XLII. A CONTRIBUTION TO THE STUDY OF KERATOMALACIA AMONG RATS.

By MARJORY STEPHENSON and ANNE BARBARA CLARK.

From the Biochemical Laboratory, Cambridge.

(*Received May 11th, 1920.*)

(With Plates IX and X.)

The association of acute mal-nutrition amongst children with a purulent condition of the eyes has long been familiar in medical practice. Mori [1904] associated a condition which he called "xerosis of the conjunctiva and keratomalacia," frequent amongst famine-stricken children, with fat starvation, and noted the curative effect of chicken liver and fish oils. He also observed that the disease occurred among populations using vegetable oils but was absent in sea-board districts where fish oils were used.

More recently Bloch [1917] associated an outbreak of corneal ulceration amongst children with a diet of highly separated milk, and noted the curative effect of whole milk and cod liver oil.

In 1917 McCollum and Simmonds [1917, 1], working with rats on deficiency diets, definitely associated a purulent condition of the eye—which they termed "xerophthalmia"—with the absence from the diet of the fat-soluble vitamine. In the above and a subsequent paper [McCollum and Simmonds, 1917, 2], these authors claim that xerophthalmia is an undoubted deficiency disease. They write: "There are then two deficiency diseases in the sense in which Funk employed this term. One of these is polyneuritis.... The other we believe is the syndrome described above in which the two most prominent features are emaciation and xerophthalmia" [1917, 1].

In order to make good the claim of keratomalacia, a term which we prefer to xerophthalmia, to rank as a deficiency disease in the strict sense of the term (due, in this case to the absence of the fat-soluble vitamine) it is necessary to establish the following points in respect of it:

1. That the determining cause is not bacterial infection.

2. That in one species, at least, the symptoms invariably result from a prolonged course of diet deficient in the fat-soluble factor.

3. That identical symptoms never occur as a result of any form of mal-nutrition in which the fat-soluble factor is adequately supplied in the diet.

4. That the symptoms invariably disappear when the factor is replaced in the diet (except in cases where death occurs before any general improvement can be manifested).

5. That the symptoms are not susceptible of cure by any treatment other than the supply of the fat-soluble factor.

As a contribution to this subject we have attempted to deal with Nos. 1, 2 and 4 of the above points, Nos. 3 and 5 falling outside the scope of our investigation.

The material dealt with in this communication, therefore, falls under two main headings.

A. Dietetic and Statistical.

B. (1) Histological. (2) Bacteriological.

A. DIETETIC AND STATISTICAL.

α. Incidence of the disease.

As has already been noted (p. 502) in order to weigh the claims of keratomalacia among rats to rank as a deficiency disease it is necessary to study statistically the cases which occur when these animals are placed on a diet deficient in the fat-soluble factor. For this purpose the results of eight experiments, including in all 46 rats, are here given. The rats were all young (between 40 and 50 g. in weight) at the beginning of the experiment. The diet consisted of the usual combination of purified caseinogen, starch, sugar, and vegetable fat (usually palm kernel oil), and McCollum's salt mixture [McCollum and Simmonds, 1917, 1], to which were added traces of sodium fluoride, potassium iodide, and manganese sulphate. The water-soluble factor was supplied by a fat-free alcoholic extract of yeast and the anti-scurvy factor by 0·5 cc. of lemon juice per rat per day. In Table I the results of these experiments are recorded; Table II summarises the results of the eight experiments. The duration of the experiment is divided into periods of ten days; in each of these periods are recorded:

1. The total number of deaths occurring in the period.
2. The number of deaths with eye disease occurring in the period.
3. The total number of survivors at the end of the period.
4. The survivors with eye disease at the end of the period.
5. The cases of eye disease occurring during the period.

Fig. 1 represents the deaths occurring in the successive periods of the experiment; the blackened portion indicates the number of those deaths preceded by eye disease. It is seen from this that no deaths occurring before the 50th day were preceded by eye disease; after that period an increasing proportion of deaths were preceded by symptoms of keratomalacia, but *in no period* are *all* the deaths so preceded.

Fig. 2 represents the number of rats surviving at the end of any one period; the blackened portion represents those survivors having eye disease. It is here seen that the incidence of the disease increased with the duration of the experiment, but that at *no* period are *all* the surviving rats afflicted with the symptoms.

Table I.

No. of rats on experiment		Day of experiment on which deaths occurred	Day of experiment on which eye disease first noted	No. of rats which had eye disease	No. of rats which had no eye disease	No. of survivors
I.	5	29	No eye disease	1	4	0
		52	„ „			
		79	„ „			
		80	64th day			
		80	No eye disease			
II.	5	11	„ „	2	3	0
		11	„ „			
		40	„ „			
		85	77th day			
		86	77th day			
III.	5	65	61st day	4	1	0
		65	61st day			
		65	No eye disease			
		80	77th day			
		86	77th day			
IV.	5	15	No eye disease	2	3	0
		15	„ „			
		31	„ „			
		82	77th day			
		86	56th day			
V.	6	47	No eye disease	4	2	0
		54	50th day			
		57	57th day			
		78	No eye disease			
		79	47th day			
		104	82nd day			
VI.	5	26	No eye disease	0	5	0
		38	„ „			
		38	„ „			
		38	„ „			
		38	„ „			
VII.	9	15	„ „	0	9	0
		27	„ „			
		30	„ „			
		30	„ „			
		31	„ „			
		31	„ „			
		31	„ „			
		41	„ „			
		43	„ „			
VIII.	6	26	„ „	0	6	1
		26	„ „			
		56	„ „			
		64	„ „			
		88 (killed)	„ „			
Total	46	—	—	13	33	1

Table II. Summary of eight experiments.

	Day											
	0 to 9	10 to 19	20 to 29	30 to 39	40 to 49	50 to 59	60 to 69	70 to 79	80 to 89	90 to 99	100 to 109	110 to 119
Total deaths	0	5	5	10	4	4	4	3	9	0	1	0
Deaths with eye disease ...	0	0	0	0	0	2	2	1	7	0	1	0
Total survivors (end of period)	46	41	36	26	22	18	14	11	2	2	1	1
Survivors with eye disease (end of period)	—	—	—	—	1	2	3	7	1	1	0	0
Cases of eye disease occurring	0	0	0	0	1	3	3	5	1	0	0	0

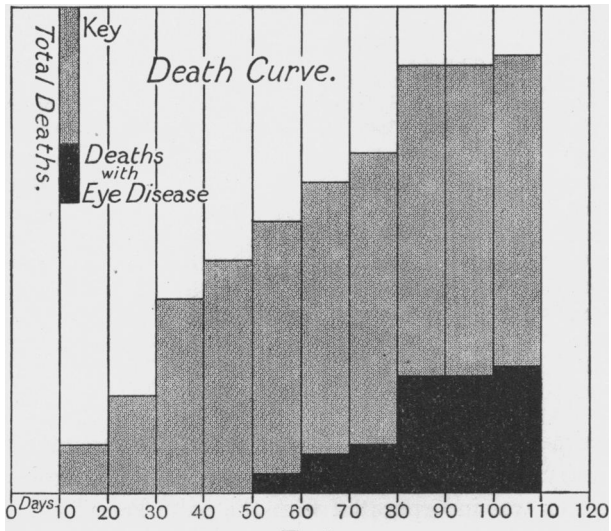


Fig. 1.

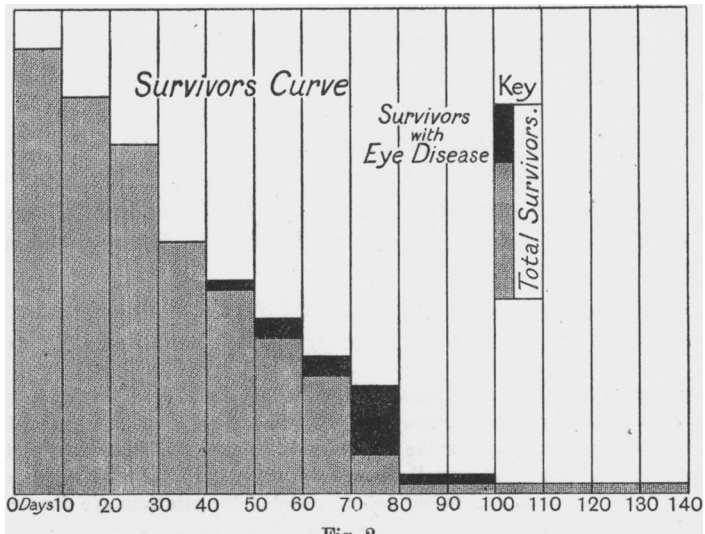


Fig 2.

These facts taken in conjunction suggest: (1) That a diet deficient in the fat-soluble factor, whilst strongly predisposing the subject to eye disease, does not invariably result in it[1]. (2) That the occurrence of the disease is not coincident with the cessation of growth, but begins at a later stage.

β. Cure of the Eye Disease.

In ten cases of eye disease (distinct from any appearing in the previous tables) cure was attempted; the results are tabulated below (Table III).

Table III.

Day of experiment on which disease was first noted	Day of experiment on which cure was attempted (*i.e.* fat-soluble factor added to the diet)	Day of experiment on which cure was complete	Day of experiment on which rat died
72nd	82nd	86th	96th
54th	82nd	93rd	106th
72nd	82nd	107th	survived
50th	54th	60th	62nd
54th	54th	67th	101st
50th	54th	60th	survived
57th	60th	64th	,,
37th	39th	46th	,,
37th	39th	46th	,,
36th	39th	43rd	,,

These experiments were made in connection with a separate investigation; the material carrying the fat-soluble factor was a light petroleum extract of dried carrot which was incorporated with the palm kernel oil. This material was shown in control experiments (including 21 rats in all) to be carrying the fat-soluble factor.

It is seen that *in every case* the eye symptoms cleared up though the time taken to effect the cure varied considerably; it is noteworthy however that four out of the ten rats failed to survive.

Thus of 46 rats on a diet deficient in the fat-soluble factor 96 % failed to survive 90 days, and 28 % contracted eye disease. Of ten rats which had contracted disease and were given the fat-soluble factor 100 % were cured of the disease, but only 60 % restored to health, 40 % dying after the eye symptoms had cleared up.

From these two sets of experiments (α and β) an apparent paradox emerges; absence of the fat-soluble factor almost invariably causes death (preceded by

[1] It is our opinion that the non-appearance of "xerophthalmia" on diets deficient in the fat-soluble factor recorded from this laboratory [Bulley, 1919] was due to the fact that for a period during the war the caseinogen used for the experimental animals was insufficiently extracted with alcohol. We have since found that a small amount of lipoid material, somewhat rich in the factor, was present in this caseinogen and in our belief protected the rats from eye disease. This, and the comparative rarity of the disease in the human subject, suggests that keratomalacia only appears when the diet is very highly deficient in the fat-soluble factor.

cessation of growth) and *sometimes* produces eye disease; replacement of the fat-soluble factor *invariably* cures the eye disease but sometimes (on the same rats) fails to restore growth and save life.

The first explanation of this apparent paradox that suggests itself is that the concentration of the fat-soluble factor in the tissues necessary to protect the eye is smaller than that necessary to ensure normal growth; this would account both for the later incidence of the disease and its more certain cure. It does not however afford a satisfactory explanation of all the facts. Supposing the concentration of the fat-soluble vitamine (in the tissues) necessary to life be x, while that necessary to protect from eye disease be y. In the case of the rats dying before they contract eye disease x is greater than y, whilst in those rats which develop eye disease before death x is less than y. Such a difference in the requirement of young animals of the same species is surprising. The facts may mean no more than that such a variation in individual resistance exists as is seen clinically when one element in a symptom complex is more prominent in one case, and some other element in another. But under the controlled conditions of experimental studies such variations within the same species are not usually seen.

An alternative hypothesis which suggests itself is that two factors are involved; one responsible for life and growth and another necessary for the nutritive integrity of a special tissue such as the cornea. Rats, which at the start of the experiment contain a relatively larger supply of the life-growth factor develop eye disease before death, whilst those which start with relatively more of the corneal-protecting factor exhaust their life-growth factor (*i.e.* die) before developing eye disease. Such a hypothesis presupposes that these two factors are present in varying proportions in different natural foodstuffs, and that the relative amounts of each accumulated in the rat at the beginning of the experiment are conditioned by its previous range of diet. The final consideration of these points must be postponed until they can be considered in relation to the histological and bacteriological results.

B. HISTOLOGICAL AND BACTERIOLOGICAL.

We have been unable to find in the literature any detailed description of keratomalacia among rats, and have therefore attempted an investigation of this disease. In planning this work the questions we had in view were as follows:

1. What microscopic degenerative changes take place in the eye during the course of this disease?

2. Are these changes directly and solely caused by bacterial invasion, or is this infection preceded by histological changes in the tissue brought about by a prolonged course of a diet deficient in the fat-soluble factor?

3. Is one particular organism predominant in the bacterial infection?

B (1). Histological.

Preparations have been made of the eyes of:

A. Normal rats on mixed diet.

B. Rats which though on a diet deficient in the fat-soluble factor showed none of the symptoms of eye disease.

C. Rats in which symptoms of disease were apparent in one eye but not in both.

D. Rats in which both eyes were in an advanced stage of the disease.

E. Rats in which a cure had been effected and sight restored by change of diet, *i.e.* by the addition of the fat-soluble factor.

F. Rats in which change of diet had cleared up the purulent symptoms and effected the healing and regeneration of tissue, but in which sight had not been restored.

Method.

The eyes were placed in 10 % formalin for 24 hours, washed in water, passed through the alcohols and xylene, and embedded in paraffin. Sections were stained by each of the following:

1. Ehrlich's haematoxylin and eosin.
2. Iron haematoxylin and van Gieson.
3. Gram-Weigert stain for bacteria.

Results.

Figs. 9–18 will be found on Plates IX and X.

A. *Normal rats on mixed diet.*

Case 1. Fig. 17 shows a section of a normal eye; Fig. 9 shows a section of a normal cornea.

B. *Rats which though on a diet deficient in the fat-soluble factor showed none of the external symptoms of eye disease.*

Case 2. *Rat No.* 53 ♀.

Diet. Laboratory synthetic diet with palm kernel oil extract of yeast and lemon juice.

Growth curve, see Fig. 3.

Note that the growth and life of this rat were unusually prolonged and that no symptoms of eye disease were shown at any time. The rat was killed on the 88th day. Sections of the cornea of this rat do not differ in any important respect from those of the normal cornea so that it is unnecessary to append a figure.

C. *Rats in which symptoms were apparent in one eye but not in both.*

Case 3. *Rat No.* 7.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

This case is one in which symptoms were apparent in one eye whilst the other seemed to be normal.

Histological examination of the apparently normal eye (Fig. 10) shows the presence of a few leucocytes (*l*) in the cornea proper.

Sections of the other eye show a swollen cornea with loosened tissue due to the infiltration of fluid; leucocytes are present in the cornea proper.

Case 4. *Rat No.* 74.

Diet. Laboratory synthetic diet.with palm kernel oil, extract of yeast and lemon juice.

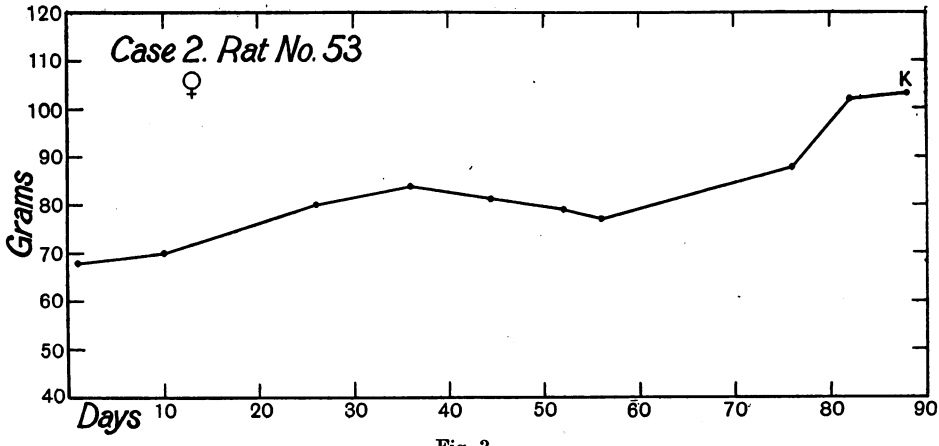


Fig. 3.

As in Case 3 (Rat No. 7) one eye was visibly diseased whilst the other was apparently normal. Histological examination of the normal eye (Fig. 11) shows that, unlike the apparently normal eye in Case 3, no abnormality can be discerned in the cornea.

In the case of the diseased eye of the same rat (Fig. 12) the cornea shows an enormously increased number of corneal corpuscles and a great many well-defined blood-vessels lined with epithelium (*b.v.*). These profound tissue changes are not uniformly distributed throughout the cornea but are more marked in some areas than in others. This observation will be referred to later in the discussion of the significance of the histological changes.

D. *Rats in which both eyes are in an advanced stage of the disease.*

Case 5. *Rat No.* 77.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

Growth curve, see Fig. 4; symptoms of disease were first noted on the 66th day (A).

Both eyes in this rat were in approximately the same state and show apparently an earlier stage of the disease than does the diseased eye of Case 4.

In Fig. 13 we notice localised increase of corneal corpuscles with well-marked blood vessels (*b.v.*). As in Case 4 these changes are not uniformly distributed throughout the cornea.

Gram-stained preparations of both these eyes show large numbers of Gram-positive cocci of the pneumococcal type, see Fig. 14 (*d*).

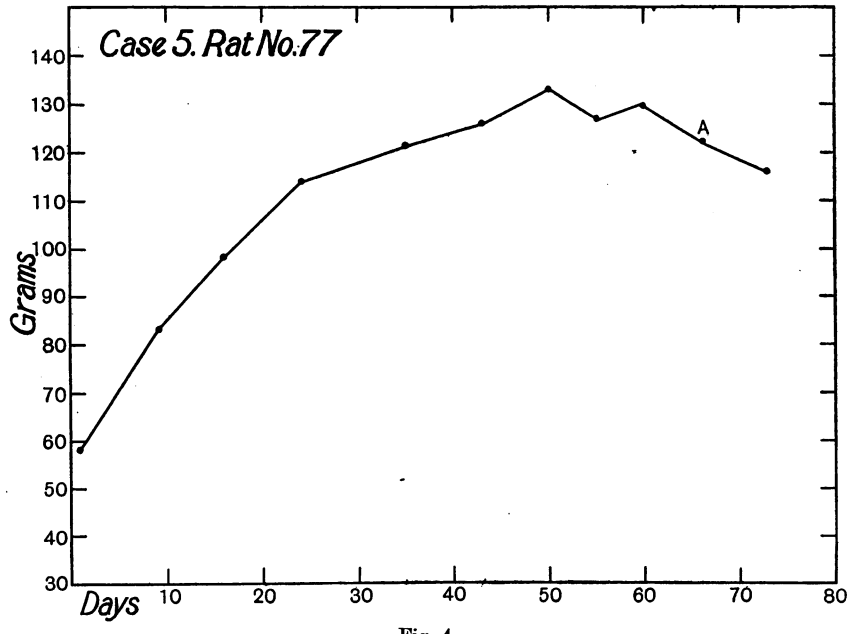


Fig. 4.

Case 6. *Rat No.* 60.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

The first symptoms of disease were noted on the 51st day (A); death took place on the 82nd day.

Growth curve, see Fig. 5.

Both eyes of this rat were in an advanced stage of the disease; both corneas were completely disintegrated exposing the front of the lens, which protruded during life.

Sections of the eye showed the lens complete but no anterior structure remaining.

Gram-stained preparations showed the presence of Gram-positive cocci of the pneumococcal type in the sclerotic.

E. *Rats in which a cure has been effected and sight restored by the addition to the diet of a fat containing the fat-soluble vitamine in place of the palm kernel oil.*

Case 7. *Rat No.* 113 ♀.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

Growth curve, see Fig. 6.

In order to interpret this growth curve it is necessary to give details of the history of this rat.

The first symptoms of eye disease were noted on the 50th day of experiment, the left eye only being affected (A).

On the 54th day both eyes were affected; the palm kernel oil was then replaced by bone marrow in order to test the vitamine content of this fat (B).

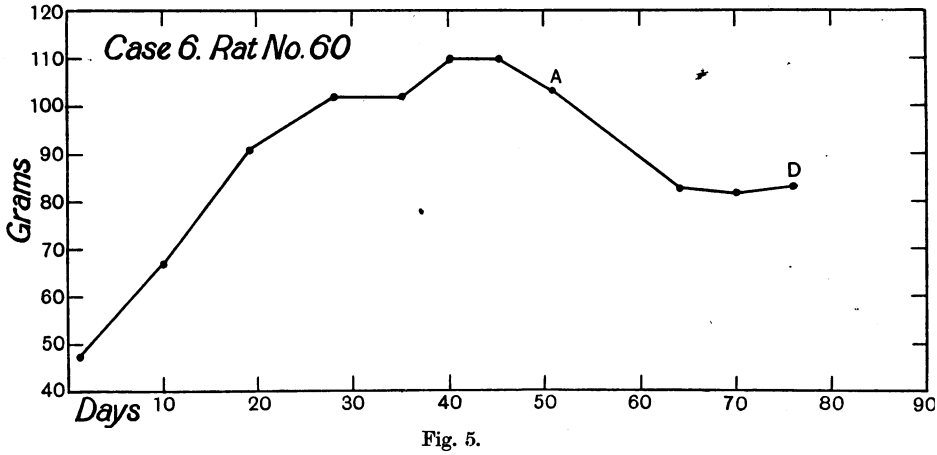


Fig. 5.

On the 57th day the eyes had improved (C), and on the 66th day all purulent symptoms had disappeared and the eyes were apparently normal (D).

Symptoms of eye disease returned however on the 93rd day both eyes becoming purulent (E).

On the 96th day the symptoms were more pronounced (both eyes were then swabbed) (F).

On the 105th day the bone marrow was replaced by butter (G).

On the 119th day the eyes were cured (H).

On the 137th day the rat was killed (K).

It is seen from the above notes that 18 days elapsed between the apparent completion of the cure by butter and the killing of the rat.

Histological examination of the sections showed that the cornea had completely recovered the normal state.

Case 8. *Rat No.* 114 ♀.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

Growth curve, Fig. 7.

The first symptoms of eye disease were noted on the 72nd day when both eyes were slightly purulent (Z). On the 82nd day both eyes were very purulent and closed (Y). On the 85th day crude light petroleum extract of carrot was added to the palm kernel oil (X); on the 95th day a marked improvement had taken place, a slight discharge only from the right eye being noticeable (W).

On the 98th day the eye was swabbed.

On the 107th day both eyes were clean showing only minute spots of discharge on the lid (V); these had disappeared on the 119th day (U). On the 142nd day the rat was killed (K).

As in Case 7, a considerable interval, in this case 23 days, elapsed between the completion of the cure and the killing of the rat. Histological examination of the sections showed that the cornea had almost completely resumed its normal condition, a slight increase in the number of corneal corpuscles only being still apparent.

F. *Rats in which supply of the fat-soluble factor had cleared up the purulent symptoms and effected the healing and regeneration of the tissue, but in which sight had not been restored.*

Case 9. *Rat No.* 5.

Diet. Laboratory synthetic diet with palm kernel oil, extract of yeast and lemon juice.

Growth curve, see Fig. 8.

The earliest symptoms of eye disease in this rat escaped observation. On the 39th day both eyes were opaque and one was bleeding (A). Light petroleum extract of carrot was then added to the palm kernel oil. On the 46th day (B), purulent symptoms had disappeared; the left eye was clean and whole but with an opaque cornea; the right eye was permanently closed. On the 251st day the rat was killed.

Sections of the left eye disclose striking abnormalities. The cornea (Fig. 15) is considerably flattened and large vessels full of blood cells (*r.b.c.*) still remain. Since 90 days elapsed between the completion of the cure and the killing of the rat, this condition of the cornea may be regarded as permanent and not as representing a stage in the cure. In the case of the right eye the cornea degenerated so completely that the lens actually fell out of the eye during life; when the diet was changed the purulent condition of the eye was cured. Histological examination of the sections (Fig. 18) shows that the disappearance of the large lens has caused collapse and shrinkage of the "eye"; in place of the cornea there is a curious regenerated tissue built up from various elements (Fig. 16). This opaque anterior structure contains an enormous number of corneal corpuscles and a mass of fibrous connective tissue that has been built up from behind by the proliferation of corneal corpuscles.

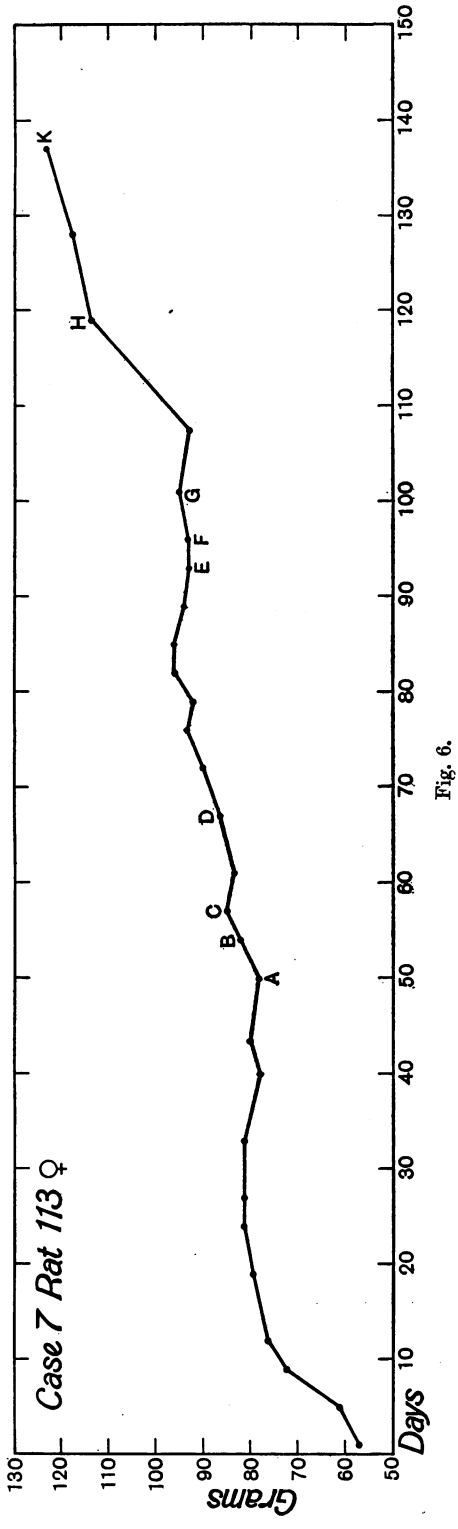

Case 7 Rat 113 ♀
Grams
Days
A
B
C
D
E
F
G
H
K

Fig. 6.

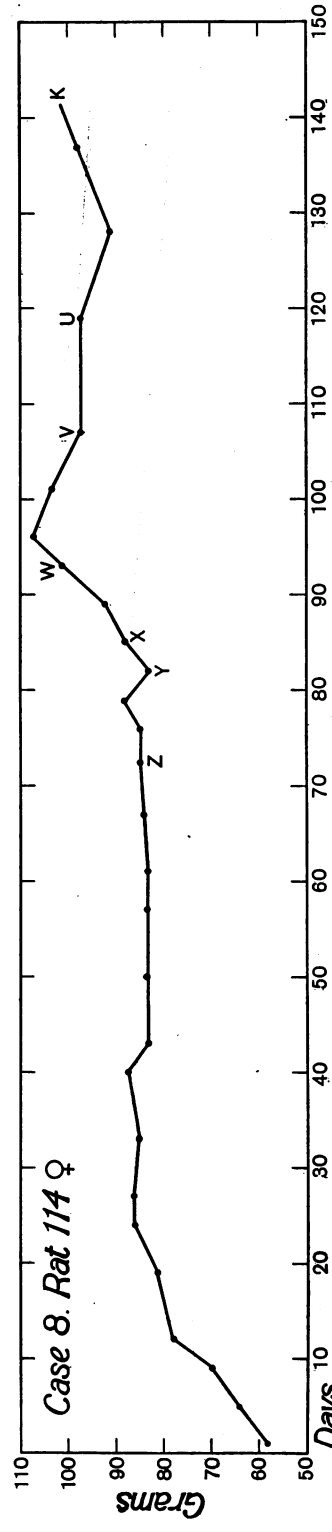
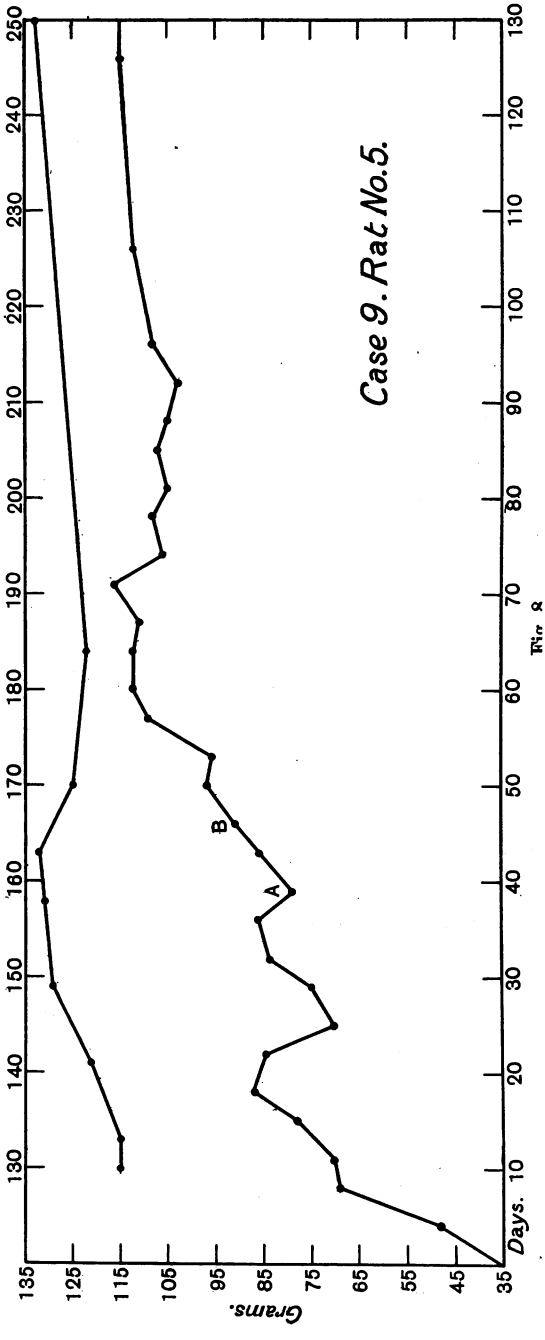

Case 8. Rat 114 ♀
Grams
Days
K
U
V
W
X
Y
Z

Fig. 7.


Case 9. Rat No. 5.
Grams.
Days.
A
B
135
125
115
105
95
85
75
65
55
45
35
130
140
150
160
170
180
190
200
210
220
230
240
250
10
20
30
40
50
60
70
80
90
100
110
120
130

Summary of Histological Results.

The earliest histological changes noticed in the cornea are those in Case 3 (the apparently normal eye of a rat whose other eye was purulent and degenerated). This apparently normal eye showed the presence of leucocytes in the cornea, a condition which is frequently associated with incipient bacterial infection. We cannot therefore deduce from the condition of such corneas that there is any demonstrable histological change directly attributable to dietary deficiency prior to bacterial invasion. A slightly later stage is shown by the other eye described in Case 3, where purulent symptoms were apparent and the cornea was swollen and oedematous. Again leucocytes are present, but no definite blood vessels are yet discernible.

In Case 5 very definite blood vessels are present, and there is marked increase in the number of corneal corpuscles.

The bad eye of Case 4 shows the same abnormalities in a much greater degree. In both Cases 4 and 5 the tissue changes are more or less localised in definite areas, thus affording strong evidence that they are the direct result of bacterial infection.

In Case 6 the cornea is completely degenerated and here, as in Case 5, organisms of the pneumococcal type were demonstrated.

The histological conditions of "cured" eyes are of different types. In the majority of cases where cure is effected by change of diet, sight is restored and the cornea completely regains its normal condition as in Cases 7 and 8.

Where degeneration has (presumably) proceeded further before cure is commenced, the cornea may remain opaque as in Case 9, left eye. Histological investigation of this cornea shows that it is flattened and contains many large blood vessels. As 105 days had elapsed between the apparent completion of the cure and the killing of the rat, it is justifiable to assume that this condition was permanent and did not represent a stage in the recovery of the cornea.

In some cases the cornea has so far degenerated before cure is begun that the lens is forced through the aperture during life, and "cure" consists in the disappearance of pus and the healing over of the injured tissues. Case 9, right eye, exemplifies this condition.

B (2). Bacteriological.

Method.

Pus was collected from rats' eyes with a sterile platinum loop, direct films were examined, and cultures were put up in tryptic broth ($P_H = 7{\cdot}4$), on tryptic blood agar ($P_H = 7{\cdot}4$), and on inspissated serum. Anaerobic broth cultures were also incubated, but in no case did they yield the growth of organisms other than those facultative anaerobes that also appeared in the aerobic cultures. The organisms were obtained in pure culture, and fermentation reactions were studied by growth for seven days in litmus milk and

sugars, etc. (1 % of the substance dissolved in diluted tryptic broth, with litmus as an indicator). The organisms present in the conjunctival sac of several normal and "cured" rats were examined in a similar way.

Results.

The following organisms were isolated:

1. Pneumococci; two types (see Table IV).
2. Staphylococci (*S. albus* and *S. aureus*).
3. Gram-positive cocco-bacilli; four types (see Table IV).
4. Bacillus subtilis.
5. Gram-positive diphtheroids; two types (see Table IV).
6. Other Gram-positive bacilli; two types (see Table IV).
7. Gram-negative bacilli; two types (see Table IV).

A list of the organisms found in 18 cases is appended:

α. Normal rats on mixed diet; eyes clean and healthy.

Case 10. (*a*) S. aureus.
(*b*) Cocco-bacillus, Type III.

Case 11. (*a*) Pneumococcus, Type I.
(*b*) Gram-positive bacillus, Type II.

Case 12. (*a*) S. albus.

β. Rats on diet deficient in fat-soluble factor.

Case 13. *Rat No.* 91; eyes purulent and bleeding.
(*a*) Pneumococcus, Type I.
(*b*) Cocco-bacillus, Type II.

Case 14. *Rat No.* 129; eyes closed; purulent.
(*a*) Pneumococcus, Type I.
(*b*) S. aureus.

Case 15. *Rat No.* 48; eyes purulent.
(*a*) Pneumococcus, Type I.
(*b*) Diphtheroid, Type I.

Case 16. *Rat No.* 202; eyes purulent.
(*a*) Pneumococcus, Type II.
(*b*) Cocco-bacillus, Type III.

Case 17. *Rat No.* 205; eyes bleeding.
(*a*) Pneumococcus, Type II.
(*b*) Gram-negative bacillus, Type II.

Table IV.

Organism	Glucose	Saccharose	Mannitol	Maltose	Lactose	Raffinose	Inulin	Levulose	Galactose	Dextrin	Dulcitol	Milk	Notes
Pneumococcus, Type I	A	A	A	A	A	A	A	A	A	A	—	A	Long chains formed in broth.
„ „ II	A	A	A	A	A	—	A	A	A	A	—	A or AC	Long chains formed in broth.
Cocco-bacillus, Type I	A	A	—	A	A	A	—	A	A	A	—	A	Very small colonies on agar.
„ „ II	A	A	A	A	A	—	A	A	A	A	—	AC	Strong growth on agar.
„ „ III	A	(A)	A	A	A	—	—	A	A	A	—	(AC)	Spreading greenish growth on agar.
„ „ IV	A	—	—	—	—	—	—	(A)	A	(A)	—	AC	Strong white viscous growth on agar; pellicle formed on broth.
Diphtheroid, Type I	No growth												Very small colonies on agar.
„ „ II	A	—	—	—	—	—	—	A	A	A	—	—	Strong growth on agar.
Gram-positive Bacillus, Type I	A	A	—	A	—	—	—	A	—	A	—	—	White lustrous growth on agar.
Gram-positive Bacillus, Type II	A	A	A	A	A	A	—	A	A	A	—	A	Strong growth on agar; non-motile.
Gram-negative Bacillus, Type I	—	—	—	—	—	—	—	A	A	A	—	A	Liquefies inspissated serum.
Gram-negative Bacillus, Type II	AG	—	—	—	—	—	—	AG	AG	AG	—	A	Strong growth on agar; non-motile.

A = acid.
AG = acid and gas.
AC = acid and clot.
(A) = acid usually but not invariably formed.

Case 18. *Rat No.* 63; eyes purulent.
(*a*) Gram-positive lancet-shaped diplococci not obtained in pure culture.
(*b*) S. albus.
(*c*) B. subtilis.

Case 19. *Rat No.* 113 (see Case 7); eyes purulent.
(*a*) S. aureus.
(*b*) B. subtilis.
(*c*) Gram-negative bacillus, Type I.

Case 20. *Rat No.* 201; eyes purulent.
(*a*) Diphtheroid, Type I.

Case 21. *Rat No.* 203; eyes opaque and bleeding; no pus.
(*a*) Cocco-bacillus, Type III.
(*b*) Gram-positive bacillus, Type I.

Case 22. *Rat No.* 204; eyes purulent and bleeding.
(*a*) Cocco-bacillus, Type III.
(*b*) Diphtheroid, Type I.

Case 23. *Rat No.* 206; eyes closed; slightly purulent.
(*a*) Cocco-bacillus, Type IV.
(*b*) Diphtheroid, Type I.

Case 24. *Rat No.* 207; eyes purulent.
(*a*) Cocco-bacillus, Type III.
(*b*) Cocco-bacillus, Type IV.

γ. Rats which developed eye disease on a diet deficient in the fat-soluble factor, and which were subsequently cured by change of diet.

Case 25. *Rat No.* 114 (see Case 8).

Right eye still purulent.
(*a*) Pneumococcus, Type I.
(*b*) S. aureus.
(*c*) B. subtilis.
(*d*) Gram-negative bacillus, Type II.

Left eye: no pus.
(*a*) Diphtheroid, Type II.

Case 26. *Rat No.* 161; eyes still showing symptoms of disease.
(*a*) S. albus.
(*b*) Diphtheroid, Type II.

Case 27. *Rat No.* 63 (see Case 1 before treatment).

The diet was changed on the 73rd day, and on the 78th day the eyes were quite clean. Two days later the eyes were swabbed.
(*a*) Cocco-bacillus, Type I.

An attempt was made to determine whether the eyes of rats which had subsisted for a prolonged period on a diet deficient in the fat-soluble factor were any more susceptible to bacterial infection than the eyes of control rats on butter diet. For this purpose four rats were selected, two normal rats and two which had not developed eye disease although they had been eight weeks on a diet deficient in the fat-soluble factor. The right eye of each of these four rats was smeared with pus just removed from a purulent eye. The experiment was repeated four times and in each case no symptoms of eye disease developed in any of the four rats.

Interpretation of Bacteriological Results.

Cases 10, 11 and 12 indicate that the normal conjunctiva of the rat has a varying flora. When interpreting the results from diseased eyes, it must be remembered that pus from the eye is open to contamination from the air, and that therefore an organism isolated from the pus is not *necessarily* concerned with the production of lesions in the eye.

Pus from the diseased eyes in many cases (13, 14, 15, 16, 17, 25) showed the presence of a Pneumococcus, but this organism was not invariably present, in fact no organism that we obtained was common to all the purulent cases, so that we have not been able to show that the specificity of the disease is related to bacterial species.

From the results given above, it appears that when the rat has been on a deficiency diet for such a length of time that the resistance of the cornea is affected, those pathogenic bacteria which happen to be present in the conjunctival sac invade the corneal epithelium and induce the destruction of tissue.

Summary and Discussion.

The histological and bacteriological evidence shows that keratomalacia among rats consists in a breakdown of the corneal tissue, caused by bacterial invasion.

The dietetic and statistical evidence shows that bacterial invasion of the cornea occurs only after a prolonged course of diet in which the fat-soluble factor is absent. The condition therefore directly attributable to dietetic deficiency is a predisposition to bacterial infection of the cornea leading to purulent symptoms and the destruction of tissue.

The question then to be decided is whether this predisposition of the cornea to infection is to be regarded as a true deficiency disease. The answer to this question is complicated by the following consideration.

We have hitherto failed to demonstrate with certainty any histological change in the cornea preceding bacterial invasion. We cannot therefore state the precise moment at which the predisposition to infection begins. The only criterion we possess for determining the preliminary change caused by the deficient diet is the appearance of the secondary symptoms caused by bacterial

invasion. Supposing a variable time to elapse between the preliminary change in the cornea and the actual invasion by bacteria—inflammation, pus, etc.—then the statistics collected in the earlier part of the paper are invalidated, although the evidence on the question of the cure is unaffected. If however we may assume that the organisms normally present in the conjunctival sac ensure the infection of the cornea as soon as the absence of the fat-soluble factor has rendered it liable to infection, then we are justified in regarding, for statistical purposes, the appearance of the purulent symptoms as an immediate notification of the preliminary change in the cornea due to dietary deficiency, and the evidence adduced on the incidence of the disease remains valid.

Assuming—as seems probable—the latter hypothesis to be the true one, the statistical evidence described in the earlier part of our paper can be applied to decide whether predisposition of the cornea to infection is a true deficiency disease. The conclusions then arrived at may be briefly recapitulated.

1. Corneal disease is not coincident with failure of growth culminating in death; it forms only 28 % of the cases examined.

2. This disease is further differentiated from the cessation of growth-death symptoms by occurring at a later stage in the experimental period and rising more rapidly to a maximum.

3. At no period in the experiment are all the deaths preceded by eye disease or all the survivors afflicted with it.

4. Cure of the corneal disease was effected by the replacement of the fat-soluble factor in 100 % of the cases attempted.

Before this evidence, however, serves to place preliminary deterioration of the cornea among deficiency diseases, it requires the acceptance of one of the three supplementary hypotheses A, B or C below:

either

A. The symptoms caused by experimental fat-soluble deficiency disease among rats vary in such a way that in some cases the cessation of growth-death symptoms predominate to such a degree that death ensues before the deterioration of the cornea commences, whereas in other cases the nutritive integrity of the cornea is disturbed before the cessation of growth symptoms reach an acute stage;

or

B. The concentration of the fat-soluble factor in the tissues of the rat necessary to protect the cornea was less than that required for life and growth in 72 % of the cases examined (*i.e.* in those dying without eye disease); whilst the reverse was true in 28 % of the cases (*i.e.* in those developing disease before death);

or

C. Two factors are involved; one responsible for the continuance of growth and the maintenance of life, and another for the protection of the cornea.

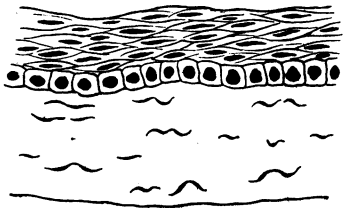


Fig. 9

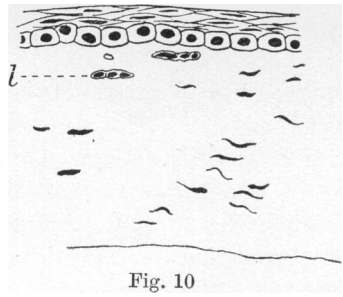


Fig. 10

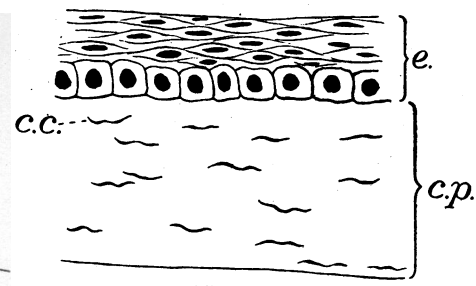


Fig. 11

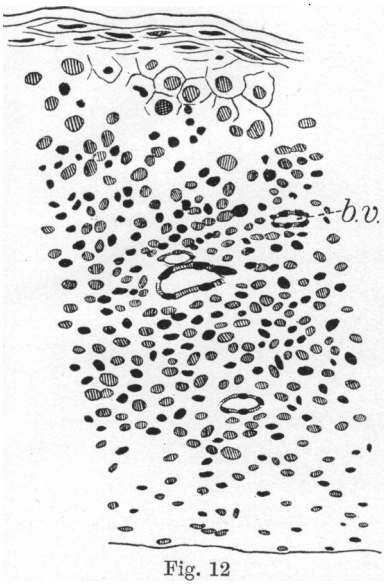


Fig. 12

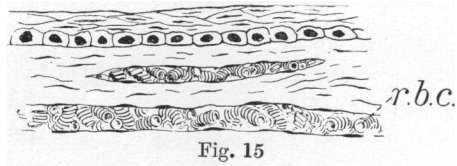


Fig. 15

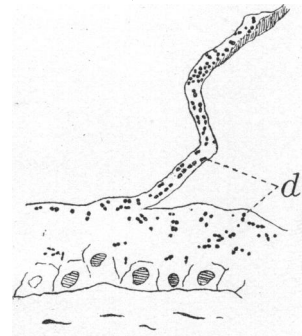


Fig. 14

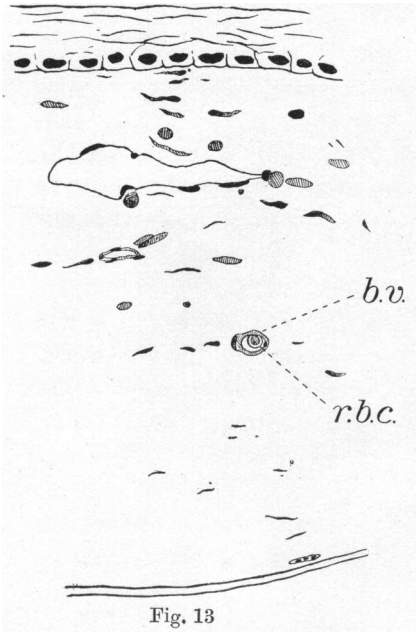


Fig. 13

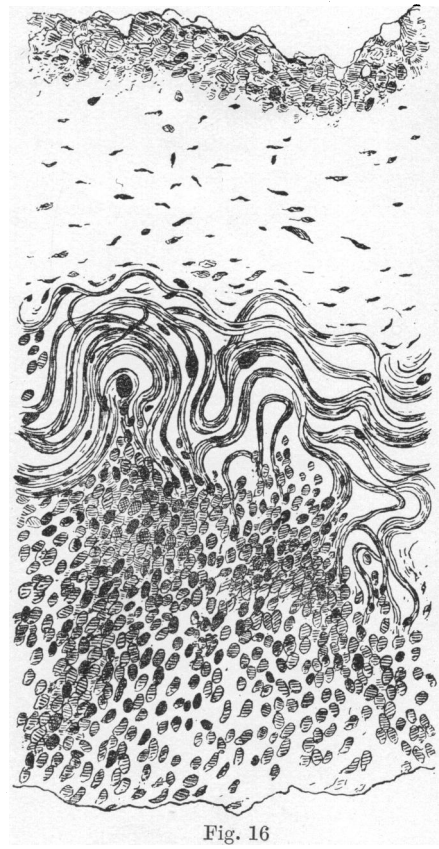


Fig. 16

0 10 20 30

Fig. 14

Figs. 9, 10, 11, 12, 13, 15, 16.

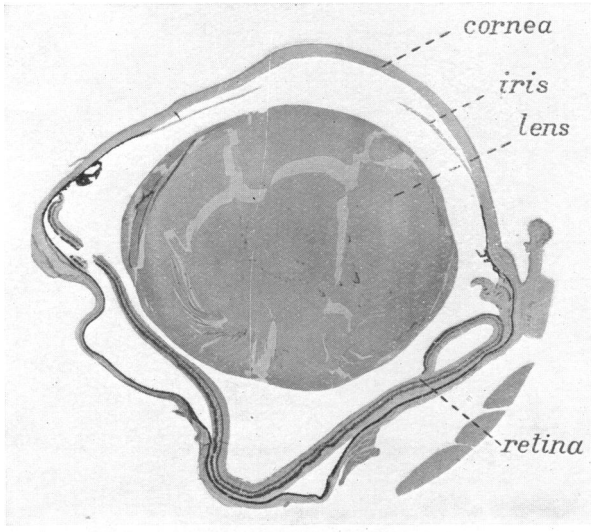


Fig. 17

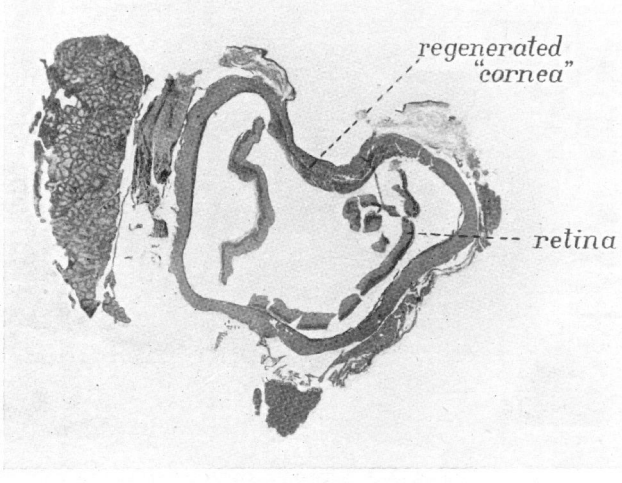


Fig. 18

The authors gladly avail themselves of this opportunity for thanking Professor Hopkins for his stimulating help and advice in the course of this investigation. They are also deeply indebted to Dr H. K. Anderson, Master of Gonville and Caius College, Cambridge, for his invaluable help in interpreting histological evidence.

The expenses of this research were defrayed from funds contributed by the Planters Margarine Co., and the Maypole Co.

The authors are also indebted to the above firms for their courtesy in permitting the results to be published.

EXPLANATION OF PLATES IX AND X.

Fig. 9. Normal cornea.
Fig. 10. Case 3, Rat 7. Cornea of apparently normal eye.
Fig. 11. Case 4, Rat 74. Cornea of normal eye.
Fig. 12. Case 4, Rat 74. Cornea of diseased eye.
Fig. 13. Case 5, Rat 77. Cornea.
Fig. 14. Case 5, Rat 77. Portion of degenerated corneal epithelium stained by Gram's method.
Fig. 15. Case 9, Rat 5. Cornea of left eye.
Fig. 16. Case 9, Rat 5. Cornea of right "eye."
Fig. 17. Microphotograph of normal eye. (× 25.)
Fig. 18. Microphotograph of Case 9, Rat 5, right "eye." (× 25.)

b.v. = blood vessel. *c.c.* = corneal corpuscle. *c.p.* = cornea proper. *d.* = Gram-positive diplococci. *e.* = epithelium. *l.* = leucocytes. *r.b.c.* = red blood corpuscles.

Microphotographs by A. C. P. Lunn, King's College, Cambridge.

REFERENCES.

Bloch, C. E. (1917). *Ugeskruft for Laeger*, **79**, 349.
Bulley, E. C. (1919). *J. Biol. Chem.*, **13**, 103.
McCollum and Simmonds (1917, 1). *J. Biol. Chem.*, **32**, 181.
—— (1917, 2). *J. Biol. Chem.*, **32**, 347.
Mori, M. (1904). *Jahrb. Kinderheilk*, **59**, 175.