EFFECTS OF IONIZING RADIATION ON THE NUCLEIC ACIDS DURING BARLEY EMBRYONIC DEVELOPMENT

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ABSTRACT

EFFECTS OF IONIZING RADIATION ON THE NUCLEIC ACIDS DURING BARLEY EMBRYONIC DEVELOPMENT

by Chong Won Chang

The aim of the present study was to investigate radiosensitivity of developing embryos *in vivo* in regard to qualitative and quantitative aspects of nucleic acids, and to determine if any disturbances are induced in the patterns of nucleic acid metabolism during embryonic development following treatment with a single dose of 450 roentgens of X-radiation applied at specific embryonic stages.

In an attempt to accomplish the purposes of this study, two different experimental methods were used. The first was a biochemical determination of the purine and pyrimidine bases in mature embryos (stage 6c) after a single dose of 450 r of X-rays was applied at each of four different periods in embryogeny, namely, early proembryos, late proembryos, mid-differentiating embryos, and late differentiating embryos. The other experimental method involved measuring the relative amounts of P-32 incorporation into the two types of nucleic acid during embryonic development. Embryos were irradiated (450 r) at the same embryonic stages as above.

In the first experimental method RNA and DNA were extracted with 1 N and 0.5 N perchloric acid, respectively, after excluding alcohol-soluble and alcohol-ether soluble compounds and acid-soluble carbohydrates. The extracts of RNA and DNA were hydrolyzed to liberate purine and pyrimidine bases. The mixture of the free bases were separated by paper chromatography and the quantity of each base was determined by ultraviolet spectroscopy.

In the second method microscope slides were prepared from embryos which were irradiated at various stages of embryo development. These sample-mounted slides were subjected to procedures for differential extraction of RNA and DNA in such a way that the first pair of slides (normal and irradiated) contained RNA, DNA, and protein; the second pair DNA and protein; the third pair protein only. Slides were coated with liquid emulsion, before photographic developing and fixing, then were stained lightly with Delafield's hematoxylin, and mounted with clarite under a coverslip. Finally, the relative incorporation of P-32 into RNA and DNA was determined by a visual counting (under oil immersion) of the grains (radioactive tracks) of the entire proembryos and over unit areas of the root, shoot, and scutellum of the differentiated embryos.

The major findings of the present study were as follows:

1. DNA was more radiosensitive than RNA during embryogeny, except for the mature embryo where DNA was more stable than RNA.
2. Altered purine-pyrimidine ratios did not return to the normal pattern of nucleic acid metabolism.

3. Uracil of RNA and thymine of DNA appeared to be more labile to X-radiation than the other bases.
4. The observed difference in RNA and DNA contents at different stages of embryogeny (with the exception of the DNA in mature embryos) indicated that the younger embryos were more affected and/or underwent less recovery during the post-irradiation period than the older embryos.
5. Stage specificity of radiation effects seemed to be referred more to effects on the DNA rather than the RNA components of the embryo cells.
6. The order of increasing radiosensitivity to X rays was scutellum, shoot, and root, if radiation was applied during the time that structural differentiation of the above three regions was occurring.
7. The difference between DNA values in three different parts of an embryo (root, shoot, and scutellum) reflected the degree of tissue heterogeneity, but RNA seemed to be independent of the level of tissue differentiation.

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INTRODUCTION

During the past decade, an increasing amount of work has been directed toward the study of radiation effects on biochemical metabolism in various organisms. Although a complete understanding of the effects of various radiations on biological systems can be determined only by studying all aspects of the diverse changes in the organism that arise as a result of irradiation, a thorough study of nucleic acid metabolism under the influence of radiation is, perhaps, of primary importance since modern concepts link fundamental aspects of growth, development, and protein formation with the synthesis and breakdown of nucleic acids.

The first historical observation of the interference of DNA metabolism by X-irradiation was discovered by Euler and Hevesy (1944) in their experiments with growing Jensen rat sarcoma. Since that time numerous investigators have reported on many aspects of the effects of ionizing radiation on nucleic acids such as the mechanisms by which ionizing radiation interferes with nucleic acid metabolism, quantitative studies on the effect of X-rays on the amount of RNA and DNA, and the effect of X-irradiation on the mitotic activity of the cell. The majority of these experiments have been carried out on animals and microorganisms with comparatively few dealing with higher plants. There has been some work which considered the effects of X-irradiation on the mitotic cycle and DNA synthesis in bean roots

(Howard and Pelc, 1951b, 1953; Pelc and Howard, 1954, 1955) and two which dealt with quantitative analysis of nucleic acids in rye and barley seedlings (Sissakian, 1955; Trudova, 1954). Thus far, however, the radiosensitivity of nucleic acid metabolism in developing plant embryos has not been investigated.

The objective of this research, therefore, was to determine whether radiosensitive stages occur in nucleic acid metabolism during developmental embryogeny. In order to do this, embryos were irradiated at different stages of development, and quantitative and qualitative studies of the composition of RNA and DNA were carried out. Barley embryos were chosen as the experimental plant material since they have been shown suitable for research work (Mericle and Mericle, 1957) and X-rays were used as the source of radiation since their dosages could be readily regulated experimentally.

HISTORICAL REVIEW

The primary purpose of the present work was to determine the relationship between the changes in the quantity of nucleic acids, the ages of embryo tissues, and eventual disturbances in the pattern of nucleic acid metabolism after the treatment of X-rays (450 r) at various embryonic stages. Most investigations involving the effect of ionizing radiation on nucleic acids have been carried out on animals and microorganisms, while higher plants have not been sufficiently studied in this respect. Although embryonic tissues of a higher plant were utilized as the material in the present investigation, it was necessary to refer not only to the literature on plants, but also the work done on animals.

The integrity of nucleic acids is of particular importance to the cell. Much data about this subject has stemmed from the earliest experiment of Hahn and Hevesy (1940), who studied the incorporation of P-32 into DNA of various organs of rabbits. According to their reports, the turnover of nucleic acids in the liver occurred at a low rate, while all other organic phosphorus compounds in the liver were found to be entirely renewed at a rapid rate. In the muscle, on the other hand, the turnover of the average nucleic acid molecule was greater than in the liver or in the thymus. In the brain the turnover rate of nucleic acids was found to be markedly lower than that of the phosphatides.

Von Euler and Hevesy (1944) observed that in unirradiated Jensen rat sarcoma about twice as much DNA became labeled as could be accounted for by the increase in total DNA, and they concluded that about half of the labeled DNA molecules were "new" DNA whereas labeling of the other half was due to renewal of "old" DNA during the process of synthesis. This conclusion has been supported by Stevens, et al. (1953) and Daoust's, et al. (1954) work on incorporation of P-32 into DNA in various rat tissues.

The first historical observation of interference of DNA metabolism due to X-irradiation was discovered by Von Euler and Hevesy (1944). They compared the rate of incorporation of labeled phosphate into DNA extracted from irradiated growing Jensen sarcoma of rat and of control rat and found that irradiation with an X ray dose of a few hundred roentgens or more reduced the rate of formation of DNA in the sarcoma by about 50%.

With similar material Hevesy (1945) studied the uptake of P-32 by DNA after doses of X rays of about 1000 r. The results indicated that isotope administered immediately after irradiation was taken up more slowly in the irradiated material than in the non-irradiated control.

A large number of papers concerning the effect of ionizing radiations on DNA synthesis followed the original discovery of the depression of DNA synthesis by Von Euler and Hevesy (1944).

Many other authors also found that relatively large doses of X rays did not depress DNA synthesis to less than 50% of the control values (Klein and Forssberg, 1954, Ehrlich ascites tumor; Forssberg

and Klein, 1954, Ehrlich ascites tumor; Lavik and Buckaloo, 1954, chick embryo).

In addition, Skipper and Mitchell (1951) repeated Hevesy's experiment (1945), which was concerned with the effect of 950 r radiation on the incorporation of $C^{14}-O_2$ into 6-carbon of DNA purine and of $HC^{14}OONa$ into 2-8-carbon of DNA purine of rat intestine. According to their reports, roentgen-ray radiation (950 r) has been shown to inhibit the incorporation of radioactive carbon into the nucleic acids and their purines by about 50% of the control for a period of six hours. Additional reports concerning the phenomena of initial inhibition of DNA synthesis caused by ionizing radiation have been reported by many other investigators, namely Hevesy (1948a, rat), Thomson,et al. (1952, rat thymus), Vermund, et al. (1953, mouse mammary carcinoma), Bennett, et al. (1954, mice), Petersen, et al. (1955, rat spleen), Harrington, et al. (1955b, rat thymus), Ord and Stocken (1956, rat), and Sherman and Quastler (1958, mice).

The fact that in many cases irradiation depressed DNA synthesis to approximately one-half led Von Euler and Hevesy (1944) to suggest that ionizing radiation may interfere with DNA synthesis but not with the high turnover of DNA. This theory of DNA synthesis depression is not accepted by Pelc and Howard (1955) on the ground that if Euler and Hevesy's interpretation of the irradiation results was correct, the labeling of single cells after irradiation should be one-half that of unirradiated ones, which was in no way supported by Pelc and Howard's experiment. The strength of the autoradiograph per cell was at least approximately equal for irradiated and unirradiated bean

roots. In addition, since Euler and Hevesy's hypothesis was proposed, many investigators have reported results of DNA depression to less than 50% of the control, which is difficult to explain in terms of the 50% DNA depression theory. For instance, Lutwak - Mann (1951), in his study of the effect of ionizing radiation (500 r as whole body exposure) on the nucleic acids in rat, reported that the greatest depression of DNA content as percent of the control was 9% and 37% in bone marrow and spleen, respectively, after 4 days post-irradiation. In recent work, Kelly, et al, (1954) found that whole-body irradiation (450 r to 800 r) of rats after administration of CCl_4 depressed DNA synthesis at its minimum to 20% of controls, if animals were irradiated well before the peak, but had no effect if given at the time of maximal synthesis. Pelc and Howard (1954), on the other hand, determined the proportion of cells in the root meristem of Vicia faba which synthesized DNA during a given period of time, as indicated by the incorporation of P-32 into DNA. According to their reports, the greatest DNA depression fell to 10% of the control at 6 to 8 days post-irradiation. They interpreted this to mean that cells were being prevented from synthesizing DNA if they were in the first part of interphase at the time of irradiation.

According to Sissakian's (1955) experiment on the effect of X-irradiation (5 kr) on the nucleic acids of growing rye seedings, the average DNA depression was recorded as 75% of the control at 3 hours post-irradiation. In a recent work, Nygaard and Potter (1959) investigated the effect of X rays (400 r) on the incorporation of

thymidine -2 -C^{14} into DNA in rats and reported 7%, 25%, and 50% depression in thymus, spleen, and small intestine, respectively, within 24 hours after the treatment of 400 r.

More attention has been paid to the fundamental mechanism implicated in the depression of DNA content due to ionizing radiation. Howard and Pelc (1951b) studied the percent of resting nuclei which showed labeling (P-32) after different periods of growth, their distribution in the bean root cells, and the delay in the appearance in mitosis of labeled nuclei. They concluded that P-32 was not incorporated into nucleic acids during cell divisions, nor during the period immediately preceding it, but during some part of interphase. The incorporation of P-32 took place in cells which were preparing for division, but not in cells which will differentiate without further divisions. In addition they reported that the P-32 incorporated remained in the nuclei for considerable periods of time and was transmitted to daughter nuclei.

The discovery of independent inhibition of mitosis and DNA synthesis due to X-irradiation was first made by Howard and Pelc (1953) in an experiment with bean root meristem. It was concluded that the sensitive period for inhibition or delay of DNA synthesis was during the first part of interphase, beginning possibly as early as 2 hours before the previous prophase and ending 2 hours before the beginning of synthesis. Cells which were already synthesizing DNA at the time of irradiation were not affected in this respect. In subsequent experiments, Pelc and Howard (1954, 1955) confirmed the

previous results by observing that the number of cells synthesizing DNA (observed by means of autoradiographs) in Vicia faba root meristem was reduced about 60% by moderate doses of X rays during the subsequent 12 hours. Reduction was the same after doses of 50 r to 200 r. These results were interpreted to be due to a greater radiosensitivity, resulting in delay or inhibition of DNA synthesis in cells which were in approximately one-third of the cell cycle at the time of irradiation (the first part of interphase).

Results obtained from the experiments with bean root meristem were supported by other authors, namely, Cater, et al. (1956, regenerating liver of the rat), Holmes (1956, rat liver), Kelly, et al. (1955, mouse liver), Kelly, et al. (1957, Ehrlich ascites cells), Kelly, et al. (1957, regenerating rat liver), Lajtha, et al. (1958, human bone marrow).

Lajtha, et al. (1958), in their study on the mechanism of radiation effects on DNA synthesis by cell cultures of human bone marrow in vitro, concluded that large doses (larger than 500 rads) or X-radiation directly inhibited the process of DNA synthesis in human bone marrow cells, while small doses (smaller than 300 rads) did not inhibit DNA synthesis in cells which already had started DNA synthesis. Low dose radiation during the presynthetic period of the mitotic cycle will produce a 40 to 50% depression of the number of cells which enter the subsequent synthetic period, but will not affect the rate of DNA synthesis in those cells that are already in the synthetic period of the cycle. These results essentially supported the original data obtained by Pelc and Howard (1955).

As already described above, considerable evidence has been accumulated which has been interpreted as indicating that period preceding DNA synthesis is more sensitive to irradiation than the synthetic period itself. In each case studied, there appears to be a delay of the onset of DNA synthesis by the cells in the presynthetic phase. Howard (1956) and Kelly (1957), however, have critically examined the data and stated that, with the possible exception of the work on regenerating liver by Holmes (1956), the inhibition of DNA synthesis could be a secondary one resulting from mitotic delay, leading to depletion of cells in the presynthetic phase. Evidence contradictory to the results of Pelc and Howard (1955) has been presented by Painter and Robertson (1959) and Painter (1960). In their recent experiment the percent of HeLa S^3 cells in DNA synthesis at various times after 500 r of X-irradiation was determined by means of autoradiography with H^3 - thymine. They found that the percent of DNA synthesis rose during the period of mitotic delay so that at 4 - 8 hours after irradiation almost twice the number of cells in the population were synthesizing DNA compared to the controls. The interpretation was that many cells synthesizing DNA at the time of X-irradiation remained in this stage for an abnormally long time and there was no effect on the rate at which cells entered DNA synthesis. Therefore, there was no effect on the cells of the presynthetic phase moving into the synthetic. In other words, the fraction of cells in the synthetic phase, as a result of mitotic delay, increased because of the uninhibited flow of cells from the presynthetic to the synthetic phase.

Nygaard and Guttes (1958), in order to find out the effect of ionizing radiation (6000 to 9000 r) on division and on DNA synthesis in slime mold, irradiated cells at different times from late prophase throughout the early part of the reconstruction period. They reported that all of the irradiated molds appeared to complete one period of DNA synthesis, even in the cases when normal nuclear division did not occur.

In addition, according to their results, irradiation during interphase caused more or less a constant delay of the subsequent division, while X-irradiation immediately after division or during late prophase gave a greatly increased effect. Gardella and Servello (1960), on the other hand, studied the labeling of P-32 into DNA in mass synchronous cultures of Tetrahymena pyriformis exposed to X-irradiation (60 K. rad). They reported that the inhibition of DNA synthesis appeared to be the primary response to irradiation and was not influenced by any particular stage of development.

Cattaneo, et al, (1960) conducted an experiment on the effect of X-rays (800 rad) on DNA of hair follicles in mice by the method of labeling with tritiated thymidine and autoradiography. According to their experimental results, the reduction of thymidine incorporation commenced immediately after irradiation and amounted to about 50% of the control within 30 minutes. This was considered to be an effect on cells in the process of synthesizing DNA at the time of irradiation. For the purpose of determining the pathway of decomposition of nucleic acids in vivo due to X-rays, Scholes, et al. (1949) performed an

experiment in which a 2% sodium nucleate solution was irradiated with doses of the order of 2 - 4 x 10^6 r. They reported that the chemical changes which occurred under the influence of X-irradiation indicated that a ring opening in the purine and pyrimidine bases had taken place. In addition, the results indicated some fission of the glycosidic linkages with the liberation of purine bases, a deamination of the constituent bases, some breaking of the ester linkages, leading to the formation of inorganic phosphate, and some splitting of the internucleotide linkages. Weiss (1952), citing the results indicated above, theorized the possible biological significance of the action of ionizing radiation on nucleic acid in similar ways. Pelc and Howard (1952), in their report on chromosome metabolism as determined by autoradiographs, theorized that the mechanism of the direct effect of X-rays on DNA synthesis involved a secondary electron. They said that this electron passes through the substrate at one or several steps in the chain of synthesis, releases electrons that can reach the enzyme, and incapacitates it either permanently or temporarily.

In contrast to the direct effect of X-irradiation, there were a number of reports which indicated an indirect effect on DNA due to X-rays. According to Von Ahlstrom, et al. (1944), the action of Roentgen rays on tumors was an indirect effect which they concluded from the following experiment. Rats were inoculated with two sarcomata. One sarcoma was irradiated with up to 2000 r, while the other sarcoma was protected by 5-mm thick lead sheaths. An investigation

An investigation of nucleic acid formation which took place in both sarcomata indicated that the shielded sarcoma exhibited reduced formation of nucleic acid.

The cells of a sarcoma protected from the direct action of Roentgen rays were thus acted upon only if other parts of the body became irradiated. Holmes (1949), in his study of DNA synthesis in irradiated Jensen rat tumor and in other tumors which were themselves not receiving irradiation, reported that DNA synthesis was reduced to 25 percent of the normal in the directly irradiated tumor. Kelly and Jones (1950), on the other hand, confirmed the existence of indirect effects of X-irradiation in their experiments by treating the liver of the animal directly and the muscle indirectly with 4.25×10^5 ergs. DNA was isolated from the livers of both animals and the specific activity of P-32 was measured. According to their results, the former showed 66 percent DNA of the control, while the latter 84 percent of the normal level.

It has been quite a common phenomenon to see overshooting of DNA content. According to the experiment of Williams, et al. (1955) on DNA synthesis of rat small bowel epithelium after X-irradiation (450 r), the mitotic index was found to be 150% of the control and the adenine and guanine of DNA 608% and 411% of the control, respectively. As to the interpretation of this overshooting phenomenon of DNA, Bennett, et al. (1954) and Howard (1956) explained that periodicity of DNA synthesis recovery might occur due to partial synchronization of DNA synthesis caused by the acute cell death or the delay of the

DNA synthesis in the surviving cells depending upon the position of the cell in the mitotic cycle at the time of irradiation. This partial synchronisation, in turn, could produce an increase of DNA content over the control level.

Concerning the initial depression and temporary recovery of DNA content due to X-radiation, Knowlton, et al. (1949 and 1950), in evaluating the results obtained from experiments involving the effect of X-rays on the mitotic activity in various mouse tissues, suggested that the time from the irradiation to the minimum of the mitotic index was a measure of duration of mitosis which usually was followed by temporary return to normal mitotic activity (abortive mitotic recovery), depending upon the doses of X rays, kinds of tissues, and period of post-irradiation.

The effects of ionising radiation on the DNA of growing tissues are quite different from that of differentiating tissues in terms of the synthesis of DNA related to the mitotic phases. However, before surveying the literature concerned with these tissues, it would be well to compare them with respect to DNA synthesis.

In investigating the relationship between the rate of DNA synthesis and mitotic phases, Pelc and Howard (1952), in their experiment on the rate of P-32 incorporation into the DNA of Vicia faba seedlings by use of autoradiographic techniques, reported that a cell which was preparing to divide synthesized DNA during the first part of the interphase, while no DNA synthesis occurred during division. In addition, they suggested that a cell which had completed

its last division and was differentiating did not synthesize DNA and retained the DNA transmitted to it by its parent cell. In contrast to the experiment of Pelc and Howard, Hevesy and Ottesen (1945) presented the similar fact that the DNA of white corpuscles and nucleated erythrocytes, in which mitosis was absent, did not interchange with P-32 present in the circulation. According to the experiment conducted by Brues, et al. (1944) on the rate of P-32 incorporation into the DNA of slow growing tissue such as kidney of rat and of partially regenerating liver, only minute amounts of tracer were found in the former, while there was a marked P-32 content in the latter. In addition, they reported that RNA turnover in slow or nongrowing tissue was considerably more active than DNA turnover.

Hevesy (1945), on the other hand, studied the uptake of P-32 by the DNA of Jensen rat sarcoma immediately after doses of X rays of about 1000 r and found 60-70% inhibition of DNA renewal compared to the normal. When the administration of labeled phosphorus into the DNA of rat liver was delayed until several days after exposure to the X rays, the difference between the specific activity of the DNA in the control and irradiated tissue was found to be very much less than when the isotope was given immediately after irradiation, while the similar degree of inhibition (60-70% of the control) occurred in the latter case as in sarcoma. He suggested that the rapid diminution in effectiveness of X rays as a means of blocking DNA renewal might well explain the greater sensitivity of growing tissues to irradiation, since, in such tissues

the frequency of mitosis and therefore the synthesis of DNA was much greater than in full grown tissues where mitosis was relatively rare. In addition, to explain the phenomenon of recovery of nucleic acid cycle from the effect of X rays on growing and full grown tissues, he hypothesizes (1946) that in the latter case, the average cell was very much further from the mitotic stages than in the growing tissue, and thus had time to recover its normal nucleic acid cycle before any appreciable change in the nuclear structure took place, if enough time was allowed; while in the former, in the absence of a normal nucleic acid cycle, an anomalous nuclear development took place with all its far reaching consequences.

In an attempt to visualize the changes of DNA occurring during the post-irradiation period, Quastler and Sherman (1958) studied the recovery of DNA synthesis in the crypts of the small intestine of rats. This was done by injecting tritiated thymidine, sacrificing the rats at various intervals, preparing high resolution autoradiographs, and determining the percent of cells in mitosis which were labeled after X-irradiation of 800 rad. According to their results, there were two waves of DNA synthesis during post-irradiation. First there was a complete blockage of DNA synthesis that took place in a half day following irradiation. The second wave of DNA synthesis was characterized by a high incorporation rate within 2 days after initial irradiation. Similar multiple effects were observed by other investigators. Williams et al. (1958) conducted an experiment on the differential effect of X-irradiation on the DNA and mitosis of rat small bowel epithelium, and Paigen and Kaufmann (1953)

observed the effect of whole body irradiation (600 r) on the nucleic acids of mouse liver at various times after treatment with X rays. In order to study the recovery after sublethal doses of irradiation (100 and 400 r), Nygaard and Potter (1960a) observed the effect of total body irradiation on DNA synthesis in thymus, spleen, and small intestine of the rat over an extended period by means of thymidine -2 -C^{14} incorporation in DNA. According to their experiments, following an initial period of inhibition the tissues recovered their ability to synthesize DNA. The time for this recovery was found to depend on the tissue and on the radiation dose. In all cases the specific activity exceeded the control value at the time of maximum recovery, usually by a factor of about 2. It was concluded that the effect of radiation on the DNA metabolism of the three tissues were not quantitatively the same, differing only in the degree of acute cell death, in the duration of the delay of DNA synthesis in the surviving cells, and in the rate of recovery resulting from accelerated cell replication during the period of regeneration.

Holmes (1947), in his study of the effect of X-irradiation (2000r) on P-32 incorporation into the nucleic acids of Jensen rat sarcoma, reported that irradiation reduced the rate of P-32 incorporation to one-half that found in the control, while there occurred a lesser degree of inhibition of phosphorus uptake by the RNA of this tissue. In contrast to this result, Abrams (1950), who treated rats with a dose of 500 r, X rays,

injected C^{14} labeled glycine, fractionated the tissues according to the methods proposed by Schmidt and Thannhauser (1945), and measured the turnover rate of RNA and DNA in terms of tracer incorporation rate into purines of both types of nucleic acids.

According to his data, the DNA and RNA were found to be decreased to 80% and 47% of the control, respectively, within 48 hours post-irradiation, indicating a more marked depression of RNA than DNA. The recovery percentages of DNA and RNA were 47% and 23%, respectively, within 96 hours following the treatment of X rays, indicating more DNA recovery than RNA. Lutwak-Mann (1951) also studied the effect of X rays (500 r) on the change in the content of RNA and DNA in the testes of the rats. He indicated more depression of RNA than DNA either in short or in long periods of post-irradiation time, and more decrease in DNA than RNA, in bone marrow at four days post-irradiation. These results were supported by Mandel, et al. (1951). Using rat spleen and mouse thymus, the same author (Lutwak-Mann, 1952) found a large reduction in the RNA and DNA contents per organ due to X ray treatment (300 - 800 r). This observation was in agreement with that of Weymouth and Kaplan (1952), who treated the mice systematically with 4 doses of 168 r at 8 day intervals.

In an attempt to find the site of action of X rays in a cell, Harriss, et al. (1952) treated the nucleus and cytoplasm of Amoeba proteus by means of the technique of nuclear transfer, and reported that the cytoplasm and nucleus were damaged independently under the influence of 100,000 to 280,000 r.

Payne, et al. (1952), in a study of the effect of total-body X-irradiation (600 r) on the relative turnover of nucleic acid in mice, observed that the incorporation of P-32 into the cytoplasmic RNA was increased after irradiation, while the uptake of isotope by nuclear RNA and DNA was greatly diminished. Thomson, et al. (1952) treated rat thymus with a dose of 800 r of gamma rays from a source of Co-60 and observed that there occurred a pronounced diminution in P-32 incorporation into DNA and to a lesser extent in RNA in 3 hours post-irradiation. Similar results were observed by Thomson, et al. (1953), who conducted an experiment with rabbit bone marrow exposed to 620 r. In addition Lavik and Buckaloo (1954) allowed chick embryos, X-irradiated with 450 r, to incorporate carbon-14 labeled formate and cytidine into purines and pyrimidines of nucleic acid. They reported a 50% inhibition of DNA bases and no change in RNA bases.

Using barley seedlings, Trudova (1954) investigated the effect of X rays on nucleic acid metabolism. He showed that irradiation with a dose of 2 kr completely and irreversibly suppressed the synthesis of DNA in the tips of the roots of barley seedlings, and the synthesis of RNA also was considerably suppressed by 73 percent. In addition, according to his data, within 5 hours after irradiation by a dose of 500 r, DNA and RNA synthesis were suppressed by 44% and 86%, respectively, but within 24 hours the activity of these fractions became equal to the activity in the control. A dose of 65 r was observed to even provoke the synthesis of DNA and RNA.

The phenomenon of transformation between RNA and DNA has been reported not only in normal tissues but also in those X-irradiated.

According to the theory of nucleic acid starvation proposed by Darlington (1947), the supply of nucleic acid controls the prophase of mitosis. If the supply of this substance is cut off, the nuclei consume their own cytoplasm and die during mitosis. According to this concept, he concluded that any factors which reduce this substance will induce the interchangeable phenomenon between cytoplasmic RNA and nuclear DNA. This theory was accepted by Davidson and Leslie (1950).

The observation that a very large quantity of ribose nucleic acid was localized in the chromosomes during cell division led Caspersson (1940) and Caspersson and Schultz (1938, 1939) to conclude that ribose nucleotides served as the supply of DNA materials in the nucleus which was the preliminary requirement of mitosis. Since the action of X rays affected cell division, he added, it might influence the cycle of nucleic acid changes and induce an interchangeable relation between RNA and DNA.

The report made by Semenenko (1958) is quite interesting. In his study of summer wheat seeds on quantitative changes of nucleic acids from the time of fertilization to mature seeds, he observed that reciprocal fluctuation existed between RNA and DNA during the first 15 days. In the same experiment, in contradiction to the result above, there was parallel relationship between the contents of RNA and DNA in maturing pea seeds. On the other hand, Prescot (1960) using Tetrahymena, labeled DNA with H^3-thymidine and RNA with C^{14}-adenine

and observed that the maximum incorporation of the former tracer into DNA preceded the minimum incorporation of the latter tracer into RNA during a period of one mitotic cycle.

The results reported by Caspersson and Schultz (1938, 1939) were demonstrated by Mitchell (1942), who found an accumulation of ultraviolet-absorbing materials which appeared to be of nucleotide nature. He indicated the transformation of nucleic acids from one type to another in irradiated tumor cells. In addition, Nicola (1950), in his experiment with the action of X rays on the metabolism of nucleic acids in proliferative and secretory cells, found that X rays induced an arrest of DNA synthesis and a conversion of DNA into RNA. He indicated some possible counteraction on the reduction process normally taking place in the transformation of RNA to DNA due to the action of substances produced by the interaction of X rays with water molecules. While surveying the literature which deals with the effects of ionizing radiations on nucleic acids, it is readily seen that very few investigators have completely followed all the changes in nucleic acid composition due to X rays.

Paigen and Kaufman (1953) observed the effect of whole body irradiation by hard X rays on the nucleic acids of mouse liver. They reported no change in nucleic acid composition when compared to non-irradiated controls. Berenbom and Peters (1956), in contradiction to Paigen and Kaufman's report above, conducted an experiment on changes in nucleic acid composition (rat spleen and thymus treated with 400 r total-body-X-radiation). According to their report, thymus RNA

composition did change slightly with a relative loss in adenine and a slight relative increase in cytosine. This change was temporary and the normal pattern was re-established within 7 days. On the other hand, a marked change in splenic DNA composition occurred, resulting in a relative increase in guanine and adenine, and a relative decrease in cytosine and thymine. This increased purine-pyrimidine ratio did not return to the normal pattern within 7 days after irradiation. These results were supported by Harrington and Lavik (1955a) who studied the phosphorus composition and P-32 incorporation in irradiated lymphatic tissue of rats.

To determine the relative sensitivity of RNA-bases to radiation, Sissakian (1955) treated growing rye seedlings with a dose of 20 kr and analyzed the base contents. He found that the effect of radiation clearly manifested itself by the decrease in content of all nitrogenous bases; guanine by 65 percent, adenine by 57 percent, cytosine by 79 percent, and uracil the lowest, by 44 percent, indicating that uraceil was more labile than other bases in this experiment.

MATERIALS AND METHODS

Biochemical Analysis of Nucleic Acids in Mature Embryos

Raising of Plants

Barley, Hordeum distichon L., var. Hannchen, a two-rowed variety, was used for the extraction of RNA and DNA in mature embryos, and incorporation of P-32 into RNA and DNA during embryogeny. This variety was chosen as the experimental material because of its uniform growth due to the availability of long inbred lines, and the fact that it produces a two-rowed "flat" head which is particularly well adapted for X-irradiation since the possibility of partial shielding by other grains is eliminated. In addition, this variety of barley produces numerous seeds per head with embryos in relatively the same stage of development, since no lateral florets develop as in a "six-rowed" variety, and provided that the four basal and the four terminal grains are discarded (Chang, 1957).

Plants were grown in the greenhouse where average temperatures were 75°F during the day and 65°F at night. Optimum temperature differentials for this strain of barley should be 10° - 15°F. It is particularly important to maintain the lower night temperature to avoid sterility problems often encountered with higher temperatures.

Since barley is a long day plant, the day length was increased to 20 hours by the use of artificial light in order to hasten flowering, thus permitting extra "crops" to be grown during a given period.

Preparation of Embryo Materials for Tissue Fractional Analysis of Nucleic Acids

The preparation of materials for quantitative determination of nucleic acids in normal and X-irradiated mature embryos was conducted as follows:

Patterned after Mericle and Mericle (1957), morphological and histological features of barley embryos, from fertilization time until "maturity" of the embryo as found in the seed, may be divided into two large groups: proembryos and differentiating embryos. The former may in turn be arbitrarily divided into three subgroups: early (stage a - c), middle (stage d - e), and late proembryos (stage f - g). Early proembryos include developmental stages from the one-celled zygote to the 8-celled stage, middle proembryos from the 16-celled stage to approximately 72 cells, and late proembryos, just prior to the initial stages of organogenesis (organ differentiation). Differentiating embryos may be grouped into six stages, namely, early (stage 1-2), middle (stage 3-4), late (stage 5-6), (plate 2). In addition, stage 6, for convenience, may be divided into 3 subgroups: early (stage 6a), middle (stage 6b), late (stage 6c).

With this in mind, approximately 200 embryos at stage 6c were dissected from the caryopses of non-irradiated barley plants, and kept

in 70% ethanol at 0°C for the purpose of fractional analysis of nucleic acids and determination of purine and pyrimidine bases in normal, control embryos.

In order to prepare analytical materials for study of the nucleic acids of X-irradiated embryos, a single dose of 450 r of X-rays (at a rate of 65 r/m with a total dose of 450 roentgens) was applied at one of four different periods in embryogeny; namely, stages a-c (Group-A), stage g (Group-B), stages 3-4 (Group-C), and stages late-5 (Group-D). The grains containing irradiated embryos were then allowed to continue development in situ in the greenhouse until reaching stage 6c. Approximately 200 X-irradiated embryos at stage 6c were dissected from each experimental group, and kept in 70% ethanol at 0°C for the purpose of quantitative determination of nucleic acids in X-irradiated embrye materials.

A G. E. Maximar 250 III therapeutic X-ray machine was used as the source of external irradiation. Physical factors for application of the doses were as follows: 200 kilo-volts of power, 15 milliamperes of current, inherent filtration of 3 mm aluminum, and 0.25 mm copper plus 1 mm aluminum added filtration. Further characteristics were: 0.75 mm copper half-value-layer, 33 cm focal spot distance, and 400 sq. cm beam size.

For determination of embryonic stages at the time of X-irradiation, histological sections were made of embryos from the middle of each head to be irradiated. Formalin - acetic acid - alcohol, FAA (Johansen, 1940), was used for killing and fixing the ovaries.

Materials were dehydrated, embedded in paraffin by standard procedures (Johansen, 1940) and serially sectioned at 12 microns. Staining was carried out by using a combination of safranin and fast green (Johansen, 1940).

Overall scheme of embryonic stages irradiated and samples analyzed chemically in this part of the experiments was as follows:

Experiment Number	Embryo Stages X-irradiated	Embryo Stages Analyzed Chemically
Group A	a - c	6c
Group B	g	6c
Group C	3 - 4	6c
Group D	Late 5	6c
Control	No Radiation	6c

Tissue Fractionation and Quantitative Determination of Purine and Pyrimidine Bases of RNA and DNA in Mature Embryos

A. Methods of Tissue Fractionation

Most of the recent studies of the nucleic acid content of various animal tissues have been carried out using the tissue fractionation methods developed by Schneider (1945) and by Schmidt and Thannhauser (1945). According to their methods, separation of RNA from DNA is accomplished by prolonged contact with alkali which degrades RNA more rapidly than DNA, leaving the latter still precipitable with acid.

Various difficulties are, however, encountered when one attempts to apply either of these methods to the study of the nucleic acids during the development of plant root tips, pollen cells, or plant embryos. Because of the small amounts of material available, making the successful quantitative precipitation at such levels somewhat questionable, and because of the presence of interfering substances (pentosans and polyuronides), neither the Schmitt and Thannhauser nor the Schneider procedures were considered adequate for the purposes of the present study. In other words, in their methods all acid-soluble compounds were first extracted with 5-10% trichloracetic acid, phospholipids, then with warm ethanol-chloroform (3 : 1), and nucleic acids as sodium nucleate with 10% NaCl. These processes, however, may possibly leave behind some alcohol-soluble compounds which are present in most plant materials and which give an intense purple coloration with diphenylamine.

Other methods in current use for tissue fractionation often begin with the extraction of homogenized tissues with cold trichloroacetic acid, followed by the hot extraction of phospholipids. Ogur and Rosen (1950), however, have modified this approach by beginning with a cold alcohol-extraction to extract as much nonnucleic acid material as possible before separation of RNA and DNA. A modification of their method was found to be most suitable for the present study and is described below.

(1) Alcohol-Soluble Compounds

Samples, each consisting of approximately 200 embryos, were collected and immediately after dissection stored in 70% ethanol at 0°C. Prior to tissue fractionation, both the control and X-irradiated "mature" embryos were dried in a dessicator over acetone (B.P. 58° C) under vacuum conditions until constant dry weights were obtained. After dry weights were determined the tissue was homogenized for 10 minutes with a small hand mortar in a cold room (4°C).

The homogenate was washed into a calibrated tube with 70% ethanol and centrifuged at 4° C. The residue was resuspended in 70% ethanol containing 0.1% perchloric acid and again centrifuged in the cold. The alcohol and acidulated alcohol extracts were combined.

(2) Alcohol-Ether-Soluble Compounds

The residue from (1) was suspended in 5 ml of 3:1 ethanol and ethyl ether, a small piece of porous clay plate added, and the mixture boiled gently for 3 minutes in a water bath. This process was repeated and the two extracts then combined.

(3) Acid-Soluble Compounds

The residue from (2) was suspended in 5 ml of cold 0.2 N perchloric acid and centrifuged. This process was repeated and the extracts combined. This step was completed as rapidly as possible, although additional controls in which the residue remained in contact with the perchloric acid for 3 hours did not lose any measurable amount of RNA.

(4) Extraction of RNA

The residue from (3) was suspended in 5 ml. of 1 N perchloric acid and stored at 4° C for 18 hours. (Roughly over-night contact in the cold is time enough to split the RNA from the tissue residue). The suspension was centrifuged and the residue extracted twice more with 5 ml. portions of cold 1 N perchloric acid and combined.

(5) Extraction of DNA

The residue from (4) was suspended in 5 ml. of 0.5 N perchloric acid and heated in a water-bath for 20 minutes at 70° C. This process was repeated and the extracts combined. More exhaustive extraction of embryos failed to reveal any additional material reacting with diphenylamine, indicating that no DNA remained in the residue.

B. Procedures for Hydrolyzing RNA and DNA Extracts

Various methods for the hydrolysis of isolated nucleic acids and the separation of the bases have been described (reviews edited by Chargaff and Davidson, 1955; Markham, 1955) but without modification the methods were not applicable to tissue extracts which contain a variety of interfering substances. A modification of the perchloric acid method (Marshak and Vogel, 1950) described by Woods (1957) and Taylor (1958) was satisfactory for separating and recovering only uracil, thymine, and guanine, but considerable loss of the base, cytosine, occurs, however, when their methods are used.

In order to separate all four bases of RNA and DNA and obtain good recovery rates, the writer modified several steps in these procedures. The methods as used in this investigation are described below.

(1) A small beaker containing 10-15 ml. of RNA, or DNA extract, prepared as described above, was placed in a hot water bath at 100° C for 160 minutes to liberate the purine and pyrimidine bases.

(2) After cooling, the mixture was diluted to 10 ml. with distilled water, then centrifuged to separate the fluid from the black particulate residue formed, presumably from the ribose or deoxyribose sugar of RNA or DNA, respectively.

(3) For the purpose of eliminating perchloric acid, the resulting clear hydrolysate was adjusted to a pH of 7.0 with 1 N solution of K_2CO_3 (with the aid of a Beckman pH-meter).

(4) The mixture was filtered through Whatman No. 1 filter paper, followed by washing with 10 ml. of distilled water and then with 50 ml. of 80% ethanol.

(5) The filtrate was concentrated to a small volume by the use of a flash evaporator at 58° C.

(6) In order to dissolve all purine and pyrimine bases (mostly water-insoluble guanine) which were possibly tied up with the salt of perchlorate, the residue from (4) was added to 15 ml. of 0.1 N HCl and agitated for one hour at 25-30° C by means of an electric shaker.

(7) The mixture from (6) was filtered through Whatman No. 1 filter paper and washed with 15 ml. of 0.1 N HCl.

(8) Filtrate from (7) was now combined with that from (5) which was already concentrated to a small volume, and dried by a flash evaporator at 58° C.

(9) The dried bases were dissolved in 1 ml. of 0.1 N HCl and diluted to 2 ml. with an additional 1 ml. of distilled water for the determination of RNA. For the determination of DNA the dried bases were dissolved in 0.5 ml. of 0.1 N HCl and diluted to 1 ml. with an additional 0.5 ml. of distilled water. The resulting solutions were used for paper chromotography.

C. Separation of Purines and Pyrimidines by Paper Chromatography

(1) Sheets of Whatman No. 1 filter paper, 20 cm. wide and 40 cm. long, were ruled into five longitudinal sections, each 4 cm. wide. A transverse line, about 8.5 cm. below the top of the sheet, indicated the starting points at which, in the center of each of four lanes, aliquots of solution from (9) were to be deposited. The fifth section was used as a blank.

(2) Prior to spotting and development of the chromatograms, the paper was electrolyzed in the following ways:

(a) Sheets were soaked for 5 minutes in 0.2% EDTA (disodium salt of ethylene dinitrilotetraacetic acid) solution, which was prepared by dissolving 2 grams of EDTA in 1000 ml. of glass-distilled water, then adjusting it to a pH of 8.5 with NaOH.

(b) The EDTA treated paper sheets were rinsed with distilled water three times.

(c) Sheets were then soaked in 1 N HCl for one minute and rinsed with distilled water until the solution checked neutral.

(d) Finally they were washed twice with glass-distilled water and dried at room temperature overnight.

(3) The dried papers were now spotted with 10 of base mixture using a micro pipette and chromatograms were run descendingly at room temperature in a solvent containing isopropanol, concentrated HCl (sp. gr. 1.19) and water in the proportions 170:41:39 (Wyatt, 1951). All five bases were resolved in the order: guanine, adenine, cytosine, uracil, and thymine.

(4) After drying in air, the base resolved chromatographic sheets were placed in a dark room under an ultraviolet lamp (253.7 m u) and the bases were located as dark spots against the white paper then lightly encircled with a pencil.

D. Methods for Determination of Purine and Pyrimidine Bases by the Beckman Quartz Spectrophotometer

(1) Elution

For elution, rectangles containing the spots of bases were cut from the chromatograms. Rectangles of equal area were cut from the blank at levels corresponding to the position of each base. Each rectangle was cut into small pieces and placed in an Erlenmeyer flask. To each flask was added 5 ml. of 0.1 N hydrochloric acid.

After thorough agitation (by means of an electric shaker) at 25-30° C for a minimum period of 60 minutes, the eluates were decanted from the paper and centrifuged (Marshak and Vogel, 1950).

(2) Ultraviolet Spectroscopy

The clarified eluate of each base was read in a Beckman quartz spectrophotometer in 1 cm. cells against the corresponding blank eluate. Acidic extracts of the filter paper itself exhibits a low,

but neither constant nor regular, absorption in the ultraviolet region. For this reason, rather than to take the absolute extinction values at the absorption maxima (adenine at 262 mμ, guanine at 249 mμ, cytosine at 275 mμ, uracil at 259 mμ, and thymine at 264 mμ) as the basis of calculation, it was preferable to estimate the purine and pyrimidine contents of the extracts by using the differences in the extinction values read at the absorption maxima and at 290 mμ.

For the standard solution, 10 μg of each of the five bases per one ml. of 0.1 N HCl, the difference, Δ, was determined as follows:

Adenine,	E 262.5	= 1.070
	E 290	= 0.046
	Δ	= 1.024
Guanine,	E 249	= 0.790
	E 290	= 0.299
	Δ	= 0.491
Cytosine,	E 275	= 0.960
	E 290	= 0.505
	Δ	= 0.455
Uracil,	E 259	= 0.740
	E 290	= 0.008
	Δ	= 0.732
Thymine,	E 264	= 0.612
	E 290	= 0.076
	Δ	= 0.536

In order to verify the position of the maximum, the ultraviolet absorption of the extracts was also determined at 5 mμ above and below the characteristic absorption maximum of the purines and pyrimidines in question, i.e. at 267.5 and 257 mμ for adenine; 254 and 244 mμ for guanine; 280 and 270 mμ for cytosine; 264 and 254 mμ for uracil; 269 and 259 mμ for thymine. In addition, the extinction of the extracts also was measured at 300 mμ, at which wave length the purines and pyrimidines absorb very little. The extinction values found at 300 mμ should, therefore, be very low, usually between - 0.010 and + 0.040. Readings outside this range are indicative of contamination, and such extracts should be discarded (Vischer and Chargaff, 1948).

Incorporation of P-32 into Nucleic Acids of Embryos During Embryogeny

Since developing embryos, especially in proembryo stages, are too small and much too difficult to obtain in adequate numbers to permit use of the methods of chemical analyses previously described for older, more mature embryos, P-32 incorporation has been used as a tracer method in order to elucidate changes in nucleic acid content following X-radiation.

In any experiment using a radioactive isotope as a tracer, an obviously important requisite for valid results is that the processes studied are not influenced by either the isotopic nature, i.e., the difference in mass number (isotope effect) or the radioactivity

(radiation effect) of the tracer used; if they are, then the results obtained apply only to the presence of the tracer element. These are very controversial questions about which much has been written. Generally speaking, however, the magnitude of the isotope effect is small enough as to be undetected (Comar, 1955) although it might be a vital consideration with elements of very low atomic number.

With regard to potential radiation effects from the use of P-32, papers so far published often report very different results, so that even for liquid cultures it does not appear permissible to set an absolute "safe" level. Scott, et al. (1949) demonstrated radiation damage in barley grown for 6 days in nutrient solution containing concentrations of radioactive phosphate as low as 10 μc per liter. Mackie, et al. (1952) detected radiation effects in shoot tip cells of barley seedlings grown for 12 days in nutrient culture containing only 4 μc per liter at a specific activity of 6,440 μc P-32/gm P-31; yet 200 μc per liter produced no detectable effects when the specific activity was lower. Levels as high as 400 μc at a specific activity of 9,500 μc/gm resulted in only microscopically detectable effects even in the highly radiosensitive barley proembryo (Mericle and Mericle, 1961).

In pot experiments, Hendricks and Dean (1948 a, b) reported that radioactive phosphorus in amounts as large as 625 μc per kilogram of soil did not influence the yield of perennial ryegrass. More recently, Strzemienski (1952) found that P-32 applied in concentrations of from 40 to 2,560 μc per gram of phosphorus did not influence either

dry matter production or distribution in short-rotation ryegrasses.

In general, there appears to be a very wide range of "safe" dose levels for P-32 especially if soil is being used as the "culture medium." In the present experiment, 200 μc of P-32 was used per kilogram soil, a level well below that at which Hendricks and Dean (1948 a, b) found no effects.

The validity of using P-32 incorporation into nucleic acids as a measure of nucleic acid content bears justification at this point.

Some years ago, Schoenheimer and his colleagues (1946) postulated that all constituents of organisms, whether functional or structural, were in a state of dynamic equilibrium. There was said to be a continuous renewal or turnover, in which the synthesis of tissue constituents was exactly balanced by their degradation. In the meantime, it has come to be tacitly assumed that this turnover not only applies to the tissues as a whole but that it occurs as an intracellular event in the various tissues.

Recently, however, the concept of intracellular turnover has been subjected to a critical examination since studies with bacteria showed that there was probably no renewal of either of the nucleic acids in these cells (Fujisawa, and Sibatani, 1954; Hershey, 1954).

The most convenient and perhaps the only certain method of determining whether or not an intracellular constituent is being renewed is to label that constituent with a radioactive isotope and to observe the fate of the incorporated label when the cell is allowed

to multiply in the absence of the extracellular isotope. If turnover of the constituent occurs, its isotope content should decrease in amount. Hershey (1954) applied this method in studying the stability of nucleic acids in E. coli and found that P-32 once incorporated into the RNA and DNA fractions, separated analytically by the Schmidt and Thannhauser method, remained there during subsequent cell multiplications. Recently Siminovitch and Graham (1956) repeated the experiments with bacteria and also showed that RNA and DNA in tissue culture cells behaved in the same manner.

Pelc and Howard (1954) used autoradiographs to investigate the proportion of cells in the root meristems of Vicia faba which synthesized DNA over 10 days, as indicated by the incorporation of P-32 into the nucleic acids.

Taylor and McMaster (1954), and Moses and Taylor (1955) also found that incorporation of P-32 into DNA is restricted to a relatively short period of the mitotic cycle which has been shown to coincide with increases in the net amount of DNA per nucleus in every instance studied at the cellular level. These data on P-32 incorporation into nucleic acids at the intracellular level of synthesis will serve as the theoretical basis in this study for applying the same method to tissues as a whole.

In the present study, P-32 was incorporated into embryos at various stages of embryogeny immediately after X-radiation and allowed to continue for extended periods of time until the last stage of the differentiating embryos was reached. During these periods of time,

several embryo samplings were made at various representative embryonic stages in order to determine relative changes in nucleic acid content as compared with control values.

Raising of Plants

Eight 7-inch clay pots were filled with 2.5 kilograms of homogeneous soil and sown with 14 seeds of the barley variety, Hannchen. The plants were later thinned so that seven plants remained in each pot. Temperatures in the greenhouse where the plants were grown averaged 75° F during the day and 65° F at night as previously described.

The plants were divided into four different groups, each group consisting of two pots of plants, one control and one X-radiated, depending upon the initial embryonic stages at the time of the irradiation. The first group was irradiated with a single dose of 450 r at stage a-c, the second group at stage g, the third group at stage 3-4, and the fourth group at stage late 5.

Overall scheme of embryonic stages irradiated and samples analyzed by P-32 grain counting in this part of the experiments was organized as follows:

Experimental Number	Embryo stages X-irradiated	Embryo stages analyzed by P-32 grain counting
Group 1	a - c	d - e, g - 1, 4, 6c
Group 2	g	g - 1, 5, 6c
Group 3	3 - 4	4, 6a, 6c
Group 4	Late 5	6a, 6b, 6c
Control	No radiation	d - e, g - 1, 4, 5, 6a, 6b, 6c

Procedures for Autoradiographs

A. Preparation of Embryo Slides

For the first experiment (Group-1), involving the youngest proembryo stages, four to five main heads from the seven plants in each of two pots were selected as replicates, one pot of plants for the control and one pot to be irradiated. In order to identify the initial embryonic stages at the time of irradiation, a single developing caryopsis was removed from the middle of each of the selected heads for histological examination. A single dose of 450 r of X rays, with the same specifications as described earlier, was given to the plants in one pot, while the other pot of plants was used as the control.

Immediately following irradiation, 0.5 mc of P-32 in 0.04 ml. of HCl, diluted to 150 ml. with glass-distilled water, was added to each of the two pots, control and treated.

The radioactive phosphorus was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, as a carrier-free separated isotope, the chemical form of which was H_3PO_4 in weak HCl solution.

The first samplings were made at 24 hours post-irradiation at stage d-e; the second samplings at stage g-1; the third at stage 4; and the fourth at stage 6c. In each sampling from the main heads of both irradiated and control plants, two caryopses borne oppositely on the rachis were removed from near the top of each spike and proceeding toward the base (except for the four terminal and four basal grains which were discarded). One grain of each sample was used for autoradiographic slides to determine P-32 incorporation into the nucleic acids of the embryos, while the other was used for the measurement of total radioactivity.

For autoradiographic purposes, the developing caryopses were killed and fixed in formalin-acetic acid-alcohol, FAA (Johansen, 1940). Materials were dehydrated, embedded in paraffin by standard methods (Johansen, 1940) and serially sectioned at 6 microns. Three successive near-median dorsi-ventral sections of each embryo were selected and separately mounted on each of three slides with Haupt's adhesive. All section-mounted slides were dried in a box with a small dish of 3-4% formalin for at least two days.

From the other caryopsis, at each sampling time, the embryo was dissected and its size measured under a binocular dissection microscope. The embryo was then squashed between 10 mm. squares of aluminum foil and saran wrap and placed in the center of a planchet for counting. Total radioactivity of the embryo was calculated by means of a Geiger-Muller counter which had been calibrated against a known P-32 simulated standard. The main purpose of the radioactivity measurements was to have a better understanding of P-32 absorption from the soil into plants, in the event that there might be some possible significant differences in the amounts and/or rates of P-32 absorption between control and irradiated plants.

In the second experiment (Group-2), the third (Group-3), and the fourth (Group-4), all procedures were carried out in the same way as in the first experiment, except for different sampling stages. The first samplings in the second experiment were made after 24 hours when the embryos were in stage g-1; the second at stage 5; the third at stage 6c. In the third experiment, the first samplings were taken after 24 hours when the embryos had reached stage 4; the second sampling made at stage 6a; and the third at stage 6c. In the last experiment, the first sample was taken at 24 hours post-radiation, at stage 6a; the second at stage 6b; the third at stage 6c.

B. Methods for Differential Extraction of RNA and DNA

As described in the previous section, the autoradiographic slides were prepared in triplicate. Differential extractions of RNA and DNA were made from the sections by procedures similar to those which

were applied to the "mature" embryo tissue described earlier in this work but with minor modifications (Taylor and Taylor, 1951; Howard and Pelc, 1951a; Taylor and McMaster, 1954; Taylor, 1958).

All sets of slides (three replicates for each radiation level along with their three replicate controls) were grouped in pairs, an irradiated embryo slide back to back with its control, and treated in the following way:

1. Paraffin was removed from the sections with xylene.
2. All slides were passed through an ethanol series, starting with 100%, down through 90%, 85%, to 70%.
3. Preparations were treated in 70% ethanol containing 0.1% perchloric acid for 5 minutes at room temperature to remove interfering substances such as pentosans and polyuronides.
4. After dehydration through another ethanol series, 85%, 90%, and 100% sample slides were treated with an ether-ethanol (1:3) solution for 5 minutes at 60° C and passed again through an ethanol series, 100%, 90%, 85%, 70% down to water.
5. For the purpose of extraction of acid soluble compounds, 0.2 N perchloric acid was applied at 2-4° C for 5 minutes. One pair of slides (irradiated and control) of a set of six was washed in cold water and stored in 70% alcohol.
6. The remaining four slides were kept in 1 N perchloric acid at 4° C overnight for the purpose of extraction of RNA. During this period of time, two changes of solution were made

with the same normality of perchloric acid. One of the pairs of slides (irradiated and control) was washed in cold water and stored in 70% alcohol.

7. The remaining pair of slides was hydrolyzed in 0.5 N perchloric acid at 70° C for 20 minutes to remove DNA, rinsed in water, and stored in 70% alcohol.

All sample preparations stored in 70% alcohol were kept at 4° C in a refrigerator until the autoradiographic emulsion was applied.

C. Application of Emulsion

Liquid emulsion (Type NTB; No. LSB-3-0) used in these processes was donated to Dr. L. W. Mericle by Eastman Kodak Company, Rochester, New York.

Before applying the emulsion, all slides with embryo sections stored in 70% alcohol were rinsed three times with glass-distilled water and processed in the following way:

1. Approximately 15 cc. of emulsion was placed in a small beaker and melted in a water bath at 40° C in the dark.
2. The sample-mounted ends of slides were dipped into the emulsion then inverted in grooved wooden stands so that any excess melted emulsion flowed evenly downward coating the samples with a very thin layer of emulsion.
3. After drying some 20 to 30 minutes, each emulsion-covered slide was placed in a horizontal position between pieces of lead (75 mm. long, 25 mm. wide, and 3 mm. thick) alternately inserted into slots of a light-tight slide box. This box

was placed within another larger case and stored for exposure under refrigeration at 4° C. Slide boxes were previously sprayed with black lacquer to minimize possible light leakage. The purpose of the alternate arrangement of lead pieces was to shield each slide to prevent possible fogging from adjacent slides.

In order to be able to make quantitative comparisons within and between sets of slides, it was mandatory that all slides have identical exposure times and subsequent photographic processing. The best exposure time could be estimated fairly closely after several extra slides had been test developed at different times. On the 21st day after the slides were coated with emulsion, photographic processing was conducted as outlined below.

D. Photographic Processing.

1. Elon-hydroquinone (Kodak D-19) was used as the developer and an acid-hardening-fixing bath (Kodak F-5) as the fixer. These solutions were adjusted and maintained in a water bath at 20° C.
2. The preparations were developed for 6 minutes, fixed for 8 minutes, and washed through three changes of glass-distilled water (each change lasting 15 minutes).
3. After being lightly stained with Delafield's hematoxylin, the slides were dehydrated in an alcohol series, alcohol-xylene, three changes of xylene, and mounted in clarite under a cover slip.

E. Track Counting and Estimation of the Relative Amount of P-32 Incorporation

Although most emulsions record only a small part of the complete path of rays or particles, it is possible to correlate the amount of radiation striking an emulsion with the density produced in the emulsion. For visible light, the relation between the intensity of the light and density produced is the well-known Hurter and Driffield curve (Mees, 1948; Fitzgerald, et al., 1953).

$$D = \log_{10} (Io / I)$$

where D = density

Io = intensity of original beam, and

I = intensity of transmitted beam.

For beta emitters, similar relationships may be drawn (Marinell and Hill, 1948; Steinberg and Solomon, 1949; Webb, 1951). According to Fitzgerald et al. (1953), there is a linear relationship between the density of the NTB emulsions and the total number of beta particles per cm^2. For this reason, it is possible to measure the relative concentration of P-32 striking the emulsion by the number of grains produced, and in turn to estimate the relative P-32 incorporation into DNA and RNA when the geometric relationships between emulsion and object have been held constant.

As already described in the previous section, three sets of slides (each comprising two slides, one for control and the other X-irradiated) were differentially treated for extraction of the two types of nucleic acids and coated with emulsion for the development of tracks so that

the first set of samples now contained RNA, DNA, and protein; the second, DNA and protein; the third, protein only.

By visual counting of the grains over a unit area of tissue, estimates of the relative incorporation of phosphorous-32 into DNA and RNA were made. The number of grains, in a unit area of 4900 sq. μ, delimited by a reticle in the eyepiece of the microscope, was separately counted over three different regions of tissues; namely, root, shoot, and scutellum under an oil immersion magnification of 1350X. The average number of tracks was obtained by counting grains in five to ten such units in each area of tissue. In those embryos smaller than a single unit of counting area, the relative number of nuclear tracks was determined by counting the total grains over the entire area of the embryos (both control and irradiated) and converting them into units of relative area (obtained by weighing cut-outs of camera-lucida drawings of the respective embryos).

Corrections for background, which were quite low, were made by counting ten areas outside the tissues on each slide and subtracting the average background count from the counts obtained over tissues.

By keeping the emulsion refrigerated it did not seem to accumulate latent tracks from cosmic ray sources during storage. The use of liquid emulsion in this work permitted a most intimate and permanent contact of specimen and emulsion, not easily obtainable in any other way. (Plate 1)

EVALUATION OF BIOCHEMICAL METHODS USED WITH EMBRYOS

Evaluation of the biochemical procedures for determination of purine and pyrimidine bases of nucleic acids applied to fully differentiated barley embryos (both normal and X-rayed) can best be done by examining the results obtained from using these same procedures on commercial samples of free bases, yeast RNA, and salmon DNA, and by comparing these results with those from other methods reported by other investigators.

Determination of Standard Extinction Values of Purine and Pyrimidine Bases

Since the bases from mature embryo samples, after separation by paper chromatography, were eluted with 0.1 N hydrochloric acid, the standard extinction values used for determination of unknown base contents in the embryo samples were obtained from spectrophotometric readings on commercial samples of free bases in 0.1 N hydrochloric acid. For each of the five bases (A grades of guanine, adenine, cytosine, uracil, and thymine, which were obtained from California Corporation for Biochemical Research, Medfort Street, Los Angeles 63, California), 10 mg. samples were dissolved in 10 ml. of 0.1 N hydrochloric acid, and an 0.1 ml. aliquot of this solution was diluted to 10 ml. with 0.1 N hydrochloric acid. Standard extinction values of

these bases were determined, as described previously, from the difference read at the absorption maximum for each base, and at 290 mμ (Vischer and Chargaff, 1948).

In Table 1, the averages of each of three determinations for standard extinction coefficients are presented for each of the five bases. Comparing these values (0.491 mμ for guanine, 1.024 mμ for adenine, 0.455 mμ for cytosine, 0.732 mμ for uracil and 0.536 mμ for thymine) with those obtained by Vischer and Chargaff (1948), (0.475, 0.900, 0.545, 0.690, and 0.545, respectively), very close agreement was observed. The standard extinction coefficients determined by the writer are, however, the ones used for calculation of unknown base content in embryo materials.

Recovery of Free Bases

Full explanation has already been made of the procedures for hydrolysis, paper chromatography, and spectrophotometry in the previous sections of materials and methods; only minor differences for this part of the experiments will be indicated here. Ten mg. each of guanine, adenine, cytosine, uracil, and thymine were added to 1.6 ml. of 12 N perchloric acid and hydrolyzed in a steam bath at 100° C for one hour. After cooling, the mixture was diluted to 10 ml. with distilled water and the resulting clear, colorless solution was used for paper chromatography. Four replicates of 10 λ aliquots of the testing solution were deposited on the paper and four bases were separated with the solvent, isopropanol-hydrochloric acid-water, in the proportions of 170:41:39 respectively (Wyatt, 1951). After

elution with 0.1 N hydrochloric acid, the clarified eluate of each base was read in a Beckman spectrophotometer and the amount of purines and pyrimidines was calculated according to the standard extinction values shown in the Table 1.

As indicated in Table 2, the percent recoveries of the five bases were ranged from 93 to 97, with an average of 96. Approximately 4% of the bases, therefore, were lost during the processes of hydrolysis, paper chromatography, eluting, and spectrophotometry.

Comparison of Two Types of Hydrolytic Processes in Yeast RNA and Salmon DNA

Since it was impossible to directly apply previously used techniques to the present work, without modification, it was necessary to compare the recovery rates obtained by previously used processes to those as modified in the present experiments.

A. Recovery Rates After Hydrolysis with 12 N Perchloric Acid

Following methods of Marshak and Vogel (1950), 80 mg. of yeast RNA in 1.6 ml. of 12 N perchloric acid or 20 mg. of salmon DNA (A grades of two commercial samples obtained from California Company for Biochemical Research) in 0.4 ml. of 7.5 N perchloric acid was heated in a steam bath at 100° C for 40 minutes.

After cooling, the mixture was diluted (10 ml. for RNA, and to 5 ml. for DNA) with distilled water, and centrifuged to separate the fluid from the black residue formed (presumably from the sugar moieties).

The resulting clear hydrolysate was used for paper chromatography with 10 λ aliquots being used for each of four replicates (25 λ for DNA). Rectangles containing each base separated on the paper were eluted in 5 ml. of 0.1 N hydrochloric acid. The extinction value of each base was determined from the difference, Δx, read at the absorption maximum and at 290 mμ, as explained previously. The quantity of each base eluted in 5 ml. of 0.1 N hydrochloric acid was calculated according to the same difference, Δ , for a standard base solution containing 10 μg of each base per ml. of similar acid solution. (The standard extinction values are listed in Table 1). From the base content in 5 ml. of acid solution, the recovery of each base in μ moles per 80 mg. yeast RNA (20 mg. for salmon DNA) and mole bases per mole phosphorus were computed according to the phosphorus value given by Marshak and Vogel (1950): 8.9% P in RNA and 9.3% P in DNA; therefore, 23×10^{-5} moles P in 80 mg. yeast RNA and 6×10^{-5} moles P in 20 mg. salmon DNA. The actual analytical values are shown in Table 3 and the recoveries were found to be 84% for yeast RNA and 79% for salmon DNA.

In order to confirm the reliability of these analytical processes, the present values for DNA-bases, corrected for 100% recovery, (20 moles for guanine, 27 moles for adenine, 23 moles for cytosine, and 30 moles for thymine per 100 g - atoms of phosphorus) were compared with those corresponding values shown by Chargaff (1955) (20, 29, 20, and 29 moles, respectively). For the same purpose, the present base recoveries of yeast RNA (0.95 for guanine, 1.05 for adenine, 0.95 for cytosine, and 1.05 for uracil) as molar ratios in a total of 4.00 were compared with

corresponding values (1.19, 1.02, 0.83, and 0.96, respectively) from Smith and Markham (1950). There was very close agreement observed in both cases of comparisons.

B. Recovery Rate after Hydrolysis with 1 N Perchloric Acid

The procedures taken in this part of experiment were exactly the same as those in part A, as previously described above, except for using 1 N perchloric acid at 100° C for 160 minutes (with an air stream to hasten evaporation). The main purpose, therefore, was to determine whether any differences in recovery rates existed as a result of the different methods of hydrolysis.

As shown in Table 4, the recovery rates in this experiment were 87% for RNA-bases and 75% for DNA-bases, while those corresponding values for the experiment with 12 N perchloric acid were 84% and 79%, respectively. Since the results in the former were only 3% higher in RNA and 4% less in DNA than those in the latter, there were no appreciable differences in recoveries observed due to the different treatments in the two experiments. These results are also in agreement with Marshak and Vogel (1950), who reported that the prolongation of heating time from 40 minutes to 160 minutes does not affect recovery.

Recovery of Yeast RNA and Salmon DNA by Processes applied to Embryo Samples

The purpose of the previous experiment was to determine the recovery rates spectrophotometrically immediately after hydrolysis following the separation of purine and pyrimidine bases by paper

chromatography. This rather simple stepwise set of procedures, however, could not be applied to quantitative determination of nucleic acid bases in embryo samples, because of the various interfering materials in the sample mixtures following hydrolysis.

As previously described earlier in the materials and methods, many steps were taken between hydrolysis and paper chromatography for the purpose of purification and elimination of a large quantity of salts and various, partially hydrolyzed organic substances. The main difficulty encountered in the processes of purification was the loss of bases due to the differences in the solubility of each base. Any single solvent always tended to cause loss of certain bases more than others. This obstacle, however, was overcome in the following ways.

The solution of bases immediately after hydrolysis was neutralized with potassium carbonate (to eliminate perchloric acid) and the mixture washed with an excess of water and 80% ethanol. A large quantity of four of the bases, adenine, cytosine, uracil, and thymine, was dissolved in the filtrate, while guanine was always tied up with potassium perchlorate due to its insolubility in water. The greatest quantity of guanine, however, was successfully recovered by dissolving the residue in 0.1 N hydrochloric acid which separated the base from the salt.

Recovery of Yeast RNA and Salmon DNA by Processes Applied to Embryo Samples

In order to find out the recovery rates of yeast RNA and salmon DNA by the procedures which were applied to the embryo samples, the

same quantities of yeast RNA (80 mg.) and salmon DNA (20 mg.) as those in the preceding experiments were subjected to the embryo sample processes which have been already described in detail in the earlier sections. The calculation of μ moles per 80 mg. yeast RNA (20 mg. salmon DNA) and mole bases per mole phosphorus were conducted in the same way as in the previous experiments in this section.

As presented in Table 5, 79% recovery for yeast RNA-bases and 73% for salmon DNA-bases were observed in this part of experiment. The ranges of recovery shown in a report on animal sources by Chargaff and Davidson (1955) are 67 to 99.5 percent. Although the percent recovery in the present data fall within these ranges they do not reach the higher range. This difference is probably due, in large part, to the different analytic procedures used, since in animal materials, it is usually not necessary to take special procedures to eliminate interfering materials following hydrolysis. Therefore, after preparing sodium nucleate, the purine and pyrimidine bases can be separated and estimated immediately after hydrolysis by paper chromatography and spectrophotometry, respectively.

In contrast, in the present work many prolonged steps have had to be taken to minimize the interfering materials which were the major difficulty encountered in the analysis of nucleic acids of embryo samples. Eventually, as a result of these long stepwise processes, the final recovery tended to be somewhat lower due, presumably, to the difference in solubility of each base. These factors are believed enough to explain rather lower recovery rates in this experiment.

Survey of Over-All Recovery Rates of Purines and Pyrimidines in Yeast RNA and Salmon DNA

From the results obtained in the three previous experiments (summarized in Table 2, 3, and 5), an average of 96% recovery was observed in the experiment utilizing free bases (Table 2). This indicates that bases once liberated from the polymerized nucleic acids will not be recovered more than 96% by the analytical processes used in the present study and that the 4% loss is mainly due to the steps taken during paper chromatography, eluting, and spectrophotometry.

As shown in Table 3, the base recovery of yeast RNA and that of salmon DNA, following hydrolysis with 12 N perchloric acid, were 84% and 80%, respectively. These data indicate that up to the process of hydrolysis (which was not followed by the steps taken for purification) 16% of yeast RNA and 20% of salmon DNA were lost. Since 4% loss was caused by the manipulation of paper chromatography, eluting, and spectrophotometry, as already explained above, approximately 12% yeast RNA and 16% salmon DNA was estimated to be lost, due mainly to incomplete liberation of bases (the processes involved in filtering the black pentose and deoxypentose residues from the mixture of bases, and probably also, in part, to possible commercial impurity).

Observing the data presented in Table 5 in which processes applied to embryo samples were used on yeast RNA and salmon DNA, 79% recovery is shown for yeast RNA and 73% for salmon DNA. Comparing

the recovery results (84% for RNA and 80% for DNA) indicated in Table 3 with those obtained from the experiment shown in Table 5 above, 5% yeast RNA and 7% salmon DNA were lost, due mainly to the different solubility of each base and to other long manipulations of the purification processes.

It is quite useful in the evaluation of recovery rates of an experiment and for comparing the data from one experiment with others, to consider some of the factors which affect the loss of sample materials during analytical processes.

First, the different solubility of each base greatly affects the recovery rate. Reviewing the properties of each base: of the purines, guanine is water-insoluble, very slightly soluble in alcohol and ethyl ether, and soluble in potassium hydroxide, while adenine is soluble in cold water (0.09 grams per 100 ml.), slightly soluble in alcohol, insoluble in ethyl ether or chloroform, and soluble in hot ammonium hydroxide; of the pyrimidines, cytosine is water soluble (1.0 gram per 130 ml.), slightly soluble in alcohol, insoluble in ethyl ether, and forms salts with acid, while uracil, on the other hand, is very slightly soluble in cold water, insoluble in alcohol, and soluble in ether and ammonium hydroxide. The last pyrimidine base of DNA, thymine, is water-soluble (0.74 grams per 100 ml.), slightly soluble in alcohol, very slightly soluble in ether, and soluble in alkali and sulfuric acid.

As described above, there are no common properties of RNA and DNA base solubilities. Any single solvent, therefore, will not be

satisfactory for the recovery of all the bases during the analytical process, unless more than one kind of solvent is applied at the same time or separately at the different steps conducted during purification in this experiment.

The second factor, which has by far the most effect on recovery rates, is the degree of base liberation from polymerized nucleic acid during hydrolysis. The possible hydrolytic products of both RNA and DNA include purine and pyrimidine bases, pentose and pentose phosphate, nucleosides, nucleotides, and oligonucleotides. It is characteristic of RNA and DNA, however, that the purine riboside or deoxyriboside linkage is unusually labile to acid hydrolysis, while the pyrimidine ribose or deoxyriboside linkage is relatively resistant. The free purine bases, adenine and guanine, are readily formed during acid hydrolysis of RNA and DNA, whereas the pyrimidine bases remain for the most part as mononucleotides, in the case of RNA, or as nucleoside diphosphates, in the case of DNA. As the purine bases are cleaved, reducing groups from the N-riboside or N-deoxyriboside linkages are liberated and, depending on the conditions used, free ribose or deoxyribose may be formed.

As to the degree of purine liberation after acid hydrolysis of RNA, apparent discrepancies in results have been reported. According to indirect evidence from the recovery of adenine (as adenylic acid) and of guanine from sodium guanylate, approximately 95% and 100%, respectively, were reported by Vischer and Chargaff (1948) after hydrolysis in 1 N hydrochloric acid at 100° C for 1 hour. Other

experiments by Abrams (1951), in which an isotope dilution method was used to estimate the purine concentration, have indicated that as much as 7-8% of both adenine and guanine may be destroyed by 1 N hydrochloric acid at 100° C. Although both experiments above were operated with the same normality of acid and at the same temperature, some differences in results were reported. Therefore, it is especially difficult to compare analytical data on purines of any two experiments when different processes are undertaken under different conditions and with different materials.

As to the liberation of pyrimidine bases of RNA, Chargaff and Davidson (1955) reported that hydrolysis of yeast RNA with 12 N perchloric acid for 1 hour at 100° C causes no appreciable destruction of either adenine, guanine, cytosine, uracil, and thymine. However, the recovery of total pyrimidine bases indicates an incomplete liberation from nucleic acid when this procedure was applied to yeast RNA (0.37 moles per mole P and 0.42 moles per mole P in the present experiment as shown in Table 3). At present, therefore, under the conditions studied, neither 20% HCl, concentrated formic acid, nor 12 N perchloric acid can be said to lead to the complete liberation of the pyrimidine components of RNA in the form of free bases.

The data analyzed on base components differ greatly depending upon the quantitative distribution of purines and pyrimidines in nucleic acids of any species. When the compositions of many specimens of DNA from different cellular sources are compared, a very striking feature emerges, as was pointed out some years ago: two principal

groups can be distinguished, namely, the "AT type," in which adenine and thymine predominate, and the "GC type," in which guanine and cytosine are the major constituents. In addition, an intermediate group was discovered in E. coli which is characterized by the presence of almost equimolar proportions of all four components. Total DNA preparations from animal sources described so far belong to the AT type while the GC type has been encountered in several microorganisms and in some insect viruses.

The molar ratio of guanine and cytosine to adenine and thymine will usually be less than 1 in the nucleic acids of the AT type but greater than 1 in those of the GC type. In other words, the analysis of purified DNA preparations isolated from a great variety of cells has led to the conclusion that the DNAs of different species of organisms have different nucleotide compositions and those of different organs of the same species have identical compositions. Because of the different distribution of purines and pyrimidines in each different species of materials, it is very difficult to compare the quantity and compositions of bases recovered from one material with that from another kind of sample.

Finally, the recovery rates may vary depending upon the different techniques utilized by different workers. In regard to this factor, Chargaff and Davidson (1955) have stated that "the comparison of analytical results obtained by different workers using procedures that are almost never identical and often radically different suffers from a great deal of uncertainty."

RESULTS AND DISCUSSION

Determination of Purine and Pyrimidine Contents of RNA and DNA in Normal Mature Embryos

The purpose of this section of the work was to determine the purine and pyrimidine contents of RNA and DNA of normal, mature embryos so that these data might be used as control values for the results to be obtained from X-raying embryos at four different developmental stages.

Four replicate embryo samples, each consisting of 138.5 mg dry weight were subjected to the analytical processes which have been described previously in Materials and Methods. The data on the electrophotometric estimation of purine and pyrimidine bases, following paper chromatography, with their quantities expressed in m μ moles per mg dry weight of embryos are presented in Table 6.

As shown in the summary of Table 6 and 12, the ratio of RNA to DNA was found to be 5.9 (calculated by dividing the total quantity of RNA bases: guanine 37, adenine 28, cytosine 32, and uracil 26 m μ moles per mg dry weight of embryos by the total content of their DNA-bases: guanine 4.4, adenine 5.0, cytosine 4.4, and thymine 7.2 m μ moles per mg dry weight). In comparison to this value, Ogur and Rosen (1950) reported RNA to DNA ratios of 5.7 in corn root tips and 1.9 in

rabbit liver (determined by direct ultra-violet absorption of polymerized nucleic acids isolated with 1 N and 0.5 N perchloric acid). Thus the ratio of RNA to DNA in both corn root tips and mature barley embryos are in close agreement. Reference to the same tables shows that the ratio of purines to pyrimidines in RNA was found to be 1.1. According to Chargaff and Davidson (1955), the range for this ratio is from 0.98 to 1.69: pregnant rabbit liver 0.98, rabbit liver 1.08, chicken liver 1.12, and calf pancreas 1.69 (determined spectrophotometrically after separation by paper chromatography). It will be noted that the present data fall within this range.

Purine and Pyrimidine Contents of RNA and DNA in Mature Embryos X-irradiated at Four Different Embryonic Stages

Four replicate embryo samples, each consisting of 160.6 mg dry weight which were X-irradiated at the youngest proembryonic stages a-c (Group-A) were subjected to the same analytical procedures as the control samples, after reaching stage 6c in embryogeny. In the same way, four replicate embryo samples, each consisting of 240.1 mg dry weight for Group-B, 273.2 mg for Group-C, and 203.2 mg for Group-D, X-radiated at stage g, 3-4, and late 5, respectively, were subjected to the same analytical methods as the control samples when these embryos in the three above groups reached stage 6c. The values for extinction at wave lengths in m μ for the spectrophotometric estimation of each base, and the base content in m μ moles per mg dry weight of embryo samples are shown in Table 7 (Group-A), Table 8 (Group-B),

Table 9 (Group-C), and Table 10 (Group-D). Those analytical data obtained from the four experimental groups and control are summarized in Table 11. This table presents the analytical purine and pyrimidine amounts of the four different irradiated groups of embryos compared with the control in m μ moles per mg dry weight. Table 12 indicates the percent nucleic acids of the control, the comparison of the ratios of RNA-DNA and purine-pyrimidine ratios of the four different experimental groups as compared with the control, while Table 13 shows the percent base content of each experimental group compared with that of the control. The effects on the total amount of RNA-bases following a 450 r dose of X-radiation given at four different stages of embryogeny may be more easily visualized by referring to Figure 1 (based on Table 11). As shown in this histogram, there was considerable depression of RNA, 48 to 42 m μ moles per mg dry weight in the X-rayed embryo groups, from the control value of 123 m μ moles. Differences within the X-irradiated groups were small, the maximum difference being only 6 m μ moles between Group-A (which showed the greatest depression) and Group-C and -D (which had the same depression). The depression occurring in Group-B was found to be intermediate between those two extremes. In general, the degree of depression of RNA, as observed at stage 6c, was greatest the earlier the stage of embryogeny at the time of irradiation, while irradiation at either stages 3-4 or late 5 of the differentiating embryo produced the same degree of RNA depression.

The amounts of DNA-bases in X-rayed and control groups of embryos (Figure 2) showed depressions of from 6.8 to 1.4 m μ moles for

the irradiated groups of embryos as a whole as compared with the control value. Comparing differences in DNA content among irradiated groups, there occurred a maximum of 5.4 m μ moles difference between Group-C and -D, and a minimum of difference (1.3 m μ moles) between Group-B and -C. The degree of depression of DNA content was greatest with Group-D, followed by lessening degrees of depression in Group-A, -B, and -C, respectively.

If the RNA-base and DNA-base contents are summed to give total nucleic acid values, the control shows 144.0 m μ moles per mg dry weight, while in the X-irradiated groups, Group-C with the least depression had 100.6 m μ moles, Group-D 95.2, Group-B 94.3, and Group-A, the greatest depression with a value of 91.9 (Figure 3).

In order to more easily assess the degree of relative differences in RNA, DNA, and total nucleic acid content in these four irradiated groups of embryos, it seems reasonable to express these analytical data on a percent basis with the control expressed as 100 percent (Table 12). The total nucleic acid content of the experimental groups showed rather large depressions of from 31% to 36% below that of the control, yet only a maximum of 5% difference among the irradiated groups. The RNA content of the experimental groups behaved much the same as the total nucleic acid, largely because the RNA makes up the major part of the total nucleic acid (80 - 85%). In terms of RNA content, among the X-rayed embryo groups, Group-C and -D showed the highest values of 66%, Group-B 62%, and Group-A the lowest value of 61%. Thus, while there was observed a 34-39% depression of RNA in the X-rayed groups as a whole compared with the control values, only a maximum

of 5% difference occurred among the irradiated groups. Also as shown in Table 12, the highest value of DNA (as percent of control) was 93% in Group-C, 87% in Group-B, 80% in Group-A, and the lowest 68% in Group-D. It would seem that there is a gradual tendency toward a decrease of DNA as younger stages are irradiated, except for Group-D. It seems important, therefore, to compare the relative changes of RNA and DNA within the tissues of barley embryos in several different ways. Two points should be brought out for the purposes of these considerations. First, the Group-A experiment underwent the longest postirradiation time, 16 days, between X-ray treatment and sampling time, Group-B 12 days, Group-C 9 days, and Group-D the shortest time, 7 days. If one considers only postirradiation time and neglects the physiological differences of the embryos at the time of irradiation in each experimental group, one might expect the percent of RNA to be the highest in Group-A, second highest in Group-B, third highest in Group-C, and lowest in Group-D. The actual order of recovery was found to be exactly reversed, Group-A lowest, Group-B next, and Group-C and -D highest.

A similar situation is found for DNA in Group-A through -C, but Group -D instead of having a DNA content equal to that of Group -C (as was the case with RNA), has instead the lowest amount. With the exception of the DNA value of Group -D, these results demonstrate that the differences in post-irradiation time cannot in themselves account for the order of the observed radiation induced differences in RNA and DNA.

The only other way in which these groups differed experimentally from one another was in the embryonic stage of development at the

time of radiation. The order of the observed differences in RNA and DNA (with the exception of the DNA value in Group -D) certainly implies that the younger embryos were more affected and/or underwent less recovery during the post-irradiation time until sampling than the older embryos. In other words, the results obtained from these experiments are in agreement with the general statement (Bergonie and Tribondeau, 1906) that the younger the tissue, the more the effect due to ionizing radiation.

The divergent results observed in Group -D, however, would appear to contradict the above general statement, since the percent DNA in this Group is seen to be the lowest among the four experiments in spite of the fact that it was the oldest tissue (stage late 5) at the time of irradiation. This could be explained by comparing the metabolic activity in the nucleus, which is the site of DNA synthesis, with that in the cytoplasm where the greatest quantity of RNA is synthesized. It is generally agreed that in more mature tissue (such as embryos in stage 6) the physiological activity of the nucleus is lower, while that of the cytoplasm is higher (Hammarsten and Hevesy, 1946). In addition, the time spent in interphase by cells comprising mature tissue is much longer than that in cells of actively growing tissues, due to the lower mitotic activity of the former. If more mature tissue, therefore, is once affected by ionizing radiation, DNA recovery is more difficult than that of RNA because of the lower metabolic activity of the nucleus as compared to the cytoplasm. In addition, it also requires a much longer time for mature tissue to recover following irradiation due to its longer time spent in interphase, which usually is the only period

in the mitotic cycle during which DNA synthesis occurs (Hevesy, 1945).

Secondly, in comparing the differences in recovery of either RNA or DNA (as percent of control) it can be seen (Table 12) that the DNA of the four experimental groups shows wider variation (6 - 25%) than does the RNA (0 - 5%). This was in spite of the fact that in all cases the percent of RNA depression exceeded that for DNA. These results lead to the important suggestion that the stage specificity of radiation effects observed by others (Mericle and Mericle, 1957; 1961) may be referred more to effects on the DNA rather than the RNA components of the embryo cells.

From Table 12 it can be seen that the ratios of RNA to DNA were the same for Group-A, -B, and -C and the values were lower than that of the control. The ratio in Group -D, however, was comparable to the control. In general, it is very difficult to determine the relative sensitivity of RNA and DNA to ionizing radiation since the ratio of RNA-DNA varies depending upon the period of post-irradiation. No conclusion may be drawn as to their comparative sensitivity to X-irradiation at any particular sampling period.

As shown in Table 13, the content of DNA-bases was lower than the control for all experimental groups. The purine-pyrimidine ratios in Group -A, -C, and -D correspond to that of the control, but the ratio in Group -B was increased by 20% as compared with the control. This increased ratio of purine-pyrimidine in this experiment did not return to the normal pattern of nucleic acid metabolism during the 12-day post-irradiation period. Analysis of the DNA compositions of

Group -B showed a considerable decrease in thymine content (65% of the control value), while the contents of guanine, adenine, and cytosine were comparable to the control. On the other hand the content of RNA-bases was also lower than the control for all experimental groups. The purine-pyrimidine ratios in Group -A, -B, and -D were comparable to the control, but the Group -C ratio was increased by 20%. This ratio did not return to the normal pattern of nucleic acid metabolism during 9 days post-irradiation. Analysis of the RNA compositions of Group-C showed a considerable decrease in uracil content (54% of the control) which was the lowest for the four experimental groups. The contents of guanine, adenine, and cytosine, in Group -C were comparable to the other treatments. The results obtained from the experiments of Group -B and -C indicate that uracil of RNA and thymine of DNA may be more labile to X-irradiation than other bases.

Comparing the present data with those by previous investigaters, Berenbom and Peters (1956), in their study of nucleic acid changes in rats after a total-body X-irradiation (400 roentgens as a single dose), reported that spleen DNA and thymus RNA showed evidence of altered composition. The increase in the purine-pyrimidine ratio of spleen DNA was accompanied by a substantial reduction in relative content of cytosine and thymine, resulting in a disproportionate increase in the guanine and adenine content. The ratio in this case did not return to the normal pattern. This irreversible alteration of purine-pyrimidine ratio was observed in the present experiments. Berenbom and Peters (1956) also noted some modification in thymus RNA

composition whereby the purine-pyrimidine ratio increased more than the control value. In their material, however, this alteration appeared to be temporary, since an essentially normal pattern was observed in 7 days after irradiation.

In an attempt to determine the effect of X rays on nucleic acid purine and pyrimidine bases of rye seedlings, Sissakian (1955) reported that uracil was most labile due to X-irradiation (20 Kr). He concluded that under the influence of X rays nucleic acid synthesis became disturbed. This lability of uracil seems to be in agreement with the results observed in the present work, although different materials and different doses of radiation were used.

Effect of X-irradiation on P-32 Incorporation Into Nucleic Acids of Embryos as a Whole

A. Changes in Nucleic Acids during the Period Following X-radiation (450r) at Youngest Proembryo Stage a-c (Group-1).

The main purpose of this experiment was to determine the contents of RNA and DNA as percent of the control values during developmental embryogeny. General methods have been already explained in Materials and Methods. Minor differences in procedures for this experiment will be explained prior to the discussion of results. After X-irradiation and treating of the plants with P-32, samplings (control and X-rayed) were made as summarized on page 38. Table 14 shows the P-32 tracks counted for RNA and DNA. These were computed according to the methods which have been described in the section of Materials and Methods. The percent of the two types of nucleic acids as compared to the control values are recorded in Figure 4.

The content of DNA fell to about 35% of the normal level in 24 hours (at stage d-e) and slightly recovered to 45% of the control in three days (at stage g-1) post-irradiation. The quantity of DNA then decreased to 28% of the normal value in 7 days (stage 4) while the final recovery value (83% of the control) was reached in 16 days post-treatment. It will be noted that there were two minima, the first 35% of the control at stage d-e and the second 28% of the normal at stage 4.

Observing the percent content of RNA compared with normal values, the first sample showed 68% at stage d-e; the second 52% at stage g-1; the third 67% at stage 4; the fourth 58% of the normal level at stage 6c.

As shown in Figure 4, there existed an interrelationship of reciprocal fluctuation between RNA and DNA throughout the 16-day post-irradiation period. The content of DNA was much lower than RNA at 24 hours post-irradiation and recovered more than RNA at the final sample stage (Table 14). Comparing the difference between minimum and maximum values of RNA with those of DNA during the 16-day post-irradiation period, RNA was found to be 16%, while DNA was 55% of the control. This indicates the relatively higher radiosensitivity of DNA as compared to RNA.

Since the results obtained in this experiment (Group-1) seemed to be most representative of the patterns of response of embryo tissue to ionising radiation, the discussion will be focused on this experiment. Results of other experiments will be discussed wherever relevant.

(1) Incorporation of P-32 as a measure of DNA synthesis.

In these and other studies (Hershey, 1954; Pelc and Howard, 1954) the incorporation of P-32 into DNA has been used as a measure of DNA synthesis. When expressed in terms of number of nuclear tracks per cell, the incorporation data acquire a meaning analogous to the mitotic index. Whereas the mitotic index indicates the relative number of cells undergoing mitosis at a given moment, the number of grains or nuclear tracks due to DNA per cell is proportional to the

relative number of cells engaged in DNA synthesis during the period of incorporation.

This phenomenon is demonstrated by data obtained for the first control samples of Group-3 and -4 (Tables 14 and 17) where 0.77 and 0.37 tracks per cell, respectively, were observed. Since these samples were taken at the same number of hours after irradiation (24 hours) the data show, as expected, that DNA synthesis is more rapid in the younger embryo tissue (Group-3) than in the older tissue (Group-4) where differentiation is more advanced, in spite of a larger reservoir of P-32 in the latter (Table 19). As shown in Table 19, the range of total radioactivity (in counts per minute) per mg dry weight of X-rayed embryos was found to be 84 to 126 percent of the controls. These differences in the amounts of P-32 between X-rayed and control plants, however, should not significantly affect the amount of nucleic acid synthesis in embryos, since the penetration of inorganic phosphorus into nuclei is so slow (Hahn and Hevesy, 1940) that a large reservoir of intracellular and extracellular inorganic phosphorus is built up.

(2) Evaluation of the Results

The interpretation of data on the labeling of DNA in a tissue is complicated by the fact that few tissues consist of a single population of cells. When a tissue contains cell populations of different mitotic activities, the contents of the isolated DNA will depend on the relative abundance of the different cell types and on their mitotic index. In studying the effect of irradiation on the tissue as a whole, we obtain the summation value for the effect on the various cell types.

Evaluation can be simplified if the assumption is made that the relatively abundant types of cells exhibit a greater rate of reproduction than the others, therefore the observed changes of P-32 incorporation following irradiation will essentially reflect the changes in these normally more active cells.

(3) Comparison of minimum values

The maximum depression of RNA and DNA in the various tissues of the rat treated with 500 r of X-ray, according to Lutwak-Mann (1951), was found to be 9% and 28% of the control in DNA and RNA of bone marrow, respectively; this maximum depression occurred four days after X-irradiation. According to Sissakian's (1955) recent report on the nucleic acid content in rye seedlings treated with a dose of 5 Kr X-radiation, the maximum depression of P-32 incorporation into DNA was found to be 25% of the control value. The relation between the time period of post-irradiation and the minimum of DNA synthesis was shown by Pelc and Howard (1954). The first depression of DNA in Vicia faba X-irradiated with 140 roentgens was down to 10% of the control value at 6 to 8 days, post-irradiation. Berenbom and Peters (1956) reported a 75% decrease of DNA at 2 days, post-irradiation, in an experiment with thymus of male rats treated with 400 r total-body X-irradiation. The maximum depression of DNA in the present study was down to 35% of the control for the first minimum and 28% for the second minimum at 24 hours and 7 days post-irradiation, respectively. This fell in the range reported by other workers even though materials were different from those of other investigators.

(4) The First Minimum

Knowlton and co-workers (1949 and 1950) have concluded that the time from irradiation to the initial minimum for the mitotic index is a measure of the duration of mitosis. Analogous to this, the time from irradiation to the minimum for the incorporation of tracer into DNA might indicate the rough duration of DNA synthesis in the more active groups of cells in the tissue.

The initial decrease in P-32 labeling of DNA can then be explained as a reduction in the number of cells entering the period of DNA synthesis. This may in part be due to a delay in the initiation of DNA synthesis (Howard and Pelc, 1953) by temporarily or irreversibly affecting the presynthetic phase in some groups of cells. In part it may be due to a direct, irreversible inhibition of DNA synthesis which in turn may be due to an interference with existing DNA in such a way that the presumed self-duplication is made impossible (Hygaard and Potter, 1959).

Although many other factors may be involved in the decrease of DNA due to X-irradiation, it was found that part of the decrease in DNA was due to the fact that there were fewer cells counted per unit area in the tissue of X-rayed embryos. The significance of this will be discussed in the section of "Theories on the Effects of Ionising Radiation on Depression of DNA."

(5) Abortive Recovery

As mentioned previously, after the first sampling the content of DNA had recovered to 45% of the control within 2 days post-irradiation. This "abortive" recovery of DNA may have been caused by cells that

were not inhibited and would have entered DNA synthesis at the time of irradiation, and also by the recovery of cells that were originally delayed by the irradiation.

(6) The Second Minimum

After this temporary recovery, the percent DNA content fell to 28% of the normal value. This second minimum in mitotic activity may be a result of the failure of DNA synthesis brought on by the previous abortive mitosis which occurred in predominant groups of active cells within the tissue. This could be possible due to the fact that DNA synthesis and mitosis do not occur simultaneously in any single cell, not within the more active groups of cells in any cell population. In other words, the replication of DNA is a necessary prerequisite for mitotic division (Howard and Pelc, 1953).

(7) The Final Recovery

The final recovery rate may depend in part on the relative number of cells which were "released" from previous suppression of DNA synthesis, and in part on the degree of metabolic disturbances which occur within individual cells as a result of the direct effects of ionizing radiation. On the other hand, the final recovery rate may be governed by the period of post-irradiation and the physiological nature of the tissue itself.

According to Nygaard and Potter (1959), any relative irregular shapes of the curves, in general, may indicate the relative cell death caused by radiation. Since in Group-1 no samplings were made between the embryonic stages, 4 and 6c, it was not possible to estimate if cell death occurred in this experiment.

(8) Theories of the Effect of Ionizing Radiation On the Depression of DNA

Many papers in the past have cited the blocking effects of ionizing radiation on mitosis as the main factor for the depression of DNA. This explanation, however, seems not to be the primary answer to this question. Since DNA synthesis precedes mitosis, major emphasis must be placed first of all on changes of the metabolic processes due to X-irradiation.

In the preliminary stages of mitosis, the transfer of nucleotides from the cytoplasm to the nucleus takes place, and this results in their conversion of ribose nucleic acid to desoxy ribose nucleic acid through the reduction process. This conclusion is based upon the very large quantities of RNA localised within the chromosomes during cell division. (Caspersson and Schultz, 1938).

As to the effects of ionizing radiation upon RNA and DNA, Mitchell (1942) found an increase in the absorption of ultraviolet radiation (wave-length, 2537 Å) within the cytoplasm of proliferating and differentiating cells following therapeutic doses of roentgen radiation. He interpreted this increase to be due to the accumulation of ribosenucleotides in the cytoplasm and as a result of disturbances in the normal metabolic processes, either by an increased rate of formation, or a decreased rate of removal. It is a widely accepted theory that oxidizing substances are produced as a result of radiation interaction with water molecules. These oxidizing reagents could counteract the reduction process of RNA to DNA and thereby cause an interference with the formation of DNA, with a concomitant increased accumulation of RNA in the cytoplasm.

According to the results reported in many papers, even relatively large doses of X rays do not depress DNA synthesis to less than about 50% of the control, therefore Von Euler and Hevesy (1944) suggested that ionizing radiation may interfere with DNA synthesis but not with the high turnover of DNA which occurs at the time of synthesis of new DNA.

In an attempt to solve the mechanism implicated in the initial drop of DNA after moderate doses of X-rays (50r to 200r), Pelc and Howard (1955) reported that the number of cells synthesizing DNA (observed by means of autoradiographs as uptake of P-32 into DNA in a form not removed by acid hydrolysis) in bean root meristems is reduced to about 60% of the control value during the subsequent 12 hours. Those cells already undergoing synthesis (or shortly before it) when irradiated, are unaffected in this respect even if subjected to 200r. This result is interpreted as being due to a greater radiosensitivity to delay or inhibition of DNA synthesis on the part of cells which are in approximately the first one-third of the cell mitotic cycle at the time of irradiation (the first part of interphase) than cells at other stages of the cycle. This contradicts Euler and Hevesy's hypothesis on the ground that, if their interpretation of the irradiation results is correct, the labeling of single cells after irradiation should be one-half that of unirradiated ones, which is in no way supported by Pelc and Howard's experiment.

The main difference existing between the two views is that the latter (Pelc and Howard) attributes the depression of DNA to the number of cells delayed or inhibited in their DNA synthesis while those cells

entering DNA synthesis, even if irradiated, are unaffected by X-irradiation. The former (Euler and Hevesy), however, insist that radiation induced decrease of DNA from the control is due to the labeling of each cell in the irradiated tissue being one-half that of unirradiated tissue.

Pelc and Howard's theory may very well apply to experiments conducted in such a way that the dose of irradiation is low enough not to affect the synthetic phase of the mitotic cycle. But since the tissue as a whole is composed of cells having different radiosensitivities, it is very difficult to say whether any dose of radiation has affected the pre-synthetic phase only. On the other hand, if one follows Euler and Hevesy's theory as to the fundamental biochemical reactions implicated in the depression of DNA, it is very difficult to explain values below 50%, which have been reported by many investigators, and which occur in the present experiments.

It is quite interesting to note that the views of some other investigators are a compromise between the above two interpretations. Kelly, et al. (1955), Holmes (1956), and Cater, et al. (1956), working with regenerating rat liver, found that once DNA synthesis starts large doses of radiation are necessary to produce a partial inhibition while small doses delivered before commencement of DNA synthesis produce a delay in onset of DNA synthesis, although not necessarily affecting its rate.

Lajtha, et al. (1958), irradiating (with absorbed doses of 300-1100 rads) cell suspensions of human bone marrow (labeling DNA by the incorporation of formate-C^{14}), reported that large doses of X-irradiation directly inhibited the process of DNA synthesis,

and that this effect appeared to be dose-dependent. Small doses did not inhibit DNA synthesis in cells which already had started the synthesis of DNA. In cells, however, which were in the presynthetic period of mitosis at the time of radiation, small doses produced a 40-50% depression on the number of cells entering the subsequent synthetic period, without affecting the rate of subsequent DNA synthesis.

It is quite difficult to define "large or small" doses of X-irradiation, since the response of any tissue to a single dose of radiation may vary depending upon the kinds of tissues and the relative number of cell types within the tissues. It seems logical to assume, however, that within the tissue of an entire barley embryo as was used in this experiment, a dose of 450 r of X-radiation will affect not only the presynthetic mitotic phases of a certain number of cells reversibly or irreversibly, but also may directly affect the DNA synthetic phases as well.

An important factor in determining DNA depression by track counting is the number of cells counted per unit area in a tissue. As shown in Table 17, fewer numbers of cells were counted per unit area in the first, second, and third samples of Group-1; the first and second samples of Group-2; and in the third sample of Group-3. These observations show that while the growth of the embryo in size was retarded due to X-radiation, the individual cell size in the X-rayed embryo increased from 110% to 120% of the normal cell size. Thus, DNA values shown in Table 15 would be even somewhat higher if recalculated on this basis of difference in cell size.

(9) The Interpretation on Interchangeable RNA and DNA

According to the theory of nucleic acid starvation proposed by Darlington (1947), an adequate supply of nucleic acid is essential for mitosis. Any factor which reduces the supply of this substance will result in the "consumption" of cytoplasmic nucleotides by the nuclei. It is well known that ionizing radiation decreases the amount of DNA either by direct effect on the DNA synthetic phase or by blocking the radiosensitive presynthetic stage of the mitotic cycle. This depression of DNA will in turn lead to two possible phenomena: one is an effect on normal cell division and the formation of anomalous mitotic products in the irradiated tissue, since the doubling of DNA synthesis is a prerequisite for mitosis; the other is an interchangeable relationship between RNA and DNA due the consumption of cytoplasmic nucleotides by starved nuclei.

From the observation that very large quantities of ribose nucleic acids are localized in the chromosomes during cell division, Caspersson and Schultz (1938, 1939), conclude that the cytoplasmic nucleotides serve as a source of nucleic acids from which the chromosomes derive their supply. The preliminary stages of mitosis involve the transfer of nucleotides from the cytoplasm to the chromosomes, their conversion from ribose into deoxyribose nucleotides, and their polymerization into long chains. Since ionizing radiation blocks cell division, it will influence this cycle and thus reciprocal interchange between RNA and DNA could occur. This expectation has already been demonstrated by Mitchell (1942) who used microspectrophotometric techniques in his study on irradiated and non-irradiated tumor tissue.

He found in the cytoplasm of the irradiated tumor cells an accumulation of ultraviolet-absorbing material which appeared to be nucleotide in nature, indicating the transformation of nucleic acids from one type to another.

Considering Caspersson's conclusion (1940) that DNA is formed from RNA and with experimental demonstration that accumulation of RNA in the cytoplasm results after X-irradiation (Mitchell, 1942), it seems reasonable that radiation may counteract the reduction process taking place normally in the transformation of RNA to DNA by means of the action of oxidising substances which are produced as a result of the interaction of ionizing radiation and water.

This interpretation is supported by experiments conducted by Nicola (1950). In studying the metabolism of nucleic acids in proliferative and secretory cells he found that X rays caused a stoppage of DNA synthesis and a conversion of DNA into RNA.

B. Changes of Nucleic Acids During the Period Following X-radiation (450 r) of Oldest Proembryo Stages, g-1 (Group-2) and at Stage 4 of Mid-differentiating Embryos (Group-3)

Tracks from P-32 counted for RNA and DNA in Group-2 are listed in Table 14 and graphed in Figure 5. It will be noted that at stage g-1 the DNA content fell to 81 percent of the control in 24 hours post-irradiation. This DNA continually decreased until stage 5 was reached, 4 days post-irradiation, where the lowest value was only 23 percent of the normal level. Between stage 5 and 6c the quantity of DNA had recovered to 97 percent of the control. On the other hand, at stage g-1, 24 hours post-irradiation, RNA content was 70 percent of the

control. RNA recovered up to 80 percent of the normal value at stage 5 and fell to 57 percent at the final stage.

As seen in Figure 5, reciprocal fluctuations between RNA and DNA occurred as in the previous (Group-1) experiment. Comparing the rate of changes in the content of DNA with that of RNA, the difference between the minimum and the final recovery value of DNA was found to be 74 percent of the control, while the corresponding value of RNA was 23 percent. When compared with Group-1, the content of DNA seems to be more variable than RNA during 12 days post-irradiation.

Data for the experiment of Group-3 are shown in Table 14 and Figure 6. It will be noted at 24 hours post-irradiation (at stage 4) that the content of DNA increased to more than the control value (110 percent of the control). Following this "over-shooting" behavior the DNA curve suddenly dropped to 22 percent of the normal at stage 6a in 4 days but recovered up to 81% by the final embryonic stage (9 days post-irradiation). When compared with DNA, the RNA content was found to be 68% of the control yet recovered slightly, up to 78%, at stage 6a (4 days post-irradiation). After this, the RNA gradually dropped to 74% of the normal value upon reaching the final stage.

As in the previous experiments (Group-1 and -2) DNA recovery was greater than RNA during the 9 days post-irradiation. The difference between maximum and minimum values was found to be 88% in DNA, while in RNA only 10%, indicating that DNA was also much more variable than RNA in this experiment. As found in the previous two experiments, the changes in contents of RNA and DNA were reciprocal. Comparing the curves of Group-2 (Figure 5) with those of Group-3 (Figure 6), the

shape in both experiments was essentially the same except for the "overshooting" DNA content at the first sample stage in the latter.

As already explained in the Group-1 experiment, there are two possible phenomena which could have occurred during the first period of post-irradiation: one being the continuous mitotic activity of some groups of cells which were not inhibited by the action of X-rays and would have entered DNA synthesis at the time of irradiation; the other, the recovery of those cells that were originally delayed by the ionizing radiation. Furthermore, a summation of DNA synthesis in the above two groups of cells, which, conceivably, could be caused by a synchronizing effect of X-irradiation, would explain the "overshoot" incorporation seen at 24 hours post-irradiation in the Group-3 experiment.

C. Changes in Nucleic Acids During the Period Following X-radiation (450 r) of Late Differentiating Embryo Stages, Late-5 (Group-4)

The experimental data for this group appears in Table 14 and Figure 7. Results obtained from this experiment were found to be quite different from those of the previous experiments, Group-1, -2, and -3.

First, the DNA content fell to only 95% of the control in the first sample (24 hours post-irradiation), yet kept more or less constant (96%) during the next 3 days, after which it decreased to 72%, lacking the ability to recover during the entire 7-day post-irradiation period. The content of RNA, on the other hand, dropped to 86% of the control at the 24-hour initial sample, continued to decrease to 34% of the normal level in 4 days post-irradiation, then recovered up to 58% of

the control value. The conspicuous feature here is the phenomenon of non-recovery in DNA but recovery in RNA. If one compares the relative changes of these two types of nucleic acids, the difference between maximum and minimum is found to be 23% in DNA and 52% in RNA, indicating that DNA is much more stable than RNA.

As seen in the previous experiments, there occurred a competitive relation between RNA and DNA for metabolic substrates. The divergent results observed in Group-4 perhaps can be explained by comparing the metabolic activity of the nucleus, which is the site of DNA synthesis, with that of the cytoplasm, where the greatest quantity of RNA is synthesized. The metabolic activity of the nucleus is lower than that of cytoplasm in more mature tissue (Hammarsten and Hevesy, 1946). In addition, the time spent in interphase by cells comprising mature tissue is much longer than that in cells of actively growing tissues, because of the lower mitotic activity of the former. When more mature tissue, therefore, is once affected by ionizing radiation, DNA recovery is more difficult than that of RNA because of the lower metabolic activity of the nucleus as compared to the cytoplasm. In addition, it also requires a much longer time for mature tissue to recover following irradiation since a longer period of time is spent in interphase during which DNA synthesis occurs (Hevesy, 1945).

D. Comparison of Total Nucleic Acids in Group-1, -2, -3, and -4

It was noted in Figures 4, 5, 6, and 7 that the changes of total nucleic acids always followed the same direction as that of DNA in all experiments during the entire post-irradiation period with the

exception of Group-4. As shown in Figure 7, the changes of total nucleic acids of Group-4 were comparable to those of RNA, they being opposite to that of the previous experiments.

Figure 8 indicates the percent total nucleic acids obtained from all four experiments. It will be seen that the total nucleic acids, as percent of the controls, was found to be 50, 72, 85, and 89 in Group-1, -2, -3, and -4, respectively, at 24 hours post-irradiation. The minimum values in these four experiments were found to be 44% at 7 days post-irradiation (Group-1), 59% at 4 days (Group-2), 54% at 4 days (Group-3), and 54% at 4 days post-irradiation (Group-4). Following these depressions, the total nucleic acid of Group-1 had recovered up to 64% of the control at 16 days post-irradiation, 69% at 12 days (Group-2), 79% at 9 days (Group-3), and 66% at 7 days (Group-4).

These results indicate that the younger the embryonic stages at the time of X-irradiation, the greater the depression of total nucleic acid at 24 hours post-irradiation. In addition, this indicates the greater radiosensitivity of younger embryonic tissues to X rays during 24 hours post-irradiation.

The pattern of susceptibility was the same for all groups except for Group-4 at the final sampling stage. Only minor differences were found in the minimum values for all four experiments.

Effect of X-irradiation on P-32 Incorporation Into Nucleic Acids of Different Parts (Root, Shoot, and Scutellum) of an Embryo

The main purpose of this part of the experiments was to determine the influence of X-radiation on the nucleic acids in three different parts of an embryo.

Table 18 indicates percent nucleic acids in three different parts (root, shoot, and scutellum) of "mature" embryos which had been previously treated with doses of 450 r at four different embryonic stages. The results obtained from Group-1 are shown in Table 18 and Figure 9. The DNA content ranged from 79% to 83% in three different parts of the embryo, while the range of RNA was found to be 52% to 58% of the control.

As shown in Table 18 and Figure 10, Group-2 had a DNA content in root, shoot, and scutellum of 98%, 95%, and 102%, respectively, while the RNA of these tissues were determined to be 43%, 88% and 77% of the normal level.

The results found for Group-3 are recorded in Table 18 and Figure 11. In this case the percent DNA of root, shoot, and scutellum was 70%, 77%, and 91%, respectively, while the RNA values in these tissues were 67%, 58%, and 81% of the control.

Finally Figure 12 (from the data indicated in Table 18) shows the results of the last experiment of the study (Group-4). DNA values are noted as 74%, 72%, and 101% of the normal level in root, shoot, and scutellum, respectively, while the RNA of these parts is observed to be 40%, 66%, and 66% of the control, respectively.

After evaluating the data shown in Table 18, it was found that the difference between maximum and minimum DNA values in the three different parts of the embryo was 6% of the control in the Group-1 experiment, 7% in Group-2, 21% in Group-3, and 29% in Group-4. The differences reflect the degree of tissue heterogeneity within the embryo at the time of X-radiation.

As shown here, the differences between DNA values in three different parts of the embryo in Group-1 and Group-2 were similar to each other and lower than those in other experimental groups. Both the embryos of Group-1 and -2 at the time of X-radiation (at stage a-c and g) were composed of the undifferentiated proembryos. Since the three parts of a "mature" embryo (at stage 6c) were derived from those morphologically homogenous groups of cells, the small differences (6% in Group-1 and 7% in Group-2) between DNA contents in the three parts of the mature embryos may be due to the homogenous effect of X-rays on the embryos at the time of X-irradiation.

From Figure 11, it can be seen that the DNA content of Group-3 was 70% in the root, 77% in shoot, and 91% in the scutellum. This indicates that the order of increasing susceptibility to irradiation is (1) scutellum, (2) shoot, and (3) root. According to Mericle and Mericle (1957), the morphological radiosensitivity of three regions (root, shoot, and scutellum) of an embryo varies with the stage of embryogeny at the time of irradiation. It was reported that the root region showed greater radio sensitivity than did the shoot region if radiation was applied during the time that structural differentiation of these regions was occurring. They explained this

increased radiosensitivity of the root region as a reflection of the fact that, since structural differentiation occurs first in the shoot region, the root region is, therefore, "ontogenetically younger."

This explanation of the morphological radiosensitivity of two parts (root and shoot) of an embryo could account for the different effects of X rays on the DNA of two parts of an embryo. In comparison with the root and shoot regions, the region of the scutellum is composed of a relatively small amount of meristematic tissue and a large quantity of differentiated tissue. Since more differentiated tissue is less radiosensitive than younger tissue (Bergonie and Tribondeau, 1906) the higher DNA value in this region could be explained in this fashion.

The results of Group-4 appear in Figure 12. The percent DNA content of the root and shoot were found to be 74% and 72%, respectively, indicating similar responses for these two tissues, while that of the scutellum (101%) indicates the highest resistance to ionizing radiation. The explanation of the results in Group-3 could be applied to those of Group-4.

The largest differences between the content of RNA in the three embryo parts are 23% in Group-1 experiment, 45% in Group-2, 23% in Group-3, and 26% in Group-4. As shown here, the response of RNA content to X rays seemed to be quite independent of degree of tissue differentiation. This confirms previous results obtained in the experiments dealing with the biochemical analysis of purines and pyrimidines in normal and X-rayed mature embryos. As seen here, the largest variation in RNA among the three tissues was in Group-2. This could be explained by pointing out the relationship between protein synthesis and RNA

content. Stage g in Group-2 is the stage just prior to morphological differentiation. The embryos undergoing differentiation are the site of marked protein synthesis, which in turn requires large quantities of RNA. Since morphological differentiation of the parts (root, shoot, and scutellum) of an embryo does not occur simultaneously, the spacial distribution of the RNA required for protein synthesis may be markedly different within an embryo at stage g.

SUMMARY

The purpose of the study was to investigate the effect of a single dose (450 r) of ionizing radiation (X rays) on the RNA and DNA content of barley embryos developing *in vivo*.

First, the purines and pyrimidines of RNA and DNA of mature embryos were separated and determined spectrophotometrically after irradiation at different stages of embryogeny. In the second technique, tracer levels of P^{32} phosphate were supplied to the roots immediately after irradiation when the embryos were at the different stages of embryogeny and histological sections of the embryo tissue were prepared and analyzed for P^{32} incorporation into RNA and DNA at subsequent stages of embryo development.

The conclusions reached during the course of the observations were as follows:

1. DNA was more radiosensitive than RNA during embryogeny, except for the mature embryo where DNA was more stable than RNA.
2. Altered purine-pyrimidine ratios did not return to the normal pattern of nucleic acid metabolism.
3. Uracil of RNA and thymine of DNA appeared to be more labile to X-radiation than other bases.
4. The observed differences in RNA and DNA contents at different stages of embryogeny (with the exception of the DNA in mature

embryos) indicated that the younger embryos were more affected and/or underwent less recovery during the post-irradiation period than the older embryos.

5. Stage specificity of radiation effects seemed to be referred more to effects on the DNA rather than the RNA components of the embryo cells.
6. If irradiation occurred prior to structural differentiation, root, shoot, and scutellum regions show similar levels of radiosensitivity with regard to the content of DNA.
7. If radiation was applied during the time that structural differentiation of the above three regions was occurring, the order of increasing radiosensitivity to X rays was scutellum, shoot, and root, as based upon DNA content.
8. The maximum difference between DNA values in the three different parts of an embryo reflected the degree of tissue heterogeneity within the embryo at the time of X-irradiation, but RNA seemed to be independent of the level of tissue differentiation.

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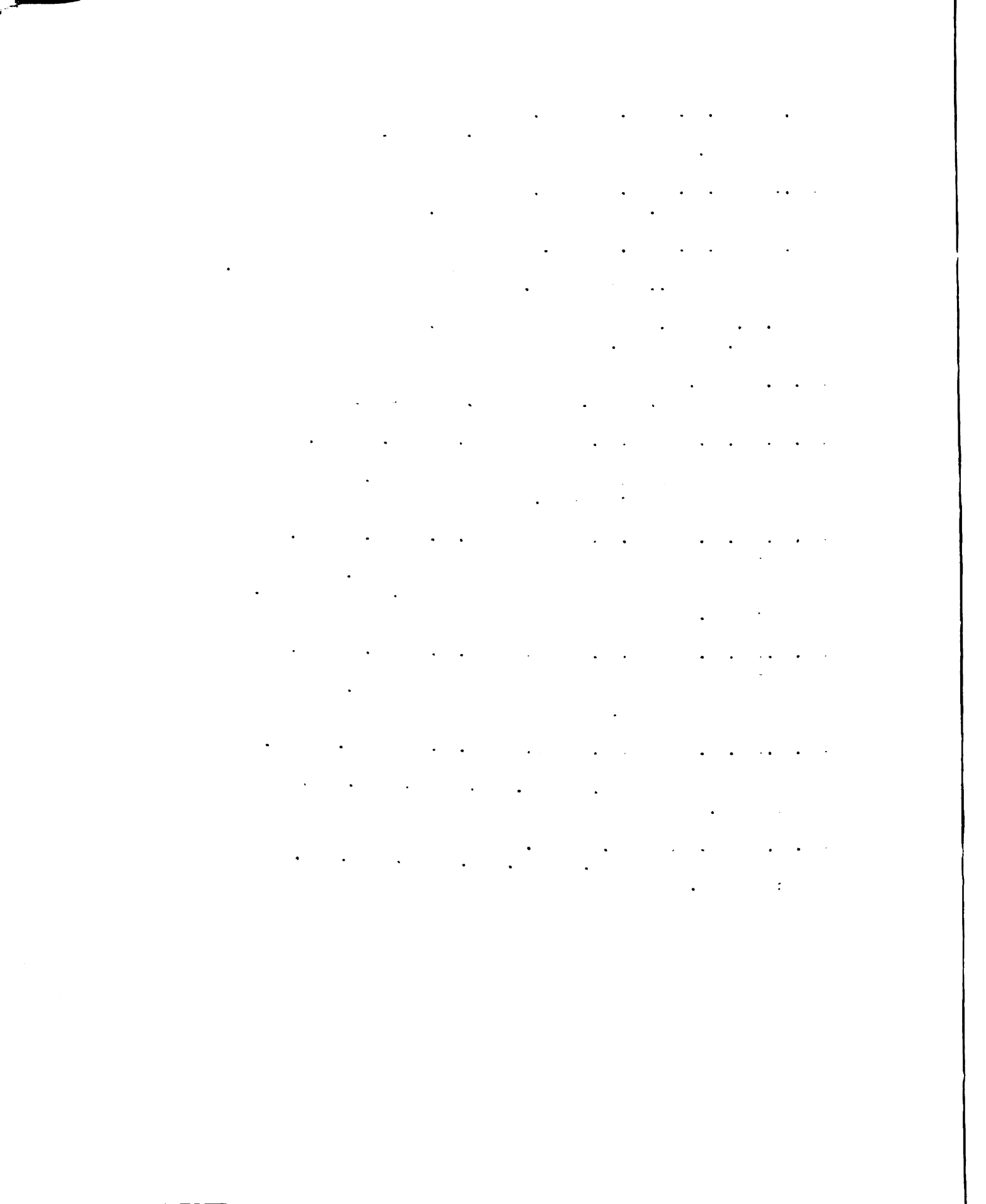

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APPENDIX A

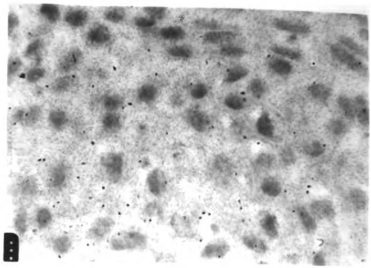
Plate 1. Photographs (x 1350 under oil immersion) of three adjacent sections (at 6 μ) of an embryo exposed to a single dose of 450 r X rays and treated with P-32 (200 μ c/kg of soil).

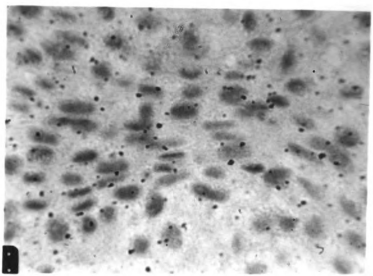
Top tissue, processed with 0.2 N perchloric acid at room temp. for 5 min. so that neither RNA nor DNA is removed.

Middle tissue, processed with 1 N perchloric acid at 4°C for 18 hours so that RNA is removed, but DNA is not.

Bottom tissue, processed with 0.5 N perchloric acid at 70°C for 20 min. so that both RNA and DNA are removed.

Time for exposure, 21 days after the slides were coated with emulsion.





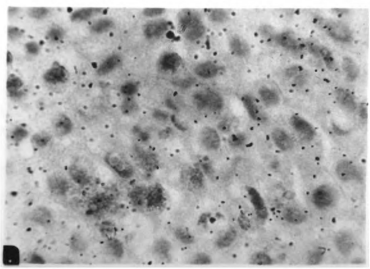


Plate 2. Developmental stages of barley embryogeny

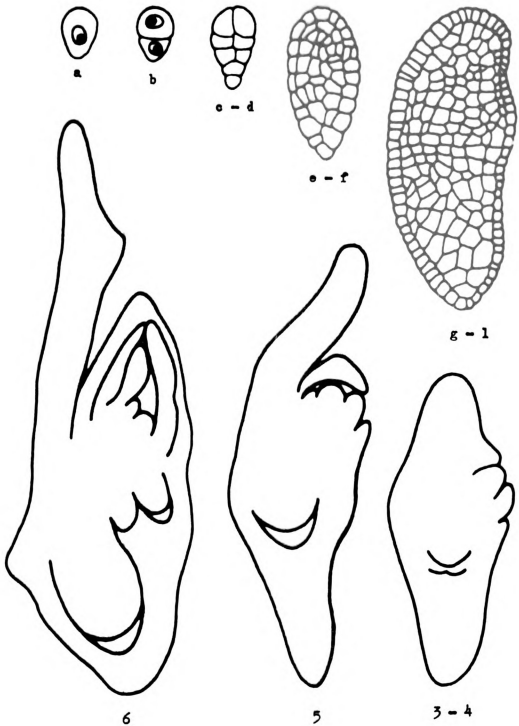
Stages a, b, and c-d are early proembryos.

Stage e-f is a mid-proembryo.

Stage g - 1 is a stage intermediate between a late proembryo and an early differentiating embryo.

Stage 3 - 4 is a mid-differentiating embryo.

Stages 5 and 6 are late differentiating embryos.


a
b
c - d
e - f
g - l
6
5
3 - 4

APPENDIX B

Fig. 1. Comparison of RNA - bases (m μ moles per mg dry weight) in normal embryos and those X-rayed at four different embryonic stages and analyzed at stage 6c, corrected for 21% loss

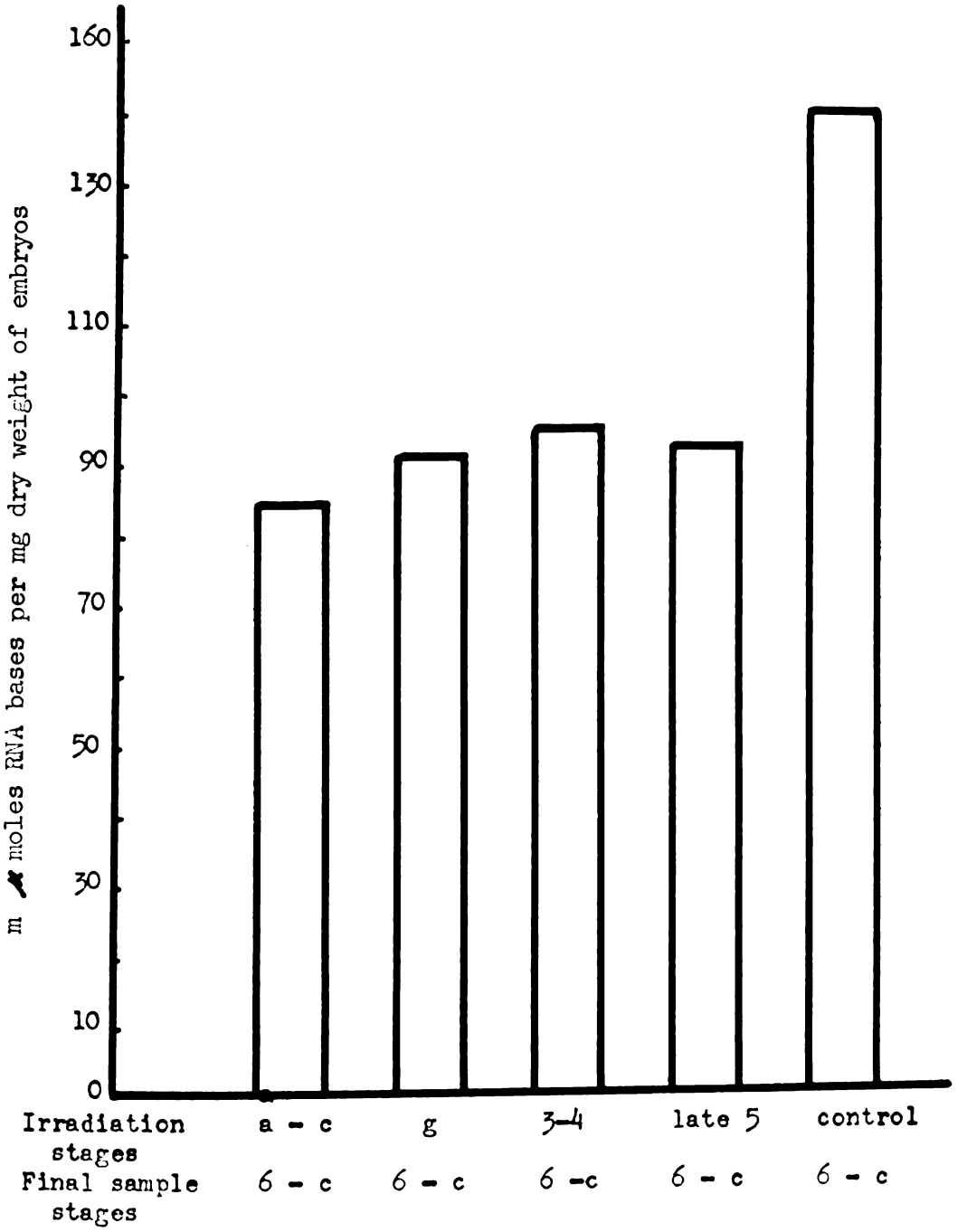

160
130
110
90
70
50
30
10
0
m moles RNA bases per mg dry weight of embryos
Irradiation stages
a - c
g
3-4
late 5
control
Final sample stages
6 - c
6 - c
6 -c
6 - c
6 - c

Fig. 2. Comparison of DNA - bases (m μ moles per mg dry weight) in normal embryos and those X-rayed at four different embryonic stages and analyzed at stage 6c, corrected for 27% loss

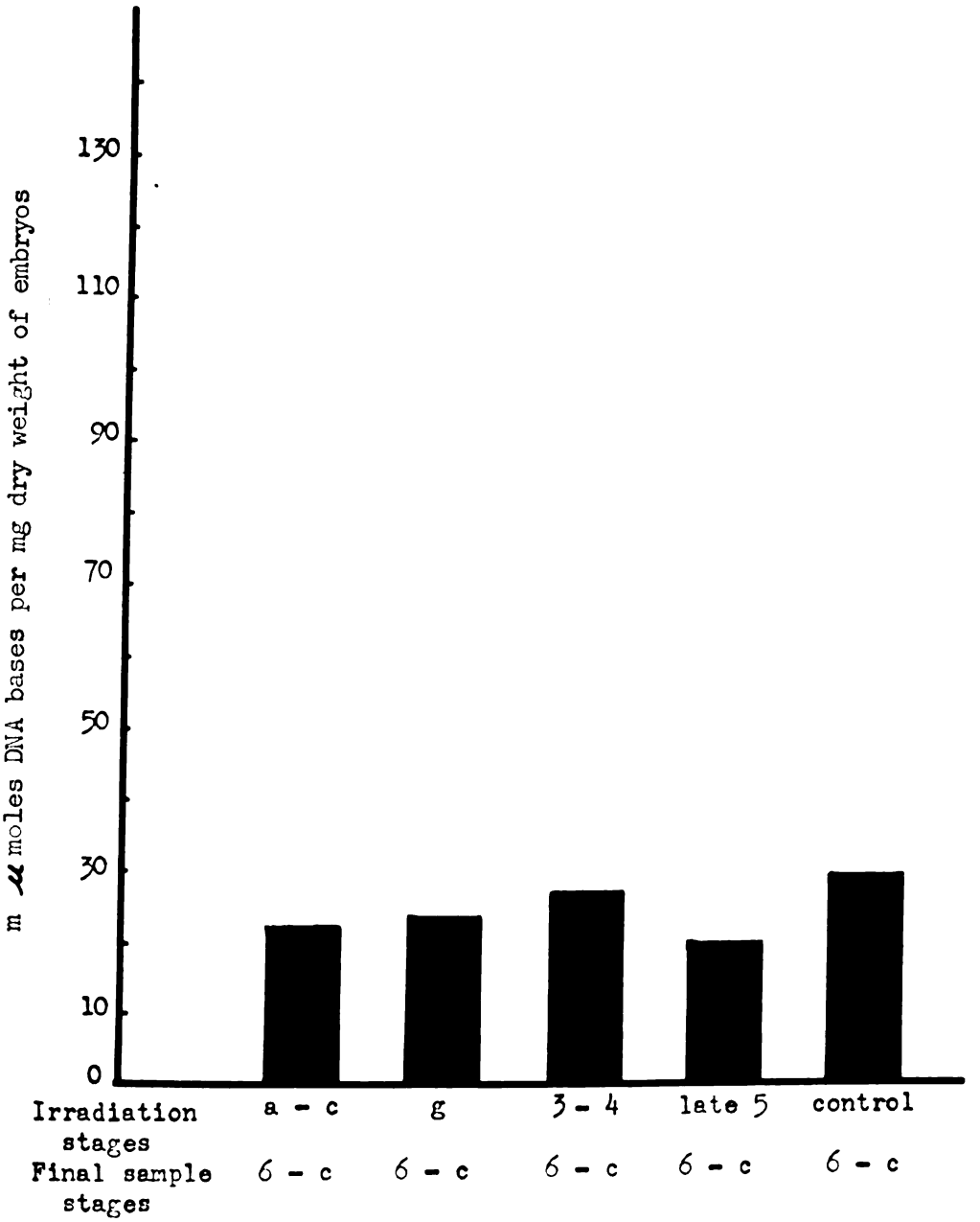

m μ moles DNA bases per mg dry weight of embryos
130
110
90
70
50
30
10
0
Irradiation stages
a - c
g
3 - 4
late 5
control
Final sample stages
6 - c
6 - c
6 - c
6 - c
6 - c

Fig. 3. Comparison of purine and pyrimidine bases of total nucleic acids (m μ moles per mg dry weight) in normal embryos and those X-irradiated at four different embryonic stages and analyzed at stage 6c, corrected for 21% loss (RNA) and 27% (DNA)

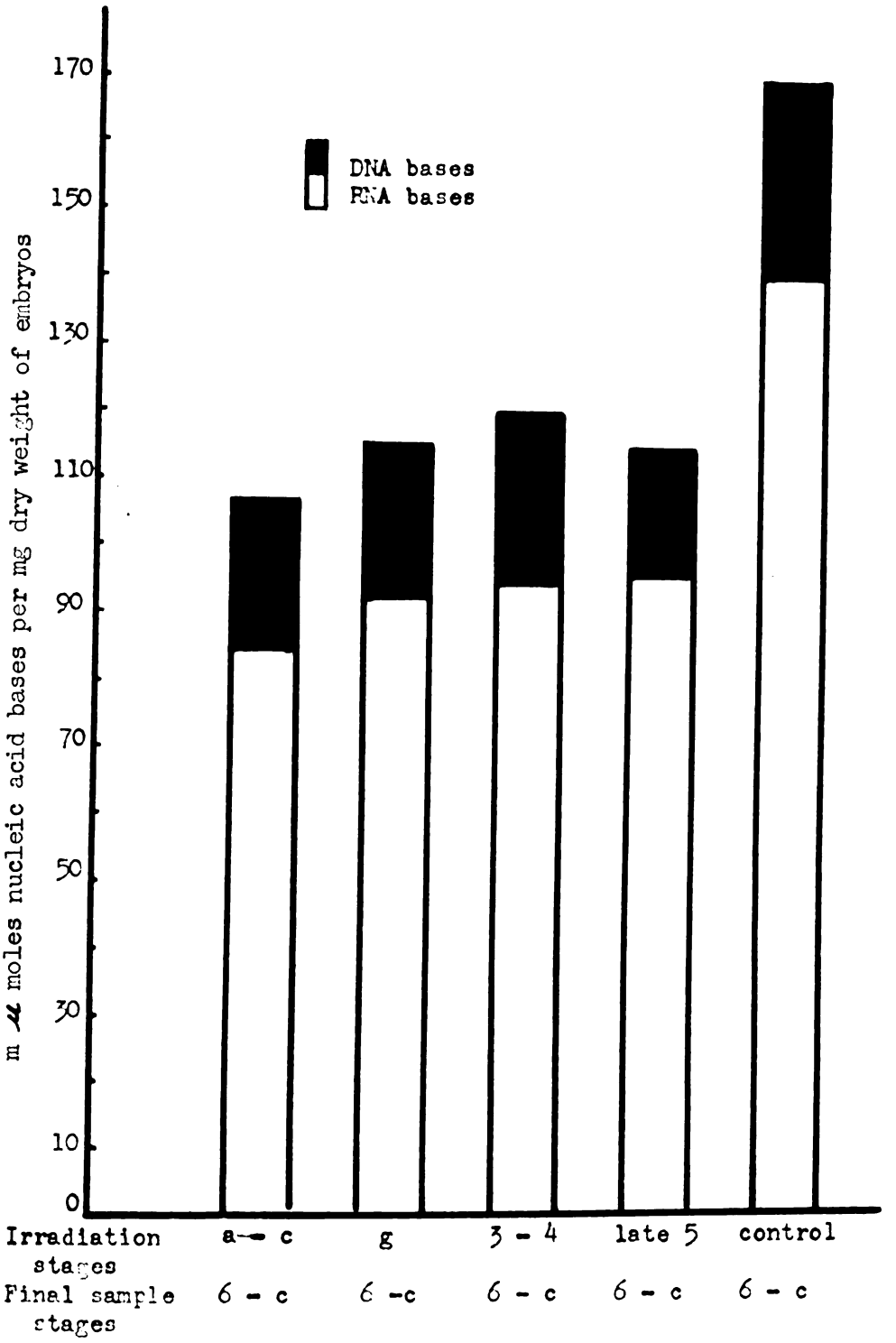

DNA bases
RNA bases
m μ moles nucleic acid bases per mg dry weight of embryos
170
150
130
110
90
70
50
30
10
0
Irradiation stages
a — c
g
3 - 4
late 5
control
Final sample stages
6 - c
6 - c
6 - c
6 - c
6 - c

Fig. 4. RNA and DNA content of irradiated embryos, as percent of controls, during 16 days post-irradiation. Embryos were treated with X rays (450 r) at embryonic stage a-c (Group - 1)

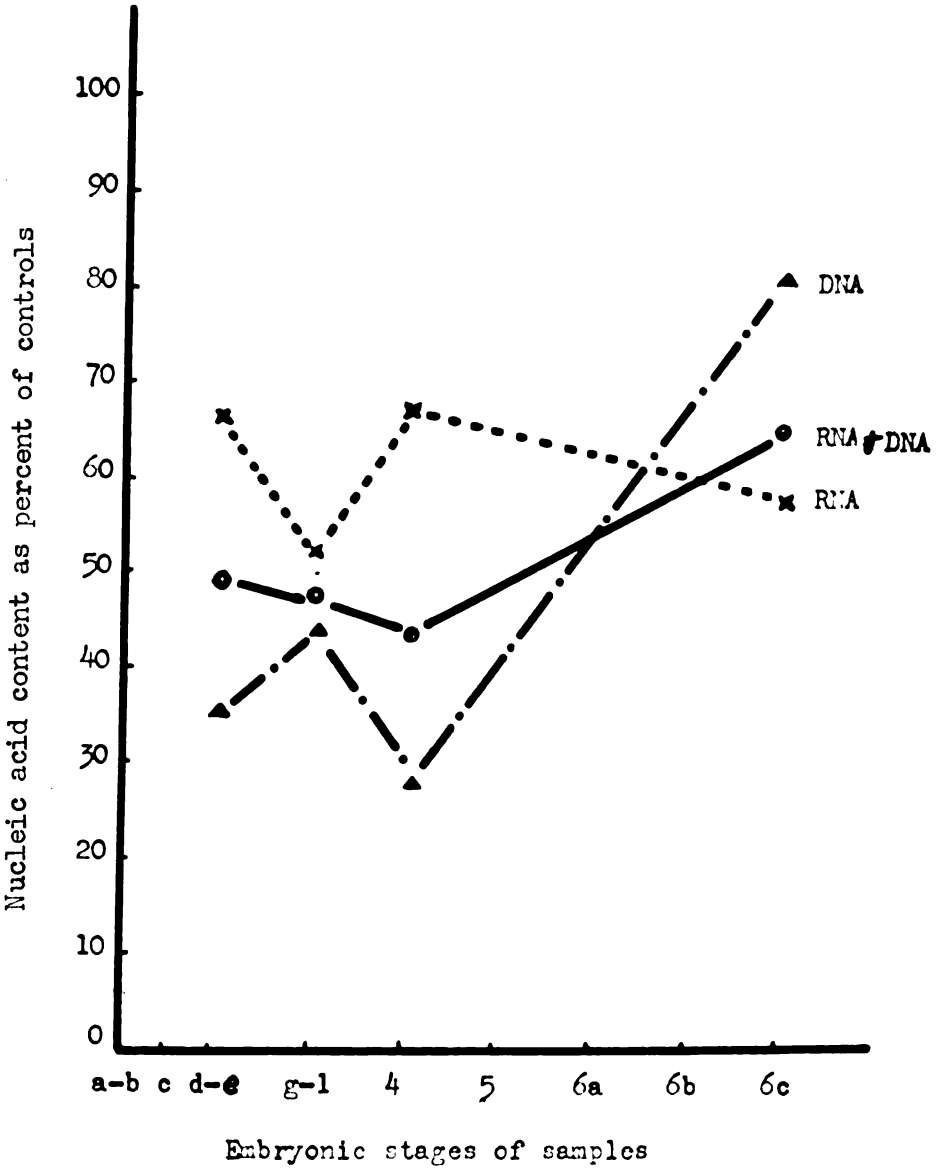

100
90
80
70
60
50
40
30
20
10
0
Nucleic acid content as percent of controls
DNA
RNA & DNA
RNA
a-b
c
d-e
g-l
4
5
6a
6b
6c
Embryonic stages of samples

Fig. 5. RNA and DNA content of irradiated embryos, as percent of controls, during 12 days post-irradiation. Embryos were treated with X rays (450 r) at embryonic stage g (Group-2)

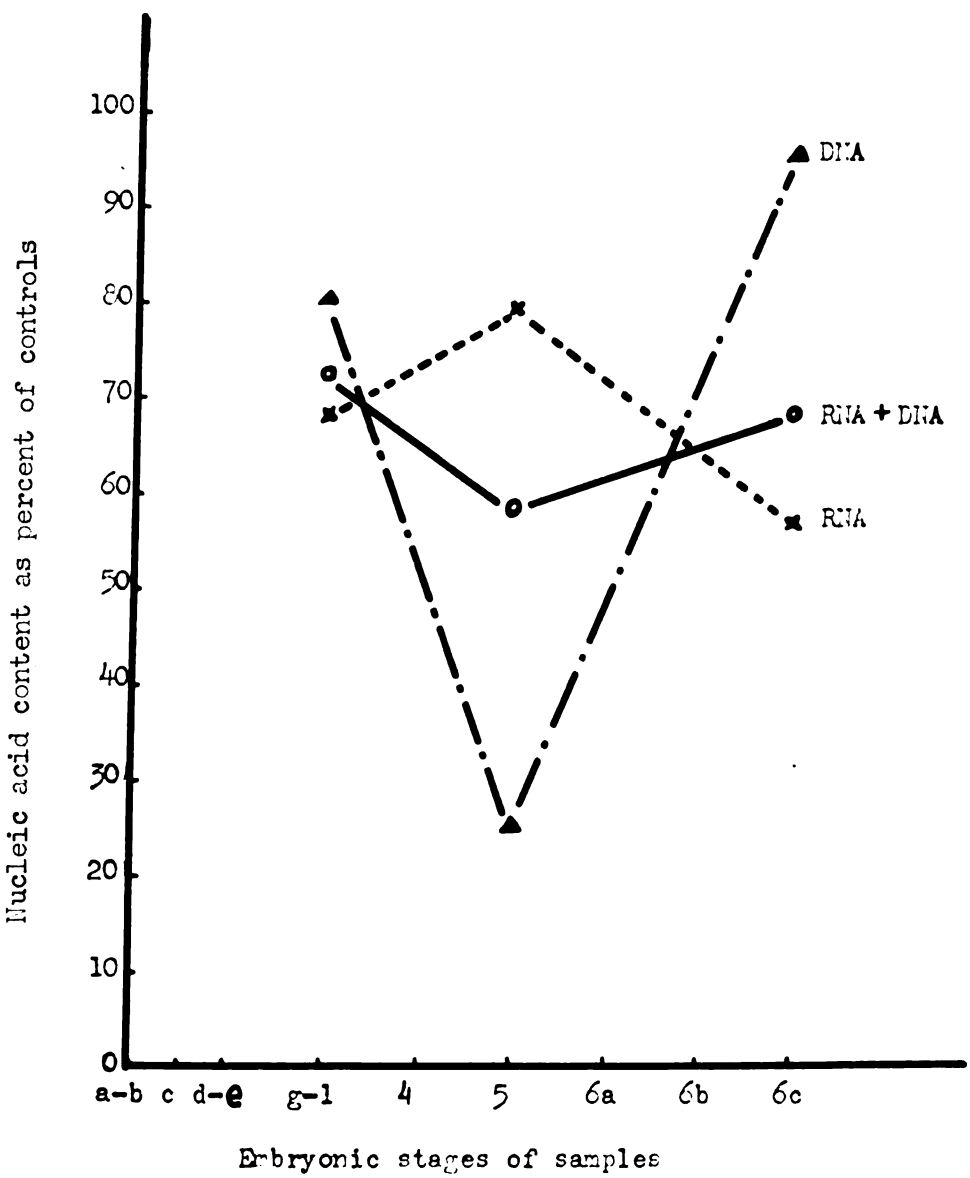

Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
DNA
RNA + DNA
RNA
a-b
c
d-e
g-l
4
5
6a
6b
6c
Embryonic stages of samples

Fig. 6. RNA and DNA content of irradiated embryos, as percent of controls, during 9 days post-irradiation. Embryos were treated with X rays (450 r) at embryonic stage 3-4 (Group - 3).

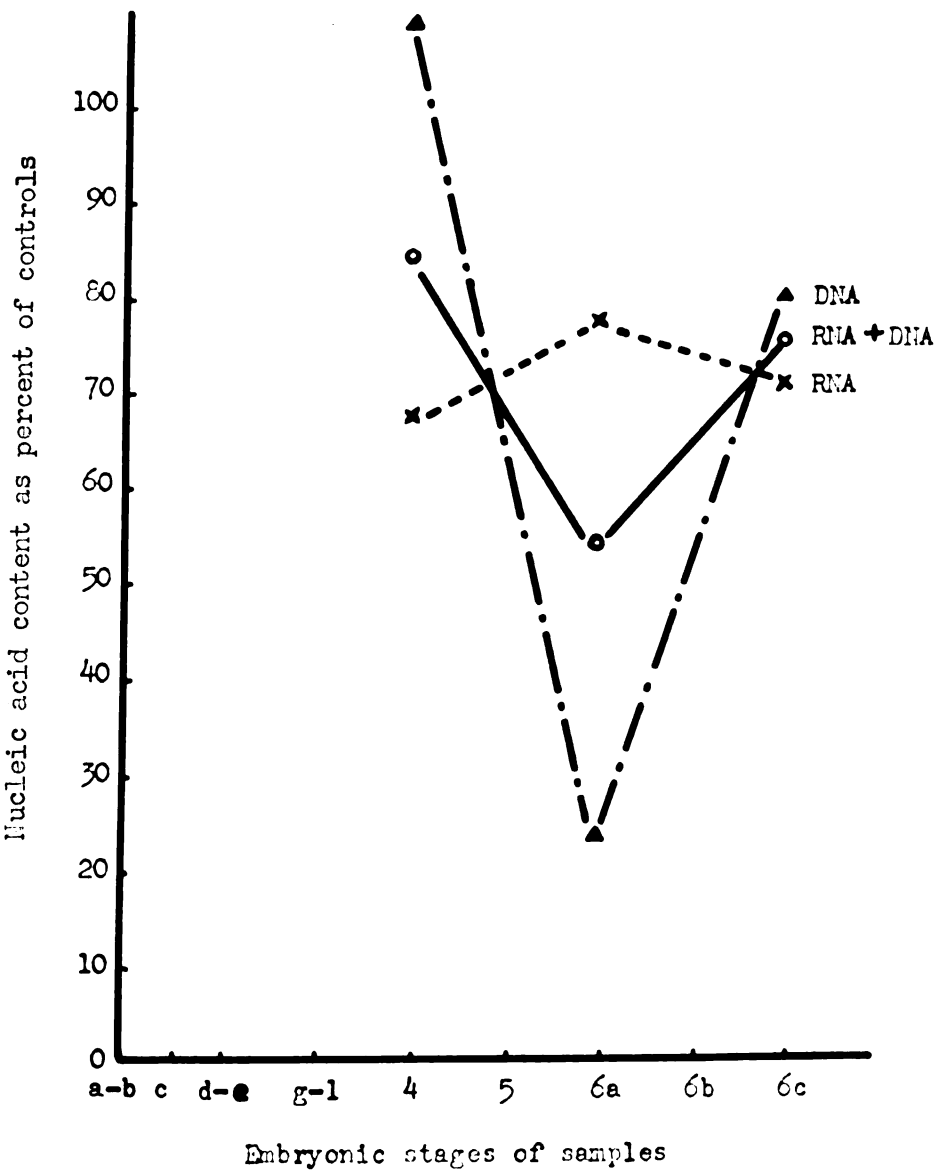

Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
a-b
c
d-e
g-l
4
5
6a
6b
6c
DNA
RNA + DNA
RNA
Embryonic stages of samples

Fig. 7. RNA and DNA content of irradiated embryos, as percent of controls, during 7 days post-irradiation. Embryos were treated with X rays (450 r) at embryonic stage late-5 (Group - 4)

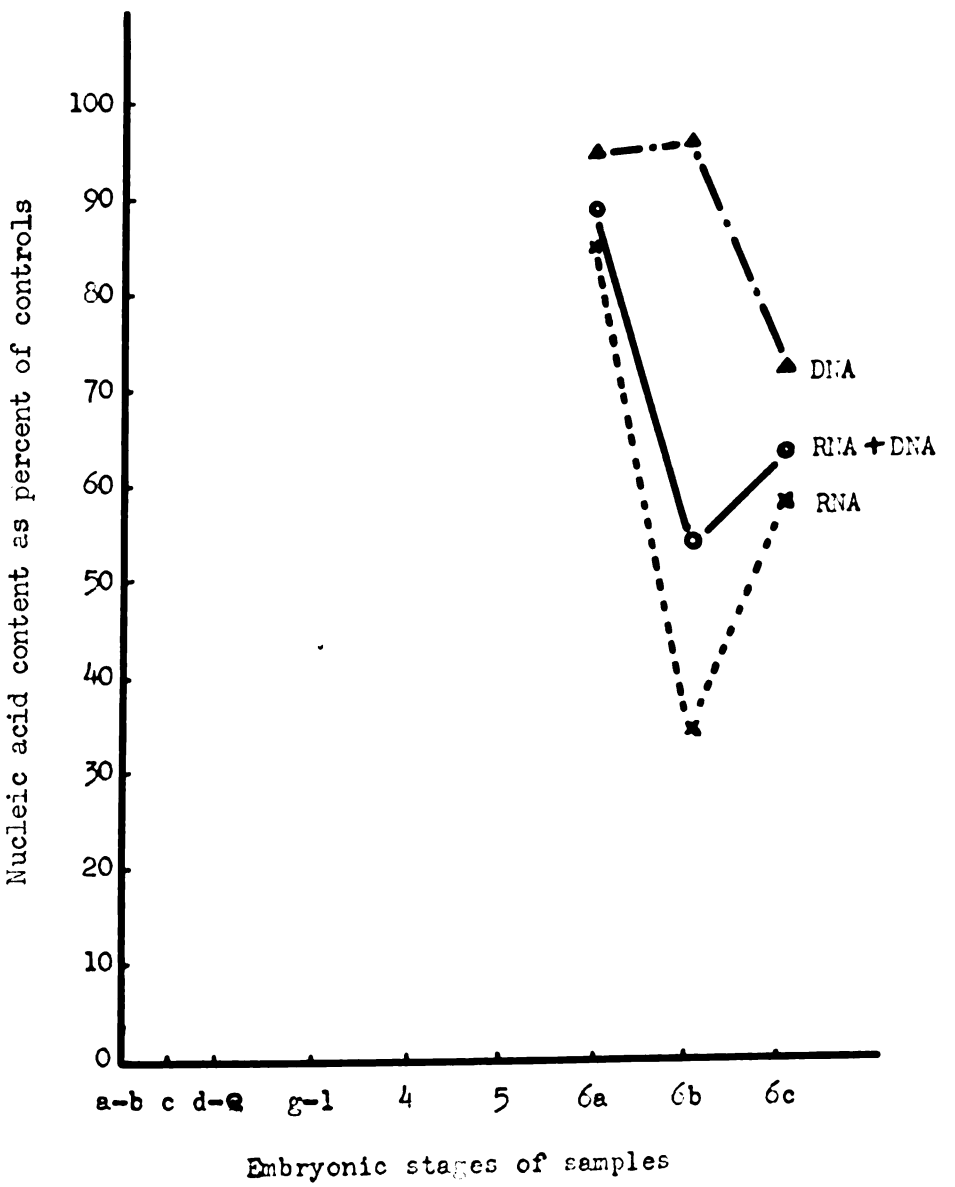

Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
DNA
RNA + DNA
RNA
a-b
c
d-e
g-1
4
5
6a
6b
6c
Embryonic stages of samples

Fig. 8. Total nucleic acid content of irradiated embryos, as percent of controls, following 450 r X rays. Embryos were X-rayed at four different stages (Group 1 at stage c, Group 2 at stage g, Group 3 at stage 3-4, and Group 4 at stage late 5)

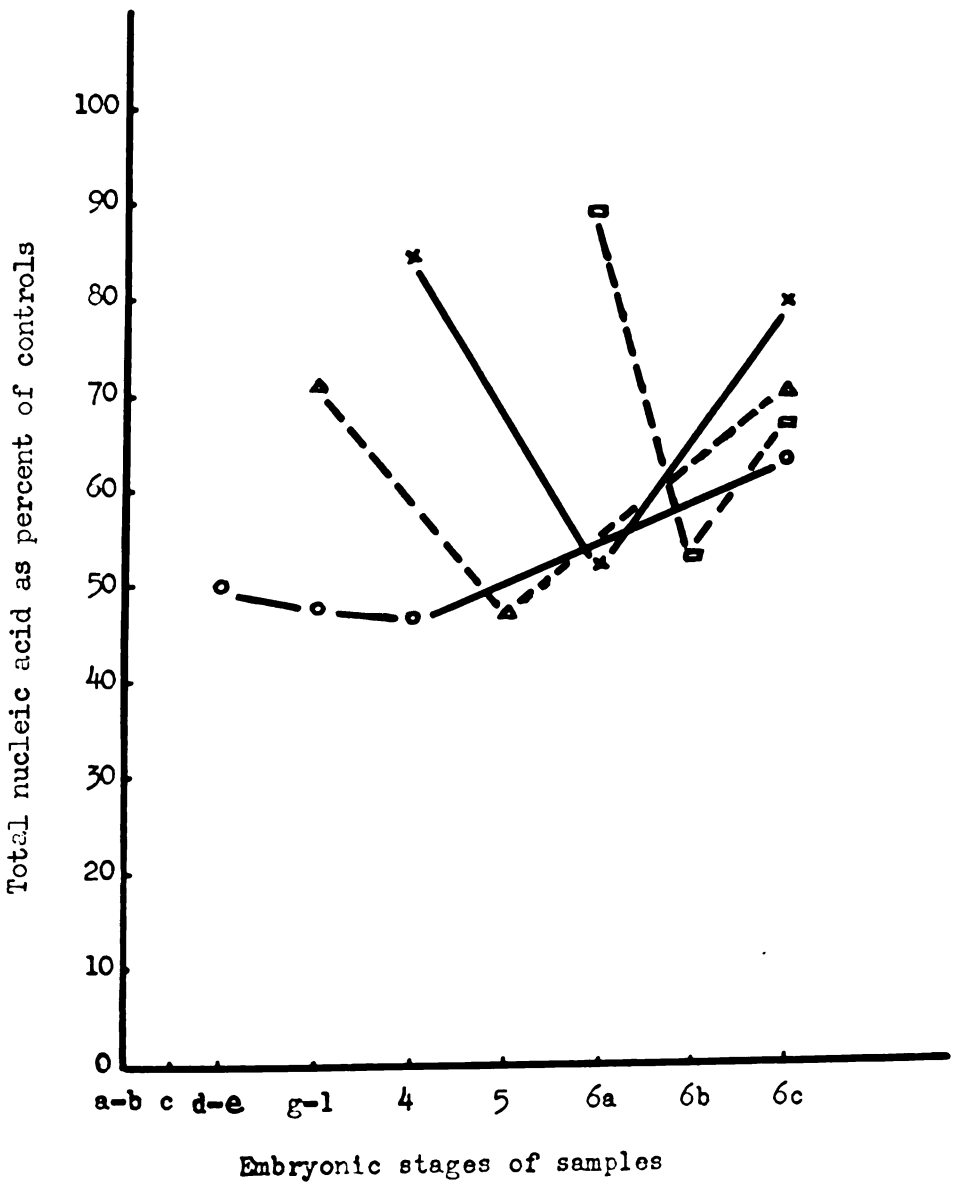

Total nucleic acid as percent of controls
100
90
80
70
60
50
40
30
20
10
0
a-b c d-e g-l 4 5 6a 6b 6c
Embryonic stages of samples

Fig. 9. Nucleic acid content, as percent of controls, of the root, shoot, and scutellum of embryos which were treated with a dose of 450 r X rays at stage a - c and sampled after complete differentiation at stage 6c (Group - 1)

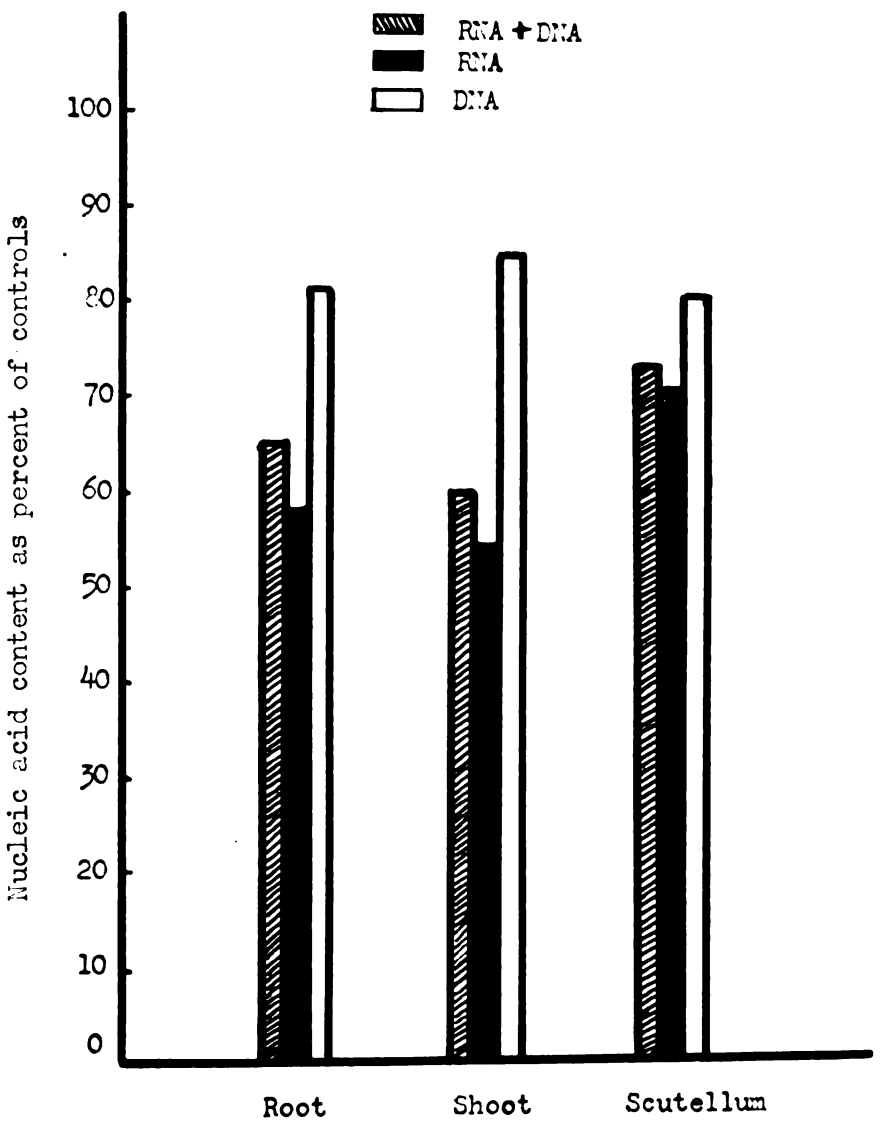

RNA + DNA
RNA
DNA
Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
Root
Shoot
Scutellum

Fig. 10. Nucleic acid content, as percent of controls, of the root, shoot, and scutellum of embryos which were treated with a dose of 450 r X rays at stage g and sampled after complete differentiation at stage 6c (Group - 2)

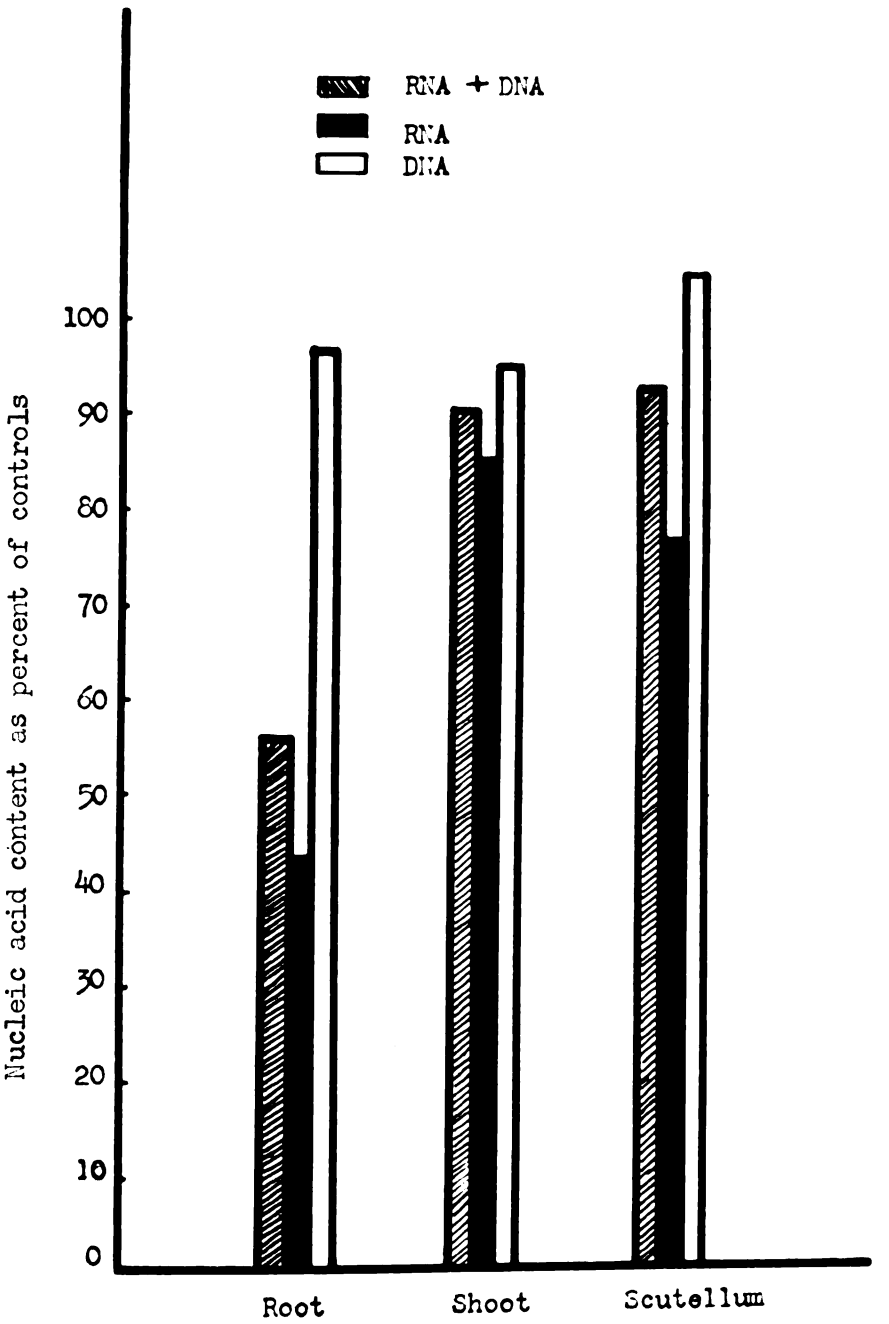

RNA + DNA
RNA
DNA
Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
Root
Shoot
Scutellum

Fig. 11. Nucleic acid content, as percent of controls, of the root, shoot, and scutellum of embryos which were treated with a dose of 450 r X rays at stage 3-4 and sampled after complete differentiation at stage 6c (Group - 3)

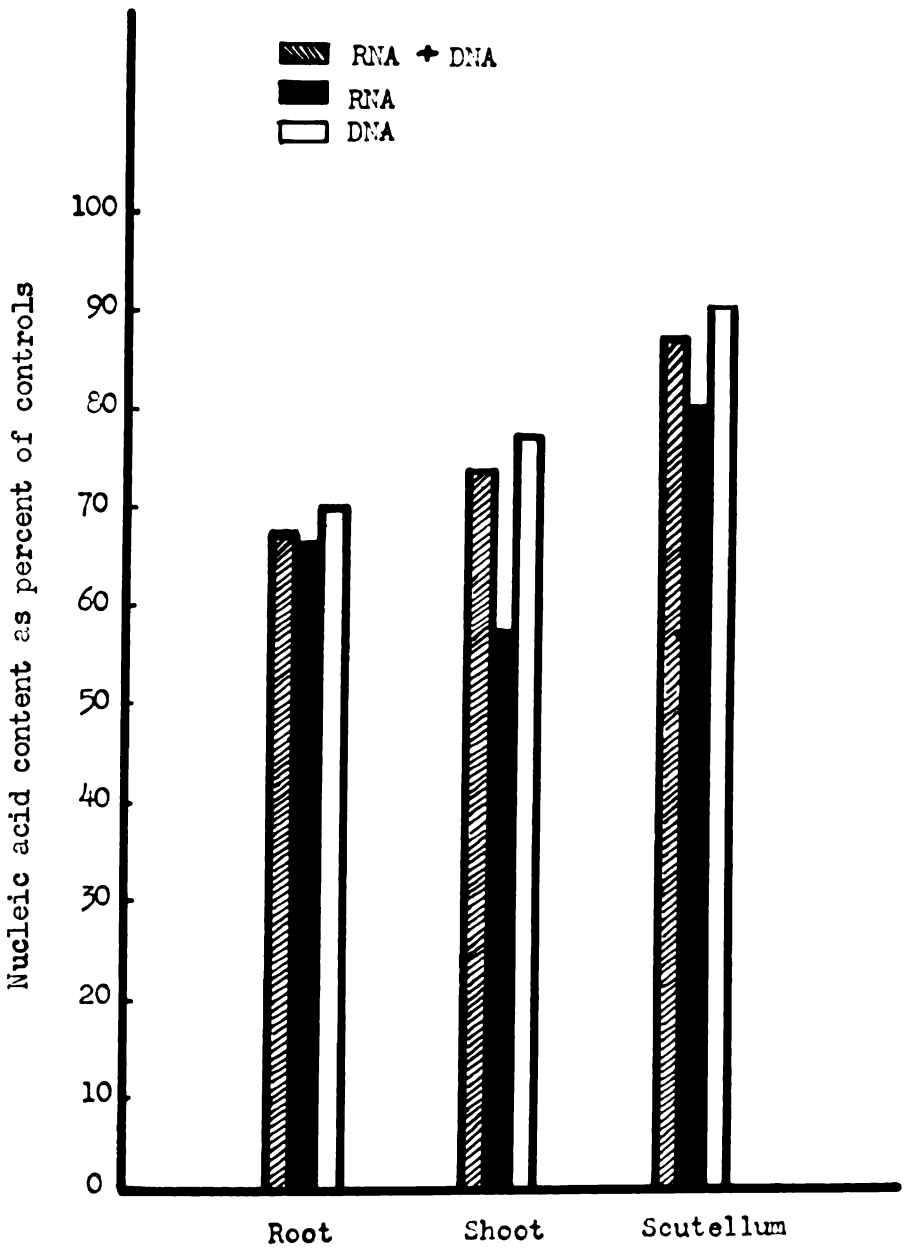
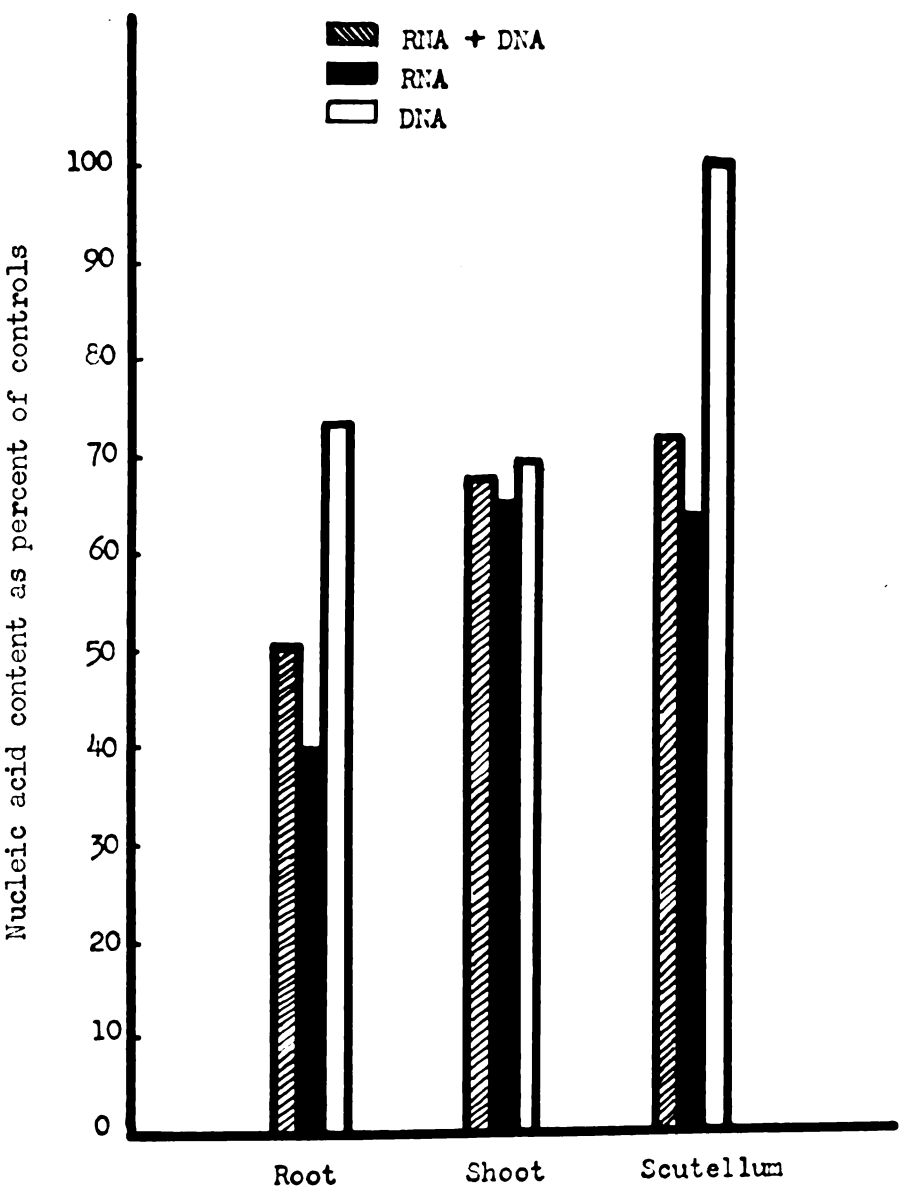

RNA + DNA
RNA
DNA
Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
Root
Shoot
Scutellum

Fig. 12. Nucleic acid content, as percent of controls, of the root, shoot, and scutellum of embryos which were treated with a dose of 450 r X rays at stage late 5 and sampled after complete differentiation at stage 6c (Group - 4)


RNA + DNA
RNA
DNA
Nucleic acid content as percent of controls
100
90
80
70
60
50
40
30
20
10
0
Root
Shoot
Scutellum

APPENDIX C

Table 1. Standard extinction values of purine and pyrimidine bases* (10 µg per ml of 0.1 N Hydrochloric acid) determined from the difference (Δ) read at the absorption maximum (guanine at 249 mµ, adenine 262.5 mµ, cytosine 275 mµ, uracil 259 mµ, and thymine 264 mµ) and at 290 mµ

Guanine

Replicates	Extinction at wave length in mµ 300	290	254	249	244	Δ	Average
1	0.031	0.306	0.723	0.794	0.721	0.488	
2	0.030	0.299	0.721	0.790	0.721	0.491	0.491
3	0.027	0.298	0.718	0.792	0.717	0.494	

Adenine

Replicates	Extinction at wave length in mµ 300	290	267.5	262.5	257	Δ	Average
1	0.001	0.048	1.022	1.070	1.025	1.022	
2	0.005	0.046	1.020	1.071	1.020	1.025	1.024
3	0.005	0.046	1.018	1.071	1.015	1.025	

Cytosine

Replicates	Extinction at wave length in mµ 300	290	280	275	270	Δ	Average
1	0.028	0.503	0.921	0.960	0.896	0.457	
2	0.028	0.504	0.919	0.960	0.895	0.456	0.455
3	0.030	0.506	0.918	0.958	0.894	0.452	

* The commercial samples of five bases (A grades) were obtained from California Corporation for Biochemical Research, Medford St., Los Angeles 63, Calif.

Uracil

Replicates	Extinction at wave length in mµ 300	290	264	259	254	Δ	Average
1	0.005	0.011	0.702	0.743	0.692	0.732	
2	0.002	0.008	0.700	0.740	0.690	0.732	0.732
3	0.003	0.010	0.700	0.742	0.691	0.732	

Thymine

Replicates	Extinction at wave length in mµ 300	290	269	264	259	Δ	Average
1	0.002	0.076	0.596	0.611	0.563	0.535	
2	0.003	0.078	0.598	0.616	0.565	0.538	
3	0.003	0.080	0.597	0.615	0.565	0.535	0.536

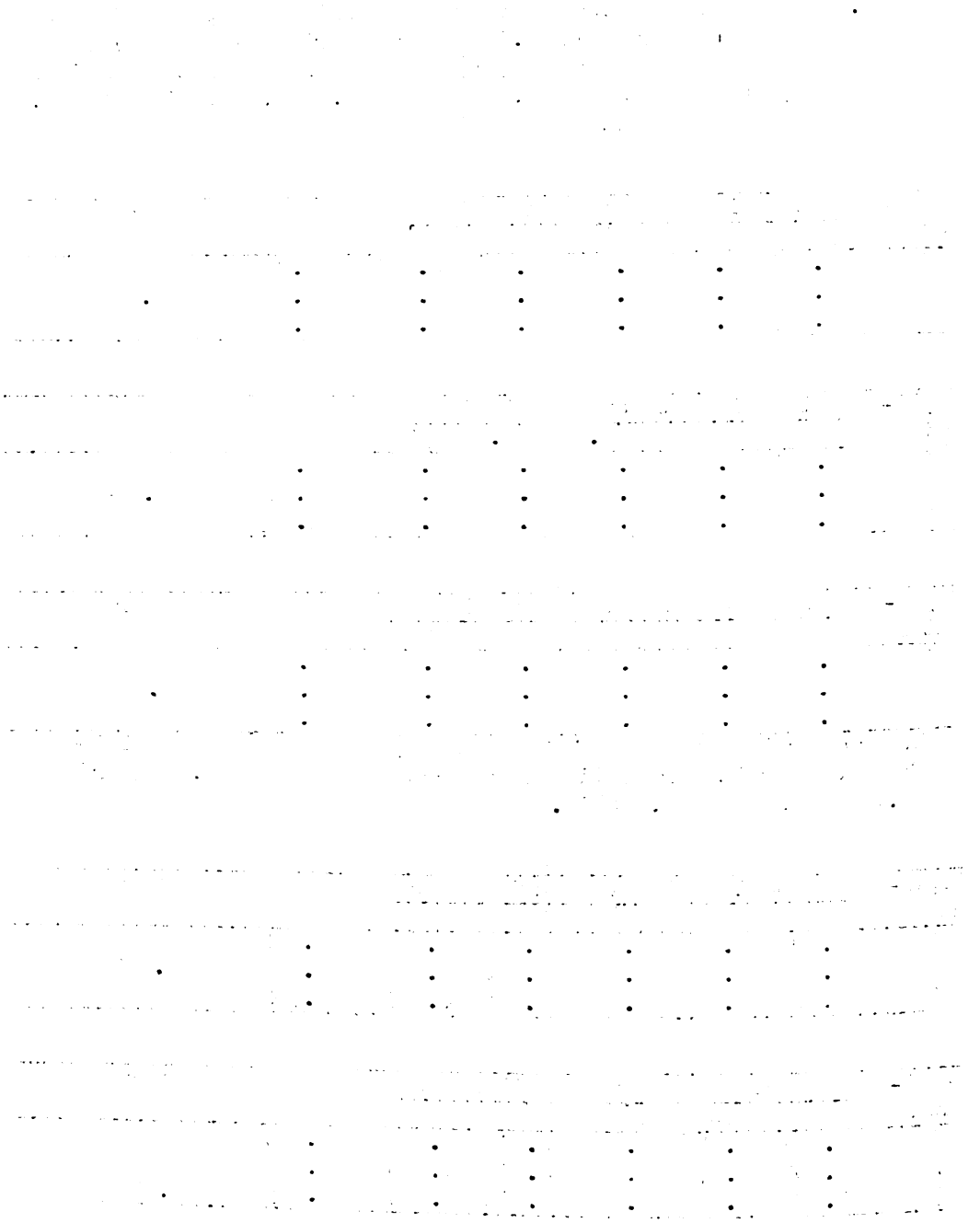


Table 2. Recoveries of five bases* after heating at 100°C in 12 N perchloric acid for 1 hour

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	Recovery **	%
1	0.013	0.051	0.140	0.148	0.137	0.097	9.90	99
2	0.005	0.037	0.127	0.130	0.127	0.094	9.55	96
3	0.002	0.033	0.123	0.126	0.123	0.093	9.55	96
4	0.005	0.037	0.127	0.130	0.127	0.094	9.55	96

Δ = 0.491 Δx= difference between maximum and 290 Extinction

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	Recovery **	%
1	0.000	0.009	0.195	0.198	0.188	0.188	9.20	92
2	0.003	0.011	0.197	0.201	0.189	0.190	9.30	93
3	0.001	0.007	0.191	0.195	0.183	0.188	9.20	92
4	0.008	0.010	0.199	0.205	0.190	0.195	9.50	95

Δ = 1.024

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	Recovery **	%
1	0.012	0.095	0.174	0.182	0.167	0.087	9.55	96
2	0.015	0.099	0.180	0.191	0.180	0.092	10.10	101
3	-	-	-	-	-	-	-	-
4	0.012	0.099	0.179	0.188	0.176	0.089	9.75	96

Δ = 0.455 * Same as in Table 1

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	Recovery **	%
1	0.005	0.009	0.150	0.152	0.132	0.143	9.8	98
2	0.006	0.007	0.133	0.150	0.131	0.143	9.75	97
3	0.008	0.015	0.150	0.155	0.130	0.140	9.55	96
4	0.004	0.010	0.159	0.148	0.129	0.138	9.45	95

Δ = 0.732

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	Recovery **	%
1	0.006	0.022	0.117	0.121	0.111	0.099	9.35	94
2	0.002	0.007	0.096	0.100	0.093	0.093	8.75	88
3	0.004	0.019	0.122	0.126	0.116	0.107	10.10	101
4	0.007	0.021	0.125	0.129	0.121	0.108	10.15	102

Δ = 0.536

** = $10\Delta x/\Delta \times 5$ μg

Summary of Table 2

	Guanine	Adenine	Cytosine	Uracil	Thymine
Average recovery	97	93	97	96	96

* Four replicates

Table 3. Base recoveries of yeast R N A* and salmon D N A* hydrolyzed with 12 N perchloric acid at 100°C

Yeast R N A

Guanine (m. wt. 151.1)

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.013	0.052	0.115	0.122	0.107	0.070	7.15	47	0.204
2	0.013	0.049	0.116	0.123	0.108	0.074	7.50	49	0.220
3	0.012	0.045	0.108	0.112	0.100	0.067	6.80	45	0.196
4	0.014	0.052	0.115	0.116	0.103	0.064	6.60	44	0.190

Δ = 0.491 Δx = same as in Table 2
P = 8.9 % by Marshak and Vogel, 1950

Adenine (m. wt. 135.1)

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.008	0.015	0.152	0.159	0.151	0.144	7.00	52	0.226
2	0.005	0.010	0.149	0.154	0.146	0.144	7.00	52	0.226
3	0.002	0.007	0.141	0.148	0.141	0.144	7.00	52	0.226
4	0.021	0.029	0.166	0.167	0.151	0.138	6.75	50	0.217

Δ = 1.024
P = same as in Guanine

Cytosine (m. wt. 111.1)

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.003	0.043	0.084	0.090	0.084	0.047	5.15	46	0.200
2	0.003	0.046	0.088	0.093	0.087	0.047	5.15	46	0.200
3	0.009	0.052	0.093	0.098	0.098	0.046	5.05	45	0.195
4	0.008	0.052	0.094	0.098	0.089	0.046	5.05	45	0.195

Δ = 0.455 Δx = same as in Table 2
P = same as in Guanine

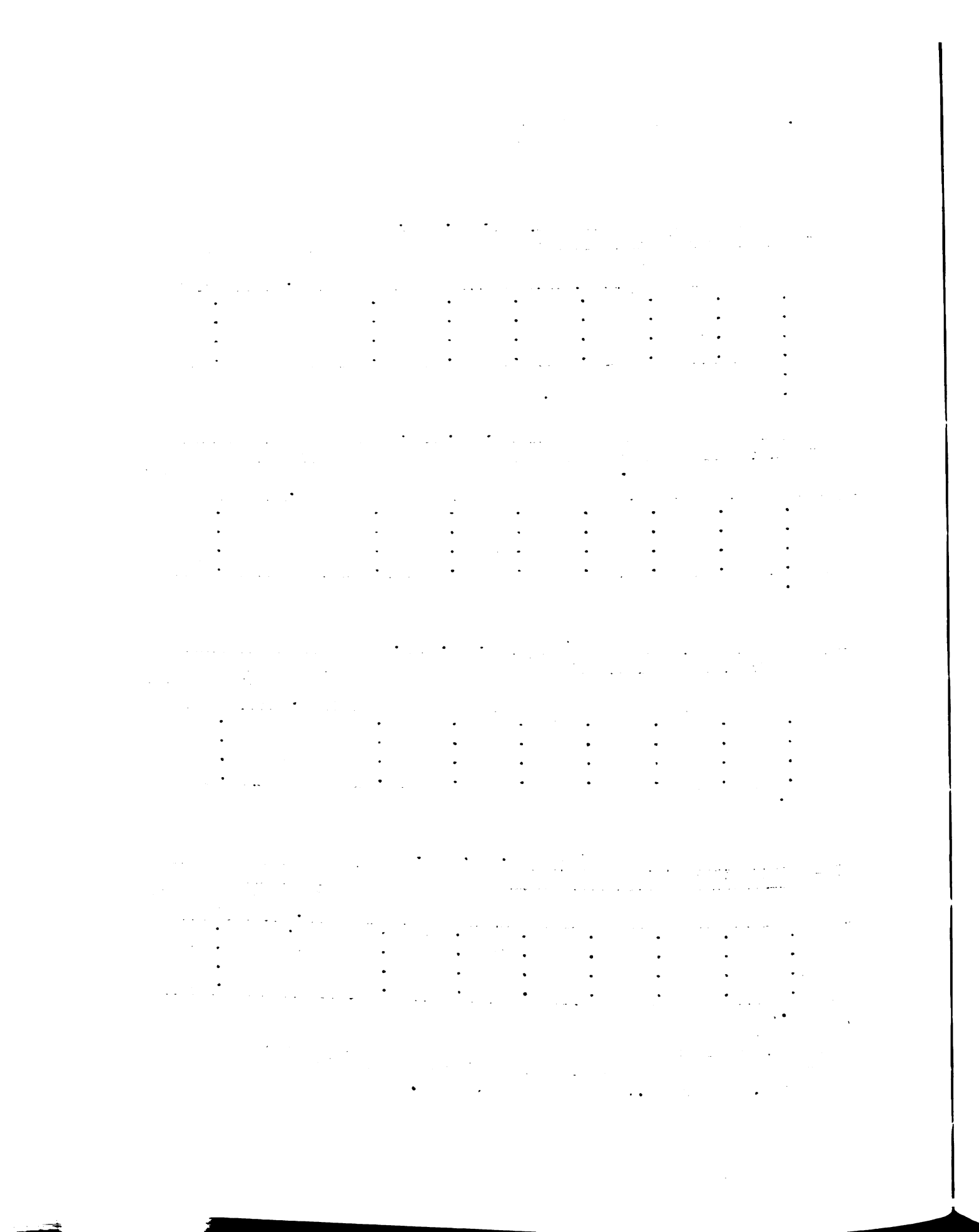
Uracil (m. wt. 112.1)

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.001	0.003	0.086	0.089	0.079	0.081	5.55	49.5	0.220
2	0.003	0.005	0.088	0.090	0.071	0.085	5.80	52	0.230
3	0.005	0.008	0.090	0.092	0.082	0.084	5.70	51	0.221
4	0.018	0.023	0.105	0.106	0.096	0.083	5.65	50	0.217

Δ = 0.732 Δx = same as in Table 2
P = same as in Guanine

* The commercial samples of yeast RNA and salmon DNA (A grades) were obtained from California Corporation for Biochemical Research, Medford St., Los Angeles 63, Calif.

** =



Summary of Table 3 on yeast R N A

Bases	Mole bases* per mole of P		
	Analytical values		Theoretical values
	Mean ± SE	Standard deviation	
Guanine	0.20 ± 0.007	0.014	-
Adenine	0.22 ± 0.008	0.017	-
Cytosine	0.20 ± 0.0005	0.001	-
Uracil	0.22 ± 0.001	0.002	-
Four bases	0.84 ± 0.016		1.00
% of recovery of four bases	84 ± 1.6		100

* Four replicates

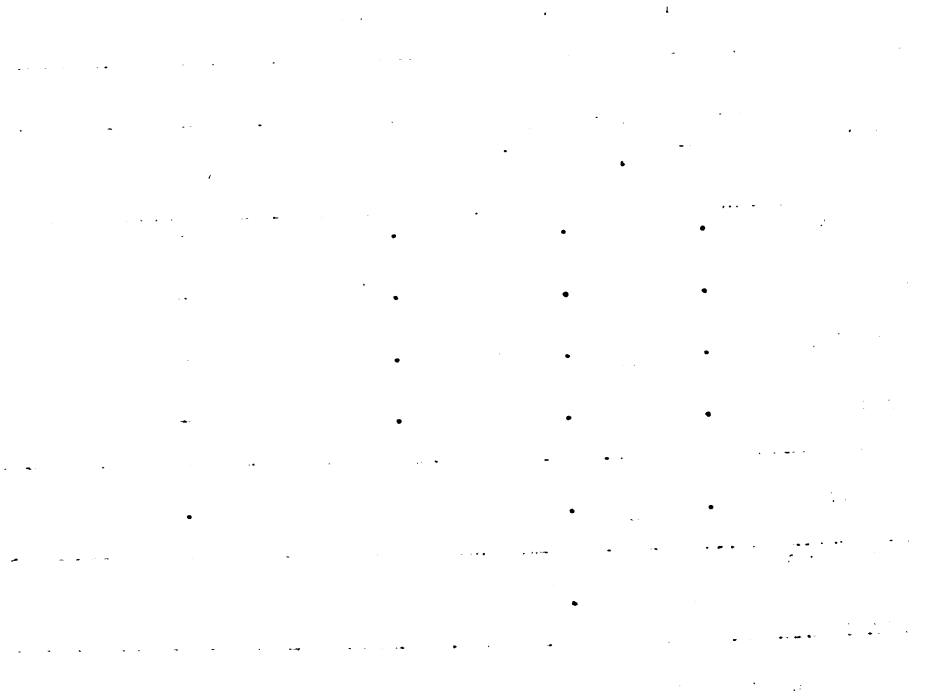


Table 3 continued

Salmon D N A

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	Recovery μ moles per mole bases 80 mg. DNA	per mole P
1	0.009	0.043	0.101	0.105	0.095	0.062	6.30	8.3	0.140
2	0.015	0.048	0.125	0.132	0.123	0.084	8.55	11.3	0.180
3	0.012	0.048	0.112	0.117	0.105	0.069	7.00	9.3	0.155
4	0.013	0.044	0.106	0.109	0.099	0.065	6.60	8.7	0.145

Δ = and Δx = same as guanine in yeast RNA (Table 3)
P = 9.3 % by Marshak and Vogel, 1950

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	Recovery μ moles per mole bases 80 mg. DNA	per mole P
1	0.000	0.008	0.168	0.177	0.167	0.169	8.25	12.2	0.200
2	0.005	0.012	0.175	0.185	0.175	0.173	8.40	12.4	0.206
3	0.005	0.012	0.177	0.185	0.177	0.173	8.40	12.4	0.206
4	0.002	0.008	0.170	0.178	0.169	0.170	8.30	12.3	0.205

Δ = and Δx = same as in adenine of yeast RNA (Table 3)
P = same as in guanine of salmon DNA (Table 3)

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	**	Recovery μ moles per mole bases 80 mg. DNA	per mole P
1	0.004	0.051	0.101	0.105	0.098	0.054	5.95	9.81	0.16
2	0.005	0.053	0.100	0.106	0.099	0.053	5.80	10.45	0.17
3	0.007	0.056	0.105	0.112	0.105	0.056	6.15	11.08	0.19
4	0.002	0.052	0.098	0.103	0.096	0.051	5.60	10.09	0.17

Δ = and Δx = same as in cytosine of yeast RNA (Table 3)
P = same as in guanine of salmon DNA (Table 3)

Thymine (m. wt. 126.1)

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	**	Recovery μ moles per mole bases 80 mg. DNA	per mole P
1	0.007	0.023	0.117	0.118	0.109	0.095	8.85	14.04	0.23
2	0.008	0.024	0.115	0.119	0.110	0.095	8.85	14.04	0.23
3	0.017	0.036	0.132	0.136	0.125	0.100	9.30	14.76	0.25
4	0.008	0.021	0.110	0.113	0.104	0.092	8.60	13.65	0.23

Δ = 0.536 Δx = same as in Table 2
P = same as in guanine of salmon DNA (Table 3)
** = $10 \Delta x / \Delta \times 5$ μg

Summary of Table 3 on salmon D N A

Bases	Mole bases* per mole of P			
	Analytical values			Theoretical values
	Mean ± SE		Standard deviation	
Guanine	0.16 ± 0.001		0.002	-
Adenine	0.21 ± 0.0008		0.0017	-
Cytosine	0.19 ± 0.007		0.014	-
Thymine	0.24 ± 0.005		0.011	-
Four bases	0.80 ± 0.014			1.00
% recovery of four bases	80 ± 1.4			100

* Four replicates

Table 4. Recoveries of yeast R N A* and salmon D N A* hydrolyzed with 1 N and 0.5 N perchloric acid, respectively, at 100° C

Yeast R N A

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.021	0.052	0.116	0.118	0.106	0.066	6.65	44	0.191
2	0.023	0.063	0.130	0.138	0.119	0.075	7.60	50	0.217
3	0.027	0.065	0.131	0.135	0.125	0.070	7.10	47	0.204
4	0.018	0.053	0.124	0.129	0.119	0.076	7.70	51	0.221

Δ , Δx , and P = same as in yeast RNA of Table 3

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	-0.002	0.005	0.140	0.145	0.120	0.140	6.85	51	0.222
2	0.004	0.015	0.156	0.160	0.155	0.145	7.10	52	0.226
3	0.006	0.012	0.146	0.160	0.150	0.148	7.25	53	0.230
4	0.008	0.020	0.160	0.170	0.163	0.150	7.30	54	0.240

Δ , Δx , and P = same as in yeast RNA of Table 3

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	0.026	0.062	0.108	0.113	0.103	0.051	5.60	50	0.217
2	0.003	0.035	0.082	0.088	0.083	0.053	5.80	52	0.226
3	0.005	0.045	0.085	0.092	0.075	0.047	5.15	46	0.200
4	0.010	0.048	0.090	0.098	0.095	0.050	5.50	50	0.217

Δ , Δx , and P = same as in yeast RNA of Table 3

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	**	Recovery μ moles per 80 mg. RNA	mole bases per mole P
1	-0.006	-0.005	0.071	0.072	0.060	0.077	5.25	47	0.204
2	0.000	0.002	0.088	0.089	0.082	0.088	6.00	53	0.230
3	0.010	0.014	0.101	0.103	0.098	0.089	6.10	54	0.240
4	0.014	0.016	0.099	0.101	0.092	0.085	5.80	52	0.226

Δ , Δx , and P = same as in RNA of Table 3

* Same as in Table 3

** $10\,\Delta x / \Delta \times 5$ mg

Summary of Table 4 on yeast R N A

Bases	Mole bases* per mole of P		
	Analytical values		Theoretical values
	Mean ± SE	Standard deviation	
Guanine	0.20 ± 0.008	0.017	-
Adenine	0.23 ± 0.001	0.002	-
Cytosine	0.22 ± 0.007	0.014	-
Uracil	0.22 ± 0.007	0.014	-
Four bases	0.87 ± 0.023		1.00
% recovery of four bases	87 ± 2.3		100

* Four replicates

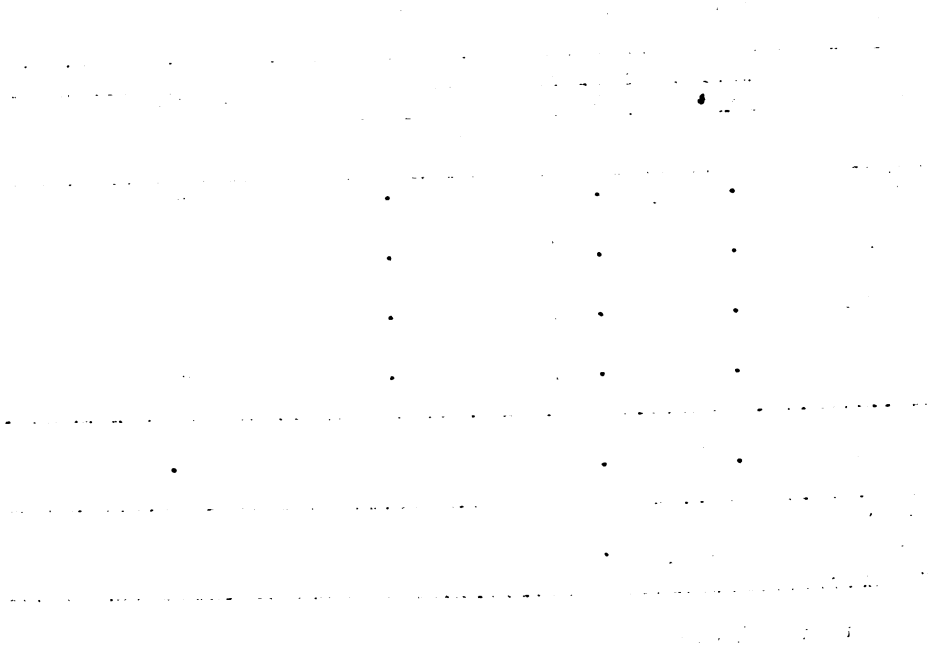


Table 4 continued

Salmon D N A

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	Recovery μ moles per 20 mg. DNA	mole bases per mole P
1	0.006	0.037	0.088	0.089	0.077	0.052	5.3	6.9	0.12
2	-	-	-	-	-	-	-	-	-
3	0.012	0.048	0.118	0.120	0.115	0.072	7.3	9.6	0.16
4	0.047	0.080	0.132	0.134	0.130	0.054	5.5	7.3	0.12

Δ , Δx , and P = same as in DNA of Table 3

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	Recovery μ moles per 20 mg. DNA	mole bases per mole P
1	0.007	0.013	0.180	0.185	0.176	0.172	8.4	12.4	0.21
2	0.002	0.009	0.181	0.183	0.170	0.174	8.5	12.5	0.21
3	0.008	0.014	0.180	0.183	0.168	0.169	8.3	12.3	0.21
4	0.040	0.050	0.200	0.230	0.222	0.180	8.7	12.8	0.21

Δ , Δx , and P = same as in DNA of Table 3

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	Recovery μ moles per 20 mg. DNA	mole bases per mole P
1	0.007	0.061	0.110	0.114	0.103	0.053	5.8	10.5	0.17
2	0.005	0.059	0.110	0.114	0.105	0.055	6.0	10.8	0.18
3	0.003	0.061	0.114	0.119	0.111	0.058	6.3	11.3	0.19
4	0.047	0.105	0.153	0.160	0.150	0.045	5.0	9.0	0.15

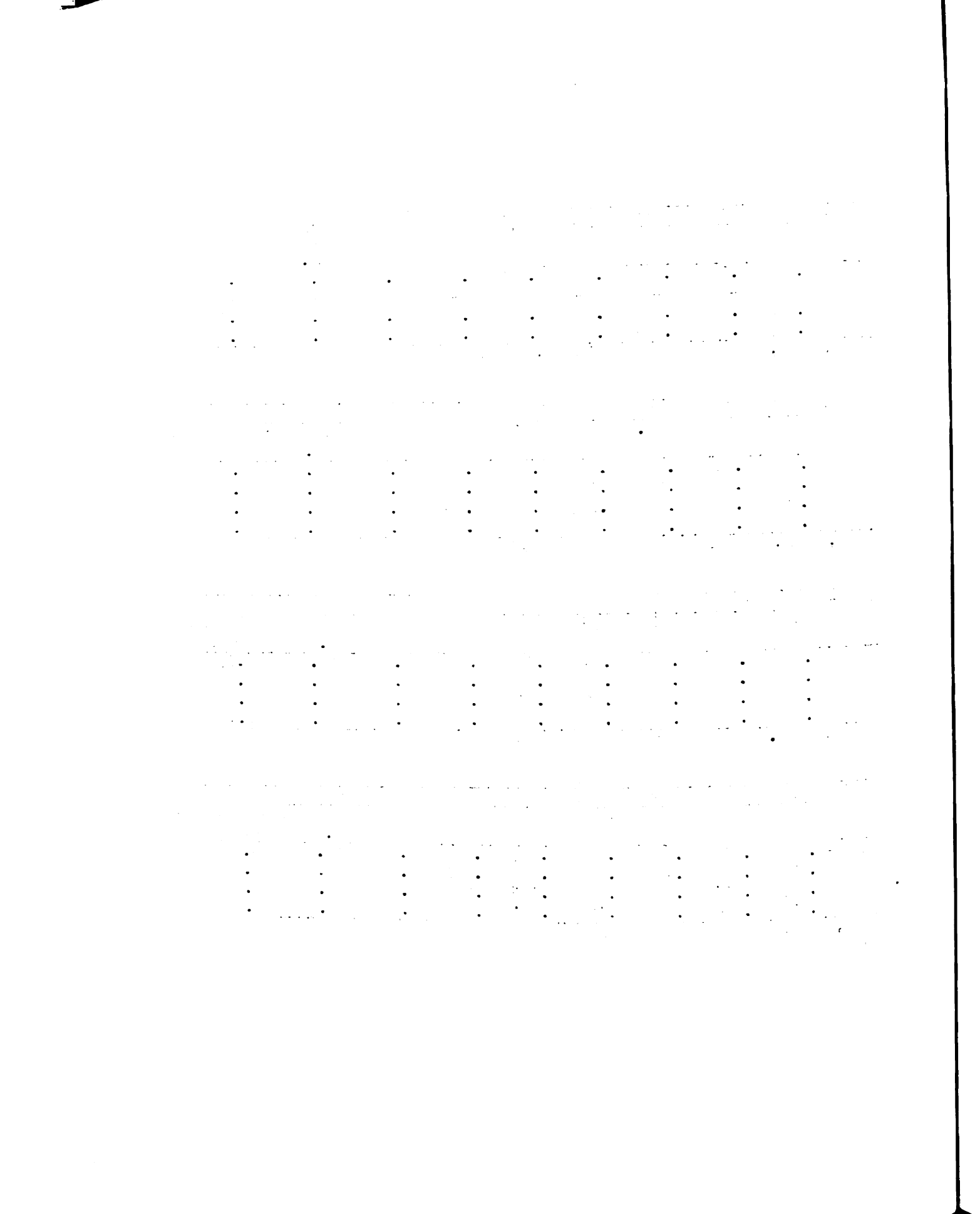
Δ , Δx , and P = same as in DNA of Table 3

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	Recovery μ moles per 20 mg. DNA	mole bases per mole P
1	-0.002	0.012	0.101	0.102	0.087	0.090	8.4	13.3	0.22
2	0.006	0.019	0.118	0.120	0.109	0.101	9.4	14.9	0.25
3	0.007	0.021	0.122	0.125	0.115	0.104	9.7	15.3	0.26
4	0.022	0.046	0.142	0.143	0.131	0.097	9.0	14.2	0.24

Δ , Δx , and P = same as in DNA of Table 3

* $10\,\Delta x / \Delta \times 5$ mg



Summary of Table 4 on salmon D N A

Bases	Mole bases* per mole of P			
	Analytical values			Theoretical values
	Mean ± SE		Standard deviation	
Guanine	0.13 ± 0.009		0.017	-
Adenine	0.21 ± 0.000		0.000	-
Cytosine	0.17 ± 0.008		0.017	-
Thymine	0.24 ± 0.008		0.017	-
Four bases	0.75 ± 0.025			1.00
% recovery of four bases	75 ± 2.5			100

* Four replicates

Table 5. Recoveries of yeast R N A* and salmon D N A* subjected to the same procedures as applied to embryo samples.

Yeast R N A

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	μ moles per 80 mg. RNA	mole bases per mole P
1	0.005	0.033	0.082	0.102	0.082	0.069	7.05	46.7	0.200
2	0.008	0.039	0.106	0.110	0.106	0.071	7.25	48.0	0.208
3	0.008	0.041	0.109	0.110	0.104	0.069	7.05	46.7	0.200
4	-	-	-	-	-	-	-	-	-

Δ, Δx, and P = same as in yeast RNA of Table 3

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	μ moles per 80 mg. RNA	mole bases per mole P
1	0.000	0.002	0.124	0.127	0.124	1.125	6.10	45	0.196
2	0.004	0.009	0.133	0.138	0.133	0.129	6.30	46.6	0.203
3	0.004	0.009	0.133	0.135	0.130	0.126	6.15	45.6	0.198
4	-	-	-	-	-	0.126	6.15	46	0.200

Δ, Δx, and P = same as in yeast RNA of Table 3

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	**	μ moles per 80 mg. RNA	mole bases per mole P
1	0.000	0.035	0.073	0.076	0.073	0.041	4.50	40.5	0.176
2	-0.004	0.033	0.075	0.079	0.070	0.046	5.05	45.5	0.199
3	0.000	0.038	0.073	0.078	0.070	0.040	4.40	39.6	0.172
4	-	-b	-	-	-	-	-	-	-

Δ, Δx, and P = same as in yeast RNA of Table 3

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	**	μ moles per 80 mg. RNA	mole bases per mole P
1	-0.005	-0.004	0.053	0.070	0.067	0.074	5.05	45	0.196
2	0.003	0.008	0.083	0.090	0.083	0.082	5.60	50	0.217
3	0.003	0.005	0.077	0.083	0.077	0.072	4.90	44	0.191
4	-	-	-	-	-	-	-	-	-

Δ, Δx, and P = same as in yeast RNA of Table 3

* Same as in Table 3

** $10 \Delta x / \Delta \times 5$ mg

Summary of Table 5 on yeast R N A

Bases	Mole bases* per mole of P			
	Analytical values			Theoretical values
	Mean ± SE		Standard deviation	
Guanine	0.20 ±	0.0012	0.002	-
Adenine	0.20 ±	0.0023	0.004	-
Cytosine	0.18 ±	0.0008	0.0014	-
Uracil	0.21 ±	0.0100	0.017	-
Four bases	0.79 ±	0.014		1.00
% recovery of four bases	79 ±	1.4		100

* Three replicates

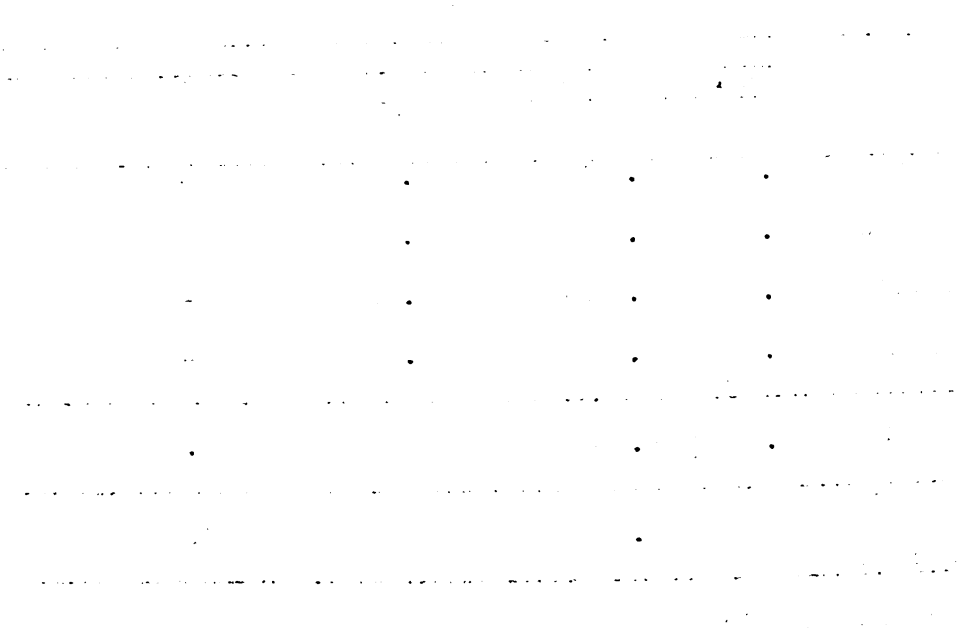


Table 5 continued

Salmon D N A

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	μ moles in 20 mg. DNA	mole bases per mole P
1	0.010	0.042	0.101	0.108	0.099	0.066	6.70	8.87	0.148
2	0.056	0.088	0.153	0.159	0.151	0.071	7.20	9.53	0.159
3	0.011	0.037	0.101	0.104	0.096	0.067	6.80	9.00	0.150
4	0.013	0.043	0.107	0.111	0.104	0.068	6.80	9.00	0.150

A, Δx, and P = same as in salmon DNA of Table 3

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	μ moles in 20 mg. DNA	mole bases per mole P
1	0.000	0.012	0.171	0.180	0.171	0.168	8.20	12.1	0.202
2	0.055	0.065	0.232	0.237	0.227	0.172	8.35	12.4	0.207
3	0.005	0.014	0.177	0.183	0.120	0.169	8.25	12.2	0.203
4	0.008	0.017	0.180	0.186	0.123	0.169	8.25	12.2	0.203

A, Δx, and P = same as in salmon DNA of Table 3

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	257	270	Δx	**	μ moles in 20 mg. DNA	mole bases per mole P
1	0.003	0.052	0.098	0.103	0.089	0.051	5.6	10.09	0.168
2	0.056	0.108	0.151	0.159	0.148	0.051	5.6	10.09	0.168
3	0.000	0.042	0.089	0.091	0.082	0.049	5.4	9.73	0.162
4	0.003	0.051	0.095	0.099	0.092	0.048	5.3	9.55	0.159

A, Δx, and P = same as in salmon DNA of Table 3

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	**	μ moles in 20 mg. DNA	mole bases per mole P
1	0.006	0.020	0.101	0.104	0.098	0.084	7.85	12.50	0.210
2	0.055	0.068	0.157	0.160	0.152	0.092	8.60	13.70	0.228
3	0.010	0.024	0.111	0.114	0.107	0.090	8.40	13.33	0.222
4	0.010	0.021	0.109	0.112	0.107	0.091	8.50	13.50	0.225

A, Δx, and P = same as in salmon DNA of Table 3

** $10 \Delta x / A \times 5$ mg

Summary of Table 5 on salmon D N A

Bases	Mole bases* per mole of P			
	Analytical values			Theoretical values
	Mean ± SE		Standard deviation	
Guanine	0.15 ± 0.0063		0.0126	-
Adenine	0.20 ± 0.0002		0.0014	-
Cytosine	0.16 ± 0.0033		0.0066	-
Thymine	0.22 ± 0.0039		0.0078	-
Four bases	0.73 ± 0.014			1.00
% recovery of four bases	73 ± 1.4			100

* Four replicates

Table 6. Determination of purine and pyrimidine bases of RNA and DNA in normal embryos (138.5 mg) analyzed at stage 6c according to methods used in Table 5

R N A of normal embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	**	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.006	0.021	0.053	0.059	0.054	0.038	3.85	0.791	37.8
2	0.008	0.028	0.058	0.066	0.058	0.038	3.85	0.791	37.8
3	0.011	0.030	0.062	0.067	0.060	0.037	3.75	0.731	35.1
4	-	-	-	-	-	-	-	-	-

Δ, and Δx = same as described in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	**	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.003	0.006	0.049	0.056	0.052	0.050	2.45	0.503	270
2	0.010	0.014	0.064	0.066	0.064	0.052	2.55	0.523	280
3	0.009	0.012	0.063	0.065	0.062	0.053	2.60	0.534	289
4	0.009	0.013	0.063	0.062	0.060	0.049	2.40	0.493	265

Δ, and Δx = same as described in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	**	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.002	0.018	0.036	0.038	0.035	0.020	2.20	0.451	29.4
2	0.003	0.023	0.045	0.048	0.043	0.025	2.75	0.564	36.8
3	-0.004	0.016	0.038	0.039	0.038	0.023	2.55	0.523	34.1
4	0.003	0.022	0.037	0.040	0.035	0.018	2.00	0.411	27.0

Δ, and Δx = same as described in Table 2

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	**	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.008	0.010	0.036	0.038	0.036	0.028	1.90	0.39	25.1
2	0.013	0.015	0.047	0.048	0.047	0.033	2.25	0.46	29.6
3	0.012	0.013	0.039	0.041	0.039	0.028	1.90	0.39	31.0
4	0.032	0.041	0.075	0.083	0.082	0.030	2.05	0.42	33.0

Δ, and Δx = same as described in Table 2

** $10 \Delta x / \Delta \times 5$ mg

Table 6 continued

D N A of normal embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.008	0.018	0.041	0.044	0.039	0.026	2.60	0.107	5.1
2	0.009	0.015	0.034	0.036	0.032	0.021	2.15	0.089	4.2
3	0.005	0.012	0.028	0.031	0.027	0.019	2.00	0.083	3.9
4	0.003	0.011	0.029	0.033	0.031	0.022	2.25	0.093	4.4

Δ , and Δx = same as described in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.012	0.013	0.060	0.063	0.060	0.050	2.45	0.101	5.4
2	0.014	0.024	0.076	0.078	0.075	0.054	2.65	0.109	5.8
3	-0.002	0.001	0.040	0.041	0.039	0.040	2.00	0.083	4.4
4	0.005	0.008	0.049	0.050	0.048	0.042	2.05	0.085	4.5

Δ, and Δx = same as described in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.007	0.026	0.038	0.040	0.037	0.014	1.55	0.064	4.1
2	0.003	0.026	0.035	0.037	0.032	0.011	1.20	0.050	4.0
3	0.010	0.029	0.045	0.046	0.041	0.017	1.85	0.077	4.9
4	0.000	0.015	0.031	0.032	0.029	0.017	1.85	0.077	4.9

Δ, and Δx = same as described in Table 2

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.018	0.029	0.065	0.069	0.057	0.037	3.45	0.142	8.0
2	0.018	0.032	0.064	0.066	0.055	0.034	3.15	0.130	7.4
3	0.016	0.022	0.052	0.053	0.046	0.031	2.90	0.120	7.0
4	0.017	0.022	0.052	0.054	0.044	0.032	3.00	0.124	7.1

Δ, and Δx = same as described in Table 2

* $10 \Delta x / A \times 5$ mg

Summary of Table 6

R N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	37 ± 0.86	1.73
Adenine	28 ± 0.71	1.41
Cytosine	32 ± 2.29	4.58
Uracil	26 ± 2.64	5.29

D N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	4.4 ± 0.77	1.55
Adenine	5.0 ± 1.00	2.00
Cytosine	4.4 ± 0.70	1.41
Thymine	7.2 ± 0.71	1.42

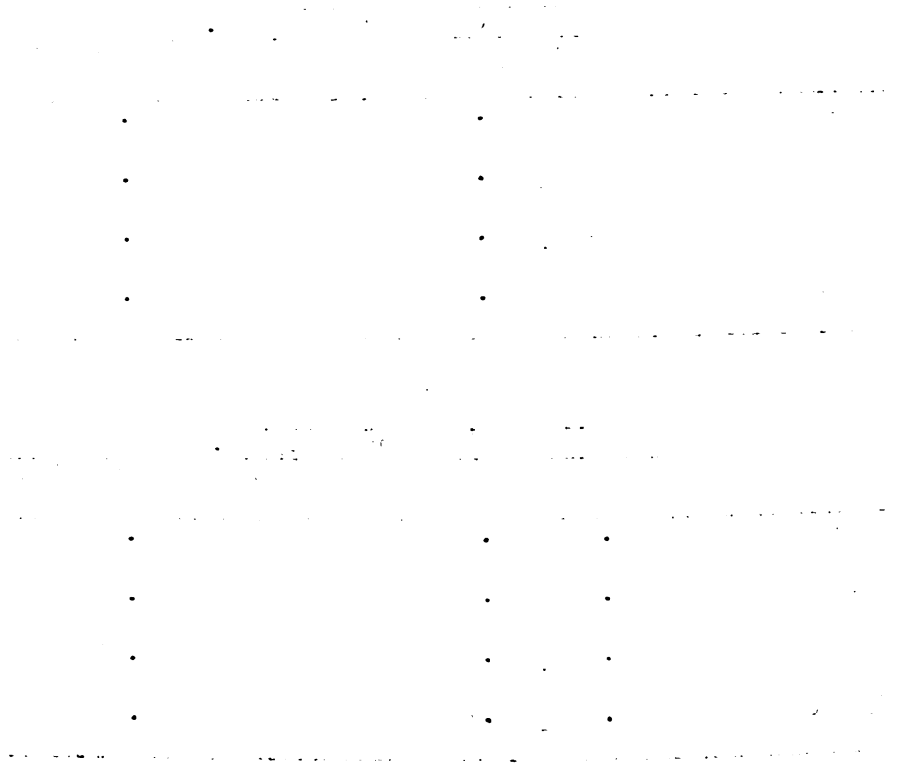


Table 7. Determination of purine and pyrimidine bases of RNA and DNA in 160.6 mg dry weight of embryos X-irradiated (450 r) at stage c and analyzed at stage 6c

R N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	* mg bases in total dry wt.	mμ moles per mg dry wt.	
1	-0.002	0.010	0.029	0.031	0.028	0.021	2.15	0.469	19.0
2	-	-	-	-	-	-	-	-	-
3	0.009	0.023	0.046	0.048	0.045	0.025	2.55	0.556	23.0
4	0.007	0.018	0.038	0.042	0.037	0.024	2.45	0.534	22.0

Δ and Δx = same as described in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	* mg bases in total dry wt.	mμ moles per mg dry wt.	
1	0.010	0.014	0.052	0.053	0.052	0.039	1.90	0.514	19.2
2	0.017	0.023	0.066	0.068	0.065	0.045	2.15	0.430	20.0
3	0.007	0.008	0.049	0.051	0.048	0.043	2.10	0.520	23.7
4	0.001	0.005	0.040	0.043	0.038	0.038	1.85	0.370	17.1

Δ and Δx = same as described in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	* mg bases in total dry wt.	mμ moles per mg dry wt.	
1	0.023	0.041	0.053	0.055	0.053	0.014	1.55	0.34	19.1
2	0.026	0.050	0.060	0.063	0.060	0.013	1.45	0.32	18.0
3	0.003	0.018	0.018	0.031	0.029	0.013	1.45	0.32	18.0
4	0.001	0.031	0.023	0.025	0.022	0.012	1.30	0.28	16.0

Δ and Δx = same as described in Table 2

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	* mg bases in total dry wt.	mμ moles per mg dry wt.	
1	0.005	0.006	0.025	0.027	0.024	0.021	1.45	0.313	17.3
2	0.007	0.008	0.024	0.027	0.023	0.019	1.30	0.282	15.6
3	0.007	0.007	0.024	0.026	0.024	0.019	1.30	0.282	15.6
4	-	-	-	-	-	-	-	-	-

Δ and Δx = same as described in Table 2

* $10 \Delta x / \Delta \times 5$ mg

Table 7 continued

D N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.013	0.018	0.036	0.037	0.036	0.019	1.95	0.081	3.37
2	0.000	0.005	0.020	0.022	0.020	0.017	1.75	0.073	3.00
3	0.003	0.016	0.033	0.035	0.030	0.019	1.95	0.081	3.37
4	0.009	0.015	0.032	0.035	0.031	0.020	2.05	0.083	3.42

Δ and Δx same as described in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.018	0.018	0.065	0.068	0.064	0.050	2.45	0.102	4.73
2	-0.002	0.000	0.040	0.041	0.039	0.041	2.00	0.083	3.79
3	0.009	0.014	0.056	0.059	0.055	0.045	2.20	0.092	4.23
4	0.005	0.008	0.050	0.053	0.049	0.045	2.20	0.092	4.23

Δ and Δx same as described in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.010	0.027	0.039	0.041	0.038	0.014	1.55	0.065	3.67
2	-0.003	0.008	0.019	0.021	0.018	0.013	1.40	0.058	3.24
3	0.010	0.029	0.039	0.041	0.038	0.013	1.40	0.058	3.24
4	-0.004	0.018	0.030	0.032	0.029	0.014	1.55	0.065	3.67

Δ and Δx same as described in Table 2

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total drywt.	mμ moles per mg dry wt.
1	0.017	0.026	0.050	0.055	0.050	0.029	2.70	0.113	5.60
2	0.005	0.007	0.034	0.038	0.033	0.031	2.90	0.121	5.97
3	0.022	0.027	0.055	0.060	0.054	0.033	3.05	0.127	6.29
4	0.015	0.021	0.045	0.049	0.045	0.028	2.60	0.108	5.35

Δ and Δx same as described in Table 2

* $10\Delta x/\Delta \times 5\,\mu g$

Summary of Table 7

	R N A m μ moles per mg dry wt. Mean ± SE	Standard deviation
Guanine	21 ± 0.86	1.73
Adenine	20 ± 1.41	2.82
Cytosine	18 ± 0.60	1.20
Uracil	16 ± 0.55	1.10

	D N A m μ moles per mg dry wt. Mean ± SE	Standard deviation
Guanine	3.4 ± 0.111	0.223
Adenine	4.3 ± 0.108	0.316
Cytosine	3.4 ± 0.111	0.223
Thymine	5.8 ± 0.173	0.346

Table 8. Determination of purine and pyrimidine bases of RNA and DNA in 240.1 mg dry weight of embryos X-irradiated (450 r) at stage 1 and analyzed at stage 6c

R N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.001	0.013	0.047	0.049	0.046	0.036	3.65	0.78	21.5
2	0.006	0.023	0.060	0.064	0.058	0.041	4.15	0.89	24.5
3	0.002	0.013	0.047	0.048	0.045	0.035	3.55	0.76	21.2
4	0.010	0.032	0.070	0.072	0.068	0.040	4.05	0.87	24.0

Δ and Δx = same as shown in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-0.002	0.000	0.047	0.049	0.046	0.049	2.40	0.516	16.4
2	0.003	0.008	0.063	0.065	0.062	0.057	2.80	0.602	18.6
3	-0.002	0.000	0.047	0.049	0.045	0.049	2.40	0.516	15.9
4	0.009	0.012	0.068	0.071	0.067	0.059	2.90	0.624	19.3

Δ and Δx = same as shown in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-0.010	0.011	0.031	0.032	0.030	0.021	2.30	0.495	18.6
2	-0.006	0.017	0.038	0.042	0.037	0.025	2.75	0.592	22.2
3	-0.009	0.009	0.026	0.029	0.024	0.020	2.20	0.473	17.8
4	0.002	0.022	0.045	0.047	0.044	0.025	2.75	0.592	22.2

Δ and Δx = same as shown in Table 2

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.001	0.004	0.030	0.032	0.028	0.028	1.90	0.409	15.2
2	0.004	0.009	0.034	0.036	0.033	0.027	1.85	0.398	14.8
3	-0.002	0.002	0.025	0.027	0.024	0.025	1.70	0.366	13.6
4	-	-	-	-	-	-	-	-	-

Δ and Δx = same as shown in Table 2

* $10\,\Delta x / 0.45$ mg

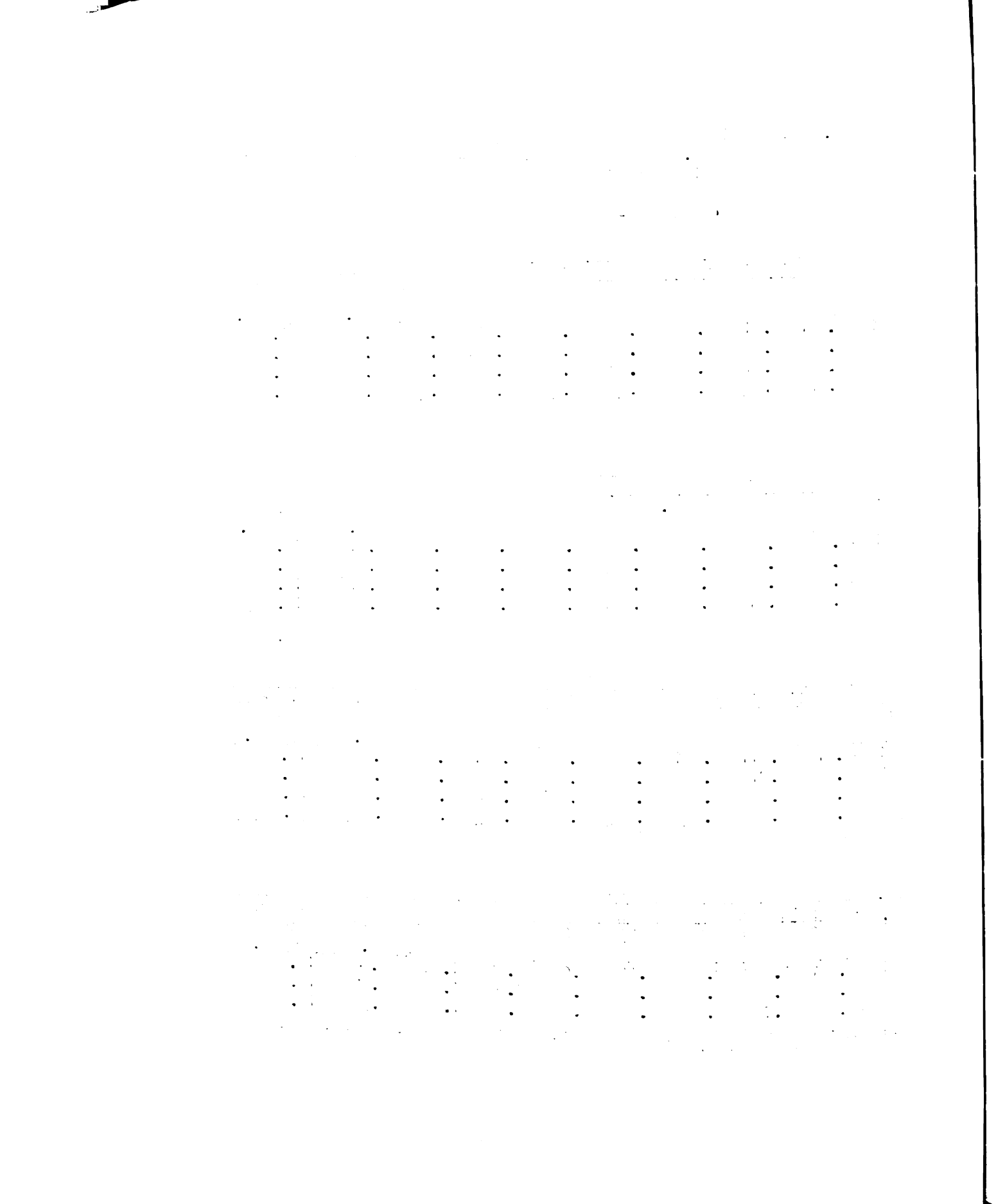


Table 8 continued

D N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δ	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-	-
2	0.014	0.025	0.042	0.045	0.040	0.020	2.05		0.154	4.1
3	0.002	0.009	0.030	0.033	0.028	0.024	2.45		0.184	5.1
4	0.012	0.022	0.039	0.042	0.037	0.020	2.05		0.154	4.1

Δ and Δx = same as shown in Table 2

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.001	0.005	0.040	0.042	0.038	0.037	1.80	0.135	4.4
2	0.007	0.009	0.051	0.053	0.050	0.044	2.15	0.162	5.1
3	0.004	0.007	0.043	0.044	0.042	0.037	1.80	0.135	4.4
4	0.002	0.005	0.040	0.044	0.038	0.039	1.90	0.143	4.5

Δ and Δx = same as shown in Table 2

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-
2	0.002	0.018	0.033	0.035	0.031	0.017	1.85	0.139	5.2
3	0.003	0.021	0.033	0.035	0.030	0.014	1.55	0.119	4.3
4	0.003	0.019	0.030	0.033	0.028	0.014	1.55	0.117	4.3

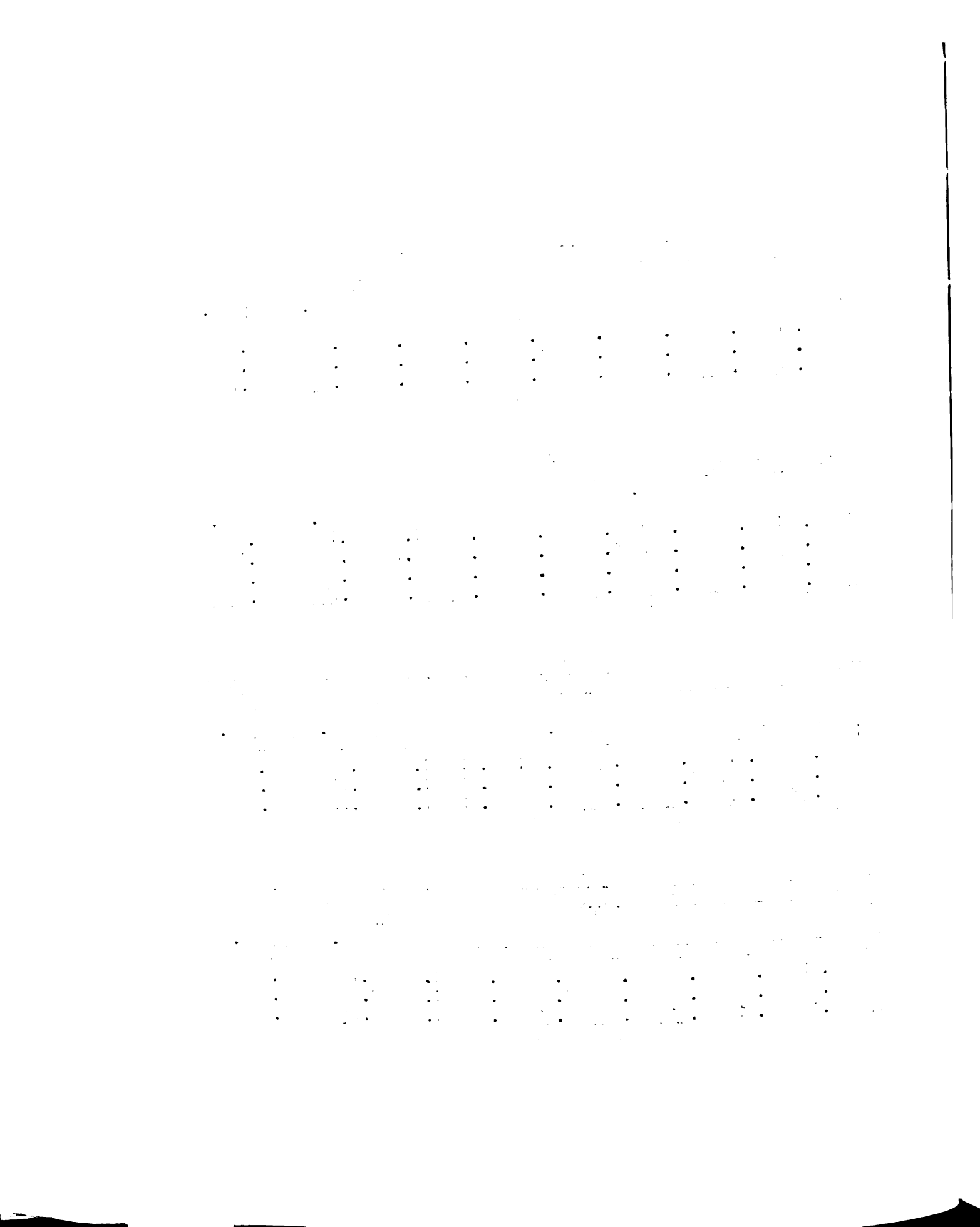
Δ and Δx = same as shown in Table 2

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-
2	0.010	0.020	0.038	0.040	0.037	0.020	1.85	0.139	4.3
3	0.008	0.017	0.035	0.038	0.033	0.021	1.95	0.147	4.9
4	0.005	0.012	0.030	0.033	0.028	0.021	1.95	0.147	4.9

Δ and Δx = same as shown in Table 2

* $10 \Delta x / A \times 5$ mg



Summary of Table 8

R N A

	mμ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	23 ± 0.866	1.73
Adenine	18 ± 0.605	1.21
Cytosine	20 ± 1.005	2.01
Uracil	15 ± 0.150	0.30

D N A

	mμ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	4.5 ± 0.234	0.469
Adenine	4.6 ± 0.160	0.331
Cytosine	4.5 ± 0.200	0.400
Thymine	4.7 ± 0.160	0.282

Table 9. Determination of purine and pyrimidine bases of RNA and DNA in 273.2 mg dry weight of embryos X-irradiated (450 r) at stage 4 and analyzed at stage 6c

R N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mu 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.013	0.027	0.057	0.059	0.056	0.030	3.05	0.963	23.4
2	0.010	0.023	0.058	0.060	0.057	0.037	3.75	1.184	28.6
3	0.009	0.023	0.047	0.051	0.046	0.028	2.85	0.886	21.5
4	-	-	-	-	-	-	-	-	-

Δ and Δx = same as previous Table

Adenine

Replicates	Extinction at wave length in mu 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.010	0.013	0.060	0.062	0.059	0.049	2.40	0.758	20.5
2	0.008	0.012	0.059	0.061	0.058	0.049	2.40	0.758	20.5
3	0.012	0.019	0.066	0.069	0.066	0.050	2.45	0.774	20.9
4	0.008	0.010	0.057	0.061	0.056	0.051	2.50	0.789	21.4

Δ and Δx = same as previous Table

Cytosine

Replicates	Extinction at wave length in mu 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.000	0.018	0.035	0.036	0.034	0.018	2.00	0.632	20.8
2	-0.002	0.015	0.032	0.035	0.032	0.020	2.20	0.695	22.9
3	0.000	0.012	0.028	0.029	0.026	0.017	1.85	0.584	19.3
4	-0.002	0.016	0.030	0.034	0.028	0.018	2.00	0.632	20.8

Δ and Δx = same as previous Table

Uracil

Replicates	Extinction at wave length in mu 300	290	264	259	254	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.009	0.012	0.031	0.033	0.030	0.021	1.45	0.458	17.3
2	0.008	0.012	0.030	0.032	0.029	0.020	1.35	0.426	13.9
3	0.005	0.010	0.028	0.030	0.027	0.020	1.35	0.426	13.9
4	0.010	0.016	0.033	0.035	0.032	0.019	1.30	0.411	13.4

Δ and Δx = same as previous Table

* $10 \Delta x / \Delta x 5$ Mg

Table 9 continued

D N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-
2	0.003	0.009	0.027	0.028	0.026	0.017	1.75	0.182	4.3
3	0.006	0.014	0.025	0.028	0.023	0.014	1.45	0.151	3.6
4	0.015	0.023	0.035	0.039	0.034	0.016	1.65	0.192	4.2

Δ and Δx = same as previous Table

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.007	0.017	0.047	0.049	0.046	0.032	1.55	0.161	4.4
2	0.007	0.017	0.051	0.053	0.051	0.036	1.75	0.182	4.9
3	0.008	0.010	0.042	0.044	0.041	0.034	1.65	0.172	4.7
4	0.008	0.017	0.047	0.049	0.046	0.032	1.55	0.161	4.4

Δ and Δx = same as previous Table

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-0.002	0.010	0.021	0.023	0.021	0.013	1.45	0.151	4.9
2	0.002	0.013	0.023	0.025	0.023	0.012	1.30	0.135	4.5
3	0.015	0.028	0.036	0.039	0.035	0.011	1.20	0.125	4.2
4	-	-	-	-	-	-	-	-	-

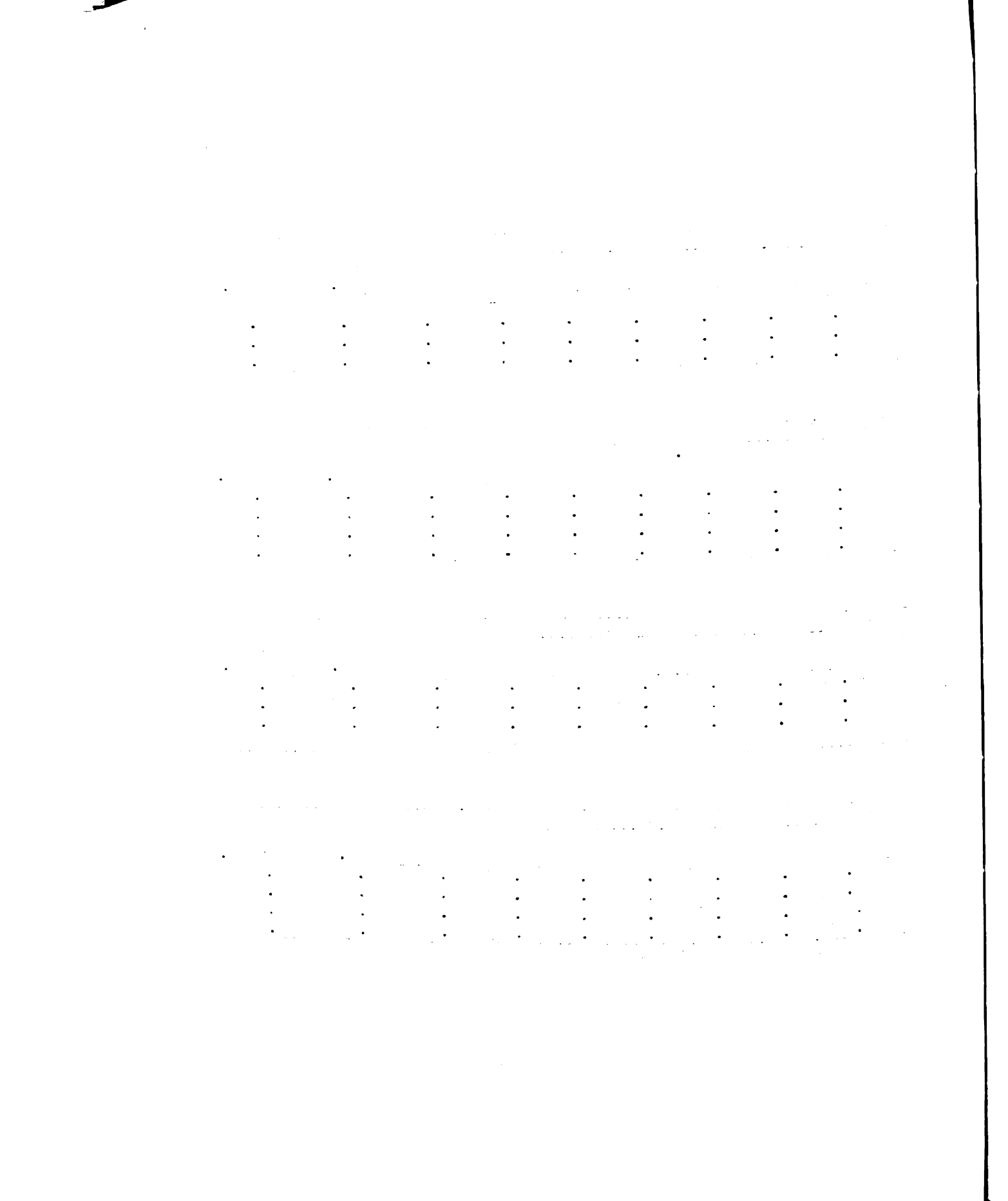
Δ and Δx = same as previous Table

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.008	0.017	0.034	0.038	0.035	0.021	1.95	0.203	5.8
2	0.007	0.013	0.035	0.038	0.034	0.025	2.30	0.239	6.9
3	0.0010	0.022	0.045	0.046	0.043	0.024	2.25	0.234	6.7
4	0.0016	0.028	0.048	0.050	0.047	0.023	2.15	0.224	6.5

Δ and Δx = same as previous Table

* $10 \Delta x / \Delta \times 5$ mg



Summary of Table 9

R N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	25 ± 1.414	0.828
Adenine	21 ± 0.234	0.469
Cytosine	21 ± 0.510	1.020
Uracil	14 ± 0.883	1.766

D N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	4.0 ± 0.2159	0.374
Adenine	4.6 ± 0.1100	0.221
Cytosine	4.5 ± 0.1520	0.361
Thymine	6.5 ± 0.2397	0.479

Table 10. Determination of purine and pyrimidine bases of RNA and DNA in 203.2 mg dry weight of embryos X-irradiated at stage late 5 and analyzed at stage 6c

R N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-
2	0.015	0.028	0.056	0.060	0.057	0.031	3.20	0.695	22.6
3	0.009	0.025	0.059	0.060	0.059	0.035	3.55	0.772	25.1
4	0.013	0.028	0.061	0.062	0.061	0.034	3.45	0.750	24.4

Δ and Δx = same as shown in the previous Table

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262.5	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.002	0.008	0.056	0.059	0.056	0.051	2.50	0.540	19.7
2	0.007	0.005	0.050	0.052	0.048	0.047	2.30	0.500	18.2
3	0.012	0.015	0.067	0.069	0.066	0.054	2.65	0.576	21.0
4	0.008	0.011	0.061	0.062	0.066	0.051	2.50	0.540	19.7

Δ and Δx = same as shown in the previous Table

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.005	0.023	0.042	0.043	0.038	0.020	2.20	0.478	21.2
2	0.010	0.027	0.044	0.045	0.041	0.018	1.95	0.424	18.8
3	0.007	0.026	0.045	0.047	0.043	0.021	2.30	0.500	22.1
4	0.009	0.028	0.047	0.048	0.047	0.020	2.20	0.478	21.2

Δ and Δx = same as shown in the previous Table

Uracil

Replicates	Extinction at wave length in mμ 300	290	264	259	254	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.015	0.019	0.041	0.045	0.041	0.026	1.80	0.391	17.1
2	0.005	0.007	0.029	0.030	0.029	0.023	1.55	0.337	14.8
3	0.007	0.010	0.032	0.035	0.032	0.025	1.70	0.370	16.2
4	0.011	0.015	0.042	0.042	0.040	0.027	1.85	0.402	17.7

Δ and Δx = same as shown in the previous Table

* $10\Delta x / \Delta \times 5$ μg

Table 10. continued

D N A of X-rayed embryos

Guanine

Replicates	Extinction at wave length in mμ 300	290	254	249	244	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	-	-	-	-	-	-	-	-	-
2	0.000	0.009	0.024	0.027	0.021	0.018	1.85	0.079	2.6
3	0.003	0.010	0.023	0.027	0.020	0.017	1.70	0.072	2.4
4	0.005	0.009	0.021	0.026	0.019	0.017	1.70	0.072	2.4

Δ and Δ x = same as shown in the previous Table

Adenine

Replicates	Extinction at wave length in mμ 300	290	267.5	262	257	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.013	0.017	0.068	0.069	0.067	0.052	2.55	0.109	3.9
2	0.007	0.008	0.056	0.060	0.052	0.052	2.55	0.109	3.9
3	0.002	0.006	0.051	0.053	0.035	0.047	2.30	0.098	3.5
4	0.006	0.007	0.051	0.052	0.050	0.045	2.20	0.094	3.4

Δ and Δx = same as shown in the previous Table

Cytosine

Replicates	Extinction at wave length in mμ 300	290	280	275	270	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.004	0.022	0.037	0.039	0.035	0.017	1.85	0.079	3.5
2	-0.002	0.014	0.029	0.031	0.027	0.017	1.85	0.079	3.5
3	-0.002	0.015	0.028	0.031	0.027	0.017	1.85	0.079	3.5
4	0.000	0.015	0.028	0.030	0.026	0.015	1.65	0.070	3.1

Δ and Δx = same as shown in the previous Table

Thymine

Replicates	Extinction at wave length in mμ 300	290	269	264	259	Δx	*	mg bases in total dry wt.	mμ moles per mg dry wt.
1	0.015	0.022	0.051	0.055	0.054	0.033	3.10	0.132	5.2
2	0.015	0.024	0.052	0.053	0.048	0.029	2.70	0.115	4.5
3	0.009	0.016	0.044	0.048	0.046	0.032	3.00	0.128	5.0
4	0.010	0.018	0.046	0.049	0.045	0.031	2.90	0.123	4.8

Δ and Δx = same as shown in the previous Table

* $10 \Delta x / \Delta \times 5$ mg

Summary of Table 10

R N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	24 ± 0.617	1.051
Adenine	20 ± 0.550	1.10
Cytosine	21 ± 0.650	1.30
Uracil	16 ± 0.605	1.21

D N A

	m μ moles per mg dry wt.	
	Mean ± SE	Standard deviation
Guanine	2.5 ± 0.050	0.100
Adenine	3.6 ± 0.132	0.264
Cytosine	3.3 ± 0.100	0.200
Thymine	4.8 ± 0.150	0.310

Table 11. Quantity in mμ moles of purine and pyrimidine bases of RNA and DNA per mg dry weight of normal and X-rayed (450 r) embryos

Experimental Groups	stages of embryos X-rayed	days post-radia-tion	RNA mμ moles per mg dry wt.				DNA mμ moles per mg dry wt.			
			G	A	C	U	G	A	C	T
Control	-	-	37	28	32	26	4.4	5.0	4.4	7.2
Group-A	a-c	16	21	20	18	16	3.4	4.3	3.4	5.8
Group-B	g	12	23	18	20	15	4.5	4.6	4.5	4.7
Group-C	3-4	9	25	21	21	14	4.0	4.6	4.5	6.5
Group-D	late-5	7	24	20	21	16	2.5	3.6	3.3	4.8

G = Guanine, A = Adenine, C = Cytosine, U = Uracil, T = Thymine

Table 12. Nucleic acid content, RNA / DNA, and purine / pyrimidine ratios, in normal and X-rayed embryos based on percent of controls

Experimental groups	stages of embryos irradiated	days after irradiation	Nucleic Acids		R N A		D N A	
			% of control	RNA / DNA	% of control	Pu / Py	% of control	Pu / Py
Control	-	-	100	5.9	100	1.1	100	0.81
Group-A	a-c	16	64	4.4	61	1.1	80	0.84
Group-B	g	12	65	4.2	62	1.2	87	0.98
Group-C	3-4	9	69	4.1	66	1.3	93	0.78
Group-D	late-5	7	66	5.7	66	1.2	68	0.75

Table 13. Purine and pyrimidine bases, as percent of controls in normal and X-rayed embryos

Experimental groups	RNA				DNA			
	G	A	C	U	G	A	C	T
Control	100	100	100	100	100	100	100	100
Group-A	57	71	56	58	77	86	77	81
Group-B	62	63	63	57	102	92	102	65
Group-C	68	75	66	54	91	92	102	90
Group-D	65	71	66	62	57	72	75	67

Table 14. Grain counts (corrected for background) per unit area (4900 μ^2) of embryos (or parts of embryos)

Group-1

1st samplings

Embryo* \ Types of nucleic acids	R N A	D N A	Total nucleic acids
Control	22	26	48
X-rayed	15	9	24
% of control	68	35	50

* Average of three determinations of an entire embryo

2nd samplings

Embryo* \ Types of nucleic acids	R N A	D N A	Total nucleic acids
Control	56	42	98
X-rayed	29	19	48
% of control	52	45	49

* Average of three determinations of an entire embryo

Table 14

Group-1 continued

3rd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Three	R N A Average	D N A Three	D N A Average	Total nucleic acids Three	Total nucleic acids Average
Root	Control	244	75	259	86	484	161
	X-rayed	86	29	81	27	204	68
	% of control	38		31		42	
Shoot	Control	239	80	259	86	497	166
	X-rayed	176	59	61	20	222	74
	% of control	74		24		45	
Scutellum	Control	130	43	246	82	340	113
	X-rayed	133	44	49	16	150	51
	% of control	102		20		45	
Total	Control	593	66	648	72	1311	146
	X-rayed	395	44	183	20	578	64
	% of control	67		28		44	

Table 14

Group-1 continued

4th samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A		D N A		Total nucleic acids	
		Three	Average	Three	Average	Three	Average
Root	Control	1794	359	460	92	2154	431
	X-rayed	1039	208	382	76	1411	282
	% of control	58		83		66	
Shoot	Control	1807	361	610	122	2417	483
	X-rayed	953	191	519	104	1472	294
	% of control	52		85		61	
Scutellum	Control	567	113	359	72	926	186
	X-rayed	428	56	286	57	714	143
	% of control	75		79		77	
Total	Control	4088	273	1419	95	5517	368
	X-rayed	2407	160	1178	79	3585	239
	% of control	58		83		64	

Table 14 continued

Group - 2

1st samplings

Embryo* \ Types of nucleic acids	R N A	D N A	Total nucleic acids
Control	87	21	108
X-rayed	61	17	78
% of control	70	81	72

* Average of three determinations of an entire embryo

2nd samplings

Parts of embryo \ Types of nucleic acids / Unit areas counted	R N A		D N A		Total nucleic acids	
	Three	Average	Three	Average	Three	Average
Root Control	76	25	37	13	113	38
Root X-rayed	37	12	6	2	43	14
Root % of control	49		16		38	
Shoot Control	57	19	29	10	86	29
Shoot X-rayed	50	17	9	3	59	20
Shoot % of control	88		31		68	
Scutellum Control	32	11	29	10	61	20
Scutellum X-rayed	45	15	7	3	52	17
Scutellum % of control	109		24		85	
Total Control	165	55	95	32	260	87
Total X-rayed	132	44	22	7	154	51
Total % of control	80		23		59	

Table 14

Group-2 continued

3rd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Ten	R N A Average	D N A Ten	D N A Average	Total nucleic acids Ten	Total nucleic acids Average
Root	Control	749	75	241	24	979	98
	X-rayed	320	32	237	23	558	56
	% of control	43		98		57	
Shoot	Control	328	33	117	12	445	46
	X-rayed	289	29	111	11	401	40
	% of control	88		95		90	
Scutellum	Control	47	16	58	19	104	35
	X-rayed	36	12	59	20	96	32
	% of control	77		102		92	
Total	Control	1120	49	416	18	1538	67
	X-rayed	645	28	407	17	1055	46
	% of control	57		97		69	

Table 14 continued

Group - 3

1st samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Two	R N A Average	D N A Two	D N A Average	Total nucleic acids Two	Total nucleic acids Average
Root	Control	159	80	92	46	751	376
	X-rayed	98	49	88	44	186	93
	% of control	61		96		74	
Shoot	Control	72	36	88	44	160	80
	X-rayed	61	31	104	52	165	83
	% of control	85		118		101	
Scutellum	Control	95	48	63	32	158	79
	X-rayed	63	32	75	38	138	69
	% of control	66		103		87	
Total	Control	326	54	243	41	569	95
	X-rayed	222	37	267	45	489	82
	% of control	68		110		85	

Table 14

Group-3 continued

2nd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Three	R N A Average	D N A Three	D N A Average	Total nucleic acids Three	Total nucleic acids Average
Root	Control	520	173	325	108	845	282
	X-rayed	371	124	35	12	406	135
	% of control	71		11		48	
Shoot	Control	273	91	186	62	459	153
	X-rayed	185	62	56	19	241	83
	% of control	68		30		53	
Scutellum	Control	164	55	174	58	338	113
	X-rayed	197	65	64	21	261	87
	% of control	120		37		77	
Total	Control	957	319	685	228	1642	547
	X-rayed	753	251	155	52	908	303
	% of control	78		22		54	

Table 14

Group-3 continued

3rd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Five	R N A Average	D N A Five	D N A Average	Total nucleic acids Five	Total nucleic acids Average
Root	Control	79	16	409	82	487	97
	X-rayed	53	11	287	57	340	68
	% of control		67		70		67
Shoot	Control	137	27	363	73	500	100
	X-rayed	80	16	290	58	370	74
	% of control		58		77		74
Scutellum	Control	289	58	381	76	670	134
	X-rayed	234	47	349	70	583	117
	% of control		81		91		87
Total	Control	505	101	1152	230	1657	331
	X-rayed	377	75	926	185	1303	261
	% of control		74		81		79

Table 14 continued

Group - 4

1st samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A		D N A		Total nucleic acids	
		Five	Average	Five	Average	Five	Average
Root	Control	72	15	13	3	84	17
	X-rayed	58	12	12	2	70	14
	% of control	81		99		83	
Shoot	Control	36	7	16	4	52	11
	X-rayed	32	6	15	3	47	9
	% of control	88		93		90	
Scutellum	Control	6	-	14	-	20	-
	X-rayed	4	-	13	-	17	-
	% of control	67		92		85	
Total	Control	109	22	42	9	156	32
	X-rayed	94	19	40	8	139	28
	% of control	86		95		89	

Table 14

Group-4 continued

2nd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	R N A Five	R N A Average	D N A Five	D N A Average	Total nucleic acids Five	Total nucleic acids Average
Root	Control	140	28	152	31	292	58
	X-rayed	121	24	107	21	228	46
	% of control		86		70		78
Shoot	Control	232	46	85	18	317	64
	X-rayed	75	15	82	16	157	32
	% of control		33		96		50
Scutellum	Control	350	70	108	22	458	92
	X-rayed	52	13	142	28	248	50
	% of control		15		130		42
Total	Control	722	145	345	69	1067	214
	X-rayed	248	50	332	66	580	116
	% of control		34		96		54

Table 14

Group-4 continued

3rd samplings

Parts of embryo	Types of nucleic acids / Unit areas counted	RNA Five	RNA Average	DNA Five	DNA Average	Total nucleic acids Five	Total nucleic acids Average
Root	Control	344	69	150	30	494	99
	X-rayed	138	28	112	23	250	50
	% of control		40		74		51
Shoot	Control	364	73	76	14	430	86
	X-rayed	242	50	55	11	297	59
	% of control		66		72		69
Scutellum	Control	249	50	85	17	334	67
	X-rayed	153	31	86	17	239	48
	% of control		66		101		72
Total	Control	947	189	351	70	1298	260
	X-rayed	533	107	253	51	786	157
	% of control		58		72		66

Table 15. Summary of Table 14. Nucleic acid content (as percent of controls) of embryos X-rayed at four different stages and sampled at intervals following irradiation.

Experimental Groups	Stages of samplings	d - e	g - 1	4	5	6 - a	6 - b	6 - c
Group 1	R + D *	50	49	44	-	-	-	64
	RNA	68	52	67	-	-	-	58
	DNA	35	45	28	-	-	-	83
Group 2	R + D *	-	72	-	59	-	-	69
	RNA	-	70	-	80	-	-	57
	DNA	-	81	-	23	-	-	97
Group 3	R + D *	-	-	85	-	54	-	79
	RNA	-	-	68	-	78	-	74
	DNA	-	-	110	-	22	-	81
Group 4	R + D *	-	-	-	-	89	54	66
	RNA	-	-	-	-	86	34	58
	DNA	-	-	-	-	95	96	72

* R + D = RNA + DNA

Table 16. Relative percent RNA and DNA of the control at each sampling stage during normal barley embrygeny

Stages sampled		R N A	D N A
d - e	number of tracks	22	26
	%	45	54
g - 1	number of tracks	56	42
	%	57	42
4	number of tracks	67	28
	%	71	29
6c	number of tracks	58	83
	%	41	58

Table 17. Average of three determinations of cell number per unit area (4900 μ^2), except stage d-ℓ of Group-1 (unit area = 1100 μ^2)

Exp. groups	Stages	number of cells counted						
		d - ℓ	g - 1	4	5	6a	6b	6c
Group 1	Control	42	34	55	-	-	-	59
	X-rayed	37	30	46	-	-	-	58
	% of control	89	89	84	-	-	-	100
Group 2	Control	-	36	-	48	-	-	59
	X-rayed	-	32	-	41	-	-	58
	% of control	-	90	-	87	-	-	100
Group 3	Control	-	-	53	-	57	-	52
	X-rayed	-	-	49	-	48	-	50
	% of control	-	-	93	-	85	-	96
Group 4	Control	-	-	-	-	60	58	56
	X-rayed	-	-	-	-	56	56	55
	% of control	-	-	-	-	93	98	99

Table 18. Summary of Table 14. Nucleic acid content (as percent of controls) in three different parts (root, shoot, and scutellum) of mature embryos X-irradiated at four different embryonic stages

Parts of embryo	Experimental groups	Group 1	Group 2	Group 3	Group 4
	Stages X-rayed	a - c	g	3 - 4	late 5
	Stages sampled	6c	6c	6c	6c
Root	R + D *	66	57	67	51
	RNA	58	43	67	40
	DNA	83	98	70	74
Shoot	R + D *	60	90	74	69
	RNA	52	88	58	66
	DNA	85	95	77	72
Scutellum	R + D *	77	92	87	72
	RNA	75	77	81	66
	DNA	79	102	91	101
Embryo	R + D *	64	69	79	63
	RNA	58	57	74	58
	DNA	83	97	81	72

* R + D = RNA + DNA

Table 19. Total radioactivity of dissected embryos at each sampling stage except for stages g-1 or younger.

Exp. groups	Stages of embryos / *	4 A	4 B	4 C	5 A	5 B	5 C	6a A	6a B	6a C	6b A	6b B	6b C	6c A	6c B	6c C
Group-1	N	0.018	162	9000										0.840	361	430
	X	0.019	153	8052										0.810	380	469
	%			89												109
Group-2	N				0.019	73	3842							0.790	320	405
	X				0.017	67	3941							0.788	343	435
	%						103									107
Group-3	N	0.020	10	500				0.040	54	1350				0.880	458	521
	X	0.019	12	631				0.037	50	1351				0.877	466	531
	%			126						100						102
Group-4	N							0.055	155	2818	0.070	252	3600	0.850	483	568
	X							0.050	147	2940	0.080	242	3025	0.890	452	508
	%									104			84			89

* A. mg dry weight of embryo.
* B. Radioactivity, in counts per minute, of entire embryo (corrected for background).
* C. Radioactivity, in counts per minute, per mg dry weight of embryo (corrected for background).

N. Control.
X. X-rayed embryos.
%. Percent of control.

ROOM USE ONLY