

VITAMIN A

and

A CLINICAL STUDY

of

VITAMIN A DEFICIENCY.

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D. P. Lambert.

In the medicine of the present century few more important discoveries have been made than that of the vitamins. Though the work of the earlier students of nutrition is of lasting value, and must still be taken into account whenever the problems of diet are considered, the new knowledge has explained much that was doubtful and has proved its value in practical work all over the world.

The pioneer work of Hopkins in England and of Osborne and Mendel in America, which established the essential properties of the fat-soluble vitamins, has been confirmed and extended by students in all parts of the world. In 1924 the Medical Research Council issued the second edition of their "Report on the Present State of Knowledge of Accessory Food Factors (Vitamins)" (66). This invaluable survey gives a very complete list of references to the earlier work on Vitamins.

In this thesis the above mentioned report is taken as a starting point, and except to illustrate particular questions or to mark an interesting early observation no references are given to work published before 1924.

THE SEPARATION OF VITAMINS A, D, and E.

Though Sherman and Smith (396) consider that Vitamins A and D had by this time been conclusively separated, British workers adopted a more conservative attitude, and though in the Medical Research Council's report (66) a distinction between "Vitamin A" and the "anti-rachitic vitamin" is drawn the authors go on to say that "This nomenclature is adopted provisionally and without prejudice to the existence of two or more fat-soluble vitamins".

Wagner and Wimberger (453) showed that oxidised cod-liver oil though deprived of much of its growth promoting power, was still a potent anti-rachitic substance, being twenty times more powerful in this regard than in growth promotion. More conclusive evidence came from the discovery of the relationship between irradiation and the anti-rachitic vitamin. The beneficial effects of sunlight in rickets had long been known; one school even believing that lack of sunlight and poor hygienic surroundings were the true cause of the disease. Hess (167, 168) and Steenbock and Black (420) discovered that the irradiation of food-stuffs and of inactive vegetable oils conferred anti-rachitic and growth promoting powers upon them.

Mellanby (283), however, noted that the growth promoting powers so conferred come to an end after about eight weeks. It was shortly found by various workers (169, 423, 421, 265, 170) that of food-stuffs, sterols, and especially cholesterol gave products particularly high in anti-rachitic power. Rosenheim and Webster (355) later reported that though the anti-rachitic effect of such irradiation was permanent, and could be produced both in the presence and in the absence of oxygen, the growth promoting powers as recorded by Drummond and Rosenheim (92) were not permanent, and that after a limit had been reached no further growth could be produced by the irradiated sterol. Hume (179) reached the same conclusion, noting that light cannot substitute cod-liver oil indefinitely for the growth and well being of the rat. Mucous membrane lesions develop and growth fails though calcification proceeds normally. Mackay and Shaw (266) and Goldblatt and Moritz (143) confirmed the statement that irradiation direct or indirect cannot protect against xerophthalmia and prolong growth indefinitely as will cod-liver oil. Peacock (326) meanwhile had found that irradiation, which confers anti-rachitic power, has a destructive effect on the growth promoting factor in cod-liver oil, as tested by the colour reactions. The accumulation of evidence is irresistible and Drummond (96) lent his authority to the statement that "It may now be definitely accepted that these two substances vitamin A and vitamin D are distinct." All later work has supported that conclusion.

Evans and Bishop (123, 124, 126) had claimed to have separated off yet another vitamin: the anti-sterility vitamin E. Sure (430, 431) and Drummond (96) confirmed the claim and Kennedy (203) in a review of the whole subject of diet and fertility admits the validity of their work. Full details are given by Evans and Burr (128) in a special memoir of the University of California.

SOURCES OF VITAMIN A.

In ascertaining the vitamin A content of previously uninvestigated foods, in checking the minimum doses required for growth and maintenance, and in elaborating a suitable technique for such investigations considerable progress has been made. Tables showing the vitamin content of foods similar to that in the already referred to Medical Research Council's Report (66) were prepared by the research institute of the American Medical Association in 1926 and by Smith (405) for the U.S.A. Department of Agriculture in 1929, and are included in *Tabulae Biologicae Periodicae* (434). The recent adoption of an interna-

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tional standard unit of Vitamin A potency should lead in the future to the compilation of much more valuable lists. The present tables which represent the values of food-stuffs in the various vitamins by plus signs are useful as guides in framing dietaries, but not for scientific work.

From the scientific point of view it may be further objected that in many cases the vitamin A value shown is due to the presence in the food in question not of Vitamin A but of carotene, whose physiological effects are identical with the vitamin into which it is ultimately converted. From the clinical point of view the distinction is academic.

The general trend of recent observations has been towards finding higher values for plant sources of the vitamin than had been previously allowed.

McCarrison (234) observed that millet and wheat grown from soil treated with farmyard manure was of higher value than grains grown on soil artificially manured. Wheat in particular contained more Vitamin A and millet more Vitamin B. Rowlands (366) confirmed this result so far as Vitamin B is concerned. Further evidence on this interesting point would be welcome. It suggests the possibility that the proper feeding of vegetables is as necessary as the proper feeding of cattle if a food of adequate vitamin content is to be secured.

The Medical Research Council report (66) stated that light is essential for the formation of Vitamin A, and the appearance of the vitamin in a culture of the marine alga *Nitzschia* was recorded. For these experiments the alga was not grown in pure culture, but Coward (69) obtained equally successful results with a pure culture of another alga, *Chlorella* -- a fresh water species. Wilson (470) in 1922 had claimed on the contrary that Vitamin A can be formed in etiolated shoots. Luce and Smedley-Maclean (228) found that Vitamin A occurred in yeast grown in the dark, and quote Southgate (409) as establishing the absence of the vitamin from barley, kilned malt, and beer which were the other substances employed in their experiments. They also observed that though their experimental animals grew, they did not keep in very good condition. Coward (70) obtained negative results, but found that Vitamin A is not used up in any process carried on by the living plant in the dark. In later experiments she (71) confirmed Wilson however, and further noted that in etiolated shoots the amount of Vitamin A present is an inverse function of the temperature at which they have been grown. Heller (166) found that some Vitamin A is always present in etiolated shoots.

Moore (295) reached similar conclusions and remarked that the anti-xerophthalmic factor was more in evidence than the growth promoting factor. Further experiments by the same worker (296) in which the experimental animals were kept in the dark to avoid the possibility of photosynthesis of the vitamin in their skins led him to decide that for the rat etiolated wheat shoots are an adequate source of Vitamin A and that "light is not essential during any stage of the formation of the vitamin from the seed." He again noted that although xerophthalmia was well cured growth and condition were comparatively bad, lung troubles being frequent. These experiments afford conclusive proof that the anti-xerophthalmic factor can be synthesised in the absence of light. Whether the less satisfactory results as regards growth and general condition are to be interpreted as a partial failure of synthesis, with the implication that the growth and the anti-xerophthalmic factors are distinct parts of a "Vitamin A complex" is a matter for further experiment. Spence (411, 412) it is interesting to remark, has deduced on clinical evidence that the anti-xerophthalmic factor, the growth factor, and the anti-infective factor are separate entities.

If light is not as essential to the formation of the vitamin as was formerly thought there nevertheless exists a very close connection between the process of photosynthesis in plants and their content in Vitamin A. Karshan, Harrow and Krasnow (200) pointed this out, and Coward (70) made the same observation, finding further that the amount of Vitamin A in a leaf increases when the leaf turns yellow, but that the vitamin is completely destroyed when the leaf shrivels and dies. Burrows and Jorstad (42) on the contrary, recorded the peculiar observation that in cultures of bacteria no Vitamin A was found till the organisms had overgrown their media and been destroyed. This observation has not been confirmed. Coward (71) found that irradiation with a mercury vapour lamp accelerated the formation of Vitamin A in plant tissues, but that the rays of such a lamp, used in conjunction with sunlight do not influence the total amount of vitamin ultimately attained in the plant tissues. Heller (166) stated that the synthesis of Vitamin A is intimately dependent on the exposure to and relative amounts of the shorter wave lengths of light, and follows closely the growth of the plant.

In view of their similar relationship to light it was natural to compare the chlorophyll content of plants with their Vitamin A potency. Dye, Medlock and Crist (104) noted a rough relationship between greenness of plants and their content of Vitamin A, and Crist and Dye (86, 87) concluded after more accurate experiments that a quantitative relationship exists

between Vitamin A and chlorophyll within the restriction of two variables; and further that chlorophyll content is a limiting factor in Vitamin A synthesis in the vegetative parts of plants. It is probable that in all these experiments carotene and not Vitamin A was the active agent. Wolff, Overhoff and Eekelen (476) have found that in plants carotene is present, but little or no Vitamin A. In view of the chemical and biological similarity between the two plant pigments it would not be surprising to find that both depended for their formation on like agents and like controlling factors.

It would be a reasonable surmise that plants grown in the months when there is a high average amount of sunshine would show more Vitamin A activity than those grown in the winter, and Coward and Eggleston (72) found that this holds good for spinach, whose most active shoots are grown in the spring and summer.

Hauge and his co-workers (159, 160, 161) found that in maize Vitamin A activity is transmitted exclusively with the yellow endosperm in crossing and segregation experiments. The heredity of food plants would seem to be important, and the assumption that any individual sample of a food contains the accepted normal amount of vitamin is obviously dangerous.

In the animal body the liver is pre-eminent as a source of Vitamin A. Sherman and Boynton (391) found that nine-tenths of the Vitamin A reserve of the body is stored in the liver, and gave the following daily doses of various organs as those required to maintain growth in the rat: Muscle 0.4 g., Lung 0.1 g., Kidney 0.1 g., and Liver 0.02 g. Rosenheim and Webster (360) found the liver oils of the fulmar petrel much more potent than the body oils. Kerppola (204) using the antimony trichloride reaction found that liver contains much more Vitamin A than lung, and that save intestine and intestinal contents all other tissues give negative results. Moore (304) observed that compared with liver, "storage fats" and lung and kidney oils are poor sources of Vitamin A, the ratio being 1/100,000. Vogt (452) noted that the foetal liver contains abundant Vitamin A though none is found in the other tissues. The properties of cod-liver oil have long been famous. In view of the prominent part played by the liver in the conversion of carotene to Vitamin A these facts are what one would expect, and are an indirect confirmation of the work on carotene metabolism.

Of all bodily tissues the retina alone compares with the liver in Vitamin A potency. Holm and Bojesen (175) recorded experiments which show that though

both the white and the grey matter of the brain are poor in Vitamin A, retinae in very small doses suffice to cure Vitamin A deficiency. As the nerve layers of the retina are properly speaking brain tissue they concluded that the vitamin is associated with the lipoids in the outer segments of the rods, and that the concentration of Vitamin A must be very great in that tissue. Smith, Yudkin and Kriss (407, 408) found that the dried retinal tissue of the pig is a potent source of Vitamin A though choroidal tissue is not. Dried retinae cured Vitamin A deficiency in doses of 30 to 50 mg. Doses of 20 mg. were not always successful in curing, though adequate as a preventative dose. The ether soluble fraction of dried retinae gave a strong antimony trichloride reaction. As hemeralopia is an early sign of Vitamin A deficiency the apparent intimate association between the vitamin and the bacillary layer of the retina is interesting.

Poulsso (344) stated that the Vitamin A reserve of the females of mammals is greater than that of the males; which agrees with Birnbacher's (23) clinical observations on acute essential hemeralopia in Vienna.

Milk as an important food has attracted less attention than might have been expected. Luce (227) found that "the diet of the cow appeared to be the main factor in determining the growth promoting power of the milk" whilst light had no special effect such as it has on the anti-rachitic factor. Chick and Roscoe (59) confirmed the above finding. Crawford et al. (85) found that the Vitamins A and D of milk are contained entirely in the milk fat. Macy and her co-workers (269, 270, 271) observed that human milk is a relatively good source of Vitamin A, being fairly comparable with the milk of cows fed on alfalfa hay and ensilage with cereals, but not on pasture. They note that a certain ration of the vitamin may be adequate for reproduction and lactation, but will leave the child with very little Vitamin A reserve. The clinical studies of Barrett, Keith-Gibson and Reece (15) led them to form a less favourable opinion of human milk as a source of Vitamin A, and upset the complacent assumption that a breast-fed child is of necessity receiving the best possible diet. By analogy with the cow, on the diet of the mother the Vitamin A content of her milk must depend; and in certain classes the diet is most unsatisfactory as far as the fat soluble vitamins are concerned.

Cod-liver oils, the classic sources of the vitamin, have been much studied and controversy exists as to which oils are the best. Zilva, Drummond and Graham (484) found that "The sexual condition and

age of the cod do not influence the Vitamin A potency of the liver oil", but Drummond (95) later arrived at the opposite conclusion, and stated that "When the fish are actively feeding their livers are usually rich in vitamins and yield pale yellow oils of high medicinal value. On the other hand when they are practically starving during the spawning period, and are at the same time transferring much fat and vitamin to the reproductive cells, the livers yield an almost white oil of low relative potency." To these facts he attributes the superiority of Newfoundland oils and of Norwegian Finmarken oils to the Norwegian Lofoten oils, these last being made from fish caught during the reproductive season. Poulsson (342) was unable to confirm the above experiments and found Newfoundland and Norwegian oils to be of equal potency. He denied that the Lofoten oil was of poorer quality than the Finmarken. Drummond and Hilditch (101) after a special study of the subject concluded that the best oils are Newfoundland oils; Icelandic and Scottish oils rank next, Norwegian oils being of still lower quality. Poulsson (343) again disagreed, upholding the oils of his native country. He observed that other fishes of the cod family yield oils equal to cod-liver oil, and that the liver oils of the shark and of the dogfish are more potent still. Whichever country's oils are the better there is no doubt that as a source of the fat soluble vitamins and of Vitamin A in particular cod-liver oil is unrivalled. Coward (77) has recently shown that various commercial Vitamin A concentrates for which considerable claims had been put forward had a content similar to but certainly not better than cod-liver oil. The warning implied in this finding is timely, as the multiplication of expensive ^{vitamin} products shows no sign of slackening.

The influence on the Vitamin A values of other substances contained in the food being tested is important. The "salt ophthalmia" of McCollum (258,259) has been shown by Jones (188) and by later work by McCollum and his collaborators (401,107) to be due to the destructive action of ferrous sulphate -- one of the constituents of their experimental diet -- on the Vitamin A of the ration. Huston, Lightbody and Ball (182) on the other hand found that hydroquinone protects the vitamin against destructive processes. Marcus (274) has noted that not only chemical nature but physical state has an influence on apparent vitamin content. He observed a destructive action of finely divided solids on Vitamin A in cod-liver oil, and suggested that this is due to the adsorption of air (oxygen) on the large surface of the particles.

GENERAL PHYSICAL AND CHEMICAL PROPERTIES OF VITAMIN A.

Heat in the absence of oxygen has little or no effect on Vitamin A. Zilva (483) found that in cod-liver oil hardened in the absence of oxygen to 32° M.P. there is no loss of vitamin potency, and that further hardening to 45° M.P. only causes a partial loss. Southgate (410) observed that if cod-liver oil is slowly heated out of contact with air to 300° both Vitamin A and Vitamin D are completely destroyed, while heating to 200° and maintenance of this temperature for some hours only results in the gradual disappearance, *pari passu*, of these two substances. At these high temperatures oxidation from external sources is unnecessary for their destruction. Drummond, Channon and Coward (93) in an attempt to isolate the vitamin found that in the distillation of a cholesterol free concentrate of cod-liver oil the vitamin passes over between 180° and 220° at a pressure of 2 to 3 mm. Hg.

Peacock (326) showed that ultra-violet radiation has a destructive effect on Vitamin A, and was confirmed by Rosenheim and Webster (358). Willimott and Wokes (460, 463) also confirmed and extended these observations, noting that irradiation sets in train a series of processes which result in the gradual destruction of the vitamin. They suggested that this action is due to the formation of volatile peroxidases by the irradiation. Ordinary bottle glass does not exclude that part of the spectrum which attacks Vitamin A, though with proper storage little or no loss need occur. Poulsson (340) has obtained marked effects with a cod-liver oil 31 years old.

It was already known that the vitamin was readily oxidised, but was stable to alkalis, being indeed found in the unsaponifiable fraction of cod-liver oils after drastic treatment with hot strong potash solution. Various attempts at its isolation were made. Steenbock and Nelson (422) found that the anti-xerophthalmic vitamin of cod-liver oil was not precipitated by digitonin. In the same year (1925) Takahashi and his co-workers (435) published an English summary of their earlier work, and claimed to have isolated the vitamin in a pure state. Their product was "a reddish yellow transparent viscous oily substance", was aldehydic in character, and a reducing substance. The provisional formula $C_{27}H_{46}O_2$ and the name "biosterin" were assigned to it. Drummond, Channon and Coward (93) later reported an extensive and important research on the same question, and succeeded in preparing a very potent fraction.

This fraction was far from being a pure substance, and they criticised the claims of Takahashi, whose supposedly pure biosterin they showed to have no greater activity than their own admittedly impure fraction. The iodine value, bromide yield, refractive index, and specific gravity of biosterin are all higher than those of their final concentrate, and they concluded that it must have contained a considerable amount of phytol. They summarised their results as follows:

"The concentrate contains no detectable traces of iodine or nitrogen so that these elements do not seem to be related to the physiological action of the oil in promoting growth.

"Approximately 50% of the unsaponifiable matter from cod-liver oil is cholesterol, which may be removed quantitatively without loss of vitamin activity.

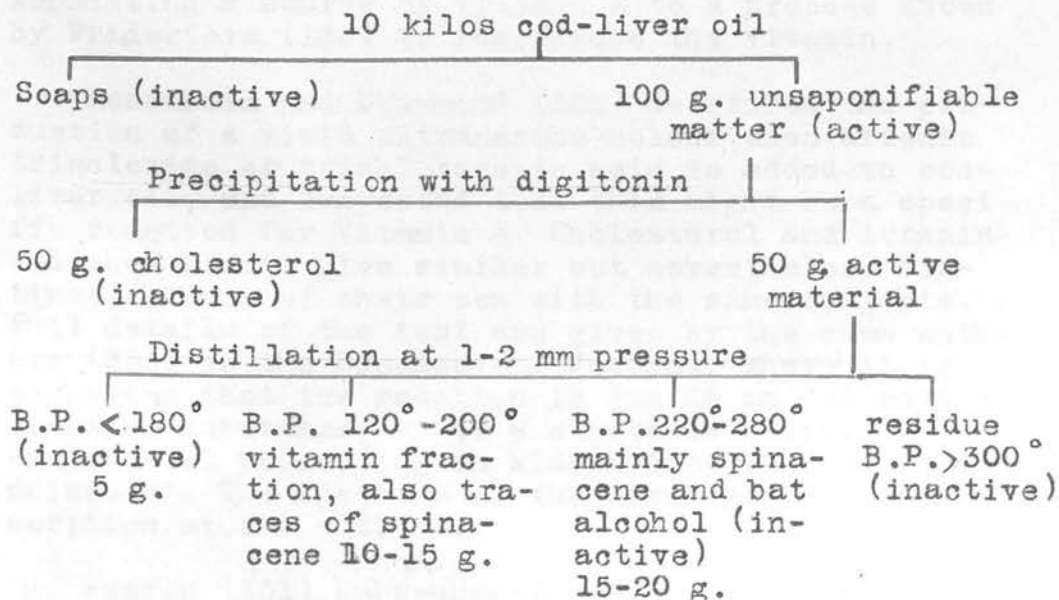
"Vitamin A is volatile in super-heated steam.

"Distillation of the cholesterol free residue did not result in a satisfactory separation of the components. Vitamin A passes mainly between 180° - 220° at 2 - 3 mm. pressure.

"Chemical examination of active distilled indicated the presence of (a) a saturated solid alcohol M.P.ca. 60° (b) the unsaturated hydrocarbon spinacene (c) one or more than one unsaturated alcohol boiling about 200° at 2 - 3 mm.

"Spinacene and the solid alcohol are without Vitamin A action."

They were unable to decide whether Vitamin A is identifiable with one of the unsaturated alcohols, but reported that certain unsaturated alcohols from other liver oils are not Vitamin A. Drummond (96) gave a diagram, copied below, showing the yield of active fraction obtained from 10 kilos of cod-liver oil,



and added the comment "We must abandon all ideas of the vitamin being a labile ill-defined compound --- when it is so readily demonstrated that it survives such drastic chemical treatment as saponification with boiling alkalis, and distillation at temperatures over 200°".

Clenshaw and Smedley Maclean (62) found that the unsaponifiable matters extracted from the two richest sources of Vitamin A (green leaves and cod-liver oil) have a parallel chemical structure being composed of

- (i) a highly unsaturated hydrocarbon.
- (ii) products obtained from higher fatty acids by condensation and reduction.
- (iii) sterols.

None of these constituents can be identified with the active agent. Drummond and Baker (97) confirmed Drummond's earlier work on the fractionation of cod-liver oil and concluded that "The active substance is present in liver oil concentrates in amounts so minute that direct attempts at its isolation by the ordinary chemical methods are of little use".

COLOUR TESTS FOR VITAMIN A AND ITS SPECTROSCOPIC PROPERTIES.

The early observations of Drummond and Watson (91) on the colour reactions obtained on adding concentrated sulphuric acid did not attract immediate attention, and Steudel and Peiser (425) threw doubts on its specificity for Vitamin A, as they found that a reaction was given by inactive cholesterol and not by a biologically tested source of the vitamin. Sjorslev (403) on the other hand demonstrated the loss of the sulphuric acid colour reaction on submitting a source of Vitamin A to a process shown by Fridericia (136) to inactivate the vitamin.

Rosenheim and Drummond (356) described the production of a vivid ultramarine colour when arsenic trichloride or trichloroacetic acid is added to cod-liver oil, and suggested that this might be a specific reaction for Vitamin A. Cholesterol and irradiated cholesterol give similar but nevertheless distinct colours of their own with the same reagents. Full details of the test are given by the same authors (354) in the Biochemical Journal, where it is suggested that the reaction is due to an "aldehydic coupling substance, or to a substance allied to cholesterol possessing an aldehydic group in its molecule". The spectrum of the chromogen shows absorption at 570 - 590 μ .

Fearon (131) subsequently described a colour reaction with pyrogallol, and also with phosphorus

pentoxide. For the pyrogallol reaction he noted that the residue after removal of the pigment had no growth promoting powers; though it proved impossible to recover the vitamin from the hydrolysis products of the pigment. Rosenheim and Webster (357, 359) v. Euler, Myrback and Karlson (108) Willimott and his co-workers (458, 459) all deny the specificity of Fearon's reaction; and Rosenheim and Webster (358) in a review of the whole question of colour tests stated "As regards Fearon's test therefore the evidence appears to be quite definitely against its having any relation to Vitamin A."

Carr and Price (53) discovered that antimony trichloride possessed certain advantages over arsenic trichloride when testing cod-liver oils whose Vitamin A was being destroyed by Peacock's (326) method. Willimott, Moore and Wokes (458) found concentrated sulphuric acid and phosphorus pentoxide less sensitive than the arsenic and the antimony trichloride reactions. Rosenheim and Webster (358) found the arsenic and the antimony trichloride reactions of value. They employed careful growth tests to check their results, but concluded with the cautious statement that "In the case of the colour reactions with arsenic trichloride (and antimony trichloride) the possibility of a direct relationship of the chromogen to Vitamin A remains open". Rosenheim (352) later devised a special colorimeter on the Lovibond principle for the testing of Vitamin A by the antimony trichloride reaction, and quoted an unpublished report of the Accessory Food Factors Committee favourable to the test. Willimott and Wokes (463) tested the colour reactions of sulphuric acid, phosphorus pentoxide, arsenic trichloride, and antimony trichloride with samples of cod-liver oil subjected to heating and oxidation against biological assays for Vitamin A and found qualitative agreement with all the tests and quantitative agreement with the last two. They also observed that the destruction of the vitamin gives rise to substances that give an immediate yellow colour with antimony trichloride. The same authors (461) later reviewed the whole question of colour tests with great attention to details of technique on the importance of which they (460) again insisted. They regarded antimony trichloride as a better reagent than arsenic trichloride, and found that the presence of Vitamin D in the substance tested was no obstacle to the test. The Accessory Food Factors Committee (2) confirmed the value of the colorimetric method with antimony trichloride for liver oils. Norris and Danielson (314) and Anderson and Nightingale (6) also found good correlation between the results of colorimetric tests and those of biological assay.

Observations to the contrary, however, are not wanting. Hawk (162) Jones et al. (189) Bailey, Cannon and Fisher (14) and Schmidt-Nielsen (374, 375, 376) failed to find the correspondence between the results of the colour tests and of biological tests, that other workers had obtained. Steudel (426) found a substance active in doses of 1.0 g. which produced no reaction with the "vitamin reagents". Drummond and Morton (98) criticised these discrepant results on various points of technique and proceeded to say that in "a wide experience" they had never met an instance where there was a difference between biological assay and the antimony trichloride reaction when absorption about 608μ was present in the chromogen; and recommended the method unhesitatingly for cod-liver oils. Schmidt-Nielsen (377) in later observations admitted that the values given by his biological technique were too low for certain of the materials tested, and that the corrected results are more in line with those obtained by colorimetry. Moore (301) in a review of the problem concluded that "For the present it seems safe to assume that materials which give no blue colouration with antimony trichloride, even after the removal of unsaponifiable matter, must be devoid of Vitamin A activity. Materials of liver oil giving absorption at 610μ may be considered active; but on the other hand materials which give absorption at other positions may be either active or inactive ---- and the biological technique still remains the only satisfactory method of assay". Drummond and Ahmed (99) found good correspondence between the two methods of testing and stress the importance of sound technique in both. Norris and Church (315, 316, 317, 318) also conclude similarly and discuss the technique of the antimony trichloride reaction at some length. Their whole conclusions on this point have not met with general acceptance. Smith and Hazley (406) make serious objections to certain of their methods. Brode and Magill (38) have also studied the technique of this test.

There still appears to be room for investigation on the colour tests and their specificity or otherwise for Vitamin A. For the present, investigations controlled solely by the colorimetric method and not by biological tests must be held suspect.

Rosenheim and Callow (364) described a "mercury reagent" which apparently reacts with the same chromogen that is concerned in the antimony and arsenic trichloride tests. The value of this new reagent remains to be estimated.

Closely associated with the question of colour tests is that of the spectroscopic properties of Vitamin A. Schultze and Morse (380) found the spectrum

of Vitamin A to be characterised by absorption at 320-328 μ and at 270-290 μ . Takahashi et al. (435) confirmed this finding as far as the former band is concerned, and Schultze, working with Zeigler, (381) obtained results similar to his earlier ones. Heilbron, Kamm and Morton (163) Woodrow (477) and Morton and Heilbron (310) confirmed the constant presence of the band in the region of 328 μ . The last quoted authors suggested that the band at 275-285 μ is probably due to decomposition products of the vitamin. It was also affirmed that the intensity of the band with its maximum at 328 μ varies directly with the antimony trichloride reaction. Morton, Heilbron Thompson et al. (312, 142) recorded an exhaustive research and concluded that the characteristic band of Vitamin A at 328 μ is free from fine structure. Nearly all cod-liver oils also show absorption bands from 260-295 μ . They discussed in detail the spectra obtained when Vitamin A concentrates are attacked by sodium ethoxide and by ordinary processes of saponification. Heilbron and his co-workers (165) after further careful studies laid down as criteria for Vitamin A that the substance should be colourless and odourless, with a maximum absorption at 328 μ , giving with antimony trichloride a blue solution with maximum absorption at 572 μ . Dihydrocarotene is described as very nearly fulfilling these postulates, but biological tests gave irregular results. V. Euler (121) has, however, recorded positive biological results with this body.

The chromogen of the arsenic trichloride reaction was described by its discoverers (354) as showing absorption at 570-590 μ . V. Euler et al. (109) found its spectrum to lie at 475-480 μ and at 535 μ . Wokes (473) observed that the substance first gives bands at 587 μ and at 475 μ but that on standing with the reagent the bands pass gradually from the longer to the shorter wave-lengths, which fact, he suggested, might explain and reconcile the earlier discrepant findings. The chromogen of the antimony trichloride reaction he found showed absorption at about 614 μ and 530 μ . V. Euler, V. Euler and Heilstrom (113) Duliere, Morton and Drummond (103) and Moore (301, 303) all confirmed the characteristic nature of the band at about 610 μ . Morton, Heilbron and Thompson (312) found that Vitamin A concentrates treated with antimony trichloride give a main band at 620-624 μ and often a lesser band at 582-593 μ . Heilbron and Morton (165) later concluded that the substance giving the band at about 610 μ was not identical with though closely allied to Vitamin A, but that the band produced with the antimony reagent at 572 μ was due to the vitamin itself.

Whilst the band at 328 μ may be held to be definitely established as characteristic of Vitamin A, the

band of the antimony trichloride reaction at 610μ must still be held doubtful. Whether or not it is finally decided that it is produced by the vitamin itself, there is no doubt that it has been of prime value in recent vitamin research.

CAROTENE AND VITAMIN A.

As early as 1919 Steenbock (416) had observed the natural association between Vitamin A and the lipochrome pigments, and had suggested that the vitamin might be a leuco form of some such substance. Subsequent observers were, however, unable to connect any one pigment directly with Vitamin A activity. Drummond, Channon and Coward (93) reported that carotene four times recrystallised at 167.5° has no Vitamin A potency, though they added that it is very liable to be contaminated with the vitamin. Lycopin from tomatoes was also found to be inactive. Javillier, Baude and Levy-Lajeunesse (185) and Willimott and Moore (462) recorded negative results for phytol and for pure crystalline xanthophyll respectively.

In 1928 the whole question was re-opened by v. Euler and his colleagues (111,112,113) when they published experiments showing that carotene has marked Vitamin A activity, a daily dose of 0.005 mg. being adequate. They also showed that the spectrum of carotene when treated with antimony trichloride gives a band at 590μ and not in the "vitamin position". They argued that if the growth obtained in their biological tests had been due to contamination with Vitamin A its characteristic band ought to have shown up. They suggested that failure to supply sufficient Vitamin D was the cause of earlier negative experiments. V. Euler and Karrer (196) obtained confirmation of the above claim for carotene and put forward the suggestion that Vitamin A activity is determined not by an integral molecule but by a particular structural arrangement, the "polyene grouping" --- a system of double bands which gives rise to colour reactions and is held to possess catalytic powers in vivo --- Carotene possesses such a system.

Further confirmation of the main claim was soon forthcoming. Moore (297,300) Kawakami and Kimm (201) v. Euler et al. (117,198) all recorded positive results with carotene. Collinson and his co-workers (63) found that purification of the pigment resulted in no loss of vitamin activity and obtained results in dosages of 0.002 - 0.005 mg. The M.P. of their purest specimen of carotene was 174° . Moore (298) gave a good account of the earlier work on the subject, and found that carotene M.P. 174° retained its activity after many recrystallisations. This carotene showed no trace of a maximum absorption band at $310-330\mu$ and with antimony trichloride gave absorption at 590μ not at

610 μ . He criticised the "polyene grouping" theory of Vitamin A activity on account of the low degree of specificity this would allow to that body, and suggested that the failure of earlier experimenters to obtain positive results with carotene was due to the lack of fat in their diets. In a later note he (299) found however that a carotene of 174° M.P. was active on a fat free diet. V.Euler and his co-workers (114, 115) also found that their explanation of these earlier failures was wrong as omission of Vitamin D from the diet does not check growth when carotene is also supplied, though faulty calcification occurs. Hume and Smedley-Maclean (180) declared that carotene was active both in the presence and in the absence of fat, but observed that the vehicle in which carotene was dissolved is of importance, as this may have a destructive action. Ethyl oleate, a solvent used in certain negative experiments, was found to be unsuitable on this account.

V.Euler, Karrer and Rydbom (118) found that different specimens of carotene varied in activity, and raised the question as to whether or not this variation might be due to the presence of "a stubbornly adhering impurity". The variations on which the above suggestion was based are, however, small and may be accounted for by experimental error. A graver criticism was entered by Duliere, Morton and Drummond (102, 103) when they announced that carotene after a very thorough purification (M.P. 184.9°) becomes much less active. They made a very careful colorimetric and spectroscopic differentiation between Vitamin A and the pigment, and suggested that the vitamin may escape detection in samples of carotene on account of the intensity of the superimposed spectrum of that substance. Capper (51) showed that this objection is without weight as regards the Vitamin A band at 328 μ , carotene M.P. 174° having insufficiently strong absorption at that wave-length to obscure it even when the vitamin is present in minimal amounts. Moore (303) confirmed this observation. Van Stolk et al. (450) found that purification of carotene enhanced rather than diminished its potency their purest product M.P. 179° being active in doses of 0.002 mg. Later experiments by Drummond, Ahmed and Morton (100) caused them to revise their opinion, and they agreed with other workers that carotene shows marked Vitamin A activity. Hume and Henderson-Smith (181) suggested that a standard carotene should be used as a unit of Vitamin A activity; and by international agreement (329) a carotene prepared by Willstatter's method 0.003 - 0.005 mg. of which will restore growth and prevent xerophthalmia in rats deprived of Vitamin A is now so accepted.

In view of subsequent work the distinctions between carotene and Vitamin A are of some importance. Moore (303) summarised them in the following table:

<u>Carotene</u>	<u>Vitamin A</u>
Synthesized in plant.	Stored in animal.
Intensely yellow.	Almost colourless.
No absorption at 328 μ .	Absorption at 328 μ .
Green-blue colour with antimony trichloride, with absorption at 590 μ .	Vivid blue colour with antimony trichloride with absorption at 610-630 μ .

Further differences were demonstrated by Wolff, Overhoff and Eekelen (476) who found that when mixtures of the two substances were shaken with petrol ether and 90% alcohol the carotene goes into the ether layer and the vitamin into the alcohol layer. Quinn and Hartley (349) found that "norit" is able to remove carotene from extracts of dried carrots, but that it fails to extract Vitamin A from cod-liver oil. Bruins, Overhoff and Wolff (39) using the method of diffusion coefficients calculated the molecular weight of Vitamin A --- represented by a highly potent fraction --- to be about 350, which being widely different from the molecular weight of carotene caused them to think that the relationship between the two substances is not a simple one.

Karrer et al. (199) confirmed by Kuhn and Lederer (213) discovered that carotene consists of a mixture of an alpha and beta form, each with different melting points and optical properties. Though one is optically active and one is not both forms are biologically potent: a curious exception to a fairly general biological rule.

V. Euler and his colleagues (115, 119) had stated that carotene given in excess is stored in the liver. Moore (299, 303) made a most important advance when he discovered that "The ingestion of excess of carotene by the albino rat leads to no increase of the pigment in the liver, but to a great intensification of the colour reaction for Vitamin A". V. Euler and Rydbom (120) corrected their observation and found Moore's claims to be well founded, and Capper (50) using spectroscopic methods also confirmed them. Liver oils from rats depleted of Vitamin A give no absorption in the vitamin position; oils from such rats fed with carotene do. Moore (304) in further researches found that though conversion of carotene to Vitamin A undoubtedly does occur in the rat's liver, the conversion is not quantitative. He emphasised

the importance of the liver in Vitamin A metabolism, and suggested that in toxic states proper conversion of carotene might not be possible, quoting Buckley et al. (40) in support of this hypothesis. These authors in an autopsy on a case of parenchymatous degeneration of the liver in a cow found that that organ was full of carotene, which it had apparently been unable to convert into Vitamin A. Cappar, McKibbin and Prentice (52) found that the hen's liver can also convert carotene to Vitamin A, and that Vitamin A deficiency in these creatures causes the syndrome of "visceral gout of poultry".

The mechanism of these changes still awaits explanation. V.Euler (122) has claimed to produce the conversion of carotene to Vitamin A in the serum of the hen in vitro, and suggested that the first steps of the conversion take place in the blood. Olcott and McCann (319) have also claimed to have achieved the in vitro conversion of carotene by means of an enzyme extracted from liver tissue.

OTHER OBSERVATIONS ON THE NATURE OF VITAMIN A.

The very interesting and very important work on carotene has somewhat overshadowed other methods of approach to this problem. V.Euler, v.Euler and Karrer (119) claimed activity for the pigment dihydro α -crocin as well as for carotene, but Karrer (197) was unable to confirm the statement by further experiments, and Drummond, Ahmed and Morton (100) also obtained negative results.

The work of Seel (384,385) is on entirely different lines. He announced the isolation of Vitamin A in a pure state in which it is stable only in oil, and concluded that it was a very labile partial product of the oxidation of cholesterol. He found that oxycholesterol gives absorption at the Vitamin A wave-length, but was unable to establish absorption for the antimony trichloride chromogen. He used anti-xerophthalmic power and not growth promoting power as his criterion of Vitamin A activity in his biological tests, and his products are not of very high potency. Rosenheim (361) had earlier observed that one of the oxidation products of cholesterol gives a blue colour with arsenic trichloride, although not the same colour as that given by Vitamin A. Feeding experiments with this substance, carried out with Webster, were negative. Wokes (472) also noted the close similarity between certain oxidation products of cholesterol and Vitamin A, when treated with the colour reagents. Rosenheim and Webster (363) recorded the fact that dehydroergosterol which is biologically inactive and fails to give the colour reactions of Vitamin A shows absorption in the same region as that

body; but Morton, Heilbron and Spring (311) found that though the spectrum of this substance is similar to that of Vitamin A it is not the same. The vitamin shows one broad intense band at 328μ ; the spectrum of dehydro-ergosterol shows four narrow bands of less intensity. Further observations by Heilbron and his co-workers (163, 312) have shown that comparison of the degradation products of Vitamin A concentrates of ergosterol and of dehydro-ergosterol yield results qualitatively, but not exactly similar.

Further work to decide whether Seel's claims are valid, and to what extent, requires to be done, and the need for extreme care in any spectroscopic work on this topic is apparent. Growth tests too, should be used in place of purely anti-xerophthalmic experiments. For the present Seel cannot be held to have established his case.

GENERAL PHYSIOLOGICAL EFFECTS OF VITAMIN A DEFICIENCY.

The failure of growth in the absence of an adequate supply of the fat soluble vitamins was the first feature to call attention to these bodies; and the promotion of normal growth still remains the most important function of Vitamin A. The term "the growth promoting vitamin" has been objected to on the grounds that other vitamins and other substances are also essential to growth. Nevertheless any substance for which Vitamin A activity is claimed must be adequate in this regard; all the accepted methods of biological assay of the vitamin are tests of this particular function.

Harris (154) contrasted the purely vegetative processes in cartilage which he said depend on the water soluble vitamins, with the differentiating functional processes of growth of bone which depend on Vitamin A. "Only in the presence of an adequate supply of the blood borne Vitamin A does one see the fully differentiated osteoblast with deeply staining nucleus and numerous basophil granules in the cytoplasm". In a later paper he (157) compared the function of Vitamin D which presides over the process of calcification in cartilage with that of Vitamin A in promoting true osteogenesis. "The differentiation of pre-existing mesenchyme cells to form active osteoblasts or marrow cells depends on the supply of fat soluble Vitamin A". In view of the abundant evidence of the effect of Vitamin A deficiency in setting in motion a train of degenerative and regressive processes in epithelial structures it is tempting to suppose that in these tissues too the vitamin has a

differentiating and a controlling function. Quinn, King and Dimit (348) found that Vitamin A deficiency affects body weight more than body length.

Though necessary also to the adult, Vitamin A is of supreme importance to the young. Observations by Cramer (79), by Sherman and Burtis (394), by Sherman and McLeod (392), and by Batchelder (16) show that the effects of an early Vitamin A deficiency are long lasting, and are indeed never fully recovered from, life itself being shortened.

Though much knowledge is available about the effects of Vitamin A deficiency on specific organs and tissues little is still known of its general effects on metabolism. Cooper (67) stated that the vitamin is present in the urine and in the gastric juice of animals to whom it is fed in excess, and suggested that it is a threshold substance. Rowntree (367) on the other hand found that when Vitamin A is abundantly supplied it is not excreted in the urine, but that a large faecal loss occurs, parallel with the faecal loss of fat. The largest loss (and the largest retention) occur when the largest amounts are ingested.

Junkersdorf and Jonen (195) found that puppies on a Vitamin A deficient diet had an abnormal chemical composition of the whole organism, particularly the liver and believed that the vitamin possesses catalytic properties. Morgan and Osborne (305), noting that whereas in the normal rat decrease in body weight is accompanied by increase in the urinary excretion of allantoin, and gain in weight by a reduced excretion, in the avitaminotic rat the reverse holds good, concluded that in the absence of Vitamin A the animal organism fails to utilise purin containing compounds from the usual sources, and instead uses over and over again such portions of discarded purin rings as are ordinarily excreted as allantoin. In this connection the observation of McCarrison and Madhava (248) is of interest. They found that a diet deficient in Vitamin A and also containing large amounts of animal protein gives rise to particularly severe deficiency effects. It is tempting to suppose that these severe effects are a reflection of the strain on the body produced by presenting it with a large amount of purin compounds which it cannot metabolise. St. Julian and Heller (370) noted that in Vitamin A deficiency the mechanical and chemical processes of digestion go on normally, and argue that the vitamin must act on the more profound metabolic processes of

the body. Sure and Smith (433) found that deficiency of Vitamin A caused no alteration in the true blood sugar, the alkali reserve of the blood, or the glycogen content of the liver. Reducing non-sugar substances in the blood were frequently increased. The total food intake of these animals was decreased, though seldom to the point of starvation, and when it was low the water intake was sometimes much increased. McCarrison (255) observed on the contrary that the amount of urine excreted in Vitamin A deficient rats was low. Burrows and Jorstad (42) stated that Vitamin A is essential to the organism in order to replace certain soaplike substances which they believe are lost in the differentiation of the cell.

Sherman and Cammack (393) have studied the storage of the vitamin. They found that the richer the diet is in Vitamin A the greater is the bodily store. Storage is rapid at first, but the maximum store of which the body is capable is only slowly completed.

INFECTION AND VITAMIN A.

The earliest workers on Vitamin A deficiency were struck by the readiness with which their experimental animals succumbed to incidental infections. The difficulty of avoiding these infections, which are often fatal, remains one of the most troublesome obstacles in certain lines of Vitamin A research, and entails much added expense in laboratory animals. Cramer (79) noted in his rats "vague ill-health and malnutrition which finally culminates in the onset of bacterial infections of an avirulent type". He considers the bacterial infections are secondary to a diminution of the local tissue resistance of the affected parts. With Kingsbury he (81) reaffirmed the belief that Vitamin A deficiency impairs the local tissue defences, and that the general humoral defence mechanisms are not appreciably affected. Findlay (134) also considered that infection is due to a breakdown of the local resistance, but noted that the onset of keratomalacia or of any acute infection is marked by a polymorphonuclear leucocytosis and a fall in the bactericidal powers of the blood. The lysozyme of the tears which has bactericidal powers is also reduced in Vitamin A deficiency. Wolbach and Howe (474) in 1925 laid the foundations of the accurate histology of Vitamin A deficiency lesions. They described a substitution of normal epithelial structures in various parts of the body by stratified keratinizing epithelium. This replacement of epithelium arises from focal proliferation of cells arising from the original epithelium and not by differentiation of pre-existing cells. "The deficiency results in loss of specific (chemical)

functions of the epitheliums concerned, whilst the power of growth becomes augmented". They found the first morphological evidence in the nucleus; and stated that infection is common, but that the characteristic changes can occur without infection; and they regarded the infections as secondary to the epithelial metaplasia. There was some doubt whether in these experiments Vitamin D might not also have been deficient. Goldblatt and Benischek (145) working with a pure Vitamin A defective diet confirmed their results fully. Cramer (83) in discussing the question summed up his position as follows: "If the supply of Vitamin A is inadequate the activity of the mucous secreting cells is impaired; they become atrophic and then the bacteria are enabled to penetrate into the mucous membranes and produce varying degrees of infection of those tissues. These infections may remain localised for a long time in the affected mucous membrane or lung, and produce a general toxæmia. If sufficiently prolonged bacteria pass into the blood stream, exhaust the humoral defensive mechanisms and set up an infection elsewhere or even a mild septicaemia". McLester (268) concurred in this view that the primary lesion is an epithelial degeneration which opens up the road to bacterial invasion.

Tyson and Smith (444) agreed with earlier workers as to the essential histological features of the epithelial degenerations, but made the important observation that following the apparent cure of this condition by cod-liver oil chronic or acute infections may nevertheless persist for quite a long time. They completely reversed the previously accepted sequence of events. "Infection is always present" they declared "even in the earliest stages, and in late cases dominates the picture. No metaplastic activity has been seen without an accompanying infection, but infection has been observed in parts where metaplasia is absent." These contrary observations require confirmation. The contradiction may, however, be more apparent than real. Different observers have different criteria by which they judge whether infection is absent or present, and the disagreement may be one of interpretation rather than of fact. It may be also mentioned here that though an epithelium may appear histologically intact there is no guarantee that its functions have not already been impaired. Seifried (386,387) supported the view that infection is a secondary process. He too confirmed the earlier work on the general nature of the changes seen, though he regards the epithelial change as a substitution rather than a true metaplasia. Cramer (84) reaffirmed his view that the lesions of Vitamin A deficiency are caused by organisms of relatively low virulence acting on already damaged tissues, whilst McCarrison summed up his long experience in

the sentence: "THE function of Vitamin A so far as it is known at present, is thus to maintain the functional integrity of the cells covering the body surfaces, thereby preventing invasion of the organism by microbic agents."

It is interesting to observe in the records of these experiments how erratic the incidence of these deficiency lesions is. Different animals are attacked to different degrees, and in the different animals different tissues appear to bear the brunt. Even in the same tissue in any one animal certain parts suffer much more than others.

Whilst the morbid histologists and the nutritional research workers have been able to arrive at a very considerable measure of agreement about the mechanism and role of infection in the production of the lesions of Vitamin A deficiency, bacteriological and immunological workers have not been so happily in accord. Webster and Pritchett (454) found that animals are made more resistant to *B. aertryke* fed by the stomach tube by a modified McCollum's diet containing 10% butter fat, as compared with animals on a stock diet rather poor in fat soluble vitamins. The number of animals used in these experiments was small and the results are not very conclusive. Later observations by Pritchett (345) upset the theory that it was the butter fat of the McCollum's diet that had been responsible for the beneficial results; for on its withdrawal from the diet even better results were obtained than in its presence. Addition to the stock diet of butter, cod-liver oil, and irradiated milk had only slight beneficial effects, and a vitamin free vegetable oil was neutral. The same objections as were made to the earlier experiments may be made to these also.

Findlay and Smedley-Maclean (133) obtained a reduction in the bactericidal power of the blood of rats on a diet deficient in Vitamins A and D, only after the onset of some severe infection, but stated that if rats are given ultra-violet light irradiation the onset of infection is delayed, and with it the reduction in bactericidal power. Rats with keratomalacia present still showed reduced bactericidal power even after ultra-violet light. Deficiency in phosphorus and in Vitamin D alone were also found to cause reduction in the bactericidal power of the blood. Orr et al. (321) found no significant variation in the serological reactions of sheep after adding cod-liver oil to their diet, though sheep on natural pasture showed higher anti-bacterial titres than sheep on artificial diets.

Verder (451) found that whereas two out of nine rats fed on a normal diet developed symptoms when given *Salmonella enteritidis* organisms by mouth, none of a like group fed on a Vitamin A deficient diet did. In the normal rats, however, cultures of the heart blood, spleen, liver, lung and kidney were sterile; in the avitaminotic group organisms normally inhabiting the alimentary canal were found in these sites, and in many cases *S. enteritidis* also. Lassen (218) reported that eight out of nine mice showing xerophthalmia died when subcutaneously injected with Breslau bacillus, whereas eight controls lived for 37 days. Further researches have led him (219) to conclude that marked decrease of resistance is found in Vitamin A deficiency, though not in deficiency of other food factors. None the less in xerophthalmic animals organisms do not invade the gut wall faster, nor can disease be produced with lower doses than in normal controls. Agglutinins are reduced but the power of vaccination to produce immunity is unaltered.

Topley, Greenwood and Wilson (439) in a study of the epidemic spread of infection in mice infected with *B. aertryke* observed no advantage from a modified McCollum's diet as compared with one composed of whole milk and water; whilst the addition to the stock diet of excess of fat, butter, lard or Vitamin A concentrate increased the mortality. Addition of cabbage or mangold did not lessen the severity of the epidemic, and addition of carrot seemed to increase it. This unfavourable action was not evident when the mice were infected by the intra-peritoneal route. Boynton and Bradford (37) stated that the survival rate of rats injected with subminimal lethal doses of a bacillus of the *mucosus capsulatus* group was markedly affected by Vitamin A deficiency. The survival rates were lowered even when the animals' vitamin reserves were not entirely depleted, and they still seemed in good health on outward inspection.

The evidence is unsatisfactory and contradictory. Deductions are often drawn on the results obtained with small numbers of experimental animals, and with diets highly unbalanced, and deficient in more than one factor. The words of an anonymous writer in the "Lancet" (216) on "Immunity and Vitamin A deficiency," are still applicable. "Experiment has not therefore been able so far to throw much light on the problem, and we must be guided chiefly by empirical knowledge." In so far as these results may be said to give any indication, they support Cramer (84) in his contention that Vitamin A has little concern in the defensive processes against acute infections.

THE EYE LESIONS OF VITAMIN A DEFICIENCY.

It has been impossible to trace the experiments referred to by Dr Livingstone when he compared the the eye symptoms of certain of his African servants on one of his journeys with the condition seen in animals fed on "pure gluten and starch", but they must be the earliest scientific observations of deficiency xerophthalmia. By 1924 xerophthalmia was well established as an early and specific sign of Vitamin A deficiency, and it has never been seriously challenged as a most valuable positive index of that condition. Some authors have suggested that it should be preferred to growth tests for biological assay; and it is noteworthy that in the recent standard unit adopted for Vitamin A the substance has to cure xerophthalmia as well as restore growth.

Mori (308) had already concluded that in the eye condition of animals on a fat soluble vitamin free diet xerosis of the lachrymal, and next of the meibomian glands was the most important change. Yudkin and Lambert (215,480) found that the chief change occurred in the harderian glands and not in the lachrymal gland, and believed that the eye changes were due to a low grade infection originating in the palpebral conjunctiva and spreading to the cornea. Yudkin (481) later noted that the secretions of the para-ocular glands first fail, and that infection follows; and Findlay (134) found the lysozyme of the tears is reduced in Vitamin A deficiency, and that the onset of keratomalacia can be prevented by washing the eye with undiluted tears. Cramer (79,82) confirmed that there was atrophy of the harderian gland, and that bacterial infections of an avirulent type also occurred.

Holm (173) observed that rats show hemeralopia when eye changes are imminent --- how one elicits this symptom in the rat is not clear --- and with Fridericia (174) found that this was related to an injury to the visual purple which is not regenerated so quickly after blanching as in normal animals. Aykroyd (9) also found that failure to synthesize rhodopsin occurs in rats on a Vitamin A deficient diet. Tansley (437) using an improved photographic technique for estimating the visual purple confirmed these claims.

Wolbach and Howe (474) in their important survey found that infection is not important in producing the eye lesions. Advanced changes occur in the cornea and conjunctiva before the harderian and other

para-ocular glands atrophy. The changes are degenerative and metaplastic as in other epithelia. In the early stages the substantia propria of the cornea becomes oedematous and vascularized. In the later stages wandering cells appear. The vascularization is not due to infection but to the call for blood made by the new metaplastic epithelium. Bowman's membrane becomes unrecognisable, but Descemet's membrane is unchanged. Goldblatt and Benischek (145) confirmed these observations in all the main features. McLester (268) placed the order of events in the avitaminotic eye as follows: loss of secreting power by the ocular and para-ocular glands, glandular atrophy, dryness and keratinization of the conjunctiva, and finally bacterial invasion leading to keratomalacia. Treacher-Collins (440) stated that first the Harderian gland atrophies and then keratinization of the conjunctiva and cornea follow. The ducts of the lachrymal and meibomian glands are later affected.

Tyson and Smith (444) found that in rats xerophthalmia is regularly preceded by changes in the tongue, and Tilden and Miller (438) reported that monkeys die of colitis before eye infections set in, though some keratinization is to be found microscopically. Wolfe and Salter (475) also found that xerophthalmia develops later than some other symptoms of Vitamin A deficiency.

Mouriquand, Rollet and Chaix (313) observed that the earliest signs of Vitamin A deficiency in the eye are to be detected by the bio-microscope. First there is general loss of transparency, then a greyish area gradually becoming more and more opaque appears in the inner portion of the horizontal diameter of the cornea. Here the epithelium appears dry and irregular but does not stain with fluorescein except perhaps as a superficial network.

Knopfmacher and Reiter (207) emphasise the importance of toxic damage to the liver, which plays the chief role in the Vitamin A exchanges, as an aetiological factor in the production of xerophthalmia.

McCullum et al. (258, 259) threw doubts on the specificity of xerophthalmia as a Vitamin A lesion when they described experiments in which a "salt xerophthalmia" indistinguishable from the other occurred on a Vitamin A rich diet. Jones (188) explained this effect as due to oxidative destruction of the Vitamin A in the ration, the speed of oxidation being increased by the ferrous sulphate in the salt mixture employed. McCullum and his co-workers

(262,107) have accepted this view, and the specificity of xerophthalmia remains unquestioned.

THE RESPIRATORY LESIONS OF VITAMIN A DEFICIENCY.

Sherman and McLeod (389) Cramer and Kingsbury (81) Cramer (82) and Jackson (183) all found that their experimental animals were prone to bronchopneumonia when on diets deficient in Vitamin A. Wolbach and Howe (474) likewise observed this condition. Professor Mellanby (285) studied this particular problem, and his results are shown in the table copied below.

	<u>Vegetable fat. Vitamin A defective.</u>	<u>Butter.</u>	<u>Cod-liver oil.</u>	<u>Heated cod-liver oil. Vit. A defective.</u>
Normal	155	55	24	3
Broncho-pneumonia	43	0	0	1
Focal collapse	23	9	0	0

The positive relationship between lung infections and Vitamin A is very clearly brought out. Macy and her co-workers (270) McCarrison (236,257,255) Goldblatt and Benischek (145) McLester (268) Mellanby and Green (287) Seifried (386,387) and Wolfe and Salter (475) may be quoted as confirmatory evidence. It is almost impossible to quote any observations where trouble has not been experienced on account of the death of the experimental animals from respiratory disease, though often other diseases too have played a part.

The upper respiratory tract is no less susceptible than the lungs themselves to Vitamin A deficiency injuries. Wolbach and Howe (474) found keratinization of the usual type in the nares, nasal sinuses, larynx and trachea, and observed that though the specific changes occur without infection, infection is nevertheless common. Dean (89) recorded a correlation between para-nasal sinus disease and infected lymphoid tissue in the upper respiratory tract and Vitamin A deficiency. He regarded the infected adenoid tissue as a more important cause of the sinus condition than Vitamin A deficiency. The idea that this in its turn might be due to vitamin deficiency does not seem to have occurred to him. Shurly and Turner (399,400) observed infection of the nasopharynx, accessory sinuses and middle ear in their Vitamin A deficient animals. Turner Anderson

and Blodgett (441) agreed with these findings and put the figure for the incidence of these conditions as high as 85%. Turner and Anderson with Loew (442) again emphasised this association of para-nasal sepsis with vitamin deficiency. Tilden and Miller (438) found the nasopharynx affected in their monkeys.

Tyson and Smith (444) considered the Vitamin A deficiency lesions found in the trachea and larynx to be atrophic rather than metaplastic in nature and Seifried (386, 387) concurred. McCarrison (244) on the contrary has found that in Vitamin A deficiency "keratinization of the tracheal epithelium was occasionally observed, but proliferative changes of greater or lesser degree of intensity dominated the histological picture". He described the formation of what he called "tracheal adenoids"; large proliferative outgrowths. Wolfe and Salter (475) also lay stress on the severity of the tracheal and bronchial lesions in Vitamin A deficiency.

Tuberculosis does not seem to have received much attention from students of Vitamin A deficiency. Smith and Hendricks (404) found that the rat can be rendered susceptible to tuberculin shock by depriving it of Vitamin A, and that rats infected with tuberculosis degenerate more rapidly on a Vitamin A deficient diet of low biological value than controls on a normal diet. Lawrynowicz and Bohdanowicz (220) using mice, confirmed the latter part of the above observation, but found that analogous results could be got by merely insufficient food. Grant et al. (147) induced tuberculosis in an otherwise resistant rat by feeding it on a rachitogenic diet deficient also in Vitamin A; an observation of which the value is not very great. Hagedorn (152) considered that generalisation of inoculated tuberculosis occurred earlier in rats deficient in Vitamin A or Vitamin C than in normally fed animals.

Rowlands (365) was able completely to eradicate tuberculosis from pigs by attention to the vitamin content of their diet; especially as regards the fat soluble vitamins; and recorded the fact that in a herd of pedigree cattle the prize animals which were being given special artificial foods of low vitamin value showed more tuberculin reactors than the remainder of the herd on a natural diet. Zilva and Schutze (485) feeding a large excess of fat soluble vitamins found that the formation in rats of certain tubercular tumours was inhibited, but they themselves admit that their results are "by no means conclusive". Pfannenstiel and Scharlau (332) using rabbits found that Vitamin A alone is of no value in curing tuberculosis. Vitamin B plus Vitamin D is of use, but the addition of Vitamin A to these gives no further benefit.

Uyei (447,448) has stated that though other substances are potent, cod-liver oil exerts an indifferent effect on the growth of tubercle bacilli in culture.

In view of the importance of the disease, and its long course, which makes it suitable for prolonged feeding experiments it is surprising that more work has not been done on the problem. At present it cannot be conclusively stated what effect, if any, Vitamin A has on the progress of this disease. It is noteworthy that in a recent extensive review of the literature of B.C.G. published in the "Lancet" no reference was made to any attempt to see whether Vitamin A deficiency made the organism more susceptible to the bacillus, or enhanced its relative virulence; though a very large number of culturing devices had been adopted in order to test its harmlessness. The point is not an academic one, as large numbers of B.C.G. vaccinations are being carried out, and in many instances on members of those classes of society whose diet is known to be deficient in the fat soluble vitamins.

THE URINARY LESIONS OF VITAMIN A DEFICIENCY.

As early as 1917 Osborne and Mendel (323) had observed the formation of phosphatic urinary calculi in animals deficient in Vitamin A. McCollum (261) also referred to this condition in a general review of vitamin deficiency. Wolbach and Howe (474) found characteristic keratinization of the epithelia of the bladder, ureters and renal pelves, and noted that in the bladder and ureters there was evidence of very rapid growth of epithelium, remarkably striking changes being produced. The parenchyma of the kidney was noted to be normal. Jackson (183) however stated that in Vitamin A deficiency spontaneous nephritis may arise. Frontali (138) found a constant infection of the urinary tract with *B. coli* and sometimes with *Staphylococcus aureus* as well. Goldblatt and Benischek (145) Tyson and Smith (444) and Wolfe and Salter (475) confirmed the occurrence of metaplastic changes in the bladder and renal pelvis, and the second named authors also noted that after apparent clinical recovery a condition of metaplasia may persist for a long time. They found that the damaged epithelium was prone to infection and that occasionally calculi were formed.

The problems of urinary calculus formation have attracted a good deal of attention. Fujimaki (141), Saiki (369) and van Leersum (222) all recorded the production of phosphatic calculi when animals were kept on Vitamin A deficient diets. In Saiki's experiments the diets were deficient in calcium and phosphorus also. Van Leersum (223) reporting further work stated that in Vitamin A deficient rats although obvious cystitis is rare, calcium oxalate con-

acid to litmus. Calcium deposits were also found in the kidney, and calcium phosphate calculi may be found in the bladders of such rats. He gave his results in tabular form as follows :

Calculi Haematuria Cylindruria Xerophthalmia

Vitamin A present	0	0	0	0
Vitamin A deficient ♂	130	46	12	92
Vitamin A deficient ♀	67			

McCarrison (237) observed that stone in the bladder can be produced by diets which have no animal protein, are entirely deficient in Vitamin A and contain an excess of earthy phosphate, with possibly some toxic substance of cereal origin. He later (239) confirmed this result and (241) found that though such a diet is "very potent in causing stone in the bladder" the addition to the daily ration of 2/3 oz. of whole milk completely prevented the development in young rats of phosphatic calculi. Further experiments led him (243) to conclude that "cystitis is not a necessary forerunner of stone in the bladder in rats" but that the condition is due to "the insufficiency in the experimental diets of a substance or substances (probably Vitamin A) which whole milk of good quality provides in abundance". With Madhava he (248) found that the ingestion of large amounts of animal protein in association with Vitamin A deficiency increased the proneness to urinary disease. In an exhaustive review of his position he (249) classified the dietetic factors concerned in stone production as positive and negative. The latter are deficiency of fats and fat soluble vitamins and in phosphates. The former are excess of lime, and an unknown toxic factor present in certain cereals. The positive factors appear to be harmless if the negative factors are adequately supplied. Malnutrition of the urinary tract is an important underlying cause of calculous disease, and superimposed infection though it may contribute, is not essential. Radiostoleum has great power to prevent both the incidence of infection and the formation of calculi. A chemical analysis of the stones found by McCarrison in his experiments is given by Ranganathan (351,352). McCarrison (246,245,247) has since confirmed various points of his beliefs and in a recent summary (256) concluded that in the production of stone in the bladder, dietetic, infective, and physico-chemical factors all had a part. The role of Vitamin A in this connection is partly as a preventer of infection, partly as a preventer of degenerative changes and partly metabolic; the amount of urine secreted is low in Vitamin A deficiency.

Perlmann and Weber (327,328) concluded that the formation of calculi was secondary to horny metaplasia. Mellanby and Green (287) noted that in pure Vitamin A deficiency cystitis and pyelonephritis occurred in 44% of the cases. Calculi were also found, but did not seem to be dependent on infection. The addition of ergot to the diet produced calculi in the kidney, where otherwise infection alone was present. Van Leersum (224) reaffirmed the power of a Vitamin A deficient diet to produce calculi. He (225) considered that the nuclei of these calculi may be provided by certain calcium impregnated cells which he found in the renal epithelium.

THE ALIMENTARY TRACT EFFECTS OF VITAMIN A DEFICIENCY.

Wolbach and Howe (474) found that in Vitamin A deficiency the teeth show "a complete sequence from the earliest demonstrable shrinkage of odontoblasts to complete disappearance". Cysts are found at the base of the tongue and the submaxillary glands show localised oedema, keratinization of the ducts and secondary cyst formation. Atrophy of the secreting epithelium occurs. The teeth have been very ^{extensively} studied by Mrs Mellanby (280,281,282) whose results are summarised in various Medical Research Council reports. Most of these reports are taken up with a discussion of the effects of Vitamin D, which controls the actual structure of the teeth and their calcification; but Vitamin A is also studied. It appears to have an influence on the periodontal tissues. "A deficiency of Vitamin A in the diet leads to hyperplasia of the subgingival epithelium and to its subsequent invasion by pathogenic organisms; a deficiency of Vitamin D on the other hand results in defects of the alveolar bone". The author has also found that "if a puppy's diet has been deficient in Vitamins A and D during the early months of life the dog is liable to develop periodontal disease at a later stage, whatever the subsequent diet. On the other hand a perfect diet in the developmental period either prevents or greatly inhibits the production of periodontal disease at a later stage, even if the diet is subsequently deficient in Vitamins A and D." Marshall (276) also noted that caries of the teeth in Vitamin A defective rats, once set going, is not checked by the subsequent administration of the missing factor.

Sherman and Munsell (390) were the first to record sublingual abscesses in Vitamin A deficiency, though it is possible that Wolbach and Howe's "cysts" were really abscesses. Goldblatt and Benischek (145) confirmed the occurrence of sublingual abscesses, and observed abscesses in the submaxillary gland as

well. Mellanby and Green (287) found macroscopic sublingual abscesses in 72% of cases, and in association with these, submaxillary abscesses and cervical adenitis were "occasionally" found. Tyson and Smith (444) considered that infection of the serous type of sublingual gland and of the glands at the base of the tongue is the first sign of Vitamin A deficiency, the submaxillary gland being next affected. Tilden and Miller (438), Wolfe and Salter (475), and McCarrison (255) all found infection of the mouth glands to be a common feature of Vitamin A deficiency. Mention may here be made of a condition described by Underhill and Mendel (445), characterised by stomatitis and enteritis and compared by them to "black tongue" in dogs. The condition is said to be curable by carotene, butter and carrots, but not by cod-liver oil. The last finding appears to contradict the others, but if supported would raise a problem of no little complexity.

Cramer (79,82) found that "in the absence of Vitamin A there is an atrophy of the intestinal mucous membrane" which disappears when Vitamin A is given. The mucous glands do not secrete, and there is bacterial penetration of the intestinal wall. McCarrison (233) in monkeys found congestion of the ^{whole} gastro-intestinal tract with ecchymoses on the peritoneal surface. Atrophic and necrotic changes were present and bacterial invasion of the bowel wall and enlargement of the mesenteric lymph glands were also seen. There was atrophy of the lymphoid elements and degeneration of the plexus of Auerbach. Ballooning of various parts of the intestinal tract occurred. In the stomach, which was dilated, shallow ulcers and papillary growths were found near the pylorus. The colon was the seat of the most advanced changes, and its muscular walls were thinned. Wolbach and Howe (474) stated that in the rat the stomach oesophagus and intestine show only a slight degree of atrophy, the liver is unaffected, but the secreting cells of the pancreas show a moderate degree of atrophy, the islands of Langerhans remaining intact. Underhill and Mendel (445) observed ulcers and congestive changes near the pylorus. McCarrison (236) laid stress on the gastro-intestinal lesions produced by a bad diet deficient in fat soluble vitamins. Goldblatt and Benischek (145) observed an enteritis, and Mellanby and Green (287) stated that enteritis was present in their series in 21% of cases, and further noted the presence in the pyloric region of "neoplasms" similar to those seen by Fujimaki (140). They concluded however that these were not truly malignant. Magee Anderson and McCollum (272) found congestion of the alimentary tract, colonic stasis and sometimes colitis, and in association with these changes, pyloric ulcers with sloping edges. McCarrison (248,257) confirmed his earlier work and in a review of his experience (255) listed the gastro-intestinal lesions he had seen in Vitamin A defic-

iciency as follows: "dilated stomach, gastric ulcer, epithelial new growth in the stomach, cancer of the stomach (in two cases only), duodenitis, enteritis, gastro-intestinal dystrophy, stasis." Seifried (386, 387) in chickens found lesions of the mucous glands and their ducts in the upper alimentary canal. Tilden and Miller (438) using monkeys found that colitis is the chief lesion of Vitamin A deficiency in these animals, proving rapidly fatal before xerophthalmia appears. The colon of the monkey appears to be peculiarly susceptible to Vitamin A deficiency.

THE REPRODUCTIVE SYSTEM LESIONS OF VITAMIN A DEFICIENCY.

Evans and Bishop (125) had already observed that Vitamin A deficiency affects oestrus, causing prolongation of the desquamative stage, and Hoffman (172) had confirmed them. Sherman and McLeod (392) noted that a supply of Vitamin A adequate for growth may nevertheless be inadequate for reproduction. Macy et al. (269) found that cornification of the vaginal epithelium is one of the earliest changes in Vitamin A deficiency. Evans (127) concluded from his wide experience of research on fertility and diet, that Vitamin A deficiency so injures the female reproductive system that fertilization and implantation fail. A constant appearance in the vaginal smear of cornified cells either exclusively or predominantly is a very early and delicate sign of this condition. This mode of action is in contrast with that of Vitamin E deficiency, where fertilization and implantation occur normally, but resorption of the ovum subsequently takes place. Coward (73) considered that the constant occurrence of cornified cells in the vaginal smear cannot be relied on to indicate Vitamin A deficiency, as the vagina in the young rat may not open till other signs of vitamin deficiency have already appeared, and when open, during the resumption of growth following the administration of the vitamin, leucocytes only may appear in the smear for many days before normal cycles are established. McCarrison (255) does not particularize but has recorded "inflammation of the uterus, ovaritis, death of the foetus in utero, premature birth and uterine haemorrhage".

In the male, whose organs have not been so closely studied, Wolbach and Howe (474) found local oedema of extreme degree in the testis, and atrophy of the tubules, leaving only cells from the sustentacular tissue. Mellanby and Green (287) found the prostate and seminal vesicles "frequent" sites of suppuration whilst epididymitis "occasionally" occurred. McCarrison (255) has observed hydroys testis. The Schmidt-Nielsons (379) stated that at puberty the body's requirements of Vitamin A increase.

THE ENDOCRINE SYSTEM LESIONS OF VITAMIN A DEFICIENCY.

That Vitamin A deficiency (as well as many other dietetic factors) has a profound effect on this important system is beyond doubt. The nature of this effect is still to seek. McCarrison has devoted much time to the study of the thyroid gland, but the other glands of internal secretion have been largely neglected. In the absence of a detailed study of the system as a whole it is unlikely that any great advance in understanding will be made. The various members of the "endocrine orchestra" act and react upon each other in so complicated and so intimate a manner that to study one gland and not the others is like attempting to make discoveries about diseases of the cardio-vascular system from observations of nothing but the apex beat.

Wolbach and Howe (474) found the thymus extremely reduced in size, owing to an almost complete disappearance of the small thymic cells. Hassall's corpuscles were enlarged. In the adrenals no changes of note were seen, whilst the thyroid, parathyroid and pituitary glands were all atrophied. The islands of Langerhans were unaffected. Magee, Anderson and McCollum (272) found on the contrary that the adrenal cortex shows hyperaemia haemorrhage and vacuolation, affecting all zones, whilst the thyroid is unaffected. Vitamin D and certain inorganic constituents were also absent from their diets. Wolfe and Salter (475) described marked atrophy of the thymus whilst the adrenals and thyroid were found normal. Harris and Smith (155) have observed changes in the thyroid. McCarrison has seen atrophy of the thymus and enlargement of the adrenal glands; but by far the greater portion of his work (229, 230, 242, 238, 240, 257, 244, 250, 252, 253) has been done on the thyroid gland. Whilst he considered the classical endemic goitre of Himalayan India to be due to an unhygienic mode of life, particularly to bacterial contamination of water supplies, with iodine deficiency as a contributory cause; he claimed that lymphadenoid goitre is related to dietary deficiency and that Vitamin A deficiency is "the chief dietetic factor responsible for or contributing to the condition". He also stated that an unknown positive factor plays a part in producing this condition. He found too that in the absence of a sufficiency of fat soluble vitamins the thyroid gland is unable to deal with iodine in a proper way, and that its administration in these circumstances may actually favour goitre production. He has claimed to have produced experimentally a different class of goitre whose early stages almost suggest primary Graves' disease. One of the faults of the diet employed in these experiments is deficiency of fat soluble vitamins. This long research leaves many problems unsolved, and many of the positive results have been

obtained with diets showing multiple and complicated faults. The peculiar "cabbage goitres" of Chesney, Clawson and Webster (57) whose findings have been endorsed by Marine, Baumann and Cirpa (275) and by McCarrison (254) --- who notices that the addition of radionutrient to the cabbage diet enhances the goitrogenic effect --- may be cited as illustrating the unknown difficulties with which this branch of research bristles, and showing the care that must be exercised in interpreting the results of experiments where the diets are unbalanced and deficient in more than one factor.

THE BLOOD PICTURE IN VITAMIN A DEFICIENCY.

The results of experiments on the blood in Vitamin A deficiency are varied and contradictory. No one feature commands general acceptance. The most carefully conducted studies are the most inconclusive; and the effects of infections which so constantly supervene are an added difficulty in the interpretation of the results.

Cramer (79) found a "great reduction in the number of blood platelets" and stated that on supplying Vitamin A the platelets became rapidly increased. He quoted Bannerman as confirming this claim, and criticised the results of Bedson and Zilva (18) who had found that protoplasmic debris is present in the blood of such animals but that there is no thrombopenia. Anaemia he stated was frequently present. Findlay (134) noted that lesions of the leucoblastic marrow were rare, but that in animals kept long on Vitamin A deficient diets aplastic changes associated with a leucopenia supervened. Keratomalacia or the onset of any acute infection produced a polymorphonuclear leucocytosis. Wolbach and Howe (474) stated that there were no characteristic changes in the blood, and that the spleen on postmortem examination showed "no trace of haematopoiesis or lymphopoiesis", but is full of haemosiderin. Stammers (414) observed no significant change in the platelet count and Falconer (129) found that such changes in the relative numbers of platelets, red blood corpuscles or white blood corpuscles as occurred in rats on a Vitamin A deficient diet were neither great enough nor constant enough to constitute a specific lesion. Cramer (83) reaffirmed the existence of a thrombopenia and an anaemia, and Sherif and Baum (388) found a pronounced thrombopenia.

Scott (383) and Koessler, Maurer and McLoughlin (208, 209) observed an anaemia in animals on a Vitamin A deficient diet, but the animals were ill when the counts were made and the actual figures are not very conclusive. The latter authors stated in addition that "blood regeneration cannot take place

without Vitamin A. The addition of Vitamin A to the diet of animals long depleted of their Vitamin A reserve brings about a rapid formation of new red blood corpuscles". In this case their observations are probably correct, but as the source of Vitamin A employed was liver, are open to quite another interpretation. Hart and his colleagues (158) and Mitchell and Schmidt (294) working on the anaemia that develops when whole milk is fed as the sole diet obtained cures with substances wanting in Vitamin A and poor results with certain rich sources. Robscheit-Robbins (353) in a careful study of the effects of diet on blood regeneration found that milk and green vegetables, both good sources of Vitamin A, are poor blood regenerators, whilst fish liver oil, the classical source of the vitamin, contains no regenerating substance at all. Cartland and Koch (54) stated that rats on a Vitamin A deficient diet, do not become anaemic and can regenerate their blood normally. Binet and Strumza (21) observed that in dogs made anaemic by venesection and kept on a constant diet the addition of carotene produced greater haemoglobin production than occurred in control periods without carotene.

Sure, Kik and Walker (432) in a carefully planned research found that from the period of onset of xerophthalmia there is a decrease in the concentration both of red blood cells and of haemoglobin, but wide variations occur and the results are inconclusive. Anhydraemia complicates the later blood picture. Wills and her co-workers in a series of experiments designed to elucidate the tropical anaemias of pregnancy, having established (466) that pregnancy alone has no effect on the red blood corpuscles or haemoglobin concluded (467, 468) that "a severe anaemia has been produced in rats by feeding on diets devised to be relatively deficient in Vitamins A and C". These conclusions are not entirely acceptable as Bartonella muris infection was present in the stock of rats employed. Later work (469) failed to support these earlier claims, and Wills has decided that Vitamins A and C are not concerned in the microcytic tropical anaemias. Turner and Boew (443) have observed leucocytosis with polymorphonuclear increase in the later stages of Vitamin A deficiency, but this most likely represents the onset of infection.

LESIONS OF THE OTHER ORGANS IN VITAMIN A DEFICIENCY.

The heart and vascular system, the nervous system and the solid organs appear to be little affected in Vitamin A deficiency. Baude and Deglaude (17) using electrocardiographic and histological methods found the heart little affected, and though Wolbach and

Howe (474) found occasional focal degeneration in the heart, more particularly in advanced cases, such changes might well have been produced by infections. In any case this finding has not been supported by subsequent workers.

Direct observations of nerve lesions in Vitamin A deficiency are lacking, except for McCarrison's (233) unconfirmed finding of degeneration of the plexus of Auerbach; but the vitamin seems to have a protective influence against certain toxins which can damage the central nervous system in its absence. A protective action against a cereal toxin potent in causing urinary calculus has been already referred to (McCarrison 249) and Professor Mellanby (284, 283, 148, 286) has recorded a like action against a cereal decalcifying toxin. Mrs Mellanby (279, 281) in her studies on the teeth has also observed such a toxin. The nerve effects were indeed first obtained in the course of the above experiments on the calcification of bone. Mellanby (286) noted that the fat soluble vitamins protect against nerve degenerations due to ergot. Later he (290) confirmed that result and recorded a like protective action against neuro-toxins present in wheat embryo and in oatmeal. He drew a comparison of these experimental findings with ergotism, lathyrism and pellagra in man, and with the degenerative nerve lesions associated with pernicious anaemia, quoting Ungley and Suzman (446) as obtaining improvement in the last named condition with whole liver, but not with a liver extract that improved the blood picture satisfactorily. With carotene and with Vitamin A he (292) again obtained similar results, and has given a full review of his work on this particular subject in a recent (September 1931) issue of "Brain". Hughes, Lienhardt and Aubel (178) found impaired vision, incoordination and spasms with degenerative nerve lesions in pigs on diet deficient in Vitamin A and also containing cereals.

NEOPLASMS AND VITAMIN A DEFICIENCY.

The question whether or not Vitamin A deficiency plays a part in the aetiology of new growths, and of carcinoma in particular has interested research workers in many fields. Cramer (80) found that no degree of Vitamin A deficiency compatible with life has any effect on malignant proliferation, and in a Ministry of Health Circular (49) published in 1924 it is stated that Vitamin A deficiency exerts no effect in retarding cancer, nor do tumours affect the deficiency effects. Wolbach and Howe (474) considered that in the epithelial changes of Vitamin A deficiency the mitotic figures seen and the connective tissue response suggested the acquisition of malignant properties, but could not establish definite evidence of malignancy.

Passey and Woodhouse (325) found that the presence or absence of Vitamin A has no effect on the induction of cancer by soot. Burrows and Jorstad with their various collaborators (41,42,43,44,45,190,191,192,193) reported a series of experiments on the effect of Vitamin A on the induction of tumours by tar, by the injection of oil, and by Roentgen rays, and arrived at an opposite conclusion. In their opinion all these carcinogenetic agents act by removing the fat soluble vitamins from their site of action, producing a focal vitamin deficiency; a rich diet in Vitamin A to some extent counteracts this effect, and a deficient diet enhances it.

McCarrison (233) observed papillary growths near the pylorus in Vitamin A deficient monkeys, and Fujimaki (139,140) reported cancerous hypertrophy in the same region in rats. Saiki (369) found that in rats on a Vitamin A deficient diet, provided just sufficient vitamin is allowed to maintain life for a long time hyperkeratosis, papillomata, and finally carcinomata develop. Mellanby and Green (287) noted neoplasms similar to those of Fujimaki but decided after closer study that they were not truly malignant. Erdmann and Haagen (105,106) found that a vitamin unbalanced diet of which Vitamin A deficiency was one feature, caused tumours to develop. These tumours did not metastasize but were definitely malignant histologically. They considered the lack of balance between the fat soluble vitamins and vitamin B, the latter predominating, to be the prime aetiological factor. De Raadt (350) has stated his opinion that vitamin injuries play no part in carcinogenesis in man, and found that the carcinogenetic effects observed in mice on Vitamin A deficient diets were due to an associated alkalosis; "without nutritional alkalosis no cancer". Sugiura and Benedict (428) in a critical review of the question of diet and cancer decided that Vitamin A deficiency has no effect on tumour genesis in rats, and Hill (171) after a like survey concluded that neither Vitamin A nor any other single dietetic factor is concerned in the aetiology of cancer.

THE POSSIBLE TOXIC EFFECTS OF AN EXCESS OF VITAMIN A.

Hopkins (176) recorded toxic effects with large doses of cod-liver oil, and Mellanby (283) found that tachycardia occurred when similar large doses were given. Takahashi et al. (435) found their Vitamin A concentrate "Biosterin" capable of powerful toxic effects, similar to those of Vitamin B deprivation, but Drummond & Channon and Coward (93) obtained no such results with their highly concentrated fraction. Magliano (273) also obtained negative results.

Agduhr (4) recorded cardiac damage with excessive doses of cod-liver oil. Plimmer and Rosedale (338) also produced toxic effects in chickens, but found that they could be prevented by increasing the ration of Vitamin B. Agduhr (5) found Vitamins B and C powerless to prevent the toxic effects he obtained. Hoyle (177) after a review of the problem concluded that "no reasons at present exist to prove that excess of Vitamin A was the poisonous factor in the observations made by Hopkins with cod-liver oil". Harris and Moore (156) found Vitamin A and D concentrates toxic in large doses, the symptoms being similar to those of Vitamin B deficiency, and preventable by adding that factor to the diet. They suggested that it was the Vitamin A and not the Vitamin D of the diet that was responsible for the toxic effects. McCarrison (254) observed that radiostoleum enhanced the effects of the goitrogenic principle resident in cabbage, though as carrots had a slight anti-goitrogenic effect Vitamin A was probably not concerned in the former result.

Though a highly impure substance like cod-liver oil may quite conceivably contain toxic principles in certain samples there is as yet little evidence that Vitamin A has such specific toxic properties as have been demonstrated for Vitamin D. The question of vitamin balance, and the effect the ingestion of large amounts of the fat soluble vitamins has on the bodily requirement of the water soluble vitamins and of Vitamin B in particular calls for further research .

CLINICAL OBSERVATIONS OF A GENERAL NATURE ON VITAMIN A DEFICIENCY.

Though clinicians may claim that they had been using cod-liver oil, in the teeth of the criticisms of sceptical pharmacologists, for many years before the conception of vitamin activity had even been formed, it cannot be denied that work on Vitamin A deficiency in man has lagged far behind the work of the nutritional research laboratories. There are many vague observations on the ill effects of various deficient diets, but in most cases the inadequacies have been multiple, and in attempted dietary cures single food factors have rarely been used. Controlled experiments are hard to find, and whilst it is admitted that the clinician must take his cases as he finds them, and can never hope to attain the precision of experimental control enjoyed by the laboratory worker, the time is past when crude observations can add much to our knowledge, and there is urgent need for clinical research on more scientific lines than have been followed in the past. Only in xerophthalmia, which the ophthalmologists have studied in some detail can it be said that clinical medicine has marched abreast of laboratory studies.

Mackay and Mackenzie (264) found that "there is undoubtedly a definite deterioration in the physique of German children aged 2 - 14 since the beginning of the war", during which period a deficiency of the fat soluble vitamins was one of the prominent dietary faults throughout the country. V.Wendt (456) in Finland found the greatest increase in weight in 15,000 children to occur from October to January, and the least from February to May, whilst the greatest increase in length occurred from June to September and the least from February to May. He considered that the variation in the increase in weight may possibly be correlated with seasonal variations in the amount of Vitamin A ingested. Cowell (78) who reviewed the question of Vitamin A deficiency in a general way believed it possible to lessen the infantile mortality from malnutrition, diarrhoea, and respiratory infections, and to raise the general standard of physique, by arranging for an adequate supply of fat soluble vitamins, beginning with the pregnant mother. The Committee on Nutritional Problems of the American Public Health Association (65) reported that Vitamin A is more seriously neglected in the modern American dietary than any other factor. They go on to suggest that since a normally adequate supply may fail to carry the individual over the strains of reproduction and lactation such relative Vitamin A deficiency may account for the fact that tuberculosis often becomes manifest about such times.

Cilento (60) observed that the native diet of Northern Melanesia is deficient in protein, fats and fat soluble vitamins and in salt, and correlated these facts with the poor physique of the people and their high death rate from respiratory and intestinal diseases. He added that a marked tendency these people show towards tuberculosis disappears when they emigrate to a land where food supplies are more adequate. Stene and Roberts (424) found that the diet of the American Sioux Indian is deficient in fat soluble vitamins, being largely composed of meat, bread and coffee. Amongst this tribe decayed teeth, rickets, sore eyes and blindness are very common. 85% of the deaths from known causes are due to tuberculosis or other respiratory infections. One third of the infantile mortality occurred in a few families who subsisted chiefly on bread and coffee only. The sanitary condition of these people was bad. McCulloch (263) found that the diet of the Hausa tribes of West Africa is notably deficient in fat soluble vitamins, and that though Vitamin D may be made up by exposure of the skin to the sun's rays Vitamin A is undoubtedly deficient. This deficiency he believed to be responsible for the low resistance shown by these people to epidemic diseases such as relapsing fever and smallpox. He found that tuberculosis and other respiratory diseases were rife in this community, and that gas-

tro intestinal troubles and chronic anaemias are common. The teeth both of adults and of children are bad, and in children xerophthalmia occurs. The average weight of the Hausa is less than that of Europeans of the same height, and they reach their maximum height earlier than do European races. Shircore (398) in Tanganyika correlated the incidence of yaws amongst the native population with a low diet deficient in animal protein, fat, calcium and Vitamin A. Orr and Gilks with their co-workers (322) compared two East African tribes, the Masai and the Kikuyu. The diet of the former contains a liberal supply of mineral elements and a large excess of protein and fat, but is low in carbohydrate and in indigestible residue. The diet of the latter is adequate in protein, but low in fat and the fat soluble vitamins, contains more than enough phosphorus, but is low in calcium and possibly also in sodium. The Masai are superior in height, weight and strength at all ages, and are typically larger in the chest and smaller in the abdomen than the Kikuyu. The following table shows the incidence of the most common diseases amongst these two tribes, expressed as a percentage of the total number of cases encountered in each tribe on a field survey:

	<u>Kikuyu</u>	<u>Masai</u>
Bronchitis	28	4
Pneumonia	3	0
Tuberculosis	6	1
Ulcers	33	3
Malaria	18	2
Gonorrhoea	1	8
Intestinal stasis	4	45
Arthritis	2	35
Helminthiasis	5	2

Conjunctivitis was less common amongst the Masai though the exact figures are not given. Hospital figures were found to agree with this general distribution of cases between the tribes, except in the case of tuberculosis, where the superiority of the Masai is not so evident. The women of these tribes who eat a diet intermediate between the diets of their respective males occupy a middle position as regards their physique and disease incidence. In Kikuyu children rickets, dental defects, anaemia and spongy gums were prevalent, as were also enlarged tonsils and cervical adenitis. Amongst Masai children bone and dental defects and anaemia were relatively uncommon. Field (132) observed that the diet of Indian immigrants into Malaya was relatively deficient in Vitamin A as compared with the local Malays and Chinese, who eat a certain amount of natural food in a fresh state. The Indians do not eat fresh natural food, and show more xerophthalmia and night blindness than the native races, and are less resistant to in-

fectious diseases. In the influenza epidemic of 1918 their mortality figures showed a very great increase whilst the rates for the other peoples showed only a small rise. He considered Vitamin A deficiency an important, though not the only factor responsible for these findings. Price (346) in America found that a correlation exists between seasonable vitamin shortage in milk and the mortality rates for such infectious diseases as pneumonia; and reported success in preventing disease in man by supplying preparations of the fat soluble vitamins during the seasons when natural foods contain relatively small quantities.

In considering these varied findings one is struck by the fact that though the diets described are faulty in various ways Vitamin A deficiency is a common factor in all; further that the diseases recorded as prevalent in the widely scattered peoples under review are those which experimental results in animals would lead one to expect Vitamin A deficiency to produce. It is surprising that xerophthalmia has not been recorded oftener in some of these surveys, as the condition is easy to recognise, and is common in other parts of the world where Vitamin A deficiency is a fault of the diet. This fact makes it wise to pause before laying too great stress on Vitamin A as the prime aetiological factor in every case.

The effect of adding milk to the diet of growing children has been very carefully studied by Corry-Mann (68). He found that the addition of one pint of milk per day to the ration of boys already on a "satisfying diet" converted an annual gain in weight of 3.85 lbs. and a like gain in height of 1.84 ins. to an annual gain in weight of 6.98 lbs. and in height of 2.63 ins. per boy. The addition of butter produced a gain in weight of 6.30 lbs. and in height of 2.22 ins. annually. This unmistakable betterment in nutrition was found by trial to be due neither to the relatively small increase in the fuel value of the dietary, nor to the extra protein supplied in the milk; but rather to more "specific qualities of milk as food". It was also noted that the boys on the extra milk diet, besides gaining in weight and height, lost the "marked tendency" to chilblains shown by the other boys. These controls showed a general roughness of the skin, sometimes amounting to ichthyosis, and a tendency to gas formation in the small intestine. "There was a complete absence of illness amongst the boys with the milk ration, although in other houses of the village the sickness rate had been somewhat higher than usual on account of naso-respiratory catarrh (influenza) and to a limited outbreak of measles and scarlatina". Cramer (83) considered these results were due to the extra Vitamin A provided by the milk and by the butter and stated that "every in-

dividual requires an average daily consumption of 40-50 g. of milk fat ----- to maintain his full health and vigour". Comparable results have been obtained by the American workers Morgan, Hatfield and Turner (306) McCollum (260) and Chaney (56). Orr (320) has also studied the same question, and found that the addition of milk to the diet of children was accompanied by an increase in both height and weight 20% greater than in the controls. The increase in growth was accompanied by improvement in the general physical condition. Separated milk was found to have great value in promoting growth. Leighton and Clark (226) continued the same work and confirmed Orr's earlier report in all particulars. The average increases in height and weight in all groups combined were actually 1.21% and 3.75% greater respectively than those recorded in Orr's first test. The value of separated milk was again stressed. Clark (61) obtained very similar results in children from a rural area --- those previously selected for these tests had been town dwellers ---. The milk fed country children showed an annual increase in height of 0.277 ins. and in weight of 0.344 lbs. more than the controls.

These results are of great value to the practical dietitian, and though, as Cramer suggested, it is possible that the Vitamin A of the milk was the factor responsible for the beneficial results obtained, the proof is by no means clear, especially as separated milk proved as valuable a growth promoter as whole milk. Similarly controlled experiments with carotene would be most valuable, and of much more scientific value.

NIGHT BLINDNESS: XEROPHTHALMIA: KERATOMALACIA.

Night blindness and its successful treatment by liver was known to the ancients, but the first accurate description of xerophthalmia and of the keratomalacia to which it leads was given by v. Graefe (146) in 1866. It is interesting to read how seriously he regarded this condition, keratomalacia, and how grave was his prognosis not only as regards vision, but as regards life itself. Mori's (307) observations on the Japanese eye disease "hikan" and its cure by fish livers attracted some attention; but it is to the Danish workers Bloch (27, 28, 29, 30, 31) and Blegvad (24, 25) that the credit must go for correlating these conditions with Vitamin A deficiency, and for demonstrating how their cure may be effected by food rich in this factor.

Widmark (457) correlated the occurrence of xerophthalmia in Denmark with the export of butter to so great an extent that the Danes were themselves deprived of the fat soluble vitamins. The incidence of the disease was checked when government restrictions were

imposed on the export of butter, and again rose when the restrictions were withdrawn. Parsons (324) observed that the condition was very rare in the Birmingham area, but gave no details of the dietary habits of his patients. Bloch (33) found that the growth of children and the occurrence of infectious disease makes great calls for Vitamin A, and that in their presence xerophthalmia is adversely affected. He recorded an interesting case where xerophthalmia developed in a child on a theoretically adequate diet. There was co-existing disease of the liver, jaundice being present, and though the necessary factor was being abundantly supplied the body was unable to use it. Wilson (471) found the liver of a child with xerophthalmia to be deficient in Vitamin A as tested by the antimony trichloride reaction. Schwartz (382) reported the development of keratomalacia in the extraordinarily short time of three weeks. The sufferer was a child undergoing a rigid dietary treatment, and the circumstances were such as to "amount to experimental conditions". The case is of value in showing that the condition can develop on a diet whose only faults are deficiency of fat and of Vitamins A and D. Neither deficiency of fat nor deficiency of Vitamin D can produce xerophthalmia, and Vitamin A remains incriminated.

Stransky (427) classified xerophthalmia as Vitamin A deficiency xerophthalmia, and non-Vitamin A deficiency xerophthalmia, recording cases where failure to obtain a cure occurred when an adequate diet was being given. Whether the adequate diet was being properly absorbed is not made clear. Blegvad (24) had already reported a case with hepatic disease where the vitamin given by mouth failed to relieve the condition, but succeeded when given parenterally. Abderhalden (1) emphasised the importance of the liver in the metabolism of Vitamin A, and the need to make sure in the treatment of xerophthalmia that this organ is functioning properly. Poulsson (341) referred to the same points, and believed that the cases of xerophthalmia recorded in jaundiced patients on adequate diets were thus explainable.

Bloch (34,35) reported two cases where neither outdoor life, sunlight, nor the Finsen arc effected the least relief. Vitamin A was potent, and thus the objections made to certain of the earlier cases, that the first named factors had been responsible for the successes was refuted. Arkle (7) described cases from Newcastle, and confirmed the relationship of xerophthalmia with Vitamin A. King-Fretts (205) found xerophthalmia common in Trinidad, especially in advanced cases of ankylostomiasis. His description of the conjunctiva in these cases as "dry and crimped, and appears like thin smoked parchment" is particularly vivid and apt. Treatment with Vitamin A was effective in the earlier stages but disappointing later on.

Adler (3) gave a full account of the condition and concurred as to its aetiology. Birnbacher (22) observed a spring peak in the figures for hemeralopia and keratomalacia and correlated this with the heightening of the physiological processes of growth and reproduction that occurs about that season and the greater demand so made for the vitamin. He did not think that seasonal changes in diet had anything to do with the increase shown. In acute essential hemeralopia he (23) noted a great preponderance of men over women, with a relatively high incidence in pregnant women, especially in the spring epidemics of 1914 - 1924. A like sex distribution was found for Vitamin D deficiency conditions. He held that the explanation of these facts lay in the greater stores of fat and of the fat soluble vitamins held by females, so enabling them to withstand deprivation better than males, and to the strain those reserves were subjected to when providing for the foetus. Aykroyd (8,9) recorded the cure of cases of hemeralopia in Newfoundland by cod-liver oil, and stated that he was unable to find any other condition associated with the vitamin deficiency.

Pillat (333, 334, 336, 337) has given a detailed account of the condition and recorded cure by proper food and cod-liver oil. He found that infection of the conjunctiva and of its glandular appendages is a frequent concomitant of xerosis. This infection is secondary; the organisms concerned live a sluggish saprophytic existence in the damaged tissues for some time before they produce clinically recognisable lesions. He contended that the pigment so frequently deposited in the conjunctiva in this condition is due to adrenal deficiency, though his argument does not explain why this should be such an early sign. Treacher-Collins (440) considered the first stage in the development of this condition to be loss of function and atrophy of the mucous secreting cells of the conjunctiva. Keratinization follows, and the ducts of the lachrymal glands become secondarily affected. In the absence of mucous secretion the surface tension of the conjunctiva becomes altered, fatty meibomian secretions adhere and form a nidus for bacteria, and the pathogenic organisms so fostered eventually produce keratomalacia. Kreiker (212) gave a full account of the clinical and histological picture in this condition. Aykroyd (10) has observed the occurrence of hemeralopia after no longer than a month on a Vitamin A deficient diet, and found a relationship between the incidence of this condition and exposure to bright sunlight. In women the occurrence of pregnancy favoured its onset. Liver oils provided a rapid cure in all cases. Spence (411,412) recorded the sporadic occurrence of cases of hemeralopia and xerophthalmia in an urban population. Vitamin D, ultra-violet light and liver extract failed to cure, but 10 cc. of cod-liver oil daily was effective. He concluded that Vita-

min A contains more than one factor, and that the anti-xerophthalmic factor is distinct from the growth promoting and the anti-infective factors. John (187) observed that in the very early stages of xerosis the sensitiveness of the cornea and conjunctiva are almost normal, but that later on the sensitiveness of the cornea is much reduced, the centre being more affected than the periphery. The conjunctiva does not suffer so much. He believed this to be due to changes in the Gasserian ganglion or in the ciliary ganglion; the corneal nutritive fibres being supposed to have a different sensitiveness to Vitamin A deficiency from those subserving sensation. The theory is more ingenious than convincing.

In India, Kirwan (206) has recorded cases in Bengal, in association with Vitamin A deficiency in the diet. Shetti (397) in Bijapur stated the condition to be common in central and southern India, and obtained good results in early cases by giving cod-liver oil. Shamsheer Singh (402) in the Punjab has been able to cure early cases in 3 - 4 months by the addition of milk to the diet; and observed the normal association with Vitamin A deficiency. Wright (478) who finds keratomalacia rampant in Madras, and who quotes the opinions of ophthalmologists in Calcutta, Bombay and Lucknow as to its frequency throughout India, has contributed an interesting discussion of its aetiology. He concluded that "the chief primary aetiological factor --- but not the only factor, in so far as our present knowledge goes --- would appear to be a lack of fat soluble Vitamin A in the diet" but later he qualified this ascription somewhat by saying that "in all probability it requires an initial multiple vitamin deficiency, with a secondary multiple dysfunction of glandular structures to account for the whole picture ----- which we see in keratomalacia". He returned however to his original statement and ended by saying that "the principal initial factor is Vitamin A deficiency". He observed that "Organismal infection as the clinician understands it is only seen as a terminal event" though organisms may be found in the damaged tissues early in the condition. An association with liver disease was likewise noted. He has obtained a cure of the condition by the use of a pure Vitamin A preparation, but considered that cod-liver oil gives superior results.

The enormous weight of evidence just recorded leaves little doubt that in man, as well as in animals, xerophthalmia is a reliable index of Vitamin A deficiency.

VITAMIN A DEFICIENCY EFFECTS IN OTHER SYSTEMS OF THE BODY.

The clinical picture of Vitamin A deficiency has very notable gaps. Records concerning other parts of the body than the eye are infrequent and often unsatisfactory. Most of them concern the respiratory tract. Bloch (27, 28, 29, 30, 31) and Blegvad (25) observed that xerophthalmic children showed a high rate of infective complications --- the association that had caused v. Graefe and the earlier observers to give such a gloomy prognosis. Nasal catarrh, otitis media, bronchitis, pneumonia, diarrhoea and pyuria are mentioned as frequent complications, and subcutaneous abscesses are said to appear. Widmark (457) correlated the high incidence of xerophthalmia with a high incidence of mortality from "congenital debility" rickets, and pulmonary tuberculosis. Dean (89) observed a connection between Vitamin A deficiency and paranasal sinus disease, though he considered the dietetic factor to be secondary to infection of the lymphoid tissues of the naso-pharynx in the aetiology of these conditions. Findlay (135) was able to check an outbreak of pneumonia in an Edinburgh school by adding cod-liver oil and raw swede juice to the diet. The food which was of good quality was being stewed in closed cauldrons for from 3-4 hours daily. Donaldson and Tasker (90) found that in pneumonias treated by massive doses of Vitamin A the mortality was 8%, whilst in a control series it was 13%. Treatment with ox liver gave a mortality of 9.09%. The authors refuse to draw definite conclusions from these results, as pneumonia is a disease with such a variable death rate. On theoretical grounds pneumonia is one of the last diseases where good results might be expected with vitamin therapy: even if the patient is known to suffer from Vitamin A deficiency there is every likelihood that the disease will have progressed to a fatal or a fortunate result before any appreciable amount of vitamin can be assimilated. Mellanby (291) suggested that such diseases as tuberculosis, bronchitis and rheumatic fever, which are most prevalent amongst the poorer classes, may be so distributed because of the low Vitamin A values of the diets eaten by them as compared with the better off members of society. Barrett, Keith-Gibson and Reece (15) claimed a correlation between Vitamin A deficiency and respiratory infections from the 2nd. to the 6th. month of life, and stated that the therapeutic administration of the fat soluble vitamins is valuable in such cases. Wright et al. (479) found intensive Vitamin A therapy did not prevent the spread of common colds in infants under two years. The controls in these experiments do not seem to have been very valuable, as they were on a Vitamin A rich diet, including cod-liver oil. Bloch (36) recorded that infantile mortality continues high in children who have suffered from xerophthalmia. Barely two-

thirds reach the age of eight years, though thereafter development proceeds normally. Respiratory conditions claim a large number of the deaths in these children.

With regard to tuberculosis Poulsson (339) testified to the value of cod-liver oil in the treatment of this condition, and Cascella (55) claimed good results by supplying the fat soluble vitamins to a small series of cases. Less striking effects were got in pneumonia, furunculosis enteritis and syphilis. His numbers are too small to be very convincing. Vaile (449) observed that in 24 families of 164 persons 101 were "fat eaters", of whom 96 were alive and well, 4 had tuberculosis, and one was doubtful. Of the remainder 61 were "fat shy", and of this number 11 were alive and well, 48 had tuberculosis in all but two cases pulmonary tuberculosis, and two were doubtful. Of two persons alive and well no information regarding their dietary habit was available. The observation is an interesting one, but in view of the high Vitamin A content of green vegetables, carrots and other non-fatty foods it cannot be claimed that the "fat shy" group were deficient in the vitamin. Lee-pattison (221) found that in bone tuberculosis, cases taking large amounts of Vitamins A and D did no better than cases on ordinary doses of cod-liver oil. Only small numbers were submitted to this experiment. Suk (429) found that in the children of pure bred Eskimos on a fat and vitamin rich diet 9.8% were tuberculin reactors. In mixed bred children on a deficient diet the percentage was 56.2%. The effect of the mixture of races might in his opinion be expected to have had an opposite effect in equal circumstances, as tuberculosis is an unusually severe and fatal disease amongst the Eskimos, whilst in Europeans it tends to run a more chronic course.

Mellanby and Green (288,290) obtained successes in the treatment of puerperal sepsis with Vitamin A rich preparations, but the number of cases treated was very small. Burton and Balmain (47) using the Dick reaction as an index, were unable to confirm the value of this form of treatment. The later work of Mellanby and Green, carried out with Pindar and Davis, (293) on the prevention of puerperal sepsis is much more important. The prophylactic administration of Vitamin A to a carefully controlled series of women in the later stages of pregnancy resulted in a statistically significant reduction in the puerperal mortality and morbidity.

Kirwan (206) found that the skin in xerophthalmia is much pigmented and roughened, and that comedones and small follicular abscesses are frequent. A non-

gonorrhoeal urethral discharge was described also, and diarrhoea was a frequent complication. Spence (411, 412) found skin sepsis of common occurrence in his series of cases, and stated that large numbers of epithelial cells are to be found in the urine. Jenner-Wright (186) described a disease due to a combined Vitamin A and Vitamin B deficiency. The features he considered attributable to Vitamin A are glazing of the tongue, and changes at the angles of the mouth. Eye changes which read like early xerophthalmia are likewise seen, and the other muco-cutaneous junctions are similarly affected to the mouth. The skin is dry and rough and a perimeatal change apt to be mistaken for gonorrhoea sometimes occurs. Kuipers (214) obtained some success in the treatment of eczema seborrhoicum with Vitamin A. The skin scaling from the diseased surfaces was tested for Vitamin A content. When the content was high healing occurred, but when low or negative the condition worsened. Improvement was also associated with a lowered alkaline reserve in the blood; and he suggested that Vitamin A acts best in a state of acidosis.

Koessler, Maurer and McLoughlin (209) reported success in the treatment of some anaemias with Vitamin A, but as liver was the source of vitamin employed their conclusions are not acceptable. Sherif and Baum (388) observed pronounced thrombopenia in children on a Vitamin A deficient diet. Keefer Chester and Yang (202) were unable to find any association between anaemia and keratomalacia, and Yang with Pillat (335) has followed up the same line of study without reaching a conclusive blood picture that can be described as that of Vitamin A deficiency. Kirwan (206) observed an anaemia both of red blood corpuscles and of haemoglobin, and sometimes a leucocytosis. In the absence of investigations as to the iron content of the diets used in such cases these observations can have little value. Mackay (267) and Davies (88) may be cited as recent authors whose work has emphasised the importance of an adequate ration of iron in the diet, and this variable must be controlled before any effects due to Vitamin A or other factors can be assessed.

Mrs Mellanby (278,282) has studied the effect of diet on dental caries in children and results comparable with those obtained in puppies have been recorded. Professor Mellanby (285) has confirmed these results, and (291) has stated that the association of well formed teeth with pyorrhoea signifies Vitamin A deficiency whilst Vitamin D was present during development. He added that if Vitamin A is adequately provided during development it is very difficult

subsequently to induce pyorrhoea. The Medical Committee on enlarged Tonsils and Adenoids (277) found a definite relationship between dental disease, rickets and tonsils and adenoids, and suggested that the last condition may be due to Vitamin A deficiency, deficiency of the similarly distributed Vitamin D being responsible for the first two defects.

King-Fretts (205) found that xerophthalmia and advanced ankylostomiasis were commonly associated.

Takasu (436) summarising a long clinical study concluded that the symptoms of alcoholic cachexia are due to deficiency of the fat soluble vitamins.

Bloch (30) observed famine oedema in some cases of xerophthalmia, and Falta (130) found a like association of hemeralopia and famine oedema. Harden and Zilva (153) had previously produced oedema in a monkey on a Vitamin A deficient diet, but the diet was otherwise incomplete as well. Schittenhelm and Schlecht (372,373), who have studied the condition carefully, do not consider that famine oedema has any causal relationship with Vitamin A deficiency; but regard it as due to excessive fluid intake coupled with protein starvation. Starling (415) had long before shown that the colloid osmotic pressure of serum is in proportion to its concentration of protein, and that decreased osmotic pressure in the serum may lead to oedema of the tissues. Kohman's (210) observation, confirmed and extended by Frisch, Mendel and Peters (137) that a diet composed mainly of carrots can produce the condition experimentally puts Vitamin A out of court as a cause of famine oedema. Aykroyd (11) after reviewing the evidence concluded that "there is, in fact, little convincing evidence to connect famine oedema with Vitamin A deficiency".

NATURE, SCOPE, AND LIMITATIONS OF THE STUDIES NOW REPORTED.

Appointment as recruiting medical officer in the Rawalpindi area of the Punjab afforded an opportunity of making a wide survey of a particular section of the population of that district. The occurrence amongst that section of xerophthalmia suggested that it would be interesting to compare the cases showing xerophthalmia (who formed about 6% of the whole) with the cases who were non-xerophthalmic, and to see what differences, if any, could be demonstrated between the two groups.

The xerophthalmia presented the same features as that described by many observers elsewhere, and now standardized in the text-books. Hemeralopia, the first symptom of the condition, was not present in this series of cases, but the strenuous denial of this symptom by the villagers who constituted the raw mat-

erial of study may be much discounted by their eagerness to enlist, which leads them to deny any matter which they think may count against them, and renders the task of eliciting an accurate history --- hard enough at any time in natives of India --- quite an impossible one. Other observations made on the same classes have convinced me that hemeralopia does occur amongst them in association with xerophthalmia. The earliest recognisable change is slight dryness of the bulbar conjunctiva which becomes greasy looking, wrinkled and pigmented "like thin smoked parchment". The pigment tends to be deposited most heavily just outside the lateral edges of the cornea. The changes are at first confined to the portion of the conjunctiva exposed to light, though whether this may have aetiological significance I am not prepared to state. Next the cornea becomes affected. At the corneo-scleral junction a thin whitish ring of irregular keratinization appears, usually broadest at the upper margin where the cornea is lapped by the upper eyelid. In this keratinized area pinpoint ulcers may later develop, again being most frequent at the upper margin. When the process has reached this stage the cornea is less sensitive than normal. In the Punjab it is very rare to see the severer changes of fully developed keratomalacia, and in the whole of the work about to be recorded no cases were seen which were further advanced than the stage of pinpoint ulcers.

From the earliest detectable change to the ulcerated stage every gradation may be observed. In order however to keep the groups for comparison as clear cut as possible, all doubtful cases and all cases in the very earliest stage were excluded from both reckonings. It must be admitted that certain of the cases considered normal may have been actually suffering from Vitamin A deficiency, and that the comparison is not between Vitamin A deficient recruits and normal controls, but between Vitamin A deficient recruits with xerophthalmia and Vitamin A deficient recruits whose conjunctivae were not so sensitive. In animal experiments xerophthalmia only occurs in from 30 - 50% of the total numbers. Nevertheless in this case the fact that there was present in the control group a number of men who passed a rigid medical examination and whose physique was very good makes it likely that if a deficiency existed in these men its effects must have been negligible. In any case the watering of the control group with a number of vitamin deficient subjects would tend to reduce the differences between the two groups, and any positive findings have therefore an added value. The control group in this case is sufficiently large to stand some dilution and still give useful results. In considering the diet of the men studied it will be seen that though some receive a Vitamin A poor diet, others are adequately supplied

with this important factor. The normal controls do therefore most probably represent a true control, and not a different form of the deficiency syndrome.

The Rawalpindi recruiting area (vide the attached map) is bounded on the North-east by the foothills of the Himalayas, on the West by the river Indus, and on the South-east by the river Chenab. It consists of a westward upland area from 1500 - 2000 feet above sea level, bounded on the South-east by the Salt Range which forms its upturned edge; and of an eastward low-lying strip watered by the rivers Jhelum and Chenab, and continuous to the south and to the east with the great plains of India. The great majority of recruits come from the upland area, which is bare and dry, and suffers from a very extreme climate the annual range being from 32°-115° Fahrenheit.

The recruits live in scattered villages throughout the area, a fact which makes accurate comparisons between different districts difficult unless very large numbers are considered; as the possession of a good well or nearness to an irrigation channel in the riverine area makes an enormous difference to the economic status of different villages, and to the mode of life and diet of their occupants. It is impossible in the ordinary way to find out how many recruits come from a rich and how many from a poor village. In general it is from the drier and poorer tracts that the recruits are drawn.

By religion they are mahomedans, and are divided into innumerable tribes, classes, and sub-classes. Of these only a certain number are considered as "fighting classes" and as such are eligible for the army. All the fighting classes are exclusively agricultural and the differences between them are slight and very much less than the resemblances. Records of the most important classes were actually kept, but no statistically significant variation could be found on analysis. For all practical purposes they may be considered as a homogeneous group. Before being sent for medical examination the recruits are seen by non-medical officers of the Indian Army, and none are passed on who are not between the ages of 16-25 inclusive, are below 5' 6" in height, below 32½" mean chest measurement with an expansion of 2", below 115 lbs. in weight, or who show such obvious defects as knock knee or varicose veins as a layman can detect.

It will be seen that only the young and physically well developed members of this small section of the Punjab population come under review and that the results obtained in such a group are by no means applicable to the province as a whole. Moreover, a large number of diseases will of necessity be excluded from the survey by the physical standard laid down. Few cases of pulmonary tuberculosis will pass a chest expansion test for example.



The diet of these recruits is monotonous. Breast fed till about one and a half years old they then begin to eat fragments of unleavened whole meal bread, and after reaching the age of two eat the ordinary adult diet. The staple of this diet is the unleavened bread just referred to, next in importance being pulses. Curry spices are used regularly. In their season sugar cane, fruits such as the mulberry, the water melon and the loquat which are abundant and cheap, and vegetables of which the most commonly eaten are onion, pumpkin and cucumber, form part of the diet. These fruits and vegetables are usually eaten raw. Meat and eggs are very rarely taken and clarified butter only occasionally. As prepared in India this last substance has little, if any, Vitamin A value, (Bacharach (10)): Wills and Taplade (465). With the exception of the small amounts of the vitamin contained in their occasional fruit and vegetable meals the sole source of Vitamin A in their diet is milk. Milk or milk curds form part of the diet of most of the richer villagers. The poorer men only obtain milk occasionally, and of the richer classes some prefer to render their milk fat down to clarified butter, the sale of which ---- particularly when the product is whole-heartedly adulterated ---- is very profitable. Accurate information with regard to the diet of individuals was very hard to get, but of those who admitted to drinking milk regularly none were xerophthalmic, and the impression was gained that the drinking of milk and the appearance of xerophthalmia were in strong negative correlation.

The conspicuous faults of this dietary are lack of fat and the fat-soluble vitamins, provided only in proportion as milk is drunk, and lack of animal protein. Carbohydrates and coarse residues are in excess. Vitamin B is abundantly and Vitamin C adequately supplied. The sun most probably makes up the deficiency of Vitamin D that the diet shows, as the skins of these people are widely and frequently exposed to its rays. The fact that even the poorest families do sometimes obtain milk explains why the severer degrees of keratomalacia are not seen in the Punjab.

As the drinking of milk and the occurrence of xerophthalmia appear to be so closely related, and as it is the richer classes who drink milk most, it may be objected that the differences shown between the xerophthalmic and the non-xerophthalmic groups are due not to Vitamin A deficiency, but to poverty and under nourishment. As the xerophthalmic group have reached a fairly high standard of physical development and appear to be well nourished on ordinary inspection it is probable that the objection has not much weight. It should also be remembered that the men come from agricultural classes; and the farmer, though he may be extremely poor in wealth or in goods, can always

feed himself from the produce of his fields, and very rarely starves except in times of general famine when the crops fail. In order to better answer this objection a grouping of the same men was made on a rough economic basis, and no such difference was demonstrated between the groups so selected as was shown by the other method of distribution.

The investigation fell into two parts. In the first an attempt was made to survey the field in a rough general sort of manner, and to find out as far as possible what particular lines of further study could be profitably followed. Observations on the general physique of the recruits were also carried out. In the second part particular diseases were studied which the results of the earlier investigation, theoretical considerations, or their own importance and common occurrence made it seem desirable to correlate with Vitamin A deficiency.

PRELIMINARY INVESTIGATION ON 3000 RECRUITS.

Of 3,000 men 179 showed xerophthalmia, i.e. 5.97%.
 AGE DISTRIBUTION: The age distribution of these cases is shown in the accompanying table. The ages are in all cases "apparent ages" only. The apparent age is estimated by the recruiting officer or his assistant, men with a considerable experience in this sort of work, and for a large series their estimates are probably as nearly correct as it is possible to have them in a country where there is no proper registration of births.

<u>Age</u>	<u>Number</u>	<u>Numbers xero- phthalmic</u>	<u>Percentage</u>
18	1306	81	6.3
19	657	40	6.1
20	481	28	5.8
21	282	17	6.0
22	69	3	4.3
23	114	6	5.3
24	91	4	4.4

None of these variations at different ages is statistically significant.

INFLUENCE OF LOCALITY: The Rawalpindi recruiting area comprises the civil districts of Rawalpindi, Jhelum, Attock, Shahpur, Gujrat, and Mianwali. The numbers from each district and the percentage of xerophthalmia cases is shown below.

<u>District</u>	<u>Number of recruits seen</u>	<u>Percentage xerophthalmic</u>
Rawalpindi	781	5.9
Jhelum	664	6.2
Attock	420	6.2
Shahpur	632	5.7
Gujjat	351	6.4
Mianwali	152	4.8

None of these variations is statistically significant.

INFLUENCE OF ECONOMIC STATE: In India it is very hard to assess a man's economic position on one inspection, as no one will admit to the possession of wealth, and very often the rich dress as badly and live as humbly as the poor. Only the wealthier men, however, educate their sons. From the larger group 200 men were selected who had passed the examination of the "eighth class" and 200 who were illiterate. Of the former 3% and of the latter 10% had xerophthalmia. Both figures are just inside the range of significant variation. It was not realised at the time these observations were made that the standard deviation would be so great or a larger number would have been studied and possibly a more conclusive result reached. (The effect of a definitely low standard of living is conclusively demonstrated if the enquiry is extended beyond the fighting classes. Of 200 sweepers --- one of the "depressed classes" --- 61% suffered from xerophthalmia, and though these men are in no wise comparable with the recruits who form the main body of this enquiry, the gross effects of a life on the famine level are evident.)

INFLUENCE OF SEASON, TEMPERATURE, AND RAINFALL: No significant variation in the incidence of xerophthalmia could be found from season to season, nor was there any correlation between temperature and rainfall, the only meteorological observations available.

THE PHYSIQUE OF NORMAL AND OF XEROPHTHALMIC RECRUITS: The average height and the average weight of the two groups are shown below. Chest measurements were also recorded, but are not included in the table as the method of measurement was not satisfactory.

	<u>Number</u>	<u>Av. height</u>	<u>Av. weight</u>
Controls	2821	5'7.8"±0.7"	125.9lbs±2.3lb.
Xerophthalmia cases	179	5'7.2"	119.1 lbs.
Educated men	200		124.2 lbs.
Non-educated men	200		126.0 lbs.

There is a significant variation in the weight of the xerophthalmic cases but not in their height. In order to test whether the variation in weight was due to poverty and under-feeding or not the weights of the

same series of educated and of non-educated men that have been previously referred to were averaged. The uneducated were found to be the heavier group, but the variation is not significant. Another cause than poverty and quantitative under-nutrition must therefore be sought for the low weight of the xerophthalmia cases.

GENERAL PHYSICAL FITNESS OF XEROPHTHALMIC AND NON-XEROPHTHALMIC RECRUITS: The figures for rejection for all medical causes are shown below.

	<u>Tot. Numbers</u>	<u>Rejected</u>	<u>Percentage</u>
Controls	2821	1885	66.8±7.7 (groups of 100)
Xerophthalmics	179	164	91.7
Recruits weighing less than 120 lbs (controls)	200	113	56.5
Educated men	200	122	61.0
Non-educated men.	200	149	74.5

It is clear from the above figures that xerophthalmia and unfitness are closely linked, the figure for the xerophthalmic group being more than three times the standard deviation removed from the figure for the controls. Comparison of this result with the figures for educated and uneducated men shows that possible under-nutrition cannot be cited as a cause of the deviation. A series was also selected from the normal group whose weights were below 120 lbs. Low weight by itself is seen not to be a cause of rejection, and the low weight of the xerophthalmic group is not the reason for their high rejection figures.

CAUSES OF REJECTION IN NORMAL AND XEROPHTHALMIC RECRUITS: The full figures for rejection for all causes are given in an appendix. The table to be given below shows only the more important and the more frequent causes of medical rejection. In considering these figures it must be borne in mind that the routine examination of recruits is not an attempt at a full assessment of their physical condition, but is devised to separate the fit from the unfit as rapidly as possible. Once a disqualifying defect is found the recruit is at once rejected and the rest of his examination is not proceeded with. It follows that conditions examined for late in the routine will have figures lower than their true incidence in the series as a whole, being examined for in a population that is growing smaller and smaller and fitter and fitter, till only the fit men are left. Moreover a number of conditions, such as cervical adenitis or conjunctivitis, are only causes of rejection when present in such severity as to be a danger to the sufferer's general health or to his vision, and a true picture of their incidence is not obtained, even when correction is made for the smaller numbers remaining

when that stage of the examination is reached in which they are searched for.

A fairly large number of cases are shown as tachycardia. This unsatisfactory diagnosis was only entered in the absence of clinically evident anaemia, enlarged spleen, or disease of the heart or chest. Simple nervousness was allowed for and only the severer degrees of tachycardia were considered cause for rejection. Fuller investigation in hospital, with laboratory aid would no doubt have cleared up the cause of many of these cases, but no such facilities were available, and a decision had to be formed on a single physical examination. No definite diagnosis has been recorded as such without good clinical evidence, doubtful cases being referred to one of the vaguer classifications of which tachycardia is the prime example. In the table below the diseases follow in the order in which they were examined for.

	Normal		Xerophthalmic	
	Numbers rejected	Percentage of normal rejections	Numbers	Percentage
Tachycardia	331	19.6	20	12.2
Anaemia	129	6.9	14	8.5
Chronic Malaria	77	4.1	12	7.3
Enlarged Spleen	342	18.6	45	27.4
Trachoma	240	12.7	14	8.5

From the above it is evident that a definite diagnosis was oftener arrived at in the xerophthalmic group than in the control group, and that the higher figures for chronic malaria and enlarged spleen suggest these conditions as suitable for further study.

Little help apart from the suggestion of a possible correlation between chronic malaria and xerophthalmia was got from this admittedly crude method of study, and most of the investigations undertaken in the second part of the study were directed to conditions which experiment has shown to have a connection with Vitamin A deficiency in animals, and which were also found frequently enough in this series to make them suitable for statistical study.

INVESTIGATION OF SPECIFIC DISEASES.

The method followed in this part of the work was to collect a large number of cases of the condition selected for study and to note how many cases of xerophthalmia occurred amongst them, comparing the result with a standard figure representing the percentage of xerophthalmia in a large number of men

taken in the order in which they appeared for examination. This standard percentage was obtained by making a statistical analysis of 5,000 recruits. Of this number 6.38% showed xerophthalmia. The standard deviations were calculated for groups of 100, 500, and 1000, and were applied whenever appropriate in the assessment of the results obtained in the case of the diseases studied. For intermediate figures the standard deviation of the grouping lower in number was applied.

For groups of 100 the standard deviation was $\pm 2.44\%$.

For groups of 500 the standard deviation was $\pm 1.79\%$.

For groups of 1000 the standard deviation was $\pm 1.06\%$.

MALARIA AND XEROPHTHALMIA: The figures for malaria are not based on an examination of the blood, and cannot have the validity of observations controlled by the laboratory. They are composed of three separate groups. In the first are cases definitely diagnosed on clinical grounds as chronic malaria. The syndrome of this condition is not readily mistaken, and as a history of fever with rigor and sweating was regarded as essential to the diagnosis I have little doubt that these cases represent malaria and no other condition. The second group comprises cases with enlarged spleens, not considered to be malarial beyond all peradventure. In the Punjab that other great cause of tropical splenomegaly Kala-azar does not exist, and of the many causes of enlarged spleen cited in the text-books none is frequent in occurrence. The majority of these spleens can therefore be considered to be malarial. (Were it not proper to make this assumption the value of the splenic index so widely used in anti-malaria work as an indication of the prevalence of malaria would be nil). The third group is formed by cases of anaemia. In these cases it is not possible to be sure that malaria is the cause of the anaemia with anything like the certainty one feels in the case of the other two groups. That malaria is the cause of the majority of them, is however, most likely. Kala-azar has been already stated to be absent from the Punjab, and ankylostomiasis, the second common cause of anaemia in the tropics is not severe or of general occurrence, though cases undoubtedly do exist. The other causes of anaemia are again conditions of infrequent occurrence, and malaria remains as the only common cause of anaemia that operates widely and generally over the area. Further proof that the two conditions are aetiologically connected is found in the fact that a marked increase in the numbers of anaemia cases occurred in the malaria season, June to September, which would not have been the case had the anaemia been largely due to other causes. I should estimate the percentage of anaemia cases due to malaria at not less than 80%. It should be noted that it is acute malaria rather than chronic malaria that is responsible for the anaemia cases, whereas with the

first two groups the reverse holds. The figures for the three groups are shown below.

	<u>Number</u>	<u>Number Xerophthalmic</u>	<u>%-age</u>
Chronic Malaria	209	29	13.9
Enlarged Spleen	795	82	10.3
Anaemia	<u>293</u>	<u>31</u>	<u>10.6</u>
Total	<u>1297</u>	<u>142</u>	<u>10.9</u>

The percentage recorded for xerophthalmia in chronic malaria and in enlarged/spleen cases is statistically significant; that for anaemia is not. The percentage for the group as a whole is likewise significant, being more than four times the standard deviation removed from the average. There appears therefore to be a definite positive correlation between malaria, particularly chronic malaria, and xerophthalmia. The problem of the connection between malaria and malnutrition is an enormous one. The good results claimed by Italian malariologists from agricultural "bonificazione" may in this connection be recalled to mind. James (184) in a recent report on malaria prevention in Uganda laid great stress on the benefit to be obtained by economic improvement, without anti-mosquito measures. The report occasioned a good deal of controversy between the advocates of the "old school" and the "new school" of malariology. The question certainly calls for further investigation, and it might prove very useful if the incidence of xerophthalmia were noted in malarial survey work, and correlated with the spleen index and the parasite rate.

SKIN DISEASES AND XEROPHTHALMIA: A general impression was obtained that the skin of the xerophthalmic men was rougher, drier, and darker than that of the control group. Such impressions are hard to evaluate, and an attempt ^{to make} a rough colour scale failed as sufficiently accurate matching could not be obtained.

The only diseases of the skin which occurred in sufficient numbers to make statistical analysis possible were acne vulgaris and seborrhoea. The percentages of xerophthalmic cases in these diseases are tabulated below.

	<u>Number</u>	<u>Number xerophthalmic</u>	<u>Percentages</u>
Acne vulgaris	300	48	16%.
Seborrhoea corporis	200	17	8.5%

The figure for acne is highly significant, and the definite association of an infective skin condition with xerophthalmia is interesting, skin lesions no less than xerosis being prominent in the Vitamin A deficiency in animals. The figure for seborrhoea is not significant, nor was it to be expected that the grease-

loving organism of seborrhoea would find a suitable nidus in the harsh dry skin of the xerophthalmic.

TONSILITIS AND CERVICAL ADENITIS AND XEROPHTHALMIA:

Chronic enlargement of the tonsils and secondary glandular involvement is very common in the Punjab. The following table shows the distribution of xerophthalmia amongst these conditions.

	<u>Number</u>	<u>Number</u> <u>Xerophthalmic</u>	<u>Percentage</u>
Enlarged Tonsils alone	150	11	7.3
Cervical Adenitis alone	71	9	12.7
Both combined	<u>779</u>	<u>103</u>	<u>13.2</u>
Total	<u>1000</u>	<u>123</u>	<u>12.3</u>

The total figure is statistically significant and is what would be expected in view of the connection between Vitamin A and mouth and throat sepsis in animal experiments.

The recording of such high figures is proof, if further proof is required, of the high correlation between unfitness and Vitamin A, even allowing for the fact that many individuals are shown in more than one series. Fit men, on the contrary, showed little xerophthalmia. In 2,000 fit recruits the percentage incidence of xerophthalmia was only 1.6%, a highly significant figure.

OTITIS MEDIA AND XEROPHTHALMIA: This disease being closely associated with naso-pharyngeal sepsis was expected to show a high percentage of xerophthalmia. Of 100 cases however, only 4 were xerophthalmic, an unexpectedly low figure but not statistically significant.

RHEUMATIC HEART DISEASE AND XEROPHTHALMIA: This condition being also associated with tonsillitis was expected to give a high xerophthalmia figure but was not found so to do. Of 104 cases of mitral disease 8 only (7.7%) had xerophthalmia, a non-significant figure. In view of the widely held belief that rheumatic heart disease is rare in India the collection of so many cases of mitral disease is interesting. In my experience, rheumatic heart disease is far from rare in the Punjab, not only chronic heart disease, but acute rheumatic fever, having been met with.

PULMONARY TUBERCULOSIS AND XEROPHTHALMIA: This disease was only diagnosed 34 times. In 6 out of 15 cases who were persuaded to produce a specimen of sputum for examination B.tuberculosis was demonstrated. Of the 34 cases 9 had xerophthalmia. The high figure is

suggestive, but the number of cases is too small to be of any value. The chest standard precluded the possibility of a large number of cases being seen, and it would be very interesting to have the xerophthalmia figures from the tuberculosis wing of one of the large civil hospitals.

TRACHOMA AND XEROPHTHALMIA: Trachoma is an almost universal disease in the Punjab. Its incidence may be compared with that of tuberculosis in Europe; almost all are infected, but comparatively few suffer. Being a disease of the conjunctiva it was thought that xerophthalmia would be highly correlated with this condition. A review of 700 cases of trachoma revealed however, only 33 cases of xerophthalmia (4.8%), a non-significant figure. Compared with other aetiological factors it would seem that Vitamin A deficiency is negligible. What these other causes may be is doubtful. Dirt and Dust, in my experience of the disease in this area, would appear to be two of them. The eastern districts appeared to show more trachoma than the western districts, but the figures were not submitted to analysis. It is worthy of note that trachoma is rampant amongst the Sikhs, whose diet is acknowledged to be a very good one. Such Sikh villages as I have seen are peculiarly filthy.

CONJUNCTIVITIS AND XEROPHTHALMIA: Trachoma is so rife that cases of simple conjunctivitis where one can be sure the graver disease is absent are rare. Of 60 such cases collected 5 were xerophthalmic (8.3%). The figures are too small to be of value, as xerosis is extremely variable in its incidence.

PTERYGIUM AND XEROPHTHALMIA: As this disease affects precisely the same area as does xerophthalmia it was considered likely to show some correlation. Unfortunately the idea of investigating this disease came late, and only 50 cases were collected. Of these 7 showed xerophthalmia (14%). It is tempting to suppose that there is a connection between the two conditions, but the series is too small to allow valid conclusions to be drawn.

PYORRHOEA AND DENTAL CARIES AND XEROPHTHALMIA: Of 400 cases of pyorrhoea alveolaris 37 showed xerophthalmia (9.4%), a non-significant figure. Dental caries of greater or lesser degree is a universal finding in recruits. In 100 normal recruits 525 carious teeth occurred. In 100 xerophthalmic recruits the number was 577. It is regretted that a longer series was not studied, as more conclusive results might then have been obtained.

ENDEMIC GOITRE AND XEROPHTHALMIA: Goitre is a rare and periodic condition in the Punjab, and obvious

cases would never be sent on for medical examination. In the hillmen of the sub-Himalayan tract immediately adjacent the condition is prevalent, and as many of these men likewise presented themselves for enlistment from time to time it was decided to study the incidence of xerophthalmia in them also. These men are not comparable with the recruits who formed the raw material of study in the preceding part of this investigation, but their diet is generally similar. In race and manner of life they differ widely, and in these cases the men were seen without any preliminary selection by the recruiting staff. Of 200 cases of goitre 176 were simple goitres whilst 24 showed toxic symptoms. None of these toxic cases showed xerophthalmia and of the others only 7 (4.0%). In 500 non-goitrous men of the same tribes 37 (7.4%) were xerophthalmic. There does not appear to be any connection between the two conditions. Locality, on the contrary, seemed to play a very large part in the aetiology of these goitres. Of the above 37 cases 11 were from one small village.

BLOOD PRESSURE AND PULSE RATE IN NORMAL AND IN XEROPHTHALMIC RECRUITS: The average figures for 500 normal cases are compared below with the average figures for 200 cases of xerophthalmia. The blood pressure readings were taken with a mercury manometer by the stethoscope method, the patient being recumbent and at rest. The pulse rate was counted just before carrying out the blood pressure estimation.

	Systolic B.P. <u>mm. Hg.</u>	Diastolic B.P. <u>mm. Hg.</u>	Pulse P. <u>mm. Hg.</u>	Pulse Rate <u>Beats/min.</u>	B.M.R. (Read's <u>formula</u>)
Normal cases.	131	84	47	76	+ 9.3
Xerophthalmics.	126	93	33	82	+ 5.8

The pulse pressure of the xerophthalmic group is somewhat lower than that of the controls, both the systolic and the diastolic pressures being nearer a mean than in the latter cases. The Basal metabolic rate as calculated by Read's formula shows little difference between the groups. The differences in blood pressure are not very striking, particularly as the range of variation in individual cases was great. The results of a larger series would be interesting.

THE BLOOD PICTURE IN XEROPHTHALMIA: Only 31 cases could be persuaded to come to hospital for a blood examination. These were all cases who had been rejected for trachoma, and who had no detectable condition likely to upset the counts. The average figures for these cases are shown below.

Red Blood Corpuscles	4,100,000	(Average to nearest 100,000)
Haemoglobin	57%	(Sahli's haemoglobinometer)
Colour Index	0.7	
White blood Corpuscles	6,400	(Average to nearest 100)
Comprising Polymorphonuclear leucocytes	3,800	(59.1%)
Lymphocytes	1,600	(25.0%)
Large Mononuclear cells	700	(11.4%)
Eosinophil cells	300	(4.5%)

No estimation of thrombocytes was unfortunately made, as at the time these blood counts were being carried out I was not aware that these cells were of importance in their relationship to Vitamin A. The figures shown are not striking, and as the number of cases is small and as the individual range was considerable little weight can be attached to them. The slight anaemia was the most constant feature. The differential leucocyte count varied more than the other features. The eosinophilia shown is due to two cases --- probably infected with helminths --- who had high eosinophil counts. The impression was gained that the counts were varying under the influence of unknown factors, past malaria and past infections being probably amongst the number.

URINE EXAMINATIONS IN XEROPHTHALMIC CASES: These were carried out on the same 31 men who were subjected to the blood examinations just mentioned. Colour and reaction were normal. The mean Specific Gravity was 1026. In one specimen a trace of albumin was detected (Cold Nitric acid test). No other abnormal constituents were found. On examination of the deposit on centrifugalization degenerated epithelial cells were seen in four cases, including the case with the trace of albumin. No pus cells or casts were seen. Whether the presence of the epithelial cells is of significance is doubtful, and the number of cases is too small to justify conclusions being drawn.

Though statistically significant correlation has been obtained between the incidence of xerophthalmia, a known Vitamin A deficiency condition, and a number of other diseases, to claim that Vitamin A deficiency was the prime cause of these conditions also would be taking too big a step. The results obtained are certainly such as a consideration of animal experiments would lead one to expect and are in conformity with the findings of clinical workers elsewhere. There are nevertheless too many uncontrolled variables in the field to allow final conclusions to be drawn. Clinical research on Vitamin A deficiency

is urgently required, and would be of the greatest value to preventive medicine, particularly in countries such as India where the diet of large sections of the people is unbalanced and deficient to a degree unknown in the West. The study recorded here is rather in the nature of a preliminary report than a detailed enquiry, and has been restricted by the nature and the circumstances of the work. Fuller investigation of the fascinating problems concerned, preferably by a fully equipped scientific team, seems to be one of the most fruitful lines that clinical scientific work can follow, and if the observations recorded here can lead towards such further researches the writer has been well repaid for the time and labour that he has given to the study now concluded.

S U M M A R Y.

1. The nature and physiological action of Vitamin A are discussed.
2. A resumé of previous clinical work on Vitamin A deficiency is given and Xerophthalmia is established as pathognomonic of Vitamin A deficiency.
3. The incidence of xerophthalmia in a selected group of men is worked out, and is correlated with the incidence of certain diseases and with their general physique.
4. Positive correlation is found between xerophthalmia and low weight, chronic malaria, enlarged tonsils and cervical adenitis, and acne vulgaris.
5. Negative correlation is found between xerophthalmia and a high state of physical fitness.
6. No correlation is found between xerophthalmia and height, trachoma, otitis media, rheumatic heart disease, seborrhoea and endemic goitre.

- A P P E N D I X

Medical rejections for all causes in 3,000 recruits.

Disease	Normal		Xerophthalmic	
	Numbers	Percentage of normal rejections	Numbers	Percentage of Xerophthalmic rejections
Tachycardia	331	19.6	20	12.2
Mitral regurgitation	39	2.1	4	2.4
Mitral stenosis	21	1.1	3	1.8
Aortic regurgitation	16	0.8	-	-
Combined valvular lesions	3	0.2	-	-
Dilatation of the heart	52	2.8	3	1.8
Myocarditis	36	1.9	3	1.8
Extra systole	86	4.6	9	5.5
High blood pressure	51	2.7	1	0.6
Heart block	2	0.1	-	-
Aneurysm of the aorta	1	0.1	-	-
Varix of the leg	9	0.5	1	0.6
Varicocoele	27	1.5	-	-
Haemorrhoids	18	1.0	2	1.2
Chronic malaria	77	4.1	12	7.3
Enlarged spleen	342	18.6	45	27.4
Anaemia	129	6.9	14	8.5
Trachoma	240	12.7	14	8.5
Conjunctivitis	29	1.5	2	1.2
Pterygium	16	0.8	-	-
Strabismus	5	0.3	-	-
Cataract	2	0.1	-	-
Malingering (failed eye test)	63	3.0	2	1.2
Tonsillitis	27	1.5	6	3.7
Bronchitis	16	0.8	1	0.6
Pulmonary tuberculosis	18	1.0	3	1.8
Cervical adenitis	31	1.7	9	5.5
Otitis media	25	1.3	2	1.2
Graves' disease	26	1.4	-	-
Colloid goitre	31	1.7	2	1.2
Thyroid adenoma	10	0.6	-	-
Hypopituitarism	1	0.1	-	-
Acquired syphilis	22	1.2	1	0.6

APPENDIX continued:

Congenital				
syphilis	7	0.4	-	-
Gonorrhoea	14	0.7	-	-
Seborrhoea	4	0.2	2	1.2
Acne vulgaris	3	0.2	1	0.6
Leucoderma	1	0.1	-	-
Psoriasis	1	0.1	-	-
Ptosis	3	0.2	-	-
Tabes dorsalis	1	0.1	-	-
Mental defect	1	0.1	-	-
Stuttering	1	0.1	-	-
Facial palsy	1	0.1	-	-
Scoliosis	19	1.0	3	1.8
Knock knee	8	0.4	-	-
Bow leg	1	0.1	-	-
Pyorrhoea				
alveolaris	4	0.2	1	0.6
Dental caries	2	0.1	-	-
Hepatitis	3	0.2	-	-
Chronic				
appendicitis	1	0.1	-	-

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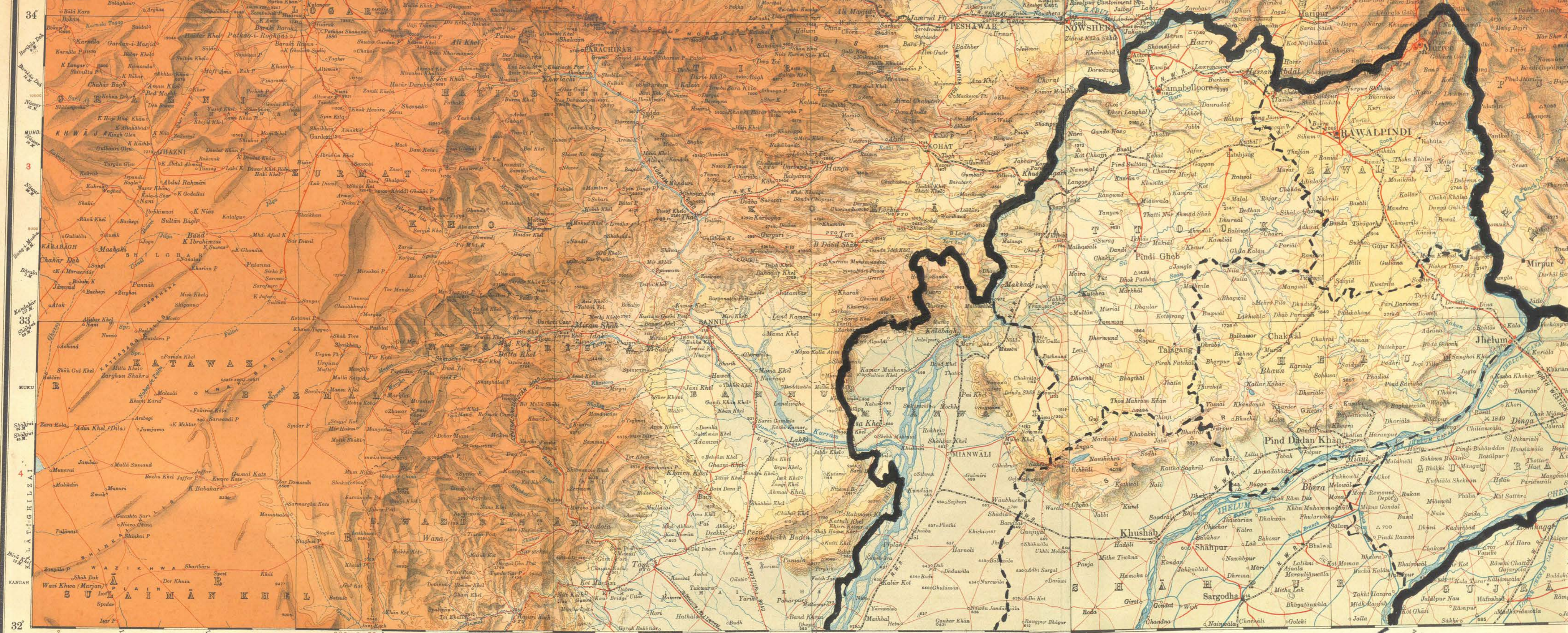
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68° E. of Greenwich. 69 70 71 72 E. of Greenwich. 73

Scale 1:1,000,000 or 1/16 inches to 16 Miles.

Scale 1:1,000,000 or 1/16 inches to 16 Miles.

Res. No. 1533 D 14 (D. O. 6) 2457-3149'18 - 3750'20-1000'24-300'25-500'28

1st edition 1917.
New edition (slight alterations) 2nd. 1926

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Administrative Index

Boundaries: International demarcated, undemarcated
Do. Province or State demarcated, undemarcated
Do. District and Tribal
Telegraph line

Administrative Centres
BOMBAY
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DEHRA
Bijnor
Jalaun

Other Towns & Villages
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Bāitlūr

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39	44	53

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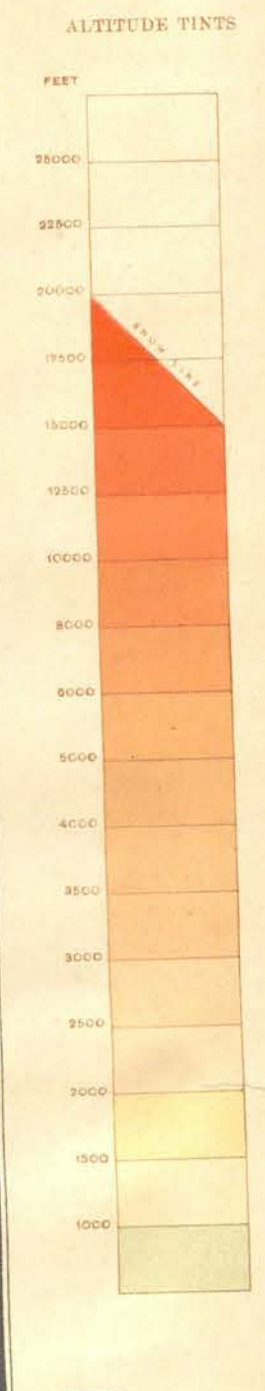
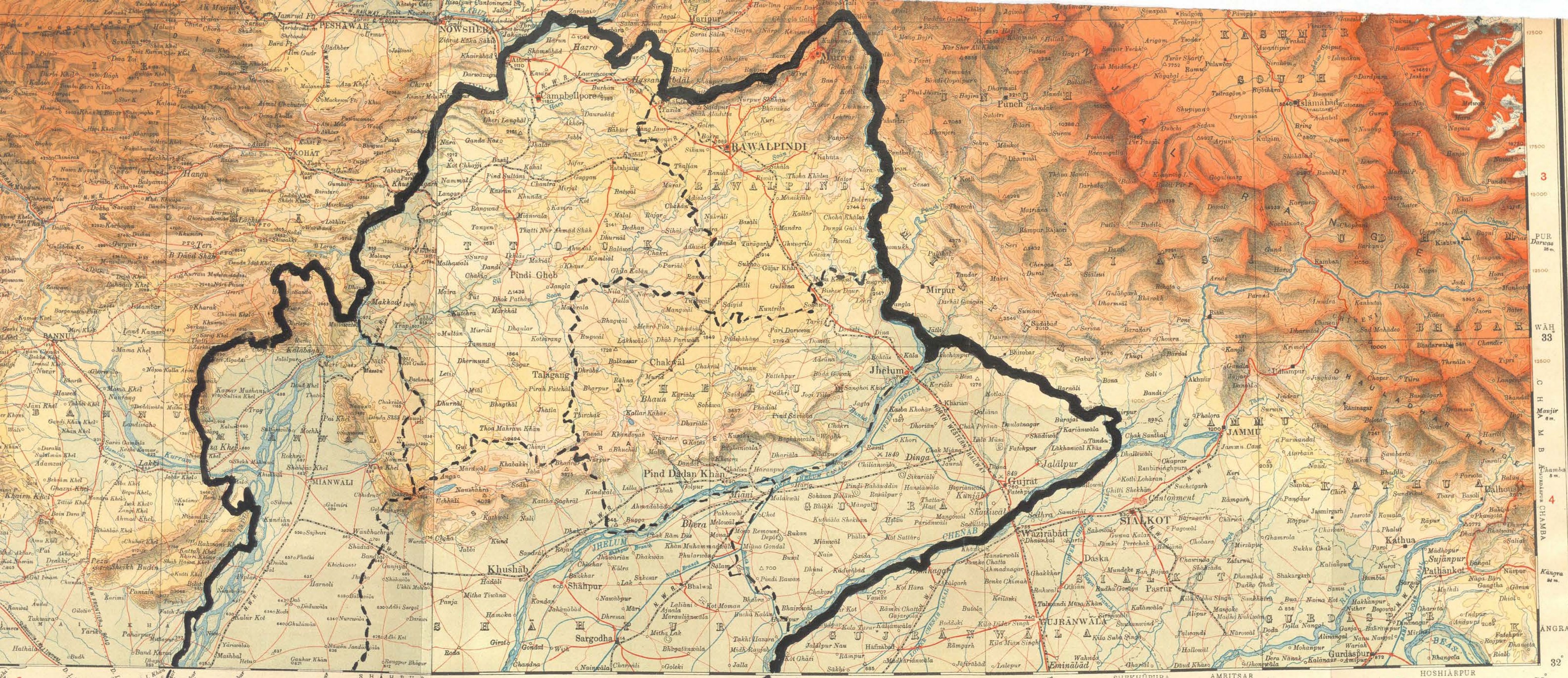
37	42	51
38	43	52
39	44	53

Scale 1:1,000,000 or 1/16 inches to 16 Miles.

Boundary of Recruiting Area

District Boundaries

Copies of this map can be obtained from the Map Record & Issue



District Boundaries
Copies of this map can be obtained from the Map Record & Issue

Administrative Index

Boundaries: International demarcated, undemarcated
Do. Province or State demarcated, undemarcated
Do. District and Tribal
Telegraph line

Head Quarters of District: or

• Do. of Tahsil: or

Administrative Centres
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Scale 1:1,000,000 or 1:1014 Inches to 16 Miles.

Miles 10 5 0 5 10 15 20 25 30 35 40 Miles

Index to Provinces

Boundaries: International demarcated, undemarcated
Do. Province or State demarcated, undemarcated
Do. District and Tribal
Railways: Broad Gauge-Double, Single, Under construction
Do. Metre Gauge, Do. Do.
Do. Other Gauges Open, Under construction
Roads: Metalled, Other Roads and Paths with Pass

Administrative Index

Boundaries: International demarcated, undemarcated
Do. Province or State demarcated, undemarcated
Do. District and Tribal
Telegraph line

