IS THE RAT DERMATITIS CONSEQUENT ON VITAMIN B_2 (G) DEFICIENCY TRUE PELLAGRA?*

By SAMUEL S. GURIN and WALTER H. EDDY, Ph.D.

With the Assistance of James Denton and Marion Ammerman

(From the Laboratory of Physiological Chemistry in Teachers College, Columbia University, New York)

Plates 40 and 41

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When rats are deprived of water-soluble vitamin B complex they quickly succumb with a variety of pathological manifestations. If the rats are adequately supplied with vitamin B_1 they usually survive much longer but many, sooner or later, develop a type of dermatitis.

The character of this dermatitis led the late Dr. Goldberger and his coworkers (1) to identify it with pellagra and to postulate the existence, in the complex we called water-soluble vitamin B, of a pellagra-preventive substance (P-P). In the years immediately following Dr. Goldberger's suggestion there accumulated evidence of the existence of a new vitamin which the English designate as vitamin B_2 and which in American literature is more commonly designated as vitamin G, also evidence supporting Goldberger's contention that this new vitamin was the pellagra-preventive factor.

At the present writing, however, the view that vitamin B_2 (G) is the significant factor concerned in pellagra prevention is being challenged. Aykroyd (2), Hunt and Wilder (3), Bliss (4), and Sure, Smith, and Kik (5), have recently published papers bearing on this point.

During the past 3 years our laboratory has been especially concerned with the chemical fractionation of the vitamin B complex. To satisfactorily assay the fractions it became necessary to use both

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pigeons and rats as test animals and to take special precautions in the purification of the basal diets of these animals, especially the protein components. We also sought as an aid in B_1 assay a source of vitamin B_2 (G) which should be completely free of B_1. The results of our studies of autoclaved yeast as a B_2 source have already appeared (6). In spite of the development of a B_1—free autoclaved yeast as a source of vitamin B_2, variations in animal behavior still developed.

We have sought for the cause of these variations in the methods used for purifying casein or other components of the basal diets or by utilizing other admitted sources of B_1 and B_2. Out of the many series of tests involved we wish to report here a series that leads us in common with the investigators already cited (2–5), to question whether the B_2-free diet resulting in rat dermatitis owes its action to lack of a factor which is identical with that preventive of human pellagra.

For our study of this series we enlisted the cooperation of Dr. James Denton, who has been identified with the study of the histology of human pellagra and black tongue of dogs (7, 8). Denton observed rarefaction of the corium, dilation of the vessels, and considerable reaction about the vessels in early cases of pellagra.[1] In all cases this was precedent to the later skin changes. Similar changes were found to take place in "black tongue." There is a corresponding fragmentation of the fibrillar material contiguous to the basal layer with very little change at this time in the epithelium itself.

His viewpoint may be stated as follows: "The distinctive lesions of pellagra and those of black tongue of dogs appear to have their origin in a failure on the part of the organism to maintain the specialized supporting tissues of epithelium in various situations." (8).

Dr. Denton separated our specimens into two distinct types of dermatitis. One he found to show the characteristic corium changes of human pellagra. The other showed the type of dermatitis that seems to always develop sooner or later on deficiency of vitamin B_2 but these animals failed to show the corium histology of early human

[1] For purposes of comparison with the photomicrographs to be discussed particular reference is made to the following photomicrographs: *J. Trop. Med.*, 1925, **5**, 182, Fig. 1; *Am. J. Path.* 1928, **4**, Plate 80.

pellagric skin. We present in evidence of these differences photomicrographs of sections made for us by Dr. Denton together with data on the diets associated with the observed effects. (See Figs. 1 to 6.)

Experiment 1.—For skin see Fig. 2; for growth curve see Chart 1.

Diet: Basal.—18 per cent raw egg white protein (commercial frozen); 68 per cent purified corn-starch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and Mendel salt mixture.

Vitamin B_1.—Supplied by 20 mg. daily of fullers' earth activated by the method of Williams and Waterman (9).

Vitamin B_2 (G).—Was supplied by feeding daily 0.5 gm. Liebig beef extract per rat. That this dosage provided B_2 adequate for growth effect is shown by the growth of this animal (Chart 1). After 60 to 70 days on this diet two of the three rats on the diet developed a dermatitis, *viz.* sore mouth, extremely red and sore paws, loss of hair on legs and chest and around joints, and to a lesser extent on the back. Nose and mouth were very sore, the eyelids and genitals inflamed, and autopsy revealed enlargement of the testes. Section of the skin of the paw of Rat 4325 is shown and reveals corium changes similar to those in human and dog pellagra. We have then in this exhibit a corium degeneration closely simulating human pellagra in spite of a dosage of vitamin B_2 in beef extract sufficient to produce excellent growth. The dermatitis of the other two rats in this series failed to clear up when the egg white protein was supplemented by 1 gm. of alkali-extracted casein daily or when 0.5 gm. daily of neutral autoclaved yeast was substituted for the beef extract.

Experiment 2.—For skin see Fig. 3; for growth curve see Chart 1.

Diet: Basal.—18 per cent extracted casein; 68 per cent purified corn-starch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and Mendel salt mixture.

Vitamin B_1.—Supplied by 0.15 mg. daily of Jansen-Donath fraction 16 prepared by the method of Williams, Waterman, and Gurin (10).

Vitamin B_2.—Was supplied as a water diffusate of beef extract. After 35 days this rat, in spite of fair growth (1.1 gm. daily), developed sore nose, sore reddened area about the mouth, bleeding at the joints, loss of hair around the eyes, and crinkled ears. The other three rats in this series also grew well but did not develop dermatitis. It must also be said that a large number of other rats have been reared on this same diet with fair growth and without dermatitis when the B_2 has been supplied by whole beef extract instead of the water diffusate. Since, however, only one of the four on the diffusate developed the skin lesions it is doubtful whether the use of the diffusate rather than whole extract as B_2 source could have been the significant factor in this result.

The section is from the skin of the chest. Like Rat 4325 it shows a true degeneration of the superficial supporting tissues in the corium. The lesion was not infected and resembled an early skin lesion of a human pellagrin. Again we had

obtained a corium degeneration and dermatitis similar to human pellagra on a diet which produced good growth and was supposedly therefore adequate in B_2.

Experiment 3.—For skin see Fig. 4.

Diet: Basal.—18 per cent acid-extracted casein; 68 per cent purified cornstarch; 10 per cent cottonseed oil; 4 to 5 drops daily of cod liver oil; 4 per cent Osborne and Mendel salt mixture.

Vitamin B_1.—Supplied as for Rat 4630 by 0.15 mg. daily of Jansen-Donath fraction 16.

Vitamin B_2.—No source given.

Two rats in this series developed, after 70 to 80 days, extreme soreness of nose and around the lower part of mouth, dermatitis of the paws, loss of hair about the joints and around the eyes, and crinkled ears. Superficially these symptoms resembled closely those of Rats 4325 and 4630. Death occurred in two animals within 2 weeks after onset of symptoms. The remaining animal held apparently normal as to skin for 20 weeks, after which nose and throat soreness developed but became no worse, and the animal was killed after 27 weeks on the diet (4342).

The skin section of Rat 4343 from this series is shown. It exhibits simple atrophic thinning of the epidermis with squamae on the surface, but with no such significant corium changes as are shown by Rats 4325 and 4630. The lesions differ markedly from those of the human pellagrin.

That this rat lacked growth-promoting vitamin B_2 was evidenced by his failure to grow.

Experiment 4.—For skin see Figs. 5 and 6.

Diet: Basal.—18 per cent acid-extracted casein; 68 per cent purified cornstarch; 8 per cent butter fat; 2 per cent cod liver oil; and 4 per cent Osborne and Mendel salt mixture.

Vitamin B_1.—Supplied by Jansen-Donath fraction 16, 0.15 mg. daily.

Vitamin B_2.—No source given.

Dermatitic symptoms similar in external appearance to those of Rats 4343, 4325, and 4630 appeared in three of the four rats on the diet in about 50 days. The rats were killed on the 85th day on the diet. No growth resulted with this series, demonstrating lack of growth-promoting vitamin B_2.

Like Rat 4343 the skin sections[2] show no corium changes comparable to the early lesions of the human pellagrin or to those of black tongue. Some scarring of the superficial corium is observed.

Summary of Cases

The outstanding feature of these cases is the production in rats of two histologically different types of dermatitis; the appearance in one

[2] In all cases care was taken when selecting skin for sectioning to avoid areas where secondary infection might have occurred.

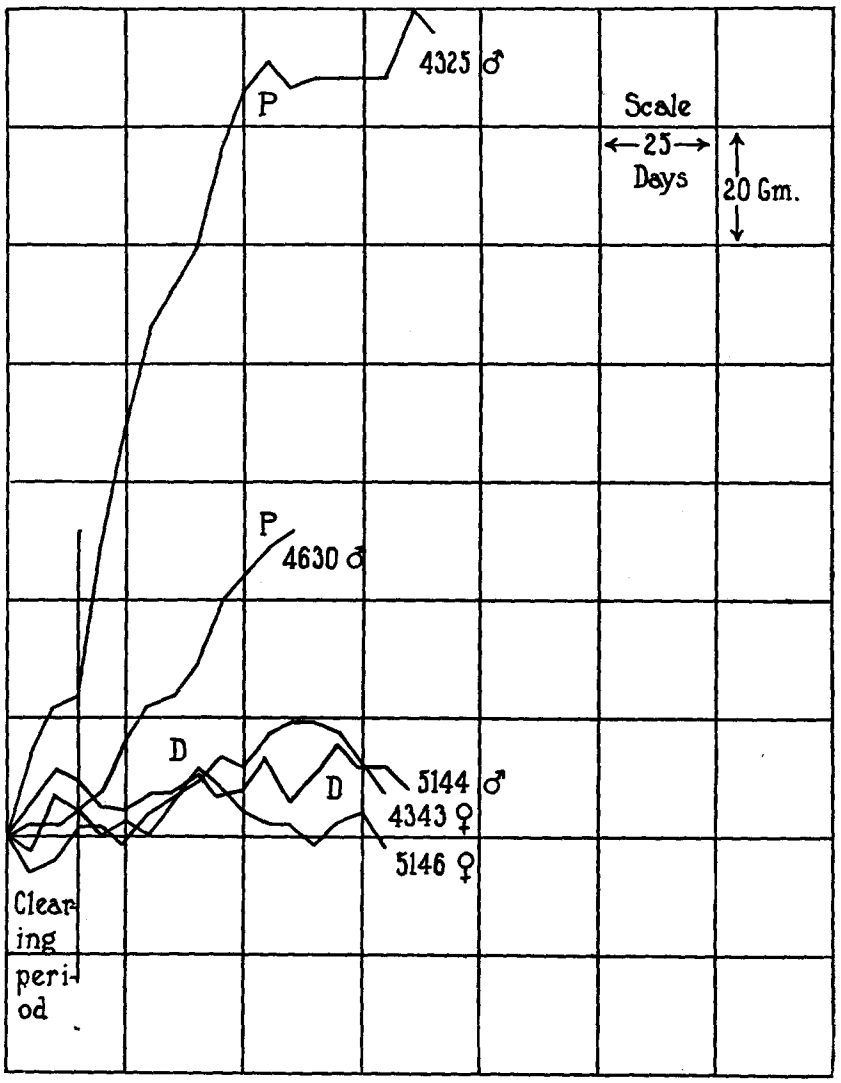

CHART 1. Growth curves of rats used in the tests reported. See pages 423 to 424.

P signifies pellagra symptoms.

D signifies dermatitis symptoms.

TABLE I

Dietary Factors Involved in the Rat Series Reported in This Article

Rats	4325	4630	4343	5143 and 5146
Diets				
Protein	18% egg white	18% acid-extracted casein	18% acid-extracted casein	18% acid-extracted casein
Carbohydrate	68% corn-starch	68% corn-starch	68% corn-starch	68% corn-strach
Fat	8% butter	8% butter	10% cotton-seed oil	8% butter
Vitamin D	2% cod liver oil	2% cod liver oil	4 drops cod liver oil daily	2% cod liver oil
Salts	4% O & M mixture	4% O & M mixture	4% O & M mixture	4% O & M mixture
B_1 source	Activated fullers' earth	0.15 mg. J-D 16	0.15 mg. J-D 16	0.15 mg. J-D 16
B_2(G) source	Liebig beef extract (0.5 gm.) and autoclaved yeast	Water diffusate beef extract	None	None
Growth	Good	Good	Maintenance only	Maintenance only
Diagnosis	Dermatitis allied to human pellagra according to Denton	Dermatitis simulating human pellagra	Dermatitis lacking corium picture of human pellagra	Dermatitis lacking corium changes of human pellagra

Acid-extracted casein was prepared as follows: 10 kilos of casein (Merck technical) was stirred into 50 liters of 20 per cent methyl alcohol solution made 0.05 per cent acid with glacial acetic acid. The mixture was stirred 24 hours by an electrically driven stirrer, allowed to stand 4 to 5 hours, and the supernatant liquid siphoned off. An equal volume of solvent was again added and procedure repeated three more times, a total of four extractions involving 96 hours of continuous stirring. The casein was then filtered on Buchner funnels, washed by stirring 20 to 30 minutes with 2 to 2½ gallons of 95 per cent ethyl alcohol, filtered, air dried, and baked at 90–100°C. for 10 hours.

The corn-starch used in the first experiments was extracted by stirring 5 hours with a large volume of 65 per cent methyl alcohol at 50°C. Three extractions of at least 5 hours each were made, the starch being filtered after each extraction, washed finally with 95 per cent ethyl alcohol, and dried in a vacuum oven at 40–50°C. until all alcohol was removed. Later this step was abandoned as no effect on the results was observable from the use of the corn-starch as purchased.

The O & M salt mixture is the classic salt mixture of Osborne and Mendel.

group of a type markedly similar to that observed in human pellagrins in spite of a diet rich in classic vitamin B_2 sources as evidenced by growth curves, and a quite different pathological picture in the other group which was deprived of vitamin B_2 source entirely.

Others have observed dermatitis in the presence of vitamin B_2 in the diet and its failure to appear in cases where B_2 was omitted. Our data supply we believe new histological evidence that vitamin B_2 deficiency or adequacy as measured by growth effect is not alone sufficient to explain the presence or absence of true pellagra.

DISCUSSION

The view that vitamin B_2(G) may not be the pellagra-preventing vitamin has been suggested by other investigators.

In a detailed study of the distribution of vitamin B_2 in foodstuffs, Aykroyd and Roscoe (11) in 1929 advanced evidence strongly supporting the idea of its identity with Goldberger's pellagra-preventive substance. But, in 1930, Aykroyd (2) from his studies of B_2 distribution in cereals and especially in corn (maize) is led to observe: "At present the association, if any, of vitamin B_2 and pellagra is obscure."

Very recently Bliss (4) notes that Goldberger's view of vitamin B_2 as the pellagra-preventive substance has: "made less of an impression upon clinicians and laboratory workers in the South who are in actual contact with the disease than might be inferred from its ready acceptance in the standard texts today." He goes on to develop the theory that the pellagra-preventive factor is not a vitamin but instead something which supplies iron deficiency. He reports a study of the response of 51 human pellagra cases to iron therapy and the cure of black tongue by intravenous iron treatment.

Sure, Smith, and Kik (5) have reported severe dermatitis accompanied by remarkable growth and have suggested that: "the growth-promoting and antidermatitis factors associated with the so-called antipellagric vitamin G are not synonymous." They have supplemented this report by several others in one of which they describe an anemia in vitamin G dermatitis which developed in spite of abundance of ferric citrate in the diet.

The activated fullers' earth used in one series was prepared by the method of Williams and Waterman and shown by growth tests to be practically B_2-free (9). Later we used a fraction of high vitamin B_1 concentration obtained in Williams, Waterman, and Gurin's (10) duplication of the Jansen-Donath procedure. It represents Jansen and Donath's $PtCl_4$ precipitate after removal of the platinum.

The egg white we used was commercial frozen raw egg white. Solids were determined and the egg white fed to provide 18 per cent egg protein in the diet.

All of these observations as well as our own experience indicate that we cannot accept the vitamin B_2 or G, as at present defined, as identical with Goldberger's pellagra-preventive substance. Whether it is a factor in the syndrome of pellagra, and what other factors are involved, are matters demanding more experimental research and comparison of results. Whether Denton's criterion for differentiating between true pellagra and non-pellagrous dermatitis is accepted or not is also immaterial, but it is at least of interest as an attempt to define sharply the pathology of pellagra.

In Table I is given the summary of the dietary factors involved in the rat series presented in this article together with data on the method of purification of the ingredients employed. Other workers may find these data of value in comparing our tests with their own. Growth curves of the rats are shown on Chart 1.

A comparison of the dietary ingredients of Rats 4325 and 4630 with that of Rats 4343, 5143, and 5146 makes it improbable that the difference in effects could have been due to either the source of vitamin B_1 used, or to the protein, fat, carbohydrate, or salt sources used. Neither do any of the diets appear to be lacking in iron.

SUMMARY

1. Isolated cases of a dermatitis resembling histologically that of human pellagra have occurred in rats supplied with sufficient vitamin B_2 (G) in the form of beef extract or neutral autoclaved yeast to produce good growth.

2. Other rats on basal diets containing similarly prepared nutrients but deprived of any known source of vitamin B_2 (G) develop a dermatitis similar in appearance to that described by other workers, but this skin effect differs in histological picture from that found in human pellagra or in black tongue of dogs. These rats showed growth failure which supports the view that they lacked growth-promoting vitamin B_2 (G).

3. It is suggested that dermatitis in rats may be of diverse type; one resulting from vitamin B_2 (G) deficiency quite different histologically from human pellagra, and one closely allied to human pellagra and black tongue in dogs due to lack of some at present unidentified factor.

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EXPLANATION OF PLATES

PLATE 40

FIG. 1. Section of the skin from foreleg of a normal rat for comparison with the coria of the pathological specimens. Note especially the intact fibrillar collagen bundles in this section.

FIG. 2. Skin from cheek of Rat 4325.

FIG. 3. Skin from cheek of Rat 4360.

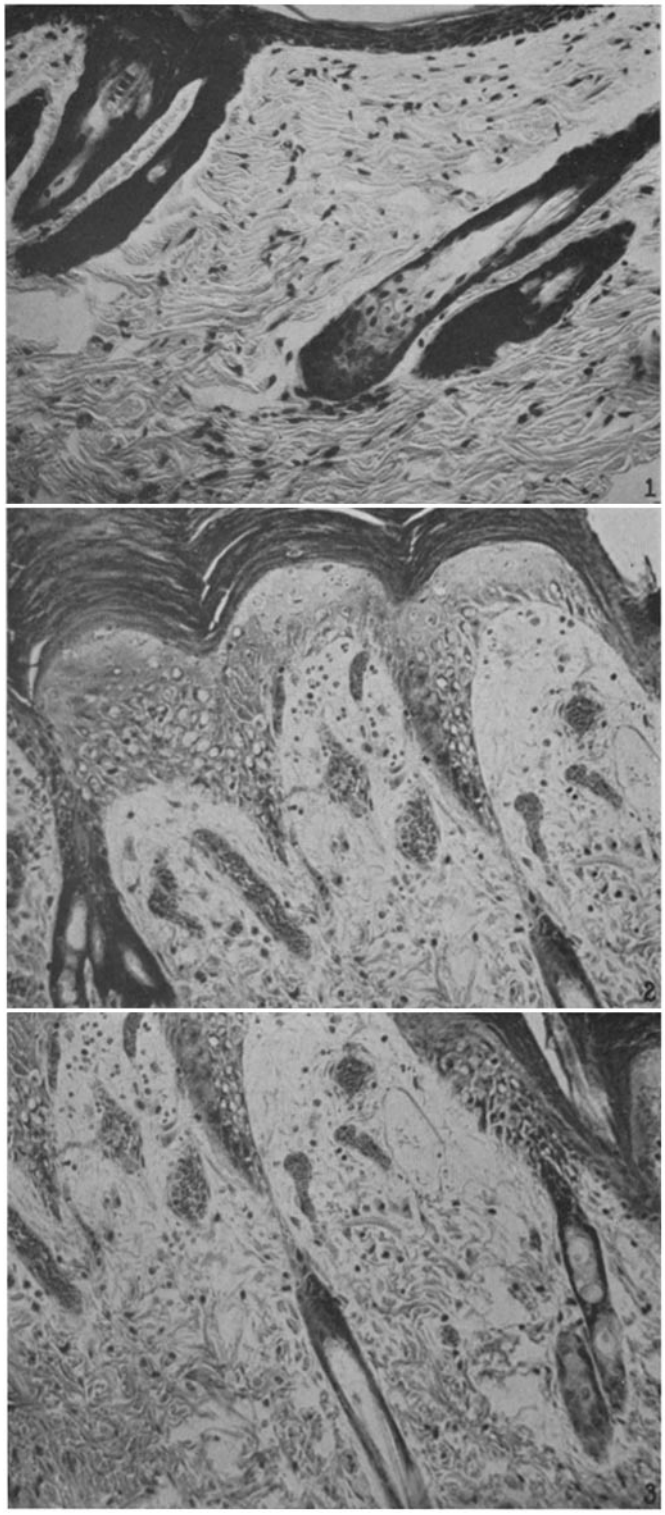
PLATE 41

FIG. 4. Skin from forearm of Rat 4343.

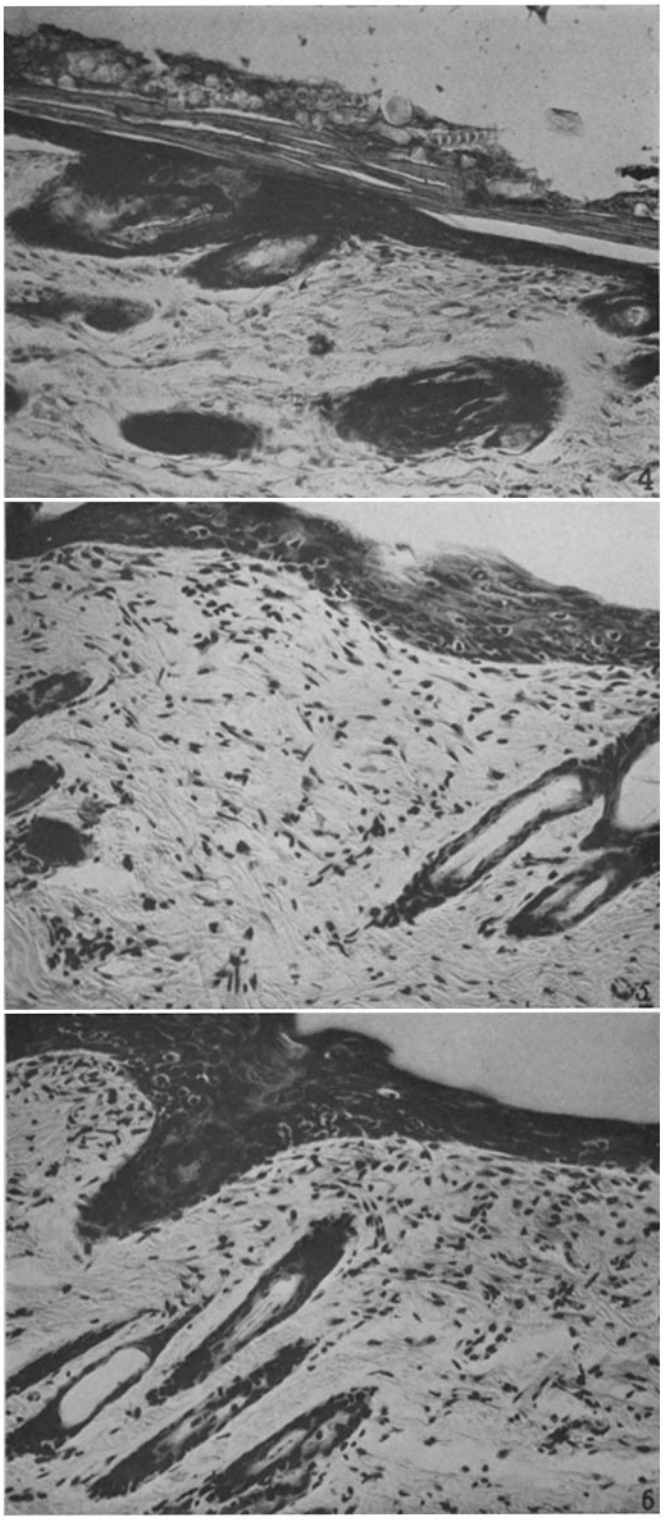
FIG. 5. Skin from forearm of Rat 5143.

FIG. 6. Skin from forearm of Rat 5148.

Giemsa stain used. Magnification = 110.



(Gurin and Eddy: Rat dermatitis and pellagra)



(Gurin and Eddy: Rat dermatitis and pellagra)