PRESIDENTIAL ADDRESS.

By Sir ROBERT ROBINSON, M.A., D.Sc., LL.D., F.R.S.

A. *Some Aspects of the Chemotherapy of Tuberculosis.**

LEPROSY and tuberculosis are analogous pathological conditions produced by infection with so-called acid-fast bacilli characterised by the possession of a fatty or waxy envelope. For this reason there has naturally been a tendency, which will probably be evident in the future also, to investigate the application of promising methods of treatment of the one disease to the other.

The date of the discovery of the beneficial effects of chaulmoogra and hydnocarpic oils in cases of leprosy cannot be stated owing to the uncertainty as to the exact botanical origin and nature of the preparations, but it is undoubtedly true that chaulmoogra has been used by the Hindus and Chinese for many centuries.

The systematic study of the mode of administration and the accumulation of accurate clinical data are, however, confined to the last 30 years. As a result it is now known that the specific properties reside in the fatty acid constituents of the oils and these may be exhibited in a variety of ways, for example, as emulsions of the purified glycerides or as ethyl esters or salts of the isolated acids.

The pure crystalline chaulmoogric acid is, however, less active than the total fatty acids of the oil and this is probably a question of diminished solubility. The addition of dispersing agents such as oleic or glycocholic acid to chaulmoogric acid has been found to increase its effectiveness to a marked degree, although these substances are very weakly leprocidic in themselves.

Thanks to the investigations of Power and of Adams it is known that chaulmoogric acid is 13-*cyclo*pentenyltridecoic acid :

$$\left.\begin{matrix}CH{=}{=}CH\\ |\\ CH_2{-}CH_2\end{matrix}\right\rangle CH\cdot[CH_2]_{12}\cdot CO_2H$$

The bactericidal properties are considered by Adams and Stanley to be due largely to the physical character of the substance and not to be connected with any particular molecular grouping. Thus the dihydro-acid is active and so is the related base in which CO_2H is replaced by $CH_2\cdot NEt_2$. Moreover, Adams and his co-workers have made an extensive study of branched-chain fatty acids of varied types and have found that the leprocidal activity *in vitro* of a number of acids is paralleled by their marked surface-tension depressing properties. The acids are placed in the same order by reference to the two phenomena and there is therefore good justification for the view that the bacilli are attacked physically and probably by an impairment of function of their fatty envelopes. They are thus weakened and made an easier prey of the defensive mechanisms of the host or, alternatively, they succumb to unfavourable conditions in experiments *in vitro*. Pharmacologists and pathologists have reached similar conclusions, especially as the result of the observation that courses of treatment must be prolonged long after the disappearance of symptoms of the disease and also from the frequency of relapses. Sometimes the hypothesis was inverted and it was suggested that chaulmoogra stimulates the defence mechanism of the organism. In the analogous case of tuberculosis the favourable results of cod-liver oil treatment have accordingly been attributed to the vitamin content and this may indeed represent an accessory factor.

An excellent account of the work of Adams, Stanley, and their co-workers is to be found in the *Journal of Pharmacology and Experimental Therapeutics* (1932, **45**, 121—162) and no attempt need be made to summarise it here apart from a mention of a few of the results. Many of the substances examined greatly surpass chaulmoogric acid in activity and in the various series there was always an optimum chain-length. C_{14} and C_{20} acids

* In the Address delivered orally, reference was included to the recent outstanding advances in chemotherapy, including the sulphanilamide group of bactericides and the remarkable results disclosed by Yorke, Lourie, and King in regard to new trypanocidal agents. The latter work has been fully described by Professor Warrington Yorke in a paper read before the Royal Society of Tropical Medicine and Hygiene on February 15th of this year.

were relatively inactive and the best results were usually obtained with acids containing 16 or 17 carbon atoms. The effect of constitution is shown well in the *n*-pentadecanecarboxylic acids. Here palmitic acid is inactive, but α-methylpentadecoic acid is leprocidal in a dilution of 1 : 62,000. The activity increases as the carboxyl is moved to the centre of the molecule and di-*n*-heptylacetic acid is active in a dilution of about 1 : 200,000. It is significant that all the active acids contain branched chains, although this may be represented by an alicyclic group or phenyl group in almost any position in the molecule. A large number of *cyclo*hexyl fatty acids were examined and one of the best was $C_6H_{11}\cdot[CH_2]_2\cdot CH(CO_2H)\cdot C_8H_{17}(n)$, which was effective in a dilution of 1 : 320,000 (chaulmoogric acid, 1 : 10,000; total chaulmoogra fatty acids, 1 : 100,000). Double bonds could be introduced and had little effect, and hydroxyl groups also, but with a lowering of activity. In the synthetic series the carboxyl could be replaced by $CH_2\cdot NEt_2$ and the bases were active, although to a smaller degree than the acids.

Seven of the acids were tested against *Mycobacterium* tuberculosis *in vitro* and four showed marked bactericidal activity. The best was $C_6H_{11}\cdot[CH_2]_2\cdot CH(CO_2H)\cdot CH_2\cdot C_6H_{11}$, active in a dilution of 1 : 50,000 (against *B. lepræ*, 1 : 100,000). It is an acid containing 17 carbon atoms and the other active compounds contained 16, 17 and 18 carbon atoms. Closely related acids with 19 and 20 carbon atoms were ineffective.

A digression may now be made to mention some other observations on the chemotherapy of tuberculosis.

In 1923 Smith (*J. Pharm. Expt. Ther.*, 1923, **20**, 419) found that acridine dyes such as acriflavine, proflavine and acridine-orange were tuberculocidal *in vitro*, but had no effect on the course of the disease in experimental animals.

Hesse, Meissner, and Quast (*Arch. exp. Path. Pharm.*, 1928, **135**, 82) found that tubercle bacilli are stained and killed by basic dyes of the azine, thiazine and triphenylmethane series. They correlated this with the negative charge on the bacilli when suspended in serum. Indamine-blue was found to give moderately good results with tubercular guinea pigs.

Meissner and Hesse (*ibid.*, 1930, **147**, 339) also tested about 2000 compounds for their bactericidal properties, using bacilli growing in blood. Inorganic salts were inactive and so were eucupin, vuzin and optochin. Among the active substances were conessine, harmine, ethylapoquinine and aminohydroquinine. In 1931 the same authors reported favourably on certain azo- and hydrazo-derivatives of pyridine and quinoline (*ibid.*, 1931, **159**, 676, 687) and in 1935 Hesse, Meissner, Sebelin, and Müller used ethyl silicylricinoleate to alleviate the tubercular symptoms of rabbits (*ibid.*, 1935, **179**, 296).

Gold compounds have frequently been suggested as remedial agents for tuberculosis and among miscellaneous compounds the salts of thiocyanic acid and diacetyl may be mentioned. The use of cod-liver and other oils has already been referred to and it is of interest, in view of the suggestion that the sole active agents in the oil are the vitamins, that Negre, Berthelot, and Bretey (*Compt. rend. Soc. Biol.*, 1936, **123**, 864) observed a retarding effect on the tuberculosis of guinea pigs produced by injections of the ethyl esters of lauric, palmitic, and stearic acids.

Finally it is encouraging to note that since 1938 several authors have claimed good results in the treatment of tuberculosis by prontosil, sulphanilamide and diaminodiphenylsulphone (see, *e.g.*, Ganapathi, *Current Sci.*, 1938, **6**, 608; Rich and Follis, *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77). If these results are later confirmed and extended, very promising lines of investigation would appear to be opened up. The quasi-physical action of fatty acid derivatives may be supposed to weaken the bacilli and it is most important to determine the precise mechanism of this process. If it has to do with the dispersion of protective fats or waxes, *the simultaneous exhibition of a fatty acid derivative and an auxiliary drug of the sulphanilamide type might be beneficial.* Such combined therapy should certainly be tried with known substances of relatively innocuous character pending the discovery of the most suitable agents. In searching for the latter, on the fatty acid side of the problem, it is natural to look for some special relation with the fatty compounds of the tubercle bacilli themselves, especially since these are known to be characterised by branched chains, and, as we have seen, such substances are effective against *B. lepræ*. A very brief summary of this subject may be attempted because it leads eventually to a slender but valuable clue which will influence further work.

In 1929 Anderson and Chargaff (*J. Biol. Chem.*, 1929, **85**, 77 and other papers) isolated tuberculostearic acid, $C_{19}H_{38}O_2$, and five years later Spielman oxidised this substance with chromic acid and isolated methyl *n*-octyl ketone, azelaic and *n*-octoic acids as products of the reaction (*ibid.*, 1934, **106**, 87).

Hence tuberculostearic acid is 10-methylstearic acid (counting CO_2H as 1), $CH_3{\cdot}[CH_2]_7{\cdot}CHMe{\cdot}[CH_2]_8{\cdot}CO_2H$. It is probably optically active and thus it is not surprising that complete identity with a synthetic inactive acid of this constitution could not be demonstrated. Several other tubercle acids have been isolated by Anderson and the scale on which his brilliant pioneering work has been conducted can best be indicated by a short description of the separation of phthioic acid, which is probably of more significance than the substance already mentioned. 3868·5 G. of dried tubercle bacilli were extracted with ether–alcohol (chloroform later took out 427 g. of wax). The extract was evaporated and the portion soluble in pure ether was separated into 253·1 g. of a phosphatide and 240 g. of a fat. The phosphatide was purified and then weighed 138·3 g. On hydrolysis with dilute sulphuric acid it afforded oleic and palmitic acids, other acids (about $C_{20}H_{40}O_2$), a sugar acid, glucose and glycerophosphoric acid.

The fatty acids were converted into lead salts, the ether-soluble part of these reconverted into acid, which was then hydrogenated * and again made into lead soaps; the ether-soluble moiety afforded crude phthioic acid. The methyl esters were distilled at $<0{\cdot}0001$ mm. and further separated by chilling of their acetone solutions. Eventually methyl phthioate was hydrolysed to phthioic acid, $C_{26}H_{52}O_2$, an amorphous mass, m. p. 21°, $[\alpha]_D^{20°} + 11{\cdot}96°$. Another component was an acid $C_{30}H_{60}O_2$, m. p. 48—60°, $\alpha_D^{20°} - 6{\cdot}16°$. Even after this tedious process it is not certain that these acids are quite homogeneous. The amide and 2 : 4 : 6-tribromoanilide of phthioic acid are, however, well-characterised crystalline substances.

Chromic acid oxidation of phthioic acid gave $C_{11}H_{22}O_2$, stated to be different from *n*-undecoic acid because two derivatives melted 20° too low, but this is inconclusive in view of the small quantity available for purification, the probability that a mixture would be obtained, and the known difficulty of separation of the constituents of such mixtures. The application of *X*-ray technique to this work would doubtless clear up many of the dubieties.

Chargaff (*Ber.*, 1932, **65**, 745) has synthesised a number of C_{26} fatty acids and concludes from the melting points that phthioic acid must contain at least three hydrocarbon chains.

By the Kuhn–Roth method of estimating side-chain methyl, Wagner-Jauregg found that tuberculostearic acid gave 1·4 mols. of $C_2H_4O_2$ and phthioic acid gave 2·4 mols. of $C_2H_4O_2$ per mol. (*Z. physiol. Chem.*, 1937, **247**, 135). This again suggests the existence of three carbon chains in the molecule. Other degradation experiments were inconclusive and the results are indeed susceptible of more than one explanation. Anderson concluded that phthioic acid bears a methyl group in the α-position to carboxyl and another such group in the neighbourhood of the eleventh carbon atom from the carboxyl group. In arriving at this view he may have been influenced by the possibility of analogy with tuberculostearic acid, because there is no clear evidence pointing to the existence of the branch chain at the eleventh carbon atom.

An important point in regard to phthioic acid is that it possesses toxic properties and produces in experimental animals, *e.g.*, guinea pigs, lesions closely resembling those of tuberculosis. It may be recalled that chaulmoogra oil and all the effective synthetic compounds of Adams produce pronounced irritation of the tissues when injected.

The study of films of phthioic acid has given more positive results and these have already been discussed in a preliminary manner at a meeting of the Society by Dr. E. Stenhagen.†

X-Ray reflections from multilayer films of the barium salt showed that the length of the molecule was that of a chain of twelve to fourteen carbon atoms.

* Hence phthioic acid *may* be derived from an unsaturated substance occurring in the micro-organisms.

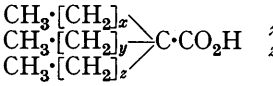
† It is understood that the details have been communicated to the Faraday Society and will shortly be published.

The acid itself differs from known fatty acids in its behaviour. It forms on water a very compressed unimolecular layer collapsing at an area of 38 A.^{2} per molecule.

On the other hand, *n*-decyl-*n*-dodecylacetic acid forms a much more expanded film collapsing at about 60 A.^{2}. The surface dipole moment of this film was much smaller than that found for phthioic acid.

This evidence suggested to Stenhagen the presence of a small alkyl group in the α-position to carboxyl, because this might account for the observed close packing of the chains.

He suggested the expression :

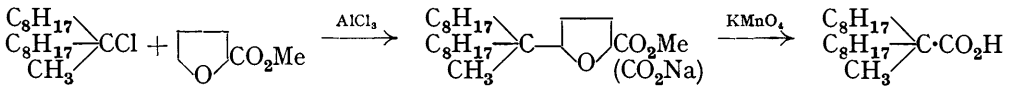


x, *y* about 10 to 12 and different.
z, 0 or 1.

The most probable formula is that of ethyl-*n*-decyl-*n*-dodecylacetic acid. Attempts to synthesise a substance of this constitution are in progress in Oxford and, although the final objective has not yet been reached, the results of my collaborator, Mr. A. J. Birch, are of interest. Only a few of the substances prepared will be mentioned.

In the first place methyldi-*n*-decylcarbinol forms closely packed films, very similar to those of phthioic acid, giving analogous compression curves and collapsing at an area of 38 A.^{2}. Di-*n*-undecylcarbinol, on the other hand, forms an expanded film similar to that of *n*-decyl-*n*-dodecylacetic acid. Neither of these carbinols exhibits physiological activity. In all cases the film studies are due to Dr. E. Stenhagen and the biological tests to his colleague Dr. T. Teorell of the University of Uppsala.

An application of a method devised by Reichstein (*Helv. Chim. Acta*, 1938, **18**, 271) enabled us to prepare a small quantity of *methyldi*-n-*octylacetic acid* :



This acid formed films of phthioic acid type and was physiologically active, producing cell reactions in animals.

The preparation of αα-dimethyl-*n*-decylacetic acid, first obtained by Haller (*Ann. Chim.*, 1914, **1**, 15), has been repeated. When injected into the peritoneum of rabbits, it produced peritonitis with leucæmia, and death ensued.

It is thus abundantly clear that αα-disubstituted long-chain fatty acids exhibit film and physiological properties that go a long way to support the essentials of Stenhagen's view of the nature of phthioic acid.

In order to go further in the synthetic field we are now relying, after many unsuccessful attempts in other directions, on the lengthening of the chain of disubstituted succinic and glutaric acids. Thus methyl *n*-octyl ketone by the Guareschi synthesis furnishes β-methyl-β-*n*-octylglutaric acid, and the ester chloride of this acid reacts with ethyl sodio-α-acetyl-*n*-heptoate to yield an ester which gives 5-keto-3-*n*-octyl-3-methyl-*n*-undecoic acid on hydrolysis. Reduction by Clemmensen's method affords 3-*methyl*-3-n-*octyl*-n-*undecoic acid* (ββ-di-*n*-octylbutyric acid), $(CH_3\cdot[CH_2]_7)_2C(CH_3)\cdot CH_2\cdot CO_2H$. This acid forms compressed films of phthioic type, a circumstance which is not really surprising because immersion in the water may well occur up to the branch. It is, however, not toxic to the animal organism and this indirectly confirms the view that phthioic acid is a trisubstituted acetic acid. In the dioctylbutyric acid we have a non-toxic fatty acid, which is physically of phthioic acid type, and therefore a substance the molecules of which might be expected to be capable of replacing those of phthioic acid or its derivatives when disposed on surfaces. Alternatively, there might be special mutual solubility relations subsisting between the acid and the components of the fat of the micro-organisms. Hence it was tested against tubercle bacilli *in vitro* and was found to be more effectively bactericidal than the C_{20} acids of Adams and Stanley. As it contains twenty carbon atoms and these authors found that C_{16} or C_{17} was the optimum in their series, we have every reason to believe that better compounds of a similar kind will eventually be encountered.

Further experiments are necessary in order to determine whether or no the three

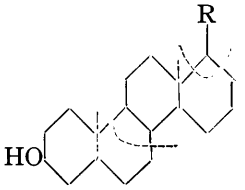
alkyl groups give any real advantage over the two occurring in the substances already studied.

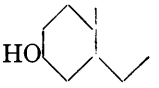
I have discussed these preliminary results in the hope that other organic chemists will be encouraged to enter this field of synthetic work.

B. *A Speculation regarding the Ring Structure of the Sterols and Related Substances.*

Comparison of the structures of the sterinoid group of substances shows that the common features are the skeleton of rings *A*, *B*, *C*, and *D* and the oxygen atom in position 3, whereas the side chain may assume a large variety of forms. Ethylene linkages, hydroxyl and keto-groups appear in several positions in individual substances and aromaticisation of a ring naturally involves extrusion of an angle-methyl group. The basic skeleton is therefore shown in the expression (I) and this contains two similar units as indicated by the dotted lines (cf. De Feu, McQuillin, and Robinson, J., 1937, 53).

(I.)



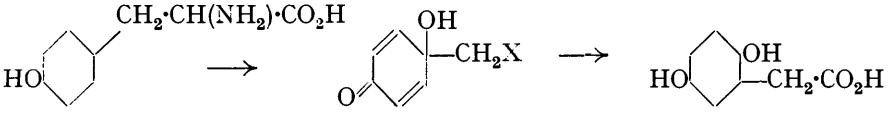


(II.)

The side chain of cholesterol suggests a di*iso*pentane make-up, but this does not apply to ergosterol and stigmasterol, so it is doubtful whether the isoprene hypothesis can be applied to the sterols. It would seem more rational to assume that two identical progenitors together with a component introducing the side chain combine to form the different members of the group.

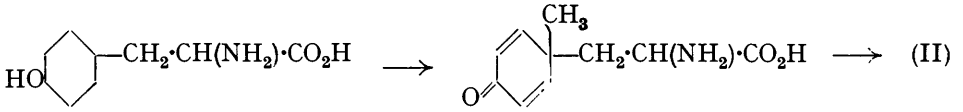
In considering the possible genesis of a group (II) it has already been suggested that it might arise from triose units which are subsequently methylated at some of the α-carbon atoms (*loc. cit.*), but attention is now drawn to a different hypothesis, namely, an origin from tyrosine or, more probably, a protein containing tyrosine residues. Such a view is not to be dismissed on account of the fact that nitrogen is not altered in function during, for example, the growth of ergosterol-producing fungi. It is already known that ammonia may be transferred from an amino-acid to an α-keto-acid. Nor is the theory necessarily disproved if it is found that added tyrosine does not increase the formation of sterols, because the tyrosine so introduced may not be in the appropriate state of combination, it may not reach the active site of the synthetic process, and it may have an unfavourable physiological effect on the natural growth of the organism. Hence it is thought worth while to put this theory on record even though Dr. E. Walker (private communication) has in fact observed an unfavourable effect of tyrosine on the formation of ergosterol by yeast.

In the metabolic error associated with homogentisinuria it has been proved that tyrosine is the source of homogentisic acid (III) and the mechanism is undoubtedly analogous to certain migrations of " chinole " examined by Bamberger.



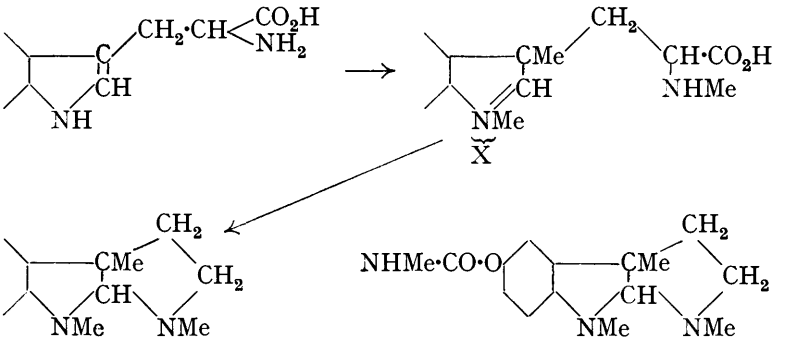
(III.)

Methylation in the *p*-position by the phenolic hydroxyl of tyrosine might by a similar migration give the structure (II) in some form.



Now it is certain that a *C*-methylation of hetero-enoid systems does occur in Nature, the clear cases being methylations of the group N–C=C to N–C–C(Me). This can be traced

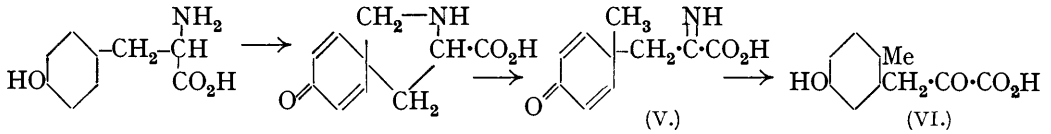
in the berberine group in the formation of such alkaloids as corydaline, but a still better example is physostigmine (eserine) (IV). Here the genesis from a substituted tryptophan is hardly to be doubted and the first approximation is indicated in the scheme :



(IV.)

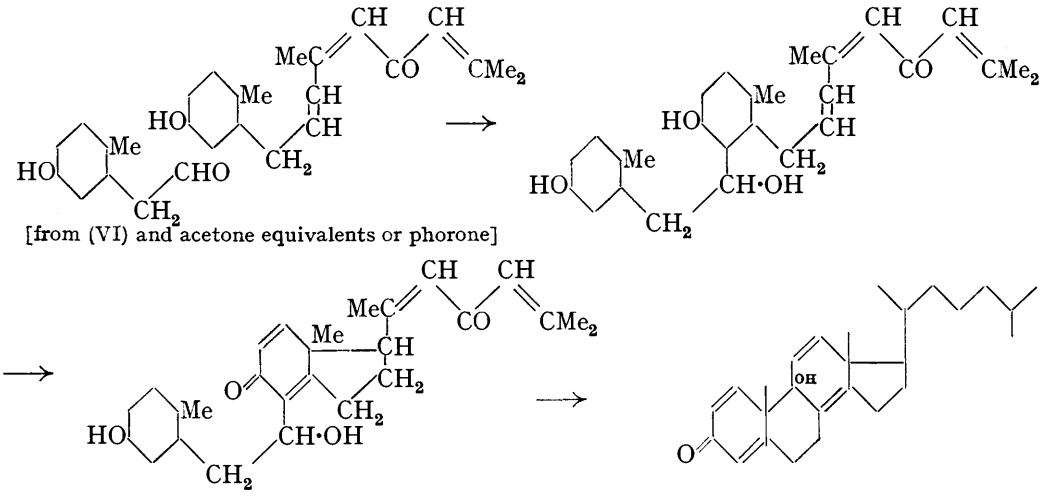
The hetero-anionoid system HO–C=C–C=C– is theoretically analogous to N–C=C and might undergo a similar *C*-methylation.

As a closer approximation it is suggested that formaldehyde (or a source or equivalent of this substance) may be the methylating agent as shown below :



(V.) (VI.)

The carbonyl group in an intermediate of type (V) may attack a *p*-cresol residue in the *p*-position to hydroxyl, whilst the carbonyl of (VI) (aldehyde or α-keto-acid) can effect the union of two molecules. The exact order of the stages cannot of course form an essential part of this theory, which is confined to the rough outlines, but it is at least necessary to show that *one* possible order of events exists. This is shown below, although no importance is attached to the assumed method of formation of ring *D* :



[from (VI) and acetone equivalents or phorone]

By subsequent oxidations, reductions, hydrations and dehydrations and by the use of starting points other than phorone (or equivalents) all the sterinoids known can be accommodated. Thus by starting with the condensation product of (VI) and dihydroxyacetone we may arrive at the expression selected by Marker (*J. Amer. Chem. Soc.*, 1938, **60**, 1725) as the probable progenitor of the sex hormones and corticosterone.