A STUDY OF THE TREATMENT OF TUBERCULOSIS CUTIS WITH CALCIFEROL.

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INTRODUCTION.

Though the recent literature contains many favourable reports on the treatment of Tuberculosis cutis with preparations of calciferol, most of these studies appear to be based on merely clinical observations (1-10) and few of them deal with the histological aspects of the disease and alterations in these arising as a result of treatment.

Vachon and Feroldi (6), Kuske (11), and Freudenthal (12) have made observations on biopsy material from patients treated with calciferol and found that the tuberculous lesions are not rapidly or profoundly affected, at least in the earlier stages of therapy.

In this study an effort has been made to follow the changes due to treatment in the pathological anatomy of the disease as assessed by the histological and clinical findings. Successive biopsies of comparable areas of the lesions under treatment provide a more accurate estimate of the mechanism of action of the drug and, in fact, it is only in the skin that such direct observation of the disease process in a single organ is so readily made.

The histology at any given time, however, is obviously of somewhat restricted value in observing the details of a continous biological process, but it does provide a definite means of evaluating any

major changes occurring in the area studied. The observed results can be interpreted only within the limits of the present available knowledge of the pathogensis of tuberculous lesions and of the various factors influencing their occurrence and fate.

Considerable advantage results from the study of the disease in man as contrasted with experimental animals since it can be assumed that the evidence, although subject to personal evaluation, is in the nature of a direct observation not conditioned by any species difference.

The picture becomes more complicated when the factors of local organ peculiarities, immunity, allergy and the nature of the bacillus come to be considered. The cellular reactions in tuberculosis arise by chemical stimulation of the tissues, the nature and severity of which determine the type of response. Tuberculosis cutis represents some phases of disease due to the tubercle bacillus and its products which produce chronic lesions capable of slow progress or regression with healing. The disease usually occurs in individuals possessing good general health and a significant degree of resistance to the tuberculous process. Toxaemia is not a feature, neither is the presence of active disease in other organs such as the lung, although there may be evidence of previous or quiescent lesions.

The clinical form of the disease varies with the route/

route of infection be it haematogenous, innoculation or from a subjacent focus and with the state and extent of the immune and allergic responses of the individual. (14)

The nature of the bacillus warrants special consideration on account of the low grade activity which Griffith (15) (16) presumed to be due to degradation in the cutaneous tissues while Frimodt-Moller (17) thought that there were attenuated strains of the bacillus in lupus.

The commonest clinical form is lupus vulgaris and in this organisms are not readily demonstrable by staining methods or by culture and animal innoculation of material from the lesions.

Reaction on the part of the host appears to destroy the organisms making demonstration difficult especially in the later stages despite persistant histological features of the disease. This is in keeping with the conclusions of Smithburn and Sabin (19) that the epithelioid cell follicles can arise as a chemical entity due to bacterial products in the presence of few, if any, viable organisms.

Lupus vulgaris consists essentially of tuberculous granulomata in the dermis involved in some degree of fibrous tissue reaction and causing secondary changes in the epidermis. It is usually localised and tends to spread at the periphery and to show central areas of healing.

Caseating areas favourable for bacterial proliferation/

proliferation are uncommon in the skin, this probably being a local feature arising in the elaborate complex of infection due to the tubercle bacillus, and rendering difficult any comparison with lesions in other organs.

Again healing in dermal tissues is slow and the metabolism of protein less than in other organs, Schoenheimer (20) finding little activity in the fixation of dietary nitrogen.

In considering the effects of treatment it is important to remember that the fate of a tubercle may be quiescence, resolution, or activity, the changes of which may be slow and not readily apparent to clinical observation.

This study covered a series of cases classified into two groups; cases of lupus vulgaris and cases of conditions commonly regarded as being of tuberculous origin.

The drug has been administered orally and by local application over long periods with few toxic manifestations. The study of the cases extends over two years and assessment has been made of each individual to the time of writing with the reservation that further treatment of some, and periodic review all the cases, will serve to determine long term results of the method of therapy.

Some small use has been made of the recent device of imitation of molecules for experiments in the biological field by the study of granulomata produced/

produced by synthetic pthioic acid in animal skin and of the effect of calciferol on these. This chemical, representing the most active granuloma producing element of the chemical fractions of the tubercle bacillus, is free of nitrogen and unlikely to produce reactions complicated by allergy.

Experiments with this and similar compounds should provide a potential source of information on the mechanism of tubercle formation and perhaps a screening method for therapeutic substances.

2. Historical Survey.

Calciferol being a synthetic compound there is no extensive history of its use as a therapeutic agent. Vitamin D in the form of Codliver oil has a traditional value in the treatment of many forms of tuberculosis and is said to have been used by the fisherfolk and first advocated by Thomas Percival in 1774. The literature contains many references to the beneficial results obtained (21) (22) (23) (24) (25) (26).

The use of codliver oil in treating lupus vulgaris is attributed by Bicknell to Devergie of Paris in 1848 and to Emery in the same year when satisfactory results were obtained from intensive and prolonged dosage. Further recommendation came from Stelwagon in 1907 since when codliver has been consistently referred to in standard works on the disease (27) (28).

A high intake of fat soluble vitamins including D is a feature of the Gerson and similar diets and of the nutritional regimes of many institutions engaged in the treatment of patients suffering from tuberculosis (29) (30) (31).

In 1928 Shope (32) based a series of chemotherapeutic experiments on the observation that cholesterol tended to accumulate in tuberculous foci and that it might act as an adjuvant to the healing process. Irradiated cholesterol was regarded as definitely/

definitely prolonging the lives of infected animals in one of these experiments. This was followed by Spies (33) finding that irradiated ergosterol produced marked calcification of the caseous and necrotic centres of tubercles in acute tuberculosis in rabbits while there was little tendency for calcium to precipitate in the absence of necrosis. Levaditi and Li Yuan Po (34) in the same year reported that irradiated ergosterol produced calcification in experimental tuberculosis and appeared to exert a favourable influence on the course of the lesions. These authors included in their paper an interesting discussion of the then available information on the effects due to vitamin D which could influence the various forms of tuberculosis and of the possible basis of action for these changes.

These works raised the possibility of promoting healing and calcification of human lesions by means of one or other form of vitamin D, irradiated ergosterol being the most readily available concentrated product.

Kramer et al (35) treated a series of children, one with skin tuberculosis and others with bone infections, over a period of a year using irradiated ergosterol and recorded that there was no detectable acceleration of the healing process.

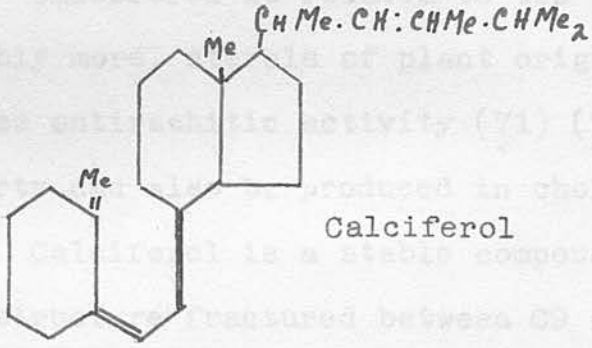
Credit for the systematic use of preparations of calciferol in skin tuberculosis must be attributed to the work of Charpy (36-41), Dowling and/

technique (62) (63) (64).

These irradiations are considered lethal to tubercle bacilli directly only to depths of 1.5. m.m. and to diminish the virulence up to 4 m.m. (65) so that the tubercles are subject to direct attack but difficult of access when situated in the deeper dermis. Vitamin D arising from the sterols in the irradiated skin is to be considered a factor in the promoting healing. Calciferol in large doses reaches a higher tissue concentration than can be achieved by irradiation but it appears sound to combine the treatments even if there is some likelihood of toxic amounts being present.

3. CALCIFEROL.

Calciferol is a sterol compound that has not been found in nature and is usually manufactured from a precursor, ergosterol, the common source of which is yeast. These two, cholesterol and various animal and plant sterols, the sex hormones, strophanthin, bufoxtoxins and certain carcinogenic hydxrocarbons have a related chemical structure (66) (67) (68)



Calciferol

This basic chemical structure is stable and difficult to rupture except by strong methods of treatment (68).

Ergosterol has a strong absorption band at 305-230 millimicrons leading to photochemical decomposition to vatious sterol compounds as follows :-

Ergosterol
↓
Lumisterol.
↓
Tachysterol.
↓
Calciferol
↓
Toxisterol
↓ ↘
Suprasterol 1. Suprasterol 2.

Wavelengths longer than 305 millimcrons can have similar effects/

There are variations due to the quantity of irradiation and to the presence of solvents and of oxygen (69).

Calciferol can also be produced from ergosterol by electrical methods. It is a crystalline stable substance which can be estimated by biological methods and prepared in a standard solution (70).

The unit is the equivalent in antirachitic activity to 0.001 gm of the standard solution, chemical methods of estimation not yet being relied upon (70).

Calciferol is related to ten other, or possibly more, sterols of plant origin known to possess antirachitic activity (71) (72) which property can also be produced in cholesterol (73) (74).

Calciferol is a stable compound having the ring structure fractured between C9 and C10 atoms. (72) (75). It is claimed that calciferol available commercially is freed of toxic bodies and is much safer than irradiated ergosterol which may contain varying proportions of unwanted products (76) (77) (78).

PHYSIOLOGY.

Much of this is still obscure and the most recent progress has come from radioactive tracer studies in relation to the sterols particularly cholesterol. There are normally wide variations in the blood levels of sterols and their determination is not at present practicable in the control of calciferol therapy. Calciferol influences the absorption of phosphorus and calcium from the intestine with/

with effects both local and general (79). It has an action on the vascular system, the thyroid, parathyroids, pituitary and nervous tissue and muscles Absorption of calciferol itself from the gut is under the influence of bile and in fistula animals Greaves (80) has shown that there is little or no uptake at all. Diarrhoeal diseases and hypofunction of the absorptive surfaces greatly reduce the intake. Skin absorption has been demonstrated to be rapid from cream bases and toxic levels can be reached by this route (81) (82) (83).

The actions of the antirachitic sterols are similar and with purified preparations of irradiated ergosterol or with pure calciferol the toxicity is low, large doses being well tolerated. (78) (77).

As an antirachitic compound and with minimum Ca and P requirements that of vitamin D2 is estimated at.

300-400 i.u./day babies.
400-600 i.u./day adults
600-800 i.u./day in pregnancy (84-

Calciferol has been shown to increase the uptake of inorganic phosphorus in bone from recently ingested material and to increase the mass of reticuloendothelial cells in experimental animals (85) (86).

Other workers have further shown that tissue cells can survive toxic levels for a short time while at lower levels the tissue O2 utilisation and the B.M.R. are increased (87) (83) (88).

The/

The action is related to that of parathyroid hormone there being some interrelationship of action since refractory cases of parathyroid tetany have been treated successfully with preparations of Calciferol (89)

The trials and usage of the drug in arthritis has been extensively studied but the results have tended to be disappointing.

PHARMACOLOGY and THERAPEUTICS.

The main use of calciferol has been as an antirachitic agent and to promote bone calcification. Large doses have been given for arthritis, various skin diseases such as acne and psoriasis, and more recently for tuberculosis, both of the skin and in other organs.

On the basis of animal experiment Spies (90) recommended calciferol as a useful agent to promote the calcification of tuberculous lesions and there have been other similar attempts to use this substance with special regard to calcium metabolism (33) (91) (92). The work of Charpy, Dowling and Prosser Thomas and others (4) (7) (93). has attracted recent attention to the usage of large doses over long periods in treating cutaneous tuberculosis.

In resistant rickets very large doses have been given or even massive single doses in the prophylaxis (94).

Toxic effects produced by excessive dosage lead to death from inanition and cardiac or renal/

renal failure conditioned by the period of administration and the diet. Alimentary and endocrine disease promote the likelihood of these effects as also do the use of impure products. The human toxic level is assessed by Steck and others (87) at.

$$\frac{\text{20-25,000 international units}}{\text{kg. body weight}} \text{ per day.}$$

and this for the ordinary preparations given by mouth.

The margin of safety is therefore large in treating rickets and much smaller in cutaneous tuberculosis where the dosage is heavy and prolonged.

The main toxic effects are cell damage and a tendency to the deposition of calcium and phosphorus prinicipally in the blood vessel walls, this process being to some extent reversible. The principal effects fall on the renal arterioles, the aorta and the small vessels of the hypophysis, thyroid, adrenal, pancreas and the gut. (95) (96) (87).

The blood calcium level may be raised, but this and that of phosphorus, do not run parallel to the harmful effects and therefore can only be used as indicators rather than absolute signs of overdosage. The blood ionsible calcium fraction is raised with large doses.

The harmful changes may be decreased by vitamin A in large doses of 250 to 1000 times the ordinarily prophylactic dosage (97).

The minimum lethal dose experimentally for oral preparations in dogs has been estimated by Goormatigh and others (98) as./

as.

13-20 X 10. μ. gms per kilo body weight or over a longer period as.

100/μ gms/kilo/ per day for 100 days.

Prolonged dosage tends to deplete the Ca and P reserves (96) (99) (100) and the toxic effects are increased by exposure to ultraviolet light, especially in children, presumably by increasing the available amount of vitamin through photochemical action in the skin (101).

The toxic tissue changes commence in the blood vessels going on to congestive and inflammatory reactions which are irreversible and the renal damage leads to nitrogen retention (102).

Extra calcium during the period of vitamin D administration tends to raise the Ca and P levels in the human and one dose of irradiated ergosterol equivalent to 10,000 i.u. vitamin D can do this. It has been recommended to control these levels at Ca 12 mgm per 100 ml. and P 4 mgm per 100 ml during the therapeutic period (90).

The changes in the blood vessels are a nonspecific arteriosclerosis commencing at toxic levels in experimental animals, with later degeneration and calcification of the elastic coats, especially in the kidney, heart, liver, lung and stomach (103) (104).

Rats fed experimentally 20 mlgms of irradiated ergosterol in alcoholic solution daily for 14 days showed vascular calcification and arterial lesions similar/

similar to Monckeberg's sclerosis, especially in the renal vessels. Degenerative changes also occured in the spleen and thymus. Death resulted from cardiac failure and the toxicity appeared to be increased by a high Ca and low vitamin diet (105) (106) (107).

Calciferol is a potentially dangerous drug and should always be administered with caution.

The toxic manifestations can be mild and a careful supervision must be made especially in children.

Recently it has been suggested that cysteine can act as an antidote to toxic doses and also can inhibit any anti-tuberculous effects (108) (41).

DOSAGE.

The doses given in rickets may be stated as follows.

Prophylactic (infant) $\frac{1}{2400}$ gr. to $\frac{1}{1200}$ gr

Therapeutic (") $\frac{1}{1200}$ gr to $\frac{1}{800}$ gr of a preparation of potency of 40,000 i.u. per mlgm (109).

Official Preparations available are :-

Liq. Calciferolis B.P. add I

Prophylactic (Infant) m. 5-10.

Therapeutic (") m. 10-15.

Liq. Vit D. Conc. B.P. Add II

0.5 to 3 minims = 250 -- 1500 i.u. D.2.

The standards for these preparations are contained.

contained in the B.P. 1932 and in Anderson's work (110).

There are various U.S. standards of potency of preparations (111) (109).

Commercial preparations as tablets or capsules are available containing 50,000 i.u. pure calciferol in each dose and these are the most convenient form of administration in treating tuberculosis cutis. Calciferol in oily solution is suitable for oral administration and with Vit. A can be given in injection by the intramuscular route (112).

Local applications can be made from oily solutions of calciferol suspended in a cold cream base at the arbitary levels of 1000 i.u. and 5000 i.u. per gm, the latter being used in this study.

In tuberculosis cutis the doses used range from 50,000 i.u. to 200,000 i.u. per day orally according to the age of the patient and the extent of the lesions. This can be supplemented by local application two or three times daily where the epidermis is intact and in some cases the patient continues to have ultraviolet irradiation systemically thereby increasing the overall total dosage.

In the absence of accurate criteria of dosage those doses used by Dowling can be taken as a guide i.e. 150,000 i.u. per day for an adult. These fall considerably short of the doses advocated on the continent, larger doses being used when results are satisfactory but slow, in an attempt to accelerate the/

the clinical progress. There appears to be little special advantage in giving calcium salts beyond preventing any possible demineralisation of the skeletal system (114) or in using the drug in alcholic solution.

The drug has been given by the intravenous and intraperitoneal routes, experimentally, when it is said to be more effective, but the method has many dangers. (115).

Warkany et al (211) studying cases receiving large daily doses of vitamin D as calciferol found on examination of the blood serum that the blood level of the drug rose rapidly to a maximum having a subsequent tendency to decrease. After abrupt withdrawal of the drug three to six months elapsed before the concentration of vitamin D in the blood serum fell to a normal level. The level during treatment was found to be roughly proportional to the daily dosage. No gross changes were observed in the phosphorus fraction. These results support a policy of administering calciferol over periods of several months followed by rest periods of at least 4 weeks before further treatment if it is indicated.

4. CALCIFEROL AS A CHEMOTHERAPEUTIC AGENT.

Chemotherapeutic substances may act against the tubercle bacillus, the exudate, or the toxins and are difficult to evaluate in vivo whatever the action in vitro, especially if the action on the natural course of the disease is not marked (116).

Generally anti-tuberculous drugs must be tested in man e.g. the use of hydnocarpates in lupus (117) and course of the reactions is often difficult to assess (118).

The principles of evaluation are to follow the aims and objects of any study by a careful check on the organisms, the exudate and the fibrous tissue reaction (119).

The strains and stages of the organisms, the effect on susceptible cells, and the physiological and pathological effects on the host are all factors that must be carefully considered in studying any anti-microbial agent (120).

Feldman and Hinshaw (121) have suggested criteria for the evaluation of therapeutic agents which may be paraphrased as follows:-

1. The agent is well tolerated.
2. It should reverse innoculation tuberculosis.
3. It should render avirulent bacilli in organs of predilection.
4. It should be controlled against a substance of known effectiveness such as Promin.
5. It should be possible to make a crucial test in selected representative material while avoiding fulminating cases.

These criteria are exhaustive and extremely difficult to attempt to work to.

In assessing results of therapy, consideration must also be given to the amount of tissue destruction the amount of resorption, fibrosis, and to calcification if this last occurs.

A chemotherapeutic should be able to reverse progressive destructive lesions and to penetrate tubercles although it may not kill the organisms when crippling of the bacterial metabolism may be sufficient (122).

In the past a great many drugs have been tried often with disappointing results as in the use of gold, despite early apparently favourable indications.

Some drugs tend to localise in tubercles (123) and some can increase the vascularity but no single one has been uniformly successful until the advent of newer antibiotics such as streptomycin.

Krause (124) considered that enquiry should be made into measures to increase fibrosis, to neutralise the toxins of the allergic reaction and to neutralise the toxins of the tuberculous foci, possibly non-specifically but that a bactericide would be best if this could be achieved.

The lipoids of the tubercle bacillus constitute its main defence by being relatively insoluble and by forming soaps which inhibit enzymes (125) both of which factors are concerned in the chronic exudative and proliferative cell responses. This lipoid may be diffuse/

diffuse in the cell and not confined to the surface layers it being shown that water soluble substances such as bile and detergents can penetrate and injure the bacillus (126) (127) (128). Some synthetic detergents are bactericidal with a special action on Gram positive organisms possibly by disorganising the resisting cell lipoids yet this action can be protected against by phospholipids (129).

Linolenic, linoleic and oleic acids normally occurring in vivo have these properties which can be modified to some degree by the sterols, and linoleic acid was found by Boissevain (130) to render the organisms non acid fast (59) (60).

Inhibition of tubercle bacilli has been recorded from unsaturated fatty acids with chains of 15-18 carbon atoms, fatty acid soaps and free fatty acids, and fatty substances derived from fungi. (131) (61) (132) (133) (134) (135) (136) (22) (137) Crim and Martos found codliver oil to be bacteriostatic at 1% concentration (138) but this was not borne out in the experiments of Stanley (132) and others.

Dowling et al (2) and Dickinson (139) record that calciferol has not been shown to have a direct action on tubercle bacilli in vitro nor to have any marked effect on the acute disease in experimental animals. Raab (140) has claimed tuberculostatic properties for vitamin D2 but this observation is as yet unconfirmed. Vitamin D 2 enhances the activity of living leucocytes (141) and accelerates the overall/

overall phosphorus metabolism in the chick embryo especially in the phosphatide fraction (142) and it is logical that this action may extend to the cellular metabolic disposal of the lipoids of the tubercle bacillus. The tuberculous process may thus be effectively acted upon by stimulation of the reticuloentothelial cells and enhancement of the neutralisation of the bacterial products (143) constituting a dynamic increase in the body defence processes.

Calciferol because of its lipoid solubility should be capable of penetrating tubercles and be able to act at the essential site of the reaction to the organisms (120).

No anti-tuberculous specificity can be claimed for this drug and more knowledge of the mode of action in relation to the individual cells can perhaps come from histochemical methods (144) (145) or from experiments on tissue cultured with isotopes (142).

Since the indication of any method of action points to an enhancement of the cellular defence mechanisms little is to be expected of the drug in the more acute forms of the disease when the resisting tissues tend to overwhelmed.

The application of the drug is therefore likely to be limited to the less active forms of the disease or as an adjuvant to other drugs.

5 Histology.

Tuberculosis in the skin has many variations and forms of which there are direct knowledge, the commonest form being Lupus Vulgaris, presenting granulomata in the dermis which tend to be grouped round the capillaries, epidermal appendages, and the lymphatic plexus. Inflammatory and exudative changes occur regularly but caseation or necrosis is uncommon. Tubercle formation consisting of the aggregation of epithelioid cells, giant cells, and lymphocytes takes place in the middle and lower dermis whence extension toward the surface leads to secondary changes in the epidermis. Some degree of fibrous tissue proliferation is usual, especially at the periphery of the tubercles and reticulum fibres are laid down in some lesions.

The morphology of the lesions both clinically and histologically can be correlated with the tuberculin sensitivity of the individual, his immune and allergic state, and the prescence of any secondary infection (146) (147) (148) (14) (149) (150). Tissue allergy and immunity are attributes of mammals with tuberculosis which result in a delayed, abortive, or more chronic form of the disease, even if it is not at present possible to make accurate measurement of these powers.

The clinical form of the disease is the outcome of the reaction in vivo between the tubercle bacillus and the reactive processes of the host (151) (14)/

(14) (152). The histological picture may, however not be identical in sections of diseased areas from the same patient since there is considerable variation in the degree of local activity. Again the strain and type of bacillus may determine the morphology of the lesions by its virulence or power to provoke destructive tissue reactions (154).

The various forms of skin diseases associated with the tubercle bacillus may be seperated by the classification of Lewandowsky (153) in which are included a number of conditions whose etiology is not fully established as being of this origin.

Lupus vulgaris presents as variable sized diseased areas, showing nodules in the dermis composed of small tubercles, which progress peripherally with scarring and a tendency to heal at the centre. Tubercles in the middle layer of the dermis may be discrete or confluent and the collagen and elastic tissue usually show some destruction. The lesions themselves are avascular but adjacent blood vessels are concerned in the disease process.

The overlying epidermis tends to be thickened, to show distortion of the rete pegs, and to be hyperkeratotic although the exudate is not usually immediately in direct contact with it, there being a clear zone in the papillary layer. Gross hyperkeratosis, follicular plugging or even necrosis of the epidermis may occur with ulcer formation.

In old cases and those treated with local caustics, healing or quiescence may show much fibrous/

fibrous tissue formation or old scar tissue. Caseation may be a feature in scrofuloderma when bacilli are numerous and the process is more widespread involving tissues deeper than the dermis.

Inflammatory polymorph exudates may be seen in forms where the epidermis is breached allowing secondary infection to occur. In the tuberculide group the lesions are localised and take the form of small necrotic areas in the corium extending upwards to involve the epidermis and showing a brisk inflammatory reaction in the later stages. There tends to be much pustular debris and tubercle formation is poor perhaps only showing in serial sections. Endophlebitis is a feature of the tuberculide while in erythema induratum perivascular epitheliod cell formation is marked.

The biological reactions to the tubercle bacillus and its products follow a constant pattern capable of experimental reproduction by both live and dead organisms (155) (121) (124) Maximow (156) showed that mammalian cells could grow in tissue culture along with tubercle bacilli, producing typical structural tubercles after a time by the formation of epithelioid and phagocytic cells capable of some, or partial disintegration of the bacilli. Kahn (157) demonstrated that histiocytes possessed some degree of bacteriostatic action toward engulfed tubercle bacilli.

In vivo the organisms are ingested by mononuclear/

mononuclear phagocytes possibly of vascular origin, and are found in the epithelioid cells when these are developed, tubercle formation consisting in part of the aggregation of these cells without obvious vascular supply (158) (124) (159) (160). Stewart et al (162) believed the mononuclear leucocyte to be capable of disposing of the products, if not being the factor lethal to ,the organisms. Failure of the bacilli to grow in the cells of the immune animal, their destruction, or inhibition, is thus a cellular property of the mononuclear phagocyte.

Tubercle formation as such appears to be related to the chemical components of the bacillus and particularly to the lipoids, occurs in both normal and allergic animals, and seems to be more than a reaction of repair yet capable of resolution in time (163). Tubercles due to dead bacilli are morphologically identical with those caused by live organisms, the fundamental type of reaction remaining the same and supporting views that the basis of virulence is chemical (208). These lesions differ only in their failure to progress by multiplication of the bacilli (155) (164) (165). Although the monocytes vary in appearance and come from the blood or local tissues they remain a fundamental part of the tubercle, together with histiocytes and fibroblasts. In the tuberculous animal the activity of these cells is increased according to the degree of reaction to the disease. The formation of tubercles is thus the essential /

basis of the disease process being the outcome of many reacting factors important among which are the chemical components of the bacillus acting singly or as complexes.

Recent work by Gerstl (166) (167) (168) (169) and others point to the capacity of the cytoplasmic enzymes occuring in the monocyte to degrade, or dispose of, the lipoid elements derived from tubercle bacilli and which have been described as maturation factors for the cells of tuberculous tissue. Cellular ingestion or disposal of the specific lipoid chemical entities such as pthioic acid identified in bacilli grown on certain media may explain the difficulty noted by Anderson (171) in demonstrating these substances in infected tissues. Chemical separation of tubercle bacilli in to their component elements and the intensive study of the biological reactions to these substances has resulted in major advances in the knowledge of the disease process. (172-180 incl)

Protein, carbohydrate, and lipoid fractions have been further refined to obtain in some cases an individual chemical compound apparently characteristically elaborated by the living bacillus and related to its disease producing properties. Much work has been done on the details of reactions produced by these fractions and it has been shown that the phosphatides of the bacillus are responsible for the formation of tubercules with epithelioid cells, giant cells/

cells, and caseation (172) (181) (182) (183) (170) (13). Alcohols and fatty acids peculiar to the organism are particularly concerned in this process. Combinations of phosphatide with protein have an enhanced activity in provoking experimental reactions (188) (181) and constitute antigenic complexes of an ill understood nature which may constitute virulence factors for the organism (208). The protein fraction experimentally produces necrosis of collagen and cells leading to a polymorphonuclear cell exudate, this reaction occurring within a few hours of injection (181) (187).

Tuberculins are complexes to which the reaction in man is usually inflammatory and does not histologically resemble the disease (190) but it is known that all tuberculins are markedly cytoxic toward the cells of tuberculous animals, a property which is inherent in these cells.

Carbohydrate fractions although toxic do not appear to cause histological changes that can be classified as specifically tuberculous. The wax and mycolic acid fractions, however, can be found to provoke inflammatory changes which, if not specific are considered to contribute to the persistence of tuberculous lesions and to the toxicity of the organism to mammalian tissues (165) (167) (199). All these fractions are difficult to obtain chemically pure, even from organisms grown on synthetic media, and the lipoids cannot be entirely freed of a residue of nitrogenous material which may in itself produce biological effects./

biological effects.

In this connection it must be noted that Boissevain (192) considered hypersensitiveness to dead bacilli to be part of the tissue response and that the protein elements of the bacillus manifested the greatest biological activity, granulomata arising in the prescence of very small amounts of bacillary material.

Dienes and Mallory (193) believed that granuloma formation developed with the allergic state after a lag period of several days, lesions arising from a small dose of bacilli being unlikely to be caused by the little bacillary lipoid present.

However the lipoids appear to be the main elements concerned in producing the capacity of the tubercle bacillus for slow hardy growth in vivo, and for a selective inhibitory action on the endocellular enzymes of growth and repair, this property being more marked in the tuberculous mammal (194) (195). The lipoids and perhaps the carbohydrate fraction tend to cause prolonged tissue reactions capable of slow repair.

The lipoids of the tubercle bacillus provoke the appearance of epithelioid cells and the formation of tubercles, this action being characteristic but not specific, since these cells may be produced by other irritants such as silica, lecithin or the lipoids of different acid fast bacteria (19) (207).

The epithelioid cel is a large pale staining cell/

cell with an oval nucleus and homogeneous cytoplasm being considered to represent the later or final stage of the reaction of a mononuclear phagocyte to certain stimuli this being bacillary lipoid in tuberculous. The cell is capable of survival for up to six months in vivo and of some degree of breakdown of ingested material by lipases, lecithinases and phosphatases. (154) (165) (168) (181) (196).

Disintegration of the epithelioid cells may liberate the irritant more or less intact leading to the formation of more such cells and perpetuation of the disease process (165) (198). This is the mechanism described by Smithburn and Sabin (186) of a vicious cycle due to the chemical make up of irritant foreign material, namely a series of higher fatty acids elaborated by the organisms and of which pthioic is the most active (170). This correlation of cellular changes and chemical structures resistant to breakdown revives the old idea that a major bar to healing in tuberculosis in inability to dispose of the bacillary lipoids and allows the inference that a chemotherapeutic agent can be judged in its capacity to alter the morphology of the lesions in the direction of resolution.

Study of this problem has led to the synthesis of pthioic acid and similar compounds and their use in biological experiments designed to provide information on the action in vivo of the compounds peculiar to the tubercle bacillus (200) (201). Synthetic

pthioic/

pthioic acid, dehydropthioic acid, and certain other fatty acid derivatives have been found capable of provoking the histological and blood changes of tuberculosis in experimental animals (55) (202) (203), and knowledge derived from these sources is applicable to the study of the pathological histology of the disease.

Mononuclear and lymphocytic cells are associated with regressive lesions and resistance of the host, since their cytoplasm has been considered a source of antibodies (204) (205) (154), and this property must be considered along with the phagocytic powers.

It would appear, however that the histology and certain chemical components of the tubercle bacillus have a sufficiently proved relationship to enable therapeutically important information to be gained from a detailed study of microscopic changes in the lesions brought about by treatment.

Changes observed in the Histology after treatment with Calciferol.

Initially there appears to be little departure from the picture seen prior to treatment but successive slides show progress toward healing and, in those cases responding clinically, it was noteworthy that this healing microscopically lagged behind the macroscopic findings. Two cases apparently clinically clear showed microscopic tubercles indicating the need for adequately prolonged treatment (Fig. 8).

The total change to cure or resolution appeared as a slow disappearance of the elements of the tuberculous exudate, commencing with swelling and in some cases vacuolation, of the epithelioid cells.

These cells tended to form less well defined tubercles and in later slides to have disappeared or to be present only in scattered collections surrounded by lymphocytic aggregations (Fig. 9).

Lymphocytes and mononuclear cells persisted in the areas occupied by the exudate and the final stage before clearing was that of a diffuse perivascular lymphocytic mantling. (Fig. 2.)

The vessels of the dermis appeared dilated, especially the lymphatics and in some slides so much so as to give the impression of new vessel formation. This effect was more noticeable in those cases responding rapidly to therapy (Fig. 11).

The reticular and fibrous tissue of the corium was observed to become more prominent with recession

of/

of the cellular exudate, there being separation of the fibrous connective tissue cells and exposure of the subjacent blood vessels (Fig. 11.).

In the bulk of cases there was no gross fibrosis but many slides showed sufficient increase of these elements to be labelled actual laying down of new fibrous connective tissue elements, even if this was not very marked and appeared to be associated with, rather than the method of healing (Fig. 2)

Some of this collagenous tissue surrounded the healing tubercles or in the resolving lesions presented as a residuum of the peripheral fibrotic reaction concerned in the previously active tubercle.

No special form of fibrosis was observed to result from the exhibition of calciferol in either cases responding or resistant to treatment. The possibility of reactivation of the lesions could only be considered unlikely when the tuberculous exudate had resolved and the sections of skin approached the normal histology leaving no trace of tuberculous architecture.

The epidermis showed no special features arising from the drug except that where broken down healing took place very readily. The overall healing of the disease process was slow except in a few instances of prompt and rapid progress yet in the ordinary responsive case progress took place more rapidly than could be expected of any spontaneous healing.

Elastic tissue visible in the healing lesions /

lesions appeared to be stretched or fragmented in the areas previously occupied by a dense exudate.

In several specimens the epithelioid cells became more prominent by recession of the mononuclear cell exudate giving rise to an appearance somewhat resembling a sarcoid. This structure came as one appearance of healing lupus vulgaris and can occasionally be seen in sections from untreated cases.

The overall picture was found to be a non-specific one and not to depart radically from the ordinary pattern of healing except in that it occurred rapidly and with uniformity in relation to calciferol treatment and to the clinical progress.

No histological calcification was demonstrated even in the occasional area of necrosis or caseation. Fat stains did not show any excess or peculiar distribution of lipoid material there being no readily available histochemical method of demonstrating the drug in the tissues and lesions. Response appeared more marked in cases of lupus vulgaris of a less active type where presumably the disease producing stimulus was minimal.

Clinical Material.

A series of 50 cases was selected from a larger bulk of available clinical material to contain as many new or untreated patients as possible thereby minimising effects due to previous or other treatments.

These were divided into two mains groups viz.

1. 40 cases of Lupus Vulgaris.

2. 10 cases comprising a number of conditions commonly considered to be of tuberculous origin. These included tuberculides, erythema induratum and sarcoidosis.

The plan of treatment was based on the methods used successfully by Dowling and others, the daily dose of calciferol for an adult being in the region of 150,000 i.u. daily by mouth supplemented by a local application to the diseased areas of calciferol in cold cream base 5000 i.u. per gm. This latter measure was designed to produce a high concentration of the drug at the desired site of action. The dosage was recorded in millions of international units of the drug given orally measured against the number of weeks of administration. To this record was added a note as to the concentration of the local application usually applied thrice daily.

The length of treatment was regulated by the clinical and histological progress of the individual case being usually continued for several weeks after/

after apparent cure of the areas affected. Alternatively some cases had the drug stopped during treatment for rest periods of up to four or six weeks. No sharp limits were set as to total dosage of calciferol or the length of treatment.

The patient's diet was not altered and the practice of prescribing extra calcium salts was abandoned as not obviously influencing the course of treatment and increasing the likelihood of the serum calcium rising to dangerous levels.

Local destructive treatments were avoided but several cases already having ultraviolet light therapy continued this satisfactorily while taking calciferol, there being no untoward effects from the combination.

Except where refused biopsy of the diseased area was made initially to establish the diagnosis, at times of significant change in the lesions under treatment, and finally to assess the results from calciferol therapy.

The series provided multiple sections from 112 relevant biopsy specimens thus allowing the histology to be accurately correlated with the clinical state of the lesions. Sample sections stained by the Ziehl-Neelsen method failed to demonstrate acid fast bacilli in the dermal tissues, this being a peculiarity of tuberculous lesions in the skin.

A series of 14 biopsy specimens on examination by concentration and cultural methods for organisms/

organisms yeilded no tubercle bacilli. Each patient was checked for active tuberculosis in organs other than the skin at the time of commencing calciferol therapy, old healed lesions in lungs or lymph glands not being regarded as a bar to treatment. The findings are recorded in Table 4.

Toxic reactions to the dosages of calciferol employed were infrequent, none of them causing serious upset, and all cases involved were able to continue the drug after a rest period or at a reduced rate of dosage. The patient's symptomatology remained the earliest manifestation of intolerance although one patient reached a level of 18.2. mgm per 100 ml in her serum/without any untoward signs. Serum calcium estimations were carried out as a precautionary measure when large amounts of the drug had been given or when any evidence of toxicity was reported.

The pattern of progress in the responsive cases of both groups took the form of a slow recession of the lesions leading to the ultimate healing. The cases of lupus vulgaris were consistently favourably affected only one case failing to benefit (Table 1). Healing was a slow process occurring over periods of weeks and accelerated by local applications of the drug.

Clinical observation showed no gross sudden changes in the process, this being confirmed by the histological changes which in themselves, tended to lag behind the gross healing. Microscopic tubercles could/

could be demonstrated (Fig. 8) in early apparent clinical cure thus revealing the need for adequate prolongation of therapy to ensure complete clearing of the lesions, and for the histological examination of any suspicious areas in the scar.

The histological changes brought about by calciferol treatment are discussed in Section 3.

Figures 5 & 6 illustrate a successful treatment in a case of lupus vulgaris of 23 years standing while the histopathological changes in the same case are shown in Figs. 3 & 4. The residual healed stage generally presented as a thin pliable scar being conditioned by the amount of tissue damage prior to treatment. No special type of scar formation was observed to arise as a result of treatment with calciferol.

One point noted was that two cases of lupus vulgaris both previously treated with tuberculin responded rapidly and effectively to calciferol.

The cases of group 2 varied in their response to the drug and the results are tabulated in Table 2. The only case of sarcoidosis treated was cleared of lesions in 15 weeks.

All patients in this series presented in a satisfactory state of general health and apart from the listed toxic manifestations there appeared to be no adverse effects due to calciferol.

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All patients in this series presented in a satisfactory state of general health and apart from the listed toxic manifestations there appeared to be no adverse effects due to calciferol.

RESULTS.

Number of cases in the series - 50.

Type of Disease. Table 1.	Group 1	Group 2.
Lupus Vulgaris.	40.	
Tuberculide - papulonecrotic		3.
Tuberculide - rosacea-like		3.
Bazin's Disease		1.
Erythema induratum		2.
Sarcoidosis		1.

Table 2.

Results of Therapy.

Lupus Vulgaris.

1. Clinical cure, confirmed microscopically	15.
2. Clinical cure	9.
3. Improved clinically	14.
4. Clinical cure and subsequent relapse	1.
5. Unimproved	1.
6. Deteriorated	Nil.
Total	40.

Tuberculide - papulonecrotic.

1. Clinical cure confirmed microscopically	1.
2. Clinical cure	1.
3. Unimproved	1.
Total -	3.

Tuberculide - rosacea-like.

1. Clinical cure.	2.
2. Clinical cure with relapse and subsequent cure.	1.
Total -	3

	Group 1	Group 2.
Erythema Induratum.		
1. Clinical cure with subsequent relapse		1.
2. Unaffected		2.
Sarcoidosis.		
Clinical and histological cure		1.

Table 3.

Toxic manifestations to calciferol.

Nausea	2.
Diarrhoea	1.
Malaise	2.
Sickness	2.
Fall in haemoglobin level	1.
Inflammatory reaction in lesions	1.
Biochemical toxicity	1.
Total. -	6.

Table 4.

Cases showing evidence of tuberculosis in other organs

	Group 1.	Group 2.
Calcified foci in lung fields	5.	
Suspicious findings " "	2.	1.
Glandular tuberculosis	5.	
History of Pleurisy	1.	
History of keratitis	1	1.
Lesions involving mucosa	2.	
Total cases -	14.	2.

Case Number and age of patient.	Calciferol	GROUP 1. Clinical Progress.	40 Cases of Lupus Vulgaris. Histological Progress	Comments.
1. A.A. Age 15. Lupus Vulgaris.	10 mill i.u. 10 weeks.	Clinical Cure	Final biopsy clear of tubercle	Good Progress but not yet terminated treatment
2. B.O. Age. 12. Lupus Vulgaris	21 mill i.u. 20 weeks	Clinical Cure	Healed; some scar tissue formation Fig. 2.	Progress to healing Still clear two months later and clear at 6 months review
3. M.M. Age. 59. Lupus Vulgaris.	15 mill i.u. 11 weeks. 2 courses	Clinical Cure	No residual lesion to biopsy	Clinical Cure - clear at 3 months review.
4. H.R. Age. 34. Lupus Vulg.	43 mill i.u. 40 weeks. plus local	Clinical cure gradually occuring	Exudate clearing and clear in latest section	Good progress & hair grew on area of alopecia. Better with local application. Clinical resolution confirmed microscopically

5. J.P. Age. 15. Lupus Vulgaris.	15.75 mill i.u. 15 weeks and local.	Good clinical resolution of lesions.	Good marked diminution of exudate to almost complete resolution in latest section	Progressing well - still has visible but resolving disease. Approaching clinical and histological healing.
6. M.H. Age. 51. Lupus Vulgaris.	60 mill i.u. 88 weeks.	Approximates to clinical cure	Suspicious exudate present but no discrete tubercles	Slow progress but continous - now healed
7. D.B. Age 13. Lupus Vulgaris.	27 mill i.u. 32 weeks.	Positive but slow	Tubercles persisting at last assess-ment.	Clinical progress. Shows response to continuation of treatment.
8. J.D. Age. 40 Lupus Vulgaris.	52.5 mill i.u. 87 weeks.	Progress but very slow - better on stepped up dosage	Histological clearing of tubercles	Still progressing slowly on continued treatment. Healing satis-factorily at latest assess-ment.

9. D.B. Age. 42. Lupus Vulgaris	50 mill i.u. 8 weeks	Clinical Cure	No tuberculosis in section	Clinical cure confirmed and under observation Clear on review.
10. J.A. Age 62.	15 mill i.u. 14 weeks	Clinical Cure	Persistent microscopic lesions	Given repeat course of drug on account of histological findings of persistant disease Fig. 8. Subsequent healing and clear on review.
11. J.G. Age. 44. Lupus Vulgaris.	24 mill i.u. 34 weeks. and local 500 i.u. gm.	Progressed to no visible nodules and quiescience	Nearly complete resolution of exudate in last section.	Slow but steady progress to clinical arrest of disease. No active disease on review.
12. J.D. Age. 43.	54.5 mill i.u. 56 weeks and continuing	Clinical Cure	Persistant granuloma microscopically - refused final biopsy.	Clinical cure not confirmed histologically and drug continued. Clear of all disease on review.

13. M.C. Age. 48. Lupus Vulgaris.	725 mill i.u. 70 weeks.	Clinical clearing of disease	Resolution of exudate to leave mononuclear cells in dermis - no tubercles.	Progressing to clinical quiesence no visible nodules in any part of scars Free of disease on review.
14. J.L. Age. 54. Lupus Vulgaris.	20.5 mill i.u. 26 weeks and local 500 i.u. gm in local application.	Clinical resolution	Resolution of epithelioid cells when assessed	Clinical resolution of disease process leaving scars. Marked effect from local application.
15. J.L. Age. 60 Lupus Vulgaris.	15 mill i.u. 15 weeks.	Clinical resolution	Histological resolution completed	Clear of all disease on review.
16. I.M. Age. 53 Lupus Vulgaris.	35 mill i.u. 1 year	Clinical cure	Sections showed small resolving tubercles and clear latest section	Lesions healed.

17. M.L. Age. 49. Lupus Vulgaris.	15. mill i.u. 20 weeks Repeat coure of 5 mill i.u. 5 weeks.	Clinical healing of lesions	Histo. recurrence of epithelioid cell which resolved with further treatment.	Clinical healing but relapsed with two nodules. General health good. Repeat course of drug caused healing.
18. T.M. Age 36. Lupus Vulgaris.	27.5 mill i.u. 30 weeks	Clinical progress to healing of ulcer	Disappearance tuberculous exudate - residual chronic inflammation.	Good clinical progress
19. S. McA Age. 32. Lupus Vulgaris.	20 mill i.u. 19 weeks and local 2000 i.u. gm.	Good with clinical healing at end of course	Satisfactory Approaching resolution.	Ulcer healed: put on weight. Healed on review.
20. T.G. Age. 26 Lupus Vulg.	40 mill i.u. 60 weeks	Consistent clinical improvement more marked after local application.	Sections showed residual tubercles with signs of partial resolution.	Slow continued clinical improvement. Therapy continued.

21. S.B. Age. 27. Lupus Vulgaris.	54.6 mill i.u. 52 weeks	Clinical cure	Sections showed healing	Clinically healed
22. R. McL. Age. 35 Lupus Vulgaris.	16.8 mill i.u. 16 weeks and local application.	Clinical disappearance granulomata area healing over cleanly	Post treatment biopsy not available	Healed. Contracted a secondary infection and hand badly deformed from old disease
23. C.S. Age 30. Lupus Vulgaris.	10.8 mill i.u. 14 weeks. Local 5000 i.u./ gm	Satisfactory progress and resolution.	Resolution of lesions progressing but not yet clear	Healing well. Appeared markedly helped by local application.
24. J.B. Age. 9. Lupus Vulgaris.	10.5. mill i.u. 30 weeks Further. 11.2 mill i.u. 16 weeks.	Negligible at first - slow on increased dosage.	No gross change after 4 months drug.	No progress on original dosage - healing commenced on stepped up dosage.

25. J.F. Age. 41 Lupus Vulgaris.	35 mill i.u 50 weeks + Local 5000 i.u./gm later.	Slow steady resolution on 100,000 i.u. day - clinical cure.	Clearing exudate	More rapid progress on increased dose of drug and local application - went to complete healing and clear on review.
26. M.D. Age. 52 Lupus Vulgaris.	15 mill i.u. 15 weeks and local 5000 i.u /gm	Good - lesions almost cleared after 12 weeks treatment.	Commencing resolution early and later well advanced	Responded from 1st weeks once surface healed. Local applications useful. Nearly healed at last assessment.
27. R.McK. Age. 10 Lupus Vulgaris.	18 mill i.u. 22 weeks local 5000 i.u./gm	Resolution commenced on increased dosage	Negligible change in sections-active granulomata persistant	An active case. Progress after increased dosage of calciferol and healing well advanced
28. H.W. Age. 30 Lupus Vulg.	15 mill i.u. 15 weeks No local applications	Clinical cure	Not available	Another course of drug to treat relapse in one part of scar.

29. I.B. Age. 40. Lupus Vulg.	15 mill i.u. 15 weeks	Marked clinical clearing - no gross visible disease	Not available biopsy refused	Good Progress continuing. Rapid resolution of nodules and now a clinical cure.
30. E.McD. Age. 16. Lupus Vulg.	22 mill i.u.	Slow but definite clinical improvement.	No change in histology.	Very little effect from treatment.
31. J.T. Age 44 Lupus Vulg.	13 mill i.u. 12 weeks and local application	Marked subsiding of lesions.	Satisfactory	Partial successful treatment not yet completed.
32. A.Y. Age. 27. Lupus Vulgaris.	10 mill i.u. 11 weeks.	Clinical cure	Residual granulomata after light therapy	Healed on calciferol combined with light therapy

33. J.D. Age. 53 Lupus Vulgaris	19 mill i.u. 18 weeks	Clinical healing	Biopsy not available	Broken down areas healed with regression of lesions - not yet complete.
34. A.R. Age. Lupus Vulg.	110 mill i.u. 100 weeks	Satisfactory	Biopsy not available from palate area	Did well but very slowly on long continued dosage lesions healed.
35. M.D. Age. 48 Lupus Vulgaris	25 mill i.u. in two courses of 20 and 16 wks with 8 weeks rest	Made clinical improvement	Persistent granulomata on review.	Recurrent lesions and given further treatment.
36. J.D. Age. 49 Lupus Vulgaris	72 mill i.u. 64 weeks	Cure	Cure	Clinical & histological cure - maintained on review

37. J.C. Age. 43 Lupus Vulgaris	10 mill i.u. 9 weeks local 5 000 i.u.gm	Healing satisfactorily	Commencing healing	Satisfactory progress
38. E.S. Age. 59 Lupus Vulgaris	7 mill i.u. 6 weeks	Satisfactory	Not yet assessed	Satisfactory progress
39. I.T. Age. 50. Lupus Vulgaris.	100,000 i.u. daily for 1 year in association with U.V.R.	Clinical cure	Histological cure	All lesions cleared on claciferol. Residual scarring near eye requires plastic repair.
40. A.C. Age. 55. Lupus Vulgaris.	104.5mill i.u. at rate of 150,000 i.u. daily	Clinical cure	Clear	Clinical healing of all lesions.

Case number and age of patient	Calciferol	GROUP 2. Clinical Progress	10 Cases of allied Conditions. Histological Progress	Comments.
41. M.H. Age. 25. Erythema Induratum	10 mill i.u. 13 weeks	No Progress	Nil	Unaffected by Calciferol
42. R.K. Age. 28 Rosacea-like Tuberculide	12.5 mill i.u. 12 weeks local application	Slow improvement	Little change in early sections but later healing.	Slow healing of disease which went to completion.

43. E.T. Age. 31 Paplonecrotic Tuberculide	8.4 mill i.u. 8 weeks and local appliction.	Clinical resolution of lesions proceeding	Biopsy showed healing lesions.	Clinical resolution of lesions on Calciferol
44. A McC Age. 36 Rosacea-like Tuberculide	17 mill i.u. 16 weeks Previous calciferol therapy	Clinical cure later relapse and further treatment.	Not available	Cleared at first, relapsed later, healed with further treatment
45. J. N. Age. Sarcoidosis	16 mill i.u. 15 weeks	Clinical subsidence of lesions	Histological diminution in size of lesions to complete healing.	Positive clearing of lesions on calciferol which went to healing.
46. E.B. Age. 30. Rosacea-like Tuberculide	22.5. mill i.u. 20 weeks	Clinical cure	Lesions cleared entirely	Clinical cure.

47 M.K. Age 27. Erythema Induratum	10 mill i.u. 13 weeks	Clinical cure	Cleared	Cleared but relapsed
48. H.B. Age. 21 Paplonecrotic Tuberculide.	47.4 mill i.u. 48 weeks plus some local application.	Clinical cure	Compatible with healing - no granuloma in latest biopsy.	Healed.
49. E.L. Age. 17. Erythema Induratum	10 mill i.u. 12 weeks	Nil	Nil	Not benefited.
50. J.W. Age. 44. Tuberculide	15 mill i.u. 20 weeks	Slight	Nil	Not benefited by treatment.

ILLUSTRATIVE CASES.

Case 1

H.R. aged 34 Lupus Vulgaris.

This patient had widespread disease present for many years, involving the face, ears, neck, trunk and left leg. There was elephantiasis of the left lobe of the ear and when first reporting he had considerable secondary infection of the chest area. Both eyelids showed marked oedema and the anterior scalp presented an area of alopecia due to the disease.

There was a history of "glands in the neck" in 1925 from which the lesions had started and gradually spread. His father had suffered from the same disease over 30 years. An x-ray chest was negative, as were W.R. and Kahn tests.

Two biopsies from different areas showed an active tuberculous infiltrate in the dermis with oedema and attenuation of the epidermis, there was no evidence of healing.

Calciferol was given at the rate of 150,000 i.u. daily by mouth and a local application of 1000 i.u. per gm of cold cream this later being increased to 5000 i.u. per gm and applied thrice daily.

At this time he was prescribed calcium lactate gr 10 t.i.d. Two progress biopsies after 9 million i.u. calciferol plus the local application showed no significant change and at this stage/

stage he had made little clinical progress.

For a time dressings were required to eliminate sepsis from necrotic and ulcerated areas on his chest but when these healed at about the twelfth week of treatment he began to improve rapidly.

After 21 million i.u. of the drug in twenty weeks he was markedly better and the diseased areas subsiding. Biopsy at this stage showed a considerable change. The epithelioid cell follicles were reduced in density and appeared slightly oedematous while the lymphocytic infiltration was reduced in amount. There were numerous giant cells still present and in some areas the fibrous tissue was more marked or increased in amount. The lymph channels were dilated and there was a diffuse perivascular mononuclear cell infiltrate. Some amorphous eosinophilic material was noticed in the diseased area but the epidermis was intact and appeared healthy.

From this stage he continued to make clinical progress and the nodules at the edges of the lesions had subsided.

It was noted that hair commenced to grow again on the scalp in the actual areas of the disease which had been entirely denuded of hair. After 38.5 million i.u. calciferol in 36 weeks mostthe areas showed healing and no active nodules could be found. There were no toxic signs, the serum calcium being 10.3. mgm per 10 ml.

At this stage he appeared to be approaching/

approaching a clinical cure and biopsy showed disappearance of the tuberculous exudate leaving scattered collections of lymphocytes in the dermis with a slight increase in the fibroblasts. Fig. 4 shows a typical section of the healed areas.

Calciferol was stopped after 43 million i.u. administered over 40 weeks combined with local applications in a cold cream base. There was now no evidence of any active disease and the scars were sound and pliable. The facial oedema subsided and the enlarged ear partly reduced in size to a deformed organ produced by previous scarring in the area.

Case 2.

A.A. Aged 15. Lupus Vulgaris.

In June 1947 he noticed nodules appearing on the left side of the forehead at the site of a "mole" present since birth. On examination a series of small nodules were visible above the left eyebrow presenting the appearance of typical tubercles and these were treated with trichloracetic acid locally. X-ray of lung fields and a Mantoux test were negative.

Biopsy in January 1948 was reported as follows:-

"This section shows a thick nodule in the dermis composed of tuberculoid follicles and many lymphocytes. Commencing caseation in some areas but no marked fibroblast proliferation. The epidermis is intact although thin and stretched."

Calciferol was then given to a total of ten million i.u. over ten weeks when biopsy of the area then showing as a clinical cure revealed that the epithelioid cell follicles has disappeared except for several poorly formed tubercles and scattered aggregations of lymphocytes. The vessels of the dermis were patent and appeared relatively increased in number and there was present some young fibrous connective tissue. One area showed a small milium surrounded by giant cells. At the site of the resolving exudate the collagen fibres were separated and a considerable amount of elastic tissue was visible.

Serum calcium at the end of treatment was 10.5 mlgms./

mlgms. % and he had no toxic manifestations.

At subsequent review in one month he was still clear of disease and was instructed to report at monthly intervals.

Case 3.

B.O. aged. 9 Lupus Vulgaris.

Reported with a three months history of an eruption on the right side of the nose, inside the nose, and on the left side of the neck starting after a "gravel rash". Six years previously he had an abscess in the neck and three years previously a strumous keratitis.

On examination the areas presented as lupus vulgaris. Biopsy showed multiple tuberculoid follicles in the dermis with necrosis and giant cell formation. There was little fibrosis and a dense infiltrate of lymphocytes and some plasma cells.

Concentration and culture of biopsy material was negative for organisms. X-ray showed an opaque right antrum and an enlarged right hilar lung shadow, the former condition clearing after drainage followed by a penicillin spray.

The mantoux test was negative to a dilution of 1:10,000 O.T.

Calciferol was given by mouth supplemented after clearing of some secondary infection with silver nitrate soaks by local application of calciferol 1000 i.u. per grm of cold cream. The oral dosage totalled 21 million i.u. administered over 20 weeks with no toxic manifestations. The neck area had almost cleared in a month and at the end of the course no nodules were visible when he was assessed as a clinical cure. Follow up biopsy showed disappearance of tuberculous exudate and follicles leaving some plasma/

plasma and mononuclear cell infiltrate around the vessels. Some areas showed traces of follicular architecture in the residual fibroblastic tissue this being sufficient in amount to be called scar formation. The epidermis was intact and showed only irregularity of the papillae.

When reviewed in a month and again at two months after cessation of treatment he was entirely clear of disease, appeared healthy and stated to be growing normally.

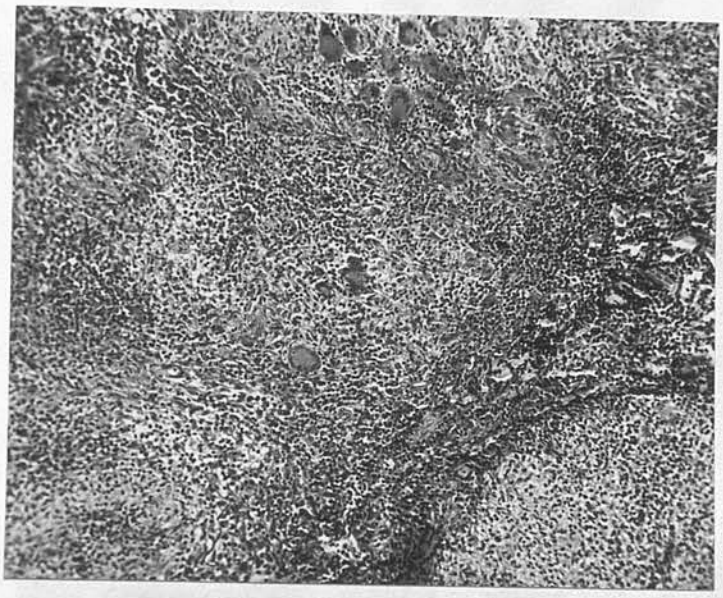

Fig. 1. H & E x 90.

A section showing active Lupus Vulgaris before any treatment. The dermis contains tubercle systems composed of epithelioid cells, giant cells and lymphocytes. No evidence of healing.

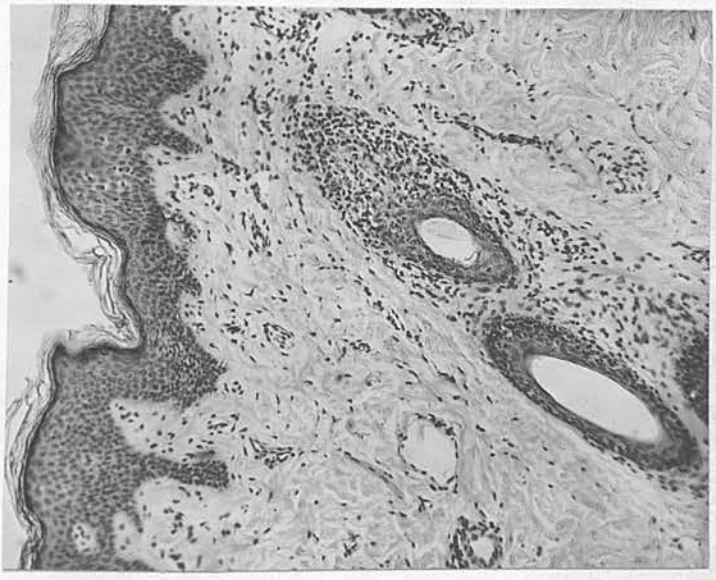

Fig. 2. H & E x 90.

A section from an adjacent area of the same case after Calciferol treatment with 21 million i.u. in 20 weeks. The granuloma has resolved leaving a scattered mononuclear cell infiltrate and a relative increase of fibrous tissue.

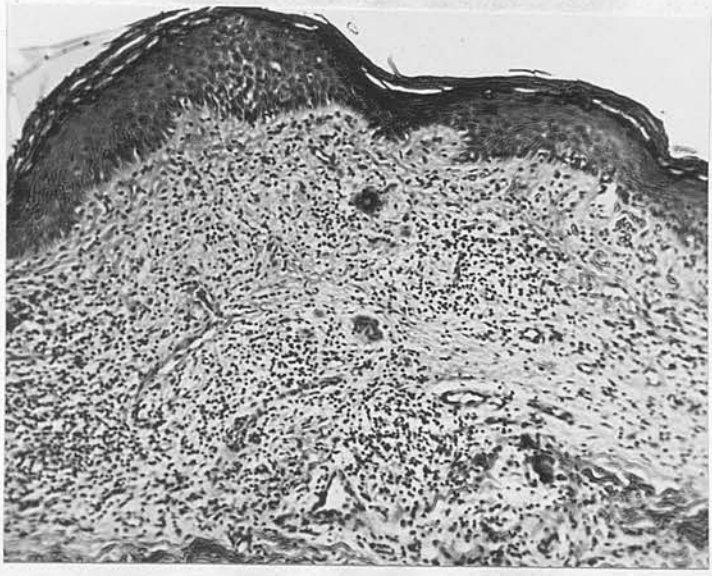

Fig. 3. X 90.

Section of a case of Lupus Vulgaris after 20 weeks treatment with Calciferol showing commencing resolution of the granuloma. Fig. 5 shows the clinical appearance before treatment. The exudate presents a loose appearance and patent vascular channels are visible.

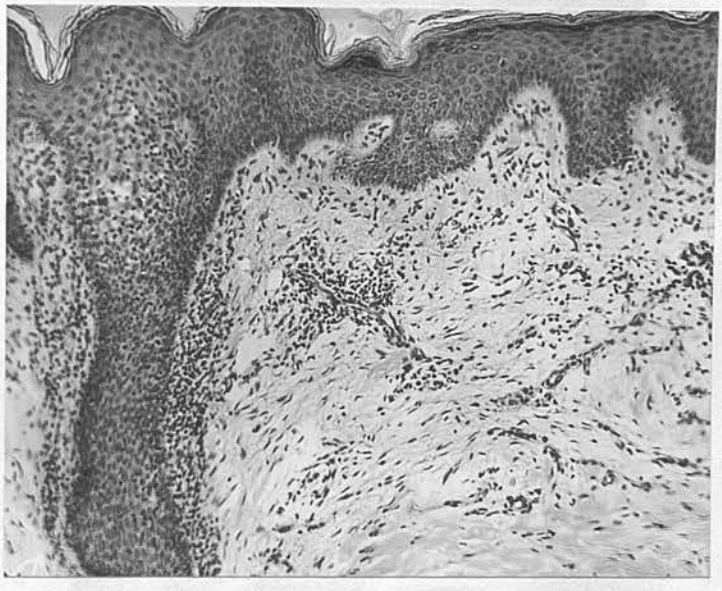

Fig. 4. X 90.

Comparable section from the same case as Fig. 3 after 36 weeks treatment when presenting as a clinical cure. The clinical appearance at this stage is shown in Fig. 6. The only remaining exudate is composed of scattered mononuclear cells round the vessels and hair follicles. This section shows advanced healing.

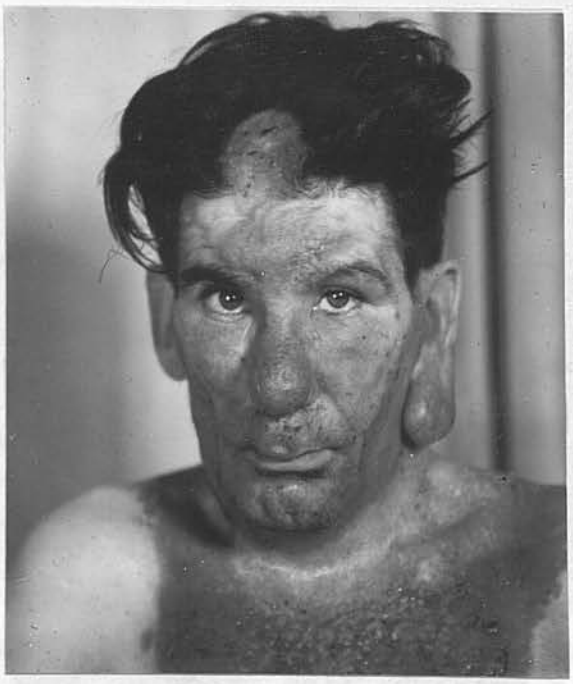

Fig. 5.

Clinical appearance of an extensive case of Lupus Vulgaris before treatment. The extension of the disease to the scalp is unusual and there is hypertrophy of the involved left ear.

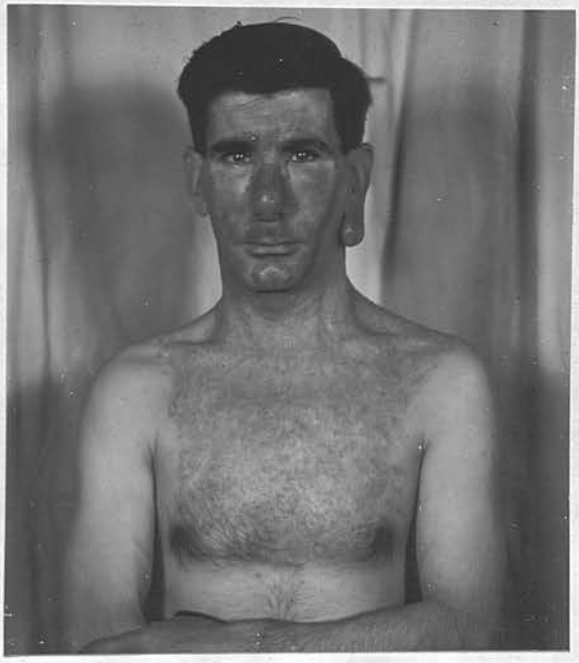

Fig. 6.

Clinical appearance of the same case after 36 weeks treatment. The growth of hair in the area of disease on the scalp is noteworthy. No active disease and residual fine scarring.

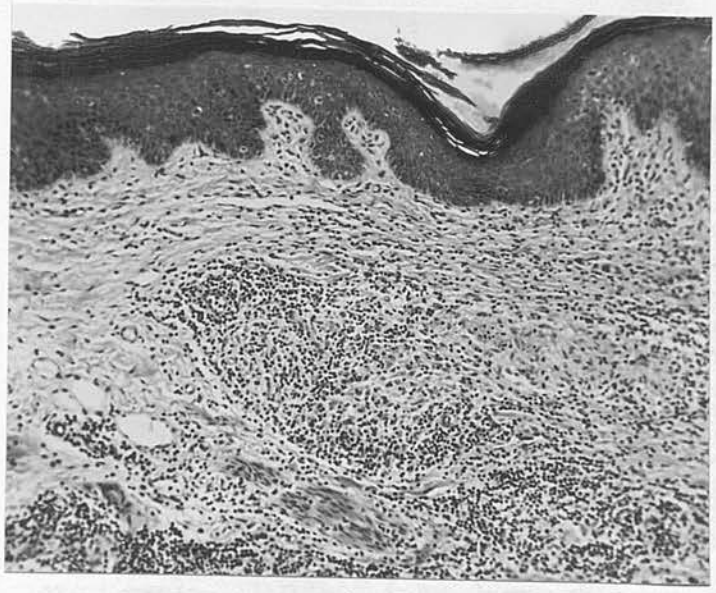

Fig. 7.

This section shows well advanced resolution in a case of Lupus Vulgaris after 70 weeks treatment with Calciferol. Clinically no sign of active disease at this stage. The dermis contains a healing tubercle with persistence of lymphocytic infiltration but there is no marked change in the fibrous connective tissue.

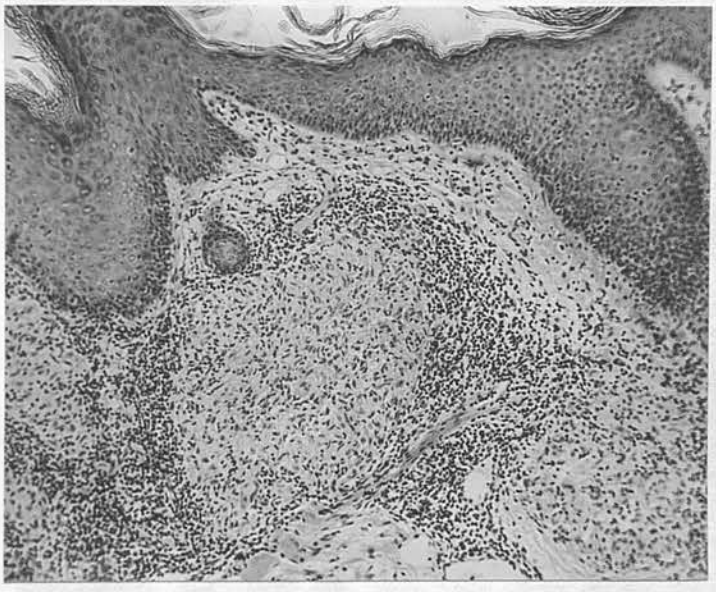

Fig. 8.

Section from a case of Lupus Vulgaris apparently clinically cured after 15 million i.u. of Calciferol. The dermis contains scattered typical tubercles indicating persistent activity of the disease at this stage of treatment.

Fig. 9. x 90.

This section shows an active Lupus Vulgaris with epithelioid and giant cells in the dermis. Untreated active disease in the same case as Fig. 10.

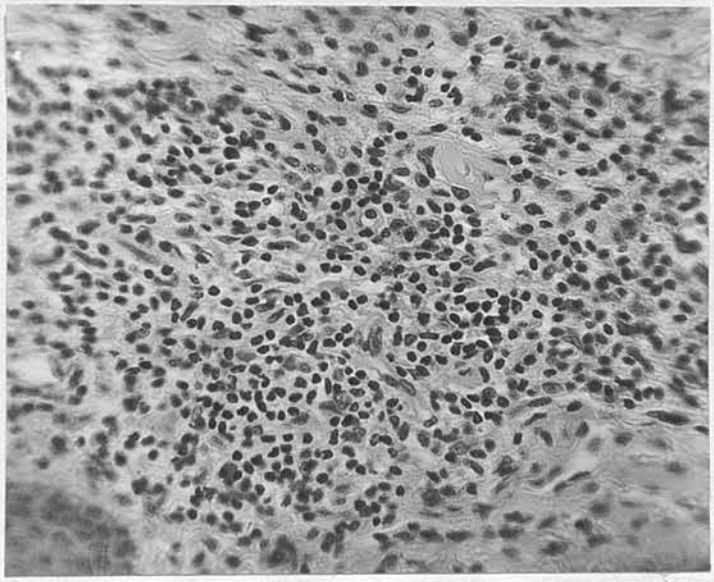

Fig. 10. x 300.

A comparable section from the same case as Fig. 9 after 10 weeks treatment with Calciferol. A higher power view to show the granuloma breaking up and diminution in the number of epithelioid cells.

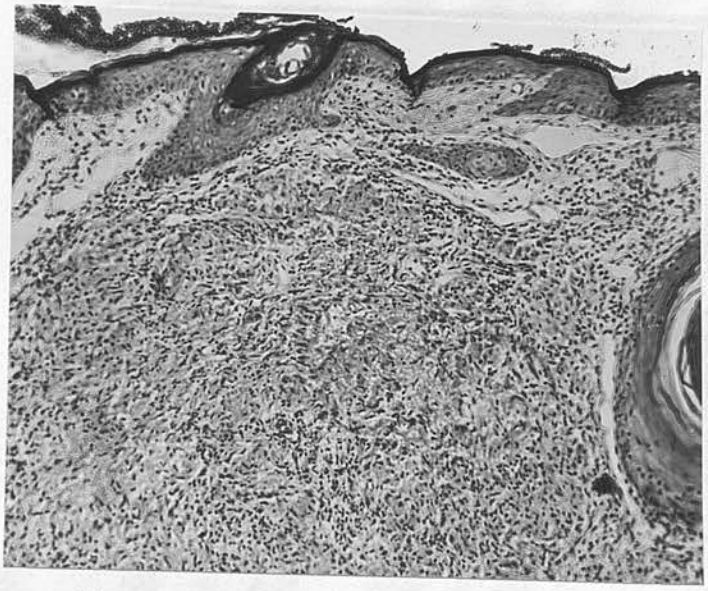

Fig. 11. x 90.
Section of untreated active Lupus Vulgaris showing the granuloma occupying the middle and deeper layers of the dermis.

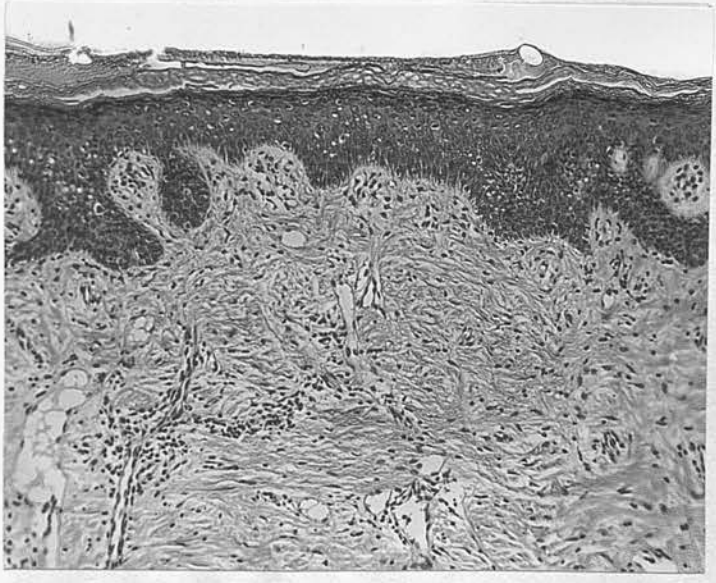

Fig. 12 .
Comparable section from the same case as Fig. 11 after treatment with Calciferol. The typical tuberculous elements have disappeared leaving a scattered mononuclear cell infiltrate. The vascular channels are patent and the dermal fibrous tissue is prominent.

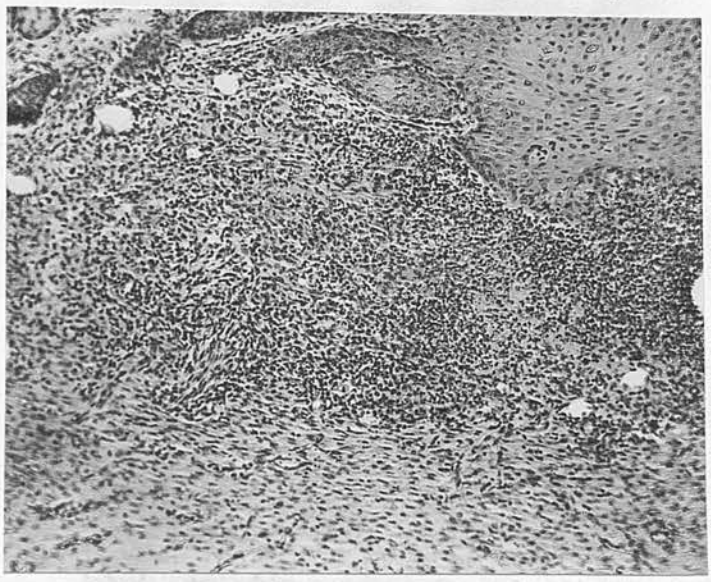

Fig. 13 . x 90.

Section of an 18 day old experimental granuloma in guinea-pig skin. Untreated control showing a necrotic central area, cells of epithelioid type, many mononuclear phagocytes and fibroblasts.

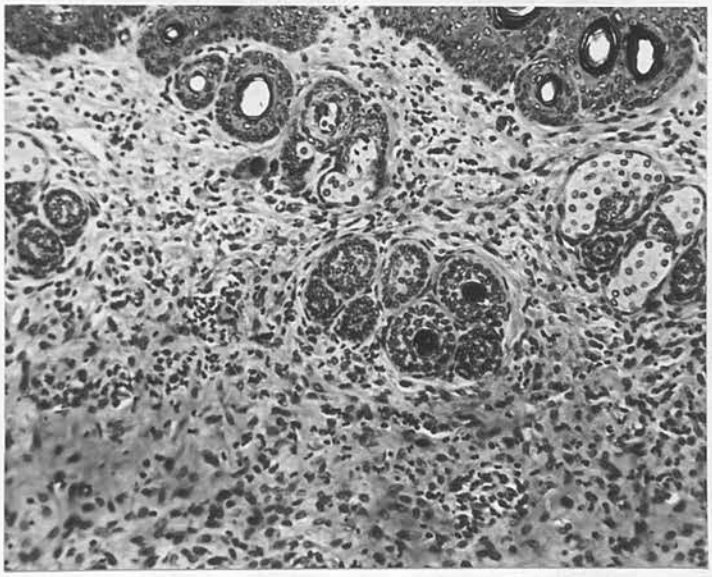

Fig. 14 . (no. 16) x 90.

Section on an 18 day old experimental granuloma in guinea-pig skin from an animal receiving 5000 i.u. calciferol per kg. daily. The granuloma has largely resolved leaving collections of epithelioid cells and mononuclear phagocytes in the dermis.

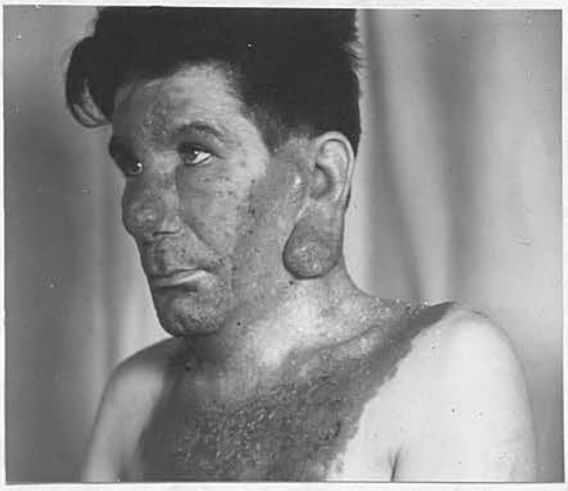

Fig.15 .

Untreated active Lupus Vulgaris with ulceration in the chest area.

Effect of Calciferol on Artificial Granulomata.

In an attempt to support the clinical and histological findings resulting from the study of human disease in this series it was decided to make a preliminary study of the effect of Calciferol on artificially created granulomata due to chemical entities recognised as being associated with the tubercle bacillus.

Chemical fractions obtained from tubercle bacilli cannot readily be obtained in a pure form and it was decided to make use of a chemical imitation of the most active granuloma producing compound as yet isolated from the lipoid moiety; viz pthioic acid.

The study of the biological activity present in synthetic analogues of such active principles has only recently received much attention although Sabin (165) experimented with long chain fatty acids resembling those found in the tubercle bacillus. By these means a duplicate form of the disease can be created which is readily available for study and subjecting to any treatment under review. There is added advantage in the absence of nitrogen from the synthetic molecule thereby removing doubts as to the activity of the residual quantity of this substance in fatty acids obtained by fractionation of bacilli.

300 mlgm of synthetic pthioic acid (T.R.C. subs 270; 3:12:15 trimethyl docosanoic acid) was kindly made available by Dr. Ungar of Messrs Glaxo.

This/

This compound was emulsified in 20% ethanol for intracutaneous injection in guinea pig skin in doses of 17 mlgms per area. Four guinea pigs received three injections each, two animals acting as controls while the other two consumed calciferol in oily solution in quantities calculated to provide an oral dosage of 5,000 i.u. per kilo body weight each day. Granulomata resulted in all cases and histological study of the lesions was made at, 8, 18 and 35 days. No suitable other fatty acid was available as a control substance.

Results.

Visible granulomata appeared in response to the compound in all animals reaching a maximum size at about the 7th to 10th day with no gross difference between the untreated or treated lesions. Resolution was observed to commence in the treated animals being macroscopically complete by the 20th to 23rd day when gross lesions were still present in the controls. All lesions resolved by the 35th day in both treated and control animals.

Sections from specimens taken on the 8th day showed.

(a) control lesions.

The injection mass surrounded by leucocytic debris peripheral to which appeared many mononuclear cells resembling epithelioid cells. Many of these cells showed vacuoles in the cytoplasm and large vesicular nuclei. There was no real tubercle formation and no giant cell formation.

(b)/

(b) Treated lesions.

A similar appearance with aggregations of mononuclear phagocytes and polymorphs at the injection site. The mononuclear cells resembled epithelioid cells but there were no discrete tubercles.

These sections confirmed the granuloma producing properties of the irritant substance but showed only a tuberculoid architecture rather than the typical lesions of tuberculosis. There was no major difference between the treated and untreated granuloma at this stage.

Sections taken on the 18th day showed.

(a) Control lesions.

The localised lesions were still present as necrotic foci in the dermis with surrounding aggregations of cells of the epithelioid type, giant cells and some fibroblasts. Many of the cells were aggregated but there was no typical tubercle formation Fig. 13.

(b) Treated lesions.

Well advanced resolution of the experimental lesions there being still a few tuberculoid aggregations of epithelioid and mononuclear cells in the dermis. No caseation and no giant cells were seen Fig. 14.

It was thus found that calciferol expedited resolution of the granulomata this being confirmed histologically on all lesions.

This experimental study limited by the amount/

amount of available synthetic chemical is not sufficient to provide any finite conclusions but has been taken to serve as an indication of the experimental results to be expected from the use of calciferol on artifically created granulomata due to fatty acids resembling those of the tubercle bacillus.

These observations made on guinea pig skin, although requiring interpretation in the light of species differences, may serve as a basis for a more extensive series of controlled studies of the biological properties of pthioic acid and similar compounds and the influence of treatment on these.

The results of calciferol therapy on the artifically created granulomata are in accord with those observed in the bulk of the human clinical material under study and point to the possibility of a chemotherapeutic basis of action in stimulation of phagocytic cells concerned in the tuberculous process.

This stimulation of the cell cytoplasmic activities leading to resolution of the cellular elements comprising the exudate can be expected to have as an underlying mechanism the speeded up breakdown of the irritant compound thus removing the stimulus which incites the localisation and activities of the phagocytic cells. If it can be confirmed that this is the case in respect of a chemically known substance representing the synthetic analogue of the most active granuloma producing entity found in tubercle bacilli, it indicates that the same/

same can occur in diseased areas with visible bacilli, although perhaps not affecting other than lipoid irritant elements.

It has long been considered that the phagocyte can slowly disintegrate such resistant products of the tubercle bacillus and facilitation of this process means that it is possible to attack these compounds when situated within the cells thus representing a chemotherapeutic advance capable of elaboration along the lines of specific action.

The resistance and capacity for slow hardy growth in vivo that characterises the tubercle bacillus is associated with the chemical structure of its products and the relative inability of the cellular defenses dispose of these substances so much valuable information can come from study of individual components obtained by fractionation or synthesis in their capacity to produce reactions in mammalian tissue.

DISCUSSION.

This series of cases confirms that tuberculosis cutis can be treated successfully with Calciferol though there are occasional lesions which remain unaffected or are only slightly altered.

The effect varies with the type of disease, lupus vulgaris being the form most readily benefited. One of the series showed minimal improvement after heavy dosage over a prolonged period. Evaluation of the results of treatment is difficult but correlation of the clinical and histological findings, at intervals, provides a definite means of recording progress. Biopsy material from the lesions shows a constant pattern of microscopic change running parallel to the clinical changes but which often lags behind in the later stages of resolution. The changes involved in healing are slow and evolve over periods of weeks yet with definite acceleration produced by local application of the drug.

The morphology of change consists of resolution of the granulomata leaving a variable residuum of formed fibrous tissue which is visible as a thin pliable scar. Macroscopically the lesions appear to subside slowly under treatment leaving a nearly normal skin or a firm scar, depending on the amount of previous tissue destruction or fibrosis. The healed area is often pigmented. Microscopically, the earliest effect associated/

associated with calciferol is some swelling of the epithelioid cells of the tuberculous follicles and some of these cells present a vacuolated cytoplasm. Patent vascular channels have been taken to indicate increased vascularity of the lesions although only a few cases showed reddening or swelling on clinical examination, and this in the first few weeks of treatment. Later the epithelioid cells tend to disappear and cannot be seen in sections from clinically healed areas.

The lymphocytic and mononuclear cell exudates tend to persist longer and to maintain the architecture of the tubercle for periods of several weeks before this too commences to break up.

In the later stages of a successful treatment it may only be possible to find small focal and perivascular aggregations of lymphocytes with no indication of any tubercles. Of course since healing does not proceed at a uniform rate in all areas of the lesion, tubercles may be microscopically visible at later stages in some sections. As will be mentioned later, such isolated foci of disease must be watched for in assessing the results of treatment and in deciding when to terminate it. The fibrous tissue elements vary in amount from case to case and in some appeared to be definitely increased. In this connection it is necessary to consider the extent of previous scarring and the tendency of the disease to heal with fibrosis. No special or peculiar form of fibrous tissue formation could/

could be found to arise from the exhibition of calciferol. In many sections the elastic tissue showed disruption or fragmentation which was revealed as the exudate subsided. A notable number of capillary vessels related to the tubercle systems in the dermis also became manifest as the cellular elements of the tubercles decreased in quantity the indication being that these existed before although they were not readily visible in sections.

The overall changes resulting from the method of treatment may be summarised as consisting of resolution of the dermal tubercles and disappearance of the cellular exudate leaving a variable degree of fibrosis and scar tissue.

These changes are not specific since they may also follow treatment with ultraviolet irradiation or even represent the course of events in spontaneous healing of a lesion. The significance lies in the uniformity with which these effects can be brought about by calciferol and in the observation that healing takes place by involution and disappearance of cellular elements which can be shown experimentally to arise in response to the tubercle bacillus and its products. Epithelioid cells can arise in other circumstances simulating the lesions of tuberculosis as in those due to silica but the cases in this series were selected as being of tuberculous origin. It is known that phagocytic cells ingest tubercle bacilli yet may fail to destroy them or their products with resulting formation/

formation of epithelioid cells and tubercle formation, so it becomes possible to infer that a substance promoting resolution of tubercles has enhanced the cytoplasmic activities which are concerned in the disposal of these irritants.

The inference that calciferol can increase the powers of phagocytes to deal with the organismal products of the tubercle bacillus is supported by the effect described on granulomata arising from the synthetic analogues of phthioic acid. In this connection the literature contains observations of the power of calciferol to stimulate leucocytes (171), to increase tissue metabolism (141) and to increase the cellular phosphatide turnover in mamnalian tissue (142).

Such a mode of action would also explain the recorded lack of direct effect against the tubercle bacillus in vitro, despite persistent reports of beneficial effects derived from calciferol in vivo.

Tuberculosis in the skin is a relatively much less active process than in other tissues and it is probable that the production of bacterial irritants is small so enhanced powers of the phagocytes become capable of dealing with these substances and this may also occur in some forms of tuberculous adenitis.

The conflicting and generally negative reports of the use of calciferol in pulmonary tuberculosis may well arise from conditions of more ready bacterial proliferation when the defensive powers of the organ are overwhelmed./

overwhelmed. Perpetuation of the disease process in the skin can be presumed to be due to the chemical resistance to cellular disintegration of the peculiar fatty acids of the tubercle bacillus and which, if released by the disintegration of the cells, are capable of inciting the production of further epithelioid cells since it may be taken that these latter arise pre-eminently in response to lipoid irritants.

The effect of calciferol on this biological reaction patently arises from alterations in the tissue chemistry when penetration of the tubercles and cells can be inferred from the resemblance to cholesterol which is itself known to accumulate in tuberculous foci.

There is no evidence that calcification was the mechanism of action and it was not observed in any section from this series of cases. Caseation and necrosis are uncommon in the skin so there is little material prone to calcify in contrast to the lesions in acute disease of experimental animals when considerable local calcification may occur. Tomlinson (167) observed that the local tissue calcium content was raised in lupus vulgaris treated with calciferol but that this occurred in a form not readily demonstrable by histological methods.

Calciferol acting on the host's powers to degrade or dispose of tubercle-producing substances need not necessarily affect the immunity or sensitivity levels with the result that on cessation of administration any residual foci may tend to reproduce the lesions

and/

and lead to reactivation of the disease.

Therapeutic response to the drug is principally observed in individuals already possessing a high level of resistance indicating the limited results to be expected of the present form and dosage. It is thus possible that a derivative or a related sterol may possess a more significant anti-tuberculous action by means of chemical effects on the phagocytic cells containing the bacillus or its products. Alternatively the best use of calciferol may be as an adjuvant to some direct bactericidal agent or even to one of the forms of tuberculin since it has been shown that inhibition of the tubercle bacillus is a cellular property of the mononuclear phagocytes of immune animals (205). A potential source of further information on the properties of the drug is study of the effect on granulomata created by fractions of the tubercle bacillus, or their synthetic analogues and having the special object in view of determining the reactions of the phagocytic cells by histological methods which enable ready evaluation of the structural changes.

The effects of this and similar chemicals on the cytoplasmic activities of the phagocytic cells is a field worthy of further study and development, especially by histochemical investigation of the enzyme systems.

Regarding the clinical aspects of the treatment, it was found that healing was slow; that it reached completion in fifteen cases of lupus vulgaris; and was proceeding satisfactorily in twenty-four cases at the time/

time of assessment.

The slow metabolism and healing in skin tissues tends to prolong any treatment and to render difficult comparison with tuberculosis in other organs. Long follow-up periods are necessary to determine any possible recurrence arising from microscopic foci of disease persisting in parts of the lesions. Serial sections of specimens from the lesions confirm the observations of Freudenthal (12) that small granulomata tend to persist and to produce subsequent relapse.

Fig. 8 illustrates such a lesion in a case free of visible active disease and under consideration for cessation of treatment. This demonstrates the value of histological assessment of cure and the need for prolonging therapy after apparent clinical cure. In this study a case was regarded as representing a clinical cure when no visible disease could be found and as a cure, at least of the skin lesions, when representative sections showed no microscopic evidence of tuberculosis. These terms are therefore relative and subject to the results of periodic review of the treated cases.

The cases of group2 represent a number of less well-defined entities yielding variable results to treatment, two cases of erythema induratum being completely unaffected. The single case of sarcoidosis showed continuous benefit and subsidence of the lesions. It is possible that here again calciferol enables the epithelioid type cell to deal more adequately with the substances provoking its occurrence.

In/

In considering dosage it has to be remembered that calciferol has dangerous potentialities and that the optimum results come from doses which may give rise to toxic effects.

On the duration and intensity of therapy depends the likelihood of these effects which must be guarded against by a watch for symptoms such as nausea, headache, gastrointestinal upset, or polyuria and by estimations of the serum calcium level. The average case however shows few if any untoward effects. Doses of 100,000 i.u. to 200,000 i.u. of calciferol daily are well tolerated and can be safely combined with local application or ultraviolet light therapy. Only six cases had toxic manifestations and none of these had any serious disability.

Local application of the drug allows of higher concentration on the diseased area and is useful both on the intact surface and in cases with ulceration provided there is no gross secondary infection. The strength of local applications can be arbitrarily set at 5000 i.u. of calciferol in a basis of cold cream and the systematic use of this preparation is capable of accelerating healing.

The period of administration usually extends over several months for the average case and is best measured by the clinical progress and by biopsy of the lesions. Clinical resolution must be confirmed histologically and this examination repeated on any suspected areas of recurrence prior to termination of treatment. No

accurate/

accurate limit can be set as a maximum safe length of treatment but cases in this series show no ill-effects after more than one year on the drug. One case showed a dangerous level of the serum calcium after 70 weeks treatment and a second a fall in the haemoglobin to 55% at the end of 100 weeks on the drug although it was not clear that this caused the anaemia.

A satisfactory policy is to prescribe continuous treatment and to review critically the progress at the end of each four week period with an assessment of the results at the end of three months and six months. This enables one to make any alterations in dosage and to keep an accurate check on the effects of treatment on the lesions.

When an apparent cure results, a three monthly periodic review is necessary to examine the healed areas and to make histological study of any suspected area of disease.

The duration of dosage required to achieve optimum results is not finite but varies with each individual case, guidance being best derived from progress while watching for any sign of toxicity.

This study of calciferol therapy shows that the drug falls short of the criteria for a successful chemotherapeutic agent but is of real value in tuberculosis cutis. Apparent clinical and histological cures of the disease assessed after short follow-up periods may not appear so favourable on longer term review when relapses should be manifest.

In/

In conclusion it may be said that this study concerns some features of only one form of tuberculosis and that the results must be interpreted with caution. An attempt has however been possible to take advantage of the readiness with which even small changes in the lesions can be inspected and subjected to microscopic scrutiny, thereby reducing the complexities of assessing chemotherapy in this disease, and with the added benefit of pathological material from human sources.

CONCLUSIONS.

1. Calciferol treatment produced clinical and histological healing in fifteen out of forty cases of tuberculosis cutis (lupus vulgaris).
2. Effective therapeutic action was found on the tubercle systems of Lupus Vulgaris.
3. The findings tend to indicate that this action is non-specific.
4. Histopathological assay of the disease at intervals during treatment revealed the structural changes involved in the chemotherapeutic effect of the drug.
5. Histologically the action of calciferol on tuberculosis cutis is found to be resolution of the tubercle systems, starting with the epithelioid cells and followed by the lymphocytic and mononuclear cell exudates, leaving a variable residuum of fibrous connective tissue.
6. The findings support the view that the mode of action of calciferol is by promoting the cellular metabolic disposal of irritant substances arising from tubercle bacilli; that it occurs in the cytoplasm of the phagocytic cells; and that this is adequate only in dealing with small quantities of such substances.
7. No special or characteristic type of fibrosis follows calciferol therapy but there is no apparent resolution of fibrous or scar tissue already created by the disease process.
8. The disease is capable of reactivation from persistent microscopic/

microscopic foci remaining after apparent clinical healing if treatment is insufficiently prolonged. It is necessary to continue treatment or to give a repeat course of the drug to ensure against such relapses.

9. Doses of 150,000 i.u. daily of calciferol are well tolerated by the average case and can be safely combined with ultraviolet light irradiation without causing toxic effects.

10. Local application of calciferol to the diseased area in a concentration of 5000 i.u. per gm. of cold cream base is found to accelerate clinical healing in lupus vulgaris.

11. In Lupus Vulgaris the period of administration is primarily governed by the clinical state of the patient. In the absence of any toxic manifestations the average case may continue treatment for 6-12 months by which time any significant benefit should have resulted.

12. Confirmation of healing is made by histopathological examination of biopsy material from the lesions.

13. All treated cases should be reviewed at intervals of three to six months for at least several years, if not for life.

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