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Title: BIOCHEMICAL INVESTIGATIONS INTO THE SUSCEPTIBILITY OF TISSUES TO RADIATION. FIRST REPORT: THE INFLUENCE OF RADIATION UPON EMBRYONAL METABOLISM IN CHICKENS (Japan)

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Biochemical Investigations into the Susceptibility of Tissues to Radiation. First Report: The Influence of Radiation Upon Embryonal Metabolism (Repair-Exchange) in Chickens.

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Manshū Igaku Zasshi
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Note: An outline of these researches was announced on the occasions of the 38th general meeting held in March 1939 by the Toco-Gynecological Medical Society and the 1st general meeting held in May 1939 by the Manchurian Gynecological Society at, respectively, a toco-gynecological class-room of the Manchurian Medical College (Professor Yuzuki in charge) and a medico-chemical class-room of the same college (Professor Toda in charge).

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0. Introductory Explanation.

Recently investigations into the effect of radiation on living organisms have been pursued ever increasingly by more and more scientists. The width and length of this field truly cannot be ascertained; already there have been numerous research reports from various areas too many to enumerate. But in looking at the results one sees mostly numerous morphological studies and success in this area has been slight. From the biochemical area, however, one hears of no observations at all on the influence of radiation upon cells in the embryonal stage.

To begin, radiational energy is undoubtedly converted into biochemical energy by means of some sort of transformation in the living individual; the physiological actions can be considered in two stages direct and indirect and include most of all the changes that we can recognize as changes after irradiation, such as changes in the composition of body fluids and functional and morphological changes in the cells, namely secondary changes that arise in an organism's responsive reaction to physical and chemical changes due to direct and then indirect physico-chemical actions.

Radiation causes morphological changes such as abnormal fission of the nucleus in living cells, and in the end can cause cells to die. Evidently radiation causes changes in the cell's physical and chemical nature which then brings about abnormalities in its metabolic mechanism and finally the phenomenon of death; therefore it can be assumed that irradiated cells and tissues must suffer changes in their chemical composition.

The susceptibility of living cells to radiation generally follows the Tribonden-Bergonie law: the more vigorous or unstable the substance metabolism (anabolism) is in living cells, the more intensely do they suffer the action of radiation.

The influence of environment upon life and internal chemical changes can be traced much more clearly and simply in single cells with uniform structure than in complex organisms. In this sense single cells — especially egg cells in the state of development in which metabolism is at its height — maintain life "ingeniously" at a critical time and produce a perfect chick. For then there is only gas exchange with the outside environment and no subtance is supplied; all the substances already within the egg cells follow during incubation their long and varied chemical courses, to form a perfect organism in a short period of 3 day in the case of chicken eggs. Obviously cell development during incubation is at its height and is greatly effected by the action of radiation. Therefore extremely valuable information should be possible in investigations of the chemical changes that occur in this case due to radiation.

The author's teacher Professor Yuzuki has undertaken similar investigations on the physiological side in his "Study of Tissues' Susceptibility to Radiation," on which he has worked for many years. The author has also depended upon the guidance of another teacher of his, Professor Toda, while investigating the influence of radiation upon metabolism, especially nitrogen metabolism.

Chapter One. Influence of Radiation upon Fetal Development in Chicks

Sec 1. Introduction.

The influence of radiation upon cells is not always constant and invariable. Thus, the more active or the more unstable the anabolism is then the greater the effect of radiation upon such cells as those in the stage of fission and the less its effect on quiescent cells. Obviously, egg cells in process of development are at the stage of highest anabolic activity and therefore their susceptibility to radiation should be highest.

The influence of radiation upon fetal development is a subject that has often been touched upon but has produced very little inspite of its early interest.

Driessen[1,2] maintained that X-rays directly impaired the development of the fetus. M. Colin[3] produced by radiation substances that hinder blood formation and argued that radiation would harm fetal development as a result of successive changes. M. Fränkel[4] caused by radiation death in fetuses due to trouble with the internal secreting glands and leucocytotoxia. Thereupon Sing[5] irradiated only the gravid uterus and thus induced abortion, although he could not recognize any changes in the ovaries; he argued that the X-rays here act directly upon the fetus. In opposition to this, Hippel and Pagenstechen[6] denied the direct damaging action of X-rays on the fetus and held that interrupted pregnancy in connection with X-ray irradiation is due to leucocytotoxia.

In addition, Bruckhard[7], Fellner and Neumann[8], Mayer[9], Flatau[10], Ganzoni and Widmer[11] also ~~demonstrated~~ impairment of fetal development. Although many still oppose this view, it is a generally recognized fact that X-rays impair fetal development, judging from the results of experiments up to now.

Gilman and Baetjer[12]; Colwel and Reginald[13]; Wakely, Colwell and Reginald[14]; Strangways and Fell[15]; Heim[16]; and YAMAMOTO[17] irradiated chicken embryos with hard X-rays and thus impaired their development, which finally caused death or teratogenesis.

Radiation, therefore, obviously must cause abnormalities in the metabolism of chicken embryos. In my biochemical experiments I considered first the influence of hard X-rays upon the development of chicken embryos.

Sec 2. Experimental Methods.

The specimens used in the experiments were fresh fertilized eggs from white Leghorns raised in Mukden; the eggs weighed 40 to 50 grams. The chickens were all raised under identical condition. Incubation was artificial, in an electric incubator. Eggs that were poorly developed on the third day of incubation were rejected; the other eggs were daily inspected thereafter, only those showing good development being selected. Every other day until the 16th day of incubation they were weighed. On the 5th day of incubation the eggs were subjected to irradiation by hard X-rays and weighed every other day in the same manner, and again inspected for the presence of teratogenesis.

Tube : manufactured by Tokyo Electric; H-type; 200; type 3; Coolidge tube.
Secondary : 175 kilovolts; 3 milliamperes.
Distance : 35 cm between focus and object.
Filter : 0.5 mm/Cu plus 3 mm/Al.
Half-value : 0.966 mm/Cu.
Field : 10 x 12 cm irradiated.
Dosage : 15.7 r/m.

On the 5th day of incubation, six of the developing chicken embryos were simultaneously subjected under the above conditions to 600-gamma radiation by hard X-rays.

The germinal embryo of the egg was always placed in its highest position if the egg lay crosswise during its initial stage of incubation; for if the eggshell had numerous pores the amount of radiation acting on the germinal embryos was not very much weaker in comparison with the dosage over the egg's surface.

Sec 3. Experimental Results

Table 1. Weights of Chicken Embryos During Incubation.

6th day	8th day	10th day	12th day	14th day	16th day
0.28 gm	1.22 gm	2.00 gm	4.25 gm	7.35 gm	12.67 gm
0.34	1.35	2.35	4.50	7.63	10.55
0.31	1.10	2.40	4.30	7.94	11.20
0.29	1.02	2.45	4.25	7.80	13.25
0.29	1.13	2.35	4.40	8.10	12.70
0.28	1.10	2.20	3.95	7.50	13.00
0.32	1.11	2.20	3.90	7.72	11.50
0.30	1.35	2.00	4.25	7.55	12.00*
0.31	1.30	2.32	4.23	7.45	12.45
0.27	1.20	1.95	4.35	7.95	11.85
0.35	1.20	1.80	4.31	7.92	10.95
0.31	1.08	1.90	4.20	7.23	10.85
0.33	1.00	2.00	4.45	7.35	13.20
0.34	1.20	2.05	4.50	8.25	12.80
0.30	1.15	2.10	4.20	8.00	11.90
0.31 av.	1.16 av.	2.14 av.	4.27 av.	7.68 av.	12.05 av.

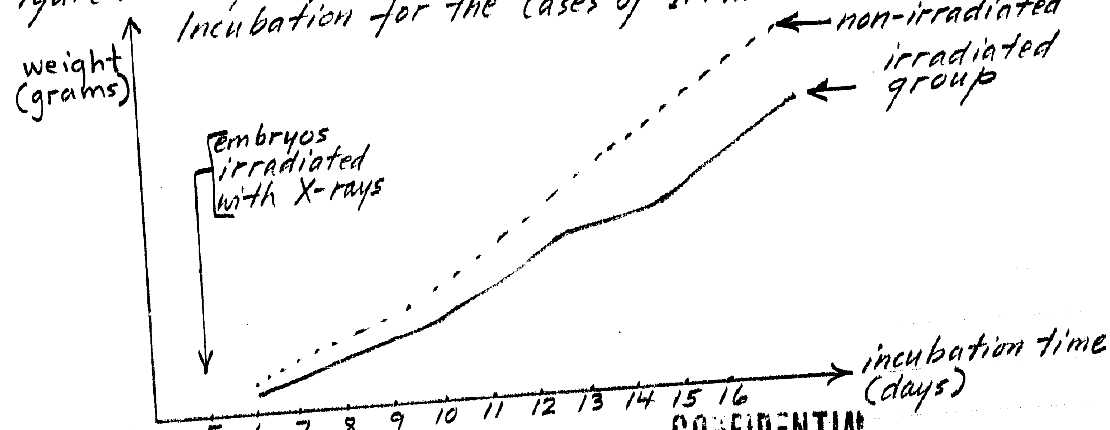
*Note: The star indicates that a monster was formed.

Table 2. Weights of Incubating Chicken Embryos After Irradiation

6th day	8th day	10th day	12th day	14th day	16th day
0.25 gm	1.20 gm	2.00 gm	3.40 gm	4.52 gm	6.80 av.
0.19	1.00	2.00	3.26	4.32	7.25
0.31	1.16	1.85	3.55	4.48	7.35
0.32	1.00	1.90	3.50	4.45 *	7.00
0.32	0.55	1.50	3.30	4.35	6.70
0.28	0.96	1.60	3.25	4.20 *	6.50
0.30	0.96	1.85	3.50	4.55	6.25
0.30	[illegible]	1.90 *	3.20	4.42	7.00 *[sic]
0.25	1.05	1.80	3.30	4.70 *	7.25
0.24	0.95	1.70	3.20	4.65	7.50
0.25	0.90	1.55	3.50	4.29	6.80 *
0.30	1.25	1.60	3.00	4.55 *	7.00
[illegible]	1.30	1.75	3.45	4.75	8.00
[illegible]	1.15	1.70	3.30	4.50	7.55
0.31	1.00	1.70	3.20	4.40	6.45 *
0.21 av.	1.04 av.	1.76 av.	3.33 av.	4.48 av.	7.03 av.

*Note: A monster was formed.

Figure 1. Graphs of the Weights of Chicken Embryos During Incubation for the Cases of Irradiation and Control.



The chicken embryos were irradiated with 600 gammas of hard X-rays on their 5th day of incubation; every other day thereafter their weights were compared with a non-irradiated control group. As shown in Tables 1 and 2 their average weight was 0.27 gram, which indicated a slight decrease due to irradiation. That is, the weights of the embryos showed a slight impairment in development; moreover, there was approximately a 20% morbidity rate among the embryos during the 24 hours after irradiation. These dead embryos revealed to the naked eye subcutaneous hemorrhage here and there; extravasated blood was especially evident in the breast and head. The weight on the 2nd day after irradiation averaged 1.04 gram; thus the increase in weight was slightly less than that of the non-irradiated group. Later the irradiated group was observed to increase gradually in weight but was still inferior to the non-irradiated group in weight and rate of increase in weight. Thus, on the 11th day after irradiation—that is, 16th day of incubation— the average weight of the irradiated was 7.03 grams and much less than that of the non-radiated group. That is, a strong impairment of weight and development was evident in the irradiated group.

The curves showing the development of the irradiated and non-irradiated groups are given in Figure 1. The difference in the two groups is especially evident in the second half of the incubation period.

In addition, terata were observed. Among 120 specimens of the irradiated group, 1 tera was discovered on the 5th day after irradiation, 4 on the 9th, and 3 on the 11th. The dysmorphosis was mainly observed in the wings, eyeballs, and beak. Embryos ordinarily begin to develop feathers in the middle of incubation, as do beak and claws. But the irradiated group showed tardy development of feathers; moreover they were rough. Especially noticeable was the poor development of beak and claws. Among the 120 specimens of the non-radiated group, one specimen was observed on the 16th day of incubation with malformed wings.

See 4. Remarks.

As is evident from the experimental results in the preceding section, hard X-rays act upon chicken embryos in the developmental stage and repress their growth and weight, or they cause dysmorphosis (teratogenesis) and even death. MIGAWA [18] irradiated with 600 gammas of hard X-rays chicken embryos on their 8th day of incubation and observed the influence on their growth. The repressive action of X-rays, he argued, is temporary and very strong within 24 hours after irradiation but has almost no effect on growth after 24 hours.

The author irradiated chicken embryos on the 5th day of their incubation with 600 gammas of hard X-rays and observed them for 11 days after irradiation; that is, up to the 16th day of incubation. According to the progress of incubation after irradiation, the rate of increase in weight of the irradiated group was observed to be slightly less than that of the non-irradiated group. According to the reports of Gilman and Bartjer, no temporary acceleration in growth has been observed. YAMAMOTO studied in detail the influence of differences in radiation time and dosage. He reported that radiation after 110 or 114 hours of incubation impaired remarkably the growth of embryos. He also observed in numerous specimens various dysmorphoses of the legs. According to many other researchers, radiation impairs the growth of chicken embryos or causes malformations (Colwell, Reginald and Wakeley, Krontowiki, Strangeways and Fell, Heim).

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Therefore it can be assumed that the impaired growth of chicken embryos exposed to hard X-rays is due directly to the radiation and this impairment results from the abnormal metabolism, induced by the radiation, of the embryonal tissues at the time of most active growth.

Sec 5. Conclusion.

Growing chicken embryos were irradiated with hard X-rays on the 5th day of incubation and their development was then under careful observation, giving the following conclusions:

1. 600 gammas of hard X-rays on the 5th day of incubation produces a morbidity rate of about 20%.

2. Hard X-rays clearly impair development, especially that of feathers, beak, and claws.

3. Already after 24 hours of irradiation the weight decreases in comparison with that of the non-radiated group. Thereupon the difference in weights of the irradiated and non-radiated groups becomes clearly apparent.

4. Hard X-rays causes dysmorphosis especially of the eyeballs, limbs, and beaks. About 8% of the specimens became malformed.

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— end of Chapter One —

Chapter Two. Influence of Radiation on Total Nitrogen Content.

Sec 1. Introduction.

If a living organism is irradiated once, a definite physico-chemical reaction occurs. Recently researches on the physiological action of such radiation and the applications of radiation physiological studies have appeared in succession. There have also been many researches on the influence of X-rays upon substance metabolism (anabolism). In the first place, the chemical components of blood or urine have been measured after irradiation by X-rays and the effects of X-rays upon metabolism (repair-exchange) have been carefully observed. Many of these studies, however, are fragmentary and the conditions of radiation were uncertain in matters of units, locus, etc; or they were studies made on patients in whom great disorders had already occurred in their anabolism. No definite views can yet be seen.

First of all let us cite works on the total nitrogen content of blood or blood protein. Hirsch and Peterson[1] and also MURAMATSU[2] reported that the rise or fall in the total nitrogen content of irradiated blood is indefinite; Mahnert and Zacher, Behrens, Alfred[4] reported that the total nitrogen content of blood after irradiation is invariable. Jugenberg[5], Gigon and Luedin[6], HISAMOTO[7], and MIZUHARA[8] reported that the total nitrogen content of blood after X-ray irradiation decreases. Mahnert and Zacher[9], Pannewitz[10], Dellepiane and Badino maintain that there is an increase; moreover, Dellepiane and Badino even found a relationship between this increase in the nitrogen of blood and the deleterious action of X-rays.

Next let us consider the nitrogen content of urine. Hall and Whipple[12], Baermann and Linser[13], Benjamin and Reuss[14], Lemmel[15], Sick[16], Krause[17], Klewitz[18], Warren and Whipple[19] proposed that the secretion of nitrogen generally increases after irradiation by X-rays; but Mueller[20], Meyer, Dodds and Webster[22], Jugenberg[5], Graefe[23], Thannhauser and Curtiss[24], FUJITA[25] advanced a "decrease" theory. Thus the two theories are completely divergent.

Next is the oft-mentioned problem of the influence of radiation upon embryonal development. As the author has already related in Chapter One, this influence is deleterious to growth and in the extreme leads to teratogenesis or even death. The cells in the embryonal period are especially susceptibility to X-rays and it is generally recognized that the nucleus of the cells possesses, the greatest susceptibility.

Generally a single cell constitutes an individual perfect organism in which marvelously complex life processes occur. The life of a cell is shown in its metabolic phenomena of breakdown, repair, exchange, etc. In the biophenomena of a chicken embryo during incubation, one observes only gas exchange with the environment; development is completely effected with substances already existing within the egg. Protein (albumen) is especially important : it is differentiated or assimilated into various chemical components in response to demands; the chemical energy stored within it is employed to build up the body's tissue components and to supply vital forces.

If radiation acts upon a living organism in which such growth is at its very height, then morphological changes obviously are easily induced, just as numerous previous experiments on the physiological action of X-rays have proved; however, the variations in the chemical processes occurring internally are still completely unknown. As the author related, he employed chicken embryos as being the most suitable in a study of the chemical composition of organisms and endeavored to study what influence X-rays exert on their metabolism (repair-exchange), especially on nitrogen metabolism.

Sec 2. Experimental Procedure.

Art 1. Lab Materials and Methods of Irradiation.

The specimens and other materials employed here were the same as those mentioned in Chapter One; since the method of X-ray irradiation also was identical its redescription will be omitted here.

Art 2. Quantitative Methods.

Normal incubating eggs, as already described, and eggs irradiated with X-rays on their fifth day of incubation were weighed every other day until the 16th day of incubation.

Special attention was paid to see that the egg contents — egg white, egg yolk, embryo, and show — were kept separate, to prevent their mixing. In the 1st experiment, we individually mixed identical contents of 5 eggs and stirred them vigorously; then we thoroughly ground up 5 chicken embryos together with emery powder in a glass mortar. The experimental results thus obtained could be regarded as the average for the contents of 5 eggs. That is, the egg white of the 5 eggs were mixed, then the egg yolks etc; at all times, however, the 4 different substances were kept separate, as was explained.

We took individual quantitative determinations of the above 4 different substances (egg white, egg yolk, embryo, show), which separately represented mixtures of identical portions of 5 eggs. The Kjeldahl method was employed in the quantitative determination of the total nitrogen content.

Sec 3. Experimental Results.

Art 1. Influence of Hard X-Rays and the Total Nitrogen Content of the Egg White.

The nitrogen content of egg white in fresh eggs is 1.356 gram/dg. At the beginning of incubation it gradually increases and on the 5th, 6th, 7th, 8th day of incubation it increases very rapidly, reaching its height on the 14th day; thereafter it seems to decrease rather slightly. The irradiated group increases slightly the next day after irradiation in comparison with the control comparison group; thereafter it varies (increases or decreases) in a manner somewhat similar to that of the control group. That is, no note-worthy variation is apparent in the total nitrogen content of egg white that has been exposed to X-rays.

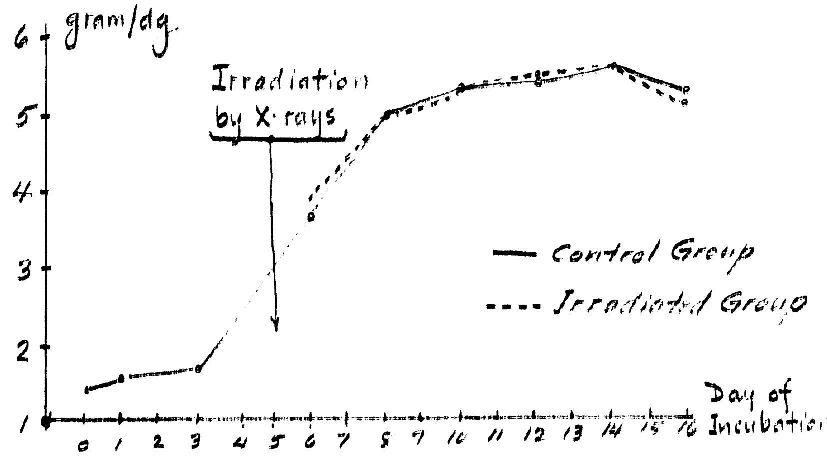
Table 1. The Total Nitrogen Content (grams/dg) of Egg White for Various Days of Incubation (0 to 16th):

Group	0th day	1st	3rd	6th	8th	10th	12th	14th	16th
1st Gp	1.379	1.648	1.625	3.621	4.742	5.239	5.353	5.456	5.154
2nd Gp	1.337	1.421	1.402	3.528	4.985	5.325	5.540	5.526	5.231
3rd Gp	1.352	1.465	1.742	3.654	4.954	5.182	5.118	5.479	5.064

Table 2. Total Nitrogen Content (gram/dg) of Egg White Irradiated by Hard X-Rays, According to Various Days of Incubation (6th to 16th):

Group	6th day	8th	10th	12th	14th	16th
1st Gp	3.765	4.994	5.523	5.372	5.470	4.882
2nd Gp	3.875	4.825	4.896	5.449	5.372	5.104
3rd Gp	3.702	4.845	5.274	5.402	5.568	4.952
Average	3.781	4.888	5.231	5.408	5.470	4.979

Figure 1. Curve Describing the Total Nitrogen Content of Egg White.



Art 2. Influence of Hard X-Rays and the Total Nitrogen Content of Egg Yolk.

As is evident from Table 3 below, the total nitrogen content in egg yolk in the initial phases of incubation gradually decreases with the progress of incubation, to reach its lowest point on the 8th day; thereafter in the middle and final phases of incubation one perceives a tendency toward gradual increase with the progress of continuing incubation. The irradiated group seems to decrease slightly in comparison with the control group on the next day following irradiation; thereafter, however, it gradually increases with time and its nitrogen content even becomes greater than that of the control group. That is, the total nitrogen content in egg yolk increases because of irradiation.

Table 3. Total Nitrogen Content (g/dg) in Egg Yolk During Incubation.

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
1st Gp	2.605	2.521	2.710	1.393	1.267	1.447	1.730	1.618	1.744
2nd Gp	2.753	2.710	2.410	1.425	1.355	1.495	1.454	1.721	1.805
3rd Gp	2.584	2.552	2.128	1.502	1.198	1.458	1.428	1.685	1.895
Average	2.647	2.594	2.416	1.440	1.273	1.466	1.530	1.675	1.814

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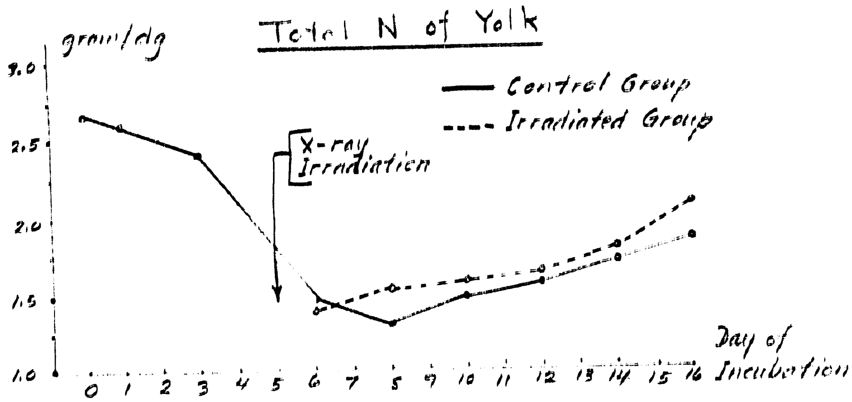
Table 4. Total Nitrogen Content (g/dg) of Egg Yolk Irradiated with Hard X-Rays for Various Days of Incubation (6 to 16th).

Groups	6th	8th	10th	12th	14th	16th
1st Group	1.344	1.541	1.562	1.527	1.657	2.073
2nd Group	1.408	1.502	1.604	1.632	1.758	1.928
3rd Group	1.352	1.481	1.512	1.625	1.852	2.105
Average	1.368	1.508	1.559	1.594	1.756	2.035

Table 2. Curve Describing Total Nitrogen of Egg Yolk.



Art 3. Influence of Hard X-Rays and the Total Content of Show.

Being very difficult to separate, the show was separated after the 6th day of incubation; when its total nitrogen content was quantitatively determined, it proved to be very much less than that of egg white or egg yolk. Although it was small and showed no noticeable change during the 1st half of incubation, it increased strongly with progress of incubation in the 2nd half. The irradiated group increased under conditions almost the same as those of the control group, but exceeded the control group in each phase of incubation. That is, the total nitrogen content in the show

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increases becauses of X-ray radiation.

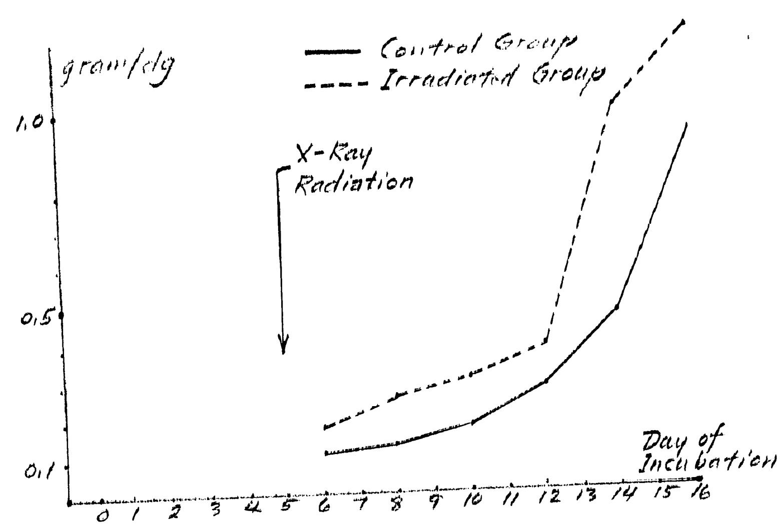
Table 5. Total Nitrogen Content (gram/dg) of Show During Incubation (6th to 16th).

Groups	6th	8th	10th	12th	14th	16th
1st Group	0.105	0.107	0.159	0.275	0.481	0.952
2nd Group	0.098	0.137	0.188	0.266	0.435	0.925
3rd Group	0.107	0.125	0.158	0.275	0.442	0.922
Average	0.106	0.123	0.168	0.272	0.452	0.933

Table 6. Total Nitrogen Content (gram/dg) of Show Irradiated by Hard X-rays, for Various Days of Incubation (6-16th).

Groups	6th	8th	10th	12th	14th	16th
1st Gp	0.152	0.256	0.295	0.392	0.964	1.175
2nd Gp	0.163	0.242	0.299	0.358	0.986	1.231
3rd Gp	0.157	0.250	0.277	0.382	1.038	1.173
Average	0.177	0.249	0.291	0.387	0.996	1.193

Figure 3. Curve Describing the Total Nitrogen of Show.



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Art 4. Influence of Hard X-Rays and the Total Nitrogen Content of Chicken Embryos.

Quantitative determinations on chicken embryos were carried out beginning on the 6th day of incubation

In the control group and the irradiated group the total nitrogen content was noticed to increase gradually with fetal development, but the irradiated group did not exceed the control group at any period. That is, the total nitrogen content in chicken embryos is diminished by irradiation. This is so because fetal development is impaired by irradiation and hence the total nitrogen content decreases.

Table 7. Total Nitrogen Content (gram/dg) of Chicken Embryos For Various Days of Incubation (6 to 16th).

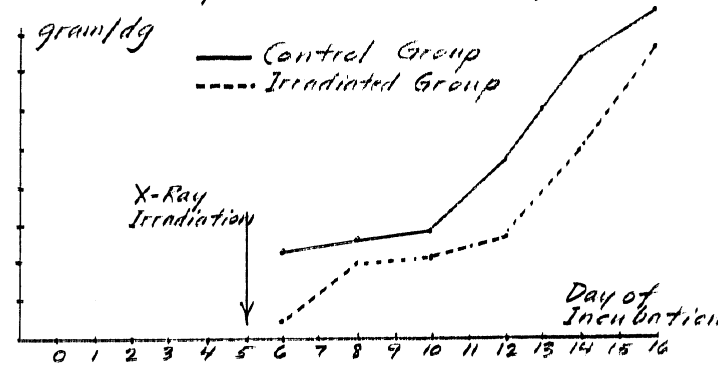
Groups	6th	8th	10th	12th	14th	16th
1st Gp	0.629	0.668	0.679	0.863	1.125	1.274
2nd Gp	0.615	0.652	0.692	0.869	1.684	1.326
3rd Gp	0.631	0.669	0.670	0.861	1.153	1.184
Average	0.625	0.663	0.680	0.864	1.121	1.261

Table 8. Total Nitrogen Content (gram/dg) of Chicken Embryos Irradiated by Hard X-Rays, for Various Days of Incubation.

Groups	6th	8th	10th	12th	14th	16th
1st Gp	0.511	0.623	0.595	0.642	0.945	1.169
2nd Gp	0.392	0.608	0.665	0.612	0.855	1.202
3rd Gp	0.432	0.548	0.581	0.7133	0.873	1.127
Average	0.445	0.593	0.614	0.666	0.891	1.166

Figure 4. Curve Describing Total Nitrogen of Chicken Embryos.

Fig 4. Total N_2 of Embryos

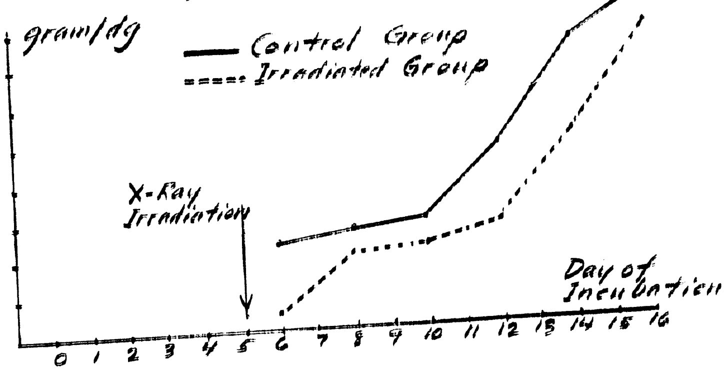


Art 5. Influence of Hard X-Rays and the Total Nitrogen Content of Incubating Chicken Embryos.

Although in the initial phases of incubation the total nitrogen content in eggs shows almost no movement, still there is embryonal develop-ment and strong catabolic activity going on in the egg; also, the total nitrogen is gradually increasing with progress of incubation. The substance of the egg is being made into an individual organism from single cells by means of complex chemical reactions; in the meanwhile, marvelous biophenomena are maintained: all this is reflected in the above behavior of nitrogen. It is regrettable that we cannot cite here the chemical transformations involved. Although the irradiated group is slightly lower than the control group 24 hours after irradiation, it recuperates after the 3rd day and thereafter increases slightly in each of the later periods. Generally, radiation can be said to have a tendency to increase the total nitrogen in the substance of the egg.

Figure 4. Curve Describing Total Nitrogen of Chicken Embryos.

Fig 4. Total N_2 of Embryos



Art 5. Influence of Hard X-Rays and the Total Nitrogen Content of Incubating Chicken Embryos.

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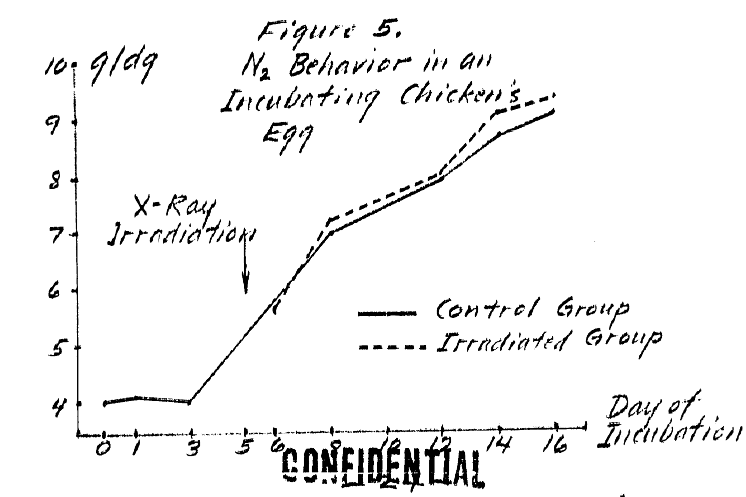
Table 9. Distribution of Nitrogen (gram/dg) in Each of the Four Different Substances Making up the Incubating Chicken Egg, With Respect to Various Days (0 to 16th) of Incubation. (Non-irradiated)

Substance	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	1.356	1.511	1.619	3.601	4.893	5.249	5.309	5.492	5.150
Egg Yolk	2.647	2.594	2.416	1.440	1.273	1.466	1.530	1.675	1.814
Embryo				0.625	0.663	0.680	0.864	1.131	1.261
Show				0.106	0.123	0.168	0.272	0.452	0.933
Total	4.003	4.105	4.035	5.712	6.952	7.563	7.975	8.750	9.158

Table 10. Distribution of Nitrogen in Each of the 4 Substances of an Incubating Chicken's Egg That Has Been Irradiated with X-Rays, with Respect to Incubation Period (6 to 16).

Substance	6th Day	8th	10th	12th	14th	16th
Egg White	3.781	4.555	5.231	5.408	5.470	4.979
Egg Yolk	1.368	1.508	1.559	1.594	1.756	2.035
Chick Embryo	0.445	0.593	0.614	0.666	0.891	1.166
Show	0.177	0.249	0.291	0.387	0.996	1.193
Total	5.611	7.238	7.695	8.055	9.113	9.373

Figure 5. The Behavior of the Total Nitrogen Content in an Incubating Chicken's Egg.



Sec 4. Summary and Remarks.

To summarize the above experimental results: The total nitrogen content in egg white increases with progress of incubation; it tends to decrease very slightly towards the final period; in the group irradiated with hard X-rays it increases slight one day after irradiation, but thereafter varies almost in the same manner as in the control group — that is, we cannot observe any note-worthy changes in fluctuations of protein in egg white because of irradiation by X-rays.

The total nitrogen in egg yolk decreases with the course of incubation during its initial phase; this is so because its nitrogenous compounds are consumed first during fetal development in the embryo. After the middle phase of incubation, the nitrogen gradually increases with the progress of incubation. If at this point irradiation by hard X-rays is employed, then the nitrogen content increases gradually and moreover becomes greater than in the control group — that is, the protein in egg yolk, because of X-ray irradiation, apparently represents a small amount taken from the the chicken embryo.

The total nitrogen content in the show shows a slight increase in the first half of incubation, but increases strongly in the second half; the irradiated group too shows a very strong increase in the second half of incubation and moreover even surpasses the control group in each phase — that is, the increase in nitrogen in the show is obviously due to irradiation.

The total nitrogen in chicken embryo gradually increases as incubation progresses in both groups, irradiated and non-irradiated; at the same time the irradiated group shows a slight increase over the control group — that is, X-ray irradiation apparently induces impairment in the structural composition of the chicken embryo.

Now let us proceed to consider the variations, rise or fall, in total nitrogen content of these 4 kinds of substances in eggs: In general the nitrogenous compounds in egg white and in egg yolk go especially to compose fetal tissues and are indispensable in the development and growth of the chicken embryo.

J. Needham [26] divided the chemical processes in fertilized eggs into the two: a) consumption of material stored in the egg and b) composition of structural tissues in a developing chick; he pursued his researches on this basis. As F. Tangl and A. von Mituch [27] showed, the nitrogen of a 54.2-gram egg before incubation is 0.929 gram and the chemical energy contained is 86.85 Calories, with 23 Calories of it going into heat during development and 38 Calories being used in the construction, or composition, of the body; they were able to prove that the remaining 26 Calories remained in the egg yolk and that the consumption of this energy follows the decrease in weight during incubation. O. Riddle [28] did research on the rate with which the components of egg yolk are consumed in the chicken embryo and thus showed that, although there is no remarkable consumption up to 12 days of incubation, after 12 days phosphatide is consumed faster than neutral fat, which are consumed at an earlier phase than protein, and that the egg white becomes a dilute liquid after 12 hours of incubation, and then the amount of the solid component contained in it increases and the aqueous component decreases. G. E. Wladimiroff [29] too observed this increase in the solid component. E. G. Schenck [30] carried out detailed studies on the transformations of protein and the movement of water that occur during incubation; thus he proved that the water in egg white and egg yolk moves during ~~the~~ formation of tissues in the chicken embryo and during energy dissipation to the environment. Sacardi, Pietro [31] investigated the aqueous component, ash, and organic content in incubating chicken embryos and thus demonstrated that in the initial phase the embryo contains much water and little salt and organic substances and that salt and organic substances increase noticeably with the course of incubation and the amount of these substances remaining in egg white and egg yolk is inverse to the amount in the chicken embryo.

The chicken embryo differs from the embryos of most animals in that it never receives nutriment from the maternal body but receives the chemical constituents necessary for formation and composition only from the egg white and egg yolk within its egg; moreover ~~the metabolic products~~, except for 1.2 [sic], cannot be excreted. Consequently, the total nitrogen content within the egg must be invariable in absolute mass according to the law of indestructibility of matter; however, one observes a considerable increase in the percentage weight as incubation progresses (see Art 5). This is so because, during the process of incubation, protein is being oxidized and reduced and sugar and fat are undergoing metabolic changes and are being consumed to supply heat energy and kinetic energy — that is, the emission of water is an important factor and thus the percentage of total nitrogen content should be considered to increase.

In observing the egg yolk and egg white which are substances that previously existed within the egg during incubation, we note that the percentage weight of total nitrogen content decreases in the initial phases of incubation in egg yolk, which fact results from the consumption of its protein during fetal development; the fact that it gradually increases slightly thereafter is probably the result of its percentage increase due to the influence of water emission, although the protein is being utilized all the while during fetal development and should by all rights decrease.

Yamada[32] reported that the total nitrogen in the blood of a chicken embryo is 1.251 on the 15th day of incubation and 1.258 on the 19th; Y. Nakamura[33] reported that the percentage weight of nitrogen in egg yolk of the second half of incubation and in chicken embryos increases.

Anna Jugenberg[34] on the basis of experiments on irradiated guinea pigs reported that decrease in nitrogen of its blood results from impairment of nitrogen secretion from the tissue or nitrogen storage in the various organs, and that because of this nitrogen storage the body weight increases slightly and thus the death of animals due to irradiation is based upon an impairment of the tissues' ability to secrete nitrogen. Nemenow[35] also states that irradiation greatly injures substance metabolism and thus causes the destruction of cells.

The author proved that the variations in the percentage weight of total nitrogen contained in an incubating egg increases as incubation progresses, as shown in Table 9 and Figure 5; but the irradiated group shows a slight decrease after irradiation (see Table 10 and Figure 5), although thereafter it increases each day in comparison with the control group, which fact is connected with the resulting decrease in egg's water content.

The total nitrogen in egg white shows no note-worthy change due to irradiation, but the fact that the irradiated group shows an evident decrease of nitrogen content in the embryo can be taken to mean that X-ray irradiation causes an impairment in the embryo's ability to absorb protein from the egg yolk. And the fact that the nitrogen in egg yolk each day is greater than in that in the non-irradiated group should enable one to understand how protein bodies in egg yolk break up and are absorbed in the chicken embryo and how the mechanism of consumption in tissue formation is disrupted by irradiation.

Next, show reveals a very sudden increase in nitrogen during the half phases of incubation, which fact is a problem of interest in inquiries into the development of show.

From the fact that, in the case of specimens irradiated by hard X-rays, the nitrogen increases all the more in the show, one can infer that this is a result of abnormal metabolism in the embryo and of changes in permeability of the epithelial cells of the show membrane.

As it appears from this, hard X-rays impair the development of chicken embryos, as already related in Chapter One; furthermore, in observing the conditions governing the metabolism of protein which are of importance to development, one notes —— no evident changes in the protein of egg white due to X-ray irradiation but does note an increase in protein of the egg yolk belonging to the irradiated group and a decrease in the embryo's total nitrogen due to irradiation in comparison with the control group, which facts evidently show that X-rays impair nitrogen metabolism in chicken embryos.

Sec 5. Conclusion.

The author irradiated incubating chicken embryos with hard X-rays, and in observing the conditions governing nitrogen metabolism in the embryos he was able to obtain the following results:

1. Total nitrogen content of egg white during incubation shows no apparent changes due to irradiation by X-rays.

2. Total nitrogen content of egg yolk during incubation increases because of irradiation by X-rays.

3. Total nitrogen in show clearly increases because of irradi-ation by X-rays.

4. Total nitrogen content in chicken embryos during development is decreased by hard X-rays; this results from the fact that irradiation impairs protein metabolism in chicken embryos.

5. Total nitrogen content in an incubating egg (egg white, egg yolk, show, embryo) has a slight tendency to increase somewhat due to irradiation by hard X-rays.

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Chapter Three. Influence of Radiation on Albumin and Globulin.

Sec 1. Introduction

In Chapter Two the author was able to demonstrate positively that hard X-ray irradiation during embryonal development impairs the embryo's nitrogen metabolism; moreover he clarified how active metabolism is maintained during incubation and how the percentage —— of total nitrogen content varies in the various substances (egg white, egg yolk, show, etc) of the egg, besides the complicated biophenomena.

The protein in egg white is mainly albumin with a smaller amount of globulin, as was already stated. Also, the main protein in egg yolk is vitellin; although it belongs to phosphoproteins, vitellin merely produces proteinaceous decomposition products and phosphoric acid when broken down and therefore it is described by the author as egg-yolk globulin. The authors intentions here is to study how these two kinds of egg protein are effected by hard X-rays according to the following experimental procedures and is not to study the precise amounts of globulin and albumin in the strict meaning. That is, the following material represents a study of how hard X-rays influence these proteins.

Sec 2. Experimental Procedure.

The materials, conditions surrounding irradiation, and also techniques have already been stated in detail in Sec 2 of Chapter Two; therefore their description will be omitted here.

The quantitative methods are as described in Art 2 in Sec 2 of Chapter Two. In the first experiment 5 eggs were used; the 4 different kinds of substances contained in the eggs—namely, egg white, egg yolk, show, embryo—were each collected and then separately mixed as thoroughly as possible. That is, the show sau from the 5 eggs was isolated and then mixed together, at all times being kept separate from the other 3 substances mentioned; similarly for the egg white, egg yolk, and embryo. At this point the egg white and yolk of incubating eggs are wrapped in a folded cloth and repeatedly crushed; the embryo is mixed with a quantity of emery powder equal in weight to the embryo and then finely pulverized — thus each substance was made as uniform as possible.

A fixed quantity of each of the substances thus obtained was measured in a balance and mixed with 40 times its amount of water and then neutralized with 3% acetic acid: Exactly at this point a precipitate was produced, which was then filtered with ashless filter paper; the resulting precipitate was completely collected on the filter paper and then thoroughly washed with water. This precipitate was globulin.

Next, the above-mentioned filtrate was made slightly acidic by the addition of 0.5% acetic acid; after a 30-minute heating in a 100° steam bath following repeated boiling, a protein finally appeared. This precipitate was filtered with ashless filter paper and then thoroughly washed with hot water after its complete collection on the filter paper. This precipitate was taken to be albumin.

The precipitates of globulin and albumin thus obtained were treated according to Kjeldahl's method to obtain the amounts of globulin-N and albumin-N contained in 100 grams; the percentage amount of albumin (abbreviated below by A) and globulin (designated G hereunder) was taken by multiplication of the above-mentioned amounts by 6.25.

Sec 3. Experimental Results.

a. The Influence of Hard X-Rays and the Total Nitrogen Content of Globulin and Albumin in Egg White.

Item 1. Total Nitrogen Content of Albumin in Egg White, and the Influence of Irradiation:

There are no evident changes in the initial phases of incubation, but when the embryo develops the nitrogen content of albumin suddenly increases, reaching its highest point in the middle phases of incubation and then tending to decrease somewhat thereafter; the situation seen here is almost like that shown by the total nitrogen curve.

The irradiated group reveals no noteworthy changes in comparison with the control group 2 to 5 days after irradiated, but on the 7th day of irradiation — that is, after the 12th day of incubation — there seems to be a decrease.

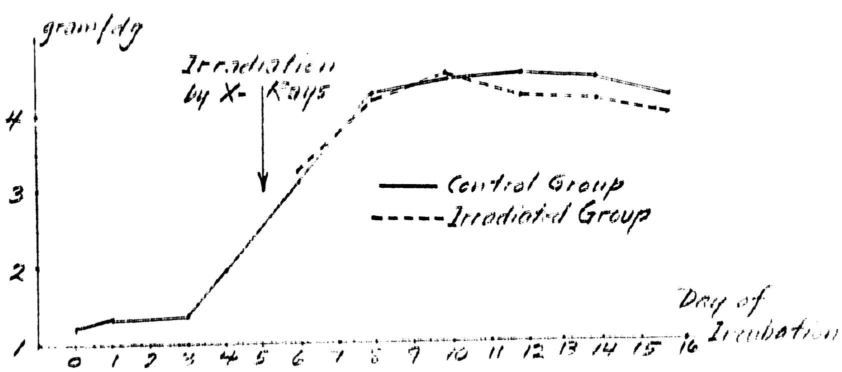
Table 1. Nitrogen Content (gram/dg) of Albumin in Egg White During Various Days of Incubation (0 to 16th Day):

Groups	0th day	1st	3rd	6th	8th	10th	12th	14th	16th
1st Gp	1.274	1.358	1.260	3.208	4.399	4.427	4.441	4.420	4.295
2nd Gp	1.190	1.342	1.534	3.085	4.121	4.425	4.448	4.412	4.134
Average	1.232	1.350	1.397	3.146	4.260	4.426	4.443	4.416	4.215

Table 2. The Nitrogen Content (grams/dg) of Albumin in Egg White Irradiated by Hard X-Rays During Incubation (6 to 16th).

Groups	6th	8th	10th	12th	14th	16th
1st Gp	3.152	4.560	4.609	4.195	4.203	4.006
2nd Gp	3.324	4.064	4.329	4.245	4.184	4.025
Average	3.238	4.312	4.469	4.220	4.193	4.016

Figure 1. Nitrogen Content of Albumin in Egg White.



b. The Nitrogen Content of Globulin in Egg White, and the Influence of Irradiation.

The nitrogen content of globulin in egg white during embryonal development increases gradually. The group irradiated with hard X-rays also shows a gradually increase; moreover it is more than for the control group, but a tendency towards decreasing amounts seems noticeable during the final phase of incubation.

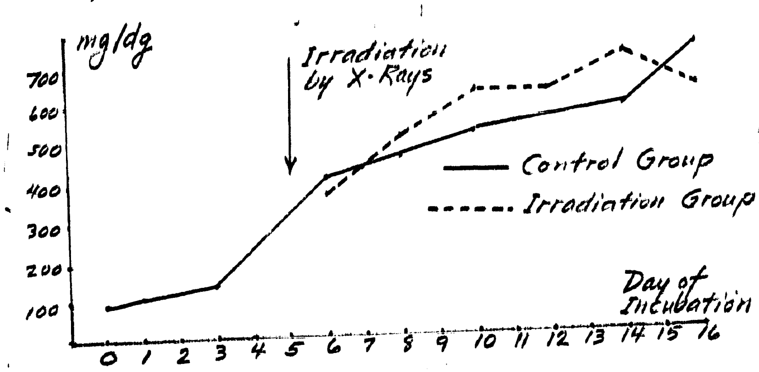
Table 3. Nitrogen Content (milligrams/dg) of Globulin in Egg White During Incubation (0 to 14th Day).

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
1st Gp	84.06	112.08	126.09	403.48	462.33	518.37	562.33	595.42	735.52
2nd Gp	98.07	108.75	155.14	418.52	471.04	529.35	549.25	581.30	751.21
Average	91.06	110.16	140.61	411.00	466.68	523.86	557.93	558.36	743.36

Table 4. Nitrogen Content (milligrams/dg) of Globulin in Egg White Irradiated with Hard X-Rays During Incubation (6 to 16th).

Groups	6th day	8th	10th	12th	14th	16th
1st Gp	376.86	523.45	683.68	581.41	770.55	630.45
2nd Gp	362.55	515.43	574.41	686.49	682.59	642.44
Average	369.70	519.44	629.05	633.95	726.57	636.44

Figure 2. Nitrogen Content of Globulin in Egg White.



c. The Ratio of Albumin and Globulin in Egg White, and its Variation Due to Irradiation.

Table 5. The Albumin/Globulin Ratio in Egg White During Incubation.

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Control	13.52	12.25	9.92	7.65	9.12	8.44	7.96	7.58	5.67
Irradiated				8.75	8.10	7.10	6.65	5.77	6.31

It is clear from Table 5 (see Tables 1,2,3,4) that, in egg white, albumin is considerably greater than globulin; albumin increases noticeably with progress of incubation, but the increase in globulin is also quite considerable, so that the ratio A/G becomes gradually smaller. The irradiated group too shows a smaller A/G ratio as incubation progresses; moreover, although it is smaller because of irradiation, the A/G ratio becomes larger on the 16th day of incubation.

Art 2. The Nitrogen Content of Albumin and Globulin in Egg Yolk, and the Influence of Hard X-Rays.

a. Nitrogen Content of Albumin in Egg Yolk, and the Influence of Hard X-Rays.

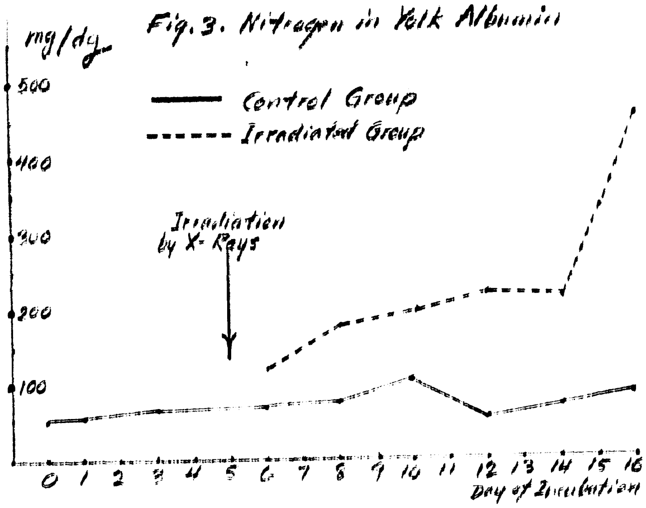
Table 6. Nitrogen Content (mg/dg) of Albumin in Egg Yolk During Incubation (0 to 16th Day).

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
1st Gp	56.04	56.04	77.05	70.051	70.88	106.09	42.03	70.05	452.33
2nd Gp	56.04	55.00	55.2	70.05	83.23	58.25	70.05	70.05	472.33
Average	56.04	57.02	66.14	70.05	77.05	82.17	56.04	70.05	462.33

Table 7. Nitrogen Content (mg/dg) of Albumin Egg White Irradiated During Incubation (6th to 16th Day).

Groups	6th	8th	10th	12th	14th	16th
1st Gp	108.05	189.13	287.20	194.06	196.78	452.33
2nd Gp	132.05	167.52	105.07	237.99	221.24	472.33
Average	120.05	178.32	196.14	221.01	214.01	462.33

Figure 3. Nitrogen Content of Albumin in Egg Yolk.



There is a slight increase with progress of incubation, but a decrease occurs about the 12th day of incubation, which is later followed by an increase again. The irradiated group gradually increases; in the final phases the increase is especially marked. In general, the albumin (A) content of egg yolk is greater in the irradiated group than in the control group.

b. The Nitrogen Content of Globulin in Egg Yolk, and the Influence of Hard X-Rays.

The control group shows a degrease in globulin nitrogen during the initial phases of incubation; later it gradually increases, starting about the 10th day of incubation, just as in the total nitrogen curve. The irradiated group too shows 'a' gradual decrease with progress of incubation, 'and' is similar to the control group.

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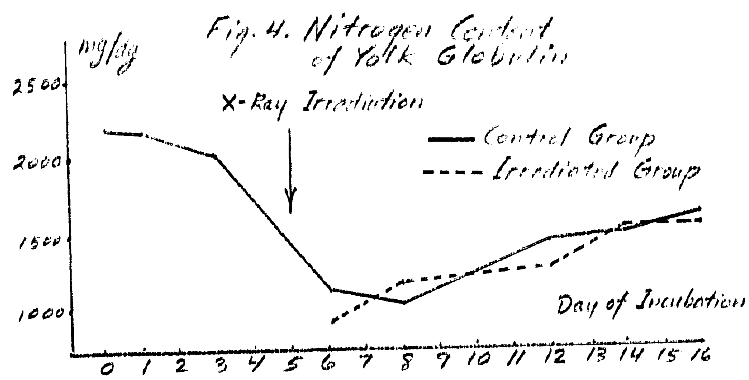
Table 8. The Nitrogen Content (mg/dg) of Globulin in Egg Yolk During Incubation (0 to 16th Day).

Groups	0th	1th	3rd	6th	8th	10th	12th	14th	16th
1st Gp	2131	2129	2031	1155	1052	1358	1555	1471	1511
2nd Gp	2285	2241	1987	1082	1006	1175	1311	1463	1621
Average	2208	2185	2009	1119	1029	1217	1433	1462	1581

Table 9. The Nitrogen Content (mg/dg) of Globulin in Egg Yolk Irradiated by Hard X-Rays During Incubation.

Groups	6th Day	8th	10th	12th	14th	16th
1st Group	905	1148	1176	1246	1443	1534
2nd Group	889	1136	1176	1218	1421	1525
Average	897	1142	1176	1232	1432	1529

Figure 4. Nitrogen Content of Globulin in Egg Yolk.



c. The Ratio of Albumin and Globulin in Egg Yolk, and Variations Due to Hard X-Ray Radiation.

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The ratio of albumin and globulin in egg yolk becomes larger with progress of incubation, but becomes smaller about the 12th day of incubation; thereafter it gradually increase again. The irradiated group suffers hardly any effects from irradiation as far as globulin is concerned, but the albumin increases noticeably; therefore the ratio of the two increases considerably more than in the control group. The A/G ratio, moreover, is less than one, and therefore albumin (A) must be less than globulin (G).

Table 10. The Albumin/Globulin Ratio for Egg Yolk, for Various Days of Incubation.

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Control	0.025	0.026	0.032	0.062	0.074	0.067	0.039	0.047	0.054
Irradiated				0.133	0.156	0.167	0.179	0.149	0.302

Art 3. The Nitrogen Content of Albumin and Globulin in Show, and the Influence of Hard X-Ray Irradiation.

a. The Nitrogen Content of Albumin in Show, and the Effect of Hard X-Rays.

In the first half of incubation, it is small and tends to increase only slightly, but in the second half it shows a strong increase. The irradiated group shows an increase 3 days after irradiation and thereafter it continues to increase gradually; in the second half of incubation it increases rapidly. Furthermore, it is always larger than the control group — that is, albumin increases markedly because of X-ray irradiation.

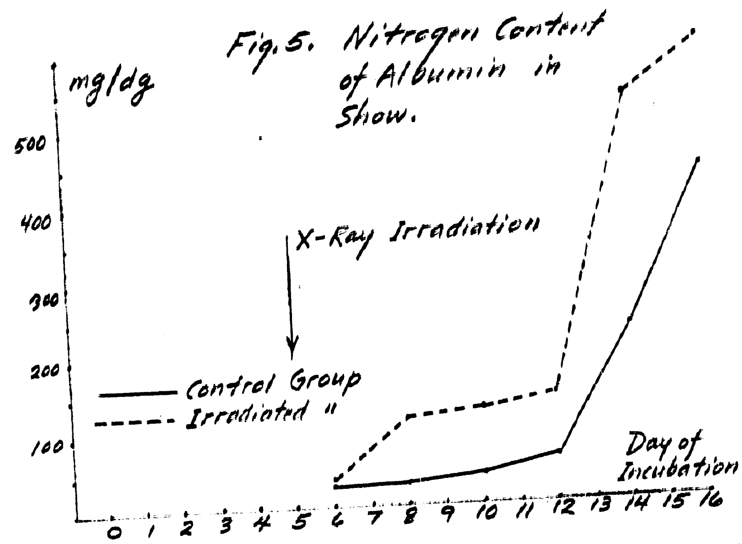
Table 11. The Nitrogen Content (mg/dg) of Albumin in Show During Various Days of Incubation (6 to 16th).

Groups	6th	8th	10th	12th	14th	16th
1st Group	27.06	28.02	42.03	55.02	248.07	434.11
2nd Group	28.23	26.34	37.28	62.03	227.25	427.25
Average	27.64	27.18	39.65	58.52	235.15	430.68

Table 12. The Nitrogen Content (mg/dg) of Albumin in Show Irradia- -ted with Hard X-Rays During Incubation.

Groups	6th Day	8th	10th	12th	14th	16th
1st Group	31.52	126.09	133.09	132.41	527.81	584.42
2nd Group	33.54	98.54	112.05	147.48	519.35	610.52
Average	32.53	112.31	122.72	139.94	523.58	597.47

Figure 5. The Nitrogen Content of Albumin in Show.



b. The Nitrogen Content of Globulin in Show, and the Influence of Hard X-Ray Irradiation.

In the first half, it gradually increased in both the control group and the irradiated group in a manner similar to albumin's; but in the second half it increased suddenly and rapidly, with the irradiated group showing the greater increase.

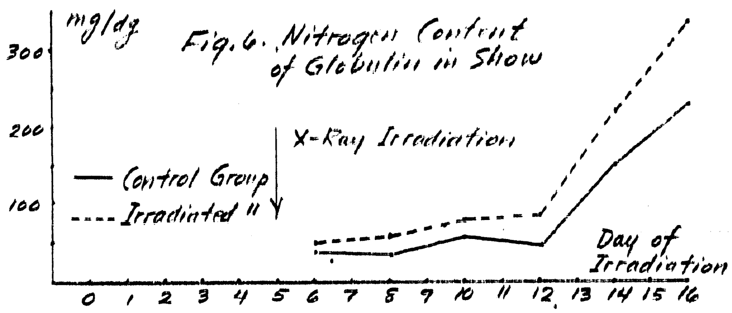
Table 13. The Nitrogen Content (mg/dg) of Globulin in Show During Incubation (6th to 16th Day).

Groups	6th	8th	10th	12th	14th	16th
1st Group	38.07	35.03	59.04	49.03	144.75	235.44
2nd Group	42.45	34.38	56.25	45.21	162.01	230.47
Average	40.26	34.70	57.64	47.13	153.39	232.95

Table 14. The Nitrogen Content (mg/dg) of Globulin in Show Irradiated by Hard X-Rays During Incubation (6-16th).

Groups	6th	8th	10th	12th	14th	16th
1st Group	37.13	62.53	84.06	54.06	218.41	339.46
2nd Group	58.25	57.35	80.25	54.06	224.54	346.65
Average	47.69	59.94	82.15	54.06	221.47	343.05

Figure 6. The Nitrogen Content of Globulin in Show.



c. The Ratio of Albumin and Globulin in Show, and the Variation Due to Irradiation.

The albumin/globulin ratio A/G increases as incubation progresses; that is, albumin becomes greater than globulin during the incubation process. In the irradiated group the A/G increases much greater than in the control group; that is, because of hard X-ray irradiation the albumin in show becomes much greater than the globulin.

Table 15. The Albumin/Globulin Ratio (A/G) in Show.

Groups	6th Day	8th	10th	12th	14th	16th
Control Group	0.686	0.783	0.687	1.241	1.533	1.868
Irradiated Group	0.682	1.873	1.493	1.664	2.492	1.741

Art 4. The Total Nitrogen Content of Globulin and Albumin in Chicken Embryos, and the Influence of Irradiation by Hard X-Rays.

a. The Nitrogen Content of Albumin of Chicken Embryos, and the Influence of Hard X-Rays.

The first half of incubation did not show any notable changes in the albumin content of chicken embryos, but in the second half it gradually increased. Already by the third day after irradiation the irradiated group showed a considerable increase and then a decrease; on the 14th day of incubation it became smaller than in the control group. On the 16th day it increased but did not exceed the control group — that is, there is a rapid increase due to irradiation followed by a gradual decrease so that on the 5th day of irradiation it has decreased more than in the control group, then it increases, but still does not exceed the control group. Thus there apparent variations due to X-rays.

Table 16. The Nitrogen Content (mg/dg) of Ablumin in Chicken Embryos During Incubation (6th to 16th Day)

Groups	6th	8th	10th	12th	14th	16th
1st Group	90.65	68.18	70.05	84.06	154.11	285.42
2nd Group	93.24	67.24	72.35	87.25	138.25	221.55
Average	91.94	67.71	71.20	85.65	146.18	253.49

Figure 17. The Total Nitrogen Content (mg/dg) in Chicken Embryos Irradiated with Hard X-Rays During Incubation.

Groups	6th Day	8th	10th	12th	14th	16th
1st Group	49.03	196.14	147.10	86.71	95.07	231.16
2nd Group	56.25	161.23	98.07	115.37	92.03	213.25
Average	52.64	178.68	122.5[illegible]	100.[illegible]	95.05	222.20

Figure [illegible]. The Nitrogen Content of Albumin in Chicken Embryos.

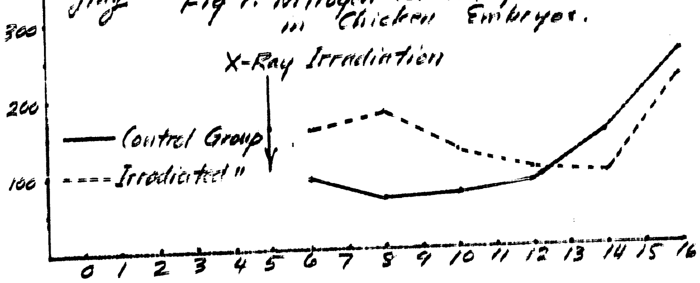


Fig 7. Nitrogen Content of Albumin in Chicken Embryos.

6. The Nitrogen Content of Globulin in Chicken Embryos, and the Influence of Irradiation by Hard X-Rays.

The control group and the irradiated group both show an increase as incubation progresses, but the irradiated group has a smaller increase than the control group; that is, irradiation causes the globulin content to decrease.

Figure 18. The Nitrogen Content (mg/dg) of Globulin in Chicken Embryos During Incubation (6th to 16th Day).

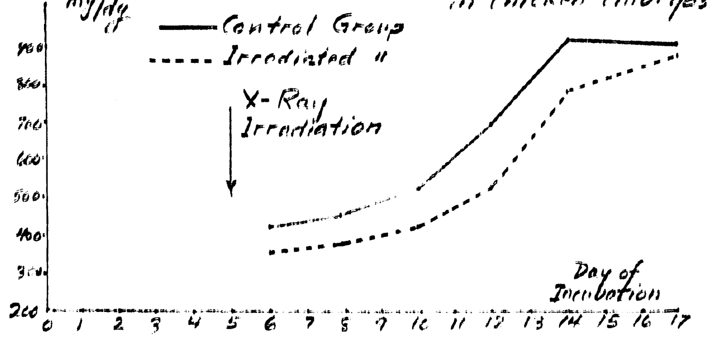
Groups	6th	8th	10th	12th	14th	16th
1st Group	443.37	482.02	507.16	686.49	910.65	896.64
2nd Group	402.95	435.84	528.25	698.25	905.62	892.49
Average	426.16	458.93	517.70	687.37	908.13	894.56

Table 19. The Nitrogen Content (mg/dg) of Albumin in Chicken Embryos Irradiated with Hard X-Rays During Incubation,

Groups	6th	8th	10th	12th	14th	16th
1st Group	357.25	364.26	432.38	535.61	770.55	882.63
2nd Group	346.85	387.23	415.26	510.51	763.49	855.21
Average	352.05	375.74	420.32	523.06	767.02	868.92

Figure 8. The Nitrogen Content of Globulin in Chicken Embryos.

Fig 8. Nitrogen Content of Globulin in Chicken Embryos



C. The Ratio of Albumin and Globulin in Chicken Embryos, and Variations Due to Irradiation.

The control group in the initial phases of incubation shows a gradual decrease in its albumin relative to globulin, but after the 14th day of incubation albumin gradually increases relative to globulin. In the irradiated group albumin possesses a greater rate of increase than globulin three days after irradiation, but later shows a gradual decrease; on the 16th day of incubation albumin increases markedly.

Table 20. The Albumin/Globulin Ratio in Chicken Embryos, For Various Days of Incubation (6 to 16th).

Groups	6th Day	8th	10th	12th	14th	16th
Control Group	0.217	0.147	0.137	0.124	0.160	0.283
Irradiated "	0.149	0.475	0.291	0.192	0.123	0.255

Art 5. The Contents of Albumin and Globulin in Incubating Chicken Embryos, and Influences Due to Hard X-Rays.

a. The Distribution of Albumin in Egg Contents, and the Influence of Hard X-Rays.

Table 21. The Distribution of Albumin Nitrogen (mg/dg) in Each of the Four Separate Substances Making up the Incubating Chicken Embryo, According to Day of Incubation.

	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	1232.	1350.	1397.	3146.	4260.	4426.	4443.	4416.	4215.
Egg Yolk	56.04	57.02	66.14	70.05	77.05	82.17	56.04	70.05	86.65
Embryo				91.94	67.71	71.20	85.65	146.18	253.48
Show				27.64	27.18	39.65	58.52	235.15	430.68
Total	1288.04	1407.02	1463.14	3335.63	4431.94	4619.02	4623.21	4734.89	4781.31

Table 22. The Distribution of Albumin Nitrogen (mg/dg) in Each of the Four Separate Substances Making Up Incubating Hen's Eggs Irradiated with Hard X-Rays, According to Various Days of Incubation (6 to 16th).

	6th	8th	10th	12th	14th	16th
Egg White	3238.	4212.	4469.	4220.	4193.	4016.
Egg Yolk	120.05	178.32	196.14	221.01	214.01	462.33
Embryo	152.64	179.68	122.58	100.79	95.05	222.20
Show	32.53	112.31	122.72	139.94	523.58	597.47
Total	3543.22	4681.31	4910.44	4681.74	5025.64	5298.00

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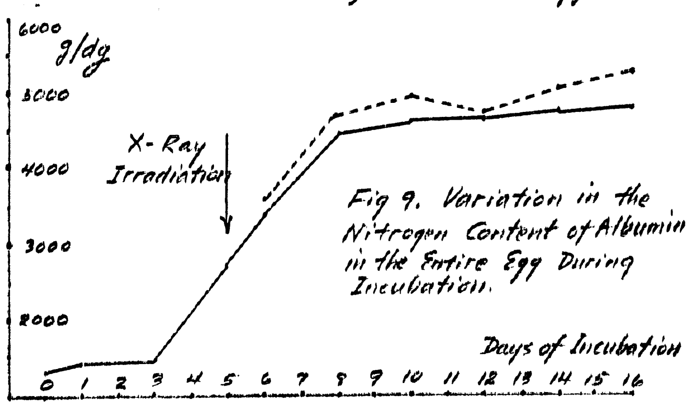
Table 23. The Albumin Content (g/dg) in Each of the Four Substances of Incubating Hen's Eggs, for Various Days.

	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	7.700	8.437	8.731	19.662	26.625	27.662	27.768	27.600	26.343
Egg Yolk	0.350	0.356	0.413	0.438	0.482	0.514	0.350	0.438	0.542
Embryo	-	-	-	0.575	0.423	0.445	0.535	0.914	1.584
Show	-	-	-	0.172	0.169	0.248	0.365	0.709	2.691
Total	8.050	8.793	9.144	20.847	27.699	28.869	29.317	29.661	31.160

Table 24. The Albumin Content in Each of the Four Substances of Incubating Hen's Eggs Irradiated with Hard X-Rays, for Various Days of Incubation (6th to 16th).

	6th	8th.	10th	12th	14th	16th
Egg White	20.238	26.325	27.931	26.375	26.206	25.100
Egg Yolk	0.750	1.115	1.226	1.319	1.338	2.890
Embryo	0.954	1.117	0.766	0.630	0.594	1.389
Show	0.203	0.702	0.767	0.874	3.272	3.734
Total	22.045	29.259	30.690	29.198	31.410	33.113

Figure 9. Variations in the Nitrogen Content of Albumin Belonging to the Entire Contents of a Hen's Egg During Incubation.



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The albumin content of egg white, egg yolk, show and embryo in an incubating egg shows almost no variation up to the 3rd of incubation, but several days thereafter it increases markedly. The irradiated group too shows an increase which is similar to that of the control group; moreover, it possesses a greater amount than the control group. In the above-mentioned four substances the egg white possesses most of the albumin; the embryo has the least, but towards the final stages it increases somewhat. It is very interesting that a comparatively large amount appears in the show in the second half of the incubation period.

b. The Distribution of Globulin in Egg and the Influence Due to Hard X-Rays.

The globulin content of the four mentioned substances in eggs decreases slightly up to the 8th day of incubation, but thereafter it increases gradually. The irradiated group increases gradually, but in comparison with the control group it there is hardly any marked variation. Among the mentional four substances, the egg yolk possesses the most globulin; but as it gradually diminishes, the globulin in egg white and embryo increases and causes a comparative increase in show too in the second half of incubation.

Table 25. The Distribution of Globulin Nitrogen (mg/dg) in the Four Substances of an Incubating Hen's Egg, According to Days of Incubation (0th to 16th).

	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	91.06	110.16	140.61	411.00	466.68	523.86	557.93	588.36	743.36
Egg Yolk	2208.	2185.	2009.	1119.	1029.	1217.	1433.	1462.	1581.
Embryo	-	-	-	423.16	458.93	517.70	687.37	908.13	894.56
Show	-	-	-	40.26	34.70	57.64	47.13	153.39	230.47
Total	2299.06	2295.16	2149.61	2038.42	1989.31	2316.70	2725.43	3112.88	3449.39

Table 26. The Distribution of Globulin Nitrogen (mg/dg) in Each of the Four Substances of an Incubating Hen's Egg Irradiated by X-Rays, for Various Days (6-16th).

	6th Day	8th	10th	12th	14th	16th
Egg White	369.70	519.44	629.05	633.95	726.57	636.44
Egg Yolk	897.	1142.	1176.	1232.	1432.	1529.
Embryo	352.05	375.74	420.32	523.06	767.02	865.92
White	47.69	59.94	52.15	54.06	221.47	343.65
Total	1666.44	2097.12	2307.52	2443.07	3147.06	3377.41

Table 27. The Globulin Content (g/dg) in Each of the Four Substances of a Hen's Egg During Incubation (0 to 16th Day).

	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	0.569	0.659	0.577	2.564	2.917	3.714	3.457	3.677	4.646
Egg Yolk	13.800	13.656	12.556	6.944	6.431	7.606	8.956	9.135	9.881
Embryo	-	-	-	2.645	2.565	3.236	4.296	5.676	5.591
Show	-	-	-	0.250	0.717	0.360	0.295	0.955	1.440
Total	14.369	14.345	13.435	14.724	12.433	14.476	17.034	19.449	21.555

Table 28. The Globulin Content (g/dg) in Each of the Four Substances of an Incubating Hen's Egg Irradiated with X-Rays, for Various Days of Incubation (6th to 16th Day).

	6th Day	8th	10th	12th	14th	16th
Egg White	2.311	3.247	3.932	3.962	4.541	3.978
Egg Yolk	5.606	7.138	7.350	7.700	8.950	9.556
Embryo	2.200	2.348	2.627	3.269	4.794	5.431
Show	0.298	0.375	0.513	0.525	1.384	2.141
Total	10.413	13.108	14.422	15.456	19.669	21.109

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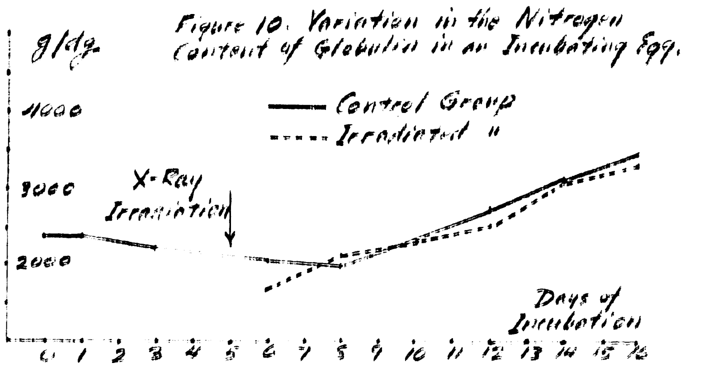
Figure 10. Variations in the Nitrogen Content of Globulin in the Entire Contents of an Incubating Hen's Egg.



c. The Ratio of Albumin and Globulin in an Egg, and the Influence Due to Hard X-Rays.

In the initial phases of incubation globulin is greater than albumin, but albumin gradually increases; on the 8th day of incubation the albumin/globulin ratio becomes large, and thereafter becomes gradually smaller, but albumin is larger than globulin. The albumin/globulin ratio becomes larger because of radiation than in the control group; with progress of incubation it becomes smaller, but as before albumin exceeds globulin.

Table 27. The Albumin/Globulin Ratio in an Entire Egg, for Various Days of Incubation (0th to 16th Day).

Groups	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Control	0.560	0.613	0.661	1.636	2.227	1.994	1.696	1.521	1.386
Irradiated			[illegible]	2.126	2.232	2.128	1.870	1.596	1.568

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Sec 4. Summary and Remarks.

Let us summarize the results of the above experiments. The nitrogen of albumin in egg white increases markedly with progress of incubation; there are no noteworthy variations due to hard X-ray irradiation in the initial period after irradiation, but starting about the 4th day after irradiation it shows a tendency to decrease slightly more than the control group. The reason that one cannot notice any notable changes in total nitrogen due to irradiation is that the albumin decreases and the globulin increases. The albumin/globulin ratio, moreover, becomes gradually smaller because of irradiation, but on the 16th day it becomes somewhat larger; egg white always contains more albumin than globulin.

The albumin nitrogen of egg yolk decreases somewhat during the middle phases as incubation progresses; otherwise, no noteworthy variations are apparent. The irradiated group clearly shows an increase directly after irradiation; thereafter it gradually continues to increase, especially towards the final phases of incubation, when it is rapid and sudden — that is, X-rays in general cause remarkable increases.

The globulin nitrogen of egg yolk decreases noticeably in the initial phases of incubation, but gradually increases after the middle phases; the irradiated group shows an increase with progress of incubation and increases slightly more than the control group with each day of incubation, but the fact that the total nitrogen content in the irradiated group is greater than in the control group is considered to be the result of an increase in the nitrogen of albumin.

Moreover, the albumin/globulin ratio A/G becomes considerably larger, because of irradiation, each day of incubation and the albumin of egg yolk increases noticeably more than the globulin because of irradiation, but in the egg yolk the albumin is always less than the globulin.

The albumin nitrogen of show has a small rate of increase in the first half of incubation, but increases considerably in the second half; the irradiated group reveals a noticeable increase after the 2nd day of irradiation and a very rapid increase much greater than in the control group during the second half of incubation. On every day of incubation it is greater than in the control group. The globulin nitrogen of show, like albumin, reveals no noteworthy variations during the first half period, but in the second half increases rapidly; the irradiated group also, like the control group, increases with the progress of incubation — moreover it is always greater than the control group. The fact that both are greater in the irradiated group proves that the total nitrogen content increases because of irradiation. Further, the albumin/globulin ratio in general becomes larger in the irradiated group — that is, we can see that albumin has a greater rate of increase than globulin; moreover albumin is less than globulin in the first half period in the case of the control group, but in the second half period albumin is greater in amount. We see that in the irradiated group the albumin is always greater except the 1st day after irradiation.

The albumin nitrogen of chicken embryo discloses no noteworthy variations in the first half of incubation, but gradually increases in the second half: Two, three days after irradiation a considerable increase is observable due to irradiation, but there is a gradual decrease with progress of incubation; then on the 6th day after irradiation there is again a rapid increase. The nitrogen of globulin gradually increases in both groups, control and irradiated; the control group is always less. Moreover, the albumin/globulin ratio reveals no regularity as to magnitude with the progress of incubation, but we can say that albumin is always less than globulin.

The albumin of egg white, egg yolk, show, and embryo during the incubation process increases considerably after the middle period of incubation; no remarkable variations due to irradiation are evident but in general there seems to be somewhat of an increase. However, globulin gradually decreases up to the 8th of incubation and thereafter increases. It is difficult to recognized any noticeable variations of the irradiated group from the control group; although the irradiated group decreases one cannot perceive any increases. The albumin/globulin ratio of the irradiated group becomes smaller with progress of incubation, but we know that albumin is smaller than globulin each day of incubation.

As for the results of the author's experiments, the influence of hard X-rays on total nitrogen has already been discussed in detail in Chapter Two. Moreover, it was proved that most of the nitrogenous substance in egg yolk is albumin and globulin. We considered how these two proteins are utilized in the metabolism of chicken embryos and how these are influenced by X-rays and found that albumin in egg white is consumed somewhat in large amounts 5 days after irradiation and that, in the case of egg yolk, the utilization of protein by the chicken embryo is markedly impaired immediately after irradiation and consequently fetal development also is impaired. The albumin in show increases because of irradiation but its mechanism is not clear; there can be no real explanation as long as the origin of the show is unknown. Next, the albumin in the various tissues composing the chicken embryo was found to decrease markedly because of irradiation; we were able to perceive a tendency to return to the former state as time passes.

Globulin in egg yolk shows no marked changes due to X-ray irradiation, and that in egg white is but little consumed. The appearance of a large amounts in the show is not explained, but as mentioned previously the

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production of show is to be considered a very interesting problem. The decrease in globulin in chicken embryos accounts for decrease in total nitrogen, as discussed in Chapter Two.

To continue, the globulin in the entire egg reveals almost no variation due to X-ray irradiation; except a slightly perceptible decrease; but albumin increases somewhat and consequently we can assert that the total nitrogen increases somewhat because of irradiation.

Sec 5. Conclusions.

The author has been able to prove experimentally in Chapter Two that hard X-ray irradiation causes variations in the total nitrogen content during irradiation; therefore in our investigations into how albumin and globulin are influenced by X-rays we can make the following conclusions:

1. In egg white, the albumin decreases slightly on about the 5th day after irradiation in comparison with the control group and the globulin tends to increase somewhat more than the control group starting the 3rd day after irradiation; consequently the albumin/globulin ratio becomes smaller than that of the control group, but on the 16th day of incubation it becomes somewhat larger — albumin, however, is always larger than globulin.

2. In egg yolk, albumin is considerably larger than in the control group, but globulin is somewhat smaller than in the control group or almost equal; consequently the albumin/globulin ratio A/G increases markedly. However, albumin is less than globulin.

3. In show, both albumin and globulin are almost more than in the control group; moreover, A/G is generally greater than in the control group — that is, albumin increases more than globulin.

4. The albumin in chicken embryos increases in the initial phases of irradiation markedly more than in the control group, but then it decreases and moreover becomes less than in the control group. Globulin is always less than in the control group; moreover, A/G increases in the initial phases after irradiation as a result of the increase in albumin, but becomes smaller in the final phases — albumin is always less than globulin.

5. The albumin in egg white, egg yolk, show, and embryo in eggs generally increases somewhat because of irradiation; globulin is less than in the control group, but does not become large. Consequently the albumin/globulin ratio A/G is always larger than in the control group; moreover, albumin is always more than globulin.

— End of Chapter Three —

Chapter Four. Influence of Radiation on Residual Nitrogen Content.

Sec 1. Introduction.

I have previously examined the influence, upon total nitrogen content, of hard X-ray irradiation of incubating chicken embryos and reported that the nitrogen content in egg white, show and embryo increases in this case with the progress of incubation and that the nitrogen content of egg yolk decreases gradually in the initial phases of incubation but gradually increases after the middle phases.

In this chapter, proceeding one step further, we shall try to determine the residual nitrogen content during the incubation process and to investigate the metabolic state.

Let us review the results of studies on residual nitrogen. First, K. Yamada (1) pointed out the remarkable fact that the total nitrogen in the blood of chicken embryos drops considerably whereas the residual nitrogen is greater than that in adult animals. Wladimiroff and Schmidt (2) reported that in the second half of development the residual nitrogen is almost fixed and moreover large; this is said to be due to the active composition and decomposition of the egg white.

Schennert and Pelchrzin (3) reported that the residual nitrogen of chicken embryo's blood is 26.3 to 46.2 mg %. M. Tomita (4) discovered that the residual nitrogen in fresh egg white and egg yolk is small and increases during incubation.

Y. Nakamura (5) found that during incubation the residual nitrogen in egg yolk and chicken embryo increases noticeably. J. Needham (6) reported that the residual nitrogen content of bird embryos during development reaches its maximum in the 5th and 13th day of incubation.

As seen above, many have observed the general increase during incubation. The author too has reëxamined the problem of residual nitrogen during incubation. It would be interesting to study the variations in the residual nitrogen content when embryos are subjected to hard x-rays. Accordingly the author carried out experiments on successive days with doses of hard x-rays of 200 and 600 gammas.

Sec 2. Procedure.

The materials used, irradiation conditions, and technique have been described already in Sec 2 of Chapter One.

The quantitative method used is the same as the method already described in Chapters Two and Three. Equal amounts of each of the egg substances found in egg contents are obtained and diluted with a 1% saline solution; then deproteinization is carried out by an improved version of Folin and Wu's (7) method for removing protein — that is, the egg white is diluted 12 times and the egg yolk is diluted 6 times. The chicken embryo too is diluted 6 times and is left for 30 minutes in cold storage, and the filtrate is used.

~~To~~ 12 cc of each of the above-mentioned dilute solutions are added 4cc each of 10% sodium tungstate and 2/3 N sulfuric acid to make a protein-free solution. Thus these dilute solutions become 20 fold in egg white and 10 fold in egg yolk, show, and embryo. A definite amount of this protein-free solution is taken and quantitatively analysed according to J. Bang's (8) micro-Kjeldahl nitrogen determination method.

Sec 3. Results.

Art 1. Influence of Hard X-rays and Residual Nitrogen Content of Egg White.

Table 1. Residual Nitrogen Content (mg/dg) of Egg White During Incubation (0 to 16th Day).

Groups	0 th	1 th	3 rd	6 th	8 th	10 th	12 th	14 th	16 th
Group I	14.25	17.28	18.55	32.57	35.34	28.02	23.15	30.45	27.31
Group II	12.95	15.36	19.03	30.24	33.58	25.31	22.58	31.26	26.95
Group III	13.63	17.92	19.00	28.95	36.00	25.48	22.65	29.67	26.80
average	13.61	16.85	18.82	30.58	34.97	26.27	22.79	30.46	27.01

Table 2. Residual Nitrogen Content (mg/dg) in Egg White Irradiated with Hard X-rays (600 r.) During Incubation.

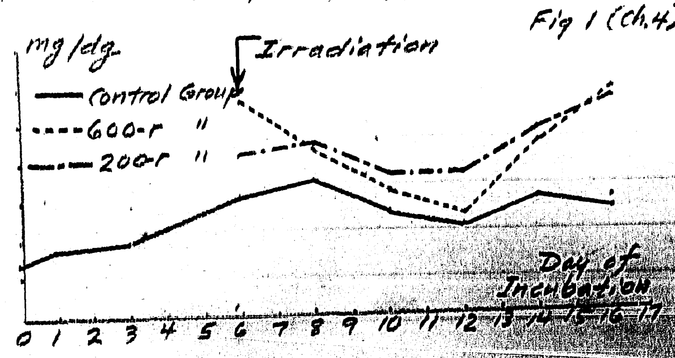
Groups	6th day	8th	10th	12 th	14th	16 th
Group I	58.02	44.02	33.77	26.42	42.25	58.05
Group II	52.55	42.21	30.55	25.01	44.59	59.63
Group III	56.00	42.05	31.28	25.20	46.05	57.04
average	55.52	42.76	31.83	25.43	44.29	58.24

Table 3. Residual Nitrogen Content (mg/dg) in Egg White Irradiated with Hard X-rays (200 r.) During Incubation.

Groups	6th day	8th	10th	12th	14th	16th
Group I	42.53	45.00	39.76	35.25	47.45	57.40
Group II	40.28	44.74	40.00	37.45	48.04	56.42
Group III	41.55	43.75	38.45	36.74	48.92	55.75
average	41.45	44.49	36.07	36.44	48.00	56.52

Figure 1. Residual Nitrogen of Egg White

Fig 1 (ch.4)



1. The Control Experiment:

In the initial period of incubation the content gradually increases somewhat, but towards the middle period it decreases slightly; on the 14th day of incubation it again increases and thereafter it tends to decrease. In general the residual nitrogen is small.

2. Influence of Hard X-Rays:

In the group irradiated with 200 gammas, the content increased the day after irradiation considerably more than the control group; the 3rd day after irradiation it increased greatly but decreased slightly in the mid-phases. Towards the final phases of incubation it increased and each day was considerably larger than the control.

The group irradiated with 600 gammas increased the day after irradiation considerably more than the control group or the 200-gamma group; thereafter it gradually decreased — moreover it decreased more than the 200-gamma group. In the second half of incubation the content increased with progress of incubation; on the 16th day of incubation it exceed that of the 200-gamma group.

Art 2. The Residual Nitrogen Content of Egg Yolk, and the Influence of Hard X-Rays.

Table 4. The Residual Nitrogen Content (mg/dg) of Egg Yolk During Incubation (0 to 16th Day).

	0	1	3	6	8	10	12	14	16
Group I	88.37	87.21	78.26	69.64	67.50	64.12	70.20	72.32	63.76
Group II	87.92	86.53	79.00	68.95	66.21	62.50	68.51	70.25	64.50
Group III	87.85	86.00	78.31	68.25	66.00	63.58	66.32	69.84	65.75
average	88.04	86.58	78.52	68.94	66.57	63.70	68.35	70.80	64.33

Table 5. The Residual Nitrogen Content (mg/dg) in Egg Yolk Irradiated (600 r.) with Hard X-Rays During Incubation.

	6th day	8th	10th	12th	14th	16th
Group I	93.33	69.54	74.99	80.87	98.49	121.30
Group II	90.25	70.00	75.00	82.05	98.50	120.84
Group III	88.65	70.22	74.80	82.31	97.85	120.65
average	90.74	69.93	74.93	81.76	98.26	120.93

Table 6. The Residual Nitrogen Content (mg/dg) in Egg Yolk Irradiated (200 r.) During Incubation.

	6th day	8th	10th	12th	14th	16th
Group I	79.59	68.50	71.27	77.78	81.76	102.28
Group II	78.94	68.50	70.85	76.95	82.55	98.44
Group III	78.95	68.34	70.69	77.00	82.45	99.02
average	79.16	68.44	70.93	77.24	82.25	99.91

Figure 2. Residual Nitrogen of Egg Yolk (Note: The irradiation time in the figure is in error; see below.)

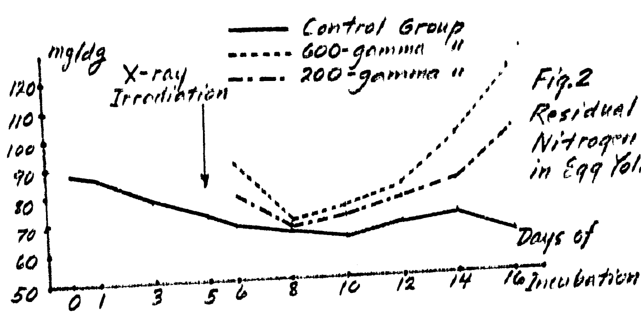


Fig. 2 Residual Nitrogen in Egg Yolk.

1. The Control Experiment:

The content fluctuated slightly with progress of incubation, but in general it decreased.

2. The Influence of Hard X-Rays:

The 200-gamma and 600-gamma groups increased considerably the day after irradiation, and on the 3rd day of irradi-ation decreased and approached the control group; thereafter, however, the content increases with progress of incubation. Moreover, each day of incubation the irradiated groups show a greater content than the control group and the 600-gamma group always exceeded the 200-gamma group.

Art 3. The Residual Nitrogen Content of Show, and the Influence of Hard X-Rays.

Table 7. The Residual Nitrogen Content (mg/dg) of Show During Incubation (6th to 16th Day).

	6th	8th	10th	12th	14th	16th
Group I	28.57	29.85	34.42	50.30	67.54	93.38
Group II	28.45	29.64	35.31	49.47	67.42	92.57
Group III	28.41	29.50	35.00	49.81	67.51	93.56
average	28.47	29.66	34.91	49.86	67.49	93.17

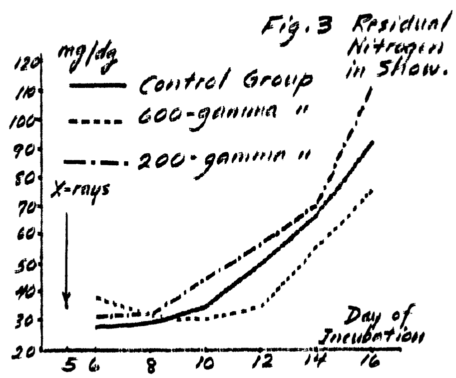
Table 8. The Residual Nitrogen Content (mg/dg) in Show Irradiated with Hard X-Rays (600 r.) During Incubation.

	6th	8th	10th	12th	14th	16th
Group I	38.18	31.66	30.53	35.18	56.58	78.22
Group II	38.25	31.54	30.47	33.45	57.45	75.43
Group III	38.41	31.60	30.48	34.64	56.47	74.97
average	38.38	31.60	30.49	34.62	56.83	76.20

Table 7. The Residual Nitrogen Content (mg/dg) in Show Irradiated with Hard X-Rays (200 r.) During Incubation.

	6th day	8th	10th	12th	14th	16th
Group I	30.52	32.26	45.25	57.54	71.76	116.36
Group II	31.24	32.56	44.96	57.00	71.63	112.34
Group III	31.15	32.67	45.00	56.97	71.45	110.57
average	30.97	32.49	45.07	57.17	71.61	113.09

Figure 3. Residual Nitrogen in Show.



1. The Control Experiment.

The content increases with progress of incubation and becomes on the 16th day of incubation about 3 times that of the 6th day.

2. Influence of Hard X-Rays.

The 200-gamma group increases gradually with progress of incubation and is always greater than the control. The 600-gamma group on the day after irradiation increases more than the control and 200-gamma groups, but gradually decreases; on the 5th day after irradiation it becomes smaller than the other 2 groups. Thereafter it increases considerably with incubation, but is less than the control and 200-gamma groups.

Art 4. The Residual Nitrogen Content of the Chicken Embryo, and the Influence of Hard X-Rays.

Table 10. Residual Nitrogen Content (mg/dg) of Chicken Embryo During Incubation (6th to 16th Day).

	6th day	8th	10th	12th	14th	16th
Group I	81.76	78.17	65.03	76.34	89.36	130.07
Group II	81.54	77.85	67.24	75.85	88.57	129.53
Group III	81.98	77.92	67.05	75.91	88.88	129.71
average	81.76	77.94	66.44	76.03	88.94	129.77

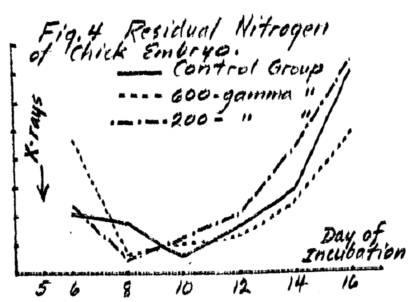
Table 11. Residual Nitrogen Content (mg/dg) in Chicken Embryo Irradiated with Hard X-Rays (600 r.) During Incubation.

	6th day	8th	10th	12th	14th	16th
Group I	108.32	65.24	69.25	73.17	83.74	109.34
Group II	108.00	66.81	70.34	73.25	82.54	108.54
Group III	108.52	66.27	70.46	74.00	82.96	108.73
average	108.28	66.10	70.16	73.47	83.80	108.86

Table 12. Residual Nitrogen Content (mg/dg) in Chicken Embryo Irradiated with Hard X-Rays (200 r.) During Incubation.

	6th day	8th	10th	12th	14th	16th
Group I	83.47	65.41	71.45	80.94	99.48	132.47
Group II	82.96	65.32	72.31	81.40	103.78	135.04
Group III	83.54	65.42	72.34	81.00	110.21	134.52
average	83.32	65.38	72.03	81.11	104.49	134.01

Figure 4. Residual Nitrogen of Chicken Embryo.



1. Control Experiment.

In the first half of incubation the content decreases, but towards the second half it increases considerably with time.

2. Influence of Hard X-Rays.

In the 200-gamma group the content decreases on the 3rd day after incubation, but thereafter it increases with time; the content is always slightly greater than that of the control group, but proceeds comparatively parallel with the control group's progress. The 600-gamma increases noticeably the day after irradiation, but the 3rd day after irradiation it is almost equal to the 200-gamma group; thereafter it gradually increases, but is smaller than the control group.

Art 5. The Residual Nitrogen Content of the Egg's Content During Incubation, and the Influence of Hard X-Rays.

Table 13. Residual Nitrogen Content (mg/dg) in Each of the Four Substances Making up the Chicken Egg, During Incubation (0 to 16th Day).

	0th	1st	3rd	6th	8th	10th	12th	14th	16th
Egg White	13.61	16.85	18.82	30.58	34.97	26.27	22.79	30.46	27.01
Egg Yolk	88.04	86.58	78.52	68.94	66.57	63.70	68.35	70.80	64.33
Embryo				81.76	77.94	66.44	76.03	88.94	129.77
Show				28.47	29.66	34.91	49.86	67.49	93.17
Total	101.65	103.43	97.34	209.75	209.14	191.32	217.03	257.69	314.28

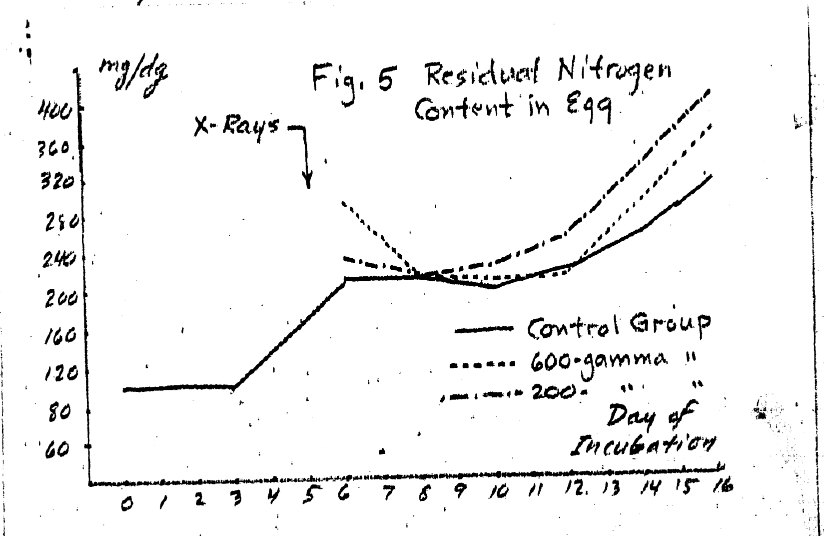
Table 14. Residual Nitrogen Content (mg/dg) in Each of the 4 Substances of an Incubating Chicken Embryo Irradiated with Hard X-Rays (600 r.).

	6th day	8th	10th	12th	14th	16th
Egg White	55.52	42.76	31.83	25.43	44.29	58.24
Egg Yolk	90.74	69.93	74.93	81.76	98.23	120.93
Embryo	108.28	66.10	70.16	73.47	83.80	108.86
Show	38.38	31.60	30.49	34.62	56.83	76.20
Total	292.92	210.39	207.41	215.28	283.15	364.23

Table 15. Residual Nitrogen Content (mg/dg) in Each of the 4 Substances of an Incubating Chicken Embryo Irradiated with Hard X-Rays (200 r.).

	6th day	8th	10th	12th	14th	16th
Egg White	41.45	44.49	36.07	36.44	48.00	56.52
Egg Yolk	79.16	68.44	70.93	77.24	82.65	99.91
Embryo	83.32	65.38	72.03	81.11	104.49	134.01
Show	30.94	32.49	45.07	57.17	71.61	113.09
Total	234.87	210.80	224.10	251.96	306.75	403.53

Figure 5. The Behavior of the Residual Nitrogen in the Egg's Contents During Incubation.



The sum of the residual nitrogen contents in the contents of an incubating egg (namely, egg white, egg yolk, show, and embryo) does not change in the initial phase of incubation, but increases considerably on about the 6th day of incubation; in the middle phases of incubation this sum shows no noteworthy variation, but in the second half of incubation it increases gradually.

The 200-gamma group showed a slight increase in its residual nitrogen content the day after irradiation; on the 3rd day after irradiation it was similar to the control group — thereafter it increased with time and moreover increased considerably more than the control group.

The 600-gamma group showed a considerably greater increase than the control group the day after irradiation, but thereafter decreased with progress of time and became almost like the control group; in general the 600-gamma group did not show a smaller increase than the 200-gamma group.

Sec 4. Summary and Remarks.

The above results of the experiments can be summarized as follows:

The residual nitrogen in egg white increases gradually in the initial phases of incubation and reaches a maximum on the 8th day of incubation; thereafter it decreases slightly. In general the irradiated groups show a greater amount of residual nitrogen than the control group on any day of incubation; in the 200-gamma group the content fluctuates comparatively parallel to that of the control group, but increases considerably in the final phases of incubation. The 600-gamma group shows a considerable increase the day after irradiation and a decrease up to the 12th day of incubation; thereafter its content gradually increases. That is, the 600-gamma group reveals a noteworthy fluctuation in its residual nitrogen content.

The residual nitrogen content in egg yolk generally decreases slightly with progress of incubation. The irradiated groups show a noticeably greater increase than the control group the day after irradiation, and approach the control group on the 3rd day after irradiation; thereafter they again show an increase. Also, the 600-gamma group varies much more than the 200-gamma; moreover, the irradiated groups generally possess a greater residual nitrogen content than the control group for any day of incubation.

The residual nitrogen content in the show increases markedly with the progress of incubation. The 200-gamma group also increases in similar manner parallel with the control group; moreover the 200-gamma group always has a greater content than the control group. The 600-gamma group increases its content slight the next day after irradiation, but thereafter decreases; towards the second half of incubation it again increases noticeably and shows a tendency to return to its former position, but in general its content is small.

The residual nitrogen content of chicken embryo decreases slightly in the first half of incubation and increases very rapidly in the second half. The 200-gamma group shows a decrease on the 3rd day of irradiation and thereafter an increase paralleling that of the control group; moreover it is slightly greater than that of the control group. The 600-gamma group shows a noticeable increase on the next day after irradiation and a decrease on the 3rd day; thereafter its content again increases gradually, but is less than that of the 200-gamma group.

As for the four above-mentioned egg

substances, the 200-gamma group fluctuates with values that are comparatively close to those of the control group and moreover generally shows greater values than the control group; but the 600-gamma group shows a considerable fluctuation and movement in its residual nitrogen in comparison with the control group and moreover, in the case of egg white and egg yolk, has greater contents generally than the control group — and in the case of show and embryo the 600-gamma group reveals a considerable increase on the day following irradiation and then a decrease and thereafter shows a tendency to increase with progress of incubation, but its contents are smaller than those of the control group. That is, mild irradiation does not damage markedly the nitrogen metabolism in chicken embryoes, but strong radiation does obviously impair greatly nitrogen metabolism.

Let us consider the sum of the residual nitrogen contents of these 4 substances in egg. In the initial phases of incubation one does not observe marked changes, but on the 6th day of incubation these contents increase noticeably and thereafter barely fluctuate; however in the second half of incubation they again increase. The 200-gamma group shows a an increase with time that parallels that of the control group; moreover it has a slightly greater content than the control group. The 600-gamma group clearly shows a greater increase than the control group on the day following irradiation and then it shows a decrease and resembles the control group; in the second half the 600-gamma group increases its content, but lies midway between the 200-gamma group and the control group. As for the entire egg, the 600-gamma group's residual nitrogen fluctuates, thus revealing the considerable movement of residual nitrogen within the egg.

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— End of Chapter Four —

[Note: Chapter Five is not translated since it was not included in the photostatic copies; its Table of Contents is given, however.]

— End —