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QUARTERLY REPORT OF
BIOLOGICAL AND MEDICAL RESEARCH DIVISION

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Director

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ANL-5247

Errata

p. 11, line 10

50r should read 50 rep.

pp. 14, 15

Acknowledgement for Figures A and B
is hereby credited to Dr. N. J. Scully.
This primary leaf effect was previously
reported in ANL-5086 (p. 12).

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QUARTERLY REPORT OF
BIOLOGICAL AND MEDICAL RESEARCH DIVISION

Argonne National Laboratory

Beginning with this issue, the Quarterly Report of the Division of Biological and Medical Research will appear in January, April, July, and October. The format is changed somewhat from that of previous reports, in that two kinds of articles are now included. In addition to the brief status reports which most of the groups submit every quarter on some phase of their work, larger and more detailed accounts by a few investigators are to be incorporated as the results of progress will warrant such inclusion.

QUANTITATIVE STUDIES OF THE GROWTH RESPONSE OF THE KREBS ASCITES TUMOR

Harvey M. Patt and Margaret E. Blackford

The initial phase of this investigation has been completed, and a detailed report is in press (Cancer Research). Further analysis of our previous data reveals that:

1. The course and rate of tumor growth in mice are similar for inocula ranging from 10^6 to 20×10^6 cells.
2. Growth appears to be more nearly exponential when the number of tumor cells is comparatively small. With increasing cell numbers, the growth rate gradually declines. An exponential fit may be applied from 10^6 to 2×10^8 tumor cells, and a cube root transformation is applicable with cell numbers from 10^7 to 4×10^8 . Neither expression accounts for the entire growth pattern which, however, appears to be a function mainly of the total number of free tumor cells.
3. Ascitic volume increases linearly with tumor cell number and exponentially with body weight.
4. Differences in survival time with varying inoculum size may be considerably in excess of the time required for attainment of equivalent cell numbers.

More recent investigations are concerned with the radiosensitivity of several varieties of ascites tumors which vary in growth rate and in chromosome number. The leakage of iodinated proteins from blood to ascites as a function of tumor cell number is being investigated by R. L. Straube. It may be remarked that the ultracentrifugal patterns of mouse plasma and early ascitic plasma are identical (kindly determined by A. L. Koch).

THE PATTERN OF RADIUM RETENTION IN MAN

W. P. Norris, T. W. Speckman, and P. F. Gustafson

A number of persons with radium in their skeletons, deposited approximately 20 years ago as a result either of therapeutic procedures or of employment in the luminous dial industry, have been studied to determine total radium burden (sum of measurements of expired Rn plus externally measured γ -ray activity), rate of excretion, and concentration in bone. In 1931, thirty-two patients of the Elgin State Hospital were injected intravenously with RaCl_2 in measured doses by Schlundt, Nerancy, and Morris,¹ who also made early measurements of retention. Nineteen of these people were located in 1951 and restudied. By combining the present results with the early data of Schlundt, et al., and using results from animal studies as a guide, it has been possible to describe mathematically as a "power function" the over-all retention (R_t) of a single intravenous dose of radium in adult humans as a function of time in days after injection (t):

$$R_t = 0.54t^{-0.52} \qquad t \geq 1 \qquad (1)$$

From equation (1) the equations of the fraction of the injected dose excreted per day vs. time and the coefficient of elimination vs. time may be determined. Previously published human data agree with this analysis. The mathematics may be extended to cover instances of continuous or repeated administrations. With this information it is possible to roughly approximate the radiation dose rate and the total dosage delivered over the period investigated.

¹Schlundt, H., J. T. Nerancy, and J. P. Morris. Detection and estimation of radium in living persons. IV. Retention of soluble radium salts administered intravenously. *Am. J. Roentgenol.* 30, 515-522 (1933).

MECHANISMS OF LEUCOCYTE REGULATION

H. M. Patt, M. A. Maloney and E. M. Jackson

It has been shown that an abrupt leucopenia, brought about by exchange transfusion with leucocyte-depleted blood, results in a rapid stimulation of blood-forming tissues with peripheral recovery in 5 to 6 hr (ANL-5200, p. 10-11). A similar recovery pattern is seen when dogs are injected with antileucocytic serum prepared from immunized rabbits. Serum dilutions ranging from 1:1 to 1:1000 have been assayed in vivo with the following results:

1. The severity of neutropenia increases with increasing concentration of antiserum, from a 99% decline in neutrophil levels with the undiluted preparation to a 10% decrease with a 1:1000 dilution.
2. The time of maximum neutropenia increases with increasing dilution of antiserum, from 10 min with undiluted serum to 60 min with dilutions in excess of about 1:100.
3. There is the suggestion that the intensity of the stab cell (nonsegmented neutrophil) response is related more to the minimal neutrophil count than to the absolute decrease in neutrophil levels. Stab cells increase within 1 to 2 hr after antiserum injection and may constitute 50% or more of the total neutrophils during the recovery period of 5 to 6 hr.
4. Lymphocytes are also diminished after injection of the antiserum, although not to the same degree as neutrophils. Erythrocyte levels are not altered significantly. The serum was absorbed against canine red cells and lymph node lymphocytes before use; the question of whether the serum contains one or more antileucocytic factors is being investigated.
5. The peripheral blood response to antiserum injection appears to be similar in intact and splenectomized dogs.
6. Total body X irradiation of a dog with 300 r (dose in air, 200 KV) immediately prior to antiserum injection decreased, but did not eliminate, the early stab cell response, and neutrophil recovery was incomplete. Further studies with other dosages and times of X irradiation are in progress.

MODIFICATION OF THE RADIATION SYNDROME IN THE CHICK BY PARTIAL BODY SHIELDING

S. P. Stearner, E. J. Christian and A. M. Brues

The relative importance of direct and indirect radiation effects in the initial response of the chick has been investigated by shielding parts of the body during X-ray exposure. The radiosensitivity of the bird kidney and its relation to the initial radiation response has received major emphasis. Three- to 4-day chicks received 1000 r X ray at 43 r/min to a part or to the entire body. Shielding the kidney region during irradiation prevented severe renal necrosis, but normal function was not maintained in those individuals that died in the initial period (within 48 hr after exposure). On the other hand, irradiation of only the kidney region (exposure of about 15% of the body mass to the standard dose) resulted in severe necrosis of the kidney tubules and, in those animals that died in the initial postirradiation period, complete renal failure. Exposure of only the abdominal region produced no alteration in renal function and little early mortality. Apparently, renal failure in the initial period following irradiation is the result of both direct radiation injury and indirect systemic effects, and is only one of multiple possible mechanisms that contribute to early radiation mortality in the bird.

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METABOLIC REQUIREMENTS FOR RECOVERY
OF IRRADIATED TETRAHYMENA

Howard S. Ducoff

Studies on radiation-induced cell division block in Tetrahymena pyriformis are continuing. Tetrahymena suspensions are maintained overnight in buffered glucose-salts solution, X-irradiated, and then transferred to synthetic media lacking in one or more essential constituents, which are restored at various time intervals afterwards. Lag after restoration of omitted amino acids to unirradiated animals is much greater than after restoration of omitted purines and pyrimidines. After restoration of amino acids to irradiated animals, however, lag appears only slightly greater than after restoration of purines and pyrimidines, and is less than the sum of the lag following irradiation in complete medium and the lag following restoration to unirradiated animals of omitted amino acids. These results suggest that some recovery may take place, even in the absence of exogenous amino acids, providing purines and pyrimidines are supplied to the irradiated cell.

Irradiation in synthetic medium followed by transfer to proteose peptone results in earlier resumption of exponential growth and, at higher doses, lower lethality, than irradiation in the proteose peptone. It was also noted that Tetrahymena is more susceptible to the toxic effects of H_2O_2 when exposed in proteose peptone than when exposed in the synthetic medium, and these two findings may be causally related.

LIGHT REFLECTANCE BY RADIATION - DAMAGED PLANTS

G. S. Rabideau

The first detailed study of the reflectance of light by leaves of plants was made by C. A. Shull.⁽¹⁾ This subject was most extensively investigated in the laboratory of A. Seybold at the University of Heidelberg.^(2,3) A large grating monochromator was designed by French et al.⁽⁴⁾ for the study of the reflectance of leaves in an Ulbricht sphere. This instrument was used by Rabideau et al.⁽⁵⁾ in a study of the absorption and reflection spectra of intact leaves, chloroplast suspensions, and a colloidal supernatant fraction obtained by centrifugation of chloroplasts ruptured by ultrasound.

Numerous investigators have found that normal-appearing green leaves usually reflect only a small fraction (10-15 per cent) of the incident light of visible wavelengths. It is known that damaged leaves, albino and variegated leaves⁽²⁾ exhibit considerably greater reflectance of visible light than do normal green leaves. Katz and Wassink⁽⁶⁾ and Billings and Norris⁽⁷⁾ studied the reflecting properties of leaves in the infrared region and found that considerably more light (50-70 per cent) is reflected from the surfaces of leaves in the infrared region than in the visible region.

The biological effects of radiation on these and other wavelengths have been summarized in Radiation Biology, A. E. Hollaender, editor.⁽⁸⁾ Radiations such as X-rays, gamma rays and fast neutrons damage young leaves of green plants and are therefore potentially harmful in view of their effects on the food-making capacity of the plant. In addition, it is conceivable that during military activity, the effects of such radiations on leaves might be of concern both in camouflage operations and as a biological indicator of areas exposed to ionizing radiation. For these reasons the following study was undertaken.

Seeds of red kidney bean, *Phaseolus vulgaris*, variety California red kidney, were surface-sterilized, air-dried, and then exposed in separate lots to various dosages of X-rays, gamma rays and fast neutrons. The seeds were then germinated, either in soil or on moist filter paper. Subsequent growth of the seedlings in soil or in nutrient culture solution revealed two leaf anomalies: (1) damage of the entire surface of the two primary leaves, and (2) damage of a relatively limited portion of an occasional secondary leaf. The nature of the appearance of damaged areas of leaves in (1) and (2) was markedly different. The primary leaves showing radiation damage were slightly smaller than normal leaves, were slightly more green, and had numerous scattered white areas at the ends of the smallest veins over the entire blade. This alternation of light and dark areas gave a speckled, or "salt and pepper," appearance to these damaged primary leaves. This is

illustrated by Figure 1. In addition to the damaged primary leaves, occasional secondary trifoliate leaves of these same plants showed solid discrete areas, often one-tenth of the area of a secondary leaflet, of uniformly light-green leaf tissue as shown in Figure 1, C. While one or more of the secondary leaves of radiated plants might be found to exhibit such a mottled appearance, these did not appear unless the level of radiation was enough to cause the appearance of the speckled primary leaves. Scully and Vogel (personal communication) have determined the approximate threshold level of seed irradiation in order to induce these effects to be as follows: X rays - 2000 r, gamma rays - 1500 r, and fast neutrons - 50 r. No differences other than threshold of radiation have been thus far observed with the different kinds of radiation in the production of these particular leaf anomalies.

Since both of these two leaf anomalies showed more striking differences (1) by reflected light than by transmitted light, and (2) upper surface location rather than lower surface, it was decided to study the reflecting ability of the upper surfaces of normal and radiation-damaged leaves. In preliminary measurements visible light from a tungsten source was passed into a Perkin-Elmer prism spectrometer, and bands of light of limited wavelength span were isolated. The leaf to be studied was superimposed on a black background and held at the critical reflecting angle of the mirror outside the exit slit. Light was reflected from the leaf surface to the active surface of a RCA S31 (Type 4) photomultiplier tube. The excitory pulse was fed through an amplification selection system and registered on a milliammeter scale. By employing darkness and suitable light baffles it was possible to reduce stray light to less than 5 per cent for all measurements. The reflection of light was determined for the upper surfaces of normal primary leaves, radiation-damaged primary leaves, and radiation-damaged areas of secondary leaves under as nearly comparable conditions as possible. Reflectance values (scale readings) were plotted against wavelength as shown in Figure 2. The reflection of radiation-damaged primary leaves is approximately the same as that of the normal primary leaves, being slightly less in the green region and slightly more in the blue and red regions. The reflectance of the lighter mottled areas of radiation-damaged secondary leaves is considerably more, being from 7-8 per cent more in the green region and increasing to 40 per cent more in the blue and red regions of the spectrum.

A possible explanation of the difference in radiation damage between the primary and secondary leaves might lie in a consideration of their comparable size at the time of seed radiation. Scully⁽⁹⁾ reports that studies have revealed that the cells of the primary leaf are largely formed at the seed-stage, whereas those of the secondary leaf are largely in an undifferentiated condition in the seed. Hence the primary leaves offer a much larger target for the radiation than the potential secondary leaf tissue in the seed. The results of primary leaf radiation resemble a roof which has been damaged by hail, whereas the secondary leaf is so small that damage to potential leaf-forming cells (nuclei ?) is subsequently carried over to much larger areas in the differentiation of the secondary leaf. It would

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be interesting to see whether or not similar irradiation of young maturing bean seeds at the stage of the beginning of differentiation of the primary leaf would produce mottled areas on the primary leaf similar to those of the secondary leaves in the present study. It is planned to investigate this effect of target size in the near future.

These reflectance measurements need to be confirmed by the use of the Ulbricht sphere technique using a monochromator with high energy output similar to that used by Rabideau et al.⁽⁵⁾ Also seeds of other species should be irradiated to ascertain whether similar effects could be obtained. Scully⁽⁹⁾ has thus far found similar radiation effects on leaves of irradiated soybean seed.

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Table I

THE REFLECTION OF LIGHT FROM THE UPPER SURFACE OF
RADIATION-DAMAGED LEAVES. NORMAL LEAVES = 100 PER CENT

Wavelength m μ	Radiation-Damaged Leaves	
	Primary Leaf	Secondary Leaf
400	+6 %	+34 %
420	+8	+30
440	-5	+20
450	-4	+13
460	-7	+14
470	-7	+ 7
475	-6	+ 8
480	-4	+13
490	-4	+11
500	0	+22
525	+3	+16
550	+7	+28
575	+5	+31
600	+3	+31
625	+6	+31
650	0	+44

Figure 1A
Control primary leaf.

Figure 1B

Radiation damaged primary leaf 5000 r, X-ray.

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C

Figure 1C

Radiation damaged secondary leaf 4000 r, X-ray.

Figure 2

ACUTE AND CHRONIC RESPONSE OF TWO HYBRID MOUSE STOCKS TO TOTAL BODY X-RAYS

Douglas Grahn, George A. Sacher and Katherine F. Hamilton

Hybrid mice are generally considered to be the best material for long range experimentation in radiobiology. They are usually characterized by superior longevity and high resistance to most infectious diseases. However, the fact is often overlooked that they may also express to some degree the less favorable qualities of their inbred parents. One hybrid, the LA F₁ (C57L ♀ x A/He ♂), has been extensively employed with favorable results. Until recently, this mouse was not commercially produced. Consequently, a test was started on two F₁ hybrids that were available commercially in order to discern which was most desirable for long-term experiments and to extend our knowledge of the utility of hybrids in general.

MATERIALS AND METHODS

The mice employed were the BA F₁ (C57BL/6 ♀ x A/Jax ♂) and the CA F₁ (BALB/c ♀ x A/Jax ♂), obtained from the Jackson Memorial Laboratory. These were split into three groups for each hybrid, one group in each being irradiated at 6 weeks, one at 8 weeks, and one at 10 weeks of age. A total dose of 800 r of whole-body X irradiation was given in 4 fractions of 200 r each with a fractionation interval of about 3 days for a protraction period of 10 days. This is a near maximal lymphoma-inducing dose, according to data of Kaplan and Brown (1952). Body weights were taken at regular intervals for the first ten months. The mice were allowed to die naturally, none being sacrificed in an apparently moribund state; actually several occasions arose wherein such mice recovered and lived several months longer or are still living. Autopsies were performed on all dead mice that were not severely autolyzed, a loss of about 5% having been incurred through this cause.

EXPERIMENTAL RESULTS

The mortality observed in the first sixty days post-irradiation for age groups 6, 8 and 10 weeks, respectively, was: BA F₁: 12%, 0% and 0%; CA F₁: 41%, 3% and 8%. This greater susceptibility of the CA F₁ mice is also reflected in their body weight response. They required from 6 to 12 weeks to recover their initial losses, while the BA F₁ mice recovered their weight loss in 4 weeks at the most. The age differences in susceptibility are similar to that reported by Abrams (1951) for C57 BL mice. Aside from the 6-week group of CA F₁ mice, wherein 67% of the males and 33% of the females died, the mortality was not high enough to permit an adequate expression of sex differences that might have existed.

The mortality from 61 to 300 days has been grossly classified into two categories: lymphoma and non-lymphoma. No attempt was made to break down the non-lymphoma deaths into their various categories or into age and sex groups, as the total incidence is still less than 10% for both hybrids.

No significant difference in the 300 day incidence of lymphoma with respect to age at time of irradiation can be detected in either hybrid (see Figure 3). Kaplan (1948) noted that one-month old C57BL mice, when exposed to 1000 r in 12 daily doses of 84 r, had a lymphoma incidence of 65%; for two-month old mice: 35%; three-month old mice: 18%; and four-month old mice: 10%, which is in contrast to the present results.

A consistent difference between the hybrids is evident, however. As seen in Figure 4, this difference is largely a function of a sex difference that is common to both hybrids but more pronounced in the CA F₁. The males are higher in total incidence of lymphoma, significantly so in the CA F₁ mice. Table 2 presents the pertinent data. This sex difference is the opposite of that found in the C57BL strain (Kaplan & Brown, 1952).

Table 2

LYMPHOMA INCIDENCES AT 300 DAYS POST-IRRADIATION

	n	Total incidence	Difference	P
BA F ₁ ♂	86	44.2 ± 5.4%		
BA F ₁ ♀	85	37.6 ± 5.3%	6.6 ± 7.5%	0.30 - 0.50
CA F ₁ ♂	32	34.4 ± 8.4%		
CA F ₁ ♀	111	16.2 ± 3.5%	18.2 ± 9.1%	0.05
BA F ₁	171	40.9 ± 5.3%		
CA F ₁	143	25.3 ± 6.4%	15.6 ± 8.3%	0.06
Males	118	39.3 ± 7.0%		
Females	196	26.9 ± 4.5%	12.4 ± 8.3%	0.10

Inspection of the data by sex, hybrid and age, though subject to larger errors due to the smaller number of mice per group, shows that the greater lymphoma incidence in the CA F₁ males is present in all three age groups. In the BA F₁ mice, the females show a higher incidence in the youngest group and a lower incidence in the two older groups. The statistical significance, or lack of it, noted in the sex differences of the two hybrids would seem to be confirmed by the independent contributions of the separate age groups.

A small group of unirradiated controls for these hybrids have not had a single lymphoma death, but altogether only about 50 mice were available for control purposes.

DISCUSSION

Of the three parent inbreds involved in these two hybrids, strain A/Jax is common to both. If we assume that its genetic contribution to each hybrid is equal and additive to the contributions of the C57BL and BALB/c parents, then the differences between the hybrids can be considered largely a function of the latter strains. The results would support this assumption qualitatively, but a deficiency of information on the three strains under comparable conditions handicaps a quantitative approach.

As observed by Kaplan (1948), 2-month old C57BL mice had an incidence of 35% lymphoma. The BA F₁ mouse, in the present study, has an average incidence of 41% lymphoma at 300 days post-irradiation, or approximately that to be expected in the C57BL parent alone. Data on strain A, exposed at 10 weeks of age to 80 r/day to a total dose of 720-880 r, indicate a 30% lymphoma incidence (Kirschbaum and Kaplan, 1944). Kaplan (1948), exposing strain A mice to 1000 r in daily fractions of 84 r, obtained a 5-10% lymphoma incidence in 2 - 3 month old mice, but observed no age dependence in mice 2 months old and beyond. Kirschbaum and Mixer (1947) exposed 10-week old A mice to a total dose of 1800 r, given in 200 r fractions either monthly or biweekly, and obtained a 10% lymphoma response. With 200 r per month for nine months, these authors observed a 60-65% incidence of lymphoma in the BALB mouse. On the basis of past findings, the presently observed results would indicate a dominant expression of the C57BL sensitivity to induced lymphoma in the BA F₁ and a dominance of the A parent expression for the absence of an age dependence for lymphoma induction. In the CA F₁, the response appears intermediate to that of its two parents. In all of these inbreds, the spontaneous incidence of lymphoma by 300-350 days is less than 5%.

The acute mortality definitely reflects the differences between the BALB/c and C57BL strains, wherein the latter is significantly more resistant to lethality after acute exposure to X-rays (Grahn, 1954).

Explanations for the sex difference in lymphoma incidence will not be attempted at this time, since present data are insufficient in this regard.

In part, then, the differences between the BA F₁ and CA F₁ hybrids are a direct expression of the BALB/c and C57BL differences. The common parent, strain A/Jax, would seem to modify the expression of the other strains, but not in the same manner for each.

Considering a low lymphoma incidence with a low acute mortality as desirable features, it would appear that neither of the two hybrids offers any particular superiority to the other or to the often used LA F₁. The latter is known to have a moderate to high lymphoma incidence under chronic gamma-ray exposure (Lorenz, 1950). It should be mentioned that the total number of possible hybrid combinations that biologists could employ has barely been touched. The proper selection of parent stocks for their most favorable qualities can undoubtedly yield one or more hybrids that could be far superior to any of those now used.

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Figure 3

Cumulative lymphoma incidence by age and hybrid.

Figure 4

Cumulative lymphoma incidence by sex and hybrid; non-lymphoma by hybrid.

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RUTHENIUM TOXICITY STUDY

H. Walton, Jr. and A. M. Brues

Although the tissue distribution and retention of ruthenium¹⁰⁶ have been studied after both injection and inhalation (MDDC-1275 and CH-3325), there are no data on the toxicity of this fission product, whose half-life is one year. Experiments have been started to determine the acute and chronic toxicity of Ru¹⁰⁶ by intravenous injection. Studies of its tissue distribution and excretion at toxic levels will be carried out at the same time.

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ACTIVATION ANALYSIS OF A TUMOR

A. M. Brues and P. F. Gustafson

A carcinoma, found in the region of the thyroid and salivary glands, of a dog (old Sr⁸⁹ series) at autopsy by Drs. Lisco and Finkel, was assayed for protein-bound iodine by activation analysis. Specimens of the tumor and of muscle of the same dog were finely divided, dialyzed against H₂O until the dialysate was chloride-free, extracted with absolute alcohol, and dried. They were then activated together with a filter paper standard containing 7.5 μ g iodine as KI. Beta-counting showed three detectable components: one of half-life less than 1 hour (including, but not necessarily limited, to I¹²⁸); one of half-life about 2.7 hours, taken as Mn⁵⁶; and a longer component with half-life and absorption curve characteristic of P³². The second component was further identified as Mn⁵⁶ by its two-component absorption curve (half-values about 65 and 160 mg/cm²) and by gamma counting in the second and third hours after activation. Calculated values based on the iodine standard are: muscle, 7.1 μ g I, 6.5 μ g Mn, and 1.7 μ g P per gram dry weight; tumor, 6.1 μ g I, 7.8 μ g Mn, and 3.0 mg P per gram dry weight. Since it was not feasible to identify I¹²⁸ by its physical characteristics or to rule out contamination with Cl, values for iodine are maximum limits of concentration.

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THE TOXICITY OF Sr^{89} IN MICE

Miriam P. Finkel, Austin M. Brues, and Hermann Lisco

An introduction to the study of the toxicity of radio-strontium in mice and an analysis of the survival of the animals that received a single injection appeared in ANL-4840 (Quarterly Report, May, June, July, 1952). The present report includes the design of the repeat-injection experiment (Table 1), an evaluation of the survival of these animals, and a comparison of the effect on longevity of single versus repeated doses.

Injection procedure and retention: The initial radioactivity injected was equal to 100% of the dose level. With the purpose of returning to this level at monthly intervals, 80% of the dose level was injected every month thereafter as long as the animals lived. However, the retention of radio-strontium by mice has been found to be 35% in 30 days, and further reduction by decay, if the material were pure strontium-89, would bring the absolute retention to 24% rather than 20% in 30 days. It can be calculated that the first reinjection would bring the radioactivity to 104% of the dose level; by the fourth reinjection 105% of the dose level would be attained, but further increase with successive reinjections would be insignificantly small.

The total beta activity of 14 animals that died 273-334 days after the beginning of the experiment was determined by ash analyses. The mice had received from 8 to 10 reinjections, the last being 25-29 (mean = 27) days before death. If all the radiostrontium they had received had been strontium-89, 27 days after an injection they should have retained 26.8% of the dose level. Instead, they showed an average retention of 28.6%. Three animals that were analyzed a year later retained 30.7% of the dose level 22 days after the last injection, rather than the calculated 28.1%. It is assumed that these differences are due to the presence of strontium-90 in the injection solutions. A preliminary estimate based on the above data suggests that the probable average composition of the solutions used during the first year was 92% strontium-89 and 8% strontium-90.

Survival: The survival of the monthly injected animals is illustrated in Fig. 5, where the control data are indicated by dashed lines. Decreased survival was indicated at 0.2 $\mu\text{c/gm/mo}$ and unquestioned at 0.5 and 1.0 $\mu\text{c/gm/mo}$.

The life expectancies of the various singly injected and monthly injected groups are presented as proportions of the life expectancy of the control group in Fig. 6. These data again illustrate the probable effect of 0.2 $\mu\text{c/gm/mo}$ and definite effects of 0.5 and 1.0 $\mu\text{c/gm/mo}$ as well as the reduction in longevity after single injections of 2.5, 5, and 7.8 $\mu\text{c/gm}$. On the other hand, mice that received 0.01 $\mu\text{c/gm/mo}$ or single injections of 0.5-1.0 $\mu\text{c/gm}$ lived longer, on the average, than the control mice.

Comparison of the effects of single versus repeated doses on longevity can be accomplished by converting the curve for singly injected animals in Fig. 6 to unity and plotting the curve for monthly injected animals accordingly. This has been done in Fig. 7, where it can be seen that the smaller the response, the greater the relative effectiveness of repeated doses, so that as levels are approached that have very little influence on longevity, repeated injections are 7 times as effective as single injections. Conversely, the greater the response, the smaller the difference between single and repeated doses. The two lines should meet at the level that leads to an expectation of life of less than one month, since at that point there would be no animals to reinject. This would be at approximately 95% reduction of life expectancy if we assume three weeks to kill by internal irradiation all of the animals that otherwise would have an expectation of living 434 days (the control figure).

It will be noted that these ratios of biological effectiveness compare the single dose with the repeated amounts. On this basis, one would expect, if no recovery occurred, that the effectiveness ratio would double at two months and rise linearly as more doses are administered. The actual curve shown indicates, therefore, that the effectiveness of repeated doses is about half that of the total dose given at one time. This may be due to recovery or may be the effect of a latent period making doses close to the time of death relatively non-contributory to mortality.

Table 3

DESIGN OF MONTHLY REINJECTION EXPERIMENT

Group	Dose level ($\mu\text{C/g}$)	Number of animals			Total
		CF#1 ♀	ABC ♀	ABC ♂	
1	1.0	15	15	15	45
2	0.5	20	20	20	60
3	0.2	30	30	30	90
4	0.1	49	50	50	149
5	0.05	80	80	80	240
6	0.02	80	81	80	241
7	0.01	80	80	80	240
8*	control	320	320	322	962
	Total	674	676	677	2027

*Same as group 8 control listed in "Design of Single Dose Experiment"

Figure 5

Number of days to various mortality percentages plotted against dose. The dashed lines represent the control data.

Figure 6

Influence of treatment on expectation of life.

Figure 7

Relative effectiveness of repeated versus single injections of strontium 89 in reducing expectation of life.

STATUS OF NEUTRON TOXICITY STUDIES IN MICE

Howard H. Vogel, Jr., John W. Clark, Donn L. Jordan,
Nicholas Bink, and Vincent M. Story

Complete survival curves for CF#1 female mice following 90-minute irradiation with Co^{60} gamma rays and with fast neutrons have been presented in a previous report.⁽¹⁾ All exposures were carried out in the gamma-neutron radiation chamber,⁽²⁾ utilizing the Argonne CP-3' Heavy Water reactor.

Figure 8 shows clearly that whether mice are irradiated during 1.5 or 24 hours with a given dose of fast neutrons there is no significant change in lethality. The LD_{50} (30 days) remains the same even if the time of exposure, and consequently the dose rate, have been changed by a factor of 16. Each point represented on this graph by an x was obtained from 24-hour neutron irradiations. These points could fit equally well the weighted adjusted regression lines of 1.5 or 24 hours of exposure. The figures in parentheses indicate the number of animals exposed at each dose level.

All neutron doses are expressed in roentgen equivalents physical (rep). Fast neutrons have been measured by means of a standard Victoreen r-chamber, calibrated against a Rossi-Failla tissue equivalent chamber of 10.1% hydrogen composition.⁽³⁾ One "n" unit on this standard 100 r chamber under our experimental conditions was equivalent to 1.89 rep of fast neutrons.⁽⁴⁾

In contrast to the results following fast neutron irradiation, it was found that after Co^{60} exposure, dose rate had a marked effect on lethality. Figure 9 shows the probit regression lines, following gamma irradiation, when the total dose is given in 1.5 or in 24 hours. In this case the LD_{50} (30 days) is increased 44% from 920 ± 5 r to 1324 ± 11 r when the total dose is given during 24 hours instead of in 90 minutes.

The relative biological effectiveness of fast neutrons and Co^{60} gamma rays can thus be calculated for lethality of the CF #1 female mouse. As indicated in Fig. 9, the RBE of these two ionizing radiations is 4.4 for 90-minute irradiation and 6.25 for 24-hour exposure.

Figure 10 presents the weighted, adjusted probit regression lines for 90-minute exposure to: (a) fast neutrons; (b) gamma rays; and (c) mixtures of these two radiations in three different proportions. The unit of dose employed in this curve is the rem (roentgen equivalent mammal). The gamma-ray component in the mixtures of radiations is expressed directly in roentgens. The neutron dose in rep, multiplied by the RBE of the two radiations (as measured by acute 30-day mouse lethality), expresses the neutron dose in rem.

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If mixtures of fast neutrons and gamma rays were completely additive in their biological effectiveness (as determined by mortality), then the probit regression lines of the mixed and pure radiations should be identical. The results show that this is not the case. The "departure from additivity" is greatest when the γ component of the mixture is lowest. A maximum departure of 11% is attained when the mixtures are made up of $1/3$ gamma and $2/3$ neutrons.

In Fig. 11 the upper 10 points (750-1100 r) represent data from the pure, 90-minute gamma irradiations. Thus, 920 r (LD_{50}) is equivalent to 210-rep fast neutrons (LD_{50}). The three remaining clusters of points represent $2/3$ gamma, the $1/2$, and the $1/3$ gamma mixture experiments, in descending order.

For each gamma component (in roentgens) of a given mixture a calculation of the rep equivalent is made as follows: the observed 30-day lethality for any mixture is referred to the pure neutron dose-mortality curve and the dose (in rep) corresponding to this lethality is read from the curve. From this figure is subtracted the measured rep of the fast neutron component of this mixture. The remaining dose in rep is that portion of the mixture dose contributed by the gamma component and can be expressed as rep equivalents.

The solid line in Fig. 11, a regression line passing through the points representing pure gamma irradiation, was extended to the origin. If there were no departure from additivity the points representing mixtures of the two radiations should lie along this line. Since this is obviously not the case, it is apparent that the two kinds of ionizing radiations are not exactly additive in producing lethality.

The dotted line, which seems to fit all the points from 200 to 1100 r, is represented by the following linear equation:

$$\text{Rep eq} = 0.259 (r - 110)$$

in which the slope of the line is 0.259 and the y-intercept is 110 r. If this linear equation actually represented the rep equivalents of the various doses of roentgens, then 110 r of gamma radiation in a mixture would be equivalent to zero rep. This would help to explain why the two radiations were not strictly additive in mixtures and why departure from additivity increased when the gamma component decreased.

Figure 12 represents the individual weighted probit regression lines for the three different mixture groups. In the top three graphs, each point marked with an X was calculated by adding the actual gamma dose in roentgens to the neutron dose in rep multiplied by the RBE of 4.39. The solid line in all six graphs represents the regression line for the pure radiation given

in 90 minutes. It is clear from the 3 upper graphs that, when the two radiations are mixed, there is a small but significant "departure from additivity" and that this departure is increased as the gamma portion of the mixture is decreased.

The three lower graphs represent the same points calculated by the linear expression developed in Fig. 11. Each point represented by o has been calculated by the formula:

$$\text{Rep eq} = 4.39 [\text{Neutron dose in rep} + 0.259 (r - 110)].$$

Through the use of this formula, all the points, both for the mixtures of varying proportions and for the pure gamma radiation, are brought to a close fit of the regression line derived from the results of the pure radiation.

An hypothesis is therefore presented that the 90-minute mixtures of Co^{60} gamma rays and fast neutrons are not strictly additive in producing lethality in CF #1 female mice, because a given gamma component in a mixture does not contribute the same proportional lethal effects over the whole dose range. This hypothesis can easily be tested, and the following experiments have already been carried out, although the final results are not yet complete:

A large group of mice was exposed to 210 rep of fast neutrons (LD_{50} - 30 days), and an equal group of mice were exposed to this same dose of neutrons in 90 minutes, but were also given 100 r of gamma rays during the exposure period. If the hypothesis is correct, the additional 100 roentgens of gamma rays should not increase significantly the lethal effect and we should expect approximately 50% of the irradiated mice to die during the 30 day period following the exposure. If, on the other hand, the proposed hypothesis does not hold, 100 roentgens of gamma rays should be equivalent to approximately 23 rep of fast neutrons (RBE: 4.39), and the mice would have received the equivalent of 233 rep (LD_{80}) rather than 210 (LD_{50}). When the data from this experiment have been analyzed, the validity of this hypothesis should be clear.

Figure 13 illustrates the patterns of death after the 90-minute irradiations. The percentage of mortality is plotted for each day after irradiation. In this graph there is no attempt made to subdivide the radiations into various dose groups. The solid line represents the death pattern of 445 mice that died during the first 30 days following a single exposure to fast neutrons over the entire lethal range from 173 to 269 rep. It is clear from this graph that there is a single large peak of mortality 4 - 9 days after the fast neutron exposure.

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Of 1154 mice exposed to Co^{60} gamma rays, in the range from 751 to 1091 r, 582 died during the first 30 days. The dotted line in Fig. 13 shows clearly that the peak of death after such exposure occurs considerably later than after a similar neutron irradiation, namely about 12 - 15 days after exposure.

It is of some interest to note that when all the mixture experiments are lumped together and plotted in this same way, there are two evident peaks of mortality: one at about the 6th day, similar to the neutron peak, and a second corresponding roughly to the gamma peak of mortality.

To see what effect different doses might have on this mortality picture, the pure radiation data have been broken into three fractions in Fig. 14. It is evident from the upper figure that the mortality peak does not shift significantly after a single exposure to gamma radiation over most of the lethal range. When a similar graph is constructed for the mortality picture after fast neutrons, however, there seems to be a shift of the mortality peak with dose. Despite the small number of animals in the low neutron dose group (173 rep), there can hardly be any doubt that on the basis of these observations the mortality curve is significantly different from that of the higher groups. By graphical integration it can be estimated that roughly 10% of the 250 - 270 rep group die later than the ninth day and 33.5% of the intermediate group (187 - 243 rep) die later than the ninth day. Calling this later proportion one-third, then the chances of no mice out of a group of ten dying earlier than the ninth day, which was the case in the 173 rep groups, are $(1/3)^{10}$ or 5.9×10^{-6} . This simple calculation is sufficient to establish the statistical significance of the difference between the low and the medium neutron dose groups.⁽⁵⁾

From the data presented in Fig. 14 it seems evident that the time of death after neutron irradiation is dependent on the dose. However, Mole has pointed out that "one can't be absolutely sure that this is peculiar to neutrons without having strictly comparable data for gamma rays. It is just conceivable that the LD_{100} (15 days) for gamma rays would give a similar time-death curve to the LD_{100} (15 days) for fast neutrons."⁽⁶⁾

In order to test this possibility 1305 r of Co^{60} gamma rays (LD_{100} - 13 days) were delivered to 54 mice. A comparison of the mortality following this irradiation with that following a similar dose of fast neutrons is illustrated in Fig. 15. It is evident that at this high dose there are still significantly different mean survival times after the two ionizing radiations.

There is little doubt that there is an earlier death after neutrons than after similar gamma irradiation of mice. It is an interesting speculation that these different time patterns might reflect different mechanisms of mortality. Some preliminary experiments, carried out in collaboration with C. P. Miller and C. W. Hammond of The University of Chicago, point to the

possible importance of bacteremia in the early mortality after neutron irradiation.⁽⁷⁾ Among neutron-irradiated mice (216 - 265 rep: LD₆₀₋₉₈) sacrificed for bacteriological examination, positive cultures of blood and/or spleen were first obtained on the 4th day, the day on which deaths began to occur. Between the 4th and the 8th day 40 % showed positive cultures. Gamma-irradiated mice (900 - 940 r) were found to have positive blood and/or spleen cultures during the second week. There thus seems to be an interesting correlation between bacteremia and time of death. Mice irradiated with fast neutrons were treated with daily subcutaneous injections of 5 or 6 mg of streptomycin. Irradiated controls received daily injections of sterile saline. The dosage of fast neutrons varied from 222 - 261 rep (LD₆₀₋₉₈:30 days). Among mice irradiated with 222 rep, streptomycin therapy reduced the mortality below that of the saline-treated controls, but the reduction was not as great as that obtained after X irradiation. It was more effective when begun on the first, rather than on the third, day postirradiation. In all experiments streptomycin therapy prolonged survival time, even though it did not reduce mortality. This suggests that the correlation between bacteremia and high mortality may have a cause-effect relationship and that bacteremia is one factor in the deaths of mice exposed to fast neutrons. It is to be hoped that similar experiments in the future may elucidate the different mechanisms that may exist following exposure to fast neutrons and to gamma rays from Co⁶⁰.

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Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 13

Figure 14

Figure 15

PRELIMINARY STUDIES ON THE EFFECTS OF N-ETHYL MALEIMIDE
ON PROTOZOAN CELLS

H. S. Ducoff

N-ethyl maleimide has been reported to be a potent antimitotic agent for chick fibroblasts in vitro.¹ Furthermore, it has been suggested that action of N-ethyl maleimide may be explained by its action in binding the -SH groups of glutathione. It therefore appeared to be of interest to investigate the action of this compound on the growth of the protozoa used for studies of the effects of radiation on cell division.

Various dilutions of ethyl maleimide (Schwarz) were added to actively growing cultures of T. pyriformis in 1% proteose peptone and of C. paramecium in ammonium acetate medium. Results were the same for both organisms: $1.6 \cdot 10^{-6}$ M ethyl maleimide caused an almost complete inhibition of growth, while concentrations of $8 \cdot 10^{-6}$ M and greater caused rapid death. Concentrations of $3 \cdot 10^{-7}$ M and lower had no effect that could be determined by turbidimetric measurements 24 hr later.

Ethyl maleimide was also administered intraperitoneally to mice. CF₁ females weighing 20-25 g were used. Of the 12 receiving 2.5 mg per mouse, all were dead in 24 hr. Mice receiving doses of 0.25 mg or less appeared completely unaffected. It has been reported that 40 mg/kg, given intravenously, is the maximum tolerated dose.

¹Friedman, E., Marrian, D. H., and Simon-Reuss, I. Antimitotic action of maleimide and related substances. Brit. J. Pharmacol., 4: 105-108 (1949).

METABOLISM OF CHLOROPLASTS

G. S. Rabideau

Investigation of the oxygen evolution in the Hill reaction was performed with: (1) *Chlorella* cells cultured on both sulfur-sufficient and sulfur-deficient nutrient medium, and (2) Chloroplasts, isolated from spinach leaves and preserved by lyophilization. Rates were slightly higher for sulfur-sufficient than for sulfur-deficient algae. Addition of 6-thioctic (lipoic) acid (6T) increased oxygen yields of sulfur-sufficient algae slightly and the yields of the sulfur-deficient algae proportionately more. Additions of glutathione to sulfur-deficient algae gave greater oxygen yields than did 6T, suggesting that 6T acceleration may be, when sulfur is lacking, more important in relation to mineral metabolism than to energy transfer as proposed by Calvin.

Results of similar additions to chloroplasts suspended in quinone as an oxidant showed the following increases in percentage over the oxygen yield of control experiments:

Substance added to chloroplasts and quinone:

<u>6-Thioctic Acid</u>	<u>Coenzyme A</u>	<u>Both 6-Thioctic Acid and Coenzyme A</u>
27	6	40
26	18	33
22	28	22
29	10	28
20		-10
44		-12

While the additions of 6-thioctic acid or Coenzyme A were followed for the most part by increased yields, additions of diphosphopyridine nucleotide, glutathione and a partially purified protein precipitate from chloroplasts did not increase yields over controls. Tests of other enzyme preparations are being made.

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STUDIES OF THE THYMOL TURBIDITY, CEPHALIN FLOCCULATION, AND THYMOL FLOCCULATION TESTS

E. J. Katz

The thymol turbidity test in conjunction with the cephalin cholesterol test has given much useful information in the diagnosis and differential diagnosis of various types of liver disorders. Preparatory to the study of screening tests of liver function in groups of people at this Laboratory working with new elements and compounds of undetermined hepatotoxic properties, work was done on "normal" individuals for the establishment of a baseline. The work has been completed; the following is a summary of the results obtained.

1. A study of four thymol buffer reagents using a precalibrated Pyrex glass standard shows that the best two-way sensitivity for screening both normal individuals and patients with liver damage is obtained by using the Huerga and Popper thymol buffer reagent at pH 7.72 with 3.0 thymol units considered as the upper limit of normal. Using this standard and this method, the range of thymol values is condensed, giving significance to an absolute value of normal.

2. Statistical analysis of thymol flocculation results shows that, regardless of test method used, this test discriminates fairly well for normal persons and discriminates poorly for patients with either liver impairment or with other diseases.

Correlation of thymol flocculation results with clinical diagnosis for both patients with liver disease and patients with other diseases showed no clear correlation between clinical diagnosis, stage of disease and the thymol flocculation result.

3. A commonly used cephalin cholesterol antigen (Difco) was found to give a significant percentage of positive results in normal individuals, and was no more sensitive than another antigen as an indicator of damage in patients with diagnosed or suspected liver disease. Another antigen (Wilson) gives fewer positive results for both normal people and patients ill with diseases other than liver disease, while retaining sensitivity for detecting patients with liver disease or involvement.

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ACID PHOSPHATASE ACTIVITY OF CYTOPLASMIC PARTICULATES OF RAT LIVER ISOLATED BY GRADIENT CENTRIFUGATION

John F. Thomson and Eleanor T. Mikuta

DeDuve *et al.*⁽¹⁾ have recently suggested that an acid phosphatase of rat liver, formerly believed to be associated with mitochondria, is actually bound to particles slightly less sedimentable than those containing succinic dehydrogenase, generally considered to be a mitochondrial enzyme. Studies by Novikoff *et al.*⁽²⁾ indicated that the acid phosphatase distribution resembled that of uricase when unwashed fractions were assayed, but appeared less sedimentable than uricase when the precipitates of cytoplasmic particles were washed after centrifugal isolation. According to Berthet and deDuve,⁽³⁾ the enzyme is inactive in the native bound state, but can be activated and solubilized by various procedures such as incubation in water, prolonged homogenization, repeated alternate freezing and thawing, or blending at high speed (we have found that exposure of mitochondrial or microsomal suspensions to 10,000 r of X radiation *in vitro* does not release the enzyme). In addition, Berthet and deDuve reported that the soluble enzyme is readily adsorbed on mitochondria and microsomes.

It was thus of interest to study the distribution of acid phosphatase among particulates separated by gradient centrifugation and to compare the distribution of this enzyme with that of others on the same sample of rat liver. The method of fractionation was the same as that previously described.⁽⁴⁾ Both fresh and frozen-thawed (15 times) samples were assayed according to the procedure of Berthet and deDuve.⁽³⁾ The difference between the two estimates was taken as the particle-bound acid phosphatase activity.

The results of a typical experiment on the comparative distribution of acid phosphatase, uricase, and succinic dehydrogenase are shown in Figures 16-18. The acid phosphatase activity of the homogenate was increased by the freezing and thawing procedure to 162 % of the activity of the fresh homogenate. Figure 16 shows the distribution of activity among the 13 fractions assayed. It is apparent that the 3 enzymes possess markedly different distributions, although the separation among them is not very precise. The curves relating activity to approximate particle size are shown in Figure 17. In Figure 18 are given the activities per unit nitrogen, it is apparent that the enrichment of acid phosphatase in fractions 7 and 8 is much higher than that observed for the other two enzymes.

It thus appears that the acid phosphatase activated after isolation is maximally associated with smaller particles than those richest in succinic dehydrogenase, but with larger particles than those with which uricase is associated. The most recent experiments of Kuff and Schneider⁽⁵⁾ have shown that particulates containing uricase differ from the rest of the

mitochondrial fraction in manifesting marked osmotic effects in strongly hypertonic (2.22 M) sucrose solutions. Whether the acid phosphatase is a property of small mitochondria, large uricase particles, or still another type of cytoplasmic particulate with similar sedimentation characteristics is not established.

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- ① URICASE
 ② ACID PHOSPHATASE
 ③ SUCCINIC DEHYDROGENASE

Figure 16

Distribution of uricase, acid phosphatase, and succinic dehydrogenase activities among rat liver fractions isolated by gradient centrifugation (2 hours at 3000 rpm, sucrose gradient 25 g/1/cm tube length, 11.5 cm radius to meniscus, 20.0 cm radius to tube bottom).

Figure 18

Activity per unit N in various fractions of enzymes: 1, uricase; 2, acid phosphatase; 3, succinic dehydrogenase.

Figure 17

Distribution in respect to approximate particle size of enzymes. See Figure 2 for legends. The dotted extension of curve 3 (succinic dehydrogenase) is based on previous data.

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EFFECTS OF IRRADIATION IN INNATE AND ACQUIRED IMMUNITY

B. Jaroslow

Mice were irradiated (550 r) 15 minutes, 2 days, 4 days, 7 days, and 14 days after infection with Trypanosoma duttoni. Irradiation of the first three groups resulted in a markedly increased reproductive activity (young and dividing forms in Figure 19) and parasitemia (thousands of trypanosomes in Figure 19). The group irradiated 7 days post-infection showed a slight rise in reproductive activity and parasitemia and also a lengthened period of reproductive activity. Irradiation 14 days after infection did not measurably alter its course. These results indicated that during the presumptive period of innate immunity (up to 4 days) irradiation decreased the resistance of the mice to the infection. When the level of acquired immunity was low (at 7 days), irradiation delayed development of a strong immunity. At 14 days when acquired immunity was strong irradiation did not visibly decrease it.

Figure 19

EFFECTS OF RADIATION-PROTECTIVE AGENTS IN ANTIBODY FORMATION

B. N. Jaroslow and W. H. Taliaferro

Acute doses of X radiation (300 r and higher) administered before the antigen greatly reduce the ability of rabbits to form hemolysins to sheep red cells. The present investigation is to ascertain whether cysteine or spleen homogenate, which have been reported to afford some protection against radiation effects (lenticular opacities in rabbits, leucopenia in mice, etc.), decreases radiation damage to the antibody-forming mechanism in the rabbit.

Six rabbits received an intravenous injection of 850 mg/kg (a dose found to inhibit the development of lenticular opacities in rabbits after exposure to acute radiation) of freshly made cysteine, pH 7, 10 to 15 min prior to 400 r whole-body irradiation. Six rabbits received an intravenous injection of 1.1 - 1.4 g freshly homogenized rabbit spleen 1/2 hr after exposure to 400 r whole-body X irradiation. These treatments were followed 2 days later by an intravenous injection of sheep red cell stromata (2×10^8 stromata/kg).

Hemolysin titers are defined as the amount of serum which produces 50 per cent hemolysis of 1 ml of 2 per cent sheep red cells. Here they are expressed as the number of hemolytic units per ml. Titers of 200 or less are within the normal range.

The results are tabulated below:

<u>Treatment</u>	<u>No. of Animals</u>	<u>Mean Peak Titer</u>
Cysteine and 400 r	6	143
NaCl and 400 r	1	60
Cysteine	1	530
NaCl	1	500
400 r and spleen homog.	6	58
400 r and kidney homog.	1	220
Spleen homog.	1	4000
Normal	1	4200

The results of these preliminary experiments indicate that cysteine and spleen homogenate in the dosages used do not decrease the damage done to the antibody-forming mechanism by 400 r whole-body X-irradiation.

ADSORPTION OF BACTERIAL VIRUSES

Arthur L. Koch and Marie Burch

When a suspension of a bacterial virus and its susceptible host are mixed the virus adsorbs on the bacteria at a rate which is a function of the bacteria and virus titer, and on the ionic constitution of the medium. In order to measure adsorption rate, previous workers have used either centrifugation or antisera to distinguish free viruses from adsorbed virus. We have adapted the millipore filter technique for this purpose and have found conditions such that, while all bacteria are retained by the filter, 93 per cent of the free virus is transmitted.

Utilizing this technique we have measured the adsorption rate of virus T_{2R} and T_{6R+} . In both stocks a new phenomenon was observed, namely, each stock was found to consist of almost equal numbers of two phage types. The first type adsorbs very rapidly (almost every collision between bacteria and virus leads to adsorption), and the second type adsorbs 20 to 30 times more slowly. As at least one of the stocks had been recently derived from a single infective particle, and as plaque morphology of both types are identical, it is concluded that these two types represent phenotypic variations.

AN HYPOTHESIS OF MITOCHONDRIAL ORIGIN IN PARAMECIUM.

E. L. Powers, C. F. Ehret, and L. E. Roth

Investigations into the nature of the previously described⁽¹⁾ large bodies of the macronucleus in two species of Paramecium and of the relationship between these and other structures in the cell have continued. Recent electron micrographs reveal several interesting similarities in structure between these and the so-called "mitochondria" which also have been described.⁽²⁾ Figure 20 is a section thru the posterior half of P. aurelia, demonstrating the gross correspondence between bodies of the macronucleus (n) and those of the cytoplasm (m) which have been called mitochondria. In size, shape, and texture they appear the same. On a finer scale in Figure 21, which is a 0.1μ section through P. aurelia, the ultrastructural details of the two kinds of inclusions appear to be quite similar.

Further investigation of the relationship between the two bodies consisted of direct observation of the macronuclear bodies in various post-zygotic and cell division stages and of application of several cytochemical tests.

Phase contrast observations on living specimens of P. bursaria of macronuclear morphogenesis from the post-conjugation synkaryon nucleus and of macronuclear "amitosis" during vegetative fission show that large bodies arise from just resolvable ($<0.2\mu$) dots and spherules (nucleoli?) which are distributed in the karyoplasm and appear recurrently through at least three generative cycles during morphogenesis, and at the time of each micronuclear mitotic metaphase-anaphase during fission. These "nucleoli" are Feulgen negative, give the "Swiss-cheese effect"⁽¹⁾ with acetocarmine, dissolve in dilute sodium hydroxide and in acetic acid, stain red with aqueous carmine, and stain a pale blue-green with Janus Green B. During the periods of our observations some were extruded through the nuclear membrane (Figure 22) where they are grossly indistinguishable from mitochondria (which also stain blue-green); some enlarged to form large bodies; some collided with other nucleoli or large bodies to form even larger bodies. These final fusion figures shrink into a fish-net like structure and ultimately disappear. Shortly before disappearance intramacronuclear generation of a new crop of nucleoli begins. Preliminary studies on the postultraviolet effect upon the macronucleus of P. bursaria indicate early "vacuolization" effects similar to those shown by Kimball in P. aurelia,⁽³⁾ but also quite similar to those seen in the normal life cycle of nucleoli as described above. Fusion of nucleoli appears to be accelerated; but the processes of disappearance of the "fish-net" agglomerates as well as generation of young nucleoli (which in some cases arise from the spherule stage in a vacuolated condition) are both somewhat retarded.

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In addition to the structural resemblances between large bodies, a second remarkable structural similarity is evident between those bodies at the bases of the cilia and the mitochondria (Figure 23). If these two structures are the same body, then in the kinetosomalciliary relationship described by Lwoff the cilium is an outgrowth of a peripherally located mitochondrion.

This evidence strongly suggests that the macronucleus of *Paramecium* acts at specific stages in development and fission in the production of particles (young nucleoli) which, when extruded, are termed mitochondria, and, when retained, large bodies or nucleoli. Appearance, location, and cytochemical reactions satisfy the application of classical terminology to such particles. The mitochondrion may migrate peripherally and there extend itself as a cilium, or remain in the cytoplasm as another site of enzymatic action. This hypothesis explains not only the relatively direct control which the nucleus exerts over the phenotype, but also the phenomenon of phenotypic lag. The data supporting the latter phenomenon suggest that some semi-autonomous replication of the mitochondria (kinetosomes) may occur,⁽⁴⁾ but the present study suggests an ultimate repopulation in the cytoplasm during morphogenesis and vegetative growth of these mitochondrial derivatives of the nucleus.

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Figure 20

Gross correspondence between large bodies (nucleoli, n) of macronucleus and those of cytoplasm (mitochondria, m) in Paramecium aurelia, 51 VIII S.

Figure 21

Ultrastructural details of nucleolus and mitochondrion,
in P. aurelia

Figure 22

Nucleolar extrusion from macronuclear anlage in a
73 hour exconjugant of P. bursaria, Variety I, B x D.

Figure 23

Section through bases of the cilia of the penniculus in P. bursaria.

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RESPONSES OF CELLS TO CONSTANT IRRADIATION

A. M. Brues and A. N. Stroud

Sr^{90} - Y^{90} point sources (about $50 \mu\text{c}$) have been embedded in a variety of mouse and rat tumors for varying lengths of time up to 8 days. We have confirmed, at least in some of these tumors, the earlier observation on the Hall tumor (SLT) that cells continue to grow in areas where mitosis is inhibited, resulting in the development of gigantic cells. Using $50 \mu\text{c}$ sources the radius of the amitotic area is about 4 mm. In the one radiosensitive lymphosarcoma so far studied, necrosis extends out to the border of this area. Because of the prevalence of necrosis in untreated tumors of most types, the material will have to be subjected to careful observation and analysis before further conclusions can be drawn.

Studies are being made of the DNA content of nuclei of normal tissues and tumors continuously irradiated by point beta ray sources by means of photometric observations on Feulgen-stained material in collaboration with Dr. H. H. Swift, University of Chicago.

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NEUTRON IRRADIATION OF PHOSPHORUS COMPOUNDS AND OF BONE

T. R. Sato and W. P. Norris

These investigations were undertaken to provide information concerning the nature of the phosphate compounds in bone. The plan of investigation was the activation of the bone phosphate with neutrons followed by resolution of the radioactive phosphate so formed by electrical migration in moist paper.

The electrical migration methods for the resolution of phosphorus compounds have several advantages over the analogous chromatographic methods. One of the principal advantages is the formation of well-defined zones of the migrating substances. The electrical migration method has now been standardized and favorable migration conditions have been selected. As an aid to others, most of this material has been published elsewhere [Analytical Chemistry, 25:438 (1954)].

Exposure of bone to neutrons, followed by dissolution and electrical migration of the products, produced several zones of radioactive material. Measurements of half-life and energy showed that all these zones contained radioactive phosphorus in different chemical combinations. One of these zones migrated at the same rate as phosphate and was assumed to be radioactive orthophosphate ion. Some of the other zones migrated at the same rates as phosphorous acid, hypophosphorous acid, and pyrophosphoric acid. Several zones migrated slowly like the synthetic polyphosphates.

Various phosphorus compounds, when irradiated, dissolved, and submitted to electrical migration, usually yielded the same series of zones obtained by neutron irradiation of bone. Although the zone containing the most activity often corresponded with the material that was irradiated, the zones with smaller amounts of activity usually separated in the same relative positions, indicating that the same minor constituents were obtained from all the phosphorus compounds.

In the light of these results, the several radioactive phosphorus compounds from bone and from phosphates may have arisen 1) from neutron splitting of the phosphate group, 2) from neutron activation of various phosphorus compounds already present in all the preparations, 3) from secondary reactions of the radioactive species, or 4) from reactions caused by γ -rays. Certain aspects of these investigations have already been summarized in the Quarterly Report (ANL-4745, 1952).

To distinguish among some of these possibilities for the formation of various radioactive species, the electrical migration has been performed with nonirradiated as well as with irradiated phosphorus compounds, and the separated zones have been located by neutron activation. As an example, a

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preparation of phosphate was irradiated with neutrons. It was dissolved and submitted to electrical migration in paper moistened with lactic acid. A portion of the same phosphate that had not been irradiated was dissolved and submitted to electrical migration under the same conditions used with the irradiated portion. The paper was then dried, and the dry paper containing the inactive phosphate was protected with aluminum foil and irradiated with neutrons. A radioautograph then revealed that the phosphate submitted to migration before irradiation yielded a single radioactive zone. This result showed that the nonirradiated specimen contained only one species of phosphate and was, therefore, homogeneous. The specimen irradiated before migration yielded a series of radioactive zones, the zone with greatest activity corresponding in location to the phosphate zone. Other phosphorus compounds such as pyrophosphate, phosphorous acid and hypophosphorous acid also yielded single zones when submitted to migration before neutron activation. In virtually every instance the major zone observed by electrical migration after irradiation corresponded with the zone obtained from material submitted to migration before irradiation. These results prove that the minor radioactive constituents were secondary irradiation products, and they do not represent normal constituents of the preparations submitted to the electrical migration.

If the minor radioactive zones were formed by nuclear reactions, only radioactive carrier-free species should be present. This conclusion was confirmed by the following procedure. The neutron-irradiated preparations were dissolved and separated by electrical migration. The paper was then dried and allowed to stand about 60 days until most of the activity had decayed. The paper was then exposed to neutrons for about 14 and 30 days, and the induced activity was located by an autograph. Only the major zones containing an abundance of inactive phosphorus were reactivated. The minor zones contained so little phosphorus that it could not be detected.

All these observations indicate that neutron irradiation disrupts the phosphorus compounds. The substances separated by electrical migration represent the end products of the reaction.

Secondary reactions of the radioactive phosphorus compounds were difficult to follow when crystals were irradiated. In aqueous solutions, however, all the phosphorus compounds were converted entirely into orthophosphate. Separate experiments with neutron-absorbing containers (Cd) showed that this oxidation to orthophosphate could be attributed to the gamma rays in the reactor.

The slowly migrating, radioactive species isolated after irradiation of crystalline phosphates resembled the synthetic polyphosphates which are formed by the action of heat. Like the thermally produced polyphosphates, they were hydrolyzed to orthophosphate when heated with mineral acids. Their formation in the irradiated phosphate crystals suggests that neutron irradiation produces condensation reactions analogous to thermal polymerization.

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In summary, these results show that neutron irradiation of phosphates and of bone produces a number of radioactive phosphorus compounds. Some of these radioactive species result from the primary disruption of the phosphate group. Others result from secondary reactions such as oxidation and condensation.

The determination of the phosphate compounds in bone has been complicated by the variety of the neutron irradiation products. It is probable that bone can be dissolved and submitted to migration before activation thus providing a better indication of the number and nature of the phosphorus components. The solution of the bone itself without chemical alteration of its phosphorus constituents also presents serious problems, but these are now subject to investigation by the use of acidic and basic solutions.

MECHANISM OF CALCIUM TRANSPORT AND DEPOSITION

Arthur Lindenbaum

In the blood of laying hens the concentrations of calcium may reach values as high as 25 mM per liter. These levels far exceed the amount of calcium known to be held in solution on the basis of solubility product and binding by protein. Consequently some additional solubilization mechanism must be involved. One such mechanism is that of complex ion formation or chelation.

Examination of the binding constants between calcium and a large number of the simpler circulating metabolites (amino acids, Krebs cycle acids, inorganic acids, etc.) as well as serum protein shows that none of these substances is capable of forming a sufficiently strong complex to account for appreciably increased calcium binding. Local pH changes cannot account for significant alterations in the amount of free or complexed calcium. The $\log K_f^*$ of glutamic acid, for example, between pH 7.1 and pH 10.4 varies only between 0.7 and 1.1.⁽¹⁾

The conclusion appears inescapable that a substance or substances capable of far greater capacity for calcium transport must be involved. Models for such compounds are to be found in the short chain polyphosphates where the binding capacity for calcium increases rapidly with chain length, so that compounds containing as few as 4 to 6 phosphate groups have enormously increased binding capacities.^(2,3) Such a compound might therefore be a polymer with functional phosphate groups and/or sulfate, hydroxyl and carboxyl groups. One such compound already characterized is the phosphoprotein serum vitellin.⁽⁴⁾

Regardless of the existence of such polymerized substances, however, it must be recognized that in the circulation all the phosphates present, even if totally complexed or chelated, could not account for much increase in calcium transport. At this point the phenomenon known to water chemists as "threshold action",^(5,6) in which small concentrations ($\sim 10^{-5}M$) of condensed phosphates are able to hold relatively large amounts of calcium (Ca: P = 100 to 300) in solution due to charge effects which stabilize the calcium micelle and hinder crystal growth, might be mentioned. Following spontaneous hydrolysis of the polyphosphate, calcium is deposited as orthophosphate. Whether there exists a physiological counterpart of this phenomenon in the circulation is an interesting conjecture.

The above hypothesis of "hypercalcemic" calcium transport and deposition may thus be regarded as having the following sequence:

*Formation quotient

(11)

1. Production, enzymatically or otherwise, of a polymer containing perhaps no more than 6 phosphate groups.
2. Transport of "hypercalcemic" calcium as a strongly bound complex and by "threshold action."
3. Deposition of calcium at sites favoring hydrolysis of the complexes.

The recent report by DiStefano, Neuman and Rouser⁽⁷⁾ of a hexoseamine phosphate involved in calcification of cartilage lends some support to the above line of reasoning. The possibility that polymers such as chondroitin sulfate, hyaluronate, glycogen, etc. - phosphorylated or with other functional groups, either free or bound to protein - may be involved in calcium transport and deposition is being investigated.

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STUDIES IN FIXATION OF CARBON

Robert W. Swick and Dorothy M. Tahara

As a part of a program to ascertain the precursor(s) of serine in the rat, preliminary studies were made with E. coli, grown on a medium containing glucose, salts, and either glycine-2-C¹⁴ or formate-C¹⁴. The protein fraction of the harvested cells was isolated, hydrolyzed, and the amino acids separated by two-dimensional paper chromatography using phenol-water-NH₃ and butanol-acetic acid-water solvent systems. The amino acid spots were located with ninhydrin, cut out, and counted.

The results of the experiment with glycine, expressed as a per cent of the activity found in the glycine spot, were: glycine, 100; serine, 16.5; valine-methionine, 7.9; arginine, 5.0; leucines, 3.84; histidine-lysine, 3.75; glutamic acid, 2.89; alanine, 1.53; proline, 0.82; threonine, 0.57; and aspartic acid, 0.33. The most striking result is that glycine is a relatively poor precursor of serine in this organism. This confirms earlier work in this Laboratory and by others that the primary source of serine is some intermediate in carbohydrate metabolism. The activity found in the valine-methionine spot may be the result of the utilization of the C-2 position of glycine for methyl group synthesis and its subsequent incorporation into methionine (or valine). The activity in the remaining amino acids is due either to contamination or to the fixation of C¹⁴O₂ arising from the metabolism of glycine.

The distribution of isotope in the amino acids from E. coli given formate indicated that oxidation of the formate to CO₂ with subsequent fixation was the predominant route of incorporation. There was almost no fixation of formate per se into the C-3 position of serine and little more into the methyl group of methionine (or valine).

Other isotope competition experiments using glucose-U-C¹⁴ and a number of nonradioactive, possible intermediates have been carried out with this organism. None of the compounds so far tested appear to have a marked effect on the incorporation of radioactivity into the serine fraction. Others are being tested at the present time.

THE DISTRIBUTION AND EXCRETION OF Sr^{90} - Y^{90} IN THE DOG

W. E. Kisielewski

The following preliminary data are presented on the retention, excretion, and tissue distribution of Sr^{90} - Y^{90} in dogs injected intravenously with carrier-free Sr^{90} in equilibrium with its Y^{90} daughter. Dog #185 received 1.96 mc (0.15 mc/kg), and Dog #183 received 2.2 mc (0.25 mc/kg). Urinary and fecal excretion measurements were made daily on both dogs for a period of 15 days after injection. The results are tabulated in Tables 4 and 5. Dog #183 died 18 days after injection; the results of a detailed bone distribution study are given in Table 6.

All of the collected samples were analyzed for radio-Sr content by the usual technique of mounting aliquots of the ash in No. 1 porcelain, Coors capsules and counting under thin mica, end window, Geiger-Muller tubes. All samples were compared against standard capsules prepared from the injection solution. In order to compensate for the possibility that Y^{90} might not remain in equilibrium with its parent after injection, because of differences in metabolism, no samples were counted sooner than 15 days after collection. This interval insures that the relatively short-lived Y^{90} ($t_{1/2} = 62$ hrs, decaying to stable Zr^{90}) will come nearly to equilibrium (94%) with its parent.

In general the preliminary data indicate that Sr^{90} is excreted very rapidly following intravenous administration. The greatest part of the excretion occurred during the first three days, and by the 10-15th day very little of the retained strontium was excreted. The average total excretion, during the first 15 days, was approximately 55% of the injected dose with a somewhat higher excretion in the feces than in the urine. The fecal excretion averaged 30% of the injected dose while the urinary excretion averaged 25% of the injected dose.

Tissue analyses on Dog #183 18 days after injection indicated that almost all of the retained Sr^{90} - Y^{90} (45% of the injected dose) was found in the skeleton. The concentration in a representative number of soft tissues was measurable but represented a very small fraction of the retained dose.

Using the Sr^{90} - Y^{90} content per gram of ash in bone as a comparison index, there appears to be a variation among different bones and portions of bones. As in the case of the humerus, the head contained 0.164% of the injected dose per gram of ash whereas the shaft contained only 0.027%. The costochondral junction of the rib contained 0.312% compared to 0.126% for bony rib. The skull and mandible, as has been determined with other

nuclides such as plutonium and yttrium, were lowest in concentration. The femurs and scapula, though structurally different, had nearly identical values (0.087% and 0.085% per gms ash weight).

Table 4

(URINARY-FECAL EXCRETION)

Exp QA - $\text{Sr}^{90}\cdot\text{Y}^{90}$ - IV - Dog #183 - 2.2 mc/Dog

Time after injection (days)	% Injected Excreted		
	Urine	Feces	
2	16.5	1.4	
3	4.7	18.7	
4	2.0	6.0	
5	0.79	2.6	
6	0.59	0.53	
7	0.41	0.80	
8	0.37	0.92	
9	0.22	0.31	
10	0.31	0.24	
11	0.12	0.11	
12	0.14	0.10	
13	0.17	0.09	
14	0.11	0.22	
Total Excreted	26.43	32.02	58.45
Cagewash			3.3
Total Retention			38.25

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Table 5

(URINARY-FECAL EXCRETION)

Exp QA - Sr⁹⁰.Y⁹⁰ - IV - Dog #185 - 1.96 mc/Dog

Time after injection (days)	% Injected dose excreted		
	Urine	Feces	
1	8.43	7.34	
2	2.40	8.37	
3	5.49	2.64	
4	1.60	1.40	
5	1.52	1.30	
6	0.53	0.97	
7	0.47	0.48	
8	0.49	0.37	
9	0.35	0.27	
10	0.21	0.24	
11	0.21	0.23	
12	0.17	0.14	
13	0.13	0.13	
14	0.12	0.11	
15	0.15	0.18	
Total excreted	22.27	24.17	46.44
Total retention			53.56

Table 6

(TISSUE DISTRIBUTION)

Exp QA - $\text{Sr}^{90}.\text{Y}^{90}$ - IV - Dog #183 - 1.96 mc/Dog - 18 D after Inj.

Tissue	Wet wght.	Ash wght.	%Inj. dose	<u>%Inj. Dose</u> g. w. wght.	<u>%Inj. Dose</u> g. a. wght.
Femur (whole)	31.53	11.50	1.0	0.032	0.087
Radii (whole)	11.83	5.03	0.25	0.021	0.050
Scapula (whole)	19.52	6.03	0.51	0.026	0.085
Humerus (head)	6.24	1.59	0.26	0.042	0.164
Humerus (shaft)	3.00	1.59	0.04	0.014	0.027
Cervical spine	9.15	2.12	0.29	0.032	0.137
Lumbar spine	11.66	2.82	0.48	0.041	0.170
Rib	2.57	0.83	0.11	0.043	0.133
Rib	2.50	0.74	0.09	0.037	0.126
Costochondral junction of rib	1.09	0.17	0.05	0.049	0.312
Skull	12.32	6.01	0.21	0.017	0.035
Tooth incisor	0.15	0.10	0.001	0.006	0.010
Mandible with teeth	33.90	15.04	0.50	0.015	0.033

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VASOCONSTRICTOR DRUGS AND RADIATION
PROTECTION IN THE CHICK

S. P. Stearner and E. J. Christian

The protective action of epinephrine against radiation mortality has been postulated to operate through its vasoconstrictor effect.^(1,2) The arteriolar constriction produced by the sustained action of large doses of epinephrine may be sufficient to produce a significant tissue anoxia. The effect of an epinephrine-induced hyperglycemia may also warrant consideration, however, in view of reports that high blood glucose levels protected rats from the lethality of X rays.⁽³⁾ In young chicks, epinephrine in peanut oil has been shown to produce a marked hypertension within 15-30 min. after administration of the drug, but the extent of the hyperglycemic response has not yet been determined.

Arterenol-HCl (nor-epinephrine), which in mammals lacks an effect on blood glucose but has a strong pressor effect, was tested for its protective action against radiation mortality in young chicks. The drug was suspended in peanut oil to prolong the time of action and was injected intramuscularly into 3-day chicks 5 min. before exposure to 1000 r at 43 r/min. A wide range of doses reduced the initial (0-48 hours) as well as later mortality (Figure 24). Although 30 mg/k gave as good protection as 20 mg/k, 40 mg/k was apparently close to the lethal range yet produced no deaths in non-irradiated birds. A maximum rise in blood pressure (mean pressure, femoral artery) occurred within 15-20 min. after administration of arterenol in peanut oil (12.5 mg/k). Normal levels were reached within 1 to 2 hr, and thereafter followed a period of sustained hypotension averaging 20% below the control level. Although the blood pressure response of arterenol-treated irradiated birds has not yet been determined, the duration of the response following drug treatment indicates that the relation to the time of postirradiation hypotension is similar to that which has been shown with epinephrine. The maximum hypertension following arterenol occurred during the time of irradiation, and the pressure had returned to normal or subnormal levels before the time at which postirradiation hypotension becomes apparent.

The reported minimal effect of arterenol on blood glucose concentration in mammals, as contrasted to that of epinephrine, indicates that a hyperglycemia is probably not involved in its protective action against radiation lethality. Nevertheless, it is planned to determine the effect of epinephrine and of arterenol on blood glucose concentration in chicks. Arterenol administered before irradiation reduced the initial as well as later mortality, apparently acting on the primary events initiated by exposure to ionizing radiations.

Other vasoconstrictor drugs that had little or no effect in reducing radiation mortality include pitressin and serotonin. Pitressin is reported to constrict arterioles and in large doses to produce a tissue hypoxia. The hypertension develops more slowly and is more prolonged than that produced by epinephrine in saline solution. In the rat, 20 units/k has been reported to substantially reduce X-ray mortality when administered within 20 min before exposure.⁽¹⁾ Young chicks tolerated large doses of pitressin and showed only a transient dyspnea and inactivity after 400 units/k. Amounts ranging from 10-400 units/k IP., given 5 min before exposure to 1000 r X rays at 43 r/min, were without effect on subsequent mortality, nor was any protection observed when 400 units/k was given 1/2, 1, or 2 hr before exposure.

Serotonin, a vasoconstrictor substance recently isolated from blood platelets, has also been reported to reduce radiation mortality in rats.⁽⁴⁾ The effects of this drug in saline solution on the mortality of irradiated chicks is shown in Figure 25. The protection afforded by 40 mg/k IP. was slight compared to that provided by epinephrine or arterenol, and larger or smaller doses had no effect.

Although serotonin and pitressin failed to provide radiation protection in the chick in a manner similar to that demonstrated in the rat, the duration and degree of the vasoconstrictor effect, upon which the extent of the tissue anoxia depends, has not yet been tested in the bird. It is also planned to determine the protective action against radiation mortality of other pressor compounds chemically related to epinephrine.

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Figure 24

Effect of various doses of arterenol (in peanut oil) on mortality following exposure to 1000 r X rays at 43 r/min. Number of animals in each group indicated in parentheses.

Figure 25

Effect of serotonin in saline on mortality following exposure to 1000 r X rays at 43 r/min. Number of animals in each group indicated in parentheses.

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BERYLLIUM TOXICITY IN YOUNG AND OLD MICE

M. R. White

A comparison of the susceptibility of mice of different ages to acute beryllium poisoning has been made as a preliminary to determining the degree of protection against beryllium poisoning by ATA in young and old animals. Using a total of sixty mice, the toxicity of three doses of beryllium sulfate (0.48, 0.52, and 0.60 mg of Be per kg), ranging around the LD₅₀ and given as a single intravenous injection, was determined in adult female CF #1 mice of two age groups - one about 85, the other about 480 days of age at injection. Little difference was observed between the survival levels of the two groups, as shown in Table 7. The results of this preliminary experiment in mice do not therefore correspond to the indication in rats that young animals are somewhat more resistant than older ones to acute Be poisoning.¹

Table 7

PER CENT SURVIVAL AT 7 DAYS

Age of Mice	Dose of beryllium (mg/kg)		
	0.48	0.52	0.60
85 ds.	90	60	0
480 ds.	60	40	20

¹ Aldridge, W. N., J. M. Barnes & F. A. Denz, Brit. J. Exptl. Pathol. 30:375 (1949).

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AN ENZYMATIC METHOD FOR BERYLLIUM ANALYSIS

Arthur Lindenbaum, Jack Schubert, and William Westfall

In a previous investigation¹ the inhibition and reactivation of plasma alkaline phosphatase has been utilized to measure the formation quotients of beryllium-organic anion complexes. It was pointed out that the large changes in alkaline phosphatase activity produced by beryllium in concentrations between 10^{-7}M and 10^{-5}M could be utilized to measure the beryllium concentrations of unknown solutions. Such a method is now being developed for the analysis of Be in urine, tissues, and air samples.

¹Studies on the mechanism of protection by aurointricarboxylic acid in beryllium poisoning. II. Equilibria involving alkaline phosphatase, by Jack Schubert and Arthur Lindenbaum, J. Biol. Chem., in press.

EFFECTS OF BERYLLIUM ON ENZYMATIC REACTIONS

Jack Schubert and J. F. Fried

Our previous investigations have shown that the interaction of beryllium, under physiological conditions, with compounds of biological importance invariably involves a phenolic -OH group; for this reason we are investigating systems associated with the metabolism of tyrosine - the only amino acid which contains a phenolic -OH group. While tyrosine itself does not react with beryllium, the compounds derived metabolically from tyrosine through enzymatic action produce compounds which contain ortho hydroxy groups on a benzene ring. In such cases, beryllium can react to form a chelate ring. We are now investigating the effects of beryllium on the enzyme tyrosinase, using tyrosine and related compounds as substrates. By this approach we hope to obtain information which will further clarify the reasons for the marked toxicity of beryllium to tissue cells.

ATTEMPTS TO REDUCE TOXICITY OF ALUMINUM, IRON, AND ATA

Marcia R. White and Jack Schubert

Since aurintricarboxylic acid (ATA) forms chelates with both aluminum and iron, as well as with beryllium, it was of interest to determine whether it could prevent death of animals acutely poisoned with either of these metals in the same way as it protects beryllium-poisoned mice. Conversely, it was also of interest to determine whether the formation of an aluminum-ATA or an iron-ATA lake would reduce the toxicity of a lethal dose of ATA. All injections were given intravenously into CF #1 mice.

The death of mice poisoned with an injection of 200 mg/kg of ammonium sulfate was not prevented by injection of an equimolar amount of ATA (200 mg/kg) immediately preceding the aluminum injection. ATA also failed to protect mice poisoned with a lethal dose of ferric ammonium citrate (150 mg/kg). The ATA (200 mg/kg, equimolar with the iron) was given either immediately preceding the iron injection, or mixed with the iron in the injection solution. In both cases, death of the treated mice occurred sooner (at an average of 1.7 hr after injection) than it did in mice receiving iron only (approximately 2.5 hr). The reasons for these failures are probably the low stability of the chelate formed under physiological conditions between ATA and either iron or aluminum, the low molar ratio of ATA to the metals, necessitated by the relatively high doses of the metals required to kill the animal, and, in the case of aluminum, the large embolic particles of the aluminum-ATA lake.

It was found that neither aluminum, in the form of ammonium aluminum sulfate, nor iron, in the form of ferric ammonium citrate, injected either immediately before ATA and also, in the case of iron, within the same injection solution, prevented the death of mice receiving the LD₅₀ of ATA (525 mg/kg). These negative results might indicate that the reactive groups of the ATA molecule, presumably inactivated by chelation with the iron or aluminum, are not the primary factor in the toxicity of ATA. However, the amount of each metal used (the maximum nontoxic) was probably too small to be effective under the conditions of the experiment. The molar ratio of Al: ATA being 1:3, and that of Fe: ATA only 1:7.

CALCULATION OF NEUTRON ACTIVATION REACTIONS

A. M. Brues and J. Gurian

Work has been completed on preparation of IBM cards covering nuclear data pertinent to slow neutron activation problems. Three sets have been prepared: one contains data by elements; another by isotopes, including data on the radiations emitted by activation products; and a third lacking radiation characteristics but including certain calculations. The third set includes atomic number and isotopic mass, thermal neutron cross section for each reaction in barns, atomic number and mass of product, half life (calculated as seconds), cross section in cm^2 per gram of natural element, curies produced at saturation per gram of natural element in a flux of 10^{11} neutrons per cm^2 per second, and curies produced by instantaneous exposure to 10^{11} neutrons per cm^2 . These cards are suitable for making additional rapid calculations of various kinds and the cards have been printed in the order of various data (e.g. half life and yields), providing useful reference material. If there is sufficient general interest, it is planned to reproduce these data in a topical report.

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ISOTOPE EFFECTS IN BIOLOGICAL REACTIONS

G. S. Rabideau

Evidence for the preferential reaction of the lighter - rather than the heavier - isotopes of a particular element used as a tracer in biological reactions is reviewed. This body of evidence has been collected largely in the last few years and has been spearheaded by endless examples of this effect in physical and chemical reactions. It is to be expected that the reactions which occur in biological systems are both physical and chemical and would therefore show the same isotope preference. In some instances, a series of one-step reactions in biological systems even enhances the discrimination against the heavier isotope-labeled tracer-substance. In the light of such preference among isotope-labeled metabolites, reinterpretation of the results of some tracer experiments is suggested.