

# THE CHEMISTRY AND METABOLISM OF BACTERIA

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It has been necessary to confine the material to be discussed in this review to certain aspects only of the very broad subject. This course has been dictated not only by the limitations of the reviewer but also necessarily by the exigencies of the moment, and by the consideration that certain portions of the subject will undoubtedly be reviewed under other headings. Thus, many of the contributions to bacterial nutrition and to the use of bacteria for purposes of bioassay can appropriately be included in the more general subjects of vitamins, amino acids, etc. The present review is limited to (a) a discussion of certain aspects of the chemical composition of the bacterial cell and some of its products, (b) that portion of the current work on bacterial nutrition which relates to new and unidentified growth requirements, and (c) some phases of chemotherapy.

## COMPLEXITY OF THE BACTERIAL CELL

One of the most remarkable properties shared by many varieties of bacteria is the existence of a multiplicity of immunological types for each species of organism. In a number of instances, for example, the pneumococci in which there are more than thirty such different types, the differences have been found to be concerned with the existence of chemically different polysaccharides which make up the mucoid capsules surrounding these organisms. Moreover, under certain conditions, these bacteria may undergo a degenerative change or "dissociation," in which the capsules and the immunological type specificity disappear. With the loss of the capsule, virulence also disappears. Colonies of such degenerated pneumococci are smaller than those formed by the encapsulated cells and have lost the glossy surface characteristic of the latter. The terms "smooth" (S) and "rough" (R) are commonly used to describe the original form and its variant. In general, rough forms from all types of pneumococci are indistinguishable either culturally or immunologically.

By certain procedures it is possible to reverse this change and to obtain smooth forms from the degenerate rough forms. Under normal

conditions the original type specificity is regained, that is, a rough Type II reverts to a smooth Type II. However, Griffith (1) in 1928 showed that a rough culture obtained from Type II could sometimes be converted to a smooth form with different specificity (e.g., Type III) by injecting mice with a small inoculum of living rough Type II and a large quantity of heat-killed smooth Type III pneumococci. The Type III smooth, virulent organisms so obtained could be maintained indefinitely on suitable media, and it thus appeared that a pneumococcus of one type could be permanently changed to another type by passing through an intermediate rough form. These experiments, adequately confirmed, were extended by Dawson & Sia (2) who devised an *in vitro* method of accomplishing the same result also using living R forms and heat-killed S forms. Still further refinements were introduced by Alloway (3) in which Berkefeld filtered, cell-free extracts of the S cells were substituted for the whole, killed organisms.

Avery, MacLeod & McCarty (4) have now announced the isolation from smooth Type III pneumococci of a substance, provisionally identified as a polymerized desoxyribonucleic acid, which in minute amount possesses the property of inducing the R  $\rightarrow$  S transformation involving change in type specificity. As little as 0.003  $\mu$ g. in 2.25 cc. of culture fluid was effective. Ultracentrifugation and cataphoresis gave results supporting the conclusion that the nucleic acid polymer was the active substance. Ultraviolet absorption spectra were characteristic of nucleic acid. A tentative estimate of the molecular weight, based on the physical data obtained, led to the figure 500,000. Solutions of the substance were relatively devoid of precipitability by immune sera capable of reacting in high dilution with pneumococcus protein or with Type III specific carbohydrate. In other words, it appears that a polymer of a nucleic acid may be incorporated into a living, degraded cell, and will endow the cell with a property never previously possessed, namely, the ability to produce a capsule composed of a complex polysaccharide entirely different in structure from that produced by the smooth organism from which the degraded form was originally derived. When thus induced the function is permanent, and the nucleic acid itself is also reproduced in cell division. The importance of these observations can scarcely be overestimated and stimulates speculation concerning such matters as the chemical basis for specificity in nucleic acids, and the genetic implications presented by the ability to induce permanent mutation in a cell by the introduction of a chemical substance. Such speculation may well include considerations of the

relation of this phenomenon to the sequence of events following the introduction of a filterable virus (or a bacteriophage particle) into a susceptible cell. A brief consideration of the facts now accumulated concerning the chemical composition of the viruses may bring this relation into perspective.

It is generally recognized that the most outstanding characteristic of filterable viruses as a group is their failure to multiply outside of the host cell. Beyond this single common property there are wide differences in size and in chemical complexity. From the upper extreme represented by the visible elementary bodies of vaccinia and psittacosis viruses and (for the sake of uniformity of concept) the bacillary forms called rickettsiae, down to the crystalline plant viruses and such agents as poliomyelitis and foot and mouth disease viruses which approach the dimensions of large protein molecules, there are representatives of a wide range of sizes. Similarly, in regard to chemical complexity, while all viruses contain nucleoprotein and the smallest may consist of single molecules of this material alone, the larger ones approach the bacterial cell in complexity, containing lipoid, polysaccharides, and even certain enzymes. Thus as a group viruses may reasonably be considered as living cells deficient in a range of vital functions which must be supplied by a suitable host cell. For example, the larger viruses may be devoid of oxidative mechanisms only, but retain many synthetic capabilities. The latter drop out progressively as size and complexity decrease until nothing remains in the smaller viruses but a large molecule of nucleoprotein multiplying as a result of the combined vital processes of the host cell.

Considerable definite information relating to the chemical composition of the largest and the smallest of the viruses has been available for some time. Information has now been provided by Taylor and others about viruses of intermediate size (5, 6, 7), namely influenza viruses A and B and swine influenza virus. Lipoid components varied from 21 to 24 per cent among the three, and included neutral fat, phospholipid, and cholesterol. The remainder consisted of polysaccharide, protein, and desoxyribonucleic acid. Even these viruses of intermediate size, therefore, are still relatively complex entities. It would be interesting to know whether they still possess certain enzymes such as phosphatase which has been found to be present in vaccinia virus (8) though absent from small viruses such as the polyhedral virus of *Lymantria monacha* L. (9), and tobacco mosaic and bushy stunt viruses (10). Williams, Schlenk & Eppright (11) re-

ported the absence of the various components of the B vitamin complex from tobacco mosaic, tobacco necrosis, and bushy stunt viruses and from a strain of influenza A virus. They believe such absence carries implications of the inanimate nature of viruses. In the light of the above discussion it seems probable that the cellular functions dependent upon these readily extractible substances may well be missing from even the largest viruses, which may yet retain so complex a make up and properties so varied as to bring their inanimate nature into considerable question.

The one component common to all viruses, therefore, is nucleoprotein, while in the case of the substance inducing variation in the pneumococcus, nucleic acid alone is involved. In the latter case a synthetic and purposeful change is brought about, i.e., the ability to produce a polysaccharide capsule rendering the cell resistant to certain destructive influences. On the other hand, so far as is known, viruses lead to degenerative changes in the host cell and eventually to its death. Conceivably, therefore, the crucial difference between the transplanted "gene" and the smallest virus may lie in the specificity of the protein which is present in the latter, or to which the former probably attaches itself in the cell. In the one case the protein heterologous to the cell, renders the particle a completely foreign parasitic molecule, which eventually becomes injurious as it accumulates. In the other instance, the cell's own protein combines with the new nucleic acid, and the resulting homologous nucleoprotein takes its place in the normal economy of the cell and confers a new property upon it. In any case, these considerations again indicate the enormous complexity of cellular and bacterial protoplasm, and emphasize our complete ignorance of the many and varied metabolic processes which proceed inside the cell wall.

As further illustrations of the great complexity of the bacterial cell a few instances from the contributions of the current year may be mentioned, selected primarily because of some bearing on medical bacteriology.

Stockinger, Ackerman & Carpenter, for example, reported extensive studies of the composition of the gonococcus (12) and its products (13). They described two nucleoprotein fractions obtained by extraction of mass cultures. One of these contained considerable combined lipid. Carbohydrate was found in three different forms, but a polysaccharide having type specific properties was not detected. A variety of lipoidal constituents were present, among which a lecithin,

a cephalin, and sphingomyelin were identified. From broth in which the gonococcus had been grown, they isolated a protein, believed to be a degradation product of the cellular nucleoprotein. It was moderately toxic to animals and possessed immunological specificity as measured by complement fixation. Boor & Miller (14) prepared carbohydrate-lipid complexes from a number of strains of gonococcus and meningococcus. These "glucolipids" were also moderately toxic for animals and were antigenic in rabbits. Sera so produced precipitated both the glucolipid itself and the carbohydrate component separated by acid hydrolysis. These complexes, therefore, have properties similar to compounds of the same sort prepared earlier by Boivin & Mesrobian (15, 16) from a variety of gram negative bacilli.

Kabat, Kaiser & Sikorski (17) have prepared a polysaccharide from Type I meningococci which is electrophoretically homogeneous, and which is type-specific. It is weakly but definitely antigenic in man. Direct comparison with an earlier product obtained by Scherp & Rake (18) indicates that the avoidance of acid or alkali during the preparation of the more recent material has resulted in a somewhat purer and more nearly natural antigenic substance.

An antigenic capsular polysaccharide has been obtained from *Cl. perfringens* by Svec & McCoy (31). It appears to be common to most members of the *perfringens* group, regardless of their toxigenic properties.

The tremendous amount of investigation on the chemistry of tuberculin carried out in the last two decades has been reviewed during the year by Seibert (19), particularly in regard to carbohydrates, nucleic acid, and protein. Corper & Cohn (20) have compared the tuberculoprotein obtained from autolyzed tubercle bacilli with that present in culture filtrates, and found similar properties, together with greater purity, in the former. Chargaff & Moore (32) described the isolation of glycogen from tubercle bacilli. The molecular weight of the material was shown to be of the order of 12 to 13 million. Anderson and his collaborators (21, 22, 23) have added to an already extensive investigation of the lipids of the tubercle bacillus, and carried out similar studies (24, 25) with *Phytomonas tumefaciens*, incited by the possibility that some of its lipid material may be responsible for the ability of the organism to induce plant galls. An unidentified fat acid with the formula  $C_{20}H_{40}O_2$  was isolated. It is believed to possess a branched chain.

The capsular material of certain hemolytic streptococci, identified

some time ago as hyaluronic acid, has aroused considerable interest as to its possible relation to the virulence of this organism. Some evidence pointing in this direction has been adduced by Kass & Seastone (26). Other bacteria and even some strains of hemolytic streptococci may secrete an enzyme, hyaluronidase, capable of hydrolyzing this acid. This enzyme appears capable of acting on the tissues of the body at the site of infection in such a way as to lead to a rapid dissemination of particulate material (India ink, bacteria) and is an important member of a group of substances called "spreading factors" which share this property. This subject was reviewed in 1942 by Duran-Reynals (27). In the current year, however, Crowley (28) has examined a considerable number of strains of hemolytic streptococci for hyaluronidase production, and finds no relation between this substance and virulence. Similar results have been obtained by Humphrey (29) for various pneumococci, and by Evans (30) for the Welch bacillus. The significance, therefore, of the production of hyaluronic acid by some bacteria, and of hyaluronidase by others is not yet clear.

#### GROWTH REQUIREMENTS OF BACTERIA

As already stated, it is not proposed to review completely the voluminous literature which continues to accumulate on bacterial growth requirements. The significant developments of the year will quite surely be reviewed elsewhere in this volume. It may not be out of place to point out at this time the accuracy of the prediction made by the writer more than twenty years ago (33) that definite knowledge of bacterial growth requirements would quite certainly supply information of considerable value in fields other than bacteriology. The incredibly close relation between the nutrition of animals and that of microorganisms, and the present concept of certain types of chemotherapeutic action more than justify the earlier optimism.

The bacterial culture still appears to offer the most convenient tool for the definition of unrecognized nutritional substances, for the existence of which there is ample evidence. The factor which seems nearest to complete elucidation is "folic acid." The rather chaotic facts bearing on the chemistry and physiology of this and related substances have been recently reviewed (34). Probably nothing can bring greater testimony to the change in status of bacterial nutrition in the last twenty-five years than the present state of affairs in which most of the pharmaceutical companies in the country are competing

for first place in clearing up this tangle. The writer recalls somewhat grimly the difficulties encountered in 1920 while attempting to enlist cooperation in getting a hundred pounds of casein hydrolyzed with sulfuric acid, from which the first of the unrecognized growth factors for bacteria (methionine) was eventually isolated.

There are a number of observations of other probably new growth factors. Their relationship to each other, to "folic acid," and to factors already more accurately defined must await chemical identification. Happold and his collaborators (35, 36) have described a substance occurring in acid-hydrolyzed casein, and more abundantly in liver extract, which is required for the growth of certain strains of *C. diphtheriae* and of *L. casei*. From liver extract a considerable degree of purification has been achieved, and certain of the properties of the material have been defined. It is not replaced by folic acid. A second factor greatly stimulating early growth of *L. casei* appears also in liver extract, and behaves in such a manner as to lead the authors to believe it may be identical with a growth factor for *L. casei* described by Pollack & Lindner (37) as present in Wilson's bacteriological peptone. The latter authors describe the substance as being stable at 100° between pH 2 and 11, but reduced in potency to half its original strength by heating at pH 12 and destroyed by 0.7 *N* sulfuric acid. Happold's factor, however, is stated to be adsorbed by norite at pH 3, whereas the substance in peptone is said not to be adsorbed to any extent by charcoal (Darco G-60) nor by a variety of other adsorbents between pH 3.0 and 8.0. A third factor for *L. casei* is described by Happold as occurring in liver extract in a combined and inactive form. Gentle hydrolysis splits it off in an active condition.

Smith (38) reported the presence in yeast extract of a growth stimulant for *Streptococcus lactis*. It failed to precipitate with the heavy metal salts and was not adsorbed by charcoal (Darco) or fullers' earth.

Sprince & Woolley (39) point out the similarity between the second Happold factor (above) for *L. casei*, the Pollack & Lindner factor for the same organism, the Smith factor for *S. lactis*, and a factor for the hemolytic streptococcus previously described by Woolley (40). Some evidence for this similarity is presented, and the name "streptogenin" is tentatively suggested for the substance. The efficacy of a number of concentrates of "solubilized liver extract" prepared in various ways was compared on the three varieties of bacteria and in each case the relative recovery of active material was the same for all

three organisms. One of the preparations employed was not made from liver extract but from partially acid-hydrolyzed vitamin-free casein. The "per cents of recovered activity" for the three organisms are stated to be 17, 19, and 18 respectively. It is not clear to the reviewer what value, in this case, is taken as 100 per cent, since both the original casein and its complete acid hydrolysate are said to be inert. In any event decision on the relationship of these several growth manifestations must await the isolation or better characterization of an active material.

Evidence for the existence in tomato juice of an unidentified stimulant for growth of *L. arabinosus* has been given by Kuiken and collaborators (41). It is prepared by adsorption on charcoal and elution with a mixture of pyridine, alcohol, and water. It is stable to strong acid hydrolysis, but its properties are not further described.

Ballentine *et al.* (42) have studied a growth factor for *Cl. perfringens* occurring in yeast extract. After precipitation by lead hydroxide, the substance was recovered with hydrogen sulfide and impurities removed with the resin Amberlite IR-4 followed by norite adsorption. It was stated to be precipitable by mercury and by picric acid but not by a variety of other reagents. It was stable to cold normal acid and alkali though inactivated by both in five minutes of boiling.

Welton, Stokinger & Carpenter (43) have presented the composition of a synthetic medium for the growth of the gonococcus. One of the components is indole-3-acetic acid, and thus by implication this substance is essential for the growth of the organism and must be considered to be a potential bacterial growth factor. So far as the reviewer is aware, this is the first available evidence that a plant auxin may function as a growth stimulant for bacteria, and the observation deserves further amplification.

Gould (44) has found that certain passage strains of the gonococcus develop a requirement for glutathione. This appears to be the first instance of the identification of this substance as a bacterial growth factor. The strains which depend upon the presence of this material for growth are inhibited by low concentrations of cystine, possibly through a blocking or competitive action in some essential system.

Finally, the presence of two chloroform-soluble components of liver extract which promote the growth of *Lactobacillus helveticus* and *Streptococcus lactis* has been described by Barton-Wright, Emery & Robinson (45). These substances are not destroyed by nitrous acid, or by acetylation or benzylation. They are unique among the

factors mentioned in this review because of their solubility in chloroform. The authors promise a further description. It is possible that fatty acids or other lipid material may be at least partially responsible for the effects noted. Oleic acid and oleates are known to act as growth stimulants or depressors (according to concentration) for the diphtheria bacillus (46) and the tetanus bacillus (47). Moreover, Kodicek & Worden (48) have recently directed attention to a variety of changes in the growth of *L. helveticus* obtainable with materials which are chloroform-soluble and almost certainly occur in liver preparations. Their results showed that growth of the organism is augmented by palmitic and stearic acids, though inhibited by oleic, linoleic, and linolenic acids. The effect of linoleic acid is reversed by either cholesterol or lecithin.

#### CHEMOTHERAPEUTIC ACTION

Evidence continues to accumulate in support of the view, first expressed by Fildes (49), that chemotherapeutic substances operate by blocking some specific chemical grouping necessary for the successful growth of the microorganism. The empirical observation of the value of sulfanilamide and other related drugs as therapeutic agents was followed by the discovery of a specific inhibitory substance in tissue extracts, and later by the provisional identification of this substance as *p*-aminobenzoic acid (50). Subsequently this compound was shown to be essential for the growth of certain bacteria, and therefore presumably to function as a component of some metabolic system. Sulfanilamide appears to be sufficiently similar in structure to combine at the same point, but the next step in the reaction fails to occur and the metabolism of the cell is arrested. The relatively low toxicity of the sulfonamides for animals may be due either to production in the cells of sufficient *p*-aminobenzoic acid to prevent the blocking or to the ability of tissues to replace the blocked function by some alternative mechanism.

A wide variety of compounds of the general nature of *p*-aminobenzoic acid and sulfanilamide, but with various substituent groups at different positions, have been tested both as bacteriostatic substances and as inhibitors of sulfonamide stasis (51 to 55), and certain general relationships appear to have been established. The nature of the process which normally involves *p*-aminobenzoic acid remains undetermined. There is evidence, however, that two of the sulfonamides,

sulfapyridine, and sulfathiazole, also block a second function which is concerned with a nicotinamide-stimulated respiratory process (56, 57, 58). This latter effect is not prevented by *p*-aminobenzoic acid. Moreover, in recent work, Reed, Orr & Reed (59) describe differences between *in vitro* and *in vivo* effectiveness of the sulfonamides on the gas gangrene group of bacteria and intimate that evidence is to be forthcoming that two types of drug inhibition may exist.

Differences between species of bacteria in relation to sulfa drug susceptibility are of considerable interest. That within the same species may be found both susceptible and resistant strains, and that the former can apparently acquire the properties of the latter either *in vitro* or *in vivo*, are facts of the utmost practical importance. Resistance to the action of one of the sulfonamides usually extends to all the others, and once established, the property seems to be permanent. In the test tube, the change may be brought about by exposure to sublethal concentrations of the drug. The same mechanism probably operates in the body. It is consequently not surprising that evidence has already begun to accumulate that certain human infections originally yielding in most instances to drug therapy may become increasingly refractory to this type of treatment. The widespread use of small doses of sulfadiazine for the prophylactic checking of such bacteria as the meningococcus, gonococcus, and streptococcus would appear especially suited to the elimination of susceptible strains and their replacement by others, equally virulent, but so altered metabolically as to be highly resistant to the sulfonamides. That this is more than a theoretical possibility is illustrated by the fact (60) that in 1940-41 about 70 per cent of cases of gonorrhoea treated in one Boston clinic were promptly cured by sulfonamide therapy, while the remaining 30 per cent were unaffected. At the present time the proportions are approximately reversed, and but for the timely introduction of penicillin it seems probable that the chemotherapy of this infection would shortly have become relatively ineffective.

Experimental demonstration of the acquisition of drug resistance *in vitro* has been made by numerous workers (e.g., 61, 62, 63). The possibly complex nature of the change is indicated by the observation (64) that whereas pneumococci which have acquired a high degree of resistance to sulfonamides appear to remain permanently in that state, partial resistance may be lost rather promptly when contact with sulfonamide is discontinued. In some instances, at any rate, resistance appears to be due to increased production of *p*-aminobenzoic acid

(65), but not all naturally resistant organisms produce extracellular inhibitors (66). Fortunately resistance to the sulfonamides does not parallel that against penicillin (67). Information on the mode of action of this antibiotic is not yet available.

A peptide containing ten or twelve glutamic acid residues linked to the carboxyl group of *p*-aminobenzoic acid has recently been isolated from yeast (68). It is not antagonistic to the sulfonamides until hydrolyzed by acid.

In view of the clinical success attending the use of the sulfonamide drugs, and of the development of a well supported theory of their mode of action, it is not surprising that a number of attempts have been made to explore the possibility of using derivatives of other known growth factors as chemotherapeutic agents. Thus, McIlwain (69) has shown that pyridine sulfonic acid inhibits the growth of organisms requiring nicotinic acid or nicotinamide and recognized three "types" of inhibition depending upon the individual circumstances. The sulfonic analogue of pantothenic acid, "pantoyltaurine," has been prepared by Snell (70, 71) and shown to inhibit the growth of bacteria which require pantothenic acid. The inhibition was reversed by additions of the latter. Similar observations were reported independently by Kuhn, Wieland & Moller (72) who also observed the specific antagonistic effect of pantothenic acid and, in addition, noted a partial annulment by  $\beta$ -alanine. McIlwain (73) has obtained similar results and shown that the effect is inhibited by pantothenic acid and by  $\beta$ -alanine. Certain strains of streptococci were resistant to the action of pantoyltaurine. This property was entirely unrelated to sulfonamide resistance (74). Neither was it related to the requirement of pantothenic acid for growth, which was common to all strains examined. Resistant strains could be rendered susceptible by salicylate and it is suggested that this fact may be evidence for the existence of alternative mechanisms for the same function, one involving pantothenic acid, the second blocked in some way by salicylate. In the case of the diphtheria bacillus, McIlwain was able to effect the transposition of strains susceptible to the action of pantoyltaurine into resistant forms by "training" those originally requiring pantothenic acid to produce their own (75). This was accomplished by gradual withdrawal of pantothenic acid and demonstrates that drug resistant strains may develop without the presence of the drug itself. Certain of the above observations have been extended by quantitative studies of the fermentation of glucose by streptococci under circumstances in which

pantothenic acid and pantooyltaurine can be shown to exert antagonistic effects (76, 77).

McIlwain & Hawking (78) have shown that in rats, pantooyltaurine acts as a moderately effective chemotherapeutic agent against hemolytic streptococci. It is ineffective in mice, which have a higher level of pantothenic acid in the blood. The possibility is pointed out that pantooyltaurine may be of use in human streptococcus infection, since the concentration of pantothenic acid in human blood is even lower than in rat blood.

Nielsen & Johansen (79, 80) have shown that with a yeast, stimulation of growth induced by  $\beta$ -alanine is prevented by  $\beta$ -amino butyric acid and by isoserine, but not by taurine.

The pyridine analogue of thiamine, "pyrithiamine," shown by Woolley & White (81) to induce symptoms of thiamine deficiency when fed to mice, was later found by the same authors (82) to inhibit the growth of bacteria requiring thiamine or its component moieties. Again, the effect was reversible by the natural vitamin. A resistant strain of yeast was obtained by growth in the presence of the inhibitor (83). This strain, however, still required thiamine or its pyrimidine portion as a growth factor.

Studies on possible modifications of biotin are beginning to appear. Desthiobiotin, described by du Vigneaud and his associates (84) can replace biotin for growth of a yeast but not of *L. casei*. It was later shown (85) that in the case of the latter organism, the substance in suitable concentration inhibits growth, acting as a specific antibiotin. Biotin sulfone and imidazolidone caproic acid exert a similar effect (86) while a number of other derivatives and related compounds either partially replaced biotin or were inert. The ability of certain of these compounds to replace biotin from its union with avidin is also reported. Lilly & Leonian (87) investigated the effect of desthiobiotin on a large number of microorganisms, which they were able to classify as to their behavior toward the compound. Antibiotin activity was manifested toward some but not all varieties unable to use desthiobiotin.

Modification of riboflavin by substitution of two methyl groups by chlorine yielded a product showing bacterial growth inhibitory properties for certain organisms (88). The inhibition was reversed by riboflavin.

That modifications even of the amino acids may result in compounds which interfere with normal bacterial growth is evident from

McIlwain's (89) experiments with sulfonic analogues of glycine, alanine, valine, leucine, and aspartic acid. Delay or inhibition of bacterial growth resulted from their presence, but the effect was readily reversed by natural  $\alpha$ -amino acids, regardless of specific structure. Fox, Fling & Bollenbach (90) obtained considerable inhibition of growth of *L. arabinosus*, which requires *l*-leucine, by means of the unnatural isomer. This observation should be amplified, since synthetic amino acids have been universally employed in bacterial growth experiments. In the work cited the proportion of *d* to *l* form is given as 200 to 1. Since general experience has led to the belief that the forms not occurring in nature are sometimes used quite as readily as the natural forms, in other instances are inert and occasionally have exhibited anomalous behavior (see for example 91-93) a further quantitative as well as qualitative investigation of the phenomenon is desirable.

The facts thus far available in connection with the effect of chemotherapeutic agents on diseases due to filterable viruses are generally in harmony both with the commonly held explanation of sulfonamide action and with the view that viruses are dependent upon host cells for many of their functions. Thus, while it has been known for some time that the sulfonamides were moderately effective against such large viruses as psittacosis and lymphogranuloma, they are completely inert in the case of the smaller agents (94 to 97). The typhus fever rickettsia appears to be moderately susceptible to penicillin, and, curiously, to *p*-aminobenzoic acid (98), a fact for which no ready explanation seems obvious. It is possible that as more knowledge of the vital processes remaining to intracellular parasites becomes available, some form of systematic approach to their chemotherapy may be found. Thus far, the empirical attack has been entirely negative although a wide variety of organic compounds of various types has been investigated (99).

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