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BIOLOGY DIVISION

QUARTERLY REPORT

JUNE, JULY, AUGUST 1948

By

ALEXANDER HOLLAENDER

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BIOLOGY DIVISION

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QUARTERLY REPORT

June, July, August 1948

by

Alexander Hollaender

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Table of Contents

	Page No.
Cytogenetics Section - - - - - - - - - - - - - - - -	6 - 23
Microbiology Section - - - - - - - - - - - - - - - -	23 - 25
Biochemistry Section - - - - - - - - - - - - - - - -	25 - 28
Radiobiochemistry Section - - - - - - - - - - - - - -	28 - 35
Mammalian Genetics Section - - - - - - - - - - - - -	35 - 36
Physiology and Pharmacology Section - - - - - -	37 - 44
Abstracts of papers to be given by members of the Biology Division at professional societies meeting in September, 1948 - - - - - - - - - - - - -	45 - 53

[redacted]

[redacted]

BIOLOGY DIVISION

QUARTERLY REPORT - JUNE, JULY, AUGUST 1948

by

Alexander Hollaender, Director

The rebuilding of the second floor of the four-story building (9207), will be completed during the next few days. Partition walls on the third floor of this building have been erected. The rebuilding of the 9210 building for the Mouse GeneticsFarm is progressing slowly. Apparently there will be aome delay on account of difficulties in the air conditioning system. It appears that the Mammalian GeneticsGroup will not be able to move into this building until January 1, 1949.

This Division has been of assistance to the Oak Ridge Institute of Nuclear Studies in connection with their classes in Radioisotope courses. We have had open house for each one of these classes. Usually this is held during the last week of the course. Many individuals from these classes have visited this Laboratory for the purpose of discussing their problems in connection with isotope work.

Dr. Norman H. Giles, Jr., spent the last ten weeks at the Donner Laboratory at the University of California to work in cooperation with Dr. Tobias and Dr. John Laurence on the effect of deuterons on Tradescantia chromosomes. The work of Dr. Giles at Donner Laboratory has turned out successfully. Preliminary results indicate that there is a pronounced effect of ionization distribution since the less energetic, more densely ionizing deuterons near the end of the Bragg curve, are much more effective in producing rearrangements.

[redacted]

A special thermal neutron treatment chamber for work on biological materials has been constructed. This makes possible the treatment of biological materials with high thermal neutron flux and very little contamination from fast neutrons and gamma rays.

It has been found that the nucleolus of the grasshopper neuroblast is extremely sensitive to ultraviolet radiation at a wavelength shorter than 3000 A. In contrast to this, the nucleolus is very resistant to x-rays.

The work on the effect of radiation on micronucleus mutations of Paramecium has given interesting results which are now being written up for publication.

It has been found that the ability of the algae Scenedesmus D. to grow is considerably more sensitive to ultraviolet radiation than to the faculty to photosynthesize.

In studying the effect of ionizing radiation on living rabbits, it was found that the desoxyribonucleic acid production in the bone marrow is extremely sensitive to this radiation, whereas the ribonucleic acid is less so. It was found in the irradiation of nucleoproteins in vitro, that this material is very resistant to ionizing radiation, whereas it is very sensitive to ultraviolet. This is in contract to the isolated sodium thymus nucleate which is very sensitive to both ionizing radiation as well as ultraviolet.

The work on the studies, separation and purification of carrier-free beryllium has been completed and is being written up for publication.

Very successful work has been done on the use of the ion-exchange methods for the separation of nucleic acid components.

A number of reports on the use of different chemicals in the prevention of radiation sickness have been completed; also a report on the

effect of local beta irradiation on the growth of bone and other tissues of rats has been written.

CYTOGENETICS SECTION

Tradescantia - Experiments are being carried on in cooperation with Dr. Cornelius Tobias of the Donner Laboratory using the 190 Mev. deuteron beam of the 184-inch cyclotron at Berkeley to produce chromosomal rearrangements in Tradescantia microspores. Preliminary results indicate that there is a pronounced effect of ionization distribution since the less energetic, more densely ionizing deuterons near the end of the Bragg curve are much more effective in producing rearrangements. (Giles, Giles, and Flynn).

Quantitative experiments with Neurospora on effect of malate and other dicarboxylic acids on microconidia production were carried out using the Yale microconidial colonial strain, 8743-45. Observations were as follows:

Microconidia do not ever form on minimum medium.
Microconidia form in approximately 12-14 days on complete medium.
Microconidia form in approximately 6 days on malate and 1.5% sucrose.
Microconidia form in approximately 9-14 days on thick malate sucrose.
Microconidia form in approximately 5-6 days on thin malate sucrose.
Succinate or fumerate will replace malate.
Glucose but not lactose will replace sucrose.

Conclusions were as follows: Dicarboxylic acids and sugar appear to be related in the formation of microconidia but not macroconidia. Nothing formed by colonies either about to conidiate or an extract of conidiated colonies has any conidia formation stimulating activity that would not be expected from the content of malate and sucrose. Apparently the earlier production of conidia on thin plates is due to drying. Crowding of colonies on a plate has a similar effect. Bacillus subtilis mutant studies carried out have indicated that the synthesis of threonine and methionine is for

the most part similar to that found in Neurospora. X-ray induced reversions from what appears to be the only easily revertible threonine-methionine mutant indicate a single gene difference as opposed to separate mutations in threonine and in methionine synthesis.

Experiments using canavanine,

$$NH_2 - \overset{\overset{NH}{\|}}{C} - \overset{\overset{H}{|}}{N} - O - CH_2 - CH_2 - CHNH_2 - COOH$$

have indicated that it can replace homoserine,

$$HO - CH_2 - CH_2 - CHNH_2 - COOH$$

Suggesting that Bacillus subtilis has enzyme systems other than the reported arginase which cause changes in guanido- and guanidino- compounds. More tests are being made with related compounds. Paper strip chromatographic techniques were developed to aid in identification of amino acids from N. filtrates and extracts. Experiments have been carried out toward developing a conidial germination assay for microgram amounts of homoserine, an amino acid for which there is no known test and of as yet unproven natural occurrence. Tergitol appears to be useful in causing a higher percent of conidia to germinate under given amino acid concentrations. (Teas and Gower).

The construction of the Biological Thermal Neutron Treatment Chamber has been completed, and some determinations of its physical characteristics have been made. These are: 1. The absolute thermal neutron flux. 2. Variation of thermal flux with variation in pile power level. 3. The ratios of thermal flux at different positions in the chamber. 4. The amount of fast neutrons contamination present - from measurements of the Cadmium Ratio. The values found agree quite well with the values predicted before construction of the unit. Absolute flux has been determined by two methods - Indium activation calibrated against the standard pile, and cobalt activa-

tion - and the agreement is good. The following remain to be determined or accomplished before the chamber can be utilized properly: 1. Measurement of the amount of gamma ray contamination present. This has proved to be the most difficult problem of all. Theoretical calculations indicate that it is quite low and will contribute only a few per cent of the total biologic effect induced. 2. Determination of the temperature (degrees Centigrade) within the chamber. 3. Determination of the approximate R-equivalent biologic effect to be expected from the neutron flux available; 4. The construction of a lead shielding wall around the access door to protect personnel inserting or removing the treatment chamber. At present, the tolerance period at the access door is only two minutes. (Conger and Stapleton).

The relative efficiency of seven different wavelengths of ultraviolet in producing a given effect on the nucleolus of the grasshopper neuroblast have been determined, and studies of other wavelengths are in progress. A Daniels-Heidt mercury vapor lamp and monochromator were used to obtain the monochromatic light. Measurements of intensity were made with a thermopile connected to a high sensitivity galvanometer. For treatment, embryos were mounted in a hanging-drop suspension on a quartz cover slip and placed in the same position of the beam that has been measured immediately before. Normally the neuroblast in interphase through late prophase has two large irregular nucleoli. Treatment with ultraviolet causes these nucleoli to form clusters of several separate spherules with increased refractility. The lower doses which do not produce an effect in all the cells do not necessarily cause the formation of these clusters, but may result only in the rounding up and increasedrefractivity of the nucleoli.

Spherical shape, whether of several small nucleoli or one large nucleolus, and increased refractivity are used as an indication of a positive effect of ultraviolet. All the neuroblasts of a treated embryo which were not covered by other cells were observed and recorded as showing or not showing a nucleolar effect. The smallest dose necessary to produce an effect, the largest dose which produces no effect and the gradation in effect with doses between have been determined for 2250 A, 2397 A, 2537 A, 2650 A, 2804 A, 2967 A, 3022 A, and 3130 A to date. (Carlson and McMaster)

The investigation of micronuclear mutations induced by radiation in Paramecium aurelia has been concentrated this quarter on two problems: (1) inheritance of the mutations; (2) comparison of the effects of 250 KV X-rays with those previously obtained with beta rays from P^{32} plaques. In addition, a paper on the induction of mutations by beta radiation was completed and submitted for publication.

The major purpose of the studies of inheritance was to clearly demonstrate that mutational changes in the micronuclei were indeed involved in producing the reduced vigor which appears in the exautogamous progeny of rayed animals. With the addition of data gathered during the quarter, the studies are now essentially completed and will be summarized in some detail in this report. The plan of the experiments was as follows. Animals were exposed to beta radiation or to X-rays, allowed to multiply by cell division, and a part of the resulting progeny were sent through autogamy. Twenty-five autogamous animals were isolated from the progeny of each rayed animal. These autogamous animals will be called the A1 generation. Another group of descendants by cell division from each rayed animal was crossed by conjugation to unrayed animals, and the two members of each conjugant pair were put in separate containers and allowed to multiply. This

exconjugant group will be called the BC1 generation. Some of the decdendants by cell division from each member of the conjugant pairs were allowed to go through autogamy and twenty-five autogamous animals were isolated from each. This group will be called the A2 generation. Unrayed animals from the same clone as the rayed ones were carried through this same procedure to serve as a control. Tests for vigor were made by allowing each exautogamous or exconjugant animal to multiply for four days in a limited quantity of culture fluid. Those which produced distinctly fewer progeny in this time than the controls were recorded as of reduced vigor.

Autogamy makes the animals homozygous for any genes which may have become heterozygous as a result of mutation. Different autogamous animals may be homozygous for different combinations of mutant genes as a result of segregation during gamete formation. Exconjugants between rayed and unrayed animals will be on the average heterozygous for some of the mutant genes from the rayed animals as a result of segregation and subsequent reciprocal fertilization of the two members of a pair by each other. The two members of a conjugant pair should have identical genotypes but different conjugants pairs from the same rayed animal may be of different genotypes as a result of segregation. The group of twenty-five autogamous animals from each rayed animal and from each exconjugant provides a test of the genotype of these animals. The more mutant genes in a rayed animal or an exconjugant, the more probable it will be that a given autogamous descendant will be homozygous for one or more of them. If reduced vigor is due to such mutants, the greater the number of mutant genes in a heterozygous animal, the greater will be the fraction of its autogamous progeny which will be of reduced vigor. In a previous report, it has been shown that the dosage data led to the conclusion that a minimum of four

homozygous mutants are needed, on the average, to produce a detectable reduction in vigor. Assuming that this is true, it is possible to calculate the mean number of mutant genes per micronucleus in a group of heterozygous animals from the fraction of their autogamous progeny that are of reduced vigor. Certain expectations concerning the quantitative and qualitative outcome of the breeding experiments follow from what has been said above. Of these, it has proven feasible to test the following. (1) The BC1 generation should include considerably fewer clones of reduced vigor than do the A1 or A2 since only mutant genes with dominant effects could express themselves in the BC1 whereas, in the A1 and A2, both dominants and recessives would have an effect. (2) A high frequency of reduced vigor should often appear in the A2 progeny of both members of the BC1 pairs despite the fact that one member gets its cytoplasm almost entirely from the unrayed ancestor, for the genes of the exconjugants are derived equally from the two members. (3) The two members of a conjugant pair should be of identical genotype after conjugation though different pairs from the same rayed animal may be different. (4) The BC1 generation should have, on the average, half as many mutant genes per micronucleus as the rayed animals from which they were derived. The first expectation is shown to be met by the data in table 1. It is clear that there are considerably fewer clones of reduced vigor in the BC1 than in either the A1 or the A2 of the rayed group. In fact the BC1 from rayed animals is not clearly different from that of controls. Thus no evidence is provided by the data for dominant effects though perhaps these occur but are sufficiently rare so as not to be detected in the present material. The second expectation is shown to be met by the data in table 2. The table shows the fraction of A2 progeny of reduced vigor for ten BC1 pairs chosen at random from rayed material and ten chosen at

[redacted]

Table 1

Comparison of the fraction of clones of reduced vigor in the A1, BC1, and A2 generations

Treatment	Generation	Number of rayed or control animals	Number of clones of generation indicated	Fraction of clones of reduced vigor
Rayed	A1	67	1673	0.77
	BC1	57	1186 (593 prs.)	0.08
	A2	49	6744	0.46
Control	A1	29	727	0.02
	BC1	22	560 (280 prs.)	0.06
	A2	17	2271	0.04

Table 2

The fractions of clones of reduced vigor in the A2 generation of the two members of BC1 pairs chosen at random from the data for ray rayed and control animals.

Treatment	Rayed		Control	
Member of pair	a	b	a	b
Fraction of clones of reduced vigor	0.96	0.68	0.04	0.05
	0.00	0.28	0.00	0.08
	0.48	0.40	0.00	0.00
	0.52	0.52	0.00	0.04
	0.56	0.40	0.04	0.00
	0.40	0.68	0.00	0.00
	0.88	0.96	0.00	0.00
	1.00	1.00	0.16	0.00
	0.52	0.96	0.00	0.00
	0.60	0.44	0.00	0.04

[redacted]

random from the controls. Data from the A2 is available for 124 BC1 pairs from rayed material and 45 from controls. In addition, there were a number of cases in which an A2 generation was gotten from one member of a pair but not from the other. All these are included in table 1. It is obvious from table 2 that the effect of radiation as expressed in high fractions of reduced vigor was transmitted to both members of the BC1 pairs. It should be noted that the designations "a" and "b" for the two members of the pairs were given arbitrarily and have no relation to the rayed or unrayed ancestry of the member. With the techniques used, this ancestry was not known though it was known that one member was from a rayed animal and one from an unrayed one. A test to demonstrate the identical genotype of the two members of a pair is somewhat more difficult to make. Even if the two members of a pair were identical after conjugation, different fractions of reduced vigor might be found in their A2 progeny for at least two reasons. In the first place, sampling variation is expected to be large with groups of 25 autogamous animals. In the second place, spontaneous mutation may occur during the two to three week period which must elapse between conjugation and autogamy. Consequently, the descendants of the two members of a pair might be of different genotype even though the exconjugants themselves were identical. However, variation due to differences between members of pairs should be less than variation due to differences between pairs derived from the same rayed animal since different pairs from the same rayed animal are likely to be of different genotype as a result of segregation at conjugation. Therefore, a test to see if this were true was carried out using the analysis of variance technique. For this purpose, the fractions of reduced vigor were converted to angles according to the method given in Snedecor's "Statistical Methods" and the

results of the analysis are given in terms of angles. It was necessary to remove from the total variance that part attributable to differences between rayed animals, and for this purpose it was desirable to have equal numbers of pairs from the different rayed animals. This was not true for the original data so two pairs were chosen at random from each rayed animal, and this random sample of the total was subjected to analysis. The total number of pairs was 74 derived from 37 rayed animals. The results are shown in condensed form in table 3. As shown by the large mean square, much of the total variation can be attributed to differences between the progeny of different rayed animals as would be expected since they receive different doses of radiation. The mean squares for differences between pairs from the same rayed animal and for differences between members of the same pair give a variance ratio of 2.35 which has a probability of occurrence of less than .01 if the two were actually the same. Thus, it can be concluded that the two members of a pair tend to be more alike in their autogamous progeny than do different pairs from the same rayed animal. In view of spontaneous mutation, this is as good confirmation as can be obtained of the expectation that the two members of a pair are of identical genotype after conjugation. The expectation that the mean number of mutations per micronucleus in the rayed animals should be twice that in the BC1 can be tested by calculating this mean number from the average fraction of clones of reduced vigor in the autogamous progeny of these two generations. The formula relating these two quantities is $F = 1 - e^{-m/2}\left[1+m/2+(1/2)(m/2)^2 + (1/6)(m/2)^3\right]$ where F is the average fraction of clones of reduced vigor and m is the mean number of mutations per micronucleus. The assumption, derived from dosage data, is made that a minimum of four homozygous mutant genes are necessary to produce a detectable reduction in vigor. The results of these calculations are shown in table 4 for the three experiments which

[redacted]

Table 3

Analysis of variance of the A2 data

	Mean Square	Degrees of Freedom
(1) Variance attributable to differences between the progeny of different rayed animals	1823.42	36
(2) Variance attributable to differences between different pairs from the same rayed animal	476.77	37
(3) Variance attributable to differences between members of the same pair	202.53	74

Ratio of mean squares of (2) to (3) - - 2.35
Probability of chance occurrence - - less than 0.01

Table 4

Comparison of the mean number of mutations per micronucleus in the rayed and B01 generations from the A1 and A2 data.

Experiment	Number of rayed animals	Generation	Average fraction of reduced vigor	Mean number of mutations per micronucleus	Ratio A1/A2
2	3	A1	0.75	10.3	3.7
		A2	0.13	2.8	
3	30	A1	0.83	9.6	2.3
		A2	0.44	4.2	
4	13	A1	0.70	6.8	1.7
		A2	0.58	4.0	

[redacted]

provide adequate data. The average fractions of reduced vigor were corrected for the fraction in the controls. The ratio of the mean number of mutations in the rayed animals as calculated from A1 data to the mean number in the BC1 as calculated from the A2 data is sufficiently close to two considering the approximations involved in deriving the equation. The greatest departure is found in experiment 2 which had the least number of rayed animals and so gives the least reliable estimate. It can be concluded that the hypothesis that reduced vigor after autogamy in the progeny of rayed animals is due to micronuclear mutations induced by the radiation is well supported by the inheritance data. The fact that the formula given above yields good results when applied to the inheritance data although it was developed in connection with dosage data is strong confirmation of the hypothesis on which it was based (see previous quarterly report). Quantitative studies on gene mutations in Paramecium are thus possible and indeed, for studying overall mutation rate as opposed to the rate for certain specific classes of mutations, it may be especially favorable since it would be expected that the character "reduced vigor" as here defined would be affected by most, if not all genes. The comparison of X-rays and beta rays is far enough along to draw some tentative conclusions although more data is still desirable. The beta radiation was from plaques of P^{32}; the X-rays were from the Maximar machine operated at 250 KV. The dosage rates used were approximately the same. The data so far gathered suggests that an r unit of X-rays was somewhat more effective than an r.e.p. of beta radiation in producing mutational changes in Paramecium. An exact estimation of the relative efficiency is not warranted as yet though it appears that the r unit as measured with a Victoreen exposure meter is distinctly less than twice as effective as an r.e.p. unit. Since there is no

reason to believe that X-rays and their true physical equivalent of beta radiation would be of different effectiveness in producing mutation, it seems probable that the calculations of the r.e.p. units delivered to the animals were in error. The X-ray data seems to be just as variable as the beta-ray data so that it would appear that any errors made in measurement of the plaques and calculations of the dose for a particular exposure to beta radiation were not large in comparison with the inherent biological variability of the material. Studies of the inactivation of kappa by ultraviolet light have been continued. The results continue to show considerable variation even after another biological variable, time since previous autogamy, was brought under control. Statistical analysis has indicated that a considerable part of the variability is probably the result of causes other than sampling variation, but the nature of these causes is not known. Previous work by a number of different investigators has shown that the reaction of paramecia to specific antisera can undergo variation within the clone. Such variations may be transmitted for many cell divisions before disappearing. There are a number of grounds for the belief that cytoplasmic elements of inheritance may be involved in this phenomenon. Preliminary trials are now underway to find out if radiation of various kinds has any effect on this phenomenon. No clear results have so far been obtained. (Kimball, Gaither, Greiss and King)

Sources of Radiation - (A) The gamma ray source was an activated sample of tantalum. There are 4 gamma rays identified, 2 of high energy, 1.12 and 1.22 Mev. (96% of the intensity) and 2 of low energy 0.12 and 0.22 Mev. The source when used for exposure is located in the center of a hexagonally-shaped lead table, some 5 feet in diameter, with lead shielding provided on the sides to protect the operator, 3 carts (of low atomic number material) are driven on precision screws to any desired distance from the source, ± 0.1 mm. The spores to be irradiated were placed in lucite tubes 3 mm. in diameter with 3 mm. walls. Since the desired intensity was available at 30 mm. from the source the spores contained in a tube received within 8% of the same dose. All exposures were made at the same location, the decay of the source changed the intensity by about 10% during the course of experimentation. (B) The source of X-rays was a Maximum Model 250. The tube and housing provides an inherent filtration of 3 mm. aluminum. Operating at 250 Kvp. and 15 m.a. the r./min. at 60 cm. from the tube port is 275 r/min. The half value layer of copper without added filtration is 0.4 mm. The X-ray exposures were carried out by placing spores in lucite tubes at about 60 cm. from the port of the tube, the position having been previously monitored with a Victoreen thimble-chamber. (100 r. type) Readings day after day fell well within the 2% claimed by the manufacturers to be the accuracy expected of the instrument.

Monitoring - Dosimetry - All dose measurements of gamma X-rays, were made with Victoreen thimble-chambers, which varied less than 3% from each other, as determined by X-ray calibration both in our laboratory and at the U.S.B.S. The reliance which one can place on such measurements is quite good for X-rays; for gamma rays, even though the thimble response

may be similar, other effects like ionization along the insulator and at the button contact tend to raise the reading one gets when measuring dosages of very penetrating radiations.

Results - Survival information for the experimentation to date is shown in Table I.

Dose (rep)	Percent Survival X-rays	Gamma rays
5,000	94.0 ± 1.7	97.5 ± 4.0
10,000	77.2 ± 1.4	88.3 ± 1.76
20,000	56.2 ± 0.8	72.0 ± 1.4
30,000	32.3 ± 0.68	41.1 ± 0.97
40,000	30.0 ± 0.5	30.0 ± 0.9
50,000	17.7 ± 0.42	28.0 ± 0.84
60,000	8.9 ± 0.18	13.0 ± 0.26
75,000	2.8 ± 0.1	7.5 ± 0.22
100,000	0.23± 0.07	1.15± 0.03

The best fit of the above points, when plotted, indicates that the X-rays are 1.2 times as effective as the gamma rays, as used. Moreover, the curve is not exponential as one would expect of killing a single hit phenomenon. The shape suggests multiple-hit type of killing, being slightly sigmoid. The recoil electrons from the 250 KV. X rays having a greater average ion-density/unit length of track, than those from gamma rays of 1.2 Mev. would be expected to be more effective per unit dose, in producing multiple hits.

The mutation information is shown in Table II.

Dose	Percent Mutation in Survivors X rays	Gamma
5,000	0.8 ± 0.2	1.0± 0.1
10,000	2.8 ± 0.105	1.0± 0.15
15,000	3.2 ± 0.14	3.6 ± 0.6
20,000	5.02± 0.21	
30,000	7.0 ± 0.3	5.1 ± 0.3
40,000	11.6 ± 0.45	6.0 ± 0.3
50,000	11.6 ± 2.6	
75,000	13.0 ± 0.9	8.0 ± 0.6
100,000	31.0 ± 2.2	18.0 ± 1.5

A plot of the mutation data shows again X-rays to be more effective than gamma rays by a factor of 1.4. This is far more difficult to explain than the comparative lethal action of these radiations. It was supposed that all the mutations were gene mutations and the rate among survivors would increase linearly with dose; moreover, the effectiveness of radiations would decrease as their ion densities increase. This point warrants further investigation with radiations of higher ion densities. (Stapleton and Langford)

Ultraviolet Irradiation of Bruchid (Coleoptera) Eggs - The problem concerns itself with the modifications produced by ultraviolet irradiation of bruchid eggs in three phases of early development, and the dynamics by which the modifications are produced. The phases of development of the eggs are physiological rather than morphological in nature and

and are as follows: (a) from time of deposition to 6 1/2 hours (30 degrees C) at which the potencies of various regions of the egg to develop in organized manner have not been determined. Cleavage is in progress but cells have not reached the cortical cytoplasm. The egg is highly susceptible to respiratory inhibiting solutions. (b) 7 - 12 1/2 hours -- early formation of blastoderm is in progress. Planes of development and the lay of the embryo are irrevocably determined. Organized development is not readily affected by inhibiting agents. (c) 12 to 16 1/2 hours -- egg is again more susceptible but inhibiting agents affect development of the neural plate principally. -- Morphologically, the embryonic plate is in process of formation. Early tests were concerned with finding effective inhibitory dosages of ultraviolet irradiation. Dosage of 20,000 ergs per egg and upward to 31,000 ergs appear effective. This is on the basis of 29,600 ergs per mm^2. Effective dosage is evidenced by inhibited development of the embryo as a whole, such as dwarfing, distortion, and on several occasions, bifida in the thoracic region. There has been no instance of unorganized development following irradiation in the first phase. This fact may be significant. Study of sectioned eggs has not been made and until this is done the specific results cannot be fully determined. Sectioning awaits arrival of suitable reagents which have been on order for four weeks. Loss of effort was experienced on the storage of eggs at refrigerator temperature (3 degrees C) when it was found that this prevents further development. A search for a physiological zero must be made at which eggs can be kept, in order to assure a constant supply. Eggs may be irradiated entire, either by letting them remain on the pea or by their removal. The second method is preferable, since the eggs can be oriented to better advantage with reference to the source of

light. Partial irradiation of the eggs is accomplished by placing them in the waxed depression of a culture slide and partially shielding them from above by two strips of cover glass placed together at right angles. Slight irregularities in the adjacent edges of the cover glasses afford gaps under which small fractions of eggs can be exposed. Indications are that the method will work. More refined versions of the apparatus are on order, and in the process of manufacture. (Brauer, visiting investigator)

MICROBIOLOGY SECTION

Photosynthesis - The study of the effects of ultraviolet radiation (2537A°) on the algae Scenedesmus D. and Chlorella pyrenoidosa has been continued. The results of many determinations of the rate of photosynthesis as a function of dosage show that with both organisms the inactivation deviates from a first order reaction to a higher order, indicating more than one component is being inactivated. In the case of Scenedesmus it was found that the amount of inactivation by a given dose was the same for each of the following systems: Warburg buffer #9, nutrient solution, and 0.005M KH_2PO_4. The rate of photoreduction by Scenedesmus is inactivated to the same extent as is photosynthesis. Although readaptation occurs, presumably due to some visible radiation from the ultraviolet lamp, readaptation back to the "reduced state" occurs after a short dark period. The curve for the effect of varying doses of ultraviolet on the rate of oxygen evolution by Chlorella pyrenoidosa in the presence of quinone and in the absence of CO_2, is similar to those obtained for the inactivation of photosynthesis. Preliminary results with fragmented Chloroplasts suspended in solutions of either phenol indophenol, ferricyanide, or a ferric oxalate-ferricyanide mixture indicate that the same amount of inactivation occurs for oxygen evolution with each of the oxidants. Survival curves have shown that the ability for Scenedesmus to produce colonies on an agar-glucose nutrient medium mixture is considerably more sensitive to inactivation than are the photochemical processes. This is shown by the fact that the survival ratio at the point where photosynthesis was reduced to 43% was 0.01% for colony formation. Colonies which were found to differ in color from the normal colonies were picked as possible mutant stocks. Preliminary observations showed differences in the color, growth rate and ability to grow on a minimal medium. Reversion has occurred in each case and replating of the

stock cultures to attempt recovery of the mutants has been carried out. (Arnold, and Holt)

Stable Isotopes - It was impractical to set up a vacuum system in our present quarters in view of the fact that we plan to move sometime in September. Considerable work has been done on a magnetic oxygen analyzer; several models were made. Analyses and calculations were made of a new design broad band amplifier that we think will be of much use to us in our Stable Isotope Program. (Stone and Love)

Intermediary Metabolism - I. Propionic Acid Fermentation: resting cells grown on glycerol utilize glycerol at a faster rate than pyruvate, although pyruvate is an intermediate in the glycerol fermentation. The autofermentation rate can be significantly decreased only by starving in buffer for 10 to 24 hours, after which time the rate of substrate dissimulation is also quite depressed (loss of soluble co-factors, etc.?). Optimum pH for glycerol dissimulation found to be 6.5; succinic acid in not fermented by resting cells, and it may be necessary to drop the pH to the pK^1 of succinic acid, unless adaptation during the growth phase will suffice. II. C^{14} Measurement: a). Solid counting: Two new G.-M. tubes have been set-up; calibration demonstrated that no coincidence correction is necessary over the following very wide range: less than 1 to 370 c/s on one tube, and up to 280 c/s on the other. This study also gave a good indication of the reliability of the pipetting, precipitation, filtration and weighing techniques employed for production of infinitely thick solid samples of $BaC^{14}O_3$. Of eleven samples at various C^{14} levels only one deviated by an amount greater than the statistical error (this sample was 4 per cent low, and the statistical error was about 1.5 per cent). b). Vibrating reed electrometer has finally been installed after a fifteen month delay. The vacuum line has been constructed for wet

oxidation of organic samples, for measurement of volume of $C^{14}O_2$, and for filling ion chambers with $C^{14}O_2$. Testing of the effects of CO_2/N_2 ratio on the ion current developed from $C^{14}O_2$ is in progress. It appears that routine measurement of 10^{-4} to 10^{2} μc of C^{14} will be possible in a thousand-fold range of total CO_2 (in 0.5 to 500 mg. CO_2). This is an enormous improvement over our present method of solid counting, which goes down only to 10^{-3} μc and in only a fifteenth-fold range (in 1 to 15 mg. CO_2).

BIOCHEMISTRY SECTION

A. Chromatographic Separation of the Components of Nucleic Acids - Suitable solvent systems have been found for the resolution of nucleic acid components on paper chromatograms. Nucleotides are resolved in a system of 5% KH_2PO_4 - amyl alcohol; nucleotides in butanol - NH_3; and free bases in a mixture of iso-butyl, isopropyl alcohol and water. Identification of the spot on the paper strip is accomplished by short wave length ultraviolet fluorescence. Using these techniques several problems are being studied (1) the products of the reaction of nucleases with nucleic acids (2) the synthesis of tissue nucleotides using C^{14} and P^{32} (3) the enzymatic degradation of nucleic acids and their components in tissues and alterations in these processes induced by radiation. B. Metabolism of Nucleic Acid in Bone Marrow - A study of the effects of radiation on the metabolism and content of nucleic acids in bone marrow has been completed. The principal results are (1) desoxyribonucleic acid content of the tissue falls following exposure to x-rays, with indication of recovery in low dosage range 100-250 r (2) the rate of synthesis of ribonucleic and desoxyribonucleic acid as indicated by incorporation of P^{32} into these molecules is moderately increased following exposure to 200-500 r (3) radiation exposure to 500-1000 r results in severe damage to respiration and loss in nucleic acids. The surviving tissue has a much higher

rate of synthesis of ribonucleic acid as compared with desoxyribonucleic acid than the normal tissue (4) there is no indication of a specific inhibition of desoxyribonucleic acid synthesis - when such inhibition is found it is always associated with severe damage to respiration systems. (Carter, Jones and Webster).

The effect of x-rays on sodium thymus nucleate (TNA) has been studied with the aid of the analytical ultracentrifuge. A 0.164% solution of TNA in 0.2 M NaCl, 0.01 M phosphate, pH 6.8 buffer was x-radiated at 830 r. per minute for measured periods of time. The samples were then sedimented in the ultracentrifuge two hours after the irradiation period. The relative centrifugal force employed was 145,000 g, and photographs were taken at 16 minute intervals. Sedimentation was carried out at room temperature, the exact temperature of the chamber being recorded throughout a run. A summary of the results obtained are given in the table, S_{20} representing the sedimentation constant in Svedberg units referred to water at 20 C.

x-ray dose in roentgens	S_{20}
0	8.17
830	8.11
1660	8.50
3320	8.45
6640	8.51
13280	8.47
19920	8.47
26560	8.57
39840	8.49

It is clear from the table that little, if any, change in sedimentation constant has resulted from x-ray doses up to 39,840 r. These results are very surprising in view of the marked drop in structural viscosity of TNA upon x-radiation. The loss in structural viscosity has been ascribed to a

depolymerization of TNA by x-rays. This hypothesis is not compatible, however, with the sedimentation data, which indicate no change in the molecular size with x-radiation. It has been observed that with increasing x-ray dosage, the sedimenting boundary of TNA, - a sharp spike throughout with the unirradiated TNA - , becomes more and more diffuse, though still essentially monodisperse. This indicates a probable change in diffusion constant with x-radiation. Diffusion measurements are to be carried out on the x-radiated TNA solutions and from the sedimentation constants and diffusion constants the change in shape of TNA with x-radiation estimated. A nucleoprotein has been prepared from rabbit bone marrow made hyperplastic with an injection of phenylhydrazine, by a modification of the Mirsky procedure whereby the inhomogeneous nucleoprotein in 1 M NaCl is precipitated from alcohol. The resulting fibrous nucleoprotein readily dissolves in 0.2 M NaCl, 0.01 M phosphate, pH 6.8 buffer. In the analytical ultracentrifuge it migrates as a single sharp spike, with a sedimentation constant, S_{20} = 9.08 Svedbergs. The nucleoprotein gave a positive test with biuret, and yielded a heavy precipitate at the interface when the aqueous solution was shaken vigorously with an equal volume of 4:1 chloroform:ocytyl alcohol, indicating the presence of protein. At wave lengths shorter than 2450 A.u. the nucleoprotein had a much greater absorption than the nucleic acid prepared from it by the chloroform;octyl alcohol extraction procedure. Above 2450 A.u. the absorption curves of the two were superimposable. The nucleoprotein had 5.0% phosphorus and 14.0% nitrogen by analysis. X-radiation apparently has little effect on the physical properties of the nucleoprotein. A dose of 33,200 r. did not result in a drop in viscosity. The x-radiated material sedimented as a sharp spike in the analytical ultracentrifuge with S_{20} = 9.02 Svedbergs. Ultraviolet irradiation, on the other hand, markedly lowered the viscosity of the nucleoprotein. the ultraviolet

irradiated material sedimented in the ultracentrifuge with a more more diffuse boundary. This work is being continued with a second, much larger preparation of nucleoprotein from rabbit bone marrow. (Volkin and Carter)

RADIOBIOCHEMISTRY SECTION

Studies related to the separation and purification of carrier-free beryllium have been completed and will be presented in detail in separate reports. Summaries are given below. I. The Preparation of Carrier-Free Radio-beryllium by Means of Acetyl Acetone. Beryllium^7 was obtained by bombarding lithium with deuterons in the Berkeley cyclotron. Carrier-free Be^7, in about 96% yield, has been successfully separated and purified from an aqueous solution of this target by means of acetyl acetone. The basic steps of the method involve: 1) Extraction of Be at pH6 into a benzene solution of acetyl acetone. This step leaves the bulk of the alkali metals in the aqueous phase; 2) Washing out impurities, such as Ag, Cr, Cu, Fe, Ni, etc. and traces of alkali metals from the benzene phase with 0.01 to 0.1 N HCl; 3) Back extracting the beryllium into an aqueous phase with 10% exalic acid, and subliming off the excess oxalic acid. No radioactive impurities were found in the final Be^7 sample. The bulk of the spectrographic impurities was made up of Ca, Fe and Mg. These metals are found in the reagents. (Bolomey and Broido - ORNL-135)

II. Studies Related to the Preparation and Purification of Carrier-Free Radio-beryllium Obtained from Lithium - Application of Thenoyl Trifluoroacetone as a Complexing Agent. Thenoyl trifluoroacetone as an extractant for Fe^{3+}, Cu^{2+}, Al^{3+}, Be^{2+}, Y^{3+}, Ca^{2+}, and Sr^{2+}, has been investigated. It was found that in slightly more than 2 hours Fe^{3+} could be almost completely extracted at pH 2.5 with insignificant contamination by the other elements studied. In one hour of shaking at pH 3.4 copper can be separated in good yield from the other ions except small

residual amounts of Fe. Al and Be are extracted together at pH 5 to 6 in 3 hours and may be separated from each other by back extracting the benzene phase with concentrated HCl. Al containing about 40% of the Be is back extracted in about 6 hours. Be takes about 80 hours for maximal extraction. It should be possible to recover the beryllium in the Al - Be fraction by recycling this fraction through the process. Yttrium extracts easily and rapidly at a pH of 7 to 8 into the benzene phase. Under our conditions Zn, Ca, and Sr were not appreciably extracted by TTA in 3 hours shaking. Fe, Sr, Y, Zn, Ca, and Cu are back extracted from the benzene phase with concentrated HCl in 15 minutes shaking. (Bolomey and Wish - ORNL 136)

III. Thenoyl Trifluoroacetone in the Preparation of Carrier-Free Radioberyllium Obtained from Lithium - Radiochemical and Chemical Purity. Carrier-free Be^7 has been successfully isolated and purified from a lithium target bombarded with deuterons in the Berkeley cyclotron. The target had decayed about 200 days before it was received for processing. Zn^{65} and Co^{59} were the only other radioactive elements found in the target solution. These isotopes were not present in the purified Be^7 sample. Spectrographic analysis of the purified Be^7 sample indicated moderate amounts of Ca and Mg, and traces of Fe, Al, Cu, and Mn. These elements are contained in trace to moderate quantities in the reagents employed. The final Be^7 solution contained about 0.05% the amount of copper found in the second acid wash indicating an excellent degree of separation in two minutes shaking time. Studies on the application of anion exchange resins for the isolation and purification of ribose nucleic acids have begun. Preliminary experiments indicate that ribose nucleic acid can probably be stripped of contaminating nucleotides and from P^{32}. (Bolomey and Wish - ORNL - 137)

Ion Exchange Studies on Nucleic Acid Components. At the time for the last quarterly report, preliminary equilibrium experiments had been performed to indicate the general absorption characteristics of the best available ion-exchangers for two nucleic acid components: nucleotides and inorganic phosphate. Since that time, attention has been turned to perfecting separation schemes for the free bases, the nucleosides and the nucleotides. As we stand now, schemes have been evaluated and demonstrated for separating the free bases and the nucleotides. After perfecting these and including the nucleosides - and devising alternate methods to be used under the various conditions which may be met with in tracer work - it is planned to proceed to other classes of compounds involved in nucleoprotein metabolism. The five bases with which we have worked are the purines, adenine and guanine, and the pyrimidines, cytosine, thymine and uracil. All but the last two have free amino groups and therefore exhibit a tendency to absorb on the cation-exchanger (Dowex-50) on the acid side of the pK of this group, (pK's circa 3) whereas thymine and uracil do not. From the resin, the three absorbable bases may be eluted by solutions sufficiently high in cation concentration and sufficiently acid to maintain all three bases in the $-NH_3^+$ form or by salt solutions at pH values such that the amino group is partially or wholly neutralized. Examples of these solutions are, respectively, 2N - HCl and 0.2 M acetate buffer at pH 5.6. In the first case, one depends exclusively upon differences in the strengths of the respective cation-resin bonds; in the second, the values of the pK's of the amino groups also plays a part. If both operate in the same direction, the second method would give the better separation; cytosine, however, is out of line in this regard. Actually, straight acid elution has been shown to be eminently satisfactory for the separation of cytosine, guanine and adenine, which are eluted in that order. (see Figure 1). Since thymine and uracil do not absorb, they

will be collected together in the effluent from the absorption step. However, since they occur in different nucleic acids which will have been separated previous to base analysis by other methods, this is not a serious handicap. Therefore, it can be stated that we are able to separate and recover quantitatively the four bases from either of the two types of nucleic acids. Thymine and uracil (and the other three bases also) exhibit weak acid properties. Therefore, at pH values alkaline with respect to their pK_a's, they will absorb on an anion-exchanger. Here we have the same possibilities outlined for the cationic separation; we may elute the anion with strong base or we may elute the free acid with buffer solutions at the proper pH value. Since the pK_a values of thymine and uracil differ sufficiently (9.94 and 9.45, respectively, according to Levene), the second possibility should easily be realized. This is being investigated and the preliminary results indicate the possibility of separating all five bases at once by such a method. Attention was then turned to the nucleotides. These exhibit, of course, the same active cationic groups as the free bases but have, in addition, ester phosphates present in the molecule. These, even in fairly strong acid (pH0), are partially ionized as anions; hence, the nucleotides, at pH's 0 - 4, are zwitterions. This fact, plus their size, makes them less strongly absorbed by cation-exchangers in the acid regions. Since the differences in cation group-resin bond strength persist, we have been able to elute the nucleotides successively and separately from the cation-exchanger by weak acid (or buffer solutions) and this has been demonstrated. (Figure 2.) The absorbing conditions used are 0.1 0.01 N - HCl; separation is accomplished with 0.01 N - HCl. Again, thymidylic and uridylic acids should not absorb (this has not been tested since samples of these acids are not available). At this point it may be mentioned that the commercially

available nucleotides are not pure; hence, we have no standards at hand with which to compare our separated nucleotides for purity and for recovery. For this reason, we have begun a program of pure compound preparation. Purity is proven by these criteria: enhancement, up to a maximum, of characteristic spectral absorption lines as a result of and adsorption-elution cycle, absence of all other known nucleic acid components in such a cycle, recovery of all material in known fractions, and absence of other components in a partition chromatographic separation. The properties used for determination are spectral absorption and fluorescence; hence, components not detected by either of these methods are not known to be either present or absent. In such a manner, 10-40 mg. amounts of "pure" nucleotides have been prepared (in one cycle on a 0.74 $cm.^2$ x 10 cm. column, containing about 3 g. resin). As is true of the free bases, the nucleotides are absorbable on anion-exchangers by means of their basic groups; however, the phosphate groups present predict a much stronger tendency in this regard. Since the phosphate pK's of all the nucleotides are very close to each other, it is likely that separation on an anion-exchanger can only be accomplished, if at all, by taking advantage of the acid properties of the other components. This has yet to be investigated. Problems for the future include (1) separation of the ribosides from each other, (2) the development of one or more complete schemes for the separation of all bases, ribosides and nucleotides from various mixtures of them, (3) the quantitative influence of common salts, (4) the investigation of means of separating desoxy from ribo - nucleosides and nucleotides (possible agents are in mind and at hand), (5) the separation of smaller fragments from the larger ones which may be encountered in biological digests. As soon as the first of these is in hand, it is planned to write a paper for publication on the separation of the bases, nucleosides and nucleotides. (Cohn).

[redacted]

Figure 1

SAMPLE SEPARATION OF FREE BASES

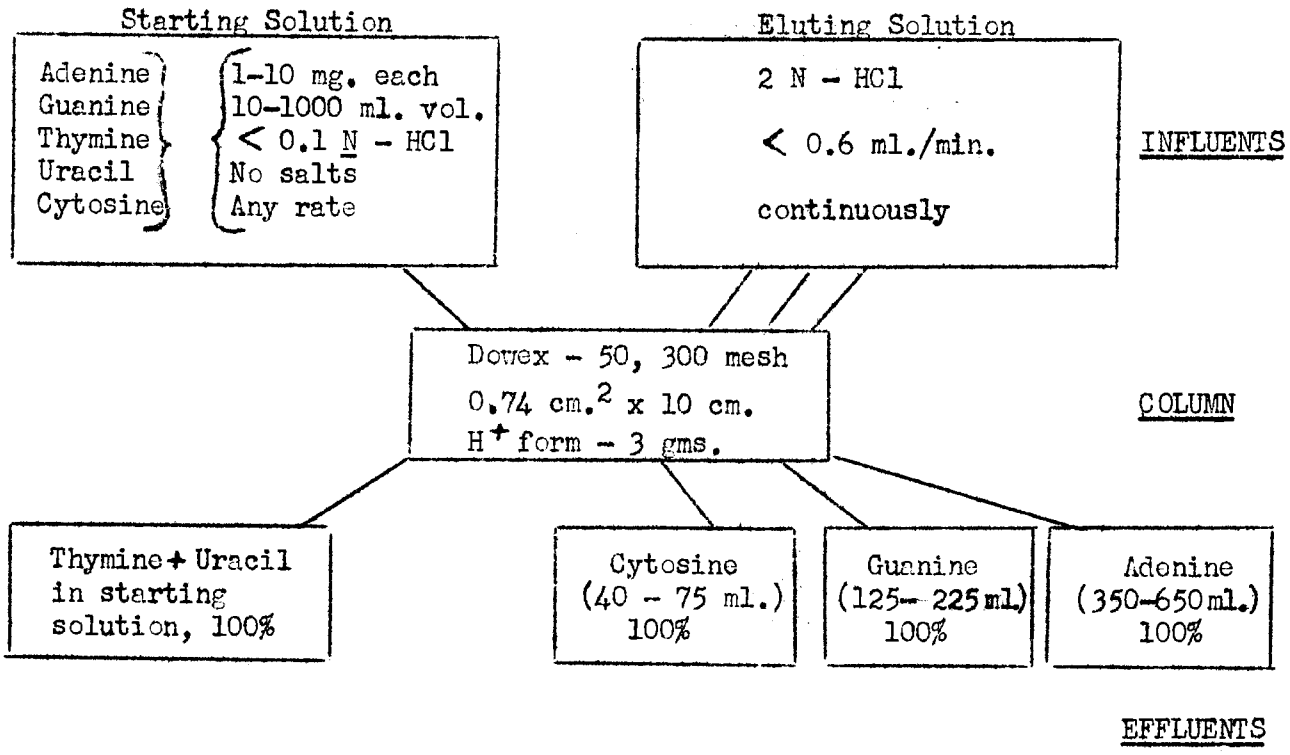
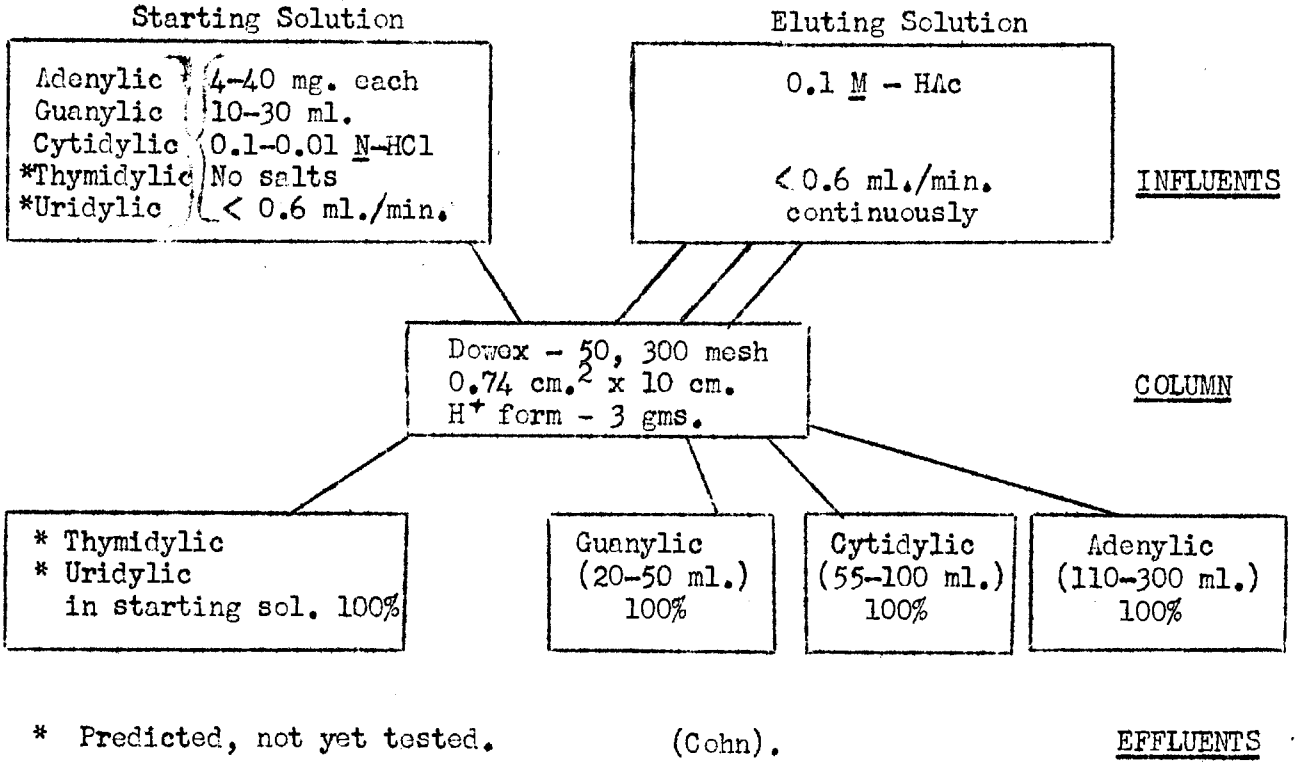


Figure 2

SAMPLE SEPARATION OF RIBO-NUCLEOTIDES



* Predicted, not yet tested.

(Cohn).

[redacted]

The Erythrocyte Project - During the last three months some time has been spent in testing the new ionization chamber for measurements of the gamma ray intensities in the gold radiation source. The instrument has responded satisfactorily and calibrations have been made with the assistance of the Health Physics Division using their equipment. We will shortly be able to make a final calibration using our own electrometer which was recently obtained. With the assistance of the Instrument Division, we have recently completed an automatic balancing device for the flame photometer which gives us a permanent record of our potassium analyses on the chart of a Brown recorder. Our activities during the quarter may be summarized as follows: 1. We have studied the exchange of potassium between plasma and cells of whole canine blood equilibrated with arterial and venous gas mixtures. No significant difference was found in the effect of the two types of gas mixture; 2. We have investigated the activity in blood cells immediately after tagging the plasma. Results show that there is little if any immediate uptake of activity by the cells; 3. In a preliminary experiment we have examined the effect of adrenal cortical hormone extracts on potassium exchange. The initial exchange is essentially the same but the cells soon disintegrate liberating the activity to the plasma. This may or may not be due to preservatives or other injurious materials present in the extracts; 4. We have studied the exchange of potassium between plasma and an ion exchange resin. The results indicate that most of the potassium in the plasma is present as ions which are free to exchange with the resin; 5. We have observed the exchange of potassium in blood from which all white cells and platelets have been removed. The results here are extremely significant since the decrease in plasma activity is very slow and proceeds essentially at about 1% per hour; we have previously reported that in whole

blood there is an initial rapid exchange followed by a slow exchange. Our previous tentative conclusion was that two fractions of potassium existed in the canine erythrocyte, one which exchanged rapidly and one slowly. We must conclude that some of the cells in whole blood (probably leukocytes) exchange their potassium rapidly whereas erythrocyte exchange is a much slower process. A more complete description of our findings will be presented at the forthcoming meeting of the Society of General Physiologists, September 8-10, 1948, Woods Hole, Massachusetts. (see abstract section). We are now beginning a series of investigations of potassium exchange in human blood. (Sheppard and Martin).

MAMMALIAN GENETICS SECTION

The one remaining strain of mice required for our first major project was finally obtained after some delay. We should now be independent of outside sources of animals. Strains obtained earlier have expanded almost to the limits of present facilities, but drawings for the remodelling of the first floor, Building 9210, Y-12 Area, have been completed and construction has begun. As mentioned in the last Quarterly Report, the somatic mutations project yielded results which suggested related projects. One of these, involving the effects of radiation on the developing embryo and fetus, is now being pursued because of its basic value to many fields of research, in addition to developmental genetics, and because of its importance in the study of radiation hazards. The current status of this work is reported in the following paragraph which is an abstract of a paper to be presented at the Annual Meeting of the Genetics Society in Washington in September, 1948. Mouse embryos in various stages of development were treated by subjecting pregnant females to 250 kvp X-rays (total body irradiation). The females used were 2-17 days gravid as timed from the observa-

tion of vaginal plugs. Using total doses of 200-500 r and intensities mostly between 72-84 r/minute, characteristic and consistent results were obtained for various stages of embryonic life treated. Raying on the 9th, 10th, 11th and 12th days, the stages most investigated at the present time, produces abnormalities which resemble, in many of their features, the effects of known gene mutations. These induced abnormalities, some of which are similar to ones described by Kaven, are being studied in greater detail. Among those which, as far as we know, have not been recorded before is a syndrome characterized by oligodactylism and resembling the description of a mutant reported by Hertwig. During this quarter, work on artificial insemination has been concerned primarily with the development of methods for the handling and irradiation of suspensions of mouse spermatozoa. In this problem we have received helpful advice from the Department of Animal Husbandry, University of Tennessee. The following technique has proved to be the best of several tried, but is probably capable of further improvement. Sperm stripped from the vas deferens are suspended in a mixture of egg yolk with a solution of NaCl, KCl and glucose. Samples 0.15 cc in volume, are irradiated in small chambers in a lucite block covered with scotch tape. Following treatment, the suspension is cooled gradually (over a period of 2 hours), to 5 - 10° C. for storage. Untreated sperm maintain their motility under these conditions for two days or longer. (Russell, Russell, Kile).

PHARMACOLOGY AND PHYSIOLOGY SECTION

The program of this section deals with mammalian non-genetic irradiation injuries, including those due to local as well as total body irradiation. Since the last quarterly report three studies have been completed and submitted for publication as O.R.N.L. or A.E.C.D. reports. They were exploratory in nature, and are briefly reviewed below under Work Completed. The major part of our program is dealt with under Work in Progress. I. Work Completed. (1) The Effect of Local Beta Irradiation from an External Source Upon the Growth of the Bone and Some Other Tissues of Rats. A.E.C.D. 2219 . These experiments were performed in order to learn how much damage might be caused in small animals as a result of beta irradiation, and also to determine whether irradiation of one region might not affect another. Stapleton (unpublished experiments) compared the acute lethal effects of total and partial body exposure to P^{32} beta rays in the one-day-old-rat. For partial body exposure, the anterior or posterior half of the animal was employed. The skin exposure on the body was about 1700 - 2000 rep., while the skin exposure on the tail, hands, feet, forearm, and leg was somewhat less than twice this amount. In collaboration with Mr. Stapleton, we have raised the survivors from some of these experiments, and have examined the relative growth of a number of organs including the viscera, and especially the bones. The animals were sacrificed at 200 days of age. The irradiated animals weighed 5 - 10 per cent less than the controls. Males and females reacted alike, and no cutaneous tumors were observed. As might be anticipated, the parts irradiated showed definite inhibitions of growth. The degree of inhibition shown by an organ correlated fairly well with its depth below the surface at the time of irradiation. The 1.7 Mev. beta ray of P^{32} is 95% absorbed in 2.9 mm of soft tissue. The effects obtained were entirely local. The "anterior" animals had

significantly smaller ears, skulls and forelimbs; the "posterior" animals had smaller tails, hindlimbs and testes. As examples of the data obtained we may consider the tail and hindleg of the male. The control tail was 21.6 cm. long. The "posterior" animals averaged 11.8 cm., while the "anterior" averaged 19.9 cm. The number of vertebrae was the same in each group. In the case of the hindlimb, the total length of the femur, tibia and foot in the controls averaged 11.8 cm., in the "anterior" animals 12.0 cm., in the "posterior" animals the length averaged 10.6 cm., the greatest inhibition occurring in the bones of the foot, then in the tibia, and least of all in the femur. The exposure in r of x-rays to inhibit the growth of the foot or tail bones of the one-day-old rat is not known. Barr, et al. (1943) reported that in the 30-day-old rat about 1000 r inhibits the proximal end of the tibia about 50%, and 1800 r by about 100%. For orientation, these figures may be compared to our estimates for the one-day-old tail and foot in which there were, respectively, 50 and 20% inhibitions at a dosage of approximately 1800 rep. of beta rays. (2) <u>The Effect of Heparin Upon the LD-50% of the X-irradiated Rat</u>. O.R.N.L. <u>116</u>. Among the mammals, the great differences in sensitivity to ionizing radiations have not been explained, no doubt because we know so little about the pathologic physiology of the irradiation injury syndromes. The recent work of Allen, et al. (1948), however, offers a new possibility in this connection. They reported that an heparinemia occurs in the dog during the second week following heavy irradiation (LD-100%, 200 KV, 450 r), that it is coincident with a remarkable rise in the clotting time as well as other hemorrhagic manifestations, and that it can be controlled by the administration of toluidine blue. Now under comparable conditions, the LD-50% for the rat is about twice that for

the dog (Prosser, 1947). Might not this difference in sensitivity be due to the failure of the injured rat to release heparin? Several points should be considered in the design and interpretation of experiments to test this hypothesis. Allen, et al. (1948) employed an exposure of 450 r, which consistently kills all of the animals in about 10 - 14 days, and which for them is 150 r more than the LD-50%. No report is given as to the extent of heparinemia at the LD-50%. Prosser (1947), in his review of the Argonne Laboratory data, states that the clotting time may be doubled at 200 r and above; presumably the changes are not great at or under the LD-50%. As matters stand at present, then, markedly prolonged clotting times occur in dogs subjected to more than the LD-50%, after the lapse of one week or more; the r-dosage for this effect, however, is only three-quarters of that equivalent to the LD-50% in the rat. As to the site of hemorrhage in the dog, Allen, et al. state that at 450 r the mouth, soles of the feet and subcutaneous tissues of the sides - sites most likely subject to trauma - bleed first. Later there may be bleeding from the rectum, vagina and urinary tract. Autopsy reveals hemorrhage along the G. I. tract, especially in the colon and the proximal four-fifths of the stomach; the heart muscle, especially along the coronaries, is frequently involved, as are the parietal pleura, diaphragm, and lungs. Field and Rekers (1948) found at the LD-50% (350 r) "generalized purpura and petechiae formation, intrapulmonary, gastric and intestional hemorrhage". Curiously, they state that less hemorrhage was seen at doses higher than at the LD-50%. This distribution of hemorrhage is somewhat different from that seen in the rat. Methods. The animals were irradiated by a G. E. Maximar outfit, run at 250 kv., 15 ma., with intrinsic filtration of 0.4 mm. of copper, and a constant potential equivalent of about 180 kv. The exposure box which held the animals was placed on a large scatter block. The exposures recorded

Table I

Heparin and the X-ray Tolerance of the Sprague-Dawley Rat

The mean weight of each group (males) varied from 275 - 277 grams, with standard deviations between 14 and 33. The animals were exposed on Day 0, and the heparin series received 2 mgm. each morning and 3 mgm. each afternoon on Days 6 and 10.

Exposure* r	Series	Survivors on day				
		0	6	11	14	22
0	Heparin	8	8	7	7	7
350		8	8	8	8	8
450		10	10	6	6	6
550		10	10	2	2	2
650		10	10	0	0	0
700	Control	10	10	9	8	8
800		10	10	6	5	4

* Includes backscatter. Air dose equals 70 per cent.

in the table are based on the readings of a Victoreen thimble chamber placed on the floor of the box, and therefore include the back-scatter. At the same level in air the reading would be only 70% as great. Experimental. The results are illustrated by the data in table 1. The dosage of heparin employed was such as to prolong the clotting time beyond 20 minutes for about 5 hours after each injection, of which there were two each day. Table 1 shows that the LD-50% of the rat was cut in half by this treatment. Autopsies were performed on all the animals which died during and shortly after the period of heparin administration. In general, there was no obvious difference in the distribution of hemorrhage between the heparin and the control animals. The heparin series showed marked hemorrhage at the site of injection in the body wall, and some showed retroperitoneal hemorrhage as well. Discussion. By the judicious use of heparin, the LD-50% of the rat has been reduced by about one-third, and it is possible that further trials could increase this figure. Measured in air, this brings the LD-50% of our albino rats from about 550 r to about 350 r; the latter figure is the LD-50% for the dog. Is heparin, then, the decisive factor which distinguishes the reaction of the dog from that of the rat? We think not. In the first place, the distribution of hemorrhage in the heparinized rat is essentially unchanged. What has occurred is more of the original type of hemorrhage, plus that in the body wall at the sites of injection. The net effect is to place an added stress upon a critically injured organism. The distribution of hemorrhage in the rat, therefore, is a direct reflection of the local injury; latent injuries requiring heparin for their development are not grossly important. In the second place, the administration of toluidine blue to the irradiated dog, according to Allen et al. (1948), dramatically diminishes hemorrhage, but does not change the fact that 450 r is an LD-100% exposure. Although the final interpretation of this

experiment must await further details, it appears now that the dog protected against circulating heparin continues to be more sensitive than the rat.

Allen, J. G., M. Sanderson, M. Milhauer, A. Kirschon and L. O. Jacobson. 1948. J. Exp. Med., 87, 71.

Barr, J. S., J. R. Longley and E. A. Gall, 1943. American J. Roent. Rad. Ther., 49, 104.

Field, J., and P. Rekers. 1948. MDDC-1672.

Prosser, C. L. 1947. Radiol., 49, 299.

(3) The Effect of Anesthesia (Ether, Amytal, Pentobarbital), of Fasting, and of Splenectomy Upon the Blood Chemistries of the Rat: Hemoglobin, NPN, Glucose, Albumin, Globulin and Chloride. O.R.N.L. 132 . In the course of our studies upon the metabolic response of the rat to whole body X-irradiation, it was necessary to chose a standardized set of conditions for the control of blood sampling. Reference to the literature, to our surprise, gave little specific information concerning the rat. In order to decide under which anesthetic and with what specific precautions blood should be obtained, it was decided to check each of the blood tests in the normal and splenectomized animal, fed or starved under ether, amytal or pentobarbital. Methods. It was decided to withdraw all blood by cardiac puncture, using a ½" No. 23 needle. The tests, except in the case of hemoglobin, were made on plasma. The animals were Sprague-Dawley males, weighing from 250 to 350 grams, and were bled between 8:30 and 10:30 a.m. Heparin prevented coagulation. All determinations were colorimetric, using the Coleman junior spectrophotometer. Hemoglobin was determined as oxyhemoglobin. A tungstic acid filtrate was used for the determination of NPN and glucose, the latter according to Nelson (1). Chloride was determined by the Van Slyke and Hiller (2) modification of the Sendroy method. Proteins were determined by Weichselbaum's (3) modification of the biuret test. Experimental. Amytal

acts more quickly and briefly than pentobarbital. These drugs cause a transient fall in hemoglobin, total protein and glucose. With pentobarbital, the fall in glucose outlasts the fall in hemoglobin and protein. As the animals recover from the anesthesia, the values of all the determinations gradually equal those obtained from brief (less than two minutes) ether anesthesia. Splenectomy slightly lowers the chloride, and perhaps some of the other levels. It abolishes the fall in total protein under amytal anesthesia. It does not prevent the transient fall in hemoglobin or glucose, but somewhat slows recovery. Withholding food, but not water, causes a parallel decrease in the total protein, NPN, and glucose, which is complete in about two days. Thereafter the levels remain constant for five days. During this later period, however, the A/G ratio rises slightly. Animals that have been starved for 1 - 3 days require about twice as many days of refeeding to recover the normal levels, and about three times as many to achieve the control weight.

(1) Nelson, N., J. Biol. Chem., 153, 375 (1944).

(2) Van Slyke, D. D., and Hiller, A., J. Biol. Chem., 167, 107 (1947).

(3) Weichselbaum, T. E., Amer. J. Clin. Path. (Tech. Sec.), 10, 40 (1946).

II. Work in Progress. (1) The Metabolic Response of the Rat to Total Body Irradiation. The changes in hemoglobin, NPN, chloride, albumin, globulin, and glucose are being studied in rats exposed to varying doses of X-rays. The first series of experiments has been completed and indicates changes in all of the tests enumerated. The second series, to confirm and extend the results of the first, is now in progress, and a report should be available within several months. (2) The Response of the Rat Tail to X-rays. This work has been delayed because the shops were slow in making a new exposure chamber. The experiments are once more under way. (3) Hormones. The first two experiments

dealing with the sensitivity of the hypophysectomized rat to X-rays indicated the animal to be much more sensitive than the normal control. The third experiment has failed to confirm the LD-50% found in the first two, and by a large margin. Further work is in progress. (4) Beta Ray. We began a study of the induction of cutaneous tumors in the rat by beta rays. Since the induction period is of the order of six months, such experiments tie up a large number of cages. Because the factories have failed to deliver our cages at the promised time, and can make no definite estimates for the future, we are temporarily abandoning this project. Meanwhile studies will be made of the acute reaction. (Kohn, Cupp, Lane, Click, Kirslis, Swingley).

The Cytogenetic Effect of Sonic Energy Applied Simultaneously With X-rays

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An experiment has been performed to test the influence of sonic energy on the yield of X-ray induced chromosomal aberrations in a clone of Tradescantia paludosa. Chromosomal aberration frequency was determined at the microspore mitosis from aceto-carmine smears made 4 days after treatment.

Inflorescences were immersed in water contained in a steel cup used for the sonic treatment. The bottom of the cup was a vibrating diaphragm which transmitted the sonic energy to the plant buds via the water. The instrument used was a Raytheon Mfg. Co. magnetostriction oscillator, run at 9,100 cycles/sec. and at a power of 30 watts. Two treatments were given to inflorescences inside the sonic cup: a) 250 r X-rays (78 r/min.) as measured in air outside the cup, and b) 250 r X-rays (as above) plus sonic treatment begun with and continued for 5 minutes after the X-radiation ceased.

The frequency of all types of chromosome aberrations was higher when the sonic treatment was given in addition to the X-rays. The ratio of X-ray plus sonic energy/X-ray aberrations was 1.3/1 for both exchanges and simple deletions. The sonic treatment alone, as given here, did not cause aberrations.

This increased yield of aberrations is probably due to an increased amount of chromosome movement caused by the transmission of sonic energy through the cells during and after the X-radiation; movement would separate the broken ends from a single chromosome break (reducing restitution) and bring together broken ends from different breaks (increasing new reunions)--both would increase aberration frequency.

Induced Reversions of Inositol-Requiring Mutants in Neurospora crassa

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Treatments of microconidial suspensions from inositolless mutants of Neurospora crassa with ultraviolet (2537 Å) or X-rays produce a greatly increased frequency of adaptation (inositol-independent cultures) in certain strains. Genetic studies of several independently produced adaptations indicate that these are the result of reverse mutation at the inositolless locus to the original wild-type allele controlling inositol synthesis. There is no evidence that gross chromosomal rearrangements are present in the induced inositol-independent stocks. The relation between frequency of reversions and radiation dosage is being investigated. Present results indicate that the percentage of reverse mutations with ultra-violet continues to increase, even at dosages well beyond the point of one percent microconidial survival. Previous studies of the frequencies of induced reversions in several independently produced, apparently allelic, inositolless mutants, have indicated that some of these strains differ markedly in their mutability. An extensive comparison has been made of two strains: S-37401, which regularly reverts with radiation treatment, and S-46802, which has failed to revert with any treatment so far attempted. Crosses of these two mutants, followed by serial ascospore isolations, yield cultures which are phenotypically indistinguishable. Such progeny can however be separated by reversion studies since, of the 8 cultures from a single ascus, four regularly adapt when conidial suspensions are treated with ultra-violet or X-rays while the remaining four do not.

Action of Ultraviolet Radiation on Green Algae

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A study of the effect of ultra-violet (2537 Å) on the green alga Scenedesmus has shown that the ability to produce colonies on an agar medium is considerably more sensitive to this radiation than are the processes photosynthesis and photoreduction, the survival ratio at the point where photosynthesis was reduced to 43% being 0.01% for colony formation. Exposure of cells adapted for photoreduction caused reversion back to photosynthesis in the case of both the irradiated and control sample (contained in pyrex) but a subsequent anaerobic dark period allowed readaptation. The rate of photoreduction of the irradiated sample was reduced the same amount as was the rate of photosynthesis. Inactivation of photosynthesis as a function of the duration of exposure to ultra-violet was found to be close to a first order reaction and was not a function of pH since curves for cells suspended in nutrient solution or in Warburg's carbonate buffer #9 were identical. The irradiation of suspensions of grana which were subsequently assayed for the ability to evolve oxygen photochemically after the addition of either phenol indophenol, ferricyanide, or a ferric oxalate - ferricyanide mixture showed equal inhibition for each of the reagents. Mutation production following ultra-violet irradiation was also observed, the mutants found differing from the controls in color, growth rate and ability to grow on a minimal medium.

The Induction and Inheritance of Mutations in Paramecium aurelia

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Beta radiation up to 13,000 r.e.p. has no immediate effects on stock 90 of variety 1 of Paramecium aurelia. When the descendants of rayed animals are sent through autogamy to make them homozygous, many are of reduced vigor. Evidence from inheritance studies indicates that the reduced vigor results from segregation into homozygous form of mutational changes induced in the micronuclei. Doses given in small daily fractions were just as effective as those given as a single exposure. Thus two-hit chromosomal aberrations could not have made a major contribution to the effect. However, the relation of the average fraction of exautogamous clones of reduced vigor to dose showed that a multiple hit phenomenon was involved. This is interpreted to mean that the majority of the effect is due to the combined action of mutant genes which are individually undetectable. A formula was developed relating the average fraction of reduced vigor to the mean number of mutations per micronucleus. The formula gives results consistent with the expectation that animals resulting from a cross of rayed to unrayed should have on the average half as many mutants per micronucleus as the original rayed animals. From the formula, it was calculated that approximately two mutations per micronucleus were induced per 1,000 r.e.p. This rate is quite high and suggests that the method can detect mutations in the great majority of all loci in Paramecium.

The Induction of Mutations in *Paramecium aurelia* by Beta Radiation

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A study of the induction of mutations in *Paramecium aurelia* has been carried out using plaques of radioactive phosphorus as an external source of beta radiation. With doses up to 13,000 r.e.p., the rayed animals and their descendants showed no clear effect of the radiation as long as they multiplied vegetatively; but quite a few of their progeny by autogamy were of reduced vigor or even non-viable. Since autogamy makes the animals completely homozyg us, it seems possible that the reduction in vigor after this process is due to the segregation into homozygous form of gene mutations or chromosomal aberrations induced in the diploid micronuclei by the radiation. Evidence from inheritance studies in support of this hypothesis will be presented in another paper. In the present paper, it is assumed to be true. It was found that radiation given within a period of about an hour before cytoplasmic division was much less effective than the same dose given during cytoplasmic division or during a period of at least two hours thereafter. This ineffective period includes or perhaps coincides with the mitosis of the micronuclei. As yet, no satisfactory interpretation can be offered. The form of the dosage curve is inconsistent with the hypothesis that gene mutations with individually detectable effects make up a major portion of all the mutational changes. The curve suggests that some multiple hit phenomenon is involved. Doses given in small daily fractions proved to be just as effective as the same doses given as single exposures. Thus two-hit chromosomal aberrations cannot be responsible for the major portion of the reduced vigor. The most plausible hypothesis seems to be that in general the re-

duction in vigor is due to the combined effect of gene mutations or perhaps small deletions with individual effects too small to be detected. On the basis of this hypothesis, a formula was developed relating the mean number of exautogamous clones of reduced vigor to the mean number of mutations per micronucleus per 1000 r.e.p. This figure is considerably higher than overall mutation rates which can be calculated from the data on Drosophila. It is suggested that this may be because the present technique detects a considerably higher percentage of all mutations.

The Production of Phenocopies in the Mouse by Means of X-ray Treatment of Embryos

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Mouse embryos in various stages of development were treated by subjecting pregnant females to 250 kvp X-rays (total body irradiation). The females used were 2-17 days gravid as timed from the observation of vaginal plugs. Using total doses of 200-500 r and intensities mostly between 72-84 r/minute, characteristic and consistent results were obtained for various stages of embryonic life treated. Raying on the 9th, 10th, 11th and 12th days, the stages most investigated at the present time, produces abnormalities which resemble, in many of their features, the effects of known gene mutations. These induced abnormalities, some of which are similar to ones described by Kaven, are being studied in greater detail. Among those which, as far as we know, have not been recorded before is a syndrome characterized by oligodactylism and resembling the description of a mutant reported by Hertwig.

Studies of the Kinetics of Potassium Exchange Between Cells and Plasma of Canine Blood in Vitro Using K^{42}

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Freshly drawn heparinized canine whole blood is equilibrated in vitro (paraffin lined vessels) at 38.1°C with a normal pulmonary atmosphere saturated with water vapor. It is then tagged by mixing with a small amount of plasma containing dextrose and radioactive KCl. The potassium content of the resulting plasma is thus raised by not more than 3% of the normal and the blood sugar to about 400 mg%. By this procedure cells are maintained in a healthy state for periods up to 10 hours as shown by minimal hemolysis, nearly constant hematocrit and minimal potassium leakage.

In whole blood the activity of the plasma decreases initially at a rapid rate which is correlated with the appearance of activity in the cells (red cells, white cells, and platelets). The initial decrease follows an exponential curve to an elevated base line, half of this change being complete in about 50 minutes. However, if the white cells and platelets be previously removed by repeated differential centrifugation the behavior is different. The plasma activity declines very slowly, at the rate of about 1% per hour.

The initial rapid decrease in plasma activity for whole blood is attributed to a rapid exchange of potassium in the fraction containing the white cells and platelets. A typical value for the amount of this easily exchangeable potassium is about 30% of the total potassium in the plasma. It is evident that studies of potassium exchange of erythrocytes must give equivocal results unless the white cells and platelets are removed in advance or otherwise taken into account.

The Genetics of Threonine Requiring Mutants of *Neurospora crassa*

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Three *Neurospora* mutants shown to differ from wild type by a single gene have growth requirements as follows: strain 51504 uses threonine plus methionine or homoserine alone; strain 44104 uses alpha-aminobutyric acid, isoleucine, or threonine; and strain 35423 uses only threonine. Mutant 51504 shows linkage with the first or sex chromosome, giving no recombinations with *aurescent* in 67 asci; mutants 44104 and 35423 show no linkage with the first or fourth chromosomes, but the genes involved in these mutants are apparently linked with each other. The latter two mutants gave centromere distances of 25 and 2 units respectively when outcrossed to one strain (12a) and 0 and 13 units respectively when outcrossed to another strain (34508, *aurescent*). Samples of asci were checked for bad spore types and an analysis was made of all the asci dissected. It was found that the failure to detect a crossover in 34 asci from the cross of 44104 with 35423 as well as the differences in centromere distance could not be explained on the basis of selection of asci for genetic analysis. It is suggested that mutants 44104 and 35423 differ in that one of them carries a suppressor of crossing over which is ineffective when homozygous.