EXPERIMENTAL CUTANEOUS LESIONS IN THE ALBINO RAT

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A peculiar, dry, earthy coloration of the skin has been mentioned in association with ocular symptoms by most of the workers on keratomalacia. A detailed clinical description of the changes in the skin in cases of keratomalacia in adults was given by Pillat (1929 *a* and *b*), emphasising that keratomalacia is a 'system disease' of the ectodermal tissues, manifesting symptoms not only in the eye but also in several other parts of the body. Infections of the skin, such as pustular eruptions, furunculosis, abscesses, impetigo, acute bed-sores (in babies), etc., have often been associated with keratomalacia.

Besides the abovementioned changes, a follicular keratosis of the skin has been described occurring with xerophthalmia and keratomalacia by Frazier and Hu (1930, 1931) in China, Loewenthal (1933 *a* and *b*) in East Africa, Nicholls (1933) in Ceylon and Radhakrishna Rao (1937 *a* and *b*) in India.

In animals, especially in rats, several workers have tried to produce uncomplicated vitamin A deficiency and proved that dietary deficiency of vitamin A alone was responsible in producing metaplasia of columnar, cuboidal and transitional epithelia to the squamous keratinizing type in some organs. Previous literature reveals that there is no confusion regarding the primary histologic alterations and resulting lesions of the eye, the paraocular glands and the respiratory, gastrointestinal and genitourinary systems. However, the question of the cutaneous lesions in vitamin A deficiency in rats was until recently not very clear.

The proof that the histological changes occurring in rat's skin are in certain respects identical with those described in human beings in cases of 'phrynoderma' comes mainly from the work of Sullivan and Evans (1943) and Moult (1943). They have independently shown that keratotic plugs

may readily be induced in rats maintained on a diet deficient in vitamin A and that these plugs are identical with those occurring in human subjects.

Most of the workers cured cases of 'phrynoderma' by administering cod liver oil. The cure brought about by cod liver oil was attributed to its high vitamin A content. But the latest work on 'phrynoderma' has thrown some doubt on this hypothesis. Gopalan (1947) has treated cases of 'phrynoderma' by combined treatment with yeast extract and linseed oil, whilst Menon, Tulpule and Patwardhan (1950) have succeeded in curing 'phrynoderma' with gingelly oil.

A review of the literature reveals that very little attention has been paid to the detailed histological study of the skin lesions occurring in the fat deficiency syndrome. Only Williamson (1941) has shown that the epidermis of rats on fat-free diets becomes thicker and more differentiated than the normal ones.

However, after the completion of the present work undertaken in 1950, Ramlingswami and Sinclair (1953) have reported plugging of the follicular openings with dense compact layers of keratin and acanthosis of their lining epithelium in albino rats maintained on fat deficient diets.

The object of the present investigation was to elucidate the comparative functions of vitamin A and essential fatty acids in maintaining the epithelium of the skin of the rat in a normal state and to study the effects of deficiencies of these factors on the structure of the skin.

TABLE I

Composition of the basal diet and vitamin supplements

(Experiment 1)

Ingredients	Parts	Vitamin supplements per rat per day		
Casein (purified) ..	18	Vitamin D	10	U.S.P. units
Starch	32·5	Vitamin E	0·43	mg.
Sucrose	32·5	Vitamin A (Group I Control)	50	I.U.
Fat (Peanut oil)	5	Vitamin A (Group II)	5	I.U.
Osborne and Mendal's Salt Mixture No. 1 332	4	Vitamin A (Group III)	1	I.U.
		Vitamin A (Group IV)	0	I.U.
Dried Brewer's Yeast (purified)	8			

Experimental

The following experiments were planned to produce uncomplicated vitamin A and essential fatty acid deficiencies in rats maintained on a synthetic diet, with a view to study the clinical manifestations and histological changes occurring in the skin, in the complete absence of vitamin A, as well as when given at suboptimal levels.

Animals

Healthy, young albino rats of the Haffkine Institute inbred strain, with an average weight of 39 gm. were selected for Experiment No. 1. In all, 24 rats (12 males and 12 females) were distributed, as far as possible, with respect to weight, littermates and sex into four different groups. For the second experiment, 30 rats with an average weight of 26 gm. were divided in five different groups.

Diet

The composition of the basal diet is indicated in Tables I and II. Cornstarch, casein and yeast used in the diet were subjected to purification.

Table II

Composition of the basal diet and vitamin supplements

(Experiment 2)

Ingredients	Group I	Group II	Group III	Group IV	Group V
Casein (purified) ..	18	18	18	18	18
Starch (purified) ..	..	..	..	..	50
Sucrose ..	65	65	65	70	20
Fat					
(1) Peanut oil ..	5	..	..	..	..
(2) Lard ..	..	5	..	..	..
(3) Hydrogenated oil (Vanaspati) ..	..	..	5	..	..
Osborne and Mendal's Salt Mixture No. 1 332	4	4	4	4	4
Dried Brewer's Yeast (purified)	8	8	8	8	8
	100	100	100	100	100

Vitamin supplements per rat per day (Incorporated in the diet) for all groups

Vitamin A (Nonsaponifiable fraction of Shark Liver Oil) ..	100 I.U.
Vitamin D	10 U.S.P. Units
Vitamin E	0·43 mg.

The required amounts of vitamin A, D and E were administered to each rat orally in olive oil.

Experiment 1

The above diet was supplied *ad libitum* and a record of the daily food consumed by each rat was maintained. The experiment lasted for 101 days.

As the experiment progressed, animals on completely vitamin A-free diet showed marked emaciation. When they became moribund, they were sacrificed by ether anæsthesia. The corresponding animals from the other groups were also sacrificed for comparative study.

Skin pieces from different parts of the body were excised for histopathological study. As the structure of the skin from the different regions was observed to be of a similar nature, the findings of sections taken from the dorsal region are presented. In this region, the stratum corneum of the skin was found to be consisting of a thin layer of cells, hence changes occurring as a result of vitamin A deficiency, like hyperkeratinization, can very easily be detected and studied.

The skin pieces were fixed in 10% formal saline, and blocked in paraffin. The sections were cut to 6–7 μ in thickness. They were stained by Ehrlich's acid hæmatoxylin and eosin, and by Weigert's iron hæmatoxylin and Van Giesson's stains.

Results

Group I: (Control) 50 I.U. vitamin A per rat per day

Throughout the period of experiment (101 days) these animals appeared very healthy, active and steadily gained in weight, till they were sacrificed. Their average gain in weight was 187 gm.

Clinical and histological observations.—Examination of the skin did not reveal any abnormality (Fig. 2).

Group II: 5 I.U. vitamin A per rat per day

All the animals steadily gained in weight. Throughout the experiment the average gain in weight was 162 gm.

Clinical signs and symptoms.—All the rats apparently remained healthy and did not show any clinical signs or symptoms of vitamin A deficiency.

Histological observations.—Though none of the rats of this group showed any signs or symptoms of vitamin A deficiency clinically, the histological examination of the skin, in 3 cases out of 6 revealed changes typical of vitamin A deficiency.

1. *Epidermis.*—Three rats showed slight hyperkeratinization of the stratum corneum. All the other layers were normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—Normal.

4. *Hair follicles.*—Few of the hair follicles showed slight dilatation and plugging of their orifices with keratinous material. This was observed in two cases where there was slight hyperkeratinization of the stratum corneum layer. The rest did not show any changes. There was a good amount of deposition of subcutaneous fat in all the animals.

Group III : 1 I.U. vitamin A per rat per day

The animals of this group, for the first 11 weeks, steadily gained in weight, the average gain in weight being 122 gm. The last three weeks showed slight decline in the weight curve.

Clinical signs and symptoms.—Coincident with the decline in weight all the animals showed signs and symptoms of vitamin A deficiency. In this group three rats developed typical xerophthalmia and other two rats showed only encrusted eyelids, photophobia and lacrymation. One did not show any eye changes throughout the period of experiment. The animals slowly became emaciated and their skin appeared loose.

Histological observations : 1. *Epidermis.*—The stratum corneum of all animals showed marked hyperkeratinization, as compared to the control rats of the group I. The keratin was present in loose lamellæ. Other layers were normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—Normal.

4. *Hair follicles.*—Though there was general hyperkeratinization only in two animals, typical dilatation and plugging of hair follicles with loose keratinous material and slight atrophy of their lining epithelium was observed. In the remaining four cases, they appeared normal. Subcutaneous fat store was diminished (Fig. 3).

Group IV : 0 I.U. vitamin A per rat per day

For the first six weeks of the experiment all the animals steadily gained in weight, the gain being comparatively less than in the other groups. The total average gain in weight was 75 gm. After the period of initial six weeks, the weight started declining till they were sacrificed. After three weeks the food consumption was lower than that of the control group.

Clinical signs and symptoms.—Coincident with the decline in weight, symptoms of vitamin A deficiency developed. All the rats started showing signs of vitamin A deficiency between the 5th and 7th week of the experiment.

The first visible manifestation of vitamin A deficiency was a brownish encrustation on the eyelids. This was followed by the eyelids becoming puffy, the animals developing photophobia and later on lacrymation. Usually both eyes were involved at about the same time. Loss of lustre and dryness of cornea soon followed. Serosanguinous exudate was found to be present round about the eyes and the animals kept on rubbing the eyes as if it caused irritation. Thus, xerophthalmia of the eyes developed (Fig. 1). Then slowly the cornea became opaque and covered with pus and finally general inflammation of the eyeball set in (panophthalmitis). It became so acute that the whole eyeball degenerated leaving a cavity filled with a serosanguinous exudate.

In some rats swelling in the areas above the snouts was observed. Side by side, the skin became very loose probably due to the loss of subcutaneous fat. Hair became coarse and showed a tendency to easy epilation. The animals became highly emaciated, so much so their weight dropped from an average of 115 gm. to an average of 73 gm. Often their bodies were found covered with blood stains probably due to their rubbing the eyes which bled on slightest touch. The animals became humped and nervous. In two rats, there was denudation of the hair around the eyes. All animals were found sneezing and on post-mortem examination, showed inflammation and congestion of the lungs.

Histological observations : 1. *Epidermis.*—The chief pathological changes were found in the corneum layer. All the rats of this group showed excessive hyperkeratinization both follicular and diffuse. The keratin was present in loose thin wavy lamellæ. All the other layers appeared normal.

2. *Corium.*—In two rats, where there was extreme hyperkeratinization, slight lymphocytic infiltration of corium especially in the perifollicular region was observed. In the other four rats, the corium appeared normal.

3. *Sebaceous glands.*—These were present in all the sections but frequently, the glands present at the root of distended plugged hair follicles were found shrivelled up. The glands often showed decrease in the number of cells and their cytoplasm.

4. *Hair follicles.*—Marked changes were found in the hair follicles of this group of rats. The orifices of the hair follicles showed marked hyperkeratinization of the lining epithelium and, as a result, the funnels of the follicles were widened and were plugged by dense masses of horny tissue. The follicular plugs consisted of lamalle of flattened cornified cells in which there were no nuclei. In extreme cases, the lining of the hair follicles were found to be atrophic. Sections of broken hair were often seen in the substance of follicular plugs. There was marked reduction in the store of subcutaneous fat (Fig. 4).

Experiment 2

The duration of the experiment was 23 weeks. For the first 12 weeks, the animals were supplied food *ad libitum*. Within 8 weeks, the deficiency symptoms became evident. On being continued on the same diet, instead of becoming moribund they went into a chronic deficiency state. At this stage, it was found necessary to bring them in an acute deficiency state by inflicting some stress on the metabolism. This was done by restricting the diet to 6 gm. per rat per day for the rest of the duration of the experiment.

When they appeared highly emaciated, the animals were sacrificed by ether anesthesia. Corresponding animals from other groups were also sacrificed and the histopathology of skin studied as in the last experiment.

Results

Group I: 5% peanut oil

Throughout the experiment the animals appeared very healthy and active. They steadily gained in weight for the first 12 weeks and then maintained a plateau on the restricted food intake regime. Their average gain in weight was 120 gm. during the course of the experiment. Since the animals were kept on a restricted caloric diet after 12 weeks, their gain in weight is less compared to the rats of the control in the last experiment.

Clinical and histological observations.—Examination of the skin did not reveal any abnormality.

Group II: 5% lard

Throughout the experiment they appeared healthy and active. Their weight curve coincided with that of the control group. The total average gain in weight at the end of the experiment was 114 gm.

Clinical signs and symptoms.—Clinically, the animals appeared healthy, and did not show any macroscopic lesions throughout the experiment.

Histological observations.—Histological examination revealed slight changes in the epidermis, though clinically the animals did not show any signs and symptoms.

1. *Epidermis.*—Out of the six rats, two rats showed considerable amount of hyperkeratinization of the stratum corneum layer. Two other rats showed slight hyperkeratinization of the stratum corneum, whilst in the remaining two stratum corneum appeared to be normal. Other layers of the epidermis were normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—Normal.

4. *Hair follicles.*—Though there was hyperkeratinization no plugging or dilatation of hair follicles was observed in any case. In all cases they were normal.

There was a good amount of subcutaneous fat deposition.

Group III: 5% hydrogenated oil

Throughout the period of experiment, their weight curve coincided with that of the control group. At the end of the experiment, the average gain in weight was 1·11 gm. Throughout the period of experiment, the animals appeared healthy and active.

Clinical signs and symptoms.—Clinically all the six rats appeared healthy and did not show any outward signs and symptoms.

Histological observations.—Histological study revealed following changes in the skin structure.

1. *Epidermis.*—All the rats showed hyperkeratinization of the stratum corneum layer as compared to the peanut oil group. In one case, it was severe whilst in other cases, it was moderate. Keratin was present in dense masses. All the other layers of the epidermis appeared normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—Normal.

4. *Hair follicles.*—Four cases out of six showed dilatation and plugging of orifices of hair follicles, with dense keratinous masses. In the other two cases, they appeared normal.

There was a marked reduction in the subcutaneous fat store in all the rats (Fig. 6).

Group IV: Fat-free diet (sucrose)

For the first 5 weeks the average gain in body weight equalled that of the control group. After that, the gain in weight was very little, followed by loss in weight on restricted food intake. At the end of the experiment, the gain in weight averaged only 44 gm.

Clinical signs and symptoms.—For the first five weeks, all the animals appeared healthy. Then they slowly started showing the deficiency symptoms. In the 9th week, typical ringed tail condition was evident. The tip of the tail and paws, especially hind ones, were erythematous and scaly. Pinnæ of the ears also showed slight scaliness. There was scanty growth of hair on the abdominal and facial regions. At a very late stage of the disease, the abdominal region of two rats were found constantly covered with a yellowish sticky exudate. Post-mortem examination showed intestinal distention accompanied by loss of subcutaneous and visceral fat (Fig. 5).

Histological observations.—Histological study of the skin revealed the following changes.

1. *Epidermis.*—All the rats showed excessive hyperkeratinization of the stratum corneum layer. Keratin was present in dense masses. All the other layers appeared normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—All animals showed normal structure.

4. *Hair follicles.*—Marked changes were found in the hair follicles of this group of rats. The orifices of the hair follicles showed marked hyperkeratinization of the lining epithelium, as a result, the funnels of the follicles were widened and plugged by dense masses of keratin. Sections of broken hairs were seen in the substance of follicular plugs.

There was complete loss of subcutaneous fatty tissue (Fig. 7).

Group IV: Fat-free diet (starch)

For the first 9 weeks, the average gain in body weight equalled that of the controlled group. After that, the gain in weight was less than that found in the first 3 groups, *viz.*, peanut oil, lard and hydrogenated oil respectively, but greater than in group IV, *i.e.*, those on fat-free diet containing 70% sucrose. The average gain in weight at the end of the experiment was 70 gm.

Clinical signs and symptoms.—Typical fat deficiency signs and symptoms were not observed in this group. For 10 weeks the animals appeared healthy. Then, they slowly started showing emaciation followed by slight

scaliness of paws and tail. Their skin appeared very loose owing to loss of subcutaneous fat. One rat from this group developed diarrhœa and died in the 11th week.

Histological observations.—Histological study revealed the following changes in the skin.

1. *Epidermis.*—Out of the 6 rats, 4 showed hyperkeratinization of the stratum corneum. In one case the hyperkeratinization was severe, whereas in other cases it was moderate. All the other layers appeared normal.

2. *Corium.*—Normal.

3. *Sebaceous glands.*—Normal.

4. *Hair follicles.*—Dilatation and plugging of the funnels of hair follicles by dense keratinous masses was observed in 4 cases. In the remaining 2 cases, they appeared normal.

There was loss of subcutaneous fat store in all the rats except one (Fig. 8).

The above results show that a fat-free diet, or a diet low in essential fatty acids gives rise to skin lesions which are microscopically very much similar to those found in vitamin A deficiency. A diet containing 5% hydrogenated oil which is low in essential fatty acids, was found to give rise to mild cutaneous lesions like slight hyperkeratinization, dilatation and plugging of hair follicles. This has been attributed to lack of essential fatty acids in the fat, since these are the only fatty acids which the animal body is found incapable of synthesising.

It was originally proposed to supplement the diet containing hydrogenated oil with pure linoleic acid and study the effect but since it was not available locally, gingelly oil was incorporated in the diet replacing part of the hydrogenated oil so as to give the concentration of about 1 gm. of linoleic acid in 100 gm. of the diet (*i.e.*, 2·5% vanaspati and 2·5% gingelly oil). When the animals were kept on this diet, their average daily intake of linoleic acid was about 63 mg. per rat per day, which is above the minimum curative dose recommended by several workers.

RESULTS

All the animals remained healthy throughout the period of experiment and histopathological examination of their skin did not reveal any abnormality.

The results of the experiment show that skin lesions like slight hyperkeratinization, dilatation and plugging of hair follicles observed in rats kept

on a diet containing 5% hydrogenated oil can be completely prevented by replacing a part of hydrogenated oil by gingelly oil, which contains 40% linoleic acid. An indirect proof is thus obtained that linoleic acid plays an important role in the maintenance of healthy skin.

Discussion

These comparative studies show that keratotic plugs can readily be induced in rats' skin when the animals are maintained on vitamin A deficient diets, or when kept on suboptimal levels of vitamin A. The cutaneous lesions produced were found to be identical in many respects with those described by Sullivan and Evans (1943) and Moult (1943) in their studies. These studies reveal the fact that the complicating deficiencies of the vitamin B complex, fat and essential fatty acids in experimental diets had been responsible for the varied interpretation of the vitamin A deficiency syndrome in the past.

Experiments on essential fatty acids also, stress the importance of the quality of fat in the diet on the production of cutaneous lesions in the rats. In 1929, Burr and Burr first described fat deficiency disease in rats and further showed that only the unsaturated fatty acids, linoleic, linolenic and arachidonic were responsible for the curative effects exerted by different fats. Since then several workers especially Graham *et al.* (1931), Turpeinen (1938), Hume *et al.* (1938) have observed typical skin symptoms of essential fatty acid deficiency in young rats. Barki *et al.* (1947) have shown that when adult rats are kept on a restricted caloric diet until they had lost one-half their weight and then placed on a fat-free diet *ad libitum*, they readily showed essential fatty acid deficiency symptoms, which were not observed when the animals were kept on the *ad libitum* regime right from the beginning. Decker *et al.* (1950) are of the opinion that mature mice on fat deficient diet fed *ad libitum*, though appear to be in a good condition remain in a chronic deficiency state. They were able to bring these mice to an acute deficiency state by imposing stresses like injuries, pregnancy or *x*-irradiation, proving that in chronic deficiency states, the animals were not able to withstand any stress. In this experiment the animals were kept on fat-free diet *ad libitum* for the initial 12 weeks and then onwards on restricted caloric diet.

The results obtained are in some respects similar to those reported by Ramlingswami and Sinclair (1953). Histological changes occurring in vitamin A deficiency are in complete agreement with the present results but in case of essential fatty acid deficiency, though hyperkeratinization and plugging of hair follicles with dense keratinous masses was prominent, acanthosis of the lining epithelium of hair follicles was not observed in any case. These

authors are of the opinion that essential fatty acids may be the cause of 'phrynoderma' in man. However, the importance of vitamin A cannot be excluded, since the main characteristic features of 'phrynoderma' were present in both types of deficiencies. A survey of the literature on 'phrynoderma' brings forth the fact that, keratin is not always present in dense masses but may be in loose meshes and acanthosis of prickle cell layer is not a marked feature (Radhakrishna Rao, 1937 *a* and *b*). The patients suffering from 'phrynoderma' are usually on a poor diet and lack of other factors may also be playing part in the causation of such changes as acanthosis, widened interpapillary processes, etc. Besides, experimentally it has been proved that acanthosis of prickle cell layer is characteristic of pyridoxine, pantothenic acid and biotin deficiencies (Follis, 1948).

The knowledge gained from such experiments have a practical application in the treatment of 'phrynoderma'. Studies, on serum fatty acids in the cases of 'phrynoderma' by Menon *et al.* (1950) indicate that iodine number of serum fatty acids were significantly lowered. Cure was brought about by gingelly oil, which is a rich source of essential fatty acids. Gopalan (1947) treated cases of 'phrynoderma' by combined treatment of linseed oil and yeast extract. The author felt, that the failure of vitamin A concentrate to appreciably influence the skin condition in cases of 'phrynoderma' and the success obtained with raw linseed oil would suggest that the curative effect claimed for the fish liver oils is probably due to their essential fatty acids content.

As far as the experimental evidence goes [Burr and Burr (1930), Salmon (1938), Hume *et al.* (1940] it is to be noted that fish liver oils were not found very effective in curing the fat deficiency dermatitis in rats. Besides Frazier and Hu (1931, 1935, 1936), Lowenthal (1933), Reiss (1936), Youmans (1937) and Goodwin (1934) have all reported the disappearance of the lesions following the administration of cod-liver oil. Radhakrishna Rao (1938) cured two advanced cases of 'phrynoderma' by administration of vitamin A concentrate. Finally, the animal experiments carried out by Moult (1943) as well as the results of the present experiment show that vitamin A is required for the maintenance of healthy skin. In its absence, lesions were produced which were found homologous to those occurring in cases of 'phrynoderma'. Rats used in the present experiment were on a diet containing peanut oil at 5% level which on the basis of average food consumption provided 130 mg. of essential fatty acids. This dose of essential fatty acids which is much above the minimum curative dose required for curing fat deficiency disease in rats, was found incapable of preventing the skin lesions in absence of vitamin A. On this point, our results tend to lend support to Radhakrishna

Rao's (1937) hypothesis that ' phrynoderma ' is a manifestation of nutritional deficiency, in which lack of vitamin A is an important factor.

It is worth mentioning here that all Gopalan's patients showed frank signs of vitamin B deficiency and the beneficial effect of yeast in all likelihood may be due to its indirect favourable influence on the utilization of essential fatty acids. The validity of this view is borne out by the finding of Menon *et al.* (1950) who have succeeded in curing ' phrynoderma ' with gingelly oil alone without the use of vitamin B supplements. Thus it is evident that clinical cases of ' phrynoderma ' (uncomplicated by other vitamin B complex deficiencies) can be cured by the administration of essential fatty acids alone, and the influence of B vitamins in this connection is only indirect. The fact that skin lesions in experimental animals paralleling ' phrynoderma ' can be produced even on yeast diets in the absence of essential fatty acids, gives further proof that the role of B complex deficiencies in the actiology of ' phrynoderma ' is secondary.

The results of the present experiment tend to show that both vitamin A and essential fatty acids are absolutely necessary in the diet and the lack of either factor gives rise to lesions which are microscopically homologous to those occurring in ' phrynoderma '.

Summary and Conclusions

This investigation was undertaken to elucidate the actiology of ' phrynoderma'. Two experiments were conducted, one to study the effect of vitamin A deficiency and the other, to study the part played by fat in the production of cutaneous lesions in rats.

It is concluded that deficiency of vitamin A in conjunction with deficiency of essential fatty acids, plays an important role in the production of cutaneous lesions in rats resembling those in ' phrynoderma '. It is suggested that ' phrynoderma ' may be due to the combined deficiencies of both these factors. The experimental evidence further points out, that lack of vitamin A plays a larger role in the production of cutaneous lesions resembling ' phrynoderma'.

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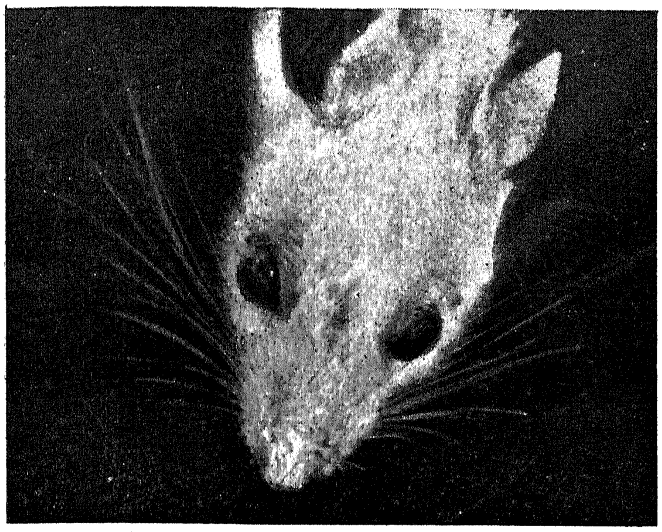


FIG. 1. Rat on a completely vitamin A deficient diet, showing typical xerophthalmia of both the eyes.

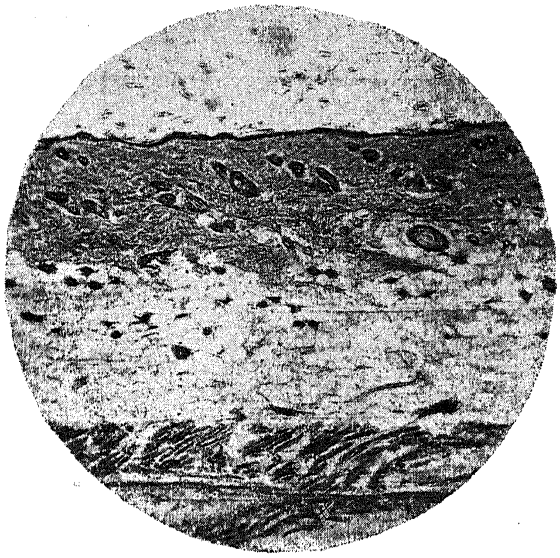


FIG. 2. Skin section of a rat receiving 50 I.U. vitamin A per day, apparently in normal health (control), ×50.

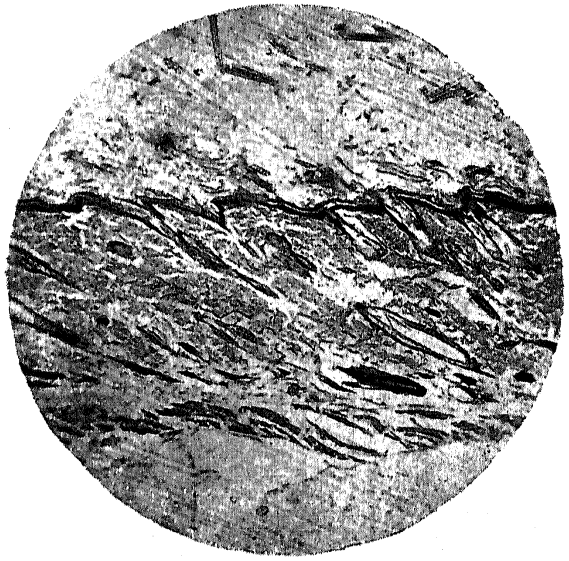


FIG. 3. Skin section of a rat receiving 1 I.U. vitamin A per rat per day, showing moderate amount of hyperkeratinization of stratum corneum layer, with dilatation and plugging of hair follicles with keratinous material. Fair amount of subcutaneous fat is present, ×50.

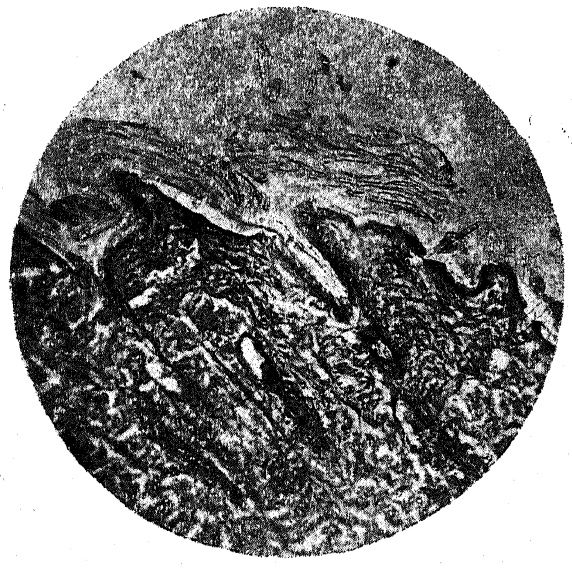


FIG. 4. Skin section of a rat on a completely vitamin A deficient diet, showing marked hyperkeratinization of stratum corneum layer, with dilatation and plugging of hair follicles with keratinous material and loss of subcutaneous fat, ×100.

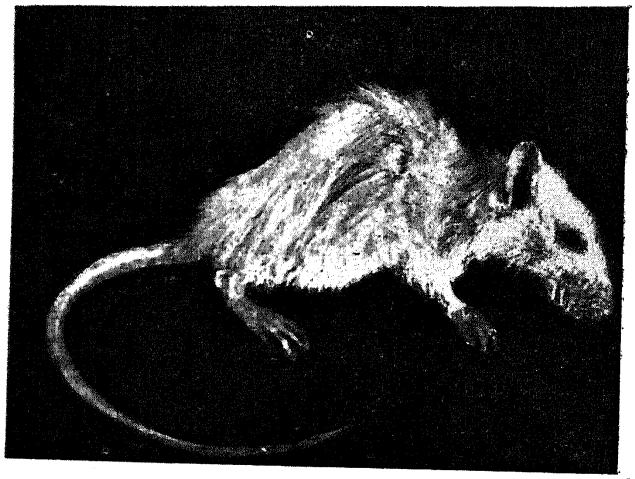
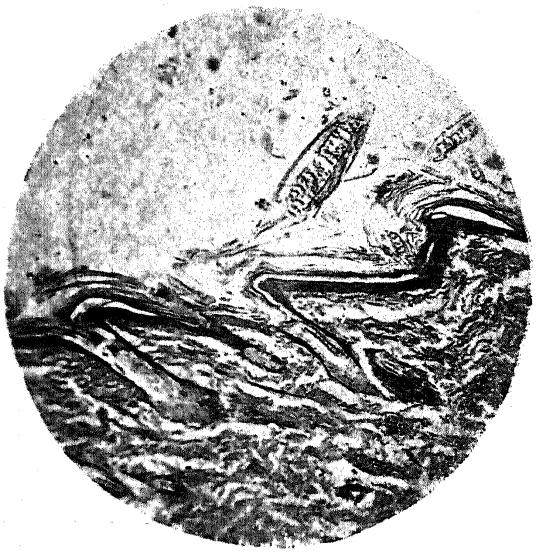


FIG. 5. Rat on a completely fat-free diet, showing y dermatitis of the paws, tail and pinnæ of the ear, evelled fur and marked emaciation. Eyes are normal.

FIG. 6. Skin section of a rat on a diet containing 5% hydrogenated oil, showing moderate amount of hyperkeratinization of stratum corneum layer, with dilatation and plugging of some hair follicles with keratinous material and loss of subcutaneous fat, × 50.



IG. 7. Skin section of a rat on a fat-free diet ining 70% sucrose, showing marked hyperkeratiniza- f stratum corneum layer, with marked dilatation and ng of hair follicles with corneum layer, with keratinous ial and loss of subcutaneous fat, ×100.

FIG. 8. Skin section of a rat on a fat-free diet, containing 50% starch and 20% sucrose, showing marked hyperkeratinization of stratum corneum layer, with slight dilatation and plugging of few hair follicles and loss of subcutaneous fat, ×50.

Figs. 2–4, 6–8. Ehrlich's acid hæmatoxylin and eosin stain.