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A. M. Brues
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TABLE II-7

Phantom Response to Ambient Low-energy X rays (10 r/min., air)

Phantom mass (gm)	Energy (pkv)	Integral body dose (gm-r/min)	Mean tissue dose (r/gm/min)
NL#1* 3.0×10^3	80	19.2×10^3	6.40
	100	23.0×10^3	7.67
	135	27.8×10^3	9.27
	150	27.9×10^3	9.30
	200	28.6×10^3	9.53
	250	28.6×10^3	9.53
S-D 2.2×10^2	80	1.68×10^3	7.64
	135	1.87×10^3	8.50
	250	1.87×10^3	8.50
BA F ₁ 22	80	1.96×10^2	8.91
	135	2.04×10^2	9.27
	250	2.04×10^2	9.27

*NL#1 $\equiv$ NLI

TABLE II-8

Change half value thickness column entries for 100
and 135 peak kilovoltage to 3.9 mm Al and 8.2 mm Al,
respectively.

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MEASUREMENT OF RADON PARTITION BY GAMMA-RAY METHOD. PART II.

P. F. Gustafson and L. D. Marinelli*

The method for determining radon partition previously described⁽¹⁾ has been improved upon by plotting the calibration data in a more meaningful manner and by the use of CsI crystals as photon detectors. Two channels were used in the work with CsI. The first contained the 186 kev Ra²²⁶ gamma, the second the three gammas from RaB. At low values of radon retention, the intensity of the 186 kev line is high compared to that of the RaB lines. As radon builds up and is retained the relative intensities shift, so that at 8 to 10 per cent retention the two intensities are comparable.

This intensity relationship itself causes no concern directly, but as the number of RaB gammas increases, the number undergoing Compton scattering in the crystal within the first channel increases as well. Similarly the Compton process occurring in the phantom or in vivo produces 186 kev photons which are indistinguishable from those emitted by Ra²²⁶. This necessitates a correction to the counts in the 186 kev channel. Experimentally, the fraction of the total counts in the RaB channel which appears to fall in the 186 kev channel is quite constant for values of radon retention between 10 and 66 per cent; there is, however, a slight shift between 1 and 10 per cent retention which may be due to lack of secular equilibrium between radon and its daughters.

The effect of the crystal Compton scattering in the 186 kev channel may be reduced by the use of crystals of higher Z. The use of thin slabs of CsI further reduces the efficiency of RaB gammas detection by both photoelectric and Compton process. Two CsI crystals, both of 1-1/2 in. diameter, one 1/8 in. thick, the other 1/4 in. thick, were used and the counting rates in the 186 kev and RaB channels were determined, keeping the counter-to-source distance fixed. The 1/8 in. crystal registered about 90 per cent of the 186 kev counts of the thicker crystal, but its relative efficiency for RaB decreased by a factor of two. Thus the method of radon partition was followed using the 1/8 in. CsI crystal; these results and those obtained with a NaI crystal are compared in Figure 1.

The data obtained with the CsI crystal were plotted using a function of the form:

$$F = \frac{B}{(Ra - k_1^B)} \quad (1)$$

* Radiological Physics Division

Figure 1

- A. Variation of the function F in the gamma ray spectrum of radium as a function of radon retention for various depths in presdwood phantom (CsI crystal).
- B. The variation of F with a NaI crystal. 186 kev channel is 30 kev wide in both instances. RaB channel is 200 kev wide with both crystals.

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Where B is the counting rate in the channel containing the three gammas from RaB, Ra is the counting rate in the 186 kev channel, and k_1 is the fraction of B which appears in the 186 kev channel due to Compton scattering. If k_1 is evaluated properly, the value of $(Ra - k_1 B)$ should be constant for any value of radon equilibrium, and should be equal to the value of Ra obtained from a completely de-emanated source. The value of F is then plotted as a function of per cent of radon equilibrium.

In the case where a NaI crystal was used, Equation (1) was modified to include the photoelectric efficiency and the relative abundance of the gamma rays involved. This modified equation has the form:

$$F = \frac{f_1 B}{f_2 (Ra - k_1 B)} \quad (2)$$

where B, Ra, and k_1 have the same meaning as in (1). The parameter f_1 takes into account the efficiency of the NaI crystal used for a gamma ray of the mean energy of the three from RaB. The parameter f_2 makes a similar correction for the 186 kev gamma including its abundance in Ra decay and its internal conversion. The crystal efficiency values were taken from the work of Maeder, *et al.* (2) With the source in air, a plot of Equation (2) versus per cent radon retained is a straight line on linear paper. The value at 100 per cent radon equilibrium should be equal to unity. Actually the experimental value is somewhat larger, which is not surprising in view of the approximations involved. The data plotted in Figure 1 for different thicknesses of presdwood between source and crystal are internally consistent and may be used for determining the radon partition in vivo or in vitro.

Values for radon retention as obtained in vivo by this method and by the radioactive growth of the carcass after sacrifice are compared in Table 1. These results suggest that the method can be applied with confidence to studies of radon partition in vivo.

Comparison of the curves in Figure 1 shows that the heavier crystal offers some experimental advantages when radon retention is greater than 30 per cent.

Table 1*

COMPARISON BETWEEN IN VIVO AND RADIOACTIVE GROWTH
VALUES OF RADON RETENTION

Animal	Radon retention at time of sacrifice	
	<u>In vivo</u> **	Radioactive growth
Dog #219	27 - 32%	29.2%
Dog #67C	15 - 18%	17.1%
Dog #67F	19 - 23%	19.0%
Rabbit # 2	20 - 24%	21.1%
Rabbit # 5	12.5 - 14%	13.6%

* Experimental data obtained with NaI (1-1/2 in. x 1/2 in.) crystal.

** Range of radon retention values obtained by use of F function curves in presdwood phantom at depths bracketing one half of the thickness of the animal.

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GEOGRAPHIC DIFFERENCES IN BONE TUMOR DEATH RATES

H. Auerbach

Average bone tumor death rates, by sex and color, for the period 1949 - 1951 were calculated for the four regions of the United States. These regions are the Northeast, which includes the New England and Middle Atlantic states; the North Central, which includes the East North Central and West North Central states; the South, which includes the South Atlantic, the East South Central and the West South Central states; and the West, which includes the Mountain and Pacific states. Table 2 lists the 3 year average bone tumor death rates.

Table 2

AVERAGE TOTAL BONE TUMOR DEATH RATES, 1949 - 1951,
UNITED STATES. DEATH RATE PER 100,000 POPULATION

Region	Male			Female			All persons
	White	Non-white	Total	White	Non-white	Total	
Northeast	1.97	1.40	1.94	1.19	1.02	1.18	1.55
North Central	1.95	1.44	1.92	1.24	0.82	1.21	1.57
South	1.60	1.17	1.51	1.27	1.07	1.23	1.37
West	1.45	0.63	1.41	0.98	0.65	0.96	1.19
United States	1.79	1.20	1.73	1.20	1.00	1.18	1.45

Cancer is predominantly a disease of older persons and differences in total death rates between regions might be due to differences in age distributions of the populations in the regions rather than real differences in tumor death rates. The death rates for the various regions were therefore adjusted to a standard population age distribution (the United States 1950 census population) and the resulting age, sex, and color adjusted tumor death rates are shown in Table 3.

The adjusted death rates show that the three regions, Northeast, North Central and South, all have similar death rates with the possible exception of a slightly higher white female death rate in the South. The tumor death rates in the West are lower than those prevailing in the other three regions and the differences are significant at the 1% level. The tumor death rates of all groups in the West are apparently lower than comparable groups in the other regions of the United States. Possible reasons for such differences are being investigated.

Table 3

AVERAGE TOTAL BONE TUMOR DEATH RATES, 1949-1951,
UNITED STATES. AGE, SEX, AND COLOR ADJUSTED TO U.S.
1950 CENSUS POPULATION. DEATH RATE PER
100,000 POPULATION

Region	Male			Female			All persons
	White	Non-white	Total	White	Non-white	Total	
Northeast	1.89	1.47	1.84	1.12	0.99	1.11	1.46
North Central	1.84	1.43	1.79	1.20	0.78	1.16	1.47
South	1.80	1.21	1.74	1.41	1.07	1.37	1.55
West	1.46	0.67	1.40	1.00	0.71	0.97	1.18
United States	1.79	1.20	1.73	1.20	1.00	1.18	1.45

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RADIUM METABOLISM IN DOGS

T. W. Speckman and W. P. Norris

Dog 219 was sacrificed 1371 days, 3.76 years, after injection of 44 microcuries of RaCl_2 ($2.6 \mu\text{c}/\text{kg}$). The dog, a male mongrel, was an adult at the time of injection.

Shortly before sacrifice two osteogenic sarcomas appeared. The larger of the tumors, about 100 grams, was located on the left 5th rib (Figure 2). A tumor of about 38 grams was on the proximal end of the left humerus. Further histological and autoradiographic studies of the tumor areas are being conducted. The tumors in this dog are of special interest since they are the first to appear in the series of radium-injected dogs.

Radium retention at sacrifice was 24.5% of the injected dose. Plotting the data in the form of a power function gives the equation for retention at any time (t) as $R_t = 0.8t^{-.18}$ and an excretion equation of $E_t = 0.144t^{-1.18}$. These values agree well with dog radium retention data already published.

At time of sacrifice it was found by gamma measurements that 29% of the radon formed was being retained within the body.

Figure 2

X ray photograph of tumor on rib of dog 3.76 years after injection of radium ($2.6 \mu\text{c}/\text{kg}$).

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COMPARATIVE EFFECTIVENESS OF SEVERAL X-RAY QUALITIES FOR ACUTE LETHALITY IN MICE AND RABBITS

D. Grahn and G. A. Sacher

The relative biological effectiveness for acute lethality of X-rays ranging from 80 to 250 kvp has been investigated. Doses were delivered in eight equal portions distributed at 45° intervals around the antero-posterior body axis. A dose rate of 10 r per minute was held for all qualities. Fore- and back-scatter boards were used for mouse exposures to insure secondary equilibrium. Mortality values have been placed on an arc-sine scale, while doses, in roentgens, are the measured mid-animal air doses.

In the NL #1 rabbit, a high incidence of deaths from 0-3 days complicated the data. This, plus the erythema produced, made tests at 80 kvp erratic. For the rabbit, LD₅₀ doses are based on the mortality from 4-30 days. The data are given in Figure 3 and Table 4, columns 2-4. The results of the tests on the BAF₁ mouse are presented in Figure 4 and Table 4, columns 2-4.

Table 4

LEAST-SQUARES ESTIMATED LD₅₀ DOSES AND MORTALITY SLOPES FOR THE NL#1
RABBIT AND BAF₁ MOUSE. MEAN TISSUE DOSE DATA ADAPTED
FROM WALTON(1).

KVP	LD ₅₀ (air)	RBE (air)	$\hat{\beta}$	Mean tissue dose (% air dose)	Est. LD ₅₀ (tissue dose)	% change in est. LD ₅₀
NL#1 rabbit						
250	805 ± 45r	1	.136 ± .085	95%	765 r	-
100	1332 ± 10r	.604	.115 ± .008	77%	1026 r	+ 34%
80	2552 ± 1700r	.315	.025 ± .049	64%	1633 r	+113%
BAF ₁ mouse						
250	634 ± 11r	1	.244 ± .075	93%	590 r	-
135	663 ± 9r	.956	.453 ± .090	93%	617 r	+ 5%
80	816 ± 8r	.777	.408 ± .065	87%	710 r	+ 20%

Figure 3

Figure 4

We can examine several hypotheses about the action of these different radiation qualities.

- I. Effectiveness per ion pair constant for all radiation qualities used. This states that equal amounts of ionization produced by different qualities would produce equal effects. The consequences of this would be:
 - A. The LD_{50} expressed as mean tissue dose should be constant for all qualities.
 - B. Increases in the LD_{50} should be accompanied by proportionate decreases in the slope.

Consequence (A) is rejected, as may be seen by examining columns 6 and 7 of Table 4. Significant increases in the tissue dose LD_{50} occur in both species tested. The data are not highly inconsistent with (B), although, in the rabbit, the slope at 100 kvp is significantly greater ($P < .05$) than expected in this hypothesis.

This hypothesis was examined first, as it is an outcome of some current radiobiological concepts, and because no alternative hypothesis based on known principles can be offered at present. The data can, however, be compared with alternative empirical hypotheses.

- II. Constant slope hypothesis. This assumes that the survival curves for all qualities have equal slopes; i.e., are parallel. The constant slope is the appropriately weighted average slope of all qualities. LD_{50} doses are obtained from fitting the constant slope through the mean of each set of observed data. Superposition of data is accomplished by subtracting a constant dose equal to the linear distance between slopes of compared qualities.
- III. Combination of hypotheses I and II. This procedure takes into account non-uniformity of depth-dosage (Walton).⁽¹⁾ The slopes are re-estimated so that they decrease proportionately to the depth-dose RBE factors in accordance with the dose-reduction principle of hypothesis I. The RBE factors, Table 5, are derived from the data in column 5, Table 4. The expected air dose LD_{50} values are relative to these RBE factors.

Table 5

RBE FACTORS AND EXPECTED LD₅₀ DOSES BASED ON DEPTH-DOSE DATA

<u>Rabbit</u>			<u>Mouse</u>		
KVP	RBE	Exp. LD ₅₀ (air)	KVP	RBE	Exp. LD ₅₀ (air)
250	1	805 r	250	1	634 r
100	.811	993 r	135	1	634 r
80	.674	1195 r	80	.942	673 r

The least-squares slope of the quality to which the depth-dose RBE factors are relative is accepted as a base-line slope. The product of the RBE for a given quality and the base-line slope provides a dose-reduction slope. Fitting these slopes through the observed data provides the LD₅₀ estimates. The difference between the LD₅₀ doses so estimated and the LD₅₀ doses expected on the basis of the depth-dose data gives a subtractive dose component as in hypothesis II.

LD₅₀ doses and pertinent RBE factors are given in Table 6 for the NL #1 rabbit along with a comparison of the slopes. The constant slope employed here was obtained from the pooled 250 and 100 kvp data, since the 80 kvp slope had a standard error nearly twice the magnitude of the slope itself. Due to the lack of precision of the estimate, the bias that would be involved by using these data is probably greater than that from their omission. Subtractive doses are 520 r at 100 kvp and 1080 r at 80 kvp.

Under hypothesis III, the 250 kvp slope was used as a base-line. Equations for superposition to 250 kvp are 0.811 (AD-343) at 100 kvp and 0.674 (AD-749) at 80 kvp, where AD is the measured air dose.

No significant deviations from the least-squares slopes are indicated for either hypothesis; however, deviations are least under hypothesis III.

The least-squares estimate of the LD₅₀ dose at 80 kvp is not considered the best estimate, since the exceptionally high estimated value of 2552 r is heavily weighted by the absence of mortality in the 2000 r test series, although about half of the exposed rabbits died in the first two days. At doses below 2000 r, only about 15% died in the first few days, while all but one rabbit died either during or shortly after exposure to 2200 r, the

remaining animal dying on the 12th day. Since the data from 1450-1800 r compare well with the main body of the data at 100 and 250 kvp when the data are superimposed, the LD₅₀ estimates at 80 kvp in the range of 1800 r to 2100 r are considered more realistic.

Table 6

LD₅₀ DOSES AND SLOPES UNDER HYPOTHESES II AND III FOR RABBIT AND MOUSE

II						III				
KVP	LD ₅₀	RBE	$\hat{\beta}$	Dev.*	P	LD ₅₀	RBE	$\hat{\beta}$	Dev.*	P
Rabbit										
250	807r	1	.123	.013	NS	805r	1	.136	-	-
100	1327r	.608	.123	.008	NS	1336r	.603	.111	.004	NS
80	1887r	.428	.123	.098	NS	1944r	.414	.092	.067	NS
Mouse										
250	634r	1	.383	.139	NS	634r	1	.453 [†]	.209	<.05
135	661r	.959	.383	.070	NS	663r	.956	.453	-	
80	818r	.775	.383	.025	NS	816r	.777	.427	.019	NS

* Deviation from least-squares estimated slope - Table 4

[†] Assumed - see text.

Table 6 gives the LD₅₀ doses, RBE factors and slope comparisons for the mouse.

For the constant slope hypothesis, the subtractive doses for superposition to 250 kvp are 27 r and 184 r for 135 and 80 kvp. No significant deviations in slope are observed.

To apply the third hypothesis, the 135 kvp slope was assumed as the base-line slope and applied to the 250 kvp data. Although this involves a significant deviation from the least-squares estimate, it is not considered serious for several reasons. The 690 r point of the 250 kvp data lies well below the other six points, and, being the highest dose, it draws the slope down. The remaining six points fall in a line parallel to the 135 kvp data

and are consistent with the constant and dose-reduction slopes (see Figure 4). In addition, the depth dose RBE for these two qualities is unity. Equations for superposition to 250 kvp are (AD-29) at 135 kvp and 0.942 (AD-143) at 80 kvp.

The biological data observed, when compared with the depth-dose data of Walton,⁽¹⁾ indicate that changes in biological effectiveness over the range of qualities used do not conform to any pre-existing hypotheses. As indicated above, the dose-reduction principle does not hold, because a comparison of the LD₅₀ doses on an integral body dose basis shows that these values increase with the lower energies instead of holding constant. The data reviewed by Packard⁽²⁾ do not support the concept that a marked variation in biological effectiveness exists for X rays in the range employed here when applied to biological test objects that have insignificant absorption.

With the non-uniformity of dose distribution obtaining at lower energies there exists a small volume of tissue which is partially shielded. If the partially shielded volume includes hematopoietic tissues, or tissues of this potentiality, a condition exists similar to that present in spleen- and marrow-shielding experiments. Bone marrow is particularly favored, since the 80 kvp radiations would be heavily absorbed by the dense bone, while 250 kvp X rays could increase the dose to marrow due to the transitory build-up of secondary radiation emitted from the bone.

Evidence suggestive of spleen or marrow shielding is given by analysis of the mouse survival data. In groups of comparable survival, the distribution of deaths at 80 kvp shows a deviation from the 250 and 135 kvp distribution. The death rate is higher from 1-10 days and lower from 21-30 days in the 80 kvp group. The accompanying mean survival times are $13.58 \pm .39$ days at 80 kvp and $14.43 \pm .30$ days at 250 and 135 kvp. These data are consistent with the more rapid recovery seen in spleen-shielded animals beyond the 15th day.

A more adequate knowledge of the dosage-mortality slope in spleen-shielded animals would permit a more critical review of the problem. If this slope were approximately parallel to that of the unshielded controls, a hypothesis similar to hypotheses II or III would thereby gain experimental validation.

In summary, significant increases in LD₅₀ doses for mice and rabbits have been demonstrated to accompany decreases in X ray peak energy. The increases are much greater than was expected on the basis of depth dose data and are inconsistent with the basic dose-reduction principle. Two hypotheses, a constant slope hypothesis and a combined dose-reduction: constant slope hypothesis have been applied to the data. It is suggested that a similarity with spleen- or marrow-shielded animals exists that may account in part for the changes in LD₅₀ doses beyond that accountable by non-uniform depth dosage with the lower energy radiations.

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THE EFFECT OF X RAYS AND TRITIUM OXIDE ON THE GROWTH OF
TYPHUS RICKETTSIAE IN THE EMBRYONATE EGG

Donald Greiff,* Henry Pinkerton* and E. L. Powers

Fertile chicken eggs, after incubation for 5 days at 37.5° C, were inoculated with Rickettsia mooseri. Two days later, groups of 30 eggs were injected with varying amounts of tritium oxide (22.5, 45, 90, and 180 millicuries per egg). Other groups were X-irradiated with total dosages in the ranges of those delivered by the tritium in single and divided (5 day) dosages. Most of the untreated embryos (no irradiation or tritium) died between the 5th and 8th day after inoculation of rickettsiae. Giemsa-stained smears of the yolk sac membrane showed 100 to 5000 organisms per microscope field. Treated eggs died at least 24 hours earlier and with much heavier infections (>5,000 organisms per field) than untreated controls. The degree of infection is correlated with dosage of tritium, the greater the dose the higher the degree of infection. All X-ray groups appear to exhibit higher rates of growth of rickettsiae than do the tritium groups.

* Medical School, St. Louis University, St. Louis, Mo.

BIOCHEMICAL CHANGES IN RATS AND BATS EXPOSED TO MASSIVE DOSES OF X-IRRADIATION

J. F. Thomson, E. M. Moss, and D. E. Smith

When animals are exposed to X-radiation at a rate of 500 r/min., death occurs after accumulation of about 100,000 r, regardless of the species of animals or, in the case of poikilotherms (bat, frog), the temperature at which the animals were exposed.⁽¹⁾

We have initiated studies of various enzyme systems in massively irradiated animals to see whether evidence could be obtained for the inactivation by radiation of a biochemical mechanism to a significant extent and to a similar degree in various species.

In studies on the tissues of 4 rats which had been exposed to 90,000 r in 3 hours and sacrificed 15 minutes after termination of exposure, we failed to find significant reduction of succinic dehydrogenase or cytochrome oxidase in the heart, kidney, liver, or brain. The ATP-ase activity of the liver, heart, and spleen was unaltered. The levels of liver tryptophan peroxidase were approximately doubled, in comparison with normal rats; however, in comparison with the appropriate controls, which were immobilized for three hours, the increase amounted to only 25 per cent. Thus one may conclude that there is not an indiscriminate denaturation of protein or inactivation of enzymes under these conditions of radiation. The fresh weights of both spleen and thymus were decreased by about 30 per cent, and the concentration of thymus DNA was 83 per cent of the control level, approximately the same reduction as observed 3 hours after an LD₅₀-30 days dose.⁽²⁾

Acting on the assumption that CNS damage is a probable cause of death in these animals, we have carried out additional studies of the effect of radiation on the glycolytic capacities of whole brain homogenates of six Sprague-Dawley male rats and four bats (*Myotis lucifugus*), along with an equal number of controls. Half of the bats were kept at about 5°C., while the others were treated at room temperature. All assays were carried out at 38°C. by the method of LePage.⁽³⁾ The results are shown in Table 7.

In rats, although there was not a significant reduction in CO₂ production or lactic acid formation, there was an appreciably diminished esterification of inorganic phosphate. This decrease was seen both aerobically and anaerobically. It may be recalled that this assay is carried out in the presence of fluoride, so that relatively little lactic acid is formed from glucose or hexose diphosphate; most of the lactate results from the reduction of added pyruvate by reduced diphosphopyridine nucleotide formed in the dehydrogenation of phosphoglyceraldehyde. Thus the phosphate uptake; lactate formation ratio is a good index of the overall

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Table 7

GLYCOLYTIC CAPACITIES OF HOMOGENATES OF WHOLE BRAINS OF
BATS AND RATS EXPOSED TO 90,000 r

	Anaerobic		Aerobic	
	Control	Exposed	Control	Exposed
CO ₂ output ($\mu\text{M}/30'$ /mg. N)				
Rats (Av. of 6 with ranges)	15.3 (13.7-15.9)	14.1 (13.2-16.1)	13.2 (11.5-15.1)	12.4 (11.0-14.3)
Bats, X-rayed at 5°C.	18.4, 16.8	17.3, 17.3	15.6, 15.6	15.2, 15.0
Bats, X-rayed at 24°C.	16.6, 15.0	18.1, 17.8	15.9, 12.2	15.2, -
Phosphate esterification ($\mu\text{M}/30'$ /mg.N)				
Rats	7.80 (7.32-8.10)	6.46 (4.99-7.54)	8.28 (7.95-8.76)	6.08 (5.78-6.28)
Bats, X-rayed at 5°C.	11.1, 11.2	11.9, 12.4	10.4, 11.9	10.9, 11.7
Bats, X-rayed at 24°C.	14.1, 11.7	14.0, 14.9	14.3, 12.7	12.8, 13.7
Lactic acid production ($\mu\text{M}/30'$ /mg. N)				
Rats	7.53 (6.69-8.26)	7.14 (5.42-7.84)	7.58 (6.92-8.28)	7.08 (6.64-7.42)
Bats, X-rayed at 5°C.	11.0, 11.7	11.3, 12.7	10.2, 11.1	11.1, 12.5
Bats, X-rayed at 24°C.	13.2, 11.5	13.7, 13.5	12.4, 10.5	12.4, 12.7
Ratio, P uptake to lactic production				
Rats	1.10 (0.98-1.18)	0.91 (0.81-0.99)	1.03 (0.95-1.08)	0.84 (0.82-0.86)
Bats, X-rayed at 5°C.	1.01, 0.96	1.06, 0.97	1.01, 1.08	0.99, 0.94
Bats, X-rayed at 24°C.	1.07, 1.02	1.01, 1.10	1.16, 1.20	1.04, 1.08

efficiency of the phosphoglyceraldehyde dehydrogenase system in esterifying inorganic phosphate. Studies on brain ATP-ase activity indicated that 90,000 r produced about 20 per cent inhibition, so that the decrease in phosphate esterification cannot be attributed to an increased breakdown of ATP. We also measured glucose disappearance, and observed some diminution after irradiation; however, the variation among individuals was much higher than that encountered with the other assays, and there was no apparent correlation between impaired glucose utilization and decreased phosphate esterification.

In bats, however, there was no indication of such an effect. Thus it seems likely that death from massive amounts of radiation is not attributable to effects on glycolysis. It is of considerable interest to note that the bat brain has about 50 per cent greater capacity for phosphate esterification than rat brain. The ATP-ase activity of bat brain we found to be 25 per cent lower than that of rat brain; radiation did not alter the level of bat brain ATP-ase.

Additional studies on the effects of radiation on the metabolism of rat and bat brain, with particular reference to oxidative phosphorylation, are contemplated.

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EFFECT OF WHOLE BODY X-IRRADIATION ON THE PHOSPHOPROTEIN PHOSPHATASE OF RAT TISSUES

R. N. Feinstein

Phosphoprotein phosphatase is an enzyme, widely distributed in mammalian tissues,⁽¹⁾ which liberates inorganic phosphate from phosphoproteins without preceding proteolysis. Because of the almost unique occurrence of phosphoproteins as food for the embryo or young organism (e.g., casein in milk, vitellin in egg yolk, ichthulin in fish eggs), this enzyme is considered of interest in all connections where the age of the organism is considered of some significance. Such a circumstance is that of the effects of ionizing radiation.

Six male Sprague-Dawley rats, about 14 weeks old, were irradiated with 750 r of X-rays to the whole body. They were then maintained for 48 hours on water but no food; at the end of this time they were sacrificed, and the testes, kidneys, spleen, small intestine, and a portion of liver were frozen in a dry ice-acetone bath and stored under deep-freeze until assay. Six other rats of the same shipment were similarly treated, but without being X-rayed.

Tissues were thawed, homogenized, and assayed for phosphoprotein phosphatase by a method described earlier,⁽¹⁾ and for protein by the method of Robinson and Hogden,⁽²⁾ as modified by Keyser and Vaughn.⁽³⁾ The results are presented in Table 8.

Table 8

EFFECT OF 750 r WHOLE BODY X-IRRADIATION ON THE PHOSPHOPROTEIN PHOSPHATASE OF RAT TISSUES

Tissue	Treatment of rats	Weight of Tissue (gm)	Mg. Protein per gm tissue	Enzyme Activity		
				Per mg. tissue	Per mg. protein	Per whole tissue
Spleen	Control	0.72 ± 0.07	165 ± 18	1.70 ± 0.12	10.3 ± 0.7	1219 ± 159
	X-Rayed	0.31 ± 0.03	167 ± 8	3.15 ± 0.24	18.9 ± 1.1	959 ± 15
Intestine	Control	5.6 ± 0.7	89 ± 5	0.92 ± 0.12	10.4 ± 1.4	5083 ± 758
	X-Rayed	4.7 ± 0.3	47 ± 9	0.72 ± 0.13	15.4 ± 1.7	3403 ± 635
Testis	Control	2.9 ± 0.1	87 ± 8	0.52 ± 0.11	6.0 ± 0.9	1481 ± 273
	X-Rayed	2.7 ± 0.2	85 ± 8	0.53 ± 0.13	6.3 ± 1.4	1384 ± 295
Liver	Control	-	287 ± 17	0.41 ± 0.11	1.5 ± 0.4	-
	X-Rayed	-	265 ± 22	0.43 ± 0.10	1.6 ± 0.5	-
Kidney	Control	2.0 ± 0.2	175 ± 13	0.36 ± 0.04	2.2 ± 0.3	757 ± 112
	X-Rayed	2.1 ± 0.1	160 ± 5	0.24 ± 0.08	1.4 ± 0.4	449 ± 271

Data are expressed as mean and average deviation from the mean. Six X-rayed and six control rats were used, except in the case of kidney, where two samples were lost from each group.

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It must be pointed out that in Table 8 the values for the enzymatic activity of liver and kidney are not strictly comparable to those for the other tissues. This is due to the use of a fresh lot of substrate (casein) for the liver and kidney, and the unexpected finding that the activity of the enzyme was less with the newly prepared casein than with the older preparation. Values are made approximately comparable by multiplying the liver and kidney enzyme activity values by 1.5.

The table indicates that in no respect measured are the testes or liver affected by the X-radiation. The kidney is affected in that the enzyme activity, on all bases, is reduced. However, the high degree of deviation and the smaller number of tissues assayed cast doubt on the significance of this effect. In the case of spleen and intestine, more significant effects are noted. Both of these tissues, of course, lose weight after whole body X-irradiation, and in each case the total phosphoprotein phosphatase activity of the whole organ is reduced. However, in the case of spleen the activity per unit weight increases greatly (85%), while the protein content per unit weight is essentially unaffected; the net result is thus a considerable increase in enzyme activity per milligram of protein.

In the case of intestine, although the enzyme activity per unit weight is decreased (22%), the protein content per unit weight is so much more greatly reduced (47%) that the enzyme activity per milligram of protein is appreciably increased (48%).

The interpretation of these results is at present uncertain. It might be considered that this enzyme is one of many proteins which are lost from the spleen and intestine upon irradiation, and that it happens merely to be less radiation-labile than some others. Or it may be considered that after whole body X-irradiation, spleen and intestine slough off whole cells of perhaps constant enzyme-protein ratio, but that a radiation-labile inhibitor of phosphoprotein phosphatase is also destroyed, so that the net result is an apparent increase in enzyme activity per milligram of protein. Further experiments are required to clarify the interpretation.

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THE FATE OF ASCORBIC ACID IN EARLY RADIATION DAMAGE

P. D. Klein, D. T. Handa and R. W. Swick

There is a paucity of information regarding the role of ascorbic acid in radiation damage. In light of its reported involvement in the conversion of folic acid to citrovorum factor and, thereby, its implication in nucleic acid synthesis, its sensitivity to irradiation and subsequent fate are of interest. Accordingly, the changes in concentration of ascorbic acid as well as total content of a variety of organs of rats after X-irradiation have been studied. Within 72 hours after a dose of 600 r, there were no gross changes in tissue concentration or organ content of ascorbic acid in brain, muscle, pancreas, brown adipose tissue, testis or liver. Within 24-28 hours there was a loss of 70, 75 and 90% of total ascorbic acid in lymph nodes, spleen, and thymus. The intestinal mucosa increased in concentration by 70%. Weight changes in the four tissues suggest that the loss or gain of ascorbic acid may be related to a particular population of cells. This is borne out by a study of the rate of change in concentration which shows an initially high rate of disappearance of ascorbic acid in relation to the weight changes, reaching a maximum at 4 hours and slowing abruptly thereafter. Studies of lymph nodes, spleen, thymus, mucosa, liver and testis at lower dosages revealed similar phenomena. At 300 r the response in mucosa was reduced, while the lymph nodes, spleen and thymus were depleted to almost the same extent as at 600 r. The influence of chloretone and aminopterin on the response of these tissues is being investigated.

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Ascorbic acid concentration (□), ~~organ~~ ^{organ} weight (○), and total ascorbic acid per organ (Δ) after 600r of x-irradiation expressed as a percent of control.

Figure 5

Early changes in tissue ascorbic acid of rats after X irradiation.

THE EFFECT OF X IRRADIATION ON ASCARIS EGGS AND THE EFFECT OF COLD TREATMENT AFTER IRRADIATION

T. N. Tahmisian, R. L. Devine and B. J. Wright

Cook⁽¹⁾ has reported that Ascaris eggs given 5000 r X rays and then allowed to develop at room temperature undergo cell division but do not form an embryo. On the other hand, if similarly-treated material, immediately after irradiation, is maintained at a temperature of 0°-5°C for a period of 2 weeks to 6 months, and then allowed to develop at room temperature, there is gradual recovery from irradiation damage. Recently, Bachofer and Pahl⁽²⁾ have shown that in Ascaris lumbricoides suum this phenomenon does not occur. We have tried, in several repeated experiments using Ascaris megalocephala, to reproduce the experiments of Cook. In no case have we found any recovery after irradiation with the same doses, although some of the eggs were in cold-storage for as long as 8 months. One thing that is evident is that 5000 r destroys the capacity of the Ascaris egg to embryonate; the fact that no worms are formed is an indication that 5000 r is a sufficient dose to interfere with tissue differentiation. Although the original fertilized egg, after treatment, undergoes several cleavages, the cells remain round. Evidently then, the primary effect of irradiation at 5000 r is to halt cell differentiation. This is in complete agreement with the results of Bachofer's experiments. It has been established from our work on the grasshopper⁽³⁾ that 2500 r in air will abolish the capacity of fertilized grasshopper eggs to form tissues. We are employing various radiation doses between 2500 r and 5000 r to determine the critical dose at which embryonation will not occur in the Ascaris eggs. At present we are not convinced that any recovery from irradiation damage at 5000 r can be found. This is in agreement with the data reported in similar experiments with Melanoplus differentialis where eggs, irradiated at a given dose level and then either left to develop at room temperature, or placed in cold storage for six months and then transferred to room temperature, showed identical irradiation effects with no evidence of recovery. The onset of the injury was kept latent for six months in the latter, but the patterns of development of the two sets of experiments produced the same phenomena. This seems to be the case, also, for Ascaris megalocephala and Ascaris lumbricoides.

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DNA SYNTHESIS DURING RADIATION-INDUCED DIVISION BLOCK
IN TETRAHYMENA

H. S. Ducoff

The Zeuthen procedure⁽¹⁾ for inducing synchronous division in Tetrahymena has been modified to permit treatment of liter volumes of culture. This is accomplished by combining culture vessel and thermostat bath functions in a single two liter round-bottom flask equipped¹ with air outlet, with two glass fingers for insertion of thermoregulators, and another glass finger for an immersion heater. One thermoregulator is set for 28°C and one for 33°C, and they are operated² by a cam-driven switch so that one or the other thermoregulator governs the circuit in alternate 30 minute periods. The apparatus is set up in a cold room to secure sufficiently rapid cooling to 28° at the end of each period at 33°. After eight to twelve hours of this cycling, the cells are slightly increased in number, greatly increased in size⁽¹⁾ and in DNA content,⁽²⁾ and, upon return to optimum or room temperature, divide synchronously for the next three divisions. Interdivision times for these synchronous divisions are somewhat shorter than the generation time in exponentially growing populations. It appeared that temperature-cycled Tetrahymena cultures might be ideal material for examining the interrelationship between radiation-induced division block and inhibition of DNA synthesis.

T. pyriformis strain W were grown at 25° in a medium consisting of two per cent proteose peptone plus 0.05 per cent liver fraction "L." The culture, at a population of about 40×10^3 /cc, was then transferred to the temperature cycling apparatus. After twelve hours, the cultures were returned to 25° and exposed to 60,000 r:

- a) immediately, so that exposure was completed before start of the first synchronous division;
- b) between the first and second synchronous divisions; or
- c) so that the unirradiated animals underwent the first synchronous division during the exposure.

Another group, d, maintained at 25°, was also irradiated as a randomly dividing control. Division in groups a and b was blocked as immediately and effectively as in d. In group c, which began to divide before the end of the exposure period, the first division was completed, but more slowly than in the corresponding unirradiated group; at the end of the exposure, therefore, most of the irradiated animals were in late division stages, and increase in numbers was slight, whereas in the unirradiated group, population had already doubled, and no dividers were seen. The subsequent delay in this irradiated group was, however, of the same duration as in the others, or about one control generation time.

¹Flask constructed by Mr. T. Doody.

²Wiring circuit designed and constructed by Mr. W. Eisler.

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Finally, samples of groups a and d were taken for DNA estimation at the time of irradiation and again five hours later. The animals were centrifuged in the cold and resuspended in distilled water, an aliquot removed for population count, and the remainder subjected to a modified Schmidt-Thannhauser procedure. After precipitation of the DNA fraction from the KOH hydrolysate, the precipitate was washed with cold perchloric acid (PCA) and then extracted with 0.5 N PCA for 20 minutes at 80°C. The residue was washed once with cold PCA; this washing and the extract were combined, and optical density was measured at 260 m μ . The results, with relative DNA/animal expressed as $D_{260}/10^6$ animals, are shown in the Table.

Table 9

DNA IN TETRAHYMENA SUBJECTED TO TEMPERATURE CYCLING
AND X-IRRADIATION

(1)	(2)	(3)	(4)	(5)
Culture	Sample	Number of cells processed ($\times 10^{-6}$)	D_{260}	Relative DNA/cell (4)/(3)
"Random"	Before irradiation	.85	.018	21
	Unirradiated, 5 hrs.			
	later	1.69	.043	25
	Irradiated, 5 hrs.			
	later	.84	.033	39
Cycled	Before irradiation	1.52	.175	115
	Unirradiated, 5 hrs.			
	later	2.71	.233	86
	Irradiated, 5 hrs.			
	later	1.55	.246	159

The increased DNA/animal in the temperature-cycled culture as compared with the randomly dividing culture, and the decline to about 75 per cent of this value after the first synchronous division, confirm the report of Zeuthen and Scherbaum.⁽²⁾ The increased DNA/animal after irradiation of the random culture was somewhat surprising, and might have been explained in part as experimental error, which is proportionately great at these low levels, and in part by the fact that at the time of sampling the irradiated animals had almost recovered and were about to resume division. These explanations are completely inadequate for the findings with the synchronized cultures, however. The DNA levels are high enough for the estimate to be reliable, and the five-hour values for the irradiated and unirradiated portions are in very good agreement, allowing for the fact

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that the unirradiated animals have doubled in number. The conclusion is inescapable, then, that in this amiconucleate, amitotic strain of T. pyriformis, X-irradiation results in a division block without any discernible effect on DNA synthesis.

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CONTROL APPARATUS FOR LOW- AND HIGH-LEVEL GAMMA ROOMS

S. S. Brar and W. J. Eisler

The independent background monitoring system (see ANL-5378, p. 6) built previously has been tested and was found to be quite satisfactory for intended use in the high-level gamma room. For the low-level gamma room a duplicate setup is contemplated if another 60 cycle vibrating reed electrometer can be found. Actually, it is planned to build another electrometer since almost all the parts are available for the job.

TOXICITY OF RADIOSTRONTIUM IN DOGS: CURRENT STATUS OF THE LONG-TERM EXPERIMENTS

M. P. Finkel, J. Lestina, G. M. Scribner, H. Lisco,
R. J. Flynn, and A. M. Brues

The dogs of the present radiostrontium toxicity experiments, and some pertinent information about them, are listed in Table 10. All but one of the animals that received strontium-89 (#163-4) are survivors of the acute and subacute studies of Swift and Prosser.⁽¹⁾ Three of these dogs have died with malignant tumors since they were last reported,⁽²⁾ and a lesion that has been diagnosed tentatively as an odontoma has developed on an upper pre-molar of one of the living animals. Although it is highly probable that the osteogenic sarcoma of the scapula in dog #77 and the osteolytic tumor of the temporal bone in dog #93, as well as the previously noted osteolytic tumor of the mandible of dog #163-4, were induced by the skeletally-deposited radiostrontium, there is some question that the adenocarcinoma in dog #107 was similarly induced. However, the tumor was buried within the deep tissues of the throat, where local irradiation from the isotope deposited in the cartilage of the larynx and trachea might have been of sufficient magnitude to initiate the neoplastic process. No additional skeletal changes, other than the above mentioned tumors, were detected radiographically during the past 15 months.

Five dogs have been added to the strontium-90 experiment at the 0.15 mc/kg level. This dose was selected with the hope that it would be small enough to permit survival beyond the acute and subacute stages but large enough to produce recognizable skeletal damage within a few years. Adult dogs tolerate the initial hematological injury produced by radiostrontium very poorly; consequently the range between acute and chronic doses is small. Although Table 10 shows only one death among the seven dogs that received 0.15 mc/kg, two animals were carried through an acute illness by prompt medication. No attempt was made to treat dog #189, and it died on the fourth day of its illness. Dog #194 displayed symptoms of acute radiation disease on the 24th day after receiving the isotope, and its temperature rose to 105.6°F. Treatment consisted of the daily injection of 600,000 units of penicillin and 100 ml of Duo-Serum® (a combination of serum immune to canine distemper, infectious canine hepatitis, and bronchisepticus and bacterins for streptococcus and typhimurium) for four days, after which time the temperature had returned to normal (101.5°F). Dog #191 developed a severe unilateral conjunctivitis and acute irradiation symptoms with a temperature of 103.5°F. on the 30th day. The daily local application of polymyxin ointment and injection of 600,000 units of penicillin was continued for six days. At present these two dogs are indistinguishable from those that did not display symptoms of severe disease. In view of the three acute illnesses, one of which terminated in death, among the seven dogs given this dose of strontium-90, it appears that 0.15 mc/kg is close to the 30-day LD₅₀.

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Table 10

CURRENT STATUS OF RADIOSTRONTIUM DOG EXPERIMENTS

#	Dose (mc/kg)	Injection		Interval to:		Major present or terminal pathology
		Age	Route	Death	Present (alive)	
Strontium-89						
107	1.0	adult	I.M.	8.3 yr.	--	Adenocarcinoma, cervical region.
77	0.63	adult	I.M.	8.5 yr.	--	Osteogenic sarcoma, scapula.
163-4	0.52	12 days	Subc.	2.3 yr.	--	Osteolytic tumor, mandible; severe skeletal abnormalities.
93	0.46	14 days	I.M.	8.5 yr.	--	Osteolytic tumor, temporal; severe skeletal abnormalities.
85	0.30	adult	I.M.	--	9.5 yr.	Slight changes, mandible; ?odontoma.
122	0.30	adult	I.M.	--	9.5 yr.	Slight changes, mandible.
87	0.30	adult	I.M.	--	9.5 yr.	Changes, mandible.
135	0.1	6 mo.	I.M.	--	9 yr.	Slight localized change, mandible.
146	0.1	adult	I.M.	5.8 yr.	--	Skeleton normal; cause of death undiag.
142	0.1	adult	I.M.	--	9 yr.	Slight change, mandible.
147	0.1	adult	I.M.	--	9 yr.	Slight localized change, mandible.
Strontium-90						
183	0.25	5 yr.	I.V.	18 days	--	Acute radiation disease.
189	0.15	6.5 yr.	I.V.	28 days	--	Acute radiation disease.
185	0.15	5.3 yr.	I.V.	--	1.6 yr.	Leucopenia.
184	0.15	6.5 yr.	I.V.	--	5 mo.	Slight leucopenia.
190	0.15	6.5 yr.	I.V.	--	5 mo.	Leucopenia.
191	0.15	6.5 yr.	I.V.	--	5 mo.	Leucopenia.
193	0.15	6.5 yr.	I.V.	--	5 mo.	Leucopenia.
194	0.15	6.5 yr.	I.V.	--	5 mo.	Leucopenia.
180	0.01	5 mo.	I.M.	--	8 yr.	None
181	0.01	5 mo.	I.M.	--	8 yr.	None
182	0.01	5 mo.	I.M.	--	8 yr.	None
Controls						
163-3	--	12 days	--	2.3 yr.	--	None; sacrificed as control for #163-4.
68	--	7 mo.	--	--	9.5 yr.	None
73	--	7 mo.	--	--	9.5 yr.	None
192	--	6.5 yr.	--	--	5 mo.	None
195	--	6.5 yr.	--	--	5 mo.	None

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The hematological findings after the administration of strontium-90 are summarized in Table 11. Although the sedimentation rate, hematocrit, hemoglobin, and erythrocyte values had returned to control levels 3-5 months after the injection of 0.15 mc/kg, the total number of leucocytes remained depressed. Nor was there any indication of recovery in the number of circulating white cells in the animal that was injected 1.6 years ago. However, the differential count shows that this continued depression does not apply to the lymphocytes. Except for the hematological symptoms of subacute radiation injury, these dogs are in apparent good health and should survive until long-term toxic effects become manifest.

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Table 11

HEMATOLOGICAL CHANGES AFTER STRONTIUM-90 ADMINISTRATION

Dose (mc/kg)	Interval	Number of observ.	Sed. rate (mm/hr.)		Hematocrit (per cent)		Hemoglobin (gm/100 ml)		Erythrocytes ($\times 10^6/\text{mm}^3$)		Leucocytes ($\times 10^3/\text{mm}^3$)		Average number of: ($\times 10^3/\text{mm}^3$)			
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Heter.	Lymph.	Mono.	Eosin.
Controls		3	0 13	4.3	36 55	48.7	11.5 18.4	15.8	4.86 7.27	6.34	6.40 7.05	6.65	4.10	1.84	0.35	0.33
0.25	15 days	1	54	54	37	37	10.6	10.6	4.61	4.61	0.60	0.60	0.04	0.53	0.02	0.00
0.15	1-2 mo.	10	30 70	44.3	24 49	39.3	8.2 14.3	12.0	3.29 5.70	4.88	2.05 5.60	3.49	1.98	1.40	0.07	0.02
0.15	3-5 mo.	6	0 4	1.5	50 55	52.2	15.2 16.8	16.0	5.98 6.97	6.56	2.25 5.25	3.49	1.75	1.60	0.06	0.06
0.15	1.6 yr.	1	1	1	55	55	17.5	17.5	7.47	7.47	3.65	3.65	1.57	1.90	0.07	0.04

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THE MUTAGENIC EFFECTS OF C^{14} ON THE SNAPDRAGON

N. D. Williams and N. J. Scully

An investigation is currently in progress which is designed to study the mutagenic effects of C^{14} incorporated into the carbon-containing compounds of snapdragon plants (*Antirrhinum majus*). Seedling plants were placed in the Argonne nutriceulture chambers and are being grown to maturity in atmospheres containing carbon dioxide of regulated concentrations held near the normal range and enriched with $C^{14}O_2$ to attain specific activities of 4, 25 and 150 $\mu c/g$ carbon. These three levels of C^{14} activity will result in maximum calculated doses of 0.5, 3.0 and 18.0 rep per day to the plant tissues.

Phenotypic changes in flower color will be the principal criterion for measurement of mutagenic action. Flower color in the snapdragon is governed by five non-allelic genes. Plants of the following two genotypes are being subjected to C^{14} treatment: 1) plants homozygous dominant with respect to the flower color genes, and 2) plants heterozygous with respect to the flower color genes. Pollen will be collected from plants of the homozygous dominant genotype and placed on the stigmas of emasculated, untreated plants homozygous recessive with respect to the flower color genes. Recessive mutations which are transmitted through the pollen will be detected in the F_1 generation of these crosses. The use of plants heterozygous with respect to the flower color genes will make possible the detection of recessive somatic mutations as chimeras, or mutant areas, in the flowers of the irradiated generation.

SHIELDING PROBLEMS AT CP-5

D. L. Jordan, J. W. Clark, and H. H. Vogel, Jr.

As it is planned to operate CP-5, Argonne's new heavy water research reactor, continuously at 1000KW, the neutron toxicity group was faced with a twofold task: The primary problem of obtaining a dose range of fission neutrons from the fixed flux resulting from this power level was solved by the construction of a diaphragm-like shutter⁽¹⁾ by which the thermal neutrons available for uranium conversion could be varied. The remaining problem of adapting the animal exposure facility⁽²⁾ was investigated during this past quarter.

Several structural changes were made to the radiation chamber to conform to the new reactor and the pile room itself. Measurements of background radiation at maximum dose were then obtained. A working area for group personnel was located at the maximum operable distance (20 ft) from the reactor. Background of slow neutrons and gamma radiation in this area was found to be within tolerance. However, the fast neutron level here was found to exceed the tolerance dose ($40 \text{ nf/cm}^2/\text{sec}$) by a factor of 60. Measurements at various other positions that personnel would occupy during operation were correspondingly higher. With all shutters closed so that zero dose was recorded at the animal position within the chamber, the flux of fast neutrons at the animal loading zone outside the chamber was 11 times tolerance.

A comprehensive survey involving nine different measurements at each of seven typical operating conditions was carried out. Analysis showed the background to be composed of a minor transmission component of fast neutrons passing through the foot-thick shield of the radiation chamber wall. A major component resulted from the unavoidable crack at which the chamber abuts the pile face.

The transmission component was dealt with by the construction of a 30-gallon 2-S aluminum water tank which fills the interior of the chamber. The eighteen Co^{60} source rods⁽²⁾ for gamma radiation pass through tubes built into the tank itself. This tank will be filled during neutron irradiation and may be emptied for pure γ -ray exposures.

The remaining component was then analyzed by a scheme utilizing a series of eight standardized and six intermediate positions for measurement at both maximum dose level (100% shutter opening) and an arbitrarily selected lower dose level (10% shutter opening). These positions were selected for consideration of exposure to personnel operating the chamber, neighboring personnel, and overall background for the instrumentation of neighboring apparatus. Each of the above series of measurements was carried out for fourteen separate conditions utilizing 200 concrete building

blocks, twenty 500-pound steel and masonite shielding blocks, and a total of 200 pounds of paraffin slabs. Each condition demanded the use of these materials both in combination or alone to form walls or to cover cracks. Six separate seven-foot walls were built in order to test both efficiency of design and comparative effectiveness of materials.

Analysis of data showed the best shielding arrangement reduced background from 60 to 2 times tolerance. At neighboring apparatus a reduction from a factor of 40 to tolerance was attained. Overall room reduction was well within the requirements for instrumentation.

Since voids and cracks occur while stacking blocks in the building of irregular walls from loose materials, it is expected that the solid nature, interlocking features, and the increased thickness of the projected design will reduce the above shielded values by at least a factor of 3.

Consultation with planning engineers resulted in the choice of concrete as the preferred shielding material and in the design of concrete walls in steel forms for the sides and front of the chamber. Construction of walls 1-1/2 ft thick and 6 ft 8 in. in height is now under way. Required shielding at the top of the chamber adjoining the reactor face will be a 1 ft slab of pressed masonite.

Although the total mass of shielding amounts to 18 tons, it is semi-permanent in nature and can be removed in sections. Most of it will be left in place for use by other investigators at the thermal column.

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MODIFIED LIVER FUNCTION TEST FOR MICE

A. J. Finkel and D. Czajka

Previously described methods for determining liver function in mice by bromsulphalein (BSP) retention in the blood have been based on a three-compartment system involving intraperitoneal injection, uptake of dye by the blood, and removal of the dye from blood by the liver. By use of intravenous injection of the dye the procedure outlined by Frommer⁽¹⁾ has been modified to a two-compartment system in a manner similar to that used in human clinical tests.

Bromsulphalein, 10 mg/ml, is injected into a lateral tail vein of a mouse at a dose of 0.1 mg per gram body weight. The mouse is bled at five-minute intervals from the contralateral tail vein and 0.025 or 0.050 ml amounts are drawn into micropipettes and added to 0.05 ml of 2.85% potassium oxalate solution. After centrifugation for 10 minutes, an 0.025 ml aliquot of this diluted plasma is added to each of two tubes containing 0.1 ml physiological saline solution. To one tube 0.025 ml of 10% NaOH is added to bring out the color of the dye; to the other a similar amount of 10% HCl is added to provide a blank. These solutions are transferred to semi-micro cuvettes and readings are made at 580 m μ in a Coleman Jr. spectrophotometer. The values are expressed in mgm per 100 ml plasma after conversion by use of a standard curve.

A comparable procedure has been developed for use with guinea pigs, although here the external jugular veins are exposed by a transverse anterior neck incision after the animal has been anesthetized with sodium pentobarbital. The injection solution contains 50 mg BSP/ml and is injected at a dose of 25 mg/kgm, and larger blood samples are withdrawn and processed.

The figure shows the results obtained with mice, each of which was given a 0.5 ml subcutaneous interscapular injection of 90% CCl₄ in mineral oil two days before the liver function tests were performed. The solid line connects the mean values while the dotted lines delimit the distance given by twice the standard error of the mean in each direction. The difference between mice with normal and diseased livers is readily apparent from this figure.

This procedure is being used to establish the pharmacologically safe level of zirconium administration to experimental animals.

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Figure 6

Bromsulphalein (BSP) test in mice

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STUDY OF THE PHOTOPERIODIC STIMULUS IN XANTHIUM

R. Watanabe

As a sequel to the earlier reports,^(1,2) the radioautographs of aqueous extracts of terminal buds of Xanthium plants are presented. In the upper row are films of the vegetative (non-induced) buds; those in the lower row are from induced plants. The ninhydrin-positive spots are circled. The paper chromatograms were run by the procedure described by Benson, et al.⁽³⁾ Examination of the radioautographs shows that the lower figures have two spots (at arrows) not clearly visible in the upper figures. This presents two questions: 1) whether the two spots really reflect differences between vegetative and induced tissues and 2) whether the spots possess the desired biological properties, i.e., whether they play a positive role in photoperiod. Further experiments are underway to answer these questions.

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Figure 7

Radioautographs of chromatograms from aqueous extracts of C^{14} -labelled Xanthium buds. First solvent, water-saturated phenol; second solvent, n-butanol: propionic acid.

VDB = Vegetative donor bud; VRB = vegetative receptor bud;
IDB = induced donor bud; IRB = induced receptor bud.

PRELIMINARY INVESTIGATIONS ON THE ROLE OF LIGHT QUALITY IN
THE WINTER FLOWERING RESPONSE OF XANTHIUM

J. Skok and N. J. Scully

Xanthium, a short-day plant, will normally remain in a vegetative condition when grown under long-day conditions and will flower only if given one or more short days or long nights. It has been previously determined that the critical night length involving one photoinductive cycle for these plants lies between 9 and 9-1/4 hours. For this reason, then, all experimental stock plants are maintained under long-day (18 hours) conditions. Incandescent filament lamps placed over greenhouse benches are employed to extend the natural day length to 18 hours. Plants grown under these conditions remain vegetative until they are properly induced.

It has been observed, however, that during the winter months, particularly during the latter part of November, and through December, January and February, a certain amount of flowering will take place even under long-day conditions. This response has been observed over a period of three winter seasons, and during this time approximately 20% of the plants grown on an 18-hour photoperiod either produced flower primordia or reached a pre-induced stage⁽¹⁾ and a few actually produced macroscopic flowers. This response has apparently been observed by other workers, too, but has not appeared in the literature.

The sunlight conditions during the winter months differ from those during the rest of the season in both intensity and quality. The light intensity is considerably less and the quality may be slightly different because of the lower zenith angle of the sun during this time. Because of the lower zenith angle the sunlight travels through a greater layer of atmosphere and differential qualitative absorption may take place. Experiments were set up to test these two factors.

In one set of experiments, Xanthium plants were grown under 18-hour day length conditions during the summer months under normal and reduced light intensities. The intensity of sunlight was reduced by shading with layers of cheese cloth. This experiment indicated that reduced light intensities during the summer months does not promote flowering.

In another experiment, plants were grown under 18-hour light periods during December, January and February. The plants were in the greenhouse and all received the prevailing sunlight of that period. In addition to the normal winter sunlight various supplemental light sources were supplied to the following four groups of plants: 1. the 18-hour photoperiod was provided by turning on incandescent filament lamps only to extend the natural day-length period; 2. light from incandescent filament lamps was supplied for the entire 18-hour light period; 3. light from a red fluorescent source

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was provided for the entire 18-hour light period; 4. light from a blue fluorescent source was provided for the entire 18-hour light period. After a period of about 8 weeks under these conditions, it was found that only the group grown under the blue light had no "winter flowering plants." In the other three groups about 20% of the plants had reached either the flower primordia or the pre-induced stage. These preliminary experiments, then, suggest that an alteration in light quality during the winter months may be responsible for this modified photoperiodic response in Xanthium. A possible deficiency in the blue end of the spectrum is further indicated.

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INDOLE-3-ACETIC ACID OXIDASE

R. E. Stutz

The mechanism of action of the indole-3-acetic acid oxidase system has been further investigated.^(1,2) The enzyme obtained from the leaves of 2-8-week-old lupines, after dialysis against water and precipitation by $(\text{NH}_4)_2\text{SO}_4$ between 25 and 100% saturation, was used except where otherwise indicated. The normal flask filling of 3 ml. contained 0.13 M orthophosphate buffer at pH 6.3, 30 micromols indole-3-acetic acid (IAA) as the potassium salt, 10^{-3} M Mn^{++} and other agents as noted.

Activators and inhibitors: The synthetic agents 2,4-dichlorophenol and β -methyl umbelliferon^(3,4) were examined. Superficially, the action of 10^{-4} M 2,4-dichlorophenol resembles the action of the natural cofactor both in decreasing the lag and in enhancing (ca. doubling) the total oxygen uptake, as is shown in Figure 8. A respiratory quotient (R.Q.) of about one is found under these conditions (see Table 12). Mn^{++} is required for maximum oxygen uptake and cannot be replaced by 10^{-4} M Mg^{++} . The addition of glycolic acid is without effect in the presence of 2,4-dichlorophenol, but the addition of 1.5 mg. of catalase per flask enhances the oxygen uptake as was also noted by Kenten.⁽⁵⁾ Parachlorophenol, 10^{-4} M, seems to be as effective as 2,4-dichlorophenol, and 0.01 M β -methylumbelliferon may be 50% as effective when used with the Mn^{++} and glycolic acid-incubated enzyme preparations. Phenol at 10^{-4} M gives a 25% stimulation, whereas, contrary to the findings of Kenten,⁽⁵⁾ neither 10^{-4} M aniline or resorcinol was found to be stimulatory (see Figure 9). Eugenol, 3,4-dihydroxyphenyl alanine, hydroquinone, 3,4-dihydrocoumarin, catechol, caffeic acid and protocatechuic acid, at 10^{-4} M, completely block oxygen uptake. The oxygen uptake was depressed about 25% by 2-methyl-1,4-naphthohydroquinone at 10^{-4} M and, at the same concentration, guaiacol and pyrogallol caused a lag in the oxygen uptake superficially identical to that induced by the naturally-occurring inhibitor (see Figures 9, 11, and 12). Thiourea, thiouracil, α -naphtholthiourea, coumarin, ethylenechlorohydrin, and tyrosine, all at 10^{-4} M, were without effect on the oxygen uptake.

Other substrates: The efficiency with which indole-3-propionic acid, indole-3-butyric acid, α -naphthalene acetic acid, and tryptophan are utilized was examined. Only indole-3-propionic and butyric acids supported oxygen uptake, although at a greatly depressed rate even in the presence of 10^{-4} M 2,4-dichlorophenol. Mn^{++} (10^{-4} to 2×10^{-2} M) does not support oxygen uptake and is probably not oxidized peroxidatively.^(5,6)

Distribution of the enzyme: The shift in pH optimum with increasing age, as reported earlier⁽¹⁾ for the crude hypocotyl preparations, was rechecked and verified with 2-, 4-, 6-, and 8-week-old lupines. An interesting region of inactivity was found in the pH curve for the crude enzyme prepared

Figure 8

The influence of activators on oxygen uptake by IAA oxidase. The 25-100% saturated $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme from the leaves of 4-week-old lupines was used in the presence of 30 micromols IAA and 10^{-3} M Mn^{++} unless otherwise indicated.

- x $\pm 10^{-4}$ M 2,4,5-trichlorophenol or $(-\text{Mn}^{++}) + 10^{-4}$ M 2,4-dichlorophenol
- + 10^{-4} M 2,4-dichlorophenol
- O 1 ml. concentrated ether extract of the dialysate
- 2 ml. concentrated ether extract of the dialysate
- Δ 10^{-4} M parachlorophenol
- ▲ $(-\text{Mn}^{++})$
- 10^{-4} M 2,4-dichlorophenol $(-\text{Mn}^{++}; \text{dialyzed crude enzyme})$
- $(-\text{IAA}) \pm 10^{-4}$ M 2,4-dichlorophenol or ± 1 ml. concentrated ether extract of the dialysate

Table 12

INFLUENCE OF ENZYME PREPARATION AND COFACTORS OR
ACTIVATORS ON THE RESPIRATORY QUOTIENT

Nature of Enzyme Preparation	R.Q.	$\mu\text{l O}_2$ uptake	Cofactor or Activator	
			R.Q.	$\mu\text{l O}_2$ uptake
Leaves - 2-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$	0.95	178		
Leaves - 4-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$	1.06	200	0.99†	323
Hypocotyls - 2-week-old Lupine - Crude enzyme	1.05	310		
Leaves - 4-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$ $6 \times 10^{-3} \text{ M Mn}^{++} + 4 \times 10^{-3} \text{ M}$ Glycolic	1.8	122		
Leaves - 4-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$	1.4	188	1.05*	395
Leaves - 4-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$ $6 \times 10^{-3} \text{ M Mn}^{++} + 4 \times 10^{-3} \text{ M}$ Glycolic	1.4	156	1.05*	323
Leaves - 4-week-old Lupines 25-100% Sat. $(\text{NH}_4)_2\text{SO}_4$ $6 \times 10^{-3} \text{ M Mn}^{++} + 4 \times 10^{-3} \text{ M}$ Glycolic	1.83	51	1.05*	365
Stems - 8-week-old Lupines Crude enzyme	0.97	244		

†1 ml concentrated ether extract of dialysate.

* 10^{-4} M 2,4-dichlorophenol.

Figure 9

The influence of activators and inhibitors on oxygen uptake of IAA oxidase. Unless otherwise indicated, 25-100% saturated $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme from 4-week-old lupine leaves, in the presence of 30 micromols IAA and 10^{-3} M Mn^{++} , was used.

- x (control)
- + 10^{-4} M 2,4-dichlorophenol
- o 10^{-4} M phenol
- 10^{-4} M hydroquinone, 3,4-dihydrocoumarin, eugenol, catechol, 3,4-dihydroxyphenylalanine, protocatechuic acid, or caffeic acid
- 10^{-4} M aniline (oxidase from the leaves of 6-week-old lupines)
- 10^{-4} M resorcinol (oxidase from the leaves of 6-week-old lupines)
- Δ 10^{-4} M guaiacol
- ▽ 10^{-4} M guaiacol and 2,4-dichlorophenol
- ▲ 10^{-4} M pyrogallol

Figure 10

The pH response of a dialyzed $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme from 4-week-old lupine leaves incubated in the presence of 6×10^{-3} M Mn^{++} and 4×10^{-3} M glycolic acid. The pH response of the crude enzyme from the leaves of 2-week-old lupines plus 10^{-3} M Mn^{++} . Thirty micromols IAA added to each flask.

- M^{++} - and glycolic acid-incubated enzyme after 1 hr.;
- crude enzyme from the leaves of 2-week-old lupines after 1 hr.;
- same after 90 min.

Figure 11

The response of the IAA oxidase from 10-day old etiolated water-grown lupine hypocotyls to 1.5 mg. catalase or dialyzed catalase per flask, 10^{-4} M guaiacol, and 10^{-4} M 2,4-dichlorophenol is shown. All flasks contain 30 micromols of IAA and 10^{-3} M Mn^{++} unless otherwise indicated. The plants had been grown in the dark and occasionally viewed with a dim green light instead of the dim red light used previously. The lights in the Warburg (550 ft. candles) were turned on after 60 minutes - all previous operations being carried out with the green safelight.

- x ($\pm Mn^{++}$)
- + 10^{-4} M 2,4-dichlorophenol
- O catalase
- catalase + 10^{-4} M 2,4-dichlorophenol
- dialyzed catalase
- dialyzed catalase + 10^{-4} M 2,4-dichlorophenol
- Δ 10^{-4} M guaiacol + 10^{-4} M 2,4-dichlorophenol
- ▲ catalase + 10^{-4} M guaiacol + 10^{-4} M 2,4-dichlorophenol
- ▽ (-IAA)

Figure 12

The dependence of the reversal of the guaiacol-induced lag on the presence of IAA. The 25-100% saturated $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme from the leaves of 6-week-old lupines was used. All flasks contained 10^{-4} M guaiacol, 30 micromols of IAA, and 10^{-3} M Mn^{++} unless otherwise indicated.

+ (-guaiacol) + 10^{-4} M 2,4-dichlorophenol

x (-IAA) + 10^{-4} M 2,4-dichlorophenol

a. Contents of the flask mixed after the 20 minute equilibration.

- enzyme in the main compartment
- enzyme in the sidearm
- Δ enzyme in the main compartment + 10^{-4} M 2,4-dichlorophenol
- ▲ enzyme in the sidearm + 10^{-4} M 2,4-dichlorophenol

b. Contents of the flask mixed after the 20-minute equilibration and 60-minute incubation period.

- enzyme in the main compartment
- enzyme in the sidearm
- ▽ enzyme in the main compartment + 10^{-4} M 2,4-dichlorophenol
- ▼ enzyme in the sidearm + 10^{-4} M 2,4-dichlorophenol

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from the leaves of two-week-old lupines in the presence of added Mn^{++} (Figure 10). It is eliminated by dialysis and $(NH_4)_2SO_4$ precipitation. A pH curve having a nearly perfect pyramidal shape can be obtained with the $(NH_4)_2SO_4$ -precipitated enzyme after incubation with 6×10^{-3} M Mn^{++} and 4×10^{-3} M glycolic acid.

When the 8-week-old lupines were examined for distribution of the oxidase in their various tissues, only the crude stem preparations were active, although leaves, petioles, buds, stems, and hypocotyls could be made active after dialysis against water and had the usual response to added Mn^{++} and 2,4-dichlorophenol.

An active and stable enzyme preparation has been obtained from greenhouse-grown cocklebur, Xanthium sp., when adequate precautions were taken to block the polyphenol oxidases by preparing the enzyme in the presence of 10^{-3} M thiourea and ascorbic acid and by removing the natural inhibitors and polyphenol oxidase substrates with dialysis against thiourea. Less stable (or less active) preparations have also been made from the leaves of 7-day-old light-grown mung beans, Phaseolus aureus Roxbg., and the tuber of white potatoes, Solanum tuberosum var. Russet.

Catalase and green light: As was previously noted,^(1,2) and contrary to the findings of Galston,^(7,8) there is no light-induced reversal of an inhibition caused by catalase; there is merely, a lag induced by the impurities contained in the catalase preparations. Catalase actually stimulates the oxygen uptake from 10-day-old etiolated lupine hypocotyls; regardless of whether the plants had been observed with a dim green light or with a dim red light as previously used,⁽²⁾ no biochemical differences were noticeable (Figure 11). Dialysis of the catalase apparently removed some naturally-occurring inhibitor; further increase in oxygen uptake was obtained by the addition of 2,4-dichlorophenol. Guaiacol introduces a lag, as in the light-grown tissue, which is extended by the addition of catalase despite the presence of 2,4-dichlorophenol.

Lag in oxidase action: Dependence of the reversal of the guaiacol-induced lag on the action of the IAA oxidase is shown in Figure 12. The lag was not overcome until 30 to 40 minutes after the introduction of IAA regardless of the conditions of incubation with guaiacol. Figure 13 shows the effect of a second addition of the enzyme and indicates enzyme limitation - not cofactor or substrate limitation. The addition of 10^{-4} M 2,4-dichlorophenol doubles the oxygen uptake.

Respiratory quotient: When the $(NH_4)_2SO_4$ -precipitated enzyme is incubated overnight with 6×10^{-3} M Mn^{++} and 4×10^{-3} M glycolic acid at pH 5.5-5.7 and at 5°C, a heavy precipitate is formed, which, upon removal, leaves the enzyme with altered characteristics (Figure 14). R.Q.'s of 1.4 and 1.8 were found for such preparations but the addition of 2,4-dichlorophenol shifts the R.Q. to 1, the value found with crude enzyme preparations (see Table 12). Fe^{++} , Co^{++} , and Al^{+++} are also effective precipitating agents, but activity of the enzyme is dependent on the addition of Mn^{++} .

Figure 13

The effect of the concentration of the enzyme and the time of addition in relation to the oxygen uptake, lag and natural inhibitor concentration. Dialyzed $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme from the leaves of 6-week-old lupines was used in the presence of 30 micromols of IAA and 10^{-3} M Mn^{++} .

x 1 ml. of enzyme

○ 2 ml. of enzyme

● 1 ml. of enzyme at $T = 0$ plus 1 ml. of enzyme at $T = 60$ min.

Figure 14

The influence of activators on the respiratory quotient and oxygen uptake. Thirty micro-mols IAA and 10^{-3} M Mn^{++} were added to each flask.

a. Mn^{++} - and glycolic acid-incubated enzyme

- x leaves of 4-week-old lupines (Dec. prep.)
- leaves of 4-week-old lupines (Feb. prep.)
- leaves of 4-week-old lupines (Feb. prep.) + 10^{-4} M 2,4-dichlorophenol
- △ leaves of 6-week-old lupines
- ▲ leaves of 6-week-old lupines + 10^{-4} M 2,4-dichlorophenol

b. $(NH_4)_2SO_4$ -precipitated enzyme from the leaves of 4-week-old lupines

- 10^{-3} M glycolic acid
- concentrated ether extract of the dialysate

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Discussion: The complexity of the IAA oxidase system and the multiplicity of the products found by paper chromatography have made an understanding of the reaction difficult. The data presented here suggest the functioning of two enzyme systems. A re-interpretation of the mechanisms encourages an effort to characterize the reaction products. A continued effort to identify the naturally-occurring cofactors, activators, and inhibitors will be made.

The two enzymes comprising the IAA oxidase system undoubtedly exist in all growing plant tissue and may be found if the necessary precautions are taken in preparation. The data seem to fit the following schematic pathway:

The hypothetical product A of step 1 could be identical with the active hormone. The second stage provides a mechanism whereby the plant may regulate the IAA or active hormone level. Such a system would permit the phenoxy acids to exert both homonal and herbicidal action, since they should be less easily destroyed than the natural cofactor. Since the reversal of the guaiacol-induced lag is retarded by the addition of catalase (even in the presence of 2,4-dichlorophenol) and is dependent on the presence of IAA, it may be presumed that guaiacol is being peroxidatively inactivated by the H_2O_2 formed by the IAA oxidase. In the absence of inhibitors and in the presence of cofactors or carriers such as 2,4-dichlorophenol, p-chlorophenol, or the natural ether-soluble cofactor, the H_2O_2 is peroxidatively utilized with greater efficiency; therefore the IAA oxidase itself is less rapidly inactivated, resulting in a greatly enhanced total oxygen uptake. Catalase may function in this manner.

In the crude enzyme preparations⁽²⁾ glycolic acid stimulation and shortening of the lag were observed, because H_2O_2 is produced by the glycolic acid oxidase⁽⁹⁾ which facilitated the peroxidative inactivation of the inhibitors. This capacity of the system seems to be lost on purification. The apparent kinetic differences noted earlier⁽²⁾ can be attributed to inhibitor inactivation.

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THE EFFECT OF LOW DOSES OF X IRRADIATION ON POST-DIAPAUSE
MELANOPLUS DIFFERENTIALIS EMBRYOS

T. N. Tahmisian, B. J. Wright and R. L. Devine

Post-diapause grasshopper embryos were given very low doses of X rays to determine the dose necessary to inhibit morphological development of the embryo. A small percentage of the eggs given 500 r will hatch. Of those that hatch a few reach adulthood. At the present time we are waiting to see whether these insects (which have been observed to copulate) are capable of reproducing. So far we have not been able to determine if they will lay eggs and, if so, whether the eggs will be fertile. It remains for further experimentation to determine how subminimal doses of irradiation will affect the stem cells of these insects and the capacity of the irradiated, sexually mature insects to produce normal offspring.

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METHYL CHOLANTHRENE CARCINOGENESIS

J. H. Pomeroy and C. A. Craig

The compound 20-methyl cholanthrene (MCA) exists in two isomeric forms which are stable (I) and unstable (II) at room temperature.⁽¹⁾ Form I exists as triclinic needles, which are transformed into rhombic plates of II between 140-144°, which then melt at 180.5°. Form II can also be obtained by crystallization from propyl alcohol or amyl acetate by evaporation of solvent near the boiling point. Crystals of II obtained in this way are stable for several days, but then spontaneously revert to I, as can be shown by either X-ray spectroscopy or infra red spectroscopy.

The infrared spectra of the two forms are essentially identical except near 740 and 795 cm^{-1} ; form I shows two sharp, closely spaced peaks near each of these frequencies but form II shows only one, somewhat broader, peak of the same magnitude. These peaks can be observed either if the MCA sample is prepared in a KBr powder disc or as a fused capillary film. The X-ray diffraction spectra of the two forms (as determined by Stanley Siegel of the Chemistry Division) are nearly completely different. These observations indicate that the two forms I and II are not molecular isomers, but differ only in the relative arrangement of the molecules in the crystal lattice. The differences in the infrared spectra are presumably due to interference with carbon-hydrogen rocking vibrations by neighboring molecules in the solid state. These differences would not exist in the liquid state or in solution.

The fact that MCA has two crystal structures with different free energy and possibly different physiological activity has not been noted previously in the biological literature. When "crystalline" MCA has been reported, it may be assumed that form I was probably referred to; when fused pellets, such as implanted by Weil and Blumklotz⁽²⁾ and others, are referred to, these could have been composed either of I or of II or of a mixture of the two.

It would appear that a comparison of the carcinogenic effect of the two forms would be of interest; implantations of the crystals alone would not be strictly comparable, since differences in surface exposed could not be controlled. Pellets of MCA weighing about 5 mg each, 1.7 mm in diameter and 2.5 mm in length can be prepared in a laboratory pellet press, using MCA which has been recrystallized from isopropyl alcohol and acetone-water. By heating to 160° for 15 minutes, the pellets are completely transformed to the II form, which is stable for several days under these conditions. These pellets are to be compared for carcinogenic activity with pellets of the unconverted form I in suitable laboratory animals, with results to be reported later. X

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THE EFFECTS OF STRONG ACIDS ON LYSOZYME

A. L. Koch, W. A. Lamont and J. J. Katz*

In a previous report⁽¹⁾ we have shown that ribonuclease can be recovered from solution in liquid HF, HF:SO₂ mixtures, formic acid, and trifluoroacetic acid still possessing the original physical and enzymatic properties if the temperature and time of exposure were short. We have now extended these studies to the enzyme lysozyme. Lysozyme is similar to ribonuclease in a number of physical properties. They are both small proteins (M.W. 15,000) and have hydrodynamic properties that suggest that they are essentially spherical. Both are resistant to heat denaturation and both can be recovered in active form after precipitation by aqueous trichloroacetic acid. It would therefore be anticipated that results similar to those found with ribonuclease would be found with lysozyme. This was found to be the case with respect to the stability of these enzymes; lysozyme being only slightly less stable in general than is ribonuclease (see Table 13). However, the process of denaturation appears to be quite different in the two cases. Preparations of ribonuclease that had lost almost all of their biological activity formed completely clear aqueous solutions, whereas almost all preparations of lysozyme contain some insoluble material and a few of the preparations had an odor characteristic of organic sulfur compounds. This difference in mode of denaturation is not simply due to a quantitative difference in sulfur-containing amino acids as it is known⁽²⁾ that both enzymes contain similar amounts of the sulfur amino acids.

Lysozyme and ribonuclease both contain considerably more sulfur than do most proteins. It has been suggested⁽³⁾ that increased proportion of S-S links is the cause of the great stability of these molecules. The only major difference in their amino acids compositions is the absence of tryptophane in ribonuclease and its presence in normal amounts in lysozyme.

One important difference between the two substances is isoelectric point. Lysozyme is quite basic (isoelectric point, pH 11) whereas ribonuclease has an isoelectric point of 7.8. The basic character of lysozyme allows it to form complexes with acidic proteins at neutral pH values.

PROCEDURE

Lysozyme samples were dried under high vacuum, dissolved in the solvent under the conditions of time, temperature, and with the addition of substances given in the table. After the solvents were quantitatively removed, the sample was suspended in $\frac{M}{15}$ phosphate buffer, pH 7.1, containing

*Chemistry Division

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Table 13

THE EFFECT OF ANHYDROUS ACID MEDIUM ON THE PHYSICAL
AND BIOLOGICAL PROPERTIES OF LYSOZYME

Exp. No.* HF	Time (hr)	Temp. (°C)	Additive	Relative activity of supernatant	% Soluble material	% Enzymatic activity remaining after treatment	Ultra- centrifugal analysis (in 10^{-13} sec.)
290	2	-78		100	86	60	1.81
233	2.5	-78		48	72	34	1.89
259	1.5	-78		80	36	30	1.83
254	2	0		2	97	1.7	Peak flattens
287	2	0		16	72	12	Peak flattens
268	2	0	Cysteine	24	24	6	Peak flattens
269	2	0	Ascorbic	39	90	35	1.88
289	2	0	Ascorbic	43	68	39	1.83
255	2	25		0.6	84	1.7	2 peaks 8.4
271	2	25	O ₂	9	86	1.3	2 peaks 13
272	2	25	Cysteine	0.3	90	1.0	2 peaks 9
273	2	25	Ascorbic	1.5	90+	1.3	1.646
274	2	25	BPA	1.6	90+	1.5	Flattens
270	2	-78	Urea·H ₂ O ₂	8.5	4	0.4	---
238	24	25		0	100	0	Peak develops then flattens
<u>Trifluoroacetic acid</u>							
275	2	0		26	60	15	1.93
292	2	0		60	34	18	---
291	2	0	Ascorbic	47	78	30	1.691
212	2	25		20	92	18	1.96
262	1	25	BPA	20	67	18	2 peaks one BPA each normal
276	2	25	Cysteine	--	70	5	1.813
277	2	25	Ascorbic	--	--	1.7	---
263	1	25	TFA anhydride	3	4	0.1	---
<u>SO₂:HF[†]</u>							
288	<0.25	-78		100	68	60	1.94
256	2	0		40	83	35	1.84
260	1.5	0		60	64	39	1.85
261	2	25		30	19	9	---
239	24	25		0	16	0	---
<u>HF:SO₂[†]</u>							
267	2	0		8.5	90+	8.5	---
<u>HCOOH</u>							
213	2	25		70	55	35	---

*In experiments 212-239, a sample of Armour lysozyme was used; in experiments 254 and on, a sample of Worthington lysozyme prep. LY551 was used.

†In these experiments the protein was dissolved in liquid SO₂ containing a small amount of HF to salt in the protein.

‡In this experiment, the bulk of the solvent was HF, with only a trace of SO₂.

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0.1N NaCl. The samples were more or less turbid with the exception of that from experiment #238. The total amount of protein and enzymic activity were determined, the solutions were centrifuged clear and the two determinations were repeated. The samples were centrifuged in the analytical ultracentrifuge as previously described.⁽¹⁾

The amount of protein present was determined by measurements of the optical density at 280 m μ . In solutions with only faint turbidity this could be determined simply by dilution in $\frac{M}{15}$ phosphate buffer. In very turbid solutions, the well-stirred samples were diluted with 0.3N NaOH to produce clear solutions. This procedure was also advantageous in those samples containing ascorbic acid. This substance absorbs in the ultraviolet at 280 m μ ; however, it is destroyed by alkali, yielding non-light-absorbing fragments. In some cases it was unnecessary to analyze for total protein because it was evident that there had been no loss of protein during the vacuum line procedures.

The assay for lysozyme activity is basically that of Shugar.⁽⁴⁾ A suspension in $\frac{M}{15}$ phosphate buffer, pH 7.1, of Difco Micrococcus lysideicticus (a lypholized preparation of organism treated with ultraviolet light to render the bacteria nonviable) was used. The suspension was placed in a plastic cuvette in a thermostatted Beckman spectrophotometer, the optical density at 450 m μ was read, the sample was added with a micropipet, and the optical density was measured at the end of a 2-minute incubation period. We found it necessary to stir the solution immediately prior to measurements because the material settled rapidly. For the reasons described below, the assay period was extended to 30 minutes for samples of low activity. However, all data presented are calculated from calibration curves prepared under identical conditions with the same preparation of enzyme used in experiments.

RESULTS

A. Solubility of the recovered material.

The samples recovered from the anhydrous acids still contained some acid. Consequently the pH of the solutions was reduced to 4-5. Under these conditions there is some insoluble material with little biological activity. At pH 7.1, more material usually precipitated. This probably results from complex formation between basic and acidic groups of the denatured protein. This precipitate, as well as that produced with milder HF treatment is soluble in acid. The precipitates formed at pH 4-5 by other agents are insoluble in acid.

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B. Effect of denatured lysozyme on the biological assay.

Since assays were made at pH 7.1, where complex formation occurs, we found it necessary to use less protein and to extend the assay period, to avoid the excess light-scattering of the precipitated material.

This appears to be the only effect of the denatured protein on the assay organism. Wilcox and Daniels⁽⁵⁾ report that high concentrations of lysozyme, while causing the bacteria to lyse, produce an increase in the light-scattering of the system by the formation of a stable "enzyme substrate" complex with cell constituents. Our denatured preparations do not do this, although active preparations do. Increased salt concentration decreases the Wilcox and Daniels reaction but has no effect on the light-scattering properties of a system containing active enzyme plus large amounts of denatured enzyme plus bacteria.

The assay of lysozyme is not influenced by addition to the assay system of bovine plasma albumin, cysteine, or ascorbic acid. This is the case with control enzyme solutions as well as with partially denatured enzyme preparations. From the above considerations, we feel that our enzymatic assays do indeed measure the amount of native enzyme remaining in solution.

C. The effect of solvent, time, and temperature on lysozyme activity.

The techniques required for the solution of protein in these anhydrous media preclude the obtaining of accurate kinetic data. First, fluorethane plastic is almost a perfect insulator, so that accurate temperature control is difficult. Second, and even more important, is the possible denaturation of the protein during the removal of the solvent by distillation or sublimation. However, in spite of these difficulties two conclusions are obvious from the data presented. First, all solvents tested are similar in their effects on the activity of lysozyme. There is no large difference between the effects of HF and trifluoroacetic acid as would be expected if hydrogen bond formation played the important role in the denaturation phenomenon. The second conclusion is that denaturation in these anhydrous solvents is much less temperature sensitive than is the denaturation of protein in aqueous solvents. If one assumes, as is reasonable, that denaturation in these anhydrous solvents is a first order process, then one may calculate that the Q_{10} for the inactivation process is at most 1.4. This is to be compared with a value of 100 commonly obtained for protein denaturation in aqueous solution.

D. The effect of various additives on lysozyme activity.

Several substances have been tested for ability to protect lysozyme from denaturation by HF and trifluoroacetic acid. It can be seen that the simple addition of extra protein in the form of bovine plasma albumin is without effect. This would tend to suggest that surface forces are not important in the denaturation processes under the conditions used here. We

have also tested two reducing agents, cysteine and ascorbic acid. At 25°, neither substance influences the activity of lysozyme in HF; both appear to decrease the activity in trifluoroacetic acid. At 0°C ascorbic acid does decrease the amount of denaturation. As will be seen below, ascorbic acid has a qualitative effect on the ultra-centrifugal diagrams.

Molecular oxygen does not increase the rate of denaturation. However, the oxidizing agent urea · H₂O₂ does destroy lysozyme. It was thought that the oxidizing action might not occur in the anhydrous medium, but would take place only after removal of solvent and the addition of the aqueous buffer. Consequently as soon as the buffer was added, catalase was added to destroy any residual H₂O₂. However, no bubbles of O₂ were formed, indicating the absence of H₂O₂ at this stage.

Trifluoroacetic anhydride added to the trifluoroacetic acid also destroys activity very rapidly.

E. Processes secondary to denaturation.

From the fact that specific enzymatic activity of the supernatant is usually less than 100 percent, it is clear that the denaturation process and the precipitation processes are independent.

The sedimentation constant of native lysozyme is 1.95. After treatment with HF for 2 hours at -78° the sedimentation constant is not significantly reduced and a sharp peak is formed. At zero degrees there is no measurable peak. A schlieren boundary starts to form but then disappears. This means that the soluble material is no longer homogeneous but consists of a distribution of particles, a considerable fraction of which are much smaller than the native lysozyme molecule. Also at zero degrees the recovered samples contain more insoluble material than is found at either -78° or 25°C. In the presence of ascorbic acid, at 0°C, a sharp homogeneous peak is found with a sedimentation constant similar to that found at -78°; this suggests that ascorbic acid prevents the processes that lead to the splitting of the lysozyme into fragments. At the higher temperature (25°C), the ultracentrifugal diagrams contain two peaks, a fast component (S = 8 - 13) which corresponds to an entity of molecular weight 200,000 to 1,000,000, depending on its shape, and a slower component with a sedimentation constant in the vicinity of 2, which corresponds to the residual lysozyme monomer, mainly enzymatically inactive. In the presence of ascorbic acid a sharp peak is formed but the sedimentation constant is considerably decreased, indicating, as in the case of ribonuclease, unfolding of the native molecule.

Finally, after 24 hours at room temperature, the product is completely water soluble, containing heterogeneous small fragments.

Thus the denaturation process in HF may be pictured as follows. First the native enzyme is unfolded and loses activity. In this material, salt linkages are formed between the basic amino acids and the acidic amino acid of the chains; this gives rise to insoluble material that is readily solubilized by adding acid to bring the pH below the isoelectric point for the acidic groups. Under more vigorous conditions the unfolded chains are cleaved and reformed to yield a heterogeneous distribution of molecular weights. Smaller fragments are formed, as well as material insoluble in acid. Under still more vigorous conditions, in HF, the insoluble material first formed is further broken down to yield fragments of weights from 200,000 to 1,000,000. Finally, all the material is broken down to considerably smaller fragments than the original material. It should be noted that all the acid-soluble stages are capable of forming complexes at neutral pH.

This sequence of events is probably applicable to the other solvents tested, with the exception of the last stage of solubilization of the acid-insoluble material after vigorous treatment. Prolonged treatment with SO_2 : HF yields material that is very insoluble and has lost sulfur in the form of small sulfur-containing compounds. Furthermore, precipitates formed by all solvents other than HF are acid insoluble.

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THE ACTIVATION OF METHIONINE FOR TRANSMETHYLATION

P. D. Klein

The process by which the terminal methyl group of methionine is transferred to acceptors such as guanidoacetic acid or nicotinamide has been shown to require an initial activation of the methionine molecule through the formation of a sulfonium pole. Cantoni⁽¹⁾ has demonstrated that this is created by the synthesis of S-adenosyl methionine from methionine and adenosine triphosphate (ATP) and that this S-adenosyl methionine is the active methyl donor. He has investigated the enzymatic formation of this compound and has succeeded in isolating a partially purified system which carries out the following reaction:

It was necessary to activate the enzyme with sulfhydryl reagents such as BAL or glutathione (GSH) for full activity. The activity of the enzyme is determined by the phosphate release in the presence and absence of methionine.

The singular use of ATP to donate an adenosyl group and the concurrent release of three moles of inorganic phosphate, as well as the unexplored relationship of transmethylation to nucleic acid synthesis prompted a closer investigation of the enzymatic nature of this reaction.

Experimental

Enzyme source: A rat is stunned and exsanguinated, and the liver is removed and chilled in crushed ice. All subsequent operations are carried out at 5°C. The liver is pulped, weighed and a portion homogenized (with rubber-covered pestle and pyrex test tube) in 10 volumes of cold 0.1 M sodium acetate, pH 7.5. The homogenate is centrifuged at approximately 18,000 x g for 30 minutes and the clear supernatant drawn off with a syringe. Usually this extract was used directly for the assay of methionine activation, although in some cases, extracts were made using an acetate buffer at pH 5.6 and the supernatant adjusted to pH 7.5 as suggested by Cantoni: these were not found to differ from the neutral extracts in activity.

Enzyme assay: The assay medium contained 250 μM MgCl_2 , 10 μM ATP (neutralized to pH 7.5) 300 μM glycyl-glycine buffer, pH 7.5, 30 μM methionine, 0.05-0.6 ml enzyme and isotonic KCl to 1.6 ml final volume. Additions such as activators or inhibitors were made at the expense of KCl. Control samples contained no methionine, and tubes deproteinized at zero time served as initial phosphate controls. The incubation was carried out at 38° for 60 minutes; at the end of this time an equal volume of cold 10%

trichloroacetic acid was added, the samples were centrifuged and an aliquot of the supernatant was taken for the determination of inorganic phosphate by the method of Lowry and Lopez.(2)

Results

One of the initial objectives was to establish a reproducible assay of the native methionine activation system in order to determine its sensitivity to irradiation. Presumably any effect would involve either a net destruction of enzymes or a decrease in the activity in the absence of such activators as glutathione. The need for multiple assays prohibits a time-consuming purification of the system. Therefore these investigations deal with the stoichiometry, reproducibility and activation of the system.

The release of orthophosphate as a function of time in the presence and absence of methionine is shown in Figure 15. The non-linearity of the enzyme action is evident. Presumably the criterion of phosphate release may contribute to this since the reported mechanism is one of initially cleaving pyrophosphate from ATP and subsequent release of inorganic phosphate from adenylic acid accompanied by hydrolysis of pyrophosphate.

Figure 15

The release of orthophosphate by methionine-activating enzyme in the presence and absence of methionine.

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The possibility of stimulating the reaction by adding a methyl recipient, in this case nicotinamide, as well as by inhibiting phyrophosphatase was next studied. Table 14 shows that fluoride completely abolishes any activation of methionine while the rate of dephosphorylation was not increased by the addition of nicotinamide.

Table 14

THE EFFECT OF FLUORIDE AND NICOTINAMIDE ON
METHIONINE ACTIVATION

Condition	- Methionine	+ Methionine	Δ	Activation*
	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	%
Control	19.4	32.1	12.7	166
F ⁻ (30 μM)	5.5	5.9	0.4	107
Nicotinamide (100 μM)	19.7	28.5	8.8	146

* Ratio of total/ATPase x 100. Conditions as described in text;
0.2 ml enzyme.

The degree of enzyme activation, i.e. the ratio of total phosphate release to ATPase phosphate release might be expected to involve the native ATPase content of the preparation, and it is not too surprising to find that blenderizing tissue and thereby disrupting nuclei would contribute to a lower enzyme activation. In Table 15, the comparison is made of the effect of the two methods on enzyme activation. Cantoni's values for a similar extract of pig liver are included for comparison: these tissues were treated in a Waring blender. It is evident that, although the enzyme concentration as measured by difference is identical, the degree of activation in the latter case is approximately half as great.

Table 16 indicates the effect of glutathione as well as the proportionality of enzyme activity at various levels of enzyme. There is no demonstrable activation by glutathione; it should be noted that the activation level is as much as fivefold greater than the values given by Cantoni. At the same time the total activity is lower, suggesting that the extraction of methionine-activating enzyme also solubilizes to a varying degree the ATPase activity of the tissue. One of the less enlightening phenomena is the lack of stoichiometry at various levels of enzyme concentration. While the summation of methionine activation and ATPase activities remains relatively constant, the higher levels of enzyme display a marked increase in the ATPase component. This situation makes the reproducibility of enzyme assay hazardous.

Table 15

THE EFFECT OF TISSUE DISRUPTION METHOD ON
METHIONINE ACTIVATION

Condition	- Methionine	+ Methionine	Δ	Activation
	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	%
Homogenized	20.4	29.4	9.0	144
Blendorized	29.4	29.4	0.0	-
Cantoni - blendorized tissue	40.9	51.1	10.2	125

Table 16

GLUTATHIONE ACTIVATION AND STOICHIOMETRY OF
METHIONINE ACTIVATION

Conditions	- Methionine	+ Methionine	Δ	Activation
Amount of Enzyme (+ GSH)	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	$\mu\text{M P/hr/ml E}$	%
0.1 ml	3.40	7.60	4.20	224
0.2 ml	4.25	9.70	5.45	228
0.4 ml	6.15	9.53	3.38	155
Amount of Enzyme (- GSH)				
0.1 ml	1.70	8.50	6.80	500
0.2 ml	4.10	8.90	4.80	217
0.4 ml	6.90	10.73	3.83	155

Present work is directed towards a closer study of the complex which carries out the methionine activation with a view to dissociating the components into isolated reactions catalysing single steps, and thus permitting the study of their variation under experimental conditions.

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361-075

THE METABOLISM OF NUCLEIC ACID PURINES IN THE RAT

R. W. Swick, A. L. Koch, and D. T. Handa

There is a general lack of accord in the literature on the nature and rate of renewal of the nucleic acids. This confusion is probably the product of a number of factors. Although the two types of nucleic acids are quite similar chemically they are markedly different metabolically in that ribose nucleic acids (RNA) turn over very rapidly while deoxyribose nucleic acids (DNA) are remarkably stable, and their formation appears concurrent only with mitosis. Mechanically the study of nucleic acid metabolism is subject to several difficulties. Most experiments have been carried out using a single dose of tracer material, producing in the appropriate intermediate pools a rapid rise and equally rapid fall in radioactivity, hence the accurate estimation of this radioactivity, required for calculations of the rate of synthesis of nucleic acids, cannot be obtained. Next, the extent to which the tracer compounds usually given become incorporated into the nucleic acids normally is not known. Even when glycine, a natural and a sole precursor of part of the purine molecule, was employed, its ultimate level of incorporation was not learned. Again, techniques for the complete separation of DNA and RNA from each other have not always been used, nor have radiochemically pure compounds been isolated and measured. Finally, the published parameters of turnover time have been estimated by comparison of the radioactivity found in groups of animals at various times after isotope administration. Thus the effects of biological variation may mask turnover phenomena in individual animals.

In order to avoid these difficulties we have studied nucleic acid metabolism in the following manner. Animals were fed, at hourly intervals, diets containing $\text{CaC}^{14}\text{O}_3$ (Table 17) for periods ranging from 7 to 59 days, thus maintaining the radioactivity of metabolic CO_2 at a constant level and one which could be determined by examination of the urea. Since we have established here that CO_2 is the sole precursor of the C-6 position of the purines and represents 12% of the carbon in the carboxyl group of glycine,⁽¹⁾ administration of isotopic CO_2 should eventually yield purines whose radioactivity is 112% of that of metabolic CO_2 . The nucleic acids isolated from the liver were separated by the Schmidt-Thannhauser procedure giving an RNA fraction entirely devoid of contamination with DNA. Because the converse is not true, the DNA fraction was hydrolyzed at pH 3 for 1 hr at 100°. Under these conditions the labile bond between the purines and deoxyribose phosphate is broken while that between purines and any ribose phosphate present is only slightly affected. Passage through an anion exchange column retains any nucleotides, permitting the isolation of the free DNA purines. After separation of all the purines by cation-exchange of Dowex 50 H^+ , adenine and guanine were isolated as the silver salts and then reconverted to the free purines in order to eliminate any extraneous carbon compounds. Their specific radioactivity was determined by wet combustion and measurement of the CO_2 by proportional gas counting.

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Table 17

GROWTH AND THE RADIOACTIVITY OF NUCLEIC ACIDS IN LIVER

Treatment	% Increase in liver		Specific activity in 10^3 c/min/mM compound				
	100 x	$\frac{\text{Final wt.} - \text{initial wt.}}{\text{initial wt.}}$	RNA			DNA	
			Urea	Adenine	Guanine	Adenine	Guanine
A. Steady exposure for 59 days, $2.2 \mu\text{c/gm}$ diet (Adult rat)		7	168	212	189	84.5	81
B. Steady exposure for 9 days, $5 \mu\text{c/gm}$ (Weanling rat)		120*	403	326	-	176	-
C. Steady exposure for 9 days, $5 \mu\text{c/gm}$ (Weanling rat)		130*	394	319	316	129	128
D. Steady exposure for 7 days, $16.8 \mu\text{c/gm}$ (Weanling rat)		87*	1426	1068	848	398	278
E. Steady exposure for 7 days, $16.8 \mu\text{c/gm}$ followed by no activity for 19 days (Weanling rat)		122*†	1439	91	210	353	333
F. Activity one day, sacrificed 21 days later (Weanling rat)		-	-	118	181	236	238
G. Activity one day, sacrificed 21 days later (Weanling rat)		-	-	53	71	122	103
H. Activity one day, sacrificed 42 days later (Weanling rat)		61*†	-	8.9	10.3	65	43.8
I. Activity one day, sacrificed 42 days later (Weanling rat)		44*†	-	1.3	1.2	94	65.4

*Weight gains were estimated from statistical analysis of a group of comparable animals.

†% increase in liver was calculated for the second 21-day period.

Wherever possible we have tried to estimate the parameters of turnover rate of nucleic acids from experimental data derived by analysis of individual rats and have made comparisons between animals only for the estimation of correction factors and for the approximate estimation of additional parameters.

Results

The results of the isotope analyses are presented in Table 17. Experiment A indicates that after 59 days exposure of an adult rat the RNA purines have reached the theoretical maximum specific activity (s.a.) while the DNA purines have slightly less than one-half of this value. Experiments B, C and D show that in growing rats after 7-9 days exposure, the RNA purines are not saturated, but that considerable activity is associated with the DNA compounds. Since the liver weights have doubled during the exposure period, one would expect the DNA to be approximately half-saturated if new growth and DNA synthesis are synonymous. This seems to be the case. Experiments D and E were performed to evaluate the conversion of adenine into guanine and their transfer from RNA to DNA. During the second period (19 days) the s.a. of the RNA purines decreased sharply, in keeping with the known rapid turnover of RNA. That the s.a. of adenine decreased more rapidly than the s.a. of the guanine is to be expected from the known ability of the rat to convert adenine into guanine but not the reverse; hence it appears that the radioactivity of RNA adenine is liberated by turnover and reincorporated as RNA guanine. The s.a. of the DNA purines did not fall to half of the original value as would have been predicted from the growth of the liver during the second phase, indicating that RNA compounds are contributing to the precursor pools from which DNA is being synthesized. Each of the four rats of experiments F - I was given 1.16 mc of $\text{NaHC}^{14}\text{O}_3$ in three portions by intraperitoneal injection during the course of one day; two were sacrificed 21 days later, the other two, 42 days after the administration of radioactivity. In contrast to the reproducibility of the data obtained in the earlier experiments, there was a marked disagreement between similarly treated animals in the single dose experiments. The possibility that the animals failed to receive identical amounts of C^{14} was eliminated by examination of the s.a. of the liver and muscle protein fractions of these animals (Table 18). These discrepancies might result from variations between animals in the rates of renewal of RNA, although, if true, the variations are not correlated with the protein metabolism of the animal (Table 18) or with the synthetic rates for DNA. The third possibility, which we tend to favor, is that there are periodic bursts of metabolic activity in the liver which are averaged out in constant exposure experiments but not in acute exposure studies. Our intention in the latter experiment was to measure changes in the radioactivity of DNA at a time when the contribution of radioactivity by RNA turnover would be minimal. During the first 21-day period the radioactivity of the RNA had decreased sufficiently to make only a small contribution to the DNA synthesized during the second phase, thus permitting

a possible distinction between the dilution of DNA radioactivity by growth and any loss of DNA activity by turnover. In spite of the large variation between duplicate animals, there is some decrease in DNA specific activity which cannot be accounted for solely by growth.

Table 18

PROTEIN RADIOACTIVITY

Expt #	Time since exposure (days)	Muscle c/min/mM C	Liver c/min/mM C
F	21	4818	1177
G	21	4916	1014
H	42	1936	189
I	42	1570	164

Model

The following metabolic scheme suggests itself from the literature and from the considerations above:

It is assumed that there are some small pools of adenine and guanine intermediates which are maintained by de novo synthesis and by the breakdown of RNA. The contribution, if any, from the breakdown of DNA necessarily is small because of the small amount of DNA present and its well-known inertness. Even in non-growing animals, where DNA breakdown must equal its synthesis, it is reasonable to assume that the DNA fragments do not enter these pools. For example, the radioactivity incorporated into human leucocytes appears to measure the life span of the leucocyte. The DNA synthesized at the birth of a cell is retained until its death and then discarded.⁽²⁾ Furthermore, it has been observed that activity present in the nucleic acids of one tissue is not transferred to other tissues.⁽³⁾

We shall use the symbol, V , as the reaction rate in μM of compound formed by the reaction per day; the capital letters, as indicated in the model, designate the specific activities of the compound at any time; and the lower case letters represent the amount of this material present. We will then derive equations for the activities in the adenine of the two types of nucleic acid under the various experimental conditions. Equations identical in form will be used for guanine. For the present purposes we will not consider the minor reaction pathway labeled $V_{A \rightarrow G}$ but will introduce the pathway in considering the final results.

At all times the activity of the "adenine" pool will be the weighted average of its two sources because the size of this pool is assumed to be small. Therefore:

$$A = \frac{V_1 P + V_3 R}{V_1 + V_3} \quad (1)$$

(subscripts referring to adenine, e.g., V_{1A} , have been dropped for the purposes of this derivation).

In the case of the growing rat, where the amount of nucleic acid is increasing continuously, it appears reasonable to assume that the increase is linear with time over the duration of the experiment. Therefore:

$$r = r_0 + (V_2 - V_3)t, \text{ and} \quad (2)$$

$$d = d_0 + V_4 t \quad (3)$$

For the first case let us assume that, for the duration of the experiment, the activity of the purine precursor pool is maintained at the definite value P , equal to 1.12 times that of the metabolic CO_2 . Then,

$$\frac{d(rR)}{dt} = V_2 A - V_3 R = \frac{r}{dt} \frac{dR}{dt} + \frac{R}{dt} \frac{dr}{dt} \quad (4)$$

now

$$\frac{dr}{dt} = (V_2 - V_3) \quad (5)$$

therefore,

$$\frac{r dR}{dt} = V_2 A - V_3 R - (V_2 - V_3) R = V_2 (A - R) \quad (6)$$

Substituting the value of A from eq (1) we obtain:

$$\frac{r dR}{dt} = V_2 \left(\frac{V_3}{V_1 + V_3} - 1 \right) R + P \frac{V_1 V_2}{V_1 + V_3} \quad (7)$$

and

$$\frac{dR}{dt} = \left(\frac{V_1 V_2}{V_1 + V_3} \right) \frac{1}{r} \cdot (P - R) \quad (8)$$

If we let r be the average amount present, we may then integrate to obtain

$$R = P \left(1 - e^{-\frac{V_1 V_2 t}{(V_1 + V_3) r}} \right) \quad (9)$$

It is interesting to note that Eq. (9) is also obtained in the case of the steady state animal although, in this case, there is no ambiguity in the significance of r.

The activity in the DNA is obtained as follows:

$$\frac{d(Dd)}{dt} = AV_4 = PV_4 \left(\frac{V_1 + V_3 - V_3 e^{-\frac{V_1 V_2 t}{(V_1 + V_3) r}}}{V_1 + V_3} \right) \quad (10)$$

and thus the total activity at time t is

$$Dd = PV_4 t - \frac{PV_4 V_3}{V_1 V_2} r \left(1 - e^{-\frac{V_1 V_2 t}{(V_1 + V_3) r}} \right) = PV_4 t - r R_t \left(\frac{V_4 V_3}{V_1 V_2} \right) \quad (11)$$

where R_t is the specific activity of the RNA at time t.

In the second type of experiment (Experiments D and E) the administration of isotope was stopped and the changes in activity of the nucleic acids noted after an interval of time. One very important property of

$C^{14}O_2$ as the tracer is that the activity of the metabolic CO_2 drops abruptly to a negligible value after cessation of administration. We may therefore assume that $P = 0$. Thus in this case, from Eq. (6),

$$\frac{r}{dt} \frac{dR}{dt} = V_2 (A - R) = \frac{-V_1 V_2}{V_1 + V_3} R \quad (12)$$

$$R = R_0 e^{-\frac{V_1 V_2 t}{(V_1 + V_3)r}} \quad (13)$$

where R_0 is the initial specific activity in the RNA. Then for the DNA,

$$\frac{d}{dt} (Dd) = A V_4 = \frac{V_3 V_4}{V_1 + V_3} \cdot R_0 \cdot e^{-\frac{V_1 V_2 t}{(V_1 + V_3)r}} \quad (14)$$

integrating,

$$(Dd)_t = (Dd)_0 + \frac{V_3 V_4}{V_1 V_2} \cdot r R_0 \left(1 - e^{-\frac{V_1 V_2 t}{(V_1 + V_3)r}} \right) \quad (15)$$

$$= (Dd)_0 + \frac{V_3 V_4}{V_1 V_2} \cdot (r R_0 - r R_t) \quad (16)$$

Computation of Experimental Parameters

In Table 19 are shown the results of estimation of the apparent rate of RNA turnover $\frac{V_1 V_2}{(V_1 + V_3)r}$. In experiments B, C, and D, one can see that about 15% of the RNA adenine is apparently replaced per day and that somewhat less guanine is being renewed. The apparent deficit in the guanine turnover rate can be attributed to the additional dilution of the guanine pool from the adenine pool. The shorter the experiment, the more noticeable would be the effect, e.g., the results obtained in experiment D.

Although the data from experiments F-I are much more variable, it is clear that all the RNA of rat liver is turning over at very nearly the same rate; i.e., there is very little, if any, RNA with a markedly longer half life than the majority of RNA.

The quantity $V_3 V_4 / V_1 V_2$ is essentially the probability that an RNA purine molecule will find its way into the DNA as a result of turnover. This quantity may be computed from Eq. (16), from the data of experiments D and E, and from a knowledge of the quantities of purines present in these animals. Because these are quantities which may fluctuate from animal to animal, we have used, wherever possible, the analytical data from the

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appropriate animal. In Table 20 are presented the average amounts of the various purines found when we were certain that, besides obtaining the substances in radiochemically pure form, we had also obtained them quantitatively. There is considerably more RNA than DNA, and guanine predominates in the RNA while adenine predominates in DNA. Thus the increased activity of the DNA guanine in experiment E is mainly a reflection of this fact rather than an increase in the probability that RNA guanine will appear in the DNA. The values obtained for V_3V_4/V_1V_2 are 0.057 for adenine and 0.030 for guanine.

Table 19

APPARENT TURNOVER RATE CONSTANTS OF RNA $\left(\frac{V_1}{V_1 + V_3} \cdot \frac{V_2}{r}\right)$			
Expt	Equation	Adenine per day	Guanine per day
B	9	0.143	--
C	9	0.143	0.144
D	9	0.160	0.108
		Avg. 0.149	0.126
D + E	13	0.129	0.074
F + H	13	0.123	0.138
F + I	13	0.228	0.240
G + H	13	0.085	0.092
G + I	13	0.187	0.193
		Avg. 0.156	0.166

Table 20

PURINE COMPOSITION OF RAT LIVER

$\mu\text{M/gm liver}$		
RNA	Adenine	3.06
	Guanine	4.97
DNA	Adenine	0.85
	Guanine	0.57

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From this value one may then calculate the quantity V_4 utilizing Eq. (11) and thence the quantity $V_4/\bar{d}$, where $\bar{d}$ is the average amount of DNA present during the experiment. $V_4/\bar{d}$ is a quantity which should be relatively independent of the inaccuracies in the estimates of material and independent of the size of the animals. Estimates of $V_4/\bar{d}$ from the radioactivity measurements as well as from the liver weights are tabulated in Table 21. That the estimate from the tracer data never exceeds that obtained from the liver weights leads to the following conclusions: first, the hypothesis of Stevens⁽⁴⁾ and of others⁽⁵⁾ that when a cell divides both daughter cells receive only new DNA is incorrect and secondly, the rate of cell death and DNA degradation is insignificant compared with synthesis in these growing rats. The estimates of $V_4/\bar{d}$ for guanine are lower than the adenine figures, presumably for the same reason as they were in the RNA.

Table 21

ESTIMATE OF GROWTH RATE $V_4/\bar{d}$

Expt	From tracer data		From liver weight per day
	Adenine per day	Guanine per day	
B	0.082	--	0.084
C	0.074	0.075	0.075
D	0.072	0.058	0.087

Let us consider next the degradation of DNA. By application of Eq. (11) to the data from experiment A, in which essentially no growth occurred ($V_2 = V_3$), one computes that $V_4/\bar{d} = 0.0102/\text{day}$. This calculation is based on the assumption that most of the breakdown of DNA during this period is occurring in unlabeled cells. As we shall see below, this assumption is not completely justified; but even if one assumes that the probability of a cell dying is independent of its length of life (i.e., true turnover kinetics) it may be calculated that this would increase the estimate of $V_4/\bar{d}$ by only 20%. Thus about 1% of the cells in the adult rat liver is replaced each day. This estimate is of the order of the value of 0.15-1%/day calculated from measurements of mitotic activity by Brues and Marble,⁽⁶⁾ by Widner *et al.*,⁽⁷⁾ and others. This appears to justify the assumption of the lack of intracellular turnover of DNA.

It is of some interest to attempt to describe the shape of the mortality curve of DNA. We can make only a crude estimate of one portion of the curve with the data from experiments F-I. In the first 21 days, the RNA had become relatively unlabeled so that its contribution to the DNA during

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the subsequent period of time was small (25% at most). One can therefore compute from experiments F and G what amount of radioactivity should be expected in the DNA after 42 days. The difference between this and the amount actually found can be attributed to attrition of the cells during the three week period. These results are listed in Table 22, and the estimations are very uncertain. However, it appears clear that significant death of cells occurs during this three week period.

Table 22

PERCENTAGE LOSS OF DNA BETWEEN THE
21st AND 42nd DAYS

Comparison between animals	Adenine %	Guanine %
F + H	58	96
F + I	40	77
G + H	36	59
G + I	11	32
	<u>11</u>	<u>32</u>
	Avg. 36	66

Thus we have two facts with which to estimate mortality of the liver cell: first, the average life span of a liver cell is approximately 100 days, and, second, an appreciable fraction dies between the 21st and 42nd day. This would suggest that there is great variation in the life span of the individual liver cell, some of the cells dying after a comparatively short time and some only after hundreds of days.

Returning now to the metabolism of nucleic acids within growing cells, one may estimate all of the various parameters indicated in the scheme from the results presented and by algebraic manipulation of the various equations. These results are summarized in Table 23. Because they are calculated on the basis of the average size of rats used in these experiments, these values are approximate and should serve only as a guide for future work.

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Table 23

PARAMETERS OF NUCLEIC ACID SYNTHESIS
IN YOUNG RATS*

	Adenine $\mu\text{M}/\text{day}$	Guanine $\mu\text{M}/\text{day}$
V ₁	2.3	2.8
V ₂	2.2	3.4
V ₃	1.6	2.5
V ₄	0.18	0.113
V ₅	1.5	1.9

* It is assumed that the composition of the rat liver nucleic acid is that given in Table 20 and that the average weight of the rat is about 70 gm.

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THE BIOSYNTHESIS OF METHIONINE IN AEROBACTER AEROGENES

S. K. Shapiro

Mutants of Aerobacter aerogenes which require methionine or methylthioadenosine (MTA) for growth have been produced by irradiation with ultraviolet light.⁽¹⁾ Tracer studies have shown that the thiomethyl group of MTA is used in the synthesis of methionine by these mutants.⁽²⁾ The mechanism of utilization of MTA for methionine synthesis is being further investigated.

Growth studies have shown that MTA and methionine sulfoxide are the only compounds so far tested which can completely replace the requirement for methionine. Homocysteine permits one-half maximal growth, while cystathionine permits only one-fourth maximal growth. Methyl methionine and methionine sulfone show no growth-promoting activity whatsoever. Attempts to investigate the activity of methyl mercaptan have thus far been difficult because of the toxicity of the compound. Complete inhibition of growth of wild type Aerobacter aerogenes occurs with 10^{-5} M methyl mercaptan. Escherichia coli B is similarly inhibited by methyl mercaptan.

Ethylthioadenosine (ETA) and ethionine will not support growth of the methionine- or MTA-requiring mutants. Further studies with these compounds as possible inhibitors are planned.

In order to investigate the enzymatic conversion of MTA to methionine, cell-free extracts of both mutant and wild type cells have been prepared. Both sonic disintegration of the cells and grinding the cells with alumina have yielded active preparations. Both mutant and wild type extracts contained a potent nucleosidase which rapidly converted MTA to methylthioribose and adenine. Hypoxanthine also was formed, but in smaller quantities. Up to 1 mg of MTA can be decomposed in 1 hour by these crude extracts. In the presence of α -aminobutyric acid and MTA the extracts produced small quantities of methionine. Homoserine, however, did not promote the production of methionine.

Further experiments are planned both with growing cells and cell-free extracts in order to determine the role of the nucleoside, MTA, in the biosynthesis of methionine. These experiments may shed some light on the role of the nucleoside in transmethylation and transthiomethylation reactions. It is also hoped to obtain some information as to the particular enzyme system that has been affected in these mutants by the ultraviolet light.

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HEMOLYSIN PRODUCTION IN X-IRRADIATED RABBITS

B. N. Jaroslow and W. H. Taliaferro

The production of hemolysin to sheep red cells in previously X-irradiated rabbits is markedly enhanced if the antigen is mixed with tissue mince (rabbit spleen, mouse spleen or HeLa cells) prior to injection.^(1,2) We found that splenic mince from irradiated (400 r) rabbits possessed little if any capacity to duplicate this phenomenon.

It would appear from our latest findings that the presence of viable cells in quantity is not required in the antigen-tissue mixture. HeLa cells suspended in a phosphate buffer (pH 7.4) were ground in an Elvehjem mill and the resulting suspension was centrifuged at approximately 20,000 x g for 30 minutes at 0°C. When the supernate was mixed with the antigen and injected into irradiated (400 r) rabbits, hemolysin production was comparable to that of unirradiated controls. The question of whether the activity is associated with small numbers of cells, sub-cellular components, or is in solution is in the process of being investigated. X

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IMAGE INTERPRETATION IN ELECTRON HISTOLOGY

L. E. Roth

It has now become common practice in electron histology to allow the embedding plastic to remain in the section while viewing in the electron microscope. However, no experimental analysis of this method has been attempted even though a proper understanding of the situation is of primary importance to image interpretation of biological sections.

The method applied here was similar to that used by Shoen and Hamilton for photographic sensitometry.⁽¹⁾ Step wedges as pictured in Figure 16 were produced by multiple-angle shadowing using sodium chloride crystals to obstruct the shadowing metal. Four evaporated layers of palladium were deposited, the first vertically and the last three at arctangents $1/1$, $1/2$, and $1/3$. Each of the four deposits was calculated to be 25 angstroms in thickness so that the result was a 100 angstrom layer at all places where crystals did not interrupt the evaporating metal. When sections were mounted by immersion in 20% ethyl alcohol on grids having many of these step wedges, the crystals dissolved, leaving the gray scale pictured in Figure 17. With this method, it is possible to compare degrees of electron scattering (density) of sectioned structures (when the plastic had been removed by solvent treatment) with standard palladium densities. Photographic variations in density are eliminated by recording a gray scale in the same photograph as the section. It is also possible, on grids with no solvent treatment, to determine the density of the plastic itself in areas where no tissue was cut. The thicknesses of the sections were estimated by a fifth shadowed layer of palladium deposited at arctangent $1/5$.

It is a basic principle in electron microscopy that regardless of the chemical nature of a structure and even if the size of the structure is considerably larger than the resolving power of the microscope, there must be a difference in electron scattering between the background and the structure if the structure is to be visualized. When the embedding plastic is allowed to remain in the section, the density differences between background and object are greatly reduced; in cases of low density structures differences may no longer exist and the structure is not resolved.

The studies of Powers, Ehret and Roth^(2,3) of sections of Paramecium aurelia have provided a notable example of low density structures. In 150 to 200 angstrom sections, the trichocyst body in the unexploded state appears to have a density nearly equal to that of 25 angstroms of palladium. The embedding plastic also has a density equal to 25 angstroms of palladium when cut 150 to 200 angstroms thick. This lack of contrast is demonstrated in Figure 18 where the trichocyst body (t) appears only as an open space in the cytoplasm and has no density difference with the area of plastic alone (p).

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Figure 16
Sodium chloride crystal showing step wedge.

Figure 17
Gray scale formed after removal of sodium chloride crystal.

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Figure 18

Thin section of *Paramecium aurelia* demonstrating lack of resolution of low density trichocyst body (t). The trichocyst body has the same density as does the embedding plastic (p).

This lack of contrast is also of importance in the electron histology of the "lamina fenestrata" (endothelial layer) of the mammalian renal glomerular capillaries.^(4,5) The lamina fenestrata is a membrane-type structure with large pores of 800 to 1000 angstroms diameter. Yet when the plastic remains in the section, these pores are difficult to resolve unless the sections are thinner than 250 angstroms.

It is possible to conclude from these density comparison studies that, if the embedding plastic is allowed to remain in the section during observation, there is a marked loss of resolution in low density structures. Since large parts of biological specimens are of low density even following osmium tetroxide fixation, this loss is of major importance.

Density differences may be improved in a limited number of cases by cutting sections thinner than one-fortieth micron. The problem may also be overcome by supplementary examination of solvent-treated sections with careful examination for low density structures.

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AN ELECTRON HISTOLOGICAL STUDY OF THE
PROTOZOAN HYPOTRICH, EUPLOTES

L. E. Roth

On the basis of studies on ciliary and pellicular structures in Paramecium ^(1,2), it seemed advisable to study the locomotor organelles in a hypotrich for purposes of comparison with ciliate protozoans. Euplotes was isolated from local soil and cultured on a lettuce infusion inoculated with Aerobacter aerogenes. The organisms were fixed for one hour in 1% osmium tetroxide to which had been added 0.9% sodium chloride and MacIlvaine's citrate-phosphate buffer (pH 7.4, 0.05M). After fixation, a short wash was given in a solution containing 0.9% sodium chloride and 0.05M buffer. The cells were dehydrated in 50%, 75% and absolute alcohol and were then infiltrated with a mixture of 40% ethyl methacrylate and 60% n-butyl methacrylate. The plastic was polymerized with a combination of ultra-violet light and chemical catalyst at 30°C. The preparation was observed with an RCA EMU-2A microscope using a wide angle pole piece with a 2 mil rear focal plane aperture.

Table 24 is a summary of the features of cirri and membranelles seen, along with other structures observed. The work of Pierson ⁽³⁾ has been of considerable help and is recommended for orientation and location of structures.

Figure 19

Anal cirri, survey micrograph of a toluene-treated section.

Table 24

STRUCTURES OBSERVED IN THE ELECTRON HISTOLOGY OF EUPLOTES

Structures	Components of Structures	See Figures	Descriptive Remarks
Cirri	Cilia Small rods and tubules	19,20	Cirri are composed of 25 to 95 cilia in four to six closely-packed rows. Small rods and tubules (20-50 m μ dia) are between the cilia.
Membranelles	Cilia Small rods and tubules	21,22	Small rods and tubules located as in cirri. Membranelles composed of two or three rows of cilia.
Cilia	Longitudinal fibrils Membrane	20,22	Cilia are typical: nine peripheral and two central fibrils, all doubled.
Pellicle	Pellicular membrane Cytoplasmic membrane	19,20,22	Both membranes are two-layered. Cytoplasmic membrane has a ribbed structure associated with it.
Mitochondria	Tubules	20,22	Structure is similar to <u>Paramecium</u> (See References 1 and 2).
Cytoplasmic Rods	No internal structure yet seen	21	Diameter is about 70 m μ ; length is up to 1.5 μ , usually appearing in gullet region.
Nucleus	Granules Ground substance Membrane	21	Granules are often in contact with the nuclear membrane. Membrane shows double structure.

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Figure 20

Higher magnification
of a cirrus to show
ciliary structure.

Figure 21

Membranelles, survey
micrograph of a toluene-
treated section.

Figure 22

Higher magnification
of membranelles to
show ciliary structure.

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STRUCTURAL CHANGES OF MITOCHONDRIA DURING SPERMATOGENESIS IN THE GRASSHOPPER

T. N. Tahmisian, H. W. Beams, E. L. Powers,
C. F. Ehret, and R. L. Devine

It has long been known that mitochondria within a given species do not possess the same definite form at all times.⁽¹⁾ During spermatogenesis in the grasshopper the mitochondria undergo several morphological changes: namely, the short rods become long filaments which, after a time, congregate in one position to form the nebenkern. This mitochondrial mass undergoes internal dissolution, but retains the thin outer membrane. Then the mitochondrial bodies coalesce, resulting in a few large compartments within the nebenkern. The structure eventually divides once and elongates to touch the centrioles, at one side of the spermatid nucleus. The filaments of the sperm tail arise from the centrioles. Concomitant with this, the nebenkern grows with the filaments to form the mitochondrial structure found in the middle or tail piece of the sperm. The present investigation has been undertaken to observe changes as they occur in the living form and to study sectioned follicles at various stages of development with the electron microscope.

Prior to the formation of the nebenkern the mitochondria line up and elongate to form what has been called the "zwischenkörper." The zwischenkörper was thought to be composed of mitochondria, but two schools of thought prevailed concerning its division; namely (1) that the whole zwischenkörper bodies were equally apportioned between the two daughter cells; and (2) that the zwischenkörper divided, each mitochondrion dividing at the time of cell division, so that one-half of each mitochondrion went to each daughter cell. We have found the second condition to be true. There is evidence that each mitochondrion divides and the halves move toward the newly formed nuclei in the primary and secondary spermatocytes. At this time the mitochondria elongate into filaments resembling long thin hairs, or long tubes. Some were observed to be as long as $150\ \mu$. In other cases the two ends could not be readily discerned with the phase microscope. These filamentous mitochondria congregate in the cytoplasm about the centrioles near the nucleus. The tubular appearance is particularly evident at this point. They coil over and around each other to look very much like the mitochondria in Paramecium.⁽²⁾ Coalition begins, preparatory to the formation of the nebenkern. Evidently, walls of adjacent tubules disintegrate at the junction, resulting in fewer partitions and larger spaces within the nebenkern body. These compartments appear very much like individual mitochondria at the short-rod stage. Adjacent compartments then fuse, with disintegration of internal structure, and this mass elongates and enters the tail of the sperm.

The significance of this observation is twofold: (1) it illustrates once more that the mitochondrion is not an unchangeable entity with constant morphology, and (2) that mitochondria, once formed in the cell, can not only divide, but be formed from other sources, e.g., from the macronuclear nucleoli of the Paramecium.⁽³⁾ This observation indicates that the mitochondrial problem is not a simple one. If the mitochondrion is considered as an organelle, its morphological description can be reconciled with the various physiological functions attributed to it.

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THE EFFECT OF STARVATION AND FEEDING UPON THE
MITOCHONDRIA OF THE GRASSHOPPER
MALPIGHIAN TUBULE

T. N. Tahmisian, H. W. Beams and R. L. Devine

In a previous report we have shown that there is migration of mitochondria from the basal to the apical end of the cells comprising the Malpighian tubule of the grasshopper.⁽¹⁾ We have shown that the mitochondria eventually congregate at the apical end of the cell and in this region leave the cell proper, moving into the brush border and from the brush border into the lumen. It has been shown by Wigglesworth⁽²⁾ that the Malpighian tubule yields a proteinaceous material which could be attributed to excreted mitochondria. Since the Malpighian tubule is an organ which eliminates wastes we undertook an experiment to study what changes in morphology might occur due to abnormal function. A grasshopper was starved for several days, after which its Malpighian tubules were dissected out and fixed. The mitochondria within the tubule cells were examined with the electron microscope. At the same time another experimental animal which had been well fed was injected with saline into the abdominal region in order to force the excretion of the saline through the Malpighian tubule. Preliminary observations indicate that mitochondrial morphology was different in the starved animal. There are fewer and more compact cytoplasmic bodies which are probably of mitochondrial origin. In the case of the well fed, saline-injected insect the mitochondria seem to be swollen and more numerous. The significance of this experiment will be examined further in the near future.

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SOME STUDIES ON THE GROWTH AND SIZE OF BACTERIA

H. E. Kubitschek and H. E. Bendigkeit

Biochemical and genetic studies of bacteria often require associated biophysical measurements for the interpretation. We present here some studies on the distributions in growth and size of Escherichia coli and Bacterium megaterium and discuss some of their implications.

Size distributions of E. Coli. The distribution of bacterial lengths in cultures of E. coli, strain B in exponential growth phase has been measured by Luria and Dulbecco,⁽¹⁾ who stained the cells with nigrosin, dried them on slides, and measured relative cell lengths with an ocular micrometer.

We obtained a rather different distribution from measurements on enlargements of electron micrographs of cells of strain B/1,t taken from exponential growth phase in a chemostat (F medium). These cells were fixed immediately with a 1% solution of OsO₄. The distribution of length L shown in Figure 23, represents 490 measurements. This distribution suggests that there are three stationary phases in the elongation of E. coli. The mean length is 2.51 μ .

We also measured the width, W, of 390 of these cells and calculated surface areas on the assumption that they are cylinders capped at both ends with hemispheres of equivalent radii, $R = W/2$. The distribution in area, Figure 24, is close to being logarithmico-normal, and has a mean of $\bar{A} = 4.83 \mu$. Notice that it has no pronounced sub-peaks.

It has been customary to use bacteria in the exponential growth state for some experiments with bacteriophage. In this case a parameter of importance is the fraction P_0 of non-infected bacteria present. Usually P_0 is calculated upon the assumption that the Poisson distribution holds for the distribution of phage absorbed. Then $P_0 = e^{-\lambda}$ where λ is the multiplicity, i.e. the average number of phage absorbed per bacterium. But this is strictly true only if all cells have the same number of surface receptor spots for phage absorption, and is certainly not true for growing bacteria. If we assume the number of receptor spots is proportional to the area A of the cell then we can estimate the deviation from the Poisson distribution from the distribution in area $f(A)$ of Figure 24. Instead of $e^{-\lambda}$ the probability for non-infection will be

$$Q = \int_0^{\infty} e^{-\lambda x} f(x) dx$$

where

$$x = A/\bar{A}$$

Figure 23

The distribution of cell lengths of E. coli in exponential growth phase.

Figure 24

The surface area distribution of cells of E. coli in exponential growth phase.

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For low multiplicity λ the Poisson distribution estimates correctly to within 5%, as is shown below:

λ	P_0/Q
1	1.054
2	1.055
3	1.012
4	.950
5	.864

The distribution in volume, Figure 25, has a mean value of $0.682 \mu^3$. Thus the average cell size may be represented by a length of 2.51μ and a width of 0.307μ .

Figure 25

The distribution of volumes of *E. coli* in exponential growth phase.

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Spore size distribution. Spores of B. megaterium were suspended in water, centrifuged, and electron micrographs taken. Relative cross sectional areas were measured at 6000 X. (We thank Eleanor Moss for these measurements.) As shown in Figure 26, a 1.7-fold range in area was observed. As a companion study the same procedure was used with spores initially in 6% sucrose solution. The observed values were:

	Number measured	Mean area, μ^2	Standard deviation, μ^2
Water suspension	365	1.59	0.16
6% sucrose suspension	109	1.42	0.13

Using the distribution for spores in water suspension, the expected distribution with a mean for that of the sucrose suspension was calculated and is superimposed upon the data observed for spores from sucrose suspension. The agreement here indicates that these spores follow the same size distribution. If this is the case, spores in 6% sucrose solution have their volume reduced by 14.7%.

The osmotic pressure calculated for the observed change in radius of these spores is 33.3 atm. Since the osmotic pressure for aged coli cells is only about 8 atm⁽²⁾ this indicates that the spore wall is rather inelastic.

Bacterial division. In any realistic description of the growth of microorganisms it is necessary to know the distribution of interdivision times. The interdivision time τ is the time between the birth by fission of an organism and the instant of its subdivision to form two daughter cells. This distribution, called the τ distribution, is also directly pertinent to the description of the kinetics of growth of cells and tissues and to the precise definition of mutation rates.

Although measurements of mean cellular interdivision times are common, there have been only a few studies of interdivision time fluctuations. The only extensive study appears to be that of Kelly and Rahn.⁽³⁾ These authors counted at 5 minute intervals the total progeny of 4- to 8-cell inocula of Bacterium aerogenes. Their results have been analyzed extensively in mathematical papers by Kendall,⁽⁴⁾ and by Finney and Martin.⁽⁵⁾ The chief results of these analyses of Rahn's experiment are: 1) for Kendall's theory, an estimate of 20 successive steps being involved in cell division in B. aerogenes. 2) for Finney and Martin's theory of cell division awaiting the slowest of parallel gene duplications, some 5 genes are involved.

Method. Using phase microscopy we photographed, usually at 10 second intervals, colonies developing, on a 1 cm² nutrient agar block in a warm (37°C) stage, from a single parent cell. Division was considered to be

Figure 26

Distribution of B. megaterium spores by area

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marked by the appearance of a septum dividing the cell into daughters. Since the cells are rod-shaped and were lying on the agar plane surface, the septa, which are normal to the plane of the agar, could be easily observed. During the sixth generation the agar surface no longer remained planar and part of the population was no longer in focus.

Results. We obtained a photographic record of divisions in four colonies, each from a single parental cell. The first three of these, No. 1, 2, and 3, were photographed at 10 second intervals and in the same field of view. No. 4 was photographed more frequently. The distribution, Figure 27, is a composite of the distributions for each cell, constructed by measuring the observed distribution times in units of the median division time. The superimposed cross-hatched areas indicate the interdivision times between the first and second divisions, which were not considered in further calculations since in some cases these cells probably had not reached fully the exponential growth phase. The first three moments of the distribution are $\bar{\tau} = 1.0471$, $\bar{\tau}^2 = 1.1438$, and $\bar{\tau}^3 = 1.3080$.

Using Kendall's theory, the distribution gives a minimal estimate of 23 sequential steps for cell division. On the other hand, Finney and Martin's theory indicates that about 400 genes are involved in cell division. It seems that Kendall's theory gives predictions which are rather insensitive to the shape of the τ distribution, and perhaps Finney and Martin's theory is to be preferred.

The question of the accuracy of this distribution, besides involving statistical considerations, also involves asking the following questions: Were the cells in the exponential growth phase after the second division? And were the growth rates of the cells within the colonies dependent upon their location? The growth curves of Figures 28 and 29 provide evidence that the colonies were in the exponential growth state from the second generation on. The mean generation time for the first three colonies is estimated to be 160 frames, and 264 for the fourth.

That the growth rate is independent of location of the cell within these small colonies is implied by the following tests. An arbitrary frame with 52 cells, Figure 30, was chosen, and the interdivision times of all of these cells noted. The cells with interdivision times greater than the median, 152 frames, appear to be distributed at random within the colony. Certainly, there is no radial dependence, with large interdivision times near the center, as might be expected for very large colonies. As a further test the 7 cells surrounded by two or more rows of cells in all directions were examined. These cells have a median division time of 140 frames, not significantly different from that above. The 31 cells on the perimeter have a median interdivision time of 156 frames.

Figure 27

The τ distribution.

Figure 28
Growth curve from a single bacterium

Figure 29
Growth curves for three neighboring colonies.

Figure 30

Interdivision times for bacteria in two contiguous colonies.

Explanation: The interdivision time (in frames) for each cell is given in parentheses after the cell number. The first digit of the cell number indicates the colony from which the cell derived. The remaining digits indicate the cell lineage, e.g. 312121 and 312122 are sisters; 321112 and 321212 had a common lineage through the second generation. Cells 222221 and 222222 did not divide during the last 190 frames of observation.

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A further question of importance in setting up a mathematical theory is that of the correlation of interdivision times for successive cell divisions. Some evidence for a negative correlation was suggested in Rahn's experiments. However, our data give very little observable correlation, suggesting that a suitable mathematical description of cell division can have successive divisions stochastically independent. There is also no evidence for a parent-granddaughter correlation.

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PROTEIN SYNTHESIS IN PANCREAS

A. K. Laird and A. D. Barton

A number of cytological studies have been made during cycles of enzyme secretion in the pancreas.^(1,2,3) Feeding and injection of pilocarpine produce a liberation of stored enzyme, which results in a stimulation of enzyme (i.e. protein) synthesis; within a few hours, characteristic changes may be observed, which include an increase in cytoplasmic basophilia, especially in the vicinity of the nucleus, and an increase in the nuclear and nucleolar volume. The basophilia appears to be due to the presence of ribonucleic acid, since the structures are no longer basophilic after the sections have been treated with ribonuclease. The results of these cytological studies have led to the conclusion that there is a large increase in the RNA content when the pancreas passes from the resting condition into active enzyme synthesis.

However, in two independent biochemical studies of whole pancreas,^(4,5) it was found that the ratio of RNA to DNA remains constant throughout the cycle of protein (enzyme) synthesis. Since there is no evidence that the DNA content changes in these cells, the authors concluded that active protein synthesis in the pancreas is not accompanied by a change in the total quantity of RNA in the average cell. The results of the cytological and biochemical studies, which at first appear to be contradictory, suggest that when active protein synthesis begins in the pancreas, there is a redistribution of the RNA within the cell, rather than an increase in quantity. The finding that secretion produces a drastic decrease in the content of digestive enzymes, but no appreciable change in the total protein of the pancreas, has been interpreted to mean that secretion of enzymes stimulates a rapid synthesis of some "precursor" protein which is converted only gradually into pancreatic enzyme.⁽⁴⁾

The present program of cell fractionations of pancreas was undertaken with a number of objectives in mind: 1) to study changes in the distribution of RNA and protein within the cell during the cycle of protein synthesis 2) to detect, if possible, the sites of formation of "precursor" and active enzyme protein within the cell and 3) to determine the time of synthesis of "precursor" and active enzyme protein in the secretory cycle.

At present only preliminary experiments have been carried out, mainly for the purpose of detecting redistribution of RNA and protein within the cell. The first two experiments were performed at another laboratory under different experimental conditions from those used here, but are nevertheless similar enough to more recent fractionations to permit their inclusion in this discussion. With the exception of these two cases, each fractionation of active pancreas has been paired with that of inactive pancreas, the two fractionations being carried out simultaneously to reduce

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to a minimum errors due to variation in technique. In order to evaluate different methods of stimulating pancreatic activity, the glands have been stimulated to activity by a variety of means: feeding alone, feeding plus pilocarpine, and pilocarpine administration to starving animals. The experimental animals have been male rats, 300 to 400 grams in weight.

The results are given in Table 25. Since no variation in the DNA content of the average nucleus has been demonstrated, the results have been expressed as concentrations relative to the DNA concentration of the whole tissue. This method of expressing the results allows us to relate the observed changes to those actually taking place within the cells. In the table, the fractionations marked "A" were done at Argonne, and those marked "X" were done elsewhere. The pairs of experiments are identified by the subscript after the "A": the members of the first pair are marked A_1 , of the second pair A_2 , etc.

In spite of the variation in the experimental conditions applied to the "active" glands, it is evident from Table 25 that, in general, the quantity of protein and of RNA in the nuclear and microsome fractions of active glands tends to be greater than that of resting glands. The increases in RNA in the nuclear and microsome fractions of active glands are to some extent offset by a decrease in the non-sedimentable RNA (supernatant fluid fraction). In agreement with the results of other authors, the total quantities of RNA and protein in the cell do not show a regular change with activity nor do the components of the other cell fractions.

From these preliminary studies therefore, it appears that a redistribution of RNA does take place within the pancreas during protein synthesis, a shift occurring mainly from the very small non-sedimentable ribonucleoprotein components toward the larger nucleoprotein particles (microsomes) and the nucleus.

Since the changes observed in the preliminary experiments reported here and those reported in the literature^(4,5,6) take place within a few hours, it is evident that rapid changes are to be expected in this system. A careful time study is planned, using pancreas which is stimulated to activity by the administration of pilocarpine to starved animals, since with this procedure timing can be most accurately controlled.

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Table 25

DISTRIBUTION OF RNA AND PROTEIN-NITROGEN
IN CELL FRACTIONS OF PANCREAS

(mgm. RNA or Protein-nitrogen per mgm. DNA)

	Inactive Gland				Active Gland					
	(Starved, no other treatment)				Fed	Fed	Fed	Fed Pilocarp.	Fasted,	
	A ₁	A ₂	A ₃	A ₄	X ₁	X ₂	A ₁	A ₂	A ₃	Pilocarp. A ₄
<u>Protein-nitrogen</u>										
Homogenate	50.8	48.5	47.4	50.3	57.5	53.0	50.0		51.8	44.8
Nuclei	6.85	2.72	7.54	4.18	7.4	8.1	5.58	4.78	5.63	6.87
Mitochondria (incl. Fluffy Layer)	4.06	5.94	6.03	4.34	4.2	4.7	4.28	5.12	5.10	4.53
Microsomes	9.70	6.12*	11.3	11.5	16.9	16.8	14.0	12.4 *	11.7	12.8
Supernatant Fluid	25.7	32.2 *	25.2	32.2	24.4	20.2	28.1	26.8 *	23.0	25.7
<u>Ribonucleic Acid</u>										
Homogenate	49.3	42.4	52.0	50.8	51.2	50.6	42.8	-	63.8	53.6
Nuclei	0.25	0.82	0.84	0.33	2.2	2.9	0.51	2.4	1.45	1.8
Mitochondria (incl. Fluffy Layer)	0.52	0.69	2.37	0.48	1.15	0.96	0.69	2.22	2.26	0.86
Microsomes	14.9	6.92*	19.3	12.0	25.3	26.0	17.4	15.3 *	20.2	18.2
Supernatant Fluid	30.7	35.2 *	29.5	31.0	21.1	17.4	23.6	31.8 *	36.3	32.9

* This pair of fractionations required about twice as long to complete as did any of the others. Apparently during this time some of the microsomes disintegrated and contributed their ribonucleoprotein to the supernatant fluid fraction.

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CENTRIFUGATION OF GIANT AMOEBAE

E. W. Daniels

In the course of studies on the effects of nonirradiated, living protoplasm after microinjection into lethally X-irradiated giant amoebae⁽¹⁾ it became clear that it would be necessary to separate various components of the normal cell. The purpose of this is to analyze the capacity of these different components to prolong life or prevent death of the lethally exposed cells after microinjection. Centrifugation is one method by which this sort of separation can be accomplished. Consequently, the need arose for a rapid and convenient method of centrifuging single cells or small groups of cells.

The type of instrument originally designed by Henriot and Huguenard^(2,3) and improved by Beams,⁽⁴⁾ Beams and Weed,⁽⁵⁾ Beams, Weed and Pickels,⁽⁶⁾ and Beams and Pickels⁽⁷⁾ was used for this purpose. Although different types of rotor chambers have been made, none of the designs which were found in the literature was entirely satisfactory for the present need. The type of rotor chamber which will hold tubes in a horizontal plane is particularly desirable for this work since: 1) direct and precise measurement of the rotating radius (distance from rotor center to cell) can be taken; 2) a larger rotating radius is obtained for a given rotor diameter; 3) direct gravitational pull is made on the cell components relative to the central axis of the tube, and circulatory movements in the tube are negligible.

Some designs of horizontal type rotor chambers have proven to be satisfactory for varying purposes.^(5,6,8,9) The novel feature of the rotor chamber which is described in this report is the removable cap into which the tubes fit. The entire rotor has been made from a high tensile strength aluminum alloy, ST 75. The rotor has a base and removable cap (Figure 31) each of which contains two grooved troughs 2.5 mm in depth. These are cut to within 2 mm of the outer edge of the rotor base and to within 3 mm of the outer edge of the rotor cap. The diameter of the cap is 36 mm and the height of the cap is 7 mm. The rotor base which contains the flutings measures 15 mm from the flat top through the apex whereas the diameter is 35 mm. When fitted together the total measurement from top of rotor to apex is 21 mm. The troughs in the cap are superimposed over those in the base and accommodate four 12 mm glass tubes or two 15 mm glass tubes all of which measure 5 mm in outside diameter. The rotor cap is held in place by a 1 mm peripheral flange and by 4 flat-top machine screws which are countersunk into the cap.

The speed of the rotor at various air pressure levels was determined by the use of methods described by Beams.^(4,10) Half of the rotor cap was dyed a dark color. A strong light was focused onto the spinning

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Figure 31

Rotor for air-driven ultracentrifuge. A, cap; B, base; C, assembled rotor.

Figure 32

Centrifugal force produced by varying air pressures at different rotating radii.

rotor and reflected into a photoelectric cell which was connected to an oscilloscope and an audio oscillator. The results obtained from the rotor which is described here are presented in Figure 32.

Figure 33 shows a Pelomyxa illinoisensis giant amoeba cell which has been centrifuged in this rotor for 10 minutes at 10,000 times gravity. The nuclei are all contained in the spherical structure at the extreme right. This is the centrifugal pole of the cell. The less dense components of the cytoplasm extend toward the left or the centripetal pole of the cell. To accomplish this result, the glass centrifuge tube is partly filled with 20% gum arabic solution, then the remaining portion is filled with a buffered salt solution. The amoeba is placed in the tube. The tube is mounted in an upright position until the organism falls to the water-gum arabic interface. The rotating radius is then measured. Surface tension forces hold the liquid in the tube as it lies horizontally in the rotor. A counterbalance tube is put in and the rotor cap is put on. The desired g force is obtained by referring to the graph (Figure 32) and by adjusting the air pressure. The timer is set for the proper interval. Following centrifugation the entire contents of the tube are easily removed with a pipette.

Figure 33
Amoeba after centrifugation.

The assistance of A. H. Akerhaugen in constructing the rotor, T. J. Doody in making the glass tubes, and Atlee S. Tracy in making the photographs is gratefully acknowledged.

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EFFECT OF NONIRRADIATED, CENTRIFUGED PROTOPLASM ON SUPRALETHALLY X-IRRADIATED GIANT AMOEBA CELLS

E. W. Daniels

The effects of nonirradiated, centrifuged protoplasm on the percent survival of supralethally X-irradiated *Pelomyxa illinoisensis* cells have been studied. These experiments are of the probing type and have not been carried out on a large scale. This is a preliminary report.

One X-ray dose level (24,000 r) was used. The rate of exposure was 4,000 r/minute. This dose is twice the LD₁₀₀ for this cell species. After the exposures, the animals were fused with nonirradiated cell portions which were cut from amoebae centrifuged at forces ranging from 700 to 30,000 g. All of the X-irradiated, nonfused cells died before the 10th post-irradiation day. There were 62 of these.

The following table presents the findings to date:

Cell portion used	Centrifugal force in gravities applied for 10 min	Percent survival 10 days after X-irradiation	Number of fused cells
Light half (enucleate)	700	86%	7
	3,000	70	10
	10,000	13	15
	15,000	0	13
	30,000	0	18
Heavy half (nucleate)	700	100%	4
	3,000	100	5
	10,000	33	3
	15,000	42	12
	30,000	100	10

These data show that the centripetal or light portion of the centrifuged cell brings about some recovery but this capacity is reduced as the gravitational force applied to the nonirradiated cell is increased. The heavy nucleated halves bring about good recovery regardless of the force applied, within the limits shown.

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THE EFFECT OF X RAY ON FERTILITY IN THE MOUSE

M. H. Sanderson and S. Phyllis Stearner

The literature contains no precise information on the maximum acute exposure to total body X ray that will not produce permanent sterility in the mature female mouse. The exposures reported to produce sterility are relatively high, some even in excess of the LD₅₀-30 days in our animals. Whether the X ray was delivered to the whole animal or to the ovaries only is not always clear.

During a twelve month period of study, we have found that doses from 50-600 r at 38 r per minute were sufficient to permanently sterilize 227 BAF₁ females in a group of 229. One individual in a group exposed to 100 r produced a small litter at the first mating only, and one that received 200 r produced five litters in eleven matings. The control females mated at the same time as treated mice have produced offspring consistently throughout this study. However their fertility is beginning to decrease somewhat with advancing age.

A group of 176 LA F₁ female mice, averaging 70 days of age, were exposed to 10, 25, 50, 75, or 100 r total body X ray at a constant exposure time of 5 minutes. The mice were mated at monthly intervals beginning one month after exposure, and are now being mated for the fourth time.

Data on litters produced from the first three matings are shown in Table 26. There was only a slight reduction in the number of litters after exposure to 10 and 25 r (in one mating group an actual increase over controls was seen). However, there was a marked reduction when females had received 50 and 75 r, and no litters were born to mice that had been exposed to 100 r. Data on abortions are not complete but it may be noted that some were found even in the lowest exposure group.

Although the mean litter size from the first mating after exposure to 10 and 25 r was slightly less than that of the controls, the average number of offspring in the two subsequent matings was larger. Groups exposed to 50 and 75 r showed a marked reduction in the size of the first litter and a further reduction after the second and third matings. The presence of sex-linked lethals is suggested by the altered sex ratio in litters produced from the first mating after exposure to 50 and 75 r.

Table 26

PERCENT PAROUS, LITTER SIZE AND SEX RATIO OF OFFSPRING
OF MICE EXPOSED TO TOTAL BODY X RAY BEFORE MATING

	Controls	10 r	25 r	50 r	75 r	100 r
Mating 1						
No. Animals	71	48	32	32	32	32
% Parous	68	58	53	25	16	0
% Observed Abortions	0	0	0	0	0	0
Average Litter Size	7.7	7.5	7.2	4.1	4.0	0
% Males	44.4	49.5	45.0	37.5	40.0	0
% Females	55.5	50.5	55.0	62.5	60.0	0
Mating 2						
No. Animals	55	48	32	32	-	32
% Parous	66	62.5	69	22	-	0
% Observed Abortions	0	0	0	0	-	0
Average Litter Size	8.0	8.7	8.1	2.3	-	0
% Males	44.8	46.2	49.7	53.3	-	0
% Females	55.2	53.8	50.3	46.7	-	0
Mating 3						
No. Animals	55	48	31	32	-	32
% Parous	66	54	55	25	-	0
% Observed Abortions	0	4	3	0	-	0
Average Litter Size	8.4	8.8	9.4	2.0	-	0
% Males	47.8	44.3	47.5	50.0	-	0
% Females	52.2	55.7	52.5	50.0	-	0
% Nulliparous in Three Matings	1.8	6.0	16.0	50.0	-	100

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STUDIES OF CHROMIUM COMPOUNDS BY NEUTRON ACTIVATION
AND ELECTRICAL MIGRATION

T. R. Sato and W. P. Norris

When bone and other phosphates are irradiated with neutrons, a variety of phosphorus compounds are formed. In order to gain an insight into these reactions by analogy, the effect of neutrons upon chromates and upon several other chromium compounds has now been investigated. Although it was necessary to irradiate crystalline substances, the analysis of the irradiated products could be performed conveniently only in solution. Of the various analytical methods that are applicable to the irradiated products, electrochromatography in moist paper offered many promising possibilities. Interpretation of the observations was, however, contingent upon consideration of reactions occurring in the solutions and during the electrical migration.

Some information regarding the irradiation and the stability of chromium compounds is already available. Muxart, et al.,⁽¹⁾ Menker and Garner,⁽²⁾ and Burgus and Kennedy⁽³⁾ found no electron exchange between trivalent chromium ion, Cr^{+++} , and hexavalent dichromate ion, Cr_2O_7^- , in aqueous solution. Muxart, et al.,⁽¹⁾ Green et al.,⁽⁴⁾ and Ottar⁽⁵⁾ investigated the Szilard-Chalmers effect in sodium dichromate irradiated by thermal neutrons ($\text{Cr}^{50} + n \rightarrow \text{Cr}^{51}$). They observed that the yield of radioactive trivalent chromium ion was extremely small and that the retention of radioactive chromium in the dichromate was high, an effect that could not be attributed to electron exchange between Cr^{+++} and hexavalent chromium ion. Recently Harbottle⁽⁶⁾ determined that in a series of alkali chromates and dichromates, the total activity retained in the irradiated hexavalent form varied from 50 to 90%. In ammonium chromate and dichromate, by contrast, it was lower (18-32%).

These studies of the radioactive products formed by neutron irradiation of chromates were based upon the use of particular chemical precipitation reactions. Each precipitation reaction was restricted to the isolation of a single chemical species such as Cr^{+++} or H_2CrO_4 . Another promising approach to the investigation of the neutron-produced radioactive chromium compounds is the electrochromatographic method. In this sensitive technique, the mixture of chromium compounds is resolved by differential electrical migration from a narrow zone in moist filter paper. With a suitable background electrolytic solution moistening the paper, all the components of the mixture separate as a series of discrete zones in one operation.

Various acidic and basic solutions, such as lactic acid, acetic acid, and ammonium hydroxide, were examined as the background electrolytic solution for the separation of chromium compounds. The separability of

the zones varied greatly with this electrolytic solution. In acid solutions, the chromate ions formed long trailing zones. In ammoniacal solutions, they formed smaller, uniform, discrete zones. The separation, the identification, and the isolation of most species were accomplished best with the ammoniacal solutions. With this electrolytic solution, the trivalent chromium ions were fixed as $\text{Cr}(\text{OH})_3$ in the paper, and the nonsorbed chromate ions migrated rapidly as a distinct zone.

The ionic species formed by the neutron irradiation were identified by comparative electrical migration with authentic radioactive chromic and chromate ions and with nonradioactive, nonirradiated preparations. To this end, the radioactive products and the authentic nonirradiated chromium compounds were submitted to electrical migration in the same sheet of paper. The radioactive species, even though present in only tracer amounts, were located by radioautography. The non-radioactive substances were located by their color or by spraying the paper with a solution of diphenylcarbazide.

With the exception of the commercial radioactive chromic ions, each of the authentic preparations yielded a single migration zone. The formation of a diffuse zone of the commercial chromic ions, as shown in Figure 34, indicates the presence of impurities.

Figure 34

Radioautograph of electrochromatogram of various neutron-irradiated chromium compounds. Unwashed paper, 2 m. long with 4 M NH_4OH . 5 v. per cm. for 7 hrs. A to F, 25 cm. A, irradiated CrO_3 ; B, irradiated $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$; C, $\text{Cr}^{51}\text{Cl}_3$ from Oak Ridge National Laboratory; D, CrO_4 from Abbott Laboratories; E, irradiated K_2CrO_4 ; F, irradiated $\text{K}_2\text{Cr}_2\text{O}_7$.

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For the irradiations, crystals of CrO_3 , $\text{Cr}(\text{NO})_3 \cdot 9 \text{H}_2\text{O}$, $\text{K}_2\text{Cr}_2\text{O}_7$, and K_2CrO_4 were exposed to neutrons in the Argonne reactor for 30 days. These irradiated chromium materials were dissolved in water and examined by electrochromatography with 4 M ammonia as the electrolytic solution. In typical experiments represented by Figure 34, only two zones representing two products were obtained, the trivalent chromic hydroxide at the starting point and the rapidly migrating chromate. The Cr^{51} both in the migrating and nonmigrating zones was determined directly on the filter paper by observation of the 325 kilovolt gamma ray with a sodium iodide (thallium-activated) scintillation crystal used in conjunction with a single channel, pulse height analyzer.

The activity of the trivalent and hexavalent chromium in the respective zones was determined with the following results.

Substance irradiated	Chromium activity	
	(trivalent)	(hexavalent)
CrO_3	42.6%	57.4%
K_2CrO_4	8.6%	91.4%
$\text{K}_2\text{Cr}_2\text{O}_7$	1.7%	98.3%
$\text{Cr}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$	100 %	00.00

These results indicate that the trivalent chromium ions were formed by a Szilard-Chalmers' reaction from chromate, dichromate and chromic acid. Irradiation of the trivalent chromium compound, $\text{Cr}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$, produced only the trivalent species. Intermediate molecular fragments as CrO_3 , CrO_2^{++} , CrO^{++++} and Cr^{6+} or even Cr^0 were not detected. Some of these substances, although temporarily stable in the irradiated solids, might not remain unaltered in the ammoniacal solutions.

These observations provide further information about the mechanism of the reactions that accompany the neutron activation of chromium compounds. They show that the hexavalent radioactive chromium is not reformed from the trivalent radioactive chromium either by electron exchange or by chemical activation and oxidation. They indicate that neutron activation may not split metallic chromium from the chromium compounds. They allow two mechanisms for the production of trivalent chromium ions from chromate; namely, by direct neutron-gamma decomposition or by chemical alteration of labile intermediates such as CrO_3 and CrO_2^{++} . Similarly, these results allow two mechanisms for the formation of radioactive hexavalent chromium, for example, direct neutron addition without decomposition and decomposition to unstable intermediates that are reconverted to chromate.

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These observations are pertinent to the interpretation of the reactions of phosphates produced by neutron irradiation. By analogy they indicate that phosphorus atoms may not be split from the phosphate molecules. From this view the various radioactive compounds produced from phosphates represent partial decomposition products.

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LIQUID SCINTILLATION COUNTING OF LOW ENERGY β -EMITTERS

W. E. Kisielecki and F. Smetana

Recently a number of publications⁽¹⁻³⁾ have appeared on the determination, by liquid scintillation techniques, of specific activity of compounds labeled with low β -emitting isotopes. This procedure provides ideal geometrical conditions and lends itself to a rapid technique of assaying biological samples. It consists of dissolving the labeled material in a liquid phosphor, such as 2,5-diphenyloxazole or terphenyl-dioxane, and measuring the scintillation pulses with a photomultiplier tube. The basic principle is the conversion of high-energy ionizing radiation to low-energy radiation in the phosphor by a fluorescence process. The fluorescent ultra-violet and visible light is then converted into electrical pulses by allowing the low-energy radiation of the phosphor to impinge on the photocathode of a photomultiplier tube. The resultant electrical signals can then be amplified and counted in a standard scaler.

The following report describes preliminary measurements on a number of low-energy β -emitters utilizing liquid scintillation techniques. All measurements were made on the "Tri-Carb" Counter Model No. 314 of the Packard Instrument Company.

In Figure 35 we have plotted counting rates against the applied voltage to the photomultiplier for the various β -emitters we measured in our physical system.

Figure 35

Plot of counting rate against voltage

For the assay of water-soluble materials satisfactory measurements were obtained using a liquid phosphor prepared from 2,5-diphenyloxazole in toluene. The following was the general procedure in preparing our samples for counting.

In the case of tritium, 1 ml of tritiated water was added to 15 ml of absolute alcohol and this added to 35 ml of the scintillation solution (2,5-diphenyloxazole at a concentration of 2 mg per ml of toluene).

In Table 27 are the results comparing the measurement of tritium as water by the scintillation method against a tritium sample calibrated by ion current measurements with a vibrating reed electrometer.

Table 27

Sample No. (Tritiated water)	μc^* conc.	cpm Scintillation Measurement	%
1	.225 μc	28,300	5.8
2	.395	50,800	5.9
3	.792	96,400	5.6

*Absolute calibration of tritium made by K. Wilzbach of Chemistry Division by ion current measurement.
(Anal. Chem., 26, 880)

The results with non-calibrated solutions indicate that the instrument detects carbon-14 with an efficiency of 65-80%, tritium as a hydrocarbon with 15% efficiency, and, as noted in Table 27, tritium as water in toluene-alcohol mixtures gave efficiencies of 6%. Calcium-45 and sulfur-35 gave efficiencies greater than 60%.

In general the measurements indicate that in selected systems the technique affords high counting efficiency, a rapid and convenient method of determining specific activity, and it affords a relative check on β - particle energy. The possibility of counting mixtures of nuclides other than just carbon and tritium is being investigated.

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EFFECT OF THORAZINE AND PHENERGAN ON SURVIVAL OF RATS AND MICE AFTER LETHAL DOSES OF WHOLE BODY X-RADIATION¹

R. N. Feinstein

Two drugs of considerable current interest because of their combined anti-histaminic and autonomic-depressant actions are thorazine² [chlorpromazine, largactil, RP 4560, SKF-2601-A, chemically 10-(γ -dimethylaminopropyl)-2-chlorophenothiazine hydrochloride] and phenergan² [promethazine hydrochloride, atosil, chemically N-(2'-dimethylamino-2'-methyl) ethyl phenothiazine hydrochloride]. These two agents are of interest to the radiobiologist because they have been used for the induction of a form of hibernation, among other uses. The literature is reviewed in (1). The drug demerol (meperidine, isonipecaine, dolantil, etc., chemically N-methyl-4-phenyl-4-carbethoxypiperidine hydrochloride) was, in some experiments, combined with the other two, because of such combined use by Bénitte⁽²⁾ in the production of an artificial hibernation. The phenomenon of hibernation is in turn of interest because of the recent demonstrations^(3,4) that the hibernating state causes a delay in the onset of the radiation syndrome. Preliminary experiments indicate that the drugs mentioned may bring about an increased survival time after whole body X-irradiation.

Development of tolerance to thorazine. When an attempt was made to determine the toxicity of repeated doses of thorazine, in order to keep the animals in a depressed state for a prolonged time, it was soon observed that animals were less affected by successive doses than by the initial dose. Thus, for example, a single dose of 15 mg/kg, intraperitoneally, completely prostrated mice beginning a minute or two after injection and lasting several hours. However, if mice were given 15 mg/kg twice in one day, the same dose on the next day merely reduced the activity somewhat but by no means prostrated the mice. Since this observation might be considered a subjective one, although quite obvious, an attempt was made to demonstrate that mice made tolerant by repeated doses of thorazine were better able to withstand an ordinarily lethal dose. Table 28 confirms this expectation. Phenergan showed no evidence of tolerance development.

¹ A portion of this work was done under the auspices of the USAF Radiation Laboratory, University of Chicago, with some technical assistance from Mrs. Sylvia L. Branch.

² I wish to thank Mr. R. Michael Buckley of Smith, Kline and French Laboratories, and Dr. Daniel L. Shaw, Jr., of Wyeth Laboratories for generous gifts of thorazine and phenergan, respectively.

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Table 28

DECREASED LETHALITY OF THORAZINE TO THORAZINE-PRETREATED MICE

Pretreatment consisted of six injections (twice a day for three successive days) of either saline (10 ml/kg) or thorazine (15 mg/kg). On fourth day (or, in case of half of each group, the seventh day) animals were challenged with a single injection of 100 mg thorazine per kg. No significant differences were noted if challenging dose was administered on the fourth or seventh day, so data are pooled as shown for simplicity. Mice are CF#1 females, approximately 60 days old, sixteen mice being used for each datum above. "Average days survival" refers only to those mice dying within 30 days.

Pretreatment	% Survivors (30 days)	Average Days Survival
Saline	25	3.4
Thorazine	69	4.6

Protection with individual drugs. In a preliminary experiment (Table 29) it was shown that phenergan, administered both pre- and post-irradiation, permitted some mice to survive an otherwise lethal dose of X-irradiation. In several experiments, no indication was found of a protective effect of thorazine. In trying to establish more effective conditions for protection of animals by phenergan, further evidence was found of the partial efficacy of the drug. Table 30 is of particular interest in that it indicates that the protective action of phenergan is therapeutic, rather than prophylactic as in the case of most radio-protective agents. Table 31 indicates that phenergan exerts its protective effects to the same slight degree at any of several doses of X-ray. (The reason for the low lethality shown in this table is not known.) An attempt to increase the effectiveness of the phenergan by increasing the number of doses of the drug (all post-irradiation) was only partially effective. Table 32 shows that more mice survived 650 roentgens of whole body irradiation if treated therapeutically with six or ten doses of phenergan than if treated with only one or none at all. On the other hand, this was not reflected in the survival times.

Protection with combinations of drugs. The drug combination that has been tested most consists of phenergan, demerol, and thorazine in a single "lytic cocktail," as originally described by French workers (discussed in (1)). In some cases the thorazine has been omitted, to permit the

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Table 29

PROTECTION AGAINST X-IRRADIATION WITH MULTIPLE DOSES OF PHENERGAN AND THORAZINE

Animals were male CF#1 mice, approximately 60 days old, 16 per group. Drug was administered twice daily as shown, for total of six days (12 doses). Seven hundred and fifty roentgens of whole body X-irradiation were administered between the fifth and sixth dose of drug. Animals treated on the same drug schedule, but without irradiation, showed no mortality. Thorazine dose: 15 mg/kg. Phenergan dose: 25 mg/kg. All injections were intraperitoneal.

Treatment	% Survivors	Average Days Survival
Thorazine twice daily	0	11.3
Phenergan twice daily	31	13.9
Thorazine a.m.; phenergan p.m.	6	10.4
1:1 thorazine-phenergan twice daily	13	11.1
Saline twice daily	0	8.8

Table 30

PROPHYLACTIC vs. THERAPEUTIC PROTECTION WITH PHENERGAN AGAINST WHOLE BODY X-IRRADIATION

Animals were CF#1 female mice, 12 per group, about 10 weeks of age. Phenergan dosage was 50 mg/kg, intraperitoneally. X ray dose = 750 roentgens.

Treatment	% Survivors	Average Days Survival
Five doses before, five doses after, irradiation	0	12.4
Five doses before irradiation	0	6.8
Five doses after irradiation	0	9.2
Saline only	0	6.3

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Table 31

EFFECT OF THERAPEUTIC PHENERGAN AGAINST VARIED DOSES OF WHOLE BODY X-IRRADIATION

Animals were female CF#1 mice, 12 per group, approximately 8 weeks old at time of experiment. Phenergan administered intraperitoneally, at 50 mg/kg, once only, between five and ten minutes after completion of irradiation.

X-ray dose, roentgens	Control		Phenergan treated	
	% Survivors	Average Days Survival	% Survivors	Average Days Survival
700	58	13.0	67	12.8
650	67	13.3	75	11.3
600	75	18.0	83	15.0
550	100	-	100	-

Table 32

EFFECT ON WHOLE BODY X-IRRADIATION LETHALITY OF MULTIPLE DOSES OF PHENERGAN, ADMINISTERED THERAPEUTICALLY

First dose of phenergan was given five to ten minutes after end of irradiation. Second dose was given about 3 hours later. Further doses were given ca. 9 a.m. and 1 p.m. on succeeding days. Size of each dose was 50 mg/kg, intraperitoneally. Mice were female CF#1, about 13 weeks of age. Eleven or twelve were used in each group.

No. of Doses of Phenergan	% Survivors (30 days)	Average Survival Time, Days
None (saline only)	18	18.6
One	17	11.6
Six	58	13.8
Ten	50	9.8

Table 33

EFFECT ON WHOLE BODY X-IRRADIATION LETHALITY OF COMBINATIONS OF DRUGS

Drug combination #1 consisted of 20 mg phenergan, 20 mg thorazine, and 40 mg demerol, all per kg animal and all dissolved in the same solution. Drug combination #2 contained 50 mg phenergan and 100 mg demerol, both per kg animal and both dissolved in the same solution. Asterisked solutions indicate dose was divided and given half before, half after irradiation. The timing varied but appears to be of no significance in these experiments.

Expt. No.	X-ray, roentgens	Species	Sex	Age, weeks	No. of Animals	Drug Combination	Therapeutic (T) or Prophylactic (P)	% Survivors	Average Days Survival
1	0	Rat	M	8	6	1	-	67	0
	750					1	P	33	0
	750					1	T	50	0
	750					Saline	-	33	14.3
2	0	Mouse	F	14	12	2	-	50	0
	750					2	P	8	0
	750					2	T	33	0
	750					Saline	-	67	16.0
	0					1	-	100	-
	750					1	P	83	16.0
3	750					1	T	83	13.0
	800	Mouse	F	16	33	1	P-T*	3	9.4
	800				12	Saline	-	0	9.1
4	800	Rat	M	9	17	1	P-T*	12	13.8
	800				6	Saline	-	17	12.4

use of higher doses of phenergan and demerol. The various combinations tested, listed in Table 33, have proved less efficacious than phenergan alone.

Discussion. The initial concept behind these experiments was the induction of an artificial hibernation. Such a state has not yet been obtained here, although others have claimed,⁽⁵⁾ with only rather vague data, to have so prepared animals (rats, and especially rabbits and dogs) as to permit survival after refrigeration. Efforts will continue in this direction. Another line of attack planned is the screening of compounds related to phenergan, if such compounds can be obtained.

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PHARMACOLOGY OF AURINTRICARBOXYLIC ACID

M. W. Rosenthal and J. F. Fried

We have compared the antipyretic effects of aurintricarboxylic acid (ATA) and salicylic acid in rats in which fever was induced by a subcutaneous injection of a suspension of dried bakers' yeast in saline. Injected intravenously when the yeast-induced fever was at its height (about 18 hours), both substances reduced the body temperature to the normal level. Compared on a molar basis, a lower dose of ATA than of salicylic acid was effective, as shown in the temperature curves from a representative experiment (Figure 36). When the doses were raised to the level where salicylic acid was effective (50 mg/kg) the comparable molar dose of ATA (145 mg/kg) not only reduced the fever, but lowered the body temperature significantly below that of unfevered controls. It was found that ATA, unlike the salicylates, was also capable of lowering the body temperature of unfevered rats significantly below the normal range. This suggests that the mechanism of antipyretic action of the two substances may be somewhat different, in spite of the similarity of their molecular structures. To investigate this, the synergistic relationships of the two are now being studied.

Figure 36

Effects of equimolar doses of ATA (100 mg/kg) and salicylic acid (34 mg/kg) on rectal temperature of rats with induced fevers. One set of controls received a subcutaneous injection of saline instead of yeast at the time of the fevering injection and an intravenous injection of saline at the time of the treatment. The second set of controls was injected with yeast and then saline. Each point represents the average from five rats.

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A MODEL OF A MULTI-COMPARTMENT SYSTEM USING ISOTOPIC TRACERS AS INDICATORS

S. A. Tyler

1. Formulation

Consider a closed, dynamic, steady state system of m chains consisting of an unknown but finite number of compartments per chain (Figure 37).^(1,2,3,4,5) For simplicity, we shall identify the compartments by their subscripts, i.e., compartments containing P_0 , P_1 , and θ_{ij} are denoted $[0]$, $[1]$, $[ij]$, respectively. Each compartment contains the same exchangeable substance which differs, except in $[0]$ and $[1]$, in species only.* Each species is identifiable and uniformly mixed within its compartment and the amounts of each species are given by the symbols shown in Figure 37. The rates of material exchange between adjacent compartments are given by K and k_{ij} 's and are assumed to be constants and equal to their correspondingly opposite rate ($k_{ij} = k_{ji}$). Species amounts and physical compartmental characteristics, i.e., volume, concentration, etc. remain constant in time.

Into $[0]$ a trace amount of radioactive $P_0(P_0')$ is introduced without appreciably altering the steady state condition and is instantaneously mixed with the P_0 already present.** We consider the problem of determining the rate of exchange, K , and obtaining information about the substance in $[1]$ under the additional assumptions that

1. P_0' and P_0 can be measured experimentally and are relatively constant over time, and
2. the substance and radioactive uptake in all other compartments can only be measured collectively.

Definitions of the symbols employed follow:

m = number of chains

n_i = number of θ 's in the i th chain ($i = 1, 2, \dots, m$)

$P_0, P_1, \theta_{ij}, P_T$ = constant amounts of transportable substance in $[0], [1], [ij]$, and $[1] + \sum [ij]$, respectively
($i = 1, 2, \dots, m; j = 1, 2, \dots, n_i$)

* Material in $[0]$ and $[1]$ is exactly the same.

** Primes are used to denote the amount of tagged species present, i.e.,
 P_0', P_1', θ_{ij}' = measure of the amount of tagged P_0, P_1 , and θ_{ij} present.

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K = exchange or escape rate between $[0]$ and $[1]$

k_{i1} = exchange or escape rate between $[1]$ and $[il]$

k_{ij} = exchange or escape rate between $[i, j-1]$ and $[i, j]$ for $j > 1$

$P_0, P_1, \theta_{ij}, P_T$ = amount of labeled P_0, P_1, θ_{ij} , and P_T present

(P_1, θ_{ij} , and P_T are functions of time)

V_0, V_T = constant volume occupied by P_0 and P_T , respectively.

$$C_0 = \frac{P_0}{V_0}, C_1 = \frac{P_1}{V_T}, C_T = \frac{P_T}{V_T} \quad (\text{concentration})$$

$$C_0' = \frac{P_0'}{V_0}, C_1' = \frac{P_1'}{V_T}, C_T' = \frac{P_T'}{V_T} \quad (\text{tagged concentration})$$

$$S_0 = \frac{P_0'}{P_0}, S_1 = \frac{P_1'}{P_1}, S_T = \frac{P_T'}{P_T} \quad (\text{specific activity})$$

If an amount K of unlabeled material is transferred from $[0]$ to $[1]$ and also from $[1]$ to $[0]$ per unit time, then the net flow of labeled material from

$[0]$ to $[1]$ in time Δt is $\left(\frac{P_0'}{P_0} K - \frac{P_1'}{P_1} K \right) \Delta t$. Likewise, the net flow of labeled atoms from compartments $[il]$ to $[1]$ in time Δt is $\sum_{i=1}^m \left(\frac{\theta_{i1}'}{\theta_{i1}} - \frac{P_1'}{P_1} \right) k_{i1} \Delta t$. The

sum of these results gives the net flow, during time Δt , of labeled material into $[1]$, and the time rate of change in labeled material in $[1]$ is given by (1). Upon extending this formulation to all $[ij]$, equations (2) result.

$$\frac{dP_1'}{dt} = \left(\frac{P_0'}{P_0} - \frac{P_1'}{P_1} \right) K - \sum_{i=1}^m \left(\frac{P_1'}{P_1} - \frac{\theta_{i1}'}{\theta_{i1}} \right) k_{i1} \quad (1)$$

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$$\begin{aligned}
 \frac{d\theta'_{i1}}{dt} &= \left(\frac{P'_1}{P_1} - \frac{\theta'_{i1}}{\theta_{i1}} \right) k_{i1} - \left(\frac{\theta'_{i2}}{\theta_{i2}} - \frac{\theta'_{i1}}{\theta_{i1}} \right) k_{i2} \\
 \frac{d\theta'_{i2}}{dt} &= \left(\frac{\theta'_{i3}}{\theta_{i3}} - \frac{\theta'_{i2}}{\theta_{i2}} \right) k_{i3} - \left(\frac{\theta'_{i2}}{\theta_{i2}} - \frac{\theta'_{i1}}{\theta_{i1}} \right) k_{i2} \\
 &\vdots \\
 \frac{d\theta'_{i,n_i-1}}{dt} &= \left(\frac{\theta'_{in_i}}{\theta_{in_i}} - \frac{\theta'_{i,n_i-1}}{\theta_{i,n_i-1}} \right) k_{in_i} - \left(\frac{\theta'_{i,n_i-1}}{\theta_{i,n_i-1}} - \frac{\theta'_{i,n_i-2}}{\theta_{i,n_i-2}} \right) k_{i,n_i-1} \\
 \frac{d\theta'_{in_i}}{dt} &= - \left(\frac{\theta'_{in_i}}{\theta_{in_i}} - \frac{\theta'_{i,n_i-1}}{\theta_{i,n_i-1}} \right) k_{in_i}
 \end{aligned} \tag{2}$$

Letting $\frac{d\psi'}{dt}$ = the total change over all θ_{ij} , which is given by the sum of rate equations (2), we have

$$\frac{d\psi'}{dt} = \frac{d}{dt} \sum_{i=1}^m \sum_{j=1}^{n_i} \theta'_{ij} = \sum_{i=1}^m \left(\frac{P'_1}{P_1} - \frac{\theta'_{i1}}{\theta_{i1}} \right) k_{i1} \tag{3}$$

Adding (1) and (3),

$$\frac{dP'_T}{dt} = \frac{d}{dt} (P'_1 + \psi') = K \left(\frac{P'_0}{P_0} - \frac{P'_1}{P_1} \right) \tag{4}$$

Thus P'_T is the tagged substance at time t in [1] and all of the compartments [ij] combined.

2. The Specific Activity Equations

By dividing equation (4) by P_T we get upon simplifying*

$$\frac{dS_T}{dt} = \gamma(S_0 - S_1), \quad \gamma = \frac{K}{P_T} \tag{5}$$

* Equation (5) with $S_1 = S_T$ has been treated in detail and used by several investigators as a biological model.(4,6,7)

Assume that at times (t) close to zero, $\frac{P_1'}{P_1} \gg \frac{\theta_{i1}'}{\theta_{i1}}$. Thus equation (1) can be approximated by

$$\frac{dP_1'}{dt} \doteq K \left(\frac{P_0'}{P_0} - \lambda \frac{P_1'}{P_1} \right), \quad \lambda = 1 + \frac{\Sigma k_{i1}}{K} \quad (6)$$

Equation (6), on dividing by P_1 , becomes

$$\frac{dS_1}{dt} \doteq \delta (S_0 - \lambda S_1), \quad \delta = \frac{K}{P_1} \quad (7)$$

which yields, for the conditions $S_1 = 0$ at $t = 0$, the solution

$$S_1 \doteq \frac{S_0}{\lambda} \left(1 - e^{-\delta \lambda t} \right) \quad (8)$$

By substituting (8) in (5), equations (9) and (10) follow

$$\frac{dS_T}{dt} \doteq \gamma S_0 \left(1 - \frac{1}{\lambda} + \frac{1}{\lambda} e^{-\delta \lambda t} \right) \quad (9)$$

$$S_T \doteq \gamma S_0 \left[\left(1 - \frac{1}{\lambda} \right) t + \frac{1}{\delta \lambda^2} (1 - e^{-\delta \lambda t}) \right] \quad (10)$$

(under the initial conditions and over t values for which the approximations can be made)

Expression (10) can be fitted empirically and the constants determined by graphical methods (Figure 38).

The model pictured in Figure 37 can also be described using concentrations as well as specific activities and additional relationships between parameters of the system can be determined. Equation (4) becomes, by dividing through by V_T and rearranging,

$$\frac{d}{dt} \left(\frac{P_1'}{V_T} \right) = \frac{K}{V_T} \left(\frac{P_0'}{P_0} - \frac{P_1'}{P_1} \right) = \frac{K}{C_0 V_T} \left(\frac{P_0'}{V_0} - \frac{C_0}{C_1} \frac{P_1'}{V_T} \right) \quad (11)$$

which can be written

$$\frac{dC_1'}{dt} = \beta (C_0' - \alpha C_1') \quad (12)$$

where

$$\beta = \frac{K}{C_0 V_T} \text{ and } \alpha = \frac{C_0}{C_1} \quad (13)$$

Figure 37

$A(t)$ = Curve of empirical points
 $B(t) = \gamma S_0 \left[\frac{1}{8\lambda^2} + \left(1 - \frac{1}{\lambda}\right)t \right]$
 $C(t) = B(t) - A(t)$

Figure 38

By using a procedure similar to that employed, with specific activity as the dependent variable, equation (14) results.

$$\frac{dC_1'}{dt} = \beta(C_0' - \omega C_1'), \quad \omega = \lambda \alpha \quad (14)$$

The solution of (14), subject to the initial condition $C_1' = 0$ at $t = 0$, is

$$C_1' = \frac{C_0'}{\omega} (1 - e^{-\beta \omega t}) \quad (15)$$

and the time pattern of C_T' , subject to the restraints on t , becomes

$$C_T' = C_0' \left[\beta \left(1 - \frac{1}{\lambda} \right) t + \frac{1}{\lambda \omega} (1 - e^{-\beta \omega t}) \right] \quad (16)$$

3. Comparison of Derived Constants

We recall that the determinable constants of the system were defined, in terms of basic quantities of the model (see Figure 37), as follows:

$$\alpha = \frac{C_0}{C_1}; \beta = \frac{K}{C_0 V_T}; \omega = \lambda \alpha; \gamma = \frac{K}{P_T}; \delta = \frac{K}{P_1}; \lambda = 1 + \frac{\sum k_{i1}}{K} \quad (17)$$

These constants, individually and in combination, give much information concerning the kinetics of this model. From (17), the relationships (18) immediately follow.

$$\frac{\gamma}{\beta} = \frac{C_0}{C_T}; \frac{\gamma}{\delta} = \frac{P_1}{P_T} = \frac{C_1}{C_T}; K = \gamma P_T; K(\lambda - 1) = \sum k_{i1} \quad (18)$$

A comparison of the computed values of α and ω with those obtained using the relationships $\alpha = \frac{\delta}{\beta}$, $\omega = \lambda \alpha$ constitutes a partial check of the consistency of the computations. For convenience, the dimensionalities of the essential quantities involved are listed in the table.

4. Comments

By mathematical means, several basic constants of a multi-compartment system are approximated that yield information concerning a single, intermediate compartment which, it is assumed, can not be acquired experimentally. The treatment uses both specific activity and concentration as the defining variable. It is seen that comparisons of the derived constants resulting from both representations are required for maximum information.

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Table 34

DIMENSIONALITY OF ESSENTIAL SYMBOLS EMPLOYED

Symbols	Dimensions	Typical Units
$P_0, P_1, P_T, \theta_{ij}$	mass	μg
$P_0', P_1', P_T', \theta_{ij}'$	$(\text{counts})(\text{time}_1)^{-1}$	cts/sec
K, k_{ij}	$(\text{mass})(\text{time}_2)^{-1}$	$\mu\text{g/hr}$
V_0, V_T	volume	ml or c.c.
C_0, C_T	$(\text{mass})(\text{volume})^{-1}$	$\mu\text{g/ml}$ or $\mu\text{g/c.c.}$
C_0', C_1', C_T'	$(\text{counts})(\text{time}_1)^{-1}(\text{volume})^{-1}$	cts/sec/ml
S_0, S_1, S_T	$(\text{counts})(\text{time}_1)^{-1}(\text{mass})^{-1}$	$\text{cts/sec}/\mu\text{g}$
α	absolute	---
β, γ, δ	$(\text{time}_2)^{-1}$	hr^{-1}

The results, as presented above, are achieved by first choosing a model and assuming that certain simplifications can be imposed to allow a tractable solution. However, the discussion is motivated by the hope that the model might apply, as a first approximation, to some biological system. Evidence for such an application is suggested by the exchange of inorganic $\text{PO}_4^{\equiv}$ ions between medium and paramecia where $[1]$ represents the cellular inorganic phosphate pool and the θ_{ij} 's correspond to the different organic $\text{PO}_4^{\equiv}$ ions in the cell.⁽⁸⁾ Recent experiments on P^{32} uptake by Chlorella pyrenoidosa⁽⁹⁾ suggest also that this model might serve as a suitable approximation. It is recognized that alternative models under varying assumptions and the behavior of this model when a different model actually holds should be mentioned. This discussion we hope to present in a future paper. Then, assuming the chosen model does empirically describe an actual phenomenon, the question as to whether, experimentally, the time restriction imposed for which results were obtained is too severe, must be answered. Experiments are now being planned to investigate this point.

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A NOTE ON KIMBALL'S METHOD OF FITTING THE
MULTI-HIT SURVIVAL CURVE .

J. M. Gurian, G. A. Sacher and S. A. Tyler

Kimball⁽¹⁾ has proposed a graphical method for evaluating the constants n and k in the equation

$$S = 1 - (1 - e^{-kx})^n \quad (1)$$

describing the survival of microorganisms receiving radiation dose x . He defines his dependent variable

$$u_i = \log (1 - S_i)^* \quad i = 1 \dots p$$

and minimizes the quantity

$$V = \sum_{i=1}^p (u_i - n \log (1 - e^{-kx_i}))^2$$

This is done by calculating

$$V_{\min} = \sum_{i=1}^p u_i^2 - n \sum_{i=1}^p u_i v_i$$

where

$$v_i = \log (1 - e^{-kx_i})$$

$$n = \frac{\sum_{i=1}^p u_i v_i}{\sum_{i=1}^p v_i^2}$$

for several values of k , and plotting $V_{\min}$ against k ; obtaining a curve from which a k , giving an absolute minimum $V_{\min}$ can be found by graphical interpolation. He also presents estimates for the errors of the constants s_n and s_k

$$s_n^2 = s^2 \frac{B}{AB - C^2}$$

* log = logarithm to the base e throughout.

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$$s_k^2 = s^2 \frac{A}{AB - C^2}$$

where

$$s^2 = \frac{V_{\min}}{p-2}$$

$$A = \sum_1^p v_i^2$$

$$B = n^2 \sum_1^p \left(\frac{x_i e^{-kx_i}}{1 - e^{-kx_i}} \right)^2$$

$$C = n \sum_1^p \left(\frac{v_i x_i e^{-kx_i}}{1 - e^{-kx_i}} \right)$$

The usual procedure of fitting equation (1) is to use the least square method, which assumes that

$$(1 - e^{-kx})^n \simeq 1 - ne^{-kx}$$

for sufficiently large values of x , and fits the equation

$$S = ne^{-kx}$$

to those points that are linear on a semi-log plot. Kimball's method has the advantage over this procedure that it makes use of all of the data, regardless of the magnitude of x . However, one drawback of this procedure is that by using a transformation of S as the dependent variable, without making corresponding adjustments of the weights, the largest S are actually given an unduly large weight. Usually experiments are designed to give equal percentage variation for all S , which means constant variance σ^2 for all $\log S$. However, when $u_i = \log(1 - S_i)$ is used, the sampling variance of the dependent variable is no longer constant, but is approximately equal to $\sigma^2 \left(\frac{S}{1-S} \right)^2$, and therefore dependent on S . The proper weight for u_i is therefore $\left(\frac{1-S}{S} \right)^2$. This weights the smallest S most heavily. However, variation in dose, dilution, temperature and other agents would exert a greater effect on the variance of small S . So as not to weight the small S too greatly, we have used

$$w_i = (1 - S_i)^2$$

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as the weighting function. The use of this weighting function assumes that the error variance of S is constant. We have modified Kimball's method by including this weight in his formulas, which thus become:

$$n = \frac{\sum_{i=1}^p w_i u_i v_i}{\sum_{i=1}^p w_i v_i^2}$$

$$V_{\min} = \sum_{i=1}^p w_i u_i^2 - n \sum_{i=1}^p w_i u_i v_i$$

The equations for s_n^2 and s_k^2 remain unchanged, with

$$A = \sum_{i=1}^p w_i v_i^2$$

$$B = n^2 \sum_{i=1}^p w_i \left(\frac{x_i e^{-kx_i}}{1 - e^{-kx_i}} \right)^2$$

$$C = n \sum_{i=1}^p w_i \left(\frac{v_i x_i e^{-kx_i}}{1 - e^{-kx_i}} \right)$$

The modified method is illustrated by evaluating the constants and their errors for the data of Pomper on the survival of Saccharomyces cerevisiae exposed to varying doses of X ray. These data were used by Kimball to demonstrate his procedure. Table 35 shows the necessary calculations for obtaining $V_{\min}$ corresponding to a pre-selected k . Figure 39 displays the curve obtained by choosing several values for k and determining the corresponding $V_{\min}$. The curve seems to reach a minimum at $k = .600$. Using this value of k , we obtain $n = 2.654$, and $V_{\min} = .0012959918$. Figure 40 exhibits the two curves obtained by using Kimball's method and the modified procedure. Table 36 gives the calculations of the errors s_n and s_k for $k = .600$. Table 37 contains the constants, their errors and their confidence intervals, as obtained by the least square method, by Kimball's procedure, and by the modified method.

By this note we wish to indicate the importance of using the appropriate weighting function in using a transformed variable to fit an equation to empirical data. The weighting function used in the example is only illustrative; the choice of the appropriate weighting function depends on one's knowledge about the nature of the variability of the data. Introduction of the appropriate weights does not vitiate the simplicity of the calculations in Kimball's method.

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Table 35

EVALUATION OF THE CONSTANTS n, k IN $S = 1 - (1 - e^{-kx})$
 BY THE MODIFICATION OF KIMBALL'S METHOD

Proportion killed $1 - S_i$	X-ray dose in $r \times 10^4$ x_i	$(1 - S_i)^2$ w_i	$\log(1 - S_i)$ u_i	$.57x_i$	$1 - e^{-.57x_i}$	$\log(1 - e^{-.57x_i})$ v_i	$w_i u_i$	$w_i v_i$
.14	1	.0196	-1.9661	.57	.4345	-.8336	-.038536	-.016338
.36	2	.1296	-1.0217	1.14	.6802	-.3854	-.132412	-.049948
.79	4	.6241	-.2357	2.28	.8977	-.1079	-.147100	-.067340
.928	6	.8612	-.0747	3.42	.9673	-.0333	-.064332	-.028678
.980	8	.9604	-.0202	4.56	.9895	-.0105	-.019400	-.010084
.9944	10	.9888	-.0056	5.70	.9966	-.0034	-.005537	-.003362
.9980	12	.9960	-.0020	6.84	.9989	-.0011	-.001992	-.001096
.99950	14	.9991	-.0004	7.98	.9997	-.0003	-.000400	-.000300

$$\sum_{i=1}^8 w_i u_i^2 = .2509550716$$

$$n = \frac{.1013931340}{.0412097510} = 2.460$$

$$\sum_{i=1}^8 w_i v_i^2 = .0412097510$$

$$V_{\min} = .2509550716 - (2.460)(.1013931340) = .0015279620$$

$$\sum_{i=1}^8 w_i u_i v_i = .1013931340$$

Subsequent trials:

k :	$V_{\min}$:
.625	.0014299329
.600	.0012959918

Figure 39

Curve for determination
of minimum $V_{\min}$.

Figure 40

$S = 1 - (1 - e^{-kx})^n$ as ob-
tained by Kimball's Method
and by Modified Kimball's
Method.

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Table 36

CALCULATION OF THE STANDARD ERRORS OF n, k

x_i	w_i	$e^{-.6x_i}$	$1 - e^{-.6x_i}$	v_i	$\frac{x_i e^{-.6x_i}}{1 - e^{-.6x_i}}$	$w_i \frac{x_i e^{-.6x_i}}{1 - e^{-.6x_i}}$
1	.0196	.5488	.4512	-.7958	1.2163	.023839
2	.1296	.3011	.6989	-.3582	.8616	.111663
4	.6241	.0907	.9093	-.9051	.3990	.249016
6	.8612	.0273	.9727	-.0277	.1684	.145023
8	.9604	.0082	.9918	-.0082	.0661	.063482
10	.9888	.0025	.9975	-.0025	.0251	.024820
12	.9960	.0008	.9992	-.0008	.0096	.009562
14	.9991	.0002	.9998	-.0002	.0028	.002797

$$k = .600$$

$$n = 2.654$$

$$V_{\min} = .0012959918$$

$$\sum_{i=1}^8 w_i \left(\frac{x_i e^{-.6x_i}}{1 - e^{-.6x_i}} \right)^2 = .25390224$$

$$\sum_{i=1}^8 w_i \left(\frac{v_i x_i e^{-.6x_i}}{1 - e^{-.6x_i}} \right) = -.0872654051$$

$$s^2 = \frac{.0012959918}{8 - 2} = .00021599863$$

$$A = .035436$$

$$B = (2.654)^2 (.25390224) = 1.788415$$

$$C = (2.654)(-.0872654051) = -.231602$$

$$AB - C^2 = (.035436)(1.788415) - (-.231602)^2 = .00974363$$

$$s_n^2 = \frac{.00021599863(1.788415)}{.00974363} = .03978378$$

$$s_n = .199$$

$$s_k^2 = \frac{.00021599863(.035436)}{.00974363} = .000788044$$

$$s_k = .0280$$

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TABLE 37

CONSTANTS, THEIR ERRORS AND THEIR 95% CONFIDENCE
INTERVALS BY THREE METHODS

	Least Squares Approximation*	Kimball's Method	Modified Kimball's Method
n	2.62	2.2205	2.654
k	.6107	.525	.600
s ²	.01254†	.001408	.000216
s _n	.13**	.14	.199
s _k	.0134	.033	.028
95% Conf. Interv. for n	1.8 - 3.7	1.9 - 2.6	2.2 - 3.1
95% Conf. Interv. for /k	.57 - .65 .49 - .56	.44 - .60	.53 - .67

* Based on 6 points

** s_{log n}

$$\dagger \sum \frac{(\log S - n e^{-kx})^2}{4}$$

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IONIZATION IN HEAVY GASES OF LOW Z

S. S. Brar and L. D. Marinelli*

In a previous report (ANL-5378, p. 5) data were given on the air equivalence of lucite chambers filled with C_4F_8 and mention was made of the difficulty in obtaining saturation currents at near atmospheric pressures.

Since the cylindrical shape of the chambers used at the time left open the question whether lack of saturation was due to weakness of the electric fields at the edges of the chambers, the latter were redesigned to assume an oval shape with approximately constant electric field throughout the volume. This modification did not yield saturation currents with p.d. as high as 2880 volts.

The relative shapes of the saturation curves, however, have proved to be identical in radiation fields varying approximately from 1 to 5000 mr/hr. This has been established for chambers of about 100 cc in volume, namely with currents of 10^{-10} to 10^{-14} amperes. This fact points to the lack of saturation in columnar ionization.

In Table 38 examples of this phenomenon are given by showing the ratio of the ionization currents obtained at "extrapolated" saturation voltage and at 360 volts under various conditions. It is seen that this ratio is essentially constant for gamma radiation of Tm^{170} , Cs^{137} , Co^{60} and Ra (B + C) and varies only slightly (less than 7%) when softer radiation is used if care is taken to draw currents smaller than 3×10^{-10} amp in our 100 cc chambers.

Since it was important to establish the validity of our extrapolation to infinite voltages (by plotting I vs $1/V$), at the suggestion of Dr. Jesse, saturation curves at different pressures were obtained and the saturation values of I were plotted as a function of pressure. The two accompanying graphs (Figures 41 and 42) indicate that our procedures were valid.

Although difficulty in saturation constitutes a definite disadvantage in many applications, use of this gas should enhance the current output of existing monitoring chambers by a factor of about 7 - with fillings at atmospheric pressure and moderate voltages, at dose rates lower than about 10 r/hr. Use in the field would not impose, in general, limitations other than gas-tightness because, with C_4F_8 , one atmosphere pressure can be maintained at ambient temperatures above $21.1^\circ F$.

*Radiological Physics Division

Figure 41

Saturation current extrapolations at various C_4F_8 pressures (mm Hg)

Figure 42

Saturated ionization currents vs absolute pressure of C_4F_8

Table 38

SATURATION RATIO WITH C_4F_8 CHAMBERS

Source	C_4F_8 Current	Ratio*	Remarks
Ra	10^{-10}	1.18	Unfiltered
	4×10^{-11}	1.18	Unfiltered
	7×10^{-12}	1.16	Unfiltered
Co^{60}	3×10^{-10}	1.19	Unfiltered
	3×10^{-11}	1.18	Unfiltered
	6×10^{-11}	1.17	Unfiltered
	6×10^{-14}	1.17	Unfiltered
Cs^{137}	2×10^{-11}	1.20	Unfiltered
	6×10^{-11}	1.18	Unfiltered
Thulium	10^{-10}	1.19	Unfiltered
	2×10^{-11}	1.19	Unfiltered
	3×10^{-11}	1.19	Unfiltered
200 Kev	5×10^{-9}	1.57	Unfiltered.
	4×10^{-11}	1.22	Filtered
150 Kev	4×10^{-11}	1.25	Filtered
100 Kev	5×10^{-11}	1.21	Filtered
86 Kev	10^{-9}	1.32	Filtered
	10^{-9}	1.35	Unfiltered
54 Kev	5×10^{-10}	1.26	Unfiltered
50 Kev	3×10^{-11}	1.23	Filtered
30 Kev	10^{-10}	1.26	Unfiltered

*Value extrapolated current at infinite voltage divided by current at 360 volt.

INFLUENCE OF ILLUMINATION ON THE CRITICAL RECOVERY TIME
OF A PHOTOPERIODIC MECHANISM

C. F. Ehret

That the diurnal rhythm of the capacity to mate among certain Paramecium bursaria populations is of an endogenous nature under constant conditions, yet is readily alterable by illumination, has been reported.⁽¹⁾ The photoperiod, (or time corresponding to it endogenously) is coincident with the reactive period, and the dark period is coincident with the non-reactive period. Under normal conditions, illumination (ca. 1000 lux) throughout the non-reactive period prevents reactivity from occurring in what would be the next reactive period, suggesting the photoinhibition of a scotophilic recovery process. From other studies of photoperiodic mechanisms in plants and vertebrates the concept that the duration of a critical dark period is a major controlling factor of the measured response has emerged.^(2,3) From such studies it is not clear how recovery time may be influenced by the irradiance dosages employed either in the photoperiod or in the recovery period interruption. To answer this question, P. bursaria populations (Variety I) were irradiated during their reactive periods with polychromatic (fluorescent) light for ten hours, at dosages varying from zero to 2000 lux. They were then retained in darkness and were tested for onset of reactivity against reactive animals of complementary mating type at intervals throughout the recovery period; immediately prior to testing, each category was irradiated for 2 hours with one of several dosages varying from zero to 2000 lux of polychromatic light. The results obtained from this matrix of variables are too complex to describe in detail here. However, some general patterns resulting from seven experiments with chlorella-containing and chlorella-less animals will be noted. The results of one such experiment are shown in Figure 43. In general, endogenous recovery time (dark followed by dark) was from 11 to about 14 hours. Low irradiance in a previous reactive period prolonged recovery time, and either high or zero dose levels shortened recovery time. Light interruption before the seventh hour of the non-reactive period prolonged recovery time, and after the eighth hour shortened it. At about this critically sensitive time, early onset of agglutinative reactivity is sometimes induced only weakly and briefly without consummation in the production of conjugants, and with a subsequent prolongation of recovery time well into the normal reactive period. These experiments show that critical recovery time is a complex function of the light intensity preceding and following (or interrupting) the recovery period. The effects of ultraviolet and visible irradiances upon the more sensitive and refractory phases are being investigated.

Figure 43

The influence of illumination throughout the previous photoperiod and as an interruptor of the dark recovery period on recovery time. The photoperiod was of 10 hours, and the recovery period interruption of 2 hours, duration. Interruption immediately preceded scoring, which was for recovery of the mating reaction in chlorella-containing stock Wu 5, mt D, Variety I animals.

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A PRELIMINARY BIOLOGICAL TEST OF THE PERFORMANCE OF THE ARGONNE SPECTROGRAPH

N. J. Scully, J. Skok and C. F. Ehret

Borthwick *et. al.*,⁽¹⁾ utilizing a two-prism, glass spectrograph, established that a reversible photoreaction is involved in the germination of lettuce seed (*Lactuca sativa* L., variety Grand Rapids). This seed normally fails to germinate in darkness but does so when water-imbibed seed is exposed to light. The action spectrum for the promotion of germination for the region of 520 to 680 $m\mu$ is illustrated in Figure 44. This germination-promoting effect of light can then be reversed by subjecting the seed to far red irradiation at approximately 730 to 780 $m\mu$.

Lettuce germination experiments were carried out with the Argonne spectrograph⁽²⁾ for the purpose of comparing the performance of this new instrument with the one at Beltsville, as well as to investigate the effectiveness of the region in the blue end about which information is scant.

While the general similarity between the two action spectra is evident, certain interesting and significant differences are shown in Figure 44. The curves illustrate that in the more efficient regions of the spectrum (600 to 680 $m\mu$) less total energy was required for promotion of germination in the case of the Argonne experiment (curves 1 and 3) as compared to the Beltsville ones (curve 2). In the less efficient region, however, (560-590 $m\mu$) just the reverse was true and the difference exceeded 100% at and near 560 $m\mu$. These differences are most likely related to differences in spectral purity of the light in the two experiments. The Beltsville spectrograph possesses a linear dispersion of 3.5 $m\mu/cm$ at 600 $m\mu$ and an effective slit width of about 9 $m\mu$ was employed. The linear dispersion of the Argonne spectrograph is 1 $m\mu/cm$ at all wavelengths and the effective slit width in this experiment was 4 $m\mu$, resulting in the isolation of narrower spectral regions. By employing still narrower slit widths, a more exact resolution of this action spectrum will be possible, although its general character may remain unchanged.

Curve No. 3, Figure 44, indicates the lesser efficiency of blue light in promotion of germination of lettuce seed.

In the course of these experiments, it was observed that elevated temperatures (30° to 35°C) are as effective in inhibiting germination as is far red irradiation. It was found, further, that the action spectrum for the promotion of germination of pre-inhibited imbibed seed was the same regardless of whether the inhibition was induced by far red irradiance or by high temperature.

Figure 44

Action spectra for promotion of germination of Grand Rapids lettuce seed.
 Curve no. 1, Argonne spectrograph (in 10^4 ergs/cm²);
 Curve no. 2, Beltsville spectrograph (in 10^4 ergs/cm²);
 Curve no. 3, Argonne spectrograph (in 10^5 ergs/cm²).

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THE QUANTITATIVE SEPARATION OF FRUCTOSE, GLUCOSE, AND SUCROSE ON A CELLULOSE COLUMN

W. Chorney

By far the most effective medium for separating a large variety of substances with very similar chemical and physical properties is the filter paper sheet. Since the advent of paper partition chromatography in 1944 with the publication of Consden, et al.⁽¹⁾ on the separation of amino acids on paper by partition, many publications have appeared describing the separation of numerous types of chemical compounds. If the conditions which are essential to effective separation on a paper sheet could be duplicated on a cellulose column, then the vast store of knowledge of the behavior of substances on paper could be transferred to the cellulose column with the resultant separation of larger amounts of material.

The quantitative separation of fructose, glucose, and sucrose having R_f values of .14, .11, and .06, respectively, in *n*-butanol saturated with water has been accomplished on a cellulose column by applying the techniques developed in separating certain flavonoid compounds.⁽²⁾ Some success had been achieved in separating the above three sugars on a cellulose column which was in equilibrium with the developing solvent, but there are slight overlapping of the individual bands.⁽³⁾

The column, 3.0 cm in diameter and 55.0 cm in length, was packed with dry cellulose powder (Whatman's Standard Grade) and washed thoroughly with the solvent systems employed both to develop and to elute the sugars. The column was then dried by drawing air through it. Seventy-five mg of each sugar were dissolved in ca. 0.5 ml of water and quantitatively transferred to the top of the column to form a narrow 1 cm band which was then thoroughly dried. The column was inverted and developed ascendingly using 95% *n*-butanol. After development was complete (22 hours), the column was again inverted and the sugars were eluted. Fructose came off first, followed by glucose and sucrose. After most of the glucose was eluted the solvent was changed to 95% *n*-butanol: 5% ethanol saturated with water,¹ in order to move the sucrose off the column faster.

Individual fractions of 100 ml were collected and assayed for sugar concentration using a modified anthrone procedure for carbohydrates.⁽⁴⁾ Figure 45 shows the elution curves which were obtained for fructose, glucose, and sucrose.

¹ To 900 ml of this solution 30 ml of *n*-butanol were added to prevent separation of phases in case temperature fluctuations occurred.

Figure 45

Behavior of fructose (1) glucose (3) and sucrose (4) on cellulose column.
Flow rate .42 to .50 ml per minute.

The purity of the individual fractions was established by chromatographing an aliquot of each fraction. Each aliquot, containing approximately 40 micrograms of sugar, was spotted on large sheets of Whatman's #1 paper and chromatographed in two different solvent systems. Known sugars were spotted at the side of every chromatogram to act as reference sugars (Figures 46-51).

Results

Table 39 shows the per cent of recovery of added sugars from the cellulose column.

Table 39

Sugar	Mg applied	Mg obtained	Per cent recovery
Fructose	75	73.3	97.7
Glucose	75	77.7	103.6
Sucrose	75	73.0	97.6
Sugar X	?	6.0	--
Total	225.0	230.0	102.2

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A B C D 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

Figure 46

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S #1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- NBA SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.C.

A B C D 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59

Figure 47

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S #1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- NBA SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.C.

A B C D 60 61 62 63 64 65 66 67 68 69 70

Figure 48

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S #1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- NBA SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.C.

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A B C D 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

Figure 49

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S#1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- PHENOL SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.G.

A B C D 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59

Figure 50

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S#1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- PHENOL SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.G.

A B C D 60 61 62 63 64 65 66 67 68 69 70

Figure 51

FRACTIONS FROM CELLULOSE COLUMN S-1
 PAPER-WHATMAN'S#1 DEVELOPMENT TIME- 48 HOURS
 DEVELOPING SOLVENT- PHENOL SATURATED WITH H₂O
 VISUALIZING AGENT- BENZIDINE-TRICHLORACETIC ACID
 REFERENCE SUGARS
 A d-FRUCTOSE B d-GLUCOSE C d-MANNOSE D- SUCROSE
 2-5-55 W.G.

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Fractions 20-29 showed fructose as the main component (Figures 46 and 49). Also showing up in fractions 21-27 were tracers of three substances which gave a positive test with benzidine-trichloroacetic acid. It appears that these compounds arise from the cellulose of the column.⁽⁵⁾ The appearance of these compounds in the fructose fraction is unexplained. On the paper chromatogram, Figure 46, three faint spots lie between glucose and sucrose and theoretically should be eluted from the column between glucose and sucrose.

Fractions 32-38 (Curve 2, Figure 45) behaved like fructose in *n*-butanol (Figure 46) but in phenol ran slightly faster than fructose, as can be seen in Figure 49. More work is needed to determine whether this compound is really fructose or is an impurity that was present in the sugars that were added to the column.

Fractions 41-59 showed only the presence of glucose (Figures 47 and 50).

Fractions 60-62 showed no sugars present when tested with anthrone, but after they had been chromatographed and sprayed with benzidine, an unknown spot that moved like glucose was found. Examination with ultraviolet light revealed that these spots had tails, which indicates that they are probably not glucose, but like the unknowns present in fractions 21-27, arise from the cellulose of the column.

Fractions 63-68 contained essentially pure sucrose except for the two extraneous spots present in fraction 65 (Figures 48 and 51).

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DEGRADATION OF TETRAMETHYL GLUCOSE

J. Glattfeld

The study of the step-wise degradation of tetramethyl glucose to products containing smaller numbers of carbon atoms has been actively pursued in the past months. The steps in the first stage of the degradation may be represented as follows:

All of these steps have been reported in the literature but, because of the meagreness of published experimental detail, much time was consumed in establishing proper experimental conditions for best results. These conditions have now been established for steps 1 and 2. More work will have to be done on steps 3 and 4 although the experiments so far carried out give promise of success.

THE USE OF Cr^{51} AND P^{32} FOR LABELLING CHICKEN ERYTHROCYTES

M. Sanderson, S. P. Stearner, and W. E. Kisielewski

Roosters exposed to X rays in the lethal range develop dilatation of the peripheral blood vessels, subcutaneous edema, and petechiae. Since these findings suggest an alteration in circulating blood volume, preliminary studies were begun to select radio-isotopes suitable for the determination of avian blood volume with labelled erythrocytes.

The use of Cr^{51} as a label for human erythrocytes was first reported by Gray and Sterling.⁽¹⁾ They obtained 80-90 per cent uptake of radioactive sodium chromate by the red cells after two hours at room temperature. There was little loss of activity from the circulating blood for several days after auto-transfusion of labelled cells. This prolonged retention of chromium by the erythrocytes makes it a more desirable tracer for the in vitro labelling of red cells than is phosphorus, which is lost, on the average, at the rate of 6 per cent per hour.

Five cc. of rooster blood were incubated with 50-100 microcuries of sodium chromate (pH 6.5) at 37.5°C for thirty minutes. The cells were washed three times with saline and then resuspended in saline. The uptake by the erythrocytes ranged from 70-90 per cent, depending on the amount of activity added. These results, obtained on nucleated erythrocytes, are very similar to those reported for non-nucleated human red cells.

Radioactive phosphorus has been used for many years in the study of red cell metabolism and as a red cell label for determinations of red cell mass. Incubation of mammalian erythrocytes for one hour with P^{32} results in 30 per cent uptake. No reports have been found in the literature of attempts to label nucleated cells in vitro with P^{32} . However, Hevesy and Ottesen have described the in vivo uptake of radioactive phosphorus by hen erythrocytes when it was administered for several days.⁽²⁾ Under these conditions the P^{32} is incorporated into the desoxyribose nucleic acid of the nucleus as the cells are formed and there is no subsequent loss of activity from cells so labelled.

Rooster blood was incubated with 500-1000 microcuries of radioactive sodium phosphate (pH 6.5) at 37.5°C for one to five hours. The cells were washed four times with iced saline and then resuspended in saline to the original volume. An uptake of 2 per cent per hour was found consistently. After five hours incubation, total uptake was approximately 10 per cent. Auto-transfusion of such labelled cells was followed by an almost immediate loss of half of the activity from the circulation. The remaining

activity, roughly 1 per cent of the original amount, was more firmly bound and the rate of loss from the circulation was similar to that reported for mammalian blood. In order to use P^{32} for the in vitro labelling of bird erythrocytes, it is essential to remove the loosely bound P^{32} before injecting the labelled cells.

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MOUSE MANGE

II. Acaricide Screening Experiments

R. J. Flynn

The following acaricides were subjected to limited experimental trials: pine oil with pyrethrins, Aramite®-15W, Pyrenone 10:1, DMC®, and the gamma isomer of benzene hexachloride (BHC).

Materials and methods: CF #1 female mice, 8-9 and 43-44 weeks of age, were housed in plastic boxes containing wood shavings and maintained on Rockland Mouse Diet and water ad libitum. The mice were treated with acaricide by placing them in a perforated aluminum box, completely submerging them in the test liquid for one second, partially submerging and force-swimming them for 15 seconds, completely submerging them again for one second, and then placing them in a clean cage with clean bedding. Observations for the presence of living mites were made 24 hours after dipping by killing the older, known infested mice and examining them for 15 minutes each with a dissecting microscope.

Results: A 2% pine oil with 0.1% pyrethrins emulsion was found to be incompletely effective against Myobia and a 3% pine oil with 0.15% pyrethrins emulsion was found to be toxic.

A 20% Pyrenone emulsion was found to be incompletely effective against Myobia and toxic.

Solutions of DMC®, 0.4%, 0.3% and 0.2%, using 50% ethyl alcohol as a wetting agent and solvent, were found to be moderately toxic to the younger mice and acutely toxic to the older mice. Stoner and Hale⁽¹⁾ using 0.4%, 0.2%, 0.1%, and 0.05% solutions with 50% ethyl alcohol found the 0.4% solution to be lethal for 11% of the mice tested (age not given), and the 0.1% and 0.05% solutions to be ineffective.

A 0.05% emulsion of BHC, using chloroform and paraffin oil as solvents, was found to be toxic, and a 0.05% solution, using ethyl alcohol as the solvent, was found to be incompletely effective against Myobia.

The 1%, 2% and 4% suspensions of Aramite®-15W (Table 40) appear to be effective for both Myobia and Myocoptes and none of these concentrations appears to be toxic to mice.

Conclusions: Although the number of animals used is small, it appears that of the substances tested, only Aramite®-15W has a broad effective non-toxic dose range and warrants further study as a possible acaricide for laboratory mice.

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Table 40

ACARICIDAL ACTIVITY OF ARAMITE® -15W AND TOXICITY TO MICE

(CF#1 female mice, 8-9 and 43-44 weeks of age, were dipped once.
Observations for effectiveness were made at 24 hours.)

Aramite® - 15W in suspension (per cent*)	Number of mice	Age (weeks)	<u>Myobia</u> observed (approx. no. per mouse)		<u>Myocoptes</u> observed (approx. no. per mouse)		Number of deaths	Group weight (in percent of original weight)				
			Dead	Alive	Dead	Alive		Days after dipping				
								0	1**	7	14	30
1	7	8-9					0	100.0	103.4	106.1	114.3	122.4
1	3	43-44	20-30	0	20-30	0	0	100.0	104.1			
2	7	8-9					0	100.0	102.8	107.7	116.2	122.5
2	3	43-44	20-30	0	20-30	0	0	100.0	99.0			
4	7	8-9					0	100.0	103.2	107.0	112.0	123.4
4	3	43-44	20-30	0	20-30	0	0	100.0	104.2			
Undipped controls	7	8-9					0	100.0	102.4	108.2	116.3	122.4
Undipped controls	3	43-44	5-10	10-20	5-10	10-20	0	100.0	100.0			

*0.2% Aerosol OT (wetting agent) added to all solutions.

**All mice 43-44 weeks of age were sacrificed at 24 hours for effectiveness observations.

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REFERENCE

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MOUSE MANGE

III. Effectiveness and toxicity of Aramite®-15W

R. J. Flynn

Aramite®-15W was tested for effectiveness against Myobia musculi and Myocoptes musculus using mice known to be heavily infested with these mites. Its toxicity for both young and old mice was also studied.

Materials and methods: Heavily-infested 18-month-old CAF₁ male and female mice were used for the effectiveness experiments. Both the older CAF₁ mice and 7-8 week old CF #1 female mice were employed in the toxicity studies. All mice were housed in plastic boxes containing shavings and were fed Rockland Mouse Diet and water ad libitum.

The mice were dipped in the test solution in the same manner as in the screening experiments. When the animals were treated twice, the second dipping was made 7 days after the initial dipping.

The observations for effectiveness were made with two different techniques. The direct observations were made by killing a mouse and searching between the hairs with the aid of a dissecting microscope. The indirect observations were made by killing a mouse and placing the carcass on black paper entirely surrounded by cellophane tape, sticky side up. As the carcass cooled the myocoptic mites, and to some extent the myobic mites, left the carcass and were confined to the paper or caught in the cellophane tape. The number of mites so trapped were counted and identified using a cross-ruled piece of plate glass and a dissecting microscope.

Results: Concentrations as low as 0.0625% (Table 41) were noticeably effective and the 2% suspension was found to be completely effective. The mites that were trapped at the 0.5 and 1% levels were practically all larval forms and could have possibly hatched after the treatment. With the addition of a wetting agent (0.1% Nacconol), concentrations as low as 0.5% were found to be completely effective.

In order to ascertain whether the mites were driven off the mice, temporarily disabled, or killed in place, a group of 30 heavily-infested mice was dipped in a 2% suspension plus 0.1% Nacconol. One mouse was sacrificed immediately and put on the paper and tape. Thereafter one mouse per day was killed and examined microscopically in addition to being placed on the paper. No mites left the carcass of the first mouse or any of the succeeding mice and only dead mites were observed microscopically

Table 41

ACARICIDAL ACTIVITY: CAF₁ MICE 18 MONTHS OLD, DIPPED ONCE,
EXAMINED AT 24 HOURS, 3 MALES AND 3 FEMALES PER GROUP.

Aramite®-15W suspension (per cent)	Living <u>Myobia</u> observed microscopically (per mouse) (approximately)	Living <u>Myocoptes</u> observed microscopically (per mouse) (approximately)	Living <u>Myobia</u> trapped (per mouse)	Living <u>Myocoptes</u> trapped (per mouse)
2	0	0	0	0
1	0	0	0	1
0.5	1-5	0	<1	5
0.25	1-5	0	1	18
0.125	5-10	5-10	<1	30
0.0625	10-20	10-20	1	55
2*	0	0	0	0
1*	0	0	0	0
0.5*	0	0	0	0
0.25*	1-5	0	0	3
0.125*	5-10	5-10	0	7
0.0625*	10-20	10-20	2	27
Controls*	TMTC	TMTC	12	1550†
Controls**	TMTC	TMTC	28	2144†
Controls***	TMTC	TMTC	36	1979†

* 0.1% Nacconol Solution

** Tap water

*** Not dipped

† Only 1 male and 1 female actually counted

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on the mice. The single dipping in the 2% suspension not only killed all living mites but either prevented the eggs from hatching or had enough residual effect to kill the larvae as they emerged.

During these studies, it was incidentally observed that Aramite®-15W, in all concentrations employed, was effective against the louse Polyplax serrata.

The results of the toxicity experiment using 7-8 week old female CF #1 mice are shown in Table 42. Concentrations as high as 4% (plus 0.1% Nacconol) applied twice were found to have no toxic effect upon these mice as measured by the weight curve.

Fifty male and 50 female 18-month-old CAF₁ mice were dipped twice in a 2% suspension plus 0.1% Nacconol. Equal numbers were used as controls. During the first 30 days after the initial dipping, 4 treated and 3 untreated mice died.

Conclusions: It appears that a single dipping in a 2% suspension of Aramite®-15W plus 0.1% Nacconol will completely free a mouse of Myobia musculi, Myocoptes musculinus, and Polyplax serrata, with no apparent toxic effects to the mouse.

Clinical trials and chronic toxicity studies appear warranted and have been initiated.

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Table 42

TOXICITY: CF #1 FEMALE MICE 7-8 WEEKS OLD,
DIPPED ONCE OR TWICE, 15 MICE PER GROUP.

Aramite®-15W suspension* (per cent)	Number of times dipped	Group weight in per cent of original weight					
		Days after initial dipping					
		0	1	7	14	21	30
1	1	100.0	100.9	101.8	105.6	109.3	115.7
1	2	100.0	101.6	101.3	107.4	110.6	118.7
2	1	100.0	101.6	105.2	112.8	118.0	123.9
2	2	100.0	100.9	105.7	111.7	117.1	125.0
3	1	100.0	101.6	104.0	112.2	117.2	126.0
3	2	100.0	101.3	104.9	113.9	120.1	127.7
4	1	100.0	100.7	105.4	115.6	120.0	128.3
4	2	100.0	102.3	105.6	113.5	118.5	126.7
Undipped Controls		100.0	103.3	104.7	107.0	117.0	123.4
Undipped Controls		100.0	102.3	105.3	114.2	117.8	125.4

* 0.1% Nacconol Solution

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