutic claim made in recent years in connection with cancer has included among its virtues the relief of pain. . . . In view of this it is probably fair to assume

[1] This communication was solicited by the Editorial Board.

[2] *Krebiozen: An Agent for the Treatment of Malignant Tumors.* Discovered by S. Durovic, M.D.; presentation by A. C. Ivy, Ph.D., M.D. Chicago: Champlin-Shealy Co. Pp. 1–106 (1951).