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The Effect of Ultra-Violet Rays on the Dermatitis Preventing Vitamin

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The Effect of Ultra-Violet Rays on the Dermatitis Preventing Vitamin

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Abstract.—It is now definitely established that the substance formerly described as vitamin B is in reality a mixture of at least two vitamins. The one now known as vitamin B prevents polyneuritis, the other, vitamin G, prevents dermatitis. Attempts in the past to produce experimental dermatitis have met with varying degrees of success, so an effort was made to evolve a technique that would produce the experimental disease consistently. The results now available indicate this has been accomplished. The most important part of this technique is the use of irradiation with a quartz-mercury arc to destroy the antidermatitis factors, though other features may also be of some significance. Forty-three animals received a ration, presumably free from vitamin G, beginning at weaning time, and were maintained on this continuously as long as they survived. Their gains in weight were insignificant. All developed dermatitis and the average time required, after weaning, for the symptoms to appear, was 34 days. The average survival period, after weaning, was 51 days.

In 1928 Hogan and Hunter[1]† reported that one of the substances then recognized as belonging to the vitamin B complex, usually designated now as vitamin G, is destroyed by irradiation with a quartz mercury arc. Several workers tried to repeat these observations, and though most of them were unable to do so, a few investigators did observe some destruction. As it seemed clear that uncontrolled factors, whose importance was not recognized, were responsible for the discrepancies an effort was made to improve the experimental procedure. It is believed that the variability encountered in the past can be eliminated by using the technique to be described, and that destruction of at least one member of the vitamin B complex by ultra-violet irradiation can be demonstrated with certainty. If this procedure should prove equally effective in the hands of other workers, it should be useful in extending our knowledge of vitamin G, and of other members of the vitamin B complex whose existence is now uncertain. Their identity, distribution, properties, and physiological functions each present difficult problems which will remain largely unsolved until more suitable techniques are developed.

HISTORICAL

The literature on vitamins up to 1930 has been thoroughly reviewed in the excellent monograph of Sherman and Smith.[2] It is unnecessary therefore to describe the earlier investigations of the vitamin B complex in detail, and only so much will be given now as has an immediate bearing on the data we are to report.

*The data presented in this paper formed part of a thesis presented by Luther R. Richardson in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of the University of Missouri, 1932.

†Reference by number to "Bibliography", page 18

In 1925 vitamin B was commonly regarded as a single substance, though various physiological functions had been ascribed to it. Apparently only a few workers had considered seriously the possibility that this vitamin might be multiple in nature until Mitchell[3] published a critical review of the literature. Since then a number of investigators have established definitely that the old vitamin B is a complex.

Smith and Hendrick[4] reported that a diet adequate in all respects other than the water-soluble vitamins is not made complete by either the inclusion of 40 per cent of the oat kernel, or by the addition of 1 to 2 milligrams of Seidell's[5] vitamin B picrate. If autoclaved yeast were also added, thus supplementing either the cereal or the picrate, the rations became entirely adequate though autoclaved yeast alone is ineffective. These observations indicate that what was then known as vitamin B is a mixture of at least two substances, and that one is supplied by autoclaved yeast, the other by either Seidell's picrate or by the oat kernel. This conclusion has been abundantly confirmed and since then American workers designate one of them, the antineuritic vitamin, as B, the other (or one of the others) as G. More recently a considerable body of evidence has accumulated which indicates that the vitamin B complex contains more than two factors, and Peters[6] argues that there are at least five. Inasmuch as the number of factors in the vitamin B complex is uncertain, it is impossible to decide definitely what physiological function should be assigned to any one of them. By common consent, however, definite symptoms have been ascribed to a deficiency of two members of the complex, B and G.

Symptoms Due to Deficiencies of Vitamins B and G

According to Sherman and Smith[2] the symptoms of vitamin B deficiency are a loss of appetite, which leads to a decreased food consumption and finally death from starvation, without definite polyneuritic symptoms. If a measurable but insufficient amount of vitamin B is included in the ration, the animals make larger initial gains. These gains are soon followed by loss in weight and development of typical symptoms of polyneuritis, such as head retraction, a tendency to roll over and over, a spastic gait, and a highly excitable nervous condition.

The symptoms of the pellagra-like condition produced in rats due to vitamin G deficiency, according to Goldberger and Lillie[7] are; "After a variable period following the arrest of growth already mentioned, there has been observed in many of the animals so fed" (80 per cent alcoholic extract of white corn as source of B) "a tendency for the lids of one or both eyes to adhere together with, in some instances, an accumulation of dried secretion on the margins of the lids. At about the time or shortly after the appearance of this ophthalmia there has developed in

nearly, if not quite, every one of the animals on the indicated diets, some loss of fur. This fur loss has in some begun in irregularly distributed patches. More commonly it has been observed to begin either at the side or over the top of the head, the sides or front of the neck, or in the region of the shoulders. From these initial sites the depilation has extended and in some of the animals has led to almost complete denudation of the head, neck, and trunk. The initially affected sites and, in the early stages, the areas involved by the spreading depilation have, in many of the animals, been sharply delimited and bilaterally symmetrical.

"With or without such loss of fur some of the animals have developed a dermatitis at one or more of the following sites: Ears, front of neck and upper part of chest, fore arms, backs of forepaws, shins, and the backs of the hind paws. This dermatitis, particularly as it has affected the paws, fore arms, neck, and ears, has been sharply outlined and bilaterally symmetrical. To the eye it has differed somewhat with the site affected. The ears seemed definitely reddened and thickened with what appeared to be a yellowish incrustation of dried serum. In healing, desquamation took place, leaving the skin of the pinna with a polished, glistening, somewhat parchment-like appearance. In one animal in which the dermatitis involved an extensive butterfly shaped area on the front of the neck and upper part of the chest, the affected skin was red and, at first, apparently superficially eroded and moist, then, like the ears, became dry, incrusted and rough. In the cases in which the backs of the forepaws were affected, the skin was red and rough and, after healing, but before the renewal of the normal fine, silky fur, the skin had a pale pink, glistening, new-skin appearance. The backs of the hind paws, when affected, presented at first an appearance as of a matting of the silky fur of this part, which then looked dull and thickened. Later this matted layer of fur began to fissure and to crack and then gradually desquamated, leaving a denuded pale pink, glistening skin. The shortest period so far recorded within which this dermatitis has appeared has been approximately seven weeks. In a few of the cases so far observed, the affected animals have presented a linear fissuring or ulceration at the angles of the mouth. In a somewhat larger number there has occurred a lesion at the tip of the tongue, which first appeared as a small, roughly circular, grayish opacity or bleb, or as an ulceration which, in some, went on to the formation of a localized yellowish slough. In one of such animals there was evidence also of an inflammation of the anterior part of the floor of the mouth. In two, diarrhea was present."

These authorities do not give either the time required for the symptoms to develop, or the percentage of animals affected, but state that after some initial growth the animals, sooner or later, declined in weight. None of the rats developed polyneuritis.

Experimental Dermatitis of the Rat

Chick and Roscoe[8] reported the development in 6 to 8 weeks of dermatitis of rats which had been on a vitamin G deficient diet. In some of their later work[9] skin symptoms did not develop, so they made an effort to improve their technique. The casein of the experimental diet was specially purified by dissolving and reprecipitating, then washing with acidulated water and finally by extraction with acid alcohol. After 12 weeks not one of the rats receiving the commercially purified casein showed any skin symptoms although two had poor coats. Five of 7 rats that received the specially purified casein displayed typical skin symptoms and none made any significant gain in weight. Even though better results were obtained with the more highly purified casein, the symptoms developed at irregular periods, from 8 to 15 weeks, and in some cases did not appear at all.

Chick and Copping[10] found that increase in weight of the animals deprived of the anti-dermatitis vitamin usually ceases within a few days, but the time required for development of dermatitis varies greatly. Sometimes it appears in 6 to 8 weeks, but on other occasions 20 or more weeks are required. The failure to grow and the development of a generalized dermatitis was more constant with the highly purified caseinogen, but the skin lesions such as inflammation of tips of ears, and oedamatous inflammation of the digits of the paws did not develop. They think that a small but inadequate amount of the anti-dermatitis vitamin is necessary for the production of these more florid symptoms. Aykroyd and Roscoe[11] used the method of Chick and Roscoe and reported that 9 of 15 young rats, deprived of the antidermatitis vitamin, developed symmetrical skin lesions. The shortest time required was 5 weeks, the longest was 22, and the average was 10.

Aykroyd[12] found that 60 per cent of the animals deprived of vitamin G developed symmetrical dermatitis. However, in later work only 2 of 11 rats that received the vitamin G deficient diet developed those symptoms. The first case developed in 11 weeks, the second in 16, and the other animals survived for 18 to 20 weeks without developing the characteristic lesions.

Findlay[13] reported that a majority of the young rats on a diet deficient in vitamin G developed a characteristic dermatitis, in an average time of 70 days. Others remained free of any unusual symptoms even after 15 to 20 weeks. In a few cases, if the symptoms were not too severe, complete recovery was observed.

Leader[14] investigated the possibility that an alcoholic extract of marmite might serve as a source of vitamin B, and yet contain negligible amounts of G. Of 6 rats which received 0.5 cc. of the extract 5 developed

mild skin lesions, but all made good growth. Of 14 rats which received 0.25 cc. of the extract 9 developed severe skin lesions in 17 to 25 days. Those that developed the pellagra-like condition made a fair gain in weight, somewhat more than was made by those which did not develop the symptoms. The basal diet contained 17 per cent of sucrose, and if this was replaced with starch pellagrous symptoms did not develop. On the other hand doubling the amount of sucrose did not hasten the onset of symptoms, or increase the percentage of animals that developed lesions. A distinct seasonal variation was also reported. During the winter 3 months were required for the lesions to appear while at other seasons symptoms were observed in 3 weeks.

Sherman and Sandel[15] have found that if diets are extremely deficient in vitamin G, dermatitis may develop around the eyes, mouth, and nose as early as the 5th week, but usually much longer periods are required. If there was a measurable but restricted amount of vitamin G in the diet there was no development of superficially visible lesions within 8 weeks. Eventually, however, the animals which were retained longer developed the pellagra-like condition with sufficient regularity to leave no doubt that this should be regarded as a characteristic "nutritional deficiency" condition. In their experience the pronounced "pellagra-like" condition, with red inflamed paws or saddle-like areas of baldness and dermatitis on the shoulders or back, is more characteristic of cases in which the deprivation of vitamin G is not complete, or is delayed, so that the survival period is lengthened. A loss of appetite was more characteristic of the lack of vitamin B than of the lack of vitamin G.

Sure et al.[16, 17a, 17b, 18], used the following technique to produce dermatitis. Young rats, 4 to 5 weeks old, were placed on the basal diet until depleted of the vitamin B complex, which usually took 2 to 4 weeks. After the cessation of growth, or after decline in body weight, the rats received in addition to the basal diet 500 mg. of irradiated rice polishings to supply vitamin B. Forty-two per cent of the animals thus treated developed dermatitis in 36 to 50 days following the administration of the irradiated rice polishings. This was the most frequent symptom observed with the exception of failure of continued growth, and loss of body weight. Loss of weight and the appearance of dermatitis did not always occur together. Some animals made good growth and at the same time had severe dermatitis, on the other hand, cessation of growth without any skin lesions was common. The appearance of these lesions was hastened by irradiating the casein. A seasonal variation was observed by these workers also. Of 12 animals on the vitamin G deficient diet during the spring and summer months, 9 developed dermatitis, whereas only 1 of 18 animals developed the disease from September to May.

Bing and Mendel[18] studied the vitamin B and G requirements of the albino mouse. Every animal that received tikitiki alone, in addition to the basal ration, developed pellagra-like lesions. These symptoms, although there was little growth, developed only after long periods, and they believe this long survival period may have been due to the presence of small amounts of vitamin G in the tikitiki preparations.

The elusive character of the pellagra-like disease is also evident from a report by Kon and Watchorn[20]. One of their rats received a basal ration containing rice starch, and in addition 50 mg. daily of Harris yeast vitamin concentrate as a source of vitamin B. Growth ceased and pellagra developed after 7 weeks on this ration. Potato starch was then substituted for rice starch in the basal ration and in 4 days after this change, the pellagra condition had improved. After 2 weeks it was completely cured and had resumed growth. This rat later declined, but there was no return of the dermatitis.

Halliday[21], in her studies on the effect of heat on the vitamin G content of skimmilk powder and protein-free milk, found that the development of dermatitis in the "negative controls" (no vitamin G) was very irregular.

Guha[22] noted depilation in rats that received an insufficient amount of vitamin G. Instead of the hair coming out in patches, large areas were denuded and lesions on the mouth and paws occurred in only a small proportion of the animals.

This survey of attempts to produce experimental dermatitis makes it clear that the workers have attained varying degrees of success. Furthermore individual investigators have not been uniformly successful. One set of experiments often resulted in a high incidence of lesions, only to be followed from the same laboratory by another in which dermatitis was rarely observed.

Irradiated Vitamin G Carriers and Vitamin G Deficiency

Since the experimental data to be presented later are limited to the effect on vitamin G of ultra-violet irradiation, special comment on previous work in this field is necessary. So far as we are aware Williams[23] was the first to observe inactivation of any part of the vitamin B complex by ultra-violet irradiation. An aqueous extract of yeast was used as the vitamin B carrier, and this was irradiated for a few hours in a layer 2-3 mm. deep. The test animals were pigeons, on a diet of white rice. Williams reported that the antineuritic vitamin was destroyed, but at that time the independent existence of the antineuritic, and of the other factors in the vitamin B complex, had not been clearly recognized.

Hogan and Hunter[1] found that yeast or yeast concentrates when exposed to a quartz mercury arc lost some essential component but

retained their antineuritic activity. The irradiated yeast or yeast concentrate relieved pigeons from an acute attack of polyneuritis, but final collapse resulted, due to lack of some other essential. Rats, after a short initial period of growth, declined in weight and finally died. A mixture of the irradiated yeast or yeast concentrates, and autoclaved yeast or yeast concentrates, was a fairly adequate source of the vitamin B complex. Although all of the animals that received only the irradiated supplement died in a few weeks, the typical lesions of vitamin G deficiency were not observed. Chick and Roscoe[24] used the method of Hogan and Hunter and found that after 6 hours of exposure to ultraviolet light the content of vitamin G was reduced about one-half. Vitamin B was partially destroyed too, for 12 hours of irradiation destroyed more than half of the amount originally present, and brought about further destruction of vitamin G. They do not report the occurrence of pellagra-like lesions in rats fed irradiated yeast extract in addition to the basal diet.

Kennedy and Palmer[25] attempted to confirm the report of Hogan and Hunter, but their observations indicated that the vitamin potency of yeast is not perceptibly reduced by irradiation.

Guerrant and Salmon[26] studied the stability of vitamin G solutions to irradiation, and estimated that the destruction was as much as 60 per cent in alkaline solution (pH 10.1) but very slight in acid solution.

Sister Rose Beatrice Walsh[27] included 15 per cent of irradiated yeast in a ration as a source of vitamin B, and was unable to detect the presence of any vitamin G. Most of the animals developed severe dermatitis, and the mortality was high. Her work was interrupted for a time, however, and when resumed she was unable to repeat her earlier observations.

Supplee and co-workers[28] reported destruction of vitamin G as well as some destruction of vitamin B by irradiation. However, they noted inconsistencies in the destructive action, and concluded that the method is unreliable.

Sure and associates[16, 17a, 17b, 18] were able to produce dermatitis by feeding irradiated rice polishings as a source of vitamin B, and they have undoubtedly been the most successful in the use of this method.

EXPERIMENTAL

Albino rats have been used exclusively as the experimental animals. When the females become pregnant, as indicated by an increase in weight, they are placed in individual cages with solid bottoms, wood shavings or paper serving as bedding. When the young are 5 days old, however, all bedding is removed and replaced by a metal screen. The experimental ration is given the mother, and litter, when the latter are

15 days old, and the young are weaned at the age of 21 days. We are as yet uncertain as to whether this preliminary preparation of the experimental animals has any specific value beyond reducing their size when weaned. We prefer using animals that weigh between 25 and 30 grams at weaning, as we have been led to believe that the experimental periods are much shortened if the initial weights of the rats are low. Several stock diets have been employed in an effort to determine whether any one may be preferable to another for our purpose. The diets of Steenbock[29] and of Sherman and Campbell, No. 13,[30] have been used more than any other, but our observations indicate that the response of the experimental animals has little if any relation to the diet of the mothers.

As soon as the rats are weaned they are confined on 2-mesh-to-the-inch screen bottoms in individual cages approximating a truncated cone in shape, 9 inches high, and with diameters at top and bottom of 8 and 9 inches respectively. These are sterilized at intervals of two weeks. Water is supplied in inverted drinking bottles, and the basal ration is fed ad libitum. It is made up as follows:

Ration 1545

Casein	20 per cent
Sucrose	71 per cent
Cellulose	3 per cent
Salt mixture (Osborne and Mendel	4 per cent
Cod liver oil	2 per cent

The sucrose, casein, cellulose, and salt mixture are mixed with an equal weight of water, and cooked for 3 hours in a double boiler, as a precaution against the possible occurrence of refection[31]. The mixture is then dried at 65°C. in a Freas oven, ground, and stored in quantities of 30 to 40 Kg. This material is mixed with the cod-liver oil in amounts that can be used in 4 to 5 days.

The sucrose is recrystallized as follows: 3½ kilos of commercial cane sugar are dissolved in 2 liters of distilled water. The thick syrup left after concentrating under reduced pressure is poured into 1.5 to 2 liters of 95 per cent alcohol and allowed to crystallize. The crystals are filtered free from alcohol, with suction on a large Buchner funnel, washed several times with 95 per cent alcohol and then dried. The casein is prepared and purified by the method of Palmer and Kennedy[32]. Dried Harris yeast is used as a source of vitamin B. It was purchased in January, 1930, in a 25 pound lot, and thoroughly mixed as further assurance of uniformity.

The procedure followed in irradiating the vitamin B carrier is the same as that described by Hogan and Hunter, except the distance from the arc to the yeast is reduced to 14 centimeters. If the arcs are new the

irradiation is continued for 10 hours, but after their intensity is reduced by long usage the time is increased to 20 hours. The yeast is spread out in a thin layer, barely enough to cover the tray that supports it, and this is stirred at least 4 times. We are convinced that some of our earlier difficulties were due to irradiation of insufficient intensity. Possibly it should be mentioned that bringing the yeast nearer the arc raises the temperature very considerably. A thermometer placed on the tray immediately beneath the arc indicated a rather constant temperature of 75 to 80°C. The effect on the vitamin B content of the irradiated yeast has not been investigated, but the response of the rats does not indicate in any case that vitamin B has become a limiting factor. Two Cooper-Hewitt mercury arc lamps have been used throughout the experiments, and as determined by the Anderson and Robinson[33] method show an intensity of 4.3×10^5 and 3.2×10^5 ergs per sq. cm. per second respectively. The material exposed to the two lamps was collected in 300 to 500 gram lots to reduce the variation in the irradiated material to a minimum, and to furnish a uniform supply of material for any one set of animals.

Our studies on the technique itself are not yet complete, though it is hoped to have them ready for publication in the near future. It is possible that further improvement may be effected, and some of the precautions now observed may be useless. It is now felt, however, that there are at least two essentials for consistent success in producing severe dermatitis. One is the use of rations that are sufficiently free from the protective factor, the other is irradiation of the vitamin with rays of sufficient intensity to destroy it.

The vitamin B supplements are given separately, in weighed amounts. The portion for each rat is placed in a small glass container, and usually it is consumed immediately and completely. In our recent trials, described in Table 2, the daily allowance was limited to 100 mg. throughout the period of observation. This was partly due to the necessity of conserving the material, but also because it was feared any reduction of vitamin G potency might be obscured if more than the minimum dosage were supplied. We are now omitting a depletion period.

The chief object at present is to merely describe the technique and present the results obtained, without entering into a discussion of the various stages of the procedure. Additional evidence of its effectiveness, however, is obtained by including a summary of the data obtained in the early stages of its development. Our preliminary results are summarized in Table 1.

Table 1 shows that a considerable degree of success had been attained. Dermatitis appeared in 85 per cent of the experimental animals, and the mortality was nearly 70 per cent. Since 15 per cent of the experimental animals did not develop dermatitis, and since some of those

TABLE 1.—RESULTS OBTAINED IN PRELIMINARY STUDIES ON THE IRRADIATION OF VITAMIN G

Animals were subjected to a depletion period

	No. of animals	Dermatitis		Mortality	
		Age at onset days[a]	Per cent	Age at death days[a]	Per cent
Controls	33	52-66	15.1[b]	63-85	15.1
Test animals	74	41-260	85.1	58-273	68.9

a. Days from birth.
b. Five rats developed lesions, three recovered.

with marked symptoms recovered spontaneously, it is obvious that the earlier technique was faulty in some respects. We have come to believe that its chief weakness was irradiation of insufficient intensity.

In the next series to be described, the experimental conditions were again not entirely uniform, but so far as we are aware the only important variable was the intensity of the ultra-violet irradiation. Of the 43 experimental animals, 29 were started on yeast that had been irradiated at a distance of 30 centimeters. Thirteen of these developed dermatitis, and four died, without changing the yeast. The other 16 were changed to yeast that had been irradiated at a distance of 14 centimeters. All of them developed dermatitis, and all of them died. The remaining 14 rats were subjected in every detail to our most recent technique, and all died, with severe lesions. These observations are summarized in Table 2.

It is at once evident that the response of the experimental animals was quite consistent. Gains in weight were insignificant, and all developed dermatitis at an early age. All of the experimental animals had developed dermatitis by the time they were 71 days old, and the average age was 55 days. Two rats had positive symptoms at the age of 35 days. The survival periods were also short. Four-fifths of the animals had died

TABLE 2.—THE EFFECT ON VITAMIN G OF IRRADIATION WITH A QUARTZ-MERCURY ARC

	No. of animals	Dermatitis		Mortality		Weight	
		Avg. age at onset days[a]	Per cent	Avg. age at death days[a]	Per cent	Avg. initial grams	Avg. final grams
Animals were subjected to a depletion period							
Controls	10	53	10	50.5	20	24.9	74.6
Experimental	10	58	100	88.0	100	26.8	35.0
Animals not subjected to a depletion period							
Controls	27	0.0	0.0	66.0	3.7	24.2	80.3
Experimental	33	54.3	100.	67.2	100	25.8	28.3

a. Days from birth.

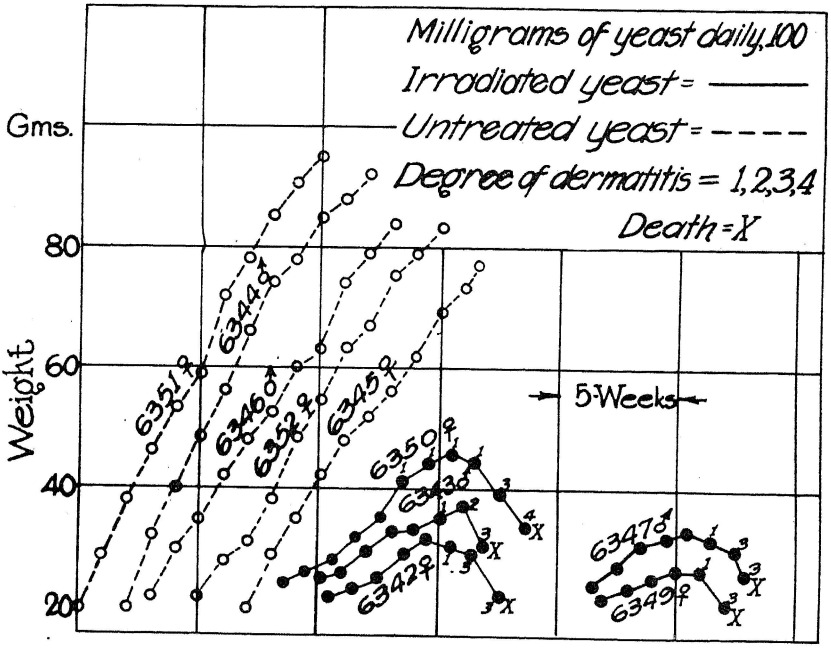

Fig. 1. Rats receiving irradiated yeast continue growing for 3 to 7 weeks, then decline.

by the eightieth day, and the average age at death was 72 days. Three lived over 100 days, and the extremes were 41-141 days. The maximum weight attained was 72 grams, and the maximum weight at death was 47 grams. The typical response of rats to irradiated yeast as indicated by body weight is illustrated in Fig. 1, and the distribution for age at which dermatitis appears, and death occurs, is shown in Fig. 2.

The rats described thus far were kept under observation as long as they lived, in order to observe the natural course of the disease. There were in addition 32 others that received irradiated yeast until they developed dermatitis, and were then used for other purposes. All of these, without exception, had pronounced lesions when the observations were interrupted, at ages ranging from 50 to 70 days. One disturbing observation shown in Tables 1 and 2, was the development of dermatitis in 6 rats that received untreated yeast. Sure[16] made a similar observation. All 6 had been subjected to a depletion period and it seemed probable that this was the explanation. The vitamin reserve was low, and as we were supplying only small amounts of the vitamin B carrier, there may not have been enough to counteract the destructive changes that were initiated during the period of depletion. With this possibility in mind we

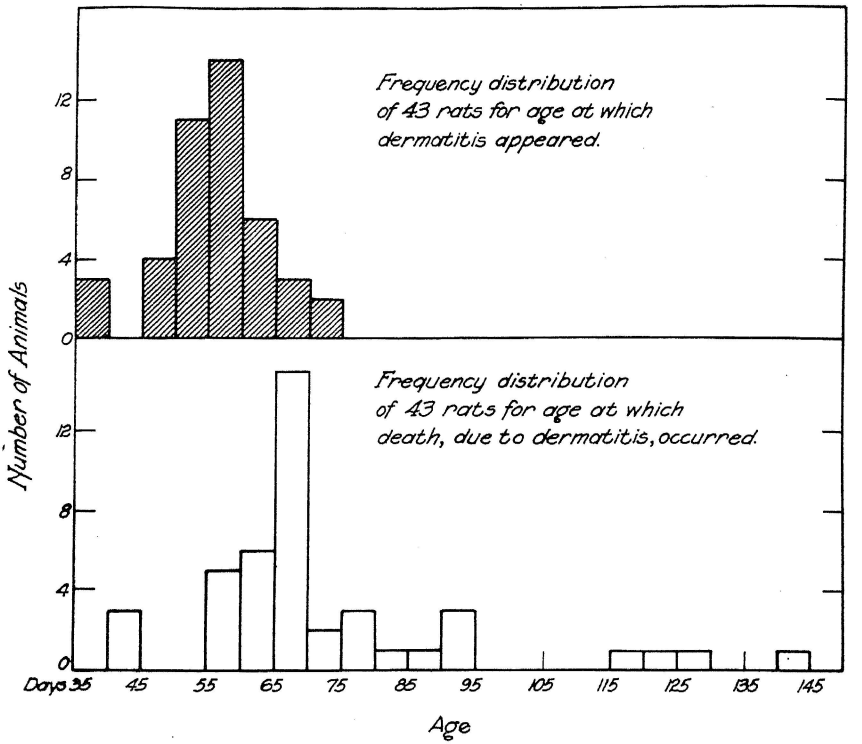

Fig. 2. None of the experimental animals developed dermatitis later than on the 71st day. Only 4 attained an age of over 95 days.

began feeding the supplements as soon as the animals were weaned, and since then not a single control has developed dermatitis.

The development of the lesions as dermatitis progresses is similar to the description from other laboratories but in many respects it differs sharply. Practically all earlier workers emphasize the loss of hair in areas that are bilaterally symmetrical, on the head, neck, or trunk. We have never observed any loss of hair except on the feet and muzzle, and this was invariably preceded by an evident dermatitis. The animals are thoroughly miserable in appearance, become emaciated, assume a humpbacked posture, and are uncertain in their movements. None of them, however, ever takes on the characteristic appearance of premature senility as shown in the photographs of Sherman and Sandels[15]. Usually the first definite sign of a lesion is the appearance of reddened spots on the front toes followed shortly by similar areas on the hind toes. The affected sites gradually become more distinct and spread until the entire foot up to the carpus or tarsus is involved. The hair falls out as the areas

advance, leaving a glistening, deep pink surface exposed, accompanied by a slight swelling. As the disease advances the swelling disappears, the surface darkens and becomes rougher, until at the end it is distinctly scaly.

Along with the lesions on the toes others appear, though the order is not definitely fixed. A very characteristic feature is the early appearance of a small, moist, and slightly reddened area beneath the nostrils. This also spreads until the entire muzzle and lips are involved. Occasionally a small gray spot is observed on the tip of the tongue, and more marked lesions may appear in the angles of the mouth. The ears frequently become slightly reddened, then thick and stiff, as if covered by a dried serous exudate, but usually the hair does not fall out. Another symptom noted in practically every case, is the early appearance of a small blood spot in the inside corners of the eyes. This is followed by a reddish exudate, and the eyelids swell and become adherent. If the animals are healed the eyes open and the hair on the eyelids falls away, giving the animals the "spectacled" appearance described by other investigators. Otherwise it has not been observed. A photograph of a severe case of dermatitis is reproduced in Fig. 3.

The urine occasionally had a reddish tint, but diarrhea was infrequent. Blood samples, drawn from the tails, were taken from 4 rats in various stages of the disease. No evidence of anemia was found, as the red cell counts fell between 7 and 8 million per c.mm. As to the type of dermatitis, a preliminary histological examination* indicates that it is similar to that described by Findlay[13], and by Thatcher and co-workers[17a]. It departs somewhat from the type described by Denton[34] as typical of pellagra. Gurin and Eddy[35] noted a histological appearance similar to that described by Denton when they used activated fuller's earth and Liebig's beef extract as a source of the vitamin B factors. When they used the Jansen-Donath fraction only, the histological appearance was apparently identical with that observed in this laboratory.

Numerous questions are still unanswered. There is no intimation as to the chemical nature of the substance affected but apparently it is not a fatty acid, as might be suggested by the work of McAmis, Anderson, and Mendel[36], Burr and Burr[37], and Evans and Lepkovsky[38]. Our basal diet contains 2 per cent of cod liver oil, and preliminary studies not yet completed have shown that rats may develop dermatitis on rations that in addition contain milk fat or lard. It is too early to decide whether there is a seasonal variation, as practically all our observations were

*We are indebted to Mr. John W. Kennedy for the preparation of the slides and for their interpretation.

made during the summer months. The question has been raised as to whether the symptoms observed are due to a single or multiple deficiency, but it is impossible to decide that point at this time. No extended effort has been made as yet to determine whether the destruction of vitamin G is complete, or whether partial destruction of other factors occurred. As a matter of fact the designation of any one factor as G must be tentative, until it becomes more certain that further differentiation can not be accomplished. It is entirely possible that there are several types of dermatitis, each due to a different deficiency.

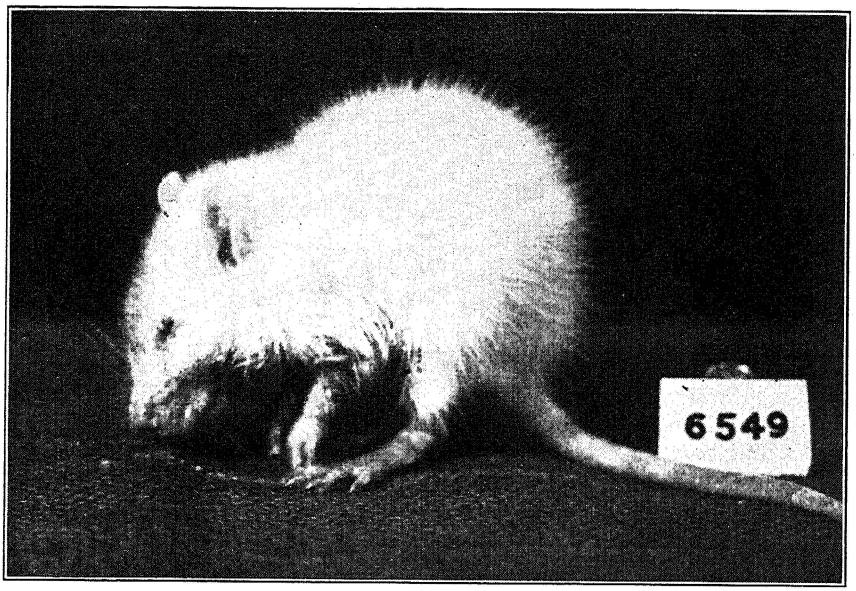

Fig. 3. Rat 6549, age 79 days, weight 38 grams, has received irradiated yeast for 58 days. Characteristic lesions have developed on the feet, nose, and mouth, but there is no loss of fur elsewhere. The eyelids are adherent, but apparently the hair does not fall out unless the animals are healed. The eyes then take on the spectacled appearance described by others.

SUMMARY

1. A portion of the vitamin B complex, now designated as G, is destroyed when yeast is strongly irradiated with a quartz-mercury arc.

2. Rats receiving irradiated yeast as the sole source of vitamin G develop a characteristic dermatitis.

3. The incidence of dermatitis and the rate of mortality have each been 100 per cent.

4. The external lesions differ from those described by other workers in being limited to the feet, muzzle, eyes, and ears. There is no loss of fur except in the affected areas.

5. The technique used is described in detail.

TABLE 3.—INDIVIDUAL HISTORIES OF EXPERIMENTAL RATS

Rat No. and sex	Onset of dermatitis days[a]	Age at death days[a]	Initial weight grams	Maximum weight grams	Final weight grams
Depleted—started on yeast insufficiently irradiated, completed on yeast irradiated by method now in use					
5733M	54	69	25	45	35
5744F	67	129	22	72	47
5745M	67	122	22	67	45
5851F	54	79	27	47	33
5852M	63	87	35	47	35
5853F	63	115	32	64	45
5928F	56	77	25	39	27
5930F	51	65	25	30	23
5939F	56	71	30	42	30
5944F	49	66	25	37	30
Not depleted—started on yeast insufficiently irradiated, completed on yeast irradiated by method now in use					
5980M	50	57	28	42	35
5982M	57	67	28	40	27
5983M	54	68	30	42	35
5986F	64	92	25	48	30
5994M	56	63	22	32	28
5996F	60	67	25	47	37
5999M	56	66	25	45	28
6004F	55	60	23	28	20
6006F	55	65	23	38	30
6010F	50	67	20	35	25
6012M	53	59	22	27	20
6038M	35	42	22	22	18
6040M	35	42	22	27	25
6053M	57	63	35	45	35
6054F	57	60	35	49	32
6055F	57	65	35	42	35
6101M	71	92	22	49	34
6103F	71	141	23	59	40
Most recent technique used throughout					
6326F	45	56	28	42	33
6328F	45	55	27	34	25
6329F	50	67	26	35	28
6330M	52	60	26	33	25
6331M	66	80	31	51	37
6332M	52	60	30	35	29
6342F	56	69	22	32	22
6343M	56	68	25	37	30
6347M	56	66	24	33	26
6349F	49	56	22	27	21
6350F	63	91	24	46	33
6394M	61	70	29	41	32
6396F	54	75	28	37	28
6298F	55	67	28	34	29

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