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*SUBCELLULAR FRACTIONATION OF MOUSE SPLEEN RADIATION PROTECTION ACTIVITY**

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Jacobson, *et al.*[1] have demonstrated that the survival of mice exposed to lethal doses of x-rays can be increased significantly by implantation of normal mouse spleens into the peritoneal cavity of irradiated mice after exposure. These findings have led to the concept of a humoral radiation recovery factor (or factors) present in, and elaborated by, normal mouse spleen tissue.[2] They were followed by experimental studies from this Laboratory,[3, 4] in which it was shown that the intraperitoneal injection of neutral phosphate buffer homogenates of normal mouse spleen into mice which had received otherwise lethal whole body x-irradiation, afforded marked protection against mortality. These studies provided a reproducible experimental procedure for obtaining active, injectable spleen preparations in which the vast majority of the cells had been disrupted, and in which the level of organization was far removed from that of the original spleen tissue. Furthermore, the experimental procedure provided a suitable biological assay for testing the protective activity of various spleen

fractions and for investigating the nature of the spleen factor. The supernatant obtained after centrifugation of the spleen homogenate at 1400 G for 10 minutes elicited no protective effect, while protective activity was retained in the sediment. In view of these results, it was considered plausible that the spleen factor—if a chemical entity—might be associated with, or bound to certain particulate or structural components comprising the spleen cells. In the present studies, normal mouse spleen tissue was subjected to subcellular fractionation by the Schneider and Hogeboom method of differential centrifugation in sucrose medium,[5, 6] and the respective subcell fractions assayed for radiation protection activity. The experimental data indicate that protective activity or mouse spleen homogenates is associated with the spleen *nuclei fraction*, under the conditions of these experiments.

Material and Methods.—The radiation procedure was essentially identical with that described previously.[4] A Westinghouse Therapy Unit was the x-ray source. Radiation factors were: 250 kvp.; 15 ma.; 0.5 mm. Cu plus 1 mm. Al filter; skin-to-target distance, 100 cm.; dose rate approximately 25 r per minute, measured in air with a Victoreen r-meter. In each experiment the control and experimental animals were matched with respect to sex, age, and body weight, irradiated simultaneously, and caged together, eight or ten per cage. The irradiated animals were LAf_1 mice of both sexes, 10 to 14 weeks old, weighing 20 to 25 g.; the spleen donors were non-irradiated young LAf_1 mice (approximately 1 week old).

Young (1-week-old) non-irradiated LAf_1 mice were sacrificed by cervical dislocation. The spleens were removed immediately (using clean but not aseptic technique), transferred to a tared chilled, sterilized Ten-Broeck glass homogenizer, weighed, and homogenized in 3 ml. of chilled 0.25 *M* sucrose solution. During this process the homogenizer was kept immersed in a water-bath containing cracked ice. The homogenate was diluted with 0.25 *M* sucrose solution to a final concentration of approximately 100 mg. spleen per ml. The diluted homogenate was then transferred to a chilled, graduated 15-ml. centrifuge tube, and centrifuged in a refrigerated International No. 2 centrifuge (5°C.) at 600 G for 10 minutes. The supernatant was withdrawn carefully with a pipette and transferred to a flask. The sediment was transferred to a clean homogenizer, rehomogenized in fresh 0.25 *M* sucrose solution, and made up to the original volume with 0.25 *M* sucrose solution. The homogenate was again centrifuged at 600 G for 10 minutes, the supernatant removed as before, and combined with that from the first centrifugation. The sediment from the second centrifugation, resuspended in 0.25 *M* sucrose solution, constitutes the *spleen nuclei* fraction. This fraction was turbid, whitish, and was easily injected through a No. 26 needle. The combined supernatants from the above

were then subjected to centrifugation in a refrigerated Model E Spinco preparatory ultracentrifuge at 8500 G for 10 minutes. The rotor was precooled to 5°C., and the vacuum chamber was refrigerated throughout the run. The supernatant fluid was carefully removed with a medicine dropper, and the sediment, constituting the *mitochondria fraction*, was resuspended in fresh sucrose solution. The supernatant from the mitochondria fraction was then centrifuged in the Spinco ultracentrifuge at 20,000 G for 90 minutes, yielding a sediment, the *microsome fraction*, which was resuspended in fresh 0.25 *M* sucrose. The supernatant from the microsome fraction was a pink, crystal-clear, optically homogeneous fluid. It constituted the *soluble fraction*. The individual subcellular fractions were administered without additional washing.

In the earlier experiments the medium used for subcellular fractionation was an aqueous solution of 0.25 *M* sucrose made up with distilled water. Since it was found that the protective activity of spleen homogenates prepared in this medium was not of a high order, it was decided to use a modified, sucrose medium containing added salts. The sucrose-salt solution had the following composition: 0.242 *M* sucrose, 0.0094 *M* KH_2PO_4, 0.0125 *M* K_2HPO_4, 0.0015 *M* $NaHCO_3$, and 0.0006 *M* adenosine triphosphate (Na ATP). This solution represents a modification of one used by Wilbur and Anderson[7] for isolating cell nuclei. It is isosmotic, fortified with potassium salts, bicarbonate, and adenosine triphosphate, and of suitable density to permit subcellular fractionation.

Thirty minutes after harvesting, the subcell fractions were injected either intraperitoneally or intravenously into the irradiated mice, usually between 2 and 3 hours after radiation exposure. Aliquots of each fraction injected never exceeded 1.0 ml., and the amount injected per mouse is expressed in terms of equivalent weight of spleen tissue from which it was derived. The control, irradiated mice, received injections of equivalent volumes of 0.25 *M* sucrose solution, or sucrose-salt solution. Survival up to 30 days post-irradiation and body weight changes were used as criteria for evaluation of protective activity in the spleen subcell fractions.

Both wet and stained dry preparations of whole spleen homogenate and of the particulate fractions were examined under the microscope. Cell counts were made using a standard hemacytometer.

Results.—It is apparent from examination of the data in table 1 that the 0.25 *M* sucrose solution was unsatisfactory as a medium for the preparation of active spleen homogenates. Whole spleen homogenates prepared in this medium exhibited some radiation protection activity, but to a far lesser degree than that reported previously for whole spleen homogenates in phosphate buffer at pH 7.2. Of the subcell fractions isolated in this medium, there was a suggestion of protective activity in the nuclei fraction, and to some extent also in the mitochondria. Whole spleen homog-

enates prepared in 0.88 *M* sucrose were completely ineffective in radiation protection.

In one experiment, spleen homogenate was prepared in a 0.25 *M* sucrose medium to which adenosine triphosphate (Na ATP, 5.7×10^{-4} *M*) had been added. Of ten mice, exposed to 750 r, each of which received a single intraperitoneal injection of this homogenate (equivalent to 56 mg. of spleen),

TABLE 1

THIRTY-DAY MORTALITY OF IRRADIATED MICE INJECTED WITH SUBCELLULAR SPLEEN FRACTIONS ISOLATED IN 0.25 *M* SUCROSE MEDIUM

RADIATION DOSE, R	FRACTION INJECTED	EQUIVALENT AMOUNT OF SPLEEN INJECTED, MG.	SURVIVAL AT 30 DAYS NO./TOTAL	PER CENT
700	Spleen homogenate in 0.25 *M* sucrose	48	6/10	60
700	Nuclei fraction	48	0/10	0
700	Supernatant from nuclei	70	0/10	0
700	0.25 *M* sucrose	..	0/10	0
750	Spleen homogenate in 0.25 *M* sucrose	80[a]	2/15	13
750	Nuclei fraction	80	2/15	13
750	Mitochondria fraction	80	1/15	7
750	Microsome fraction	80	0/6	0
750	Soluble supernatant fraction	72	0/6	0
750	0.25 *M* sucrose	..	0/15	0
750	Spleen homogenate in 0.25 *M* sucrose	42	2/13	15
750	Spleen homogenate in 0.88 *M* sucrose	42	0/13	0
750	0.25 *M* sucrose	..	0/6	0
750	0.88 *M* sucrose	..	0/6	0
750	Spleen homogenate in 0.25 *M* sucrose	42	1/10	10
750	Supernatant—70 G for 10 min.	42	0/12	0
750	Residue from above	42	2/12	17
750	0.25 *M* sucrose	..	0/12	0
750	Spleen homogenate in 0.25 *M* sucrose[b]	56	8/10	80
750	Nuclei fraction	280	3/9	33
750	Mitochondria fraction (intravenous)	250	0/5	0
750	Mitochondria fraction	220	2/7	29
750	0.25 *M* sucrose + ATP	..	0/10	0

[a] Donors, 2 weeks old.

[b] Contains 6×10^{-4} *M* adenosine triphosphate (ATP).

All injections were intraperitoneal, except as otherwise indicated.

eight were alive 30 days after radiation exposure, whereas none of the irradiated control mice receiving sucrose plus ATP survived. The spleen nuclei fraction, isolated in this medium, exhibited some protective activity: three out of nine survived of the irradiated mice each injected with the nuclei fraction (equivalent to 280 mg. spleen) the average survival time of the non-survivors was 15.5 days, compared to 9.2 days for the su-

crose controls. The mitochondria fraction when injected intraperitoneally (equivalent to 220 mg. spleen) also afforded a small degree of protection: two survivors out of seven; average survival time of non-survivors was 9.4 days. Intravenous injection of mitochondria did not afford protection against death; average survival time was 11.2 days.

TABLE 2

THIRTY-DAY MORTALITY OF IRRADIATED MICE INJECTED WITH SPLEEN SUBCELLULAR FRACTIONS ISOLATED IN SUCROSE-SALT MEDIUM

RADIATION DOSE, R	FRACTION INJECTED	EQUIVALENT AMOUNT OF SPLEEN INJECTED, MG.	SURVIVAL AT 30 DAYS NO./TOTAL	SURVIVAL AT 30 DAYS PER CENT
750	Spleen homogenate in sucrose-salt	56	7/17	41
750	Nuclei fraction	280	9/9	100
750	Mitochondria fraction	250	0/8	0
750	Microsomes + soluble supernatant	140	0/8	0
750	Sucrose-salt solution	..	0/10	0
750	Spleen homogenate in sucrose-salt	56	10/10	100
750	Nuclei fraction	280	10/10	100
750	Mitochondria (intravenous)	250	0/8	0
750	Nuclei fraction	196	5/7	71
750	Nuclei fraction	98	2/3	67
750	Sucrose-salt solution	..	0/8	0
750	Spleen homogenate in sucrose-salt	39	5/5	100
750	Nuclei fraction	168	3/8	38
750	Nuclei fraction in 1 *M* NaCl	150	0/6	0
750	Controls (no injection)	..	0/11	0
750	Spleen homogenate in sucrose-salt	56	7/8	87
750	Nuclei fraction	196	2/4	50
750	Salt-sucrose solution	..	0/7	0
750	Spleen homogenate in sucrose-salt	56	3/5	60
750	Nuclei fraction in 0.5 *M* NaCl	280	0/4	0
750	Salt-sucrose solution	..	0/5	0
750	0.5 *M* NaCl solution	..	0/5	0
750	Nuclei fraction in 1 *M* NaCl, diluted to 0.75 *M* NaCl	250	0/6	0
750	0.75 *M* NaCl solution	..	0/10	0

All injections were intraperitoneal, except as otherwise indicated.

Subcell Fractionation in Sucrose-Salt-ATP Medium.—The results indicate that the modified sucrose-salt-ATP solution provided an excellent medium for the preparation of protective spleen homogenates, which could then be subjected to subcellular fractionation. The survival data are summarized in table 2. The degree of post-irradiation protection afforded by the injection of spleen homogenates prepared in the sucrose-salt medium was greater than that observed with comparable homogenates prepared in

0.25 *M* sucrose medium. This is also illustrated by comparative body weight changes of mice exposed to 750 r and treated with spleen homogenates prepared in both media. (See figure 1.)

In a series of five separate experiments, marked protection against radiation mortality was afforded by a single injection of the spleen nuclei fraction. In two experiments 100 per cent of the irradiated mice (750 r), each injected with nuclei fraction from ten spleens, survived the 30-day observation period, whereas all of the irradiated controls (750 r) injected with the sucrose-salt solution died. Apparently, the amount of spleen substance in the form of the nuclei fraction required for protective activity

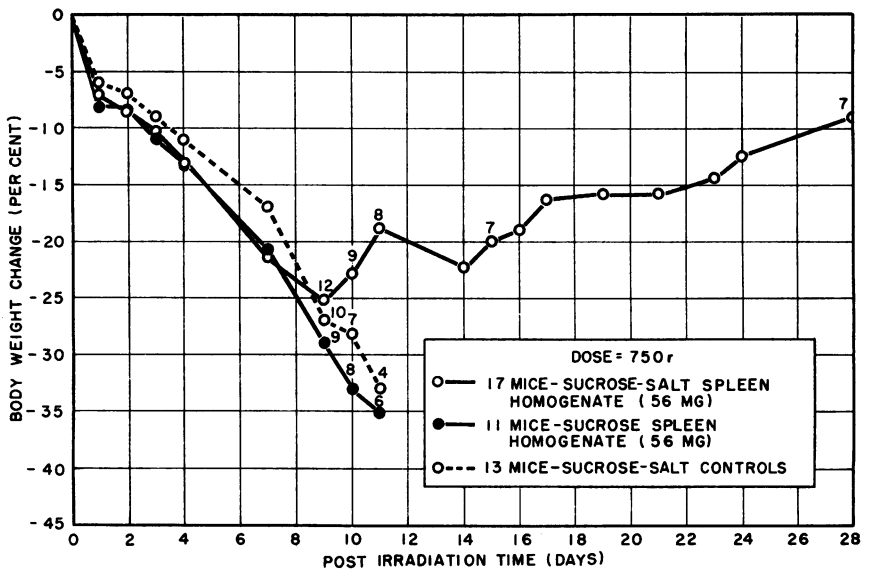

FIGURE 1

Body weight changes in x-irradiated mice treated with spleen homogenates prepared in sucrose-salt medium and in 0.25 *M* sucrose

is greater than that in the form of whole spleen homogenate. However, some activity was observed with the nuclei fraction derived from as little as 3.5 spleens (98 mg.) was administered to irradiated mice.

No evidence of radiation protection activity was observed in the mitochondria fraction obtained by differential centrifugation in the sucrose-salt medium (table 2), in contrast with a small increase in the number of survivors in the group of irradiated mice injected with the mitochondria fraction isolated in 0.25 *M* sucrose medium. Since it is known that mitochondria become aggregated and agglutinated in the presence of salts, and as a result are sedimented with the nuclei,[9] it seems likely that the nuclei fraction isolated in the sucrose-salt medium contains a considerable

admixture of aggregated mitochondria. These considerations suggest that a small degree of radiation protection activity may well be associated with the mitochondria fraction, but that the apparent lack of activity in the mitochondria isolated in sucrose-salt medium may be ascribable to the smaller amount of mitochondria injected.

Desoxyribonucleic acids, in association or combination with protein, comprise the major portion of the cell nucleus. Furthermore, Schneider[10] has shown that all of the desoxyribonucleic acid in liver, kidney, and tumors is recovered in the nuclei fraction. Desoxyribonucleic acid constitutes as much as 48.5 per cent by weight of the dry, fat-free salmon sperm nuclei[11] and indeed, among the various tissues of the animal body, the spleen is one of the richest in desoxyribonucleic acid content.[12] On the basis of the present finding that the radiation protection activity of mouse spleen homogenates is associated with the cell nuclei fraction, an attempt was made to determine whether crude nucleic acid extracts from the nuclei fraction would exhibit radiation protection activity. The procedure of Mirsky and Pollister[13] involving the extraction of nucleoprotein by 1 *M* NaCl was employed. The spleen nuclei fraction was first prepared in sucrose-salt medium as described above; one-half of the nuclei suspension was injected into a group of eight mice approximately 1 hour after exposure to 750 r. Solid NaCl, to a final concentration of 1 *M* NaCl, was added to the remaining half of the spleen nuclei suspension in a flask immersed in an ice-water bath. Immediately upon addition of the salt, the nuclei suspension became very viscous and quite resistant to flow. Since it could not be readily subjected to centrifugation, the viscous mass was administered directly to a second group of irradiated mice (750 r). The material was administered by intraperitoneal injection through a No. 23 needle, after packing it into the barrel of the hypodermic syringe with a glass rod. The data in table 2 show that the viscous 1 *M* NaCl suspension of the nuclei fraction was devoid of radiation protection activity, as was the spleen nuclei fraction suspended in 0.5 *M* NaCl solution.

Inactivation of Spleen Homogenates by in Vitro Exposure to X-Rays and to Ultrasonic Vibrations.—Additional experimental data pertinent to the present discussion show the effect of x-radiation, and of ultrasonic vibrations on the radiation protection activity of mouse spleen homogenates. The homogenate was prepared and divided into two equal portions. One portion was exposed to the physical agent while the other portion, although kept under otherwise identical conditions, was not exposed. The treated and untreated homogenates were then respectively injected into two groups of irradiated animals. The source of the ultrasonic radiations was a crystal Ultra-Sonorator (Crystal Research Laboratories). The spleen homogenate, contained in a Pyrex test-tube, was immersed in the oil bath of the Ultra-Sonorator during the exposure. In one experiment, the

homogenate was exposed continuously for 5 minutes at 1010 v. and 160 ma. In the second experiment, the ice-chilled homogenate was exposed at 750 v. and 100 ma. for a total time of 4 minutes; the exposure was alternated 1 minute in the Ultra-Sonorator and 1 minute in an ice-water bath. It seems clear from the data presented in table 3 that both x-ray exposure (750 r), and ultrasonic vibrations, in vitro, inactivate the protective activity of mouse spleen homogenates. Relative body weight changes in x-irradiated mice treated with normal spleen homogenate, and with spleen homogenate exposed to 750 r are shown in figure 2.

Microscopic Observations.—An attempt was made to ascertain the number of whole cells, broken cells, and cell nuclei in whole spleen homogenates and in nuclei fraction. The attainment of this objective is far more difficult than would appear at the outset, since adequate microscopic criteria for distinguishing intact spleen cells from partially disrupted ones and from nuclei, are not readily established. In order to obtain base line criteria for the normal appearance and size of intact spleen cells, imprints of young mouse spleens were prepared. The freshly cut surface of the spleen was touched lightly on a glass slide; the slide was then air-dried and stained with Wright-Giemsa stain. Lymphocytes of all sizes were seen, as well as reticulum cells, megakaryocytes, normoblasts, polymorphonuclear leukocytes, blast cells, and an occasional eosinophil. The vast majority of the cells ranged in diameter from 6 μ to 13 μ. A few macrophages 19–20 μ in diameter were seen. The cell nuclei stained dark purple and comprised the largest proportion of the cell diameter. The cytoplasm appeared as pale blue rims around the nuclei.

TABLE 3

INACTIVATION OF SPLEEN HOMOGENATE RADIATION PROTECTION ACTIVITY BY IN VITRO EXPOSURE TO X-RAYS, OR TO ULTRASONIC VIBRATIONS

RADIATION DOSE R	MATERIAL INJECTED	SURVIVAL AT 30 DAYS NO./TOTAL	PER CENT
650	Spleen homogenate (32 mg.)[a]	10/10	100
650	Spleen homogenate (32 mg.) exposed to ultrasonic vibrations	8/17	47
650	Phosphate buffer	8/13	62
700	Spleen homogenate (36 mg.)	14/15	93
700	Spleen homogenate (36 mg.) exposed to ultrasonic vibrations	3/15	20
700	Phosphate buffer	1/10	10
750	Spleen homogenate (56 mg.)	7/10	70
750	Spleen homogenate (56 mg.) exposed to 750 r	0/10	0
750	Phosphate buffer	0/10	0

[a] Equivalent amount of spleen.

Microscopic examination of the stained preparations of phosphate buffer spleen homogenate revealed that the vast majority of cells in this preparation are obviously disintegrated or disrupted. A few apparently intact cells resembling small lymphocytes were seen; the remainder of the preparation consisted of a great number of deeply staining, free nuclei, a number of partially disintegrated cells in which portions of the cell surface had been torn away, and granular cell debris. Cell counts indicated the presence of approximately 4.3×10^7 cellular bodies, i.e., whole cells plus disrupted cells plus nuclei, in sucrose-salt homogenates derived from 100 mg. of spleen. Of these bodies, approximately 3.6×10^6 appeared to be

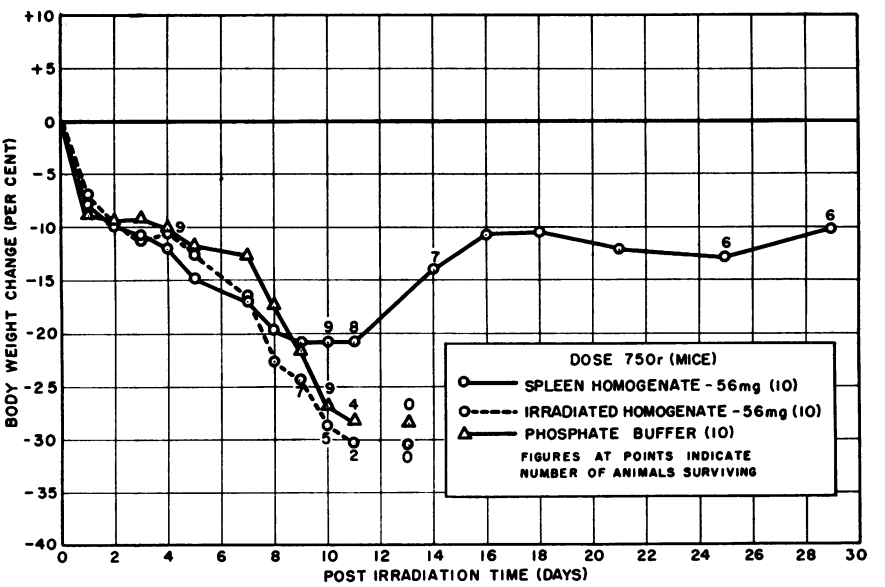

FIGURE 2

Effect of in vitro exposure of spleen homogenates to x-rays (750 r) on radiation protection activity

intact cells. On the basis of the value of 22.9×10^5 cells per milligram of normal mouse spleen recently reported by Mizen and Petermann,[14] it was calculated that the single homogenization involved in the preparation of whole spleen homogenates had disrupted approximately 80 per cent of the spleen cells beyond recognition as cells or cell bodies. Of the remaining 20 per cent recognizable as cell bodies, approximately one-fifth appeared to be whole cells. Thus, the spleen homogenate contains about 2 per cent of the original number of cells in seemingly whole form. It will be recalled that the whole homogenate derived from one mouse spleen will afford marked protection against radiation mortality. Assuming that the

remaining whole cells after homogenization are representative of the spleen population, implantation of one-fiftieth of one spleen would almost certainly not provide the same order of radiation protection (if any) as that afforded by spleen homogenate derived from one spleen and containing the same number of whole cells as that contained in one-fiftieth of one spleen. The additional protection, then, must have come from the disrupted cells.

Microscopically, the nuclei fraction consisted largely of nuclei which were, in many instances, disrupted. The number of seemingly whole cells in this fraction was very low, and indeed, many microscopic fields were entirely devoid of whole cells. Visual comparison of the smear of the whole homogenate and of the nuclei fraction indicates that the procedure for nuclei isolation, involving, as it does, two separate homogenization steps in sucrose-salt medium, results in a great reduction of the number of apparently intact cells as compared with the original homogenate. Smears were also prepared from normal unhomogenized spleen fragments, and treated identically with the smears of spleen homogenates and the nuclei fraction. Practically all the cells in such smears appeared to be intact, indicating that the method of smear preparation *per se* did not disrupt the normal appearance of the cells.

Discussion.—The present finding of the association of radiation protection activity of mouse spleen homogenates with the cell nuclei fraction bring to the fore the problem of the radiosensitivity of the cell nucleus, and the role of nucleic acids and nucleoproteins as determinants in the biological effects of x-rays. Certainly, the earliest damaging effects of ionizing radiations, such as inhibition of cell division, are most readily observed in the nucleus. The well-known induction of chromosome breaks and mutations by ionizing radiations provide additional examples of the great vulnerability of the cell nucleus to radiation damage. An excellent review of the problem of radiation sensitivity of the nucleus has been written by Sparrow.[15]

The experimental evidence is available to support the supposition that the primary disturbance—on the biochemical level—elicited by ionizing radiations may be an aberration in the chain of metabolic events leading to the biosynthesis or utilization of nucleoproteins. Thus, Hevesy[16] observed that x-irradiation inhibits the incorporation of C^{14} carboxy-labeled acetate into desoxyribonucleic acid (DNA) purines. He stated that the finding "that the rate of formation of a main constitutent of cell nuclei is reduced appreciably after irradiation helps to explain the blocking effect of irradiation on cell division and the formation of anomalous mitotic products in the irradiated tissue." Skipper and Mitchell[17] demonstrated a similar inhibition of incorporation of radioactive (C^{14}) bicarbonate and formate into viscera nucleic acids and nucleic acid purines and mice, 6 hours after ex-

posure to x-rays (950 r). Furthermore, the work of Abrams[18] and of Holmes[19] shows that the rates of biosynthesis of nucleic acids are reduced markedly by x-rays, *even though the synthesis of proteins is relatively unaffected.* Also pertinent to this discussion is the observation of x-ray-induced depolymerization of thymonucleohistone and of Na thymonucleate.[20, 21] Limperos and Mosher[22] attempted to demonstrate this effect in vivo. Rats receiving 1000 r whole-body irradiation, were sacrificed either immediately or 24 hours after exposure, and the thymus DNA was isolated and analyzed. The results indicated that a splitting off of either purine or pyrimidine bases had occurred in the DNA isolated from the rats immediately sacrificed; the thymus DNA isolated from animals sacrificed 24 hours after irradiation was almost completely depolymerized, as revealed by the structural viscosity, streaming birefringence, and sedimentation constants of the DNA solutions. Also of considerable significance to the present discussion is the experimental demonstration of nucleic acid depolymerization by chemically produced hydroxyl radicals,[23] and the evidence for an oxygen effect in the action of x-radiation on nucleic acid in vitro.[24]

The present finding of radiation protection activity in the spleen nuclei fraction and the destruction of this activity by treatment of the nuclei fraction with 1 *M* NaCl, or by x-irradiation of spleen homogenate in vitro appears to be in line with above observations when viewed in the light of the following hypothesis: It is postulated (*a*) that the cell nuclei of normal mouse spleen contain a specific macromolecular nucleoprotein complex which is required for the cell growth and division, regeneration, or maturation of critical hematopoietic tissue (bone marrow, spleen); (*b*) that the biological activity of this nucleoprotein is inhibited by ionizing radiations. The inhibition may result from interference with the biosynthesis of this compound, or as a consequence of a physico-chemical alteration of the nucleic acid moiety which may result in depolymerization. According to the above hypothesis, the exposure of mice to an LD_{100} dose of total body x-irradiation, would result in a cessation or inhibition of the new-formation of the specific nucleoprotein complex. A deficiency in the supply or biological activity of this complex would then lead to a defect in the production or new-formation of leukocytes and erythrocytes and, since the biological half-life of the circulating leukocytes is known to be much shorter than that of the erythrocytes, the defect in hematopoiesis at the site of the formation would be manifested in the characteristic leukopenia of the acute radiation syndrome—the essential features of which can indeed be largely explained on the basis of cell damage leading to deficiencies that are first apparent in areas of rapid cell turnover, e.g., hematopoietic and intestinal epithelial tissues.

The biological activity of the postulated nucleoprotein may, therefore,

be the limiting factor in the recovery of mice exposed to LD_{100} total body x-rays; and the administration of the spleen nuclei fraction (obtained from normal, non-irradiated mice) to irradiated mice would provide a new source of the intact, non-degraded, biologically active nucleoprotein complex, able to stimulate or elicit hematopoietic recovery in the irradiated host mouse, and thus prevent death.

The present observations that exposure of spleen homogenates to x-radiation (750 r) and to ultrasonic vibrations, in vitro, results in loss of radiation protection activity are consistent with the above hypothesis, since both these physical measures are known to induce degradation and depolymerization of nucleic acid macromolecules.[25]

In view of the functional similarities betweeen the spleen and bone marrow in the mouse,[26] and the fact that injection of mouse bone marrow suspensions into irradiated mice affords them marked protection against death,[27] it seems likely that the protective factors in spleen and bone marrow are closely related in nature, or even identical. This conclusion finds some support in the observation that the sensitivity of splenectomized mice to total body x-irradiation does not differ appreciably from that of intact, non-splenectomized mice.[28] Presumably the spleen and bone marrow factors can replace each other. It is significant, too, that spleen and bone marrow, both tissues of high cell turnover, appear to be the major sources of the radiation protection factor in mice. In this connection it is of interest that Kelly and Jones[29] were able to demonstrate an alteration in the turnover of desoxypentose nucleic acids in the liver, spleen and kidneys of mice bearing growing mammary carcinoma transplants. These workers suggested "that the presence of a rapidly dividing tissue mass may influence the rate of cell division (as evidenced by the nucleic acid turnover rate) in the other body tissues."

Finally, it seems pertinent to note at this point that thiourea inhibits the depolymerization of DNA by x-radiation and by chemically generated free radicals, whereas ascorbic acid (another reducing agent) does not have this action.[23] Since thiourea protects rats against lethal x-ray doses when given prior to irradiation[30] while ascorbic acid administration confers no radiation protection,[31] it would appear plausible that preirradiation protection by compounds such as thiourea or cysteine may be related to their ability to inhibit DNA depolymerization by x-rays. Thus, radiation protection by these compounds would involve "protection" of the postulated spleen nucleoprotein factor against depolymerization and resultant loss of biological activity. Cronkite, *et al.*,[26] have previously suggested that the accelerated recovery of the bone marrow and of splenic myelopoiesis in glutathione-pretreated irradiated mice "may be due to protection of humoral factors which control orderly hemopoietic regeneration."

The experimental data presented in this paper strongly suggest that the

post-irradiation protection factor present in the spleens of young LAf_1 mice is non-cellular in nature and is associated with the cell nuclei. Studies aimed at the isolation and further identification of the factor are now in progress.

Summary.—The subcellular particulate fractions of mouse spleen were isolated by differential centrifugation of spleen homogenates in sucrose media. The experimental data indicate that post-irradiation protection activity of mouse spleen homogenates is associated with the cell nuclei fraction. The highest degree of protective activity was exhibited by the nuclei fraction isolated in a modified, isosmotic sucrose-salt medium fortified with adenosine triphosphate. In two of the experiments 100 per cent of the irradiated mice (750 r), each injected with the nuclei fraction derived from 280 mg of spleen tissue, were alive 30 days after exposure, whereas all of the irradiated controls (750 r) died. No activity was observed in the mitochondria, microsome, or soluble supernatant fractions isolated in this medium. A low level of protective activity was observed in whole spleen homogenates and in the spleen nuclei fraction prepared in 0.25 *M* sucrose medium. Crude nucleic acid extracts prepared by treating the nuclei fraction with 1 *M* NaCl were inactive. The data strongly suggest that the radiation protection spleen factor is non-cellular in nature. It is proposed that the factor may be a nucleoprotein.

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THE MECHANISM OF PROPIONIC ACID FORMATION BY SUCCINATE DECARBOXYLATION. I. THE ACTIVATION OF SUCCINATE

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The studies of Johns[1–8] and Delwiche[4] with suspensions of anaerobic micrococci (*Micrococcus lactilyticus* or *Veillonella gazogenes*) and *Propionibacterium* spp. showed that these bacteria produced propionate by the decarboxylation of succinate. This decarboxylation can also be accomplished by cell-free extracts of *M. lactilyticus* and the requirement for specific cofactors in this reaction has been briefly described.[5] The present paper contains detailed evidence for the participation of these cofactors in the activation of succinate and for the nature of the active product.

Methods.—M. lactilyticus (strain 221) was grown in tap water with 2–3% sodium lactate, 2% peptone, 1% yeast extract, salts, and 0.0015% thiamin. The latter was added routinely to all media, except when otherwise noted, because some batches of yeast extract, apparently deficient in thiamine, yielded small cell crops and extracts of such cells had low decar-