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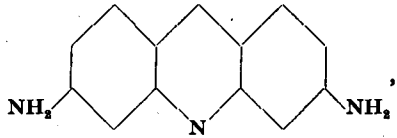
The compound referred to as 2:7-diaminoacridine is



normally designated 2:8-diaminoacridine.

147. A NUTRITIONAL INVESTIGATION OF THE ANTIBACTERIAL ACTION OF ACRIFLAVINE

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STUDIES of the mode of action of chemotherapeutic agents, based on the hypothesis that they interfere with metabolites essential to pathogenic organisms, have recently been successful in interpreting the antibacterial action of sulphanilamide [Woods, 1940] and mercuric salts [Fildes, 1940, 1], and in suggesting lines for the development of further therapeutic agents [Fildes, 1940, 2; McIlwain, 1940, 2, 3]. In these papers [see also McIlwain, 1941] it is shown that inhibitory agents cause fresh nutritional needs in the affected organisms, and that when these are satisfied growth can take place in the presence of an otherwise inhibitory concentration of therapeutic agent ('reversal' of the inhibitor). There is a general realization that therapeutic agents may act by affecting metabolic processes, and the present method of investigating their action is possible only because more exact knowledge of bacterial nutrition is now available. Thus Ehrlich [see Muir, 1915–16] suggested that the normal function of the 'receptors' was nutritive, though he later abandoned, or failed to emphasize, this view [Ehrlich, 1909; 1913]. Metabolic ideas have more recently been applied to acridine compounds by Quastel & Wheatley [1931] and by Dickens [1936]. Analogous conceptions of enzyme interference have also successfully explained the pharmacological actions of eserine as due to blocking of choline esterase.

The acriflavine drugs were developed by Ehrlich as trypanocides, but their main current use is as antiseptics. *Bact. coli* and *Streptococcus haemolyticus* were the organisms mainly used in the present work. Acriflavine (here used as a general term) is a mixture of 2:7-diamino-acridine, proflavine, and its 9-methochloride which is sometimes, as in this paper, referred to as euflavine. Slight differences in the actions of the two compounds have been described. The present work was mainly carried out with euflavine; the conclusions were found applicable to proflavine.

EXPERIMENTAL

Each test comprised a tube in which the organisms were grown from a small inoculum in a chemically defined liquid medium, a second in which growth was prevented for several days by a suitable concentration of acriflavine component, and further tubes which contained all the above materials together with different concentrations of additional nutrients whose relation (as 'reversing factors') to acriflavine inhibition was being studied.

Bact. coli will grow in a simple medium containing per tube of 10 ml.: KH_2PO_4, 45 mg.; $(NH_4)_2SO_4$, 5 mg.; NH_4Cl, 5 mg.; Na lactate, 28 mg.; Fe and Mg salts, traces; NaOH to *p*H 7·6. For specific reversal effects it was necessary to add further nutrients; in the present experiments these comprised: glucose, 12 mg.; aneurin, nicotinamide and β-alanine, 12μg.; pimelic acid, uracil, cytosine, thymine and guanine, 25μg.; methionine and tryptophan, 125μg.; cystine,

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600μg. The inoculum of about 1000 organisms, grown in the simple medium, was added last, and the tubes were incubated in a 5% CO_2-air mixture at 37°. *Streptococcus haemolyticus* (groups A and C) was grown according to McIlwain [1940, 1] in media containing thiolacetate, pantothenate concentrates (which contain other nutrients, probably biotin) and $2 \times 10^{-8} M$ riboflavin.

Differentiation of reversing effects

Various extracts of natural materials, of which some 30 were made, removed the inhibition of *Bact. coli* caused by addition of euflavine to the medium described above; such reversal was specific in the sense that the concentrations of extracts causing it were without visible effect on uninhibited growth (Table 1).

Table 1. *Differentiation of reversing factors*

Euflavine M ($\times 10^{-5}$)	Reversing factor: Material	Reversing factor: mg./ml.	Growth* after: Hours: 16	Growth* after: Hours: 24	Growth* after: Days: 2	Growth* after: Days: 3
0	0; or, (*a*) (see below)	150	+ + +	+ + + +	+ + + +	+ + + +
	(*b*) (see below)	4				
3 or 6	0	—	0	0	0	0
3	(*a*) Alkaline yeast† preparation (Type I)	150	+ + +	+ + + +	+ + + +	+ + + +
3	,,	30	+	+ + +	+ + +	+ + + +
3	,,	6	0	0	+ + +	+ + + +
6	,,	150	+ + +	+ + + +	+ + + +	+ + + +
6	,,	30	0	+	+ + +	+ + + +
6	,,	6	0	0	0	+ + +
3	(*b*) Tryptic casein (Type II)	4 or 0·8	+ +	+ + +	+ + +	+ + +
3	,,	0·16	+	+ + +	+ + +	+ + +
3	,,	0·032	0	+ +	+ + +	+ + +
6	,,	4	0	+	+ +	+ +
6	,,	0·8 or less	0	0	0	0

* Here and in other tables: organism, *Bact. coli*. Plus signs are proportional to mass of growth, estimated visually. Concentrations are those in final media.

† An alcohol precipitate of an alkaline extract of yeast; the figures refer to the weights of yeast from which the addenda were prepared.

The reversing activity was, however, complex in that it appeared due to at least two types of material differing in occurrence and in chemical and biological properties. Thus marmite or casein hydrolysates were very active (little material being necessary for reversal) against $3 \times 10^{-5} M$ euflavine, while an alkaline extract of yeast was much less active; against $6 \times 10^{-5} M$ euflavine, however, the alkaline extract of yeast was almost equally active, while more than 100 times the previous quantity of tryptic casein was not. The interrelations between inhibitor and reversing agents thus approximate to those named Types I and II in a previous nutritional study of inhibitory action [McIlwain, 1940, 2]. Analogy with the above types is not complete, as the reversing factors in the former investigation were also essential for uninhibited growth, but the points of similarity are: in the case of Type I action, the effects of increased inhibitor concentration can be reversed by a comparable increase in reversing agent, while in the case of Type II action such increase does not lead to comparable reversal.

Nucleic acid and nucleotides as Type I factors

The sources and modes of extraction which yielded most material reversing according to Type I, and the preliminary chemical reactions of the material, led to attempts to remove the inhibition of euflavine with nucleic acid. This was immediately successful. Purification of commercial yeast nucleic acid according to Gulland & Jackson [1938] and by Na acetate-alcohol precipitation, did not afford fractions of marked difference in activity. Thymus nucleic acid was equally active. Processes which degrade nucleic acid greatly reduced its activity (Table 2). It was concluded that the intact nucleic acid molecule is most effective in reversal, though this necessarily lacks the final confirmation of synthetic material. A lesser activity on the part of smaller nucleotides was confirmed by experiments with crystalline specimens of adenylic and guanylic acids, and with cozymase (Table 2).

Table 2. *Nucleotides as Type I reversing factors*

Substance added	Approx. molarity	Fluorescence before mg./ml.	inoculation	Growth after (days) 1	2	3	5
0	—	—	+ + +	0	0	0	0
Na nucleate*	4×10^{-4}	0·5	0	+ + + +	+ + + +	+ + + +	+ + + +
	8×10^{-5}	0·1	+	+	+ + + +	+ + + +	+ + + +
	$1{\cdot}6 \times 10^{-5}$	0·02	+ +	0	+ +	+ + + +	+ + + +
Nucleic acid NH_4OH hydrolysate†	—	0·5	+	0	+ +	+ + + +	+ + + +
	—	0·1	+ +	0	0	+ +	+ + + +
	—	0·02	+ + +	0	0	0	0
Nucleic acid H_2SO_4 hydrolysate‡	—	0·5	+ + +	0	+ +	+ + +	+ + +
	—	0·1 or 0·02	+ + +	0	0	0	0
Adenylic or guanylic acids	$1{\cdot}5 \times 10^{-3}$	0·5	+ +	0	+ +	+ + +	+ + +
	3×10^{-4}	0·1	+ + +	0	0	+ + +	+ + +
Cozymase§	10^{-5}	—	+ +	0	+ + +	+ + + +	+ + + +
	2×10^{-6}	—	+ + +	0	0	+ +	+ +
Uninhibited control			+ + + +				

Inhibiting concentration of euflavine, $4 \times 10^{-5} M$.
* Commercial or purified yeast sodium nucleate, or thymus nucleate.
† By 2·5% NH_4OH autoclaved in aqueous solution at 10 lb./sq. in. for ¾ hr.
‡ By 2% aqueous H_2SO_4 refluxed for 2 hr.; concentrations given refer to the initial nucleate.
§ Not pure material.

Reversal of euflavine inhibition with purified nucleic acid took place also with *Bact. coli* in ordinary broth and in the simple ammonium lactate medium, and with *Streptococcus haemolyticus*. Inhibition due to proflavine was also removed.

Relation between Type I activity and euflavine fluorescence

The familiar greenish fluorescence of acriflavine solutions in visible light is shown well at the concentrations used in these tests. It was very noticeable in the present colourless media that this fluorescence sometimes disappeared with growth. The disappearance of fluorescence was not caused by removal of euflavine from the solution, which remained yellow, though such removal was caused to some extent by the bacterial cells. It could be simulated by the addition of acid, though the *p*H necessary was much lower than that attained during growth.

In mixing test solutions prior to inoculation it was observed that Type I sources, and nucleates, removed or reduced acriflavine fluorescence and that there was a direct correlation between such removal and the rapidity of subsequent growth (Table 2). As removal of fluorescence in acid solution is attributed to salt formation, it was considered that acriflavine probably formed with nucleic acids complex salts which, unlike those with simple acids, were stable at neutral *p*H. Mixing solutions of proflavine and nucleate showed this to be the case. In strong solutions a brown precipitate formed, with maximal precipitation from 4 mols. of proflavine to one of nucleate. To proflavine (0·5 g.) in water (100 ml.) was added yeast sodium nucleate (0·65 g.) in water (20 ml.). The precipitate was filtered after ½ hr., washed with water, dried *in vacuo*, ground to a red powder and further dried at 60° *in vacuo*; the product contained P, 5·98 % (calc. for $C_{38}H_{43}O_{28}N_{15}P_4.4C_{13}H_{11}N_3$, P, 5·85 %). In common with other nucleates, it was not crystalline, but a crystalline salt was prepared from adenylic acid. Proflavine base (0·1 g.) was added to a hot suspension of yeast adenylic acid (0·13 g.; found, P, 8·89 %; $C_{10}H_{14}O_7N_5P$ requires P, 8·94 %) in water (4 ml.) and the resulting brown solution filtered. Rosettes of small brown crystals which formed on cooling were filtered, washed with water and dried *in vacuo* at 60°: M.P. 189–191°; P, 5·54 % (calc. for $C_{10}H_{14}O_7N_5P.C_{13}H_{11}N_3$, P, 5·58 %). Qualitative evidence of complex formation was also obtained with thymus nucleic acid and guanylic acid.

Removal of fluorescence of dilute acriflavine solutions by nucleate does not occur with equivalent concentrations, but requires an excess of nucleate, as does also the reversal of acriflavine inhibition. Thus with $2{\cdot}5 \times 10^{-4}M$ proflavine; 1·2 equiv., with $10^{-4}M$, 1·6 equiv., and with $10^{-5}M$, 3·2 equiv. of yeast nucleate were required for removal of visible fluorescence. This is attributed to dissociation of the salt. Salts of acridine itself with adenosine phosphoric acids are already known [Wagner-Jauregg, 1936].

Amino-acids as Type II factors

As fractionation proceeded it was found that Type II factor, unlike Type I, did not remove the fluorescence of acriflavine solutions. Marmite was the most readily available good source; the following were active, in decreasing order: casein hydrolysates; liver, heart and testicle extracts, especially press juice or cytolysates; potato, blood, serum and milk.

Marmite was fractionated by the techniques familiar in nutritional work, purification being followed by comparing the dry weight or amino-N (see (*d*) below) of fractions corresponding to 1 g. of marmite, with their reversing activity (see Table 3). The following successive processes yielded material of the activity described:

(1) *Starting material*: dry wt., 800 mg.; amino-N, 42 mg.

(2) *Lead acetate precipitation* at *p*H 3. Filtrate: amino-N, 36 mg.

(3) *Mercuric acetate precipitation* at *p*H 7. Decomposed precipitate: dry wt., 290 mg.; amino-N, 18 mg.

(4) *Butyl alcohol extraction* of dried material from Hg precipitate: amino-N, 7 mg.

(5) *Adsorption to alumina* from butyl alcohol-ether solution and elution with water. Eluate: dry wt., 27 mg.; amino-N, 3·6 mg.

The active material had the following properties.

(*a*) *Stability*. At stage 5, but not earlier, it was stable to autoclaving (120°, 20 min.). It was throughout stable to boiling with *N* or 20 % H_2SO_4 in an inert atmosphere, but not in air; thus though many protein hydrolysates were active

Table 3. *Concentrates and amino-acids as Type II factors*

Substance added	NH_2-N molarity	mg./ml.	Growth after: Hours 16–18	Hours 24	Days 2	Days 3
0	—	—	0	0	0	0
Marmite	3×10^{-3}	1	+ + +	+ + +	+ + +	+ + +
	6×10^{-4}	0·2	+	+ +	+ + +	+ + +
	$1{\cdot}2 \times 10^{-4}$	0·04	0	+ +	+ +	+ + +
Al_2O_3 eluate (stage 5)	$2{\cdot}6 \times 10^{-4}$	0·027	+ +	+ +	+ + +	+ + +
	$5{\cdot}2 \times 10^{-5}$	0·0054	+	+ +	+ + +	+ + +
	10^{-5}	0·0011	0	0	+ +	+ + +
Amino-acid mixture (see text)	10^{-2}	1·3	0	+	+ + +	+ + +
Phenylalanine	5×10^{-3}	0·84	0	0	+ + +	+ + +
	10^{-3}	0·168	0	0	+	+ + +
	Uninhibited control		+ + +	+ + + +	+ + + +	+ + + +

Inhibitory concentration of euflavine, 3×10^{-5} *M*.

when produced in N_2, they were little so when normally produced in air. Boiling with 10*N* NaOH in N_2 reduced the activity to ½. It was not affected by catalytic reduction in acid solutions, by $NaHSO_2$ in acids or alkalis, nor by HIO_4.

(*b*) *Solubility.* The material could not easily be removed from aqueous solution by amyl or butyl alcohols, but these solvents, or ethyl alcohol, extracted it from dried material; ether or chloroform did not. The extractions were not markedly affected by *p*H between 3 and 10.

(*c*) *Adsorption* did not occur from aqueous solutions of *p*H 7, 10 or 3 (when this could be used) by norite, alumina, or fuller's earth when shaken with the adsorbent, but adsorption took place to columns of permutite from aqueous solution and to alumina from butyl alcohol.

(*d*) *Nitrous acid* destroyed activity in 2 hr. at room temperature but not in ½ hr. at 0°. For this reason, amino-N determinations were used in following the concentration of the factor.

(*e*) *Acetylation* with acetic anhydride in slightly alkaline solution removed almost all activity; this was not restored by standing in dilute alkali but was restored by acid hydrolysis.

(*f*) *Esterification.* Alcoholic HCl produced at stage 5 an ether-soluble substance with delayed activity which became fully active on hydrolysis. On distillation of the ether-soluble substance no activity was found in fractions of B.P. (bath temperatures) $< 100°/10$ mm. and very little $> 100°/0{\cdot}002$ mm.; the most active fraction was that of B.P. 95–100°/0·008 mm.

(*g*) *Salts.* Phosphotungstic acid partly precipitated the material, but flavianic and rhodanilic acids did not. Ca, Ba and Cu′ salts yielded no insoluble products in water or aqueous alcohol. Ag′ partly precipitated the material, and Cu″ salts, prepared according to Town [1936], were less soluble in methyl alcohol than was the original material.

Properties (*d*) and (*e*) are interpreted as probably due to an amino group, and (*f*) and the Cu″ salt (*g*) to a carboxylic acid; the whole, including the separation 2–5 above, is consistent with the material being an amino-acid. The loss of one half of the activity with alkali may be due to racemization. Recombination of separated fractions, performed in each test, gave no indication of separation of the active material into two fractions of which both were necessary for reversal.

Marmite and the concentrates (5) were active in similar concentrations against streptococci inhibited by $8 \times 10^{-7} M$ euflavine, and marmite reversed the inhibitions of *Bact. typhosum* caused by $8 \times 10^{-6} M$, and of *Staphylococcus aureus* caused by $1{\cdot}6 \times 10^{-6} M$ euflavine.

The basal medium (p. 1311) already contains those amino-acids of greatest importance in bacterial nutrition. A further mixture of amino-acids, containing glycine, alanine, valine, leucine, *iso*leucine, lysine, arginine, histidine, proline, hydroxyproline, phenylalanine, tyrosine, aspartic and glutamic acids was active, with delay, as Type II factor (Table 3). Individually most of the acids were inactive or deleterious and the effect of the mixture was due mainly to phenylalanine. The properties of phenylalanine would probably cause it to be present in the active concentrates described above and phenylalanine acts in the Type II manner, but the concentrates were more active weight for weight than was this amino-acid. The following derivatives of phenylalanine were inactive or slightly active: tyrosine, 3:4-dihydroxyphenylalanine (delayed activity owing to removal of acriflavine from solution), thyronine, thyroxine. Many less related compounds, and also most of the substances described as promoting growth of organisms, had little or no reversing activity.

Further reversal and an interpretation of Types I and II activity

Throughout this work it had been borne in mind that acriflavine might act by interfering with structurally related compounds of importance to the inhibited organisms. Reversal with riboflavin and with phenazine derivatives [cf. Weil-Malherbe, 1937] was accordingly tried at several stages, but these were without activity under the conditions recorded in Tables 2 and 3. In the presence of different sources of Type II factor they were however immediately active (Table 4) in reversing $6 \times 10^{-5} M$ euflavine. No increase in the concentration of Type II factor brought about such reversal. This activity was however not at all specific, being given by methylene blue, brilliant cresyl blue, to some extent by phenosafranine, as well as riboflavin, pyocyanine and phenazine methosulphate (Table 4). As in animal nutrition, riboflavin as a bacterial growth

Table 4. *Hydrogen carriers in presence of Type II factors*

Special feature of media	Substance added	Molarity	Growth after: Hours 16–18	Hours 24	Days 2	Days 3
Marmite treated with norite,* 10 mg./ml.	0	—	0	0	0	0
	Pyocyanine or phenazine methosulphate	2×10^{-5}	+ + +	+ + + +	+ + + +	+ + + +
		4×10^{-6}	0	+ + +	+ + + +	+ + + +
	Riboflavin	10^{-4}	+ + +	+ + + +	+ + + +	+ + + +
	,,	2×10^{-5}	0	+ + +	+ + + +	+ + + +
Al_2O_3 eluate, 0·27 mg./ml.	0	—	0	0	0	0
	Pyocyanine	2×10^{-5}	+ + + +	+ + + +	+ + + +	+ + + +
	,,	4×10^{-6}	0	0	+ + + +	+ + + +
	Brilliant cresyl blue	5×10^{-5}	+ + +	+ + + +	+ + + +	+ + + +
	,,	5×10^{-6}	0	0	+ + + +	+ + + +
	Riboflavin	10^{-4}	0	+ +	+ + + +	+ + + +
	Uninhibited control on both media		+ + +	+ + + +	+ + + +	+ + + +

Inhibiting concentration of euflavine, $6 \times 10^{-5} M$.

* Marmite (25 g.) in water (to 125 ml.) stirred with norite (5 g.) at room temperature for 1 hr., filtered and neutralized.

factor is reported as being fairly specific [Snell & Strong, 1939], though attempts at replacement by the above hydrogen carriers are not recorded. This was accordingly tried by omission of riboflavin from the medium used for growth of *Streptococcus haemolyticus* and substitution of a wide range of concentrations of the above compounds. Growth did not occur, but it could be caused by further addition of $10^{-9}M$ and more of riboflavin, with groups A and C streptococci. Even 6:7-dimethylalloxazine was inactive. The above dyestuffs are thus not replacing riboflavin, but all, including riboflavin, may be replacing a hydrogen transporting system inactivated by the higher concentration of inhibitor.

These experiments, in which inhibited organisms can function only in the presence of such artificial redox systems and of a Type II factor, are reminiscent of many experiments with bacterial suspensions in which hydrogen carriers are found necessary for enzyme activity in the presence of added substrates. When such additions are necessary for growth of inhibited organisms, but not at all for normal ones, it is considered that the inhibited organisms lack both a substrate and a normal hydrogen transporting system. Lack of substrate (need for Type II factor) occurs first at a lower euflavine concentration ($3 \times 10^{-5}M$) and is presumably due to disturbance of the systems involved in its formation, since organisms such as *Bact. coli* do not normally need it pre-formed, and it is produced by many other normal cells. Interference with metabolizing enzymes, replaceable by the artificial carriers, occurs at euflavine concentrations of $6 \times 10^{-5}M$.

Acriflavine is known to inactivate many enzyme systems [Scheff & Hasskó, 1936; Quastel & Wheatley, 1931], and the previous experiments with nucleotides provide a mechanism for this interference. It is imagined that acriflavine forms complex salts with similar groupings essential to enzyme systems, and that the complexes are inactive. There was no simple relation between the inhibitory concentration of euflavine and the reversing concentration of pyocyanine. In an experiment in which $4 \times 10^{-6}M$ pyocyanine allowed growth in the presence of $6 \times 10^{-5}M$ euflavine, up to $2 \times 10^{-4}M$ pyocyanine gave a delayed effect only with $8 \times 10^{-5}M$ euflavine. This is interpreted as the inactivation of still further systems, which are pictured as being affected successively in order of stability of their different acriflavine complexes. Results with redox systems apply to *Bact. coli* but not to *Strep. haemolyticus*. Pyocyanine, and to a lesser degree riboflavin, contributed also to the reversal of inhibition of *Bact. coli* caused by proflavine, rivanol and crystal violet in the presence of marmite preparations.

Discussion

The results of the present studies of inhibitory action bear some fairly close relation to those which Fildes [1940, 1] obtained with mercuric compounds. These compounds are imagined to inhibit by combination with essential —SH groups in the cell, and can be reversed by added thiol compounds. No analysis of natural products which might lead to Type II reversal was carried out by him.

The removal of acriflavine inhibition by methylene blue etc. is not novel, but has previously been termed an example of chemotherapeutic interference [see Findlay, 1939], the interpretation of which has been realized as of importance in understanding the action of drugs. The present explanation does not necessitate combination of the interfering substance with the same group ('receptor') in the cell as combines with the inhibitory agent [see Wright & Hirschfelder, 1930], but rather the short-circuiting of such a group.

It has been asserted that the action of acriflavine is little affected by the growth medium, and is irreversible. Such views are no longer tenable. Thus,

normal bacteriological technique compares growth in peptone water, broth or serum media, but does not contrast growth in simple chemically defined solutions with that in others containing nucleate. Again, in metabolic experiments, reversal has been attempted by washing the inhibitor from the affected tissue, but not always by the addition of antagonistic substances. It is possible to 'resurrect' inocula of *Bact. coli*, rendered apparently non-viable on ordinary media by allowing them to stand for 30 min. in *M*/200 euflavine, with Type I or II factors.

A structural relationship between inhibitor and reversing agent, useful in modelling further therapeutic substances, has not yet emerged from this work, and the extent of the present knowledge of the enzymes and complex salts considered to be involved in acriflavine inhibition makes understandable the empiricism of current chemotherapy. Published work makes it probable that styryl 430 [Pourbaix, 1939] and germanin (Fourneau 309, Baeyer 205) [Oesterlin, 1935] act also by similar combination with enzyme systems, though the present technique of analysis of new nutrient needs has not yet been applied to them. Results with crystal violet and with rivanol have already been mentioned; in addition, marmite preparations and nucleate contributed to reversal of *Bact. coli* and *Bact. typhosum* inhibited by suitable concentrations of rivanol. The weakest step in the present arguments is the assumption that reversing agents characterized in the above manner are those normally involved in the blocked processes of the inhibited organisms. This is rendered more likely by using a variety of natural sources of the factors, and the assumption is one made in most straightforward nutritional work. At the least, the results would have significance as examples of 'detoxication mechanisms'; an instance of such a finding in animal nutrition is vitamin H.

Summary

A chemotherapeutic agent is regarded as depriving the inhibited organism of the use of enzymes or metabolites by various types of interference. The organism thus becomes nutritionally more exacting than in its normal state, and its new demands can, with due consideration for extraneous effects, be analysed by the usual techniques of bacterial nutrition.

Bact. coli and *Streptococcus haemolyticus*, inhibited by acriflavine components, required for further growth two types of material not normally needed. Type I (so called from a previous nutritional study of inhibitory action) was best replaced by nucleotides, and Type II by a concentrate of amino-acids, but partly by phenylalanine. In the presence of Type II compounds, but not without, artificial hydrogen carriers were further active against inhibition of *Bact. coli*. Type I compounds form complex salts with acriflavine components, and it is considered that the inhibitors inactivate enzyme systems of which Type I compounds are essential parts, of which Type II compounds are substrates or products, and of which some can be replaced by the hydrogen carriers.

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